Supplemental methods

Histology

The Abs used for histology staining were as follows: anti-periostin serum, anti-proliferating cell nuclear antigen (PCNA) Ab (Dako), anti–phospho-Akt Ab (Cell Signaling), anti-cytokeratin 10 (CK10) Ab (Dako), anti-cytokeratin 14 (CK14) Ab (Chemicon International), anti-filaggrin Ab (Covance Research Products), and anti-human integrin $\alpha_v\beta_3$ Ab (Chemicon International).

Cells

Mouse keratinocytes and dermal fibroblasts were isolated from skin removed from the back and abdomen of 2-day-old mice. The skin was cut and treated with 2 U/ml dispase (Invitrogen) at room temperature for 2 h. After washing, the epidermis was peeled off from the dermis by forceps and gently stirred in 0.05% trypsin at 37°C for 2 min. The keratinocytes were prepared by filtration. Fibroblasts were isolated from the remaining dermis by trypsinization.

RT-PCR

For quantitative RT-PCR analysis, RNA of mouse ears and lymph nodes was isolated

using an RNeasy Mini kit (Qiagen), and the RT reaction was performed using QuantiTect Reverse Transcription Kit (Qiagen). Quantitative analysis was performed using the ABI PRISM™ 7700 sequence detection system (Perkin-Elmer, Japan). The primer and probe sequences are shown in Supplemental Table 2.

Measurement of serum periostin

SS18A (2 μg/ml) was incubated overnight at 25°C in 96-well plates (Thermo Fisher Scientific). After blocking with 0.5% casein in Tris-buffered saline, diluted (1/200) serum samples or recombinant periostin standards were incubated overnight at 25°C. Then peroxidase-labeled SS17B (50 ng/ml) was added followed by incubation for 1.5 h at 25°C. The signals were developed by the reaction solution (0.8 mM 3,3',5,5'-tetramethylbenzidine and 2.5 mM H_2O_2) and the different absorbances at 450 nm and 550 nm were evaluated. We performed the ELISA assay on duplicated samples.

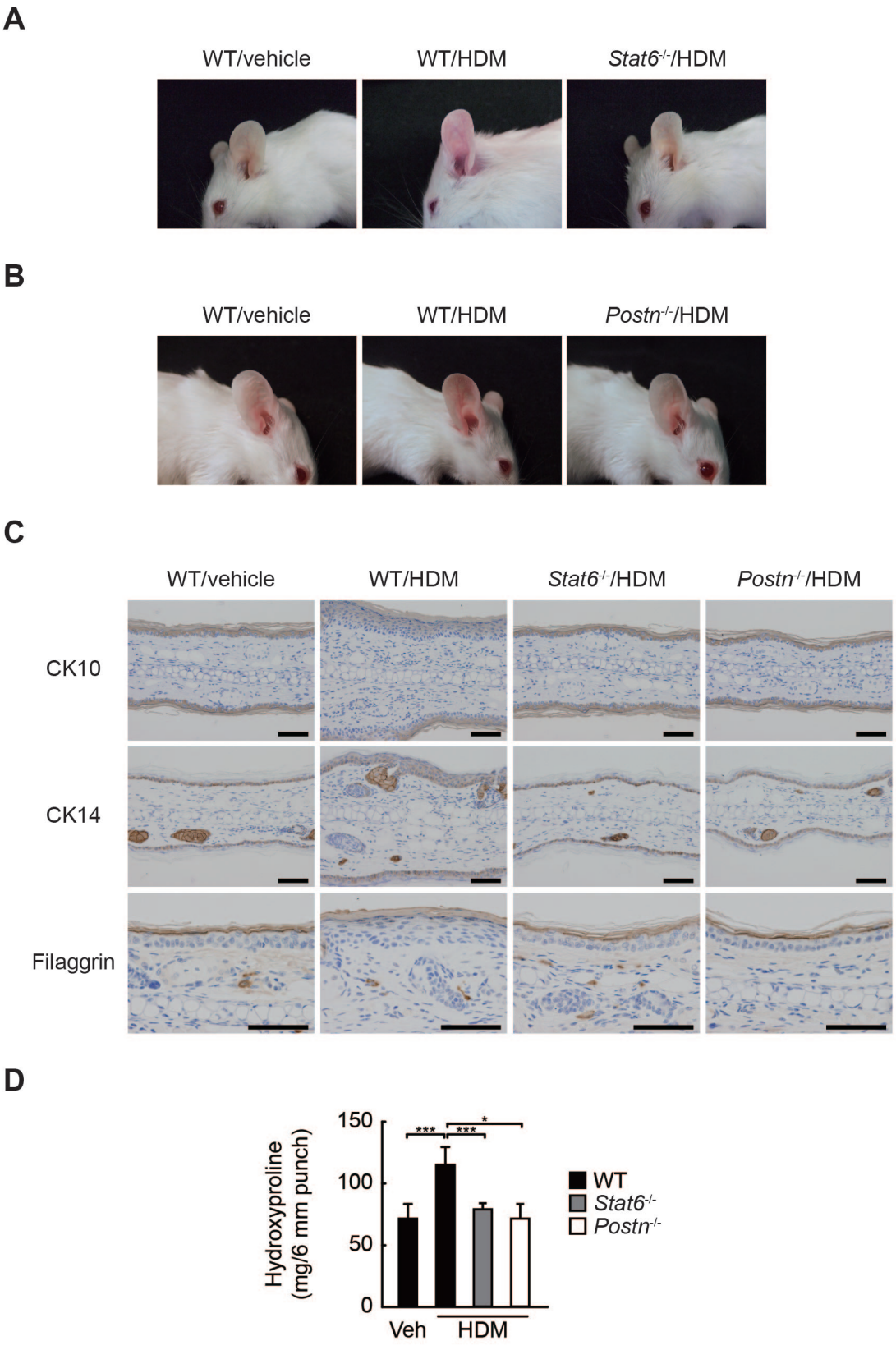
Supplemental table 1
Composion of hematopoietic cells in spleen and lymph nodes of wild-type and periostin knockout mice

	spleen		lymph nodes	
	wild type	periostin knockout	wild type	periostin knockout
total cell number	526 X 10E3	534 X 10E3		
dendritic cell	3.2%	2.8%	0.9%	0.8%
T cell	25%	29%	76%	76%
B cell	50%	48%	22%	21%
macrophage	1.6%	1.5%	n.d.	n.d.
c-kit+ cell	1.8%	1.8%	2.0%	1.4%
neutrophil	0.03%	0.03%	n.d.	n.d.
NK cell	3.9%	3.5%	n.d.	n.d.
NKT cell	0.73%	0.73%	n.d.	n.d.

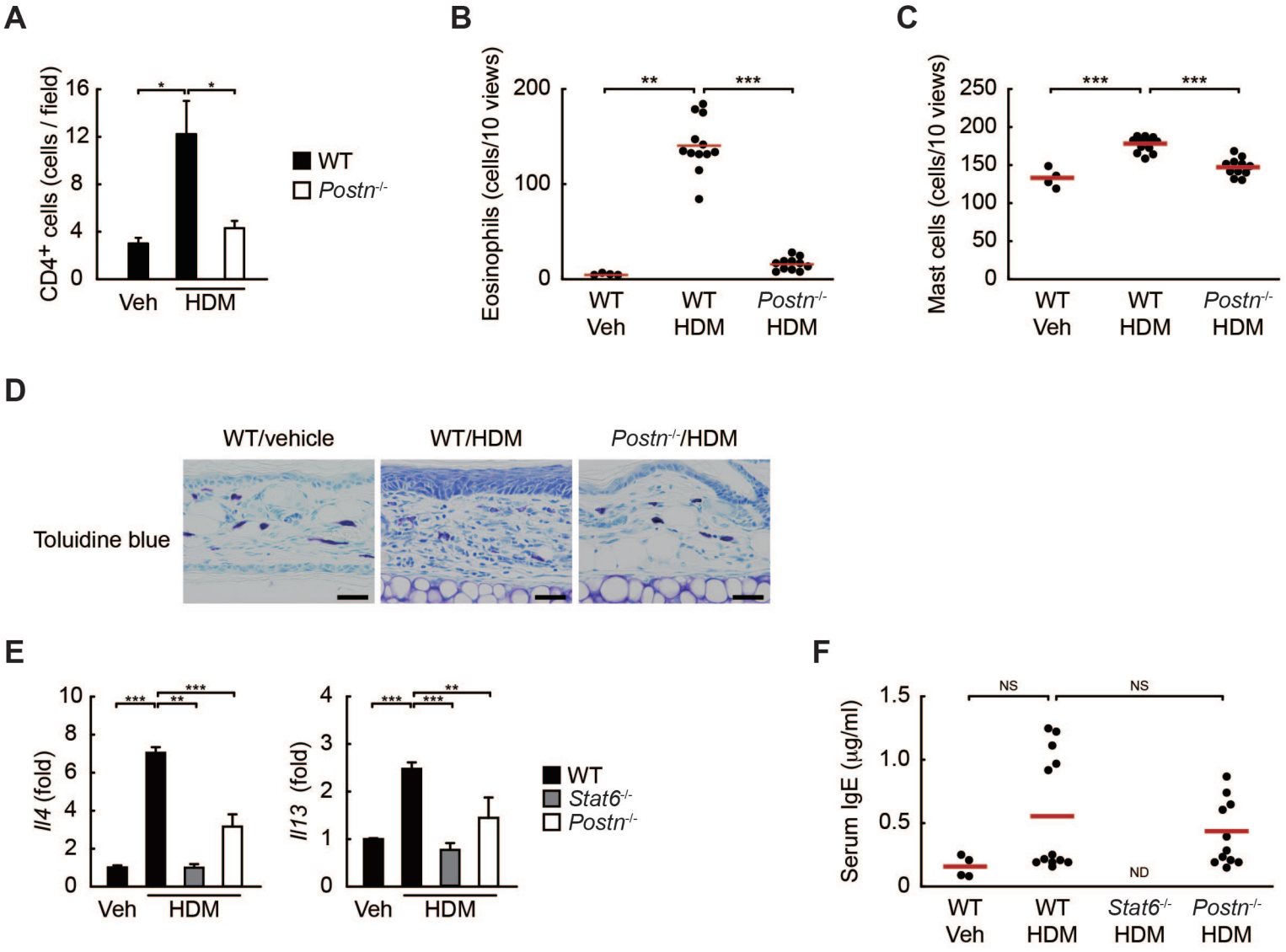
Supplemental table 2
Sequences of the promes and probes for PCR

	Forward	Reverse
POSTN	CTGCCAAACAAGTTATTGAGCTGGC	AATAATGTCCAGTCTCCAGGTTG
GAPDH	GCACCACCAACTGCTTAGCC	GATGCAGGGATGATGTTCTGG
Postn	TAGCCCAATTAGGCTTGGCATCC	TAAGAAGGCGTTGGTCCATGCT
Gapdh	GCACCACCAACTGCTTAGCC	GATGCAGGGATGATGTTCTGG
Itgav	GTGCCAGCCCATTGAGTTTGATTC	TTCACCACCATAGGGAGCAGCAAT
Itgb1	GTGACCCATTGCAAGGAGAAGGAC	GTCATGAATTATCATTAAAAGTTT
Itgb3	CTGGTGTTTACCACTGATGCCAAG	TGTTGAGGCAGGTGGCATTGAAGG
Itgb5	ACTTGGAGAACATCCGGAGC	TTGAAGCTGTCGACTCTGTC
Itgb6	GCTTGGCTCCCGGCTGGC	AGTTAATGGCAAAATGTGCT
Itgb8	ATGCACAATAATATAGAAAAA	TCCTTGTACCAATGAAACTG

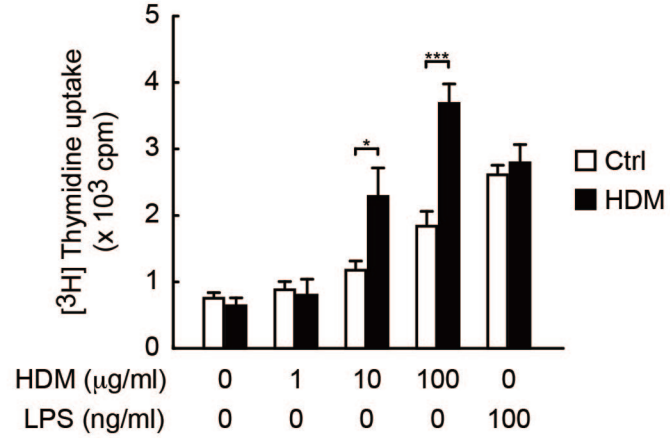
	Forward	Reverse	Probe
Postn	TAGCCCAATTAGGCTTGGCATCC	TAAGAAGGCGTTGGTCCATGCT	FAM-TAGCACCTGTGAACAATGCGTTCTCTGATG-TAMRA
Il4	GCTTTTCGATGCCTGGATTC	GCTTTCCAGGAAGTCTTTCAG	FAM-CGATAAGCTGCACCATGAATGAGTCCAA-TAMRA
Il13	CAGAGGCCATGCAATATCCTC	CAGCATGGTATGGAGTGTGGA	FAM-TAGCCCTGGATTCCCTGACCAACATCTC-TAMRA
Tslp	Applied biosystems	Mm00498739_m1	
Ccl17/Tarc	GAGCTGGTATAAGACCTCAGTGGAG	TGGCCTTCTTCACATGTTTGTC	FAM-TCTGACTGTCCAGGGCAAGCTCATCTGT-TAMRA
Ccl11/Eotaxin	CCCAACACACTACTGAAGAGCTACAA	TTTGCCCAACCTGGTCTTG	FAM-CAACAACAGATGCACCCTGAAAGCCA-TAMRA
Ifng	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCATG	FAM-TCACCATCCTTTTGCCAGTTCCTCCAG-TAMRA
Il17a	GCTCCAGAAGGCCCTCAGA	AGCTTTCCCTCCCCATTGA	FAM-CTCTCCACCGCAATGAAGACCCTGA-TAMRA
Actb	ACTATTGGCAACGAGCGGTTC	GGATGCCACAGGATTCCATACC	FAM-CCTGAGGCTCTTTTCCAGCCTTCCTTCT-TAMRA



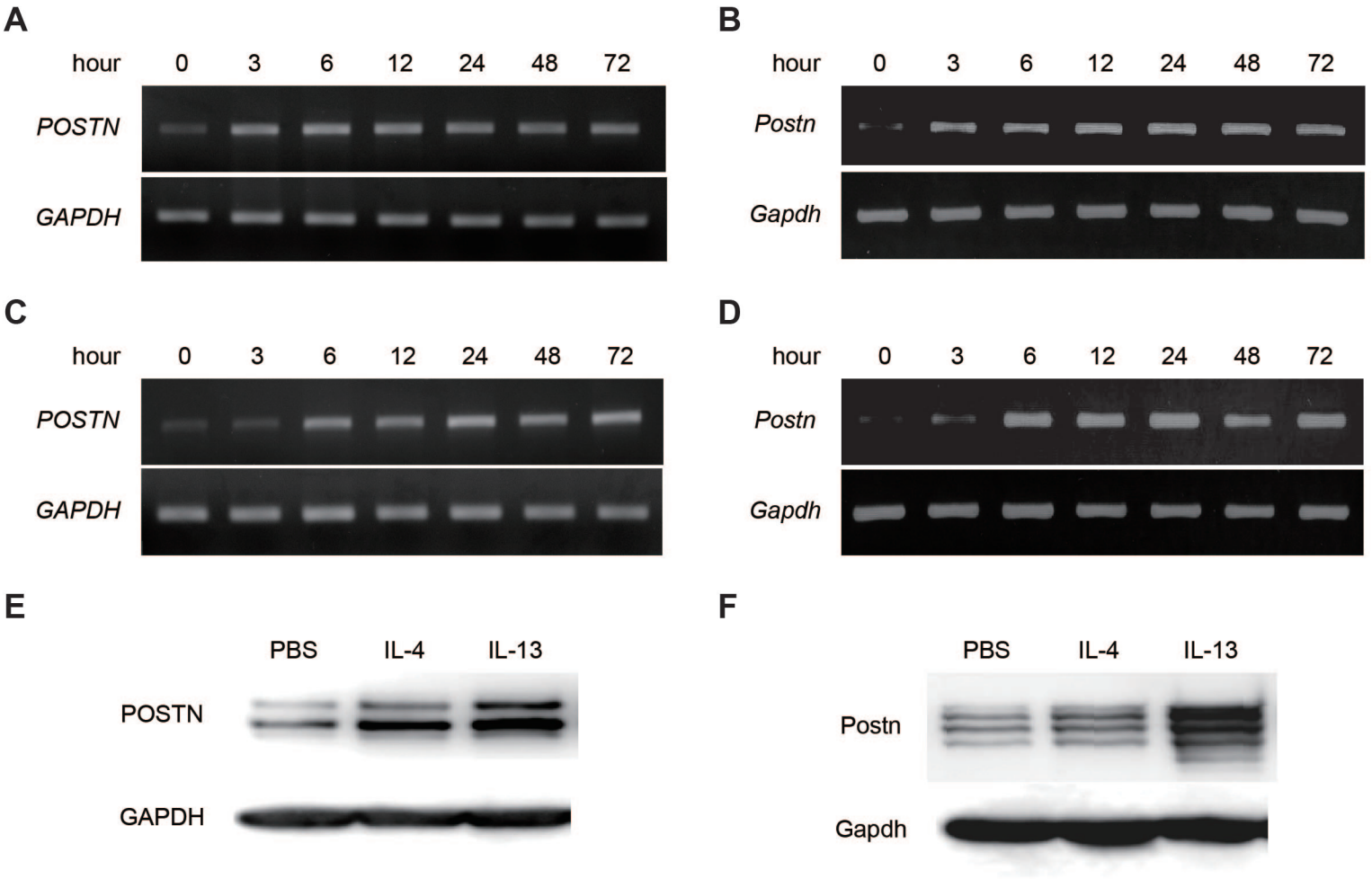
Supplemental Figure 1. Allergic skin inflammation is Stat6/periostin-dependent in model mice.
Mice were painted with HDM once a week for 7 weeks. (A, B) Macroscopic views of ears on day 50 in wild-type (WT), Stat6-deficient (*Stat6*$^{-/-}$), or periostin-deficient (*Postn*$^{-/-}$) mice treated with vehicle or HDM. (C) Skin tissues with immunostaining for CK10, CK14 or filaggrin. Scale bar, 50 μm. (D) Measurement of hydroxyproline in skin tissues on day 50 was performed according to Stegemann's procedure as modified by Woessner (Arch Biochem Biophys, 1961;93:440-447). *P < 0.05, ***P < 0.001.



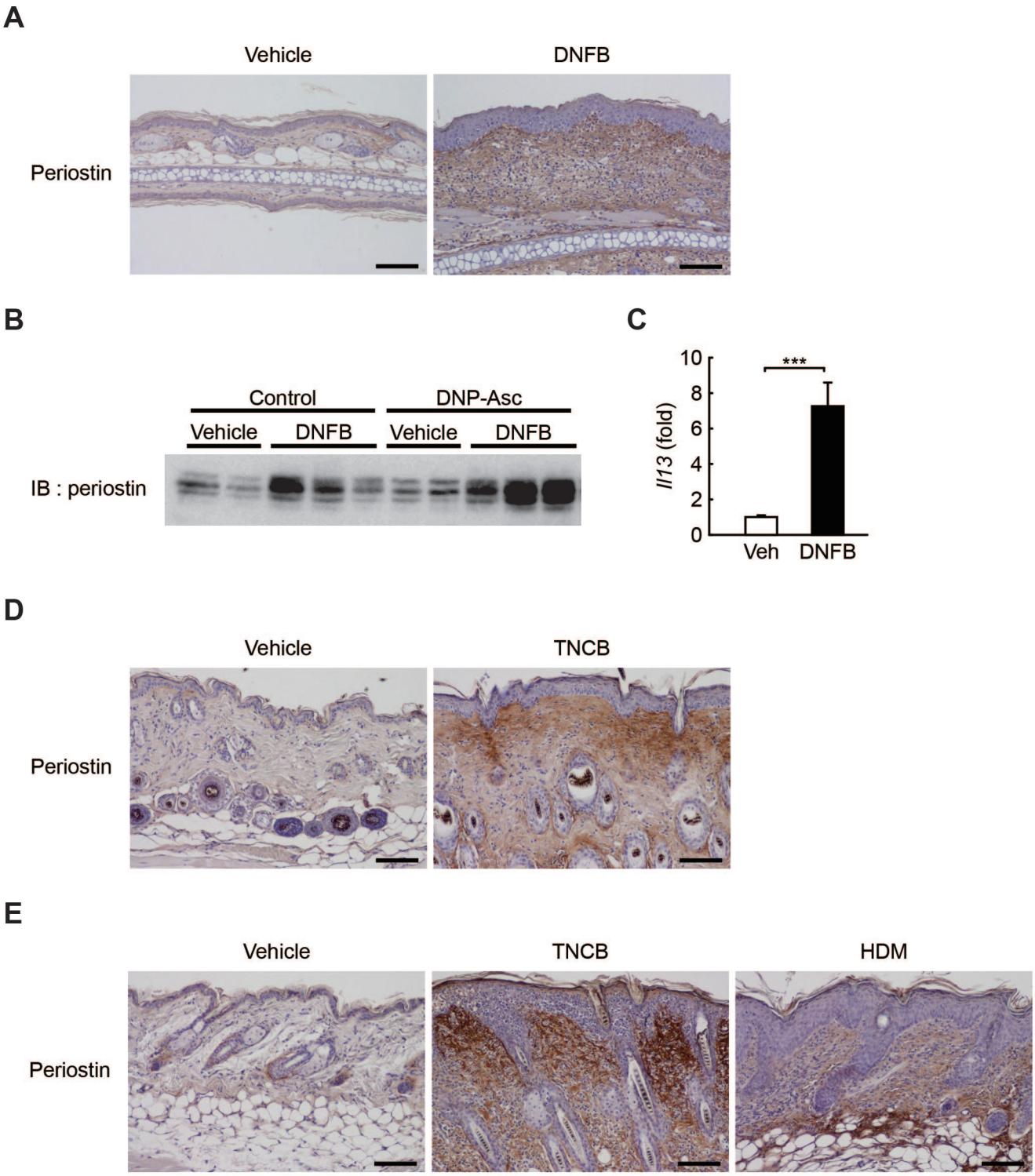
Supplemental Figure 2. Allergic skin inflammation is Stat6/periostin-dependent in model mice.
Mice were painted with HDM once a week for 7 weeks. The numbers of CD4+ cells (A), eosinophils (B), or mast cells (C) in the skin tissues on day 50. (D) Skin tissues stained with toluidine blue. Scale bar, 25 μm. (E) mRNA levels of the indicated cytokines in draining lymph nodes of each mouse on day 50. (F) Serum IgE levels on day 50 were analyzed with an ELISA kit for total IgE (Bethyl Laboratories). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. NS, not significant. ND, not detected.



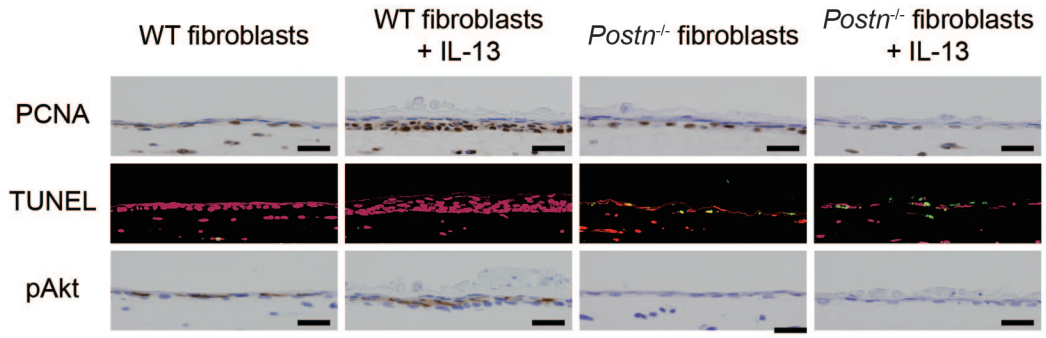
Supplemental Figure 3. Splenocytes of HDM-treated mice respond to HDM *in vitro*.
The ears of BALB/c mice were painted with vehicle or HDM at day 0 and 7. Splenocytes were harvested at day 8 and 2.0 × 10^5 cells were incubated with the indicated concentration of HDM or LPS. After two days culture, cells were pulsed with [^{3}H]thymidine for 4 hours and the [^{3}H]thymidine incorporation were measured. *P < 0.05, ***P < 0.001.



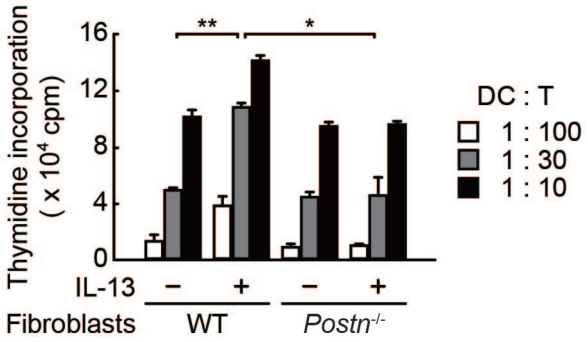
Supplemental Figure 4. Periostin is induced by IL-4 or IL-13 in dermal fibroblasts *in vitro*.
Human skin fibroblasts (A, C, and E) or mouse dermal fibroblasts isolated from skin of 2-day-old BALB/c mice (B, D, and F) were stimulated with IL-13 (A and B) or IL-4 (C and D). Expression of mRNA of periostin and GAPDH at the indicated times were analyzed with RT-PCR (A-D). The amount of periostin in supernatant or GAPDH in cell lysates 24 hours after the indicated stimulation were analyzed with Western blotting analysis (E and F).



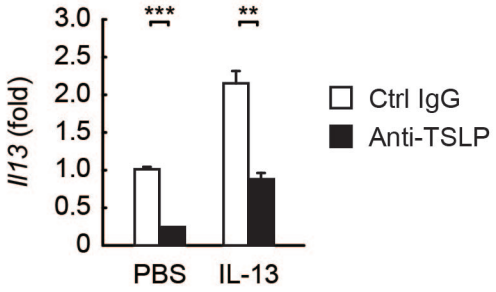
Supplemental Figure 5. Periostin accumulates in the dermis of Hapten- or HDM-induced model mice of allergic skin inflammation.
(A-C,) BALB/c mice were immunized by injecting intraperitoneally with 10 μg of 2,4-dinitrophenylated Ascaris Extract (LSL, Tokyo) mixed with 1 mg of alum on day 0. The Mice were challenged with 25 μl of 0.15% 2, 4-dinitrofluorobenzene (DNFB, Sigma) acetone solution onto each surface of both ears 5 times every other day from day 14 (days 14, 16, 18, 20, 22).The mice received a final DNFB challenge similarly on day 36 and the ear specimens harvested 24 hours after the final challenge were subjected to immunostaining for periostin (A), Western blotting analysis (B) and quantitative RT-PCR analysis (C). (D-E) Shaved back skin of C57BL/6NCrj mice (D) or NC/Nga mice (E) were painted every other day (days 0, 2, 4, 6, 8, 10, 12) with 2,4,6-trinitrochlorobenzene (TNCB, Sigma) solution of 50 μl or twice a week with HDM (Biostir, Japan) for 4 weeks. The skin samples were harvested 24 hours after the final painting and subjected to immunostaining for periostin. Scale bar, 100 μm. ***$P < 0.001$.



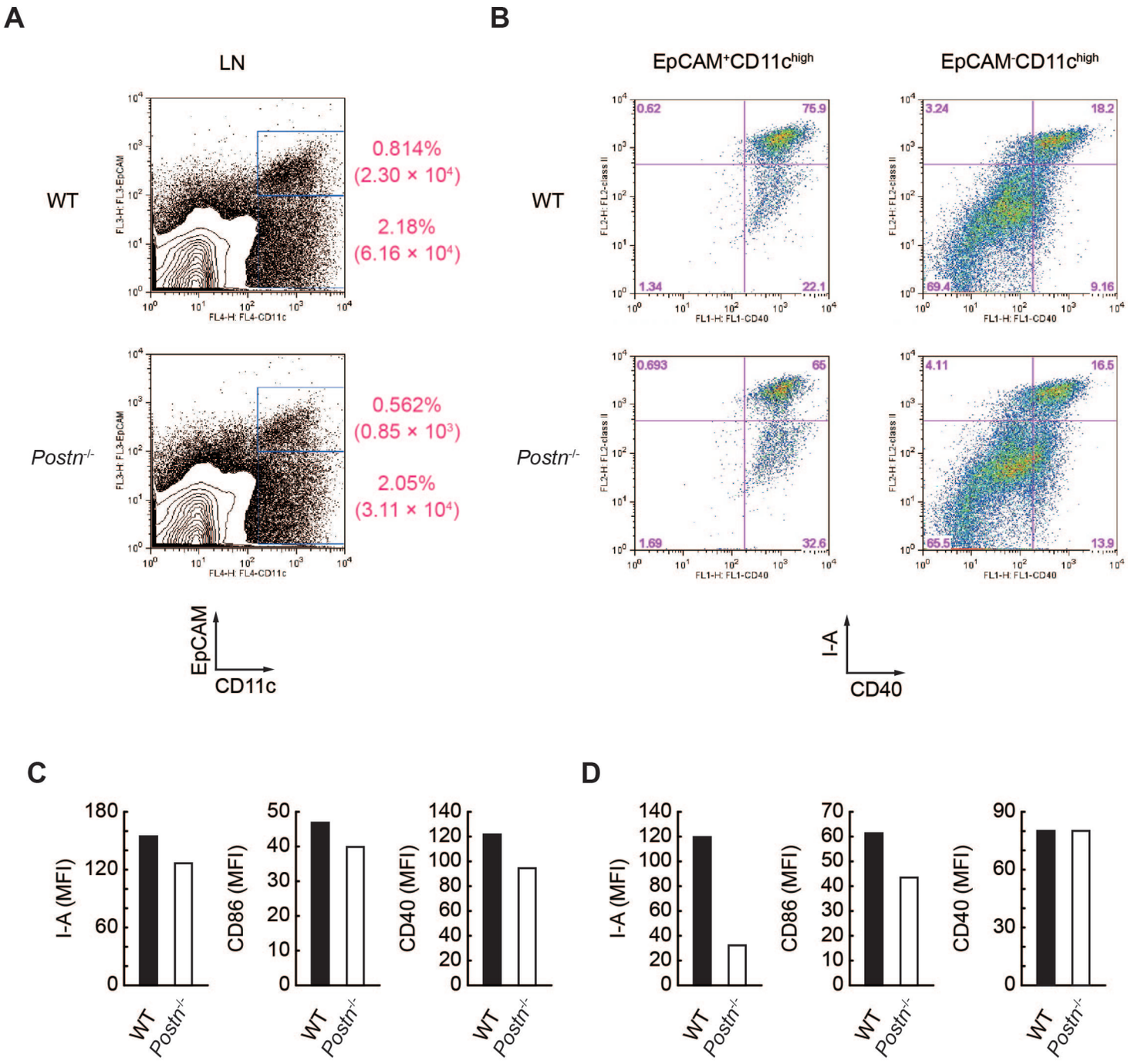
Supplemental Figure 6. Enhancement of proliferation and survival of keratinocytes by periostin in a 3-dimensional organotypic coculture system.
Keratinocytes were coculture with periostin-deficient (*Postn*$^{-/-}$) mouse-derived or the littermate control (WT) mouse-derived fibroblasts (2.0×10^5 cells) in the presence or absence of 10 ng/ml IL-13 for 7 days. Representative photomicrographs of TUNEL staining, immunostaining for PCNA, or immunostaining for phospho-Akt are shown. Scale bar, 25 μm.



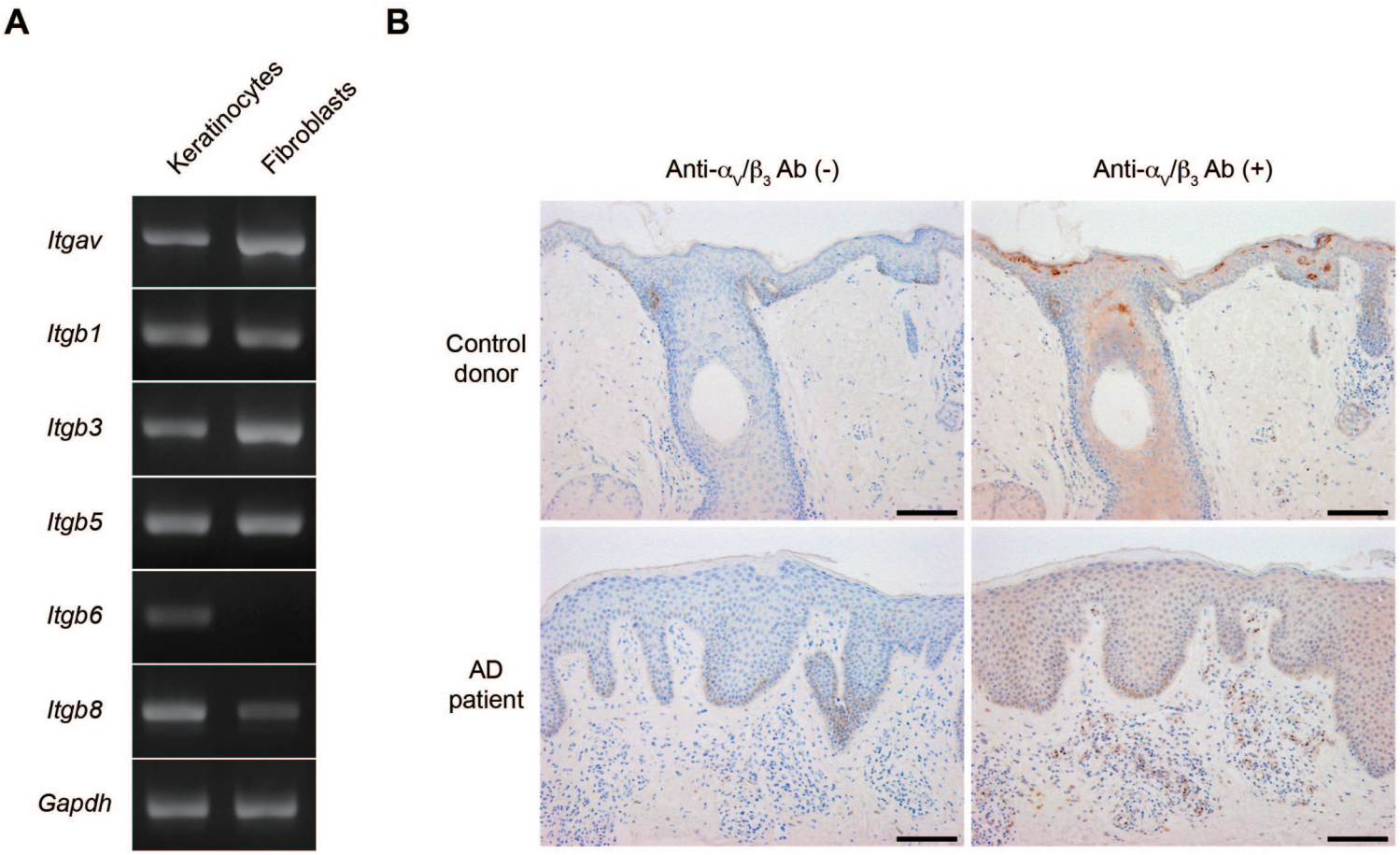
Supplemental Figure 7. Induction of proliferation of naive T cells by periostin-activated keratinocytes via DCs. Allogeneic $CD4^+$ T cells were cocultured with the indicated ratio of BMDCs conditioned by the supernatants from 3-dimensional organotypic coculture of keratinocytes with periostin-deficient (*Postn*$^{-/-}$) mouse-derived or their littermate control (WT) mouse-derived fibroblasts in the presence or absence of IL-13. [^{3}H]thymidine incorporation is shown. *P < 0.05, **P < 0.01.



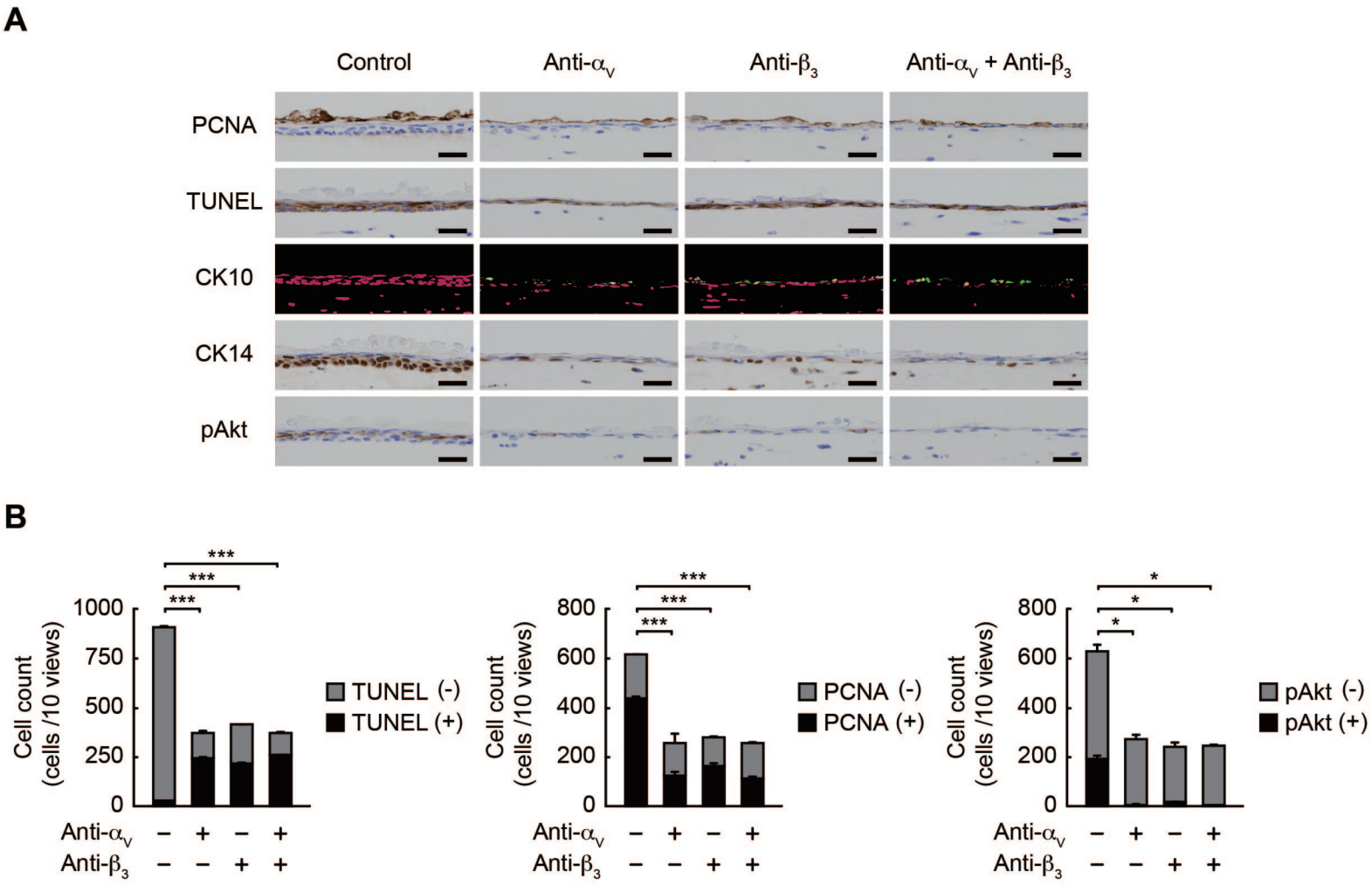
Supplemental Figure 8. Neutralizing anti-TSLP antibodies partially inhibit Th2 skewing induced by the conditioned medium of keratinocytes cocultured with fibroblasts.
BMDCs treated with each KCM with control antibody or anti-TSLP antibody (R&D Systems) were mixed with $CD4^+$ T cells at a ratio of 1:100. Cells were harvested 5 days after culture and subjected to quantitative RT-PCR analysis of IL-13 mRNA. $^{**}P < 0.01$, $^{***}P < 0.001$.



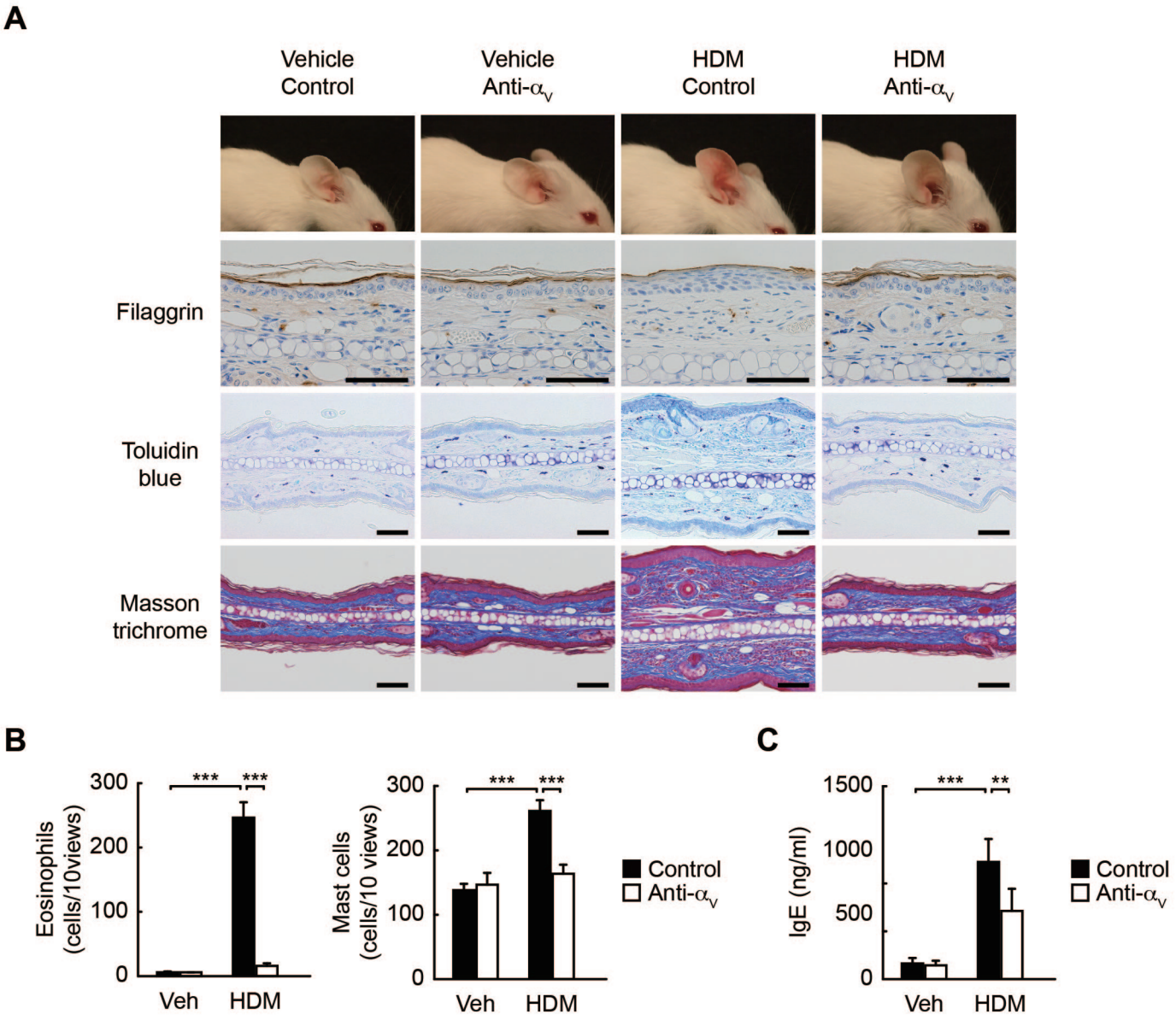
Supplemental Figure 9. The activation of skin DCs by HDM-sensitization partially depends on periostin. Periostin-deficient mouse (*Postn*$^{-/-}$) or the littermate control mouse (WT) was painted with HDM once a week for 2 weeks (day 0, 7, 14). Flow cytometric analysis of draining lymph nodes or skin cell suspensions from HDM-sensitized mice was performed 24 h after last sensitization. Skin cell suspensions were pretreated with Fc blocker (2.4G2) and stained with FITC-conjugated anti-CD40 (HM40-3, eBioscience), PE-conjugated anti-I-A (M5/114 15.2, eBioscience), biotin-conjugated anti-EpCAM (G8.8, BioLegend) or biotin-conjugated anti-CD86 (GL1, eBioscience), APC-conjugated anti-CD11c (N418, eBioscience), and SAv-PerCP-Cy5.5 (BD Pharmingen). (A) Numbers and those in parenthesis on the right side of outlined areas indicate percent cells and absolute numbers per draining lymph nodes from the indicated mouse, respectively. (B) Expression of MHC class II (I-A) and CD40, gated with EpCAM^{+}CD11c^{high} (Langerhans cells) or EpCAM^{-}CD11c^{high} (dermal DCs). Numbers in quadrants indicate percent cells in each. (C, D) Geometric mean fluorescence intensity (MFI) of MHC class II, CD86, and CD40 of CD11c^{high} cells from draining lymph nodes (C) or skin cell suspensions (D) was calculated with FlowJo software. Each result indicated in this figure is representative of two experiments.



Supplemental Figure 10. Expression of integrin on keratinocytes and skin tissues.
(A) Expression of mRNA of integrin α_V and β_1, β_3, β_5, β_6, and β_8 and GAPDH in mouse keratinocytes and dermal fibroblasts. (B) Skin histology of atopic dermatitis patient and normal donor with immunostaining for $\alpha_V\beta_3$ integrin. Scale bar, 100 μm.

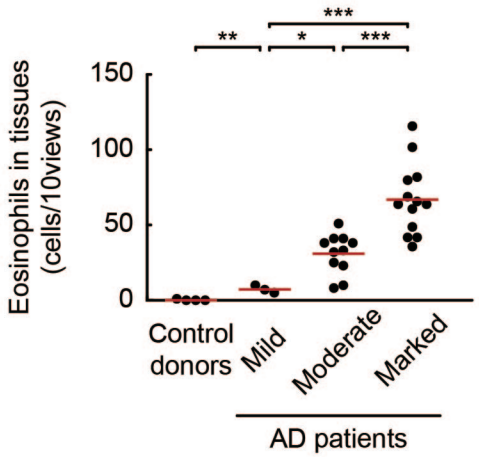


Supplemental Figure 11. Proliferation, survival, and differentiation of keratinocytes in a 3-dimensional organotypic coculture system are dependent on the integrin/periostin interaction.
Keratinocytes were cocultured with control mouse-derived fibroblasts in the presence of IL-13 and the indicated Ab for 7 days. (A) Representative photomicrographs of TUNEL staining or immunostaining for CK10, CK14, PCNA, or phospho-Akt are shown. Scale bar, 25 μm. (B) The numbers of TUNEL-, PCNA-, or phospho Akt-positive/negative cells were counted in 10 views by 200-fold magnitutes for each section. **P* < 0.05, ****P* < 0.001.

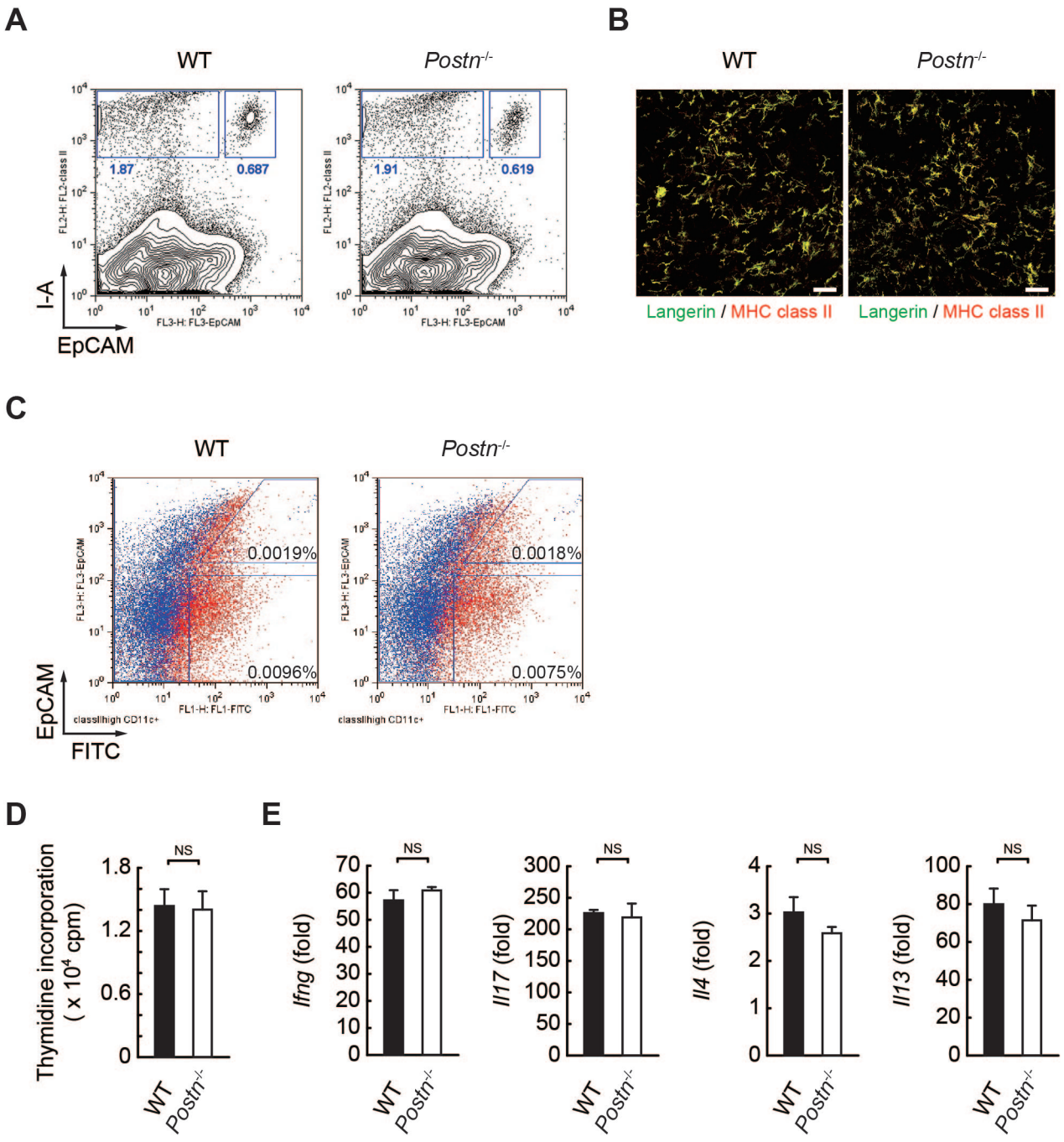


Supplemental Figure 12. Th2-type inflammation in HDM-sensitized mice is dependent on the integrin/periostin interaction.

BALB/c mice were painted with HDM once a week for 4 weeks and injected subcutaneously with the indicated Ab twice a week. (A) Representative photomicrographs of immunostaining for filaggrin, toluidine blue staining, or Masson trichrome staining of ears on day 29 are shown. Scale bar, 50 μm. (B) The numbers of eosinophils or mast cells in ear skin tissues on day 29 are shown. (C) Serum IgE level of the mice on day 29 are measured with ELISA. $^{**}P < 0.01$, $^{***}P < 0.001$.



Supplemental Figure 13. High expression of periostin in AD patients is correlated with the numbers of infiltrated eosinophils.
The numbers of eosinophils in the dermis in healthy controls or patients (periostin expression level: mild, n = 3; moderate, n = 11; marked, n = 13) were counted in 10 views by 400-fold magnitude for each sample. Red lines represent mean. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



Supplemental Figure 14. The defect of Skin DCs were not observed in periostin-deficient mouse in steady state. (A) Whole skin cell suspension of periostin-deficient mouse (*Postn*$^{-/-}$) or the littermate control mouse was prepared by incubating split ears with 2.6 units/ml Liberase TL (Roche) for 60 minutes at 37°C after removal of cartilage. Cell suspensions were pretreated with Fc blocker (2.4G2) and stained with PE-conjugated anti-I-A (M5/114 15.2, eBioscience), biotin-conjugated anti-EpCAM (G8.8, BioLegend), APC-conjugated anti-CD11c (N418, eBioscience), and SAv-PerCP-Cy5.5 (BD Pharmingen). Numbers in outlined areas indicate percent cells of EpCAM^{+}I-A^{high} (Langerhans cells) or EpCAM^{-}I-A^{high} (dermal DCs). (B) Epidermal sheets were separated from each mouse and stained with MHC class II (red) and Langerin (green) to visualize Langerhans cells. Scale bar, 100 μm. (C) WT mouse or *Postn*$^{-/-}$ mouse was painted on ears with 20 ml of 1% FITC (Sigma) dissolved in a 1:1 (v/v) acetone / dibutyl phthalate (Sigma) mixture and the number of migrated cutaneous DCs into draining lymph nodes 24 hours after painting was enumerated by flow cytometry. Red and blue dots indicate CD11c^{high}I-A^{high} cells from FITC-treated WT or *Postn*$^{-/-}$ mouse and vehicle-treated WT mouse, respectively. Numbers in outlined areas indicate percent cells in each. (D, E) 5.0 × 10^3 skin DCs prepared from ears of each mouse using MACS column with CD11c beads (Miltenyi Biotec) were cocultured with 2.0 × 10^5 allogeneic CD4$^+$ T cells. [^{3}H]thymidine incorporation on day 3 (D) and mRNA levels of IFN-γ, IL-17, IL-4, or IL-13 in CD4$^+$ T cells stimulated with PMA and ionomycin for 4 hours (E) are shown. NS, not significant.