**Supplementary Material**

**Congenital Deficiency of Conventional Dendritic Cells Promotes the Development of Atopic Dermatitis-Like Inflammation**

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**SUPPLEMENTARY MATERIALS AND METHODS**

**Tissue and Cell Isolation**

Leukocytes were prepared from Spl, EDLNs, and BM as described previously (4, 5, 9, 33-37). In brief, Spl and EDLNs were digested with 400 U/ml collagenase type III (Worthington Biochemical) at 37 °C for 20 to 30 min, and were ground between glass slides. BM cells were flushed from the femurs and tibias. Cell suspensions of Spl and BM were further treated with RBC lysis buffer (Sigma-Aldrich). Ear skin tissue was obtained by separating the dermal sheets of the ear and digesting with 400 U/ml collagenase type III at 37 °C for 60 min and cut into pieces. Epidermis was obtained by separating the dermal sheets of the ear and digesting in 0.1% Trypsin-EDTA (Gibco) and 10 mg/ml DNase (Roche) at 37 °C for 50 min. Single-cell suspensions were obtained by forcing through a 40- or 100-μm cell strainer (BD Biosciences). CD4+ T cells or CD8+ T cells were purified from splenocytes of B6.CD45.1+OT-II mice (CD45.1+Vα2+OT-II CD4+ T cells) or B6.CD45.1+OT-I mice (CD45.1+Vα2+OT-I CD8+ T cells) with mouse CD4 T lymphocyte Enrichment Set-DM and (BD Biosciences) or mouse CD8 T lymphocyte Enrichment Set-DM (BD Biosciences).

**Flow Cytometry**

Cells were stained with fluorescein-conjugated monoclonal Abs (mAbs) listed in Table S1. For the intracellular expression of cytokines (4, 5, 9, 33-37), cells were incubated for 4 hrs with phorbol 12-myristate 13-acetate (PMA, 50 ng/ml; Sigma-Aldrich) and ionomycin (IoM, 500 ng/ml; Sigma-Aldrich) plus GolgiPlug (BD Biosciences) during the final 2 hrs. Subsequently, the cells were resuspended in Fixation-Permeabilization solution (eBiosciences) and intracellular cytokine staining was carried out according to the manufacturer's directions. Fluorescence staining was analyzed with a FACSVers flow cytometer (BD Biosciences) and FlowJo software (Tree star).
**Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)**

Total RNA from cells was extracted by using ISOGEN II (Nippon Gene) and the first-strand complementary DNA (cDNA) was synthesized from 100 ng of total RNA with oligo(dT)20 primer using the PrimeScript RT Master Mix (Takara) according to the manufacturer’s instructions. Transcriptional expression levels were analyzed as described previously (9, 36) by using SYBR® Premix Ex Taq II on Thermal Cycler Dice (Takara) with specific primer pairs listed in TABLE S1 after normalization for bactin expression.

**Measurement of Serum Ig, Fms-Related Tyrosine Kinase 3 Ligand (Flt3L), and Cytokines**

Serum total IgG and total IgE were assayed by using Mouse IgG enzyme-linked immunosorbent assay (ELISA) kit (Bethyl Laboratories) and Mouse IgE ELISA MAX kit (BioLegend). Serum Flt3L was measured by using Mouse/Rat Flt-3L Ligand/FLT3L Quantikine ELISA kit (R&D Systems). Serum TNF-α, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17A, and IL-22 were analyzed by flow cytometry using LEGENDplex™ MU Th Cytokine Panel (13-plex) (BioLegend). All experiments were performed according to the manufacturer’s instructions.

**AD-Like Inflammation**

To induce AD-like skin lesions (13, 15, 38-40), mice received topical application of 2 nmol of MC903 (calcipotriol, Sigma-Aldrich) in 20 µl of ethanol (vehicle) on the left ear skin for five consecutive days a week followed by two consecutive days a week during last week for 16 days. As vehicle control, the same amount of ethanol was applied. The development of AD-like inflammation was determined by ear thickness at every day after topical application with MC903 for 16 days using digital calipers (PK-1012CPX; Mitsutoyo) (9, 37). In some experiments, Spl, EDLNs, and ear were obtained from the mice before or after the first topical application of MC903 at 16 days. For the improvement of the compromised skin barrier function, petrolatum (Wako Pure Chemicals) was topically applied twice daily at the days of MC903 application to the ventral and dorsal portions of the ear for the duration of the treatment with MC903. For antibiotic treatment, mice were treated topically with 1% Acuatim ointment (Nadifloxacin, Otsuka) on the ventral and dorsal portions of the ear twice daily at the days of MC903 application for the duration of the treatment with MC903.

**Adoptive Transfer**

For Ag-specific priming of CD4+ T cells or CD8+ T cells in vivo(4,33-36), CD45.1+OT-II CD4+ T cells or CD45.1+OT-I CD8+ T cells were labeled with eFluor™ 670 (Thermo Fisher Scientific ; 2.5 µM) at 37 °C for 10 min, and washed twice with cold PBS. Subsequently, eFluor™ 670-labeled CD45.1+OT-II CD4+ T cells or CD45.1+OT-I CD8+ T cells (each 5x10⁶/mouse) were intravenously (i.v.) injected into mice 24 hrs before intraperitoneal (i.p.) injection with OVA protein (A5503, Sigma-Aldrich). After 3 days, the gated CD45.1+OT-II CD4+ T cells or CD45.1+OT-I CD8+ T cells
in Spl and EDLNs were analyzed for eFluor™ 670 dilution to detect the dividing cells by flow cytometry.

**Histopathologic Assessment**

Tissues from the ear were fixed with 4% paraformaldehyde (PFA) in PBS and embedded in paraffin. The tissue sections (5 µm thickness) were stained with H&E and toluidine blue to detect MCs. The stained slides were examined with a bright-field microscopy (BX53; Olympus). The areas of the epidermis and dermis of ear were quantified by thickness using ImageJ (National Institutes of Health) by a blinded observer as described previously (9, 37).

**Immunohistochemical Analysis**

Spl and ear skin were embedded in OCT compound (Sakura Finetec) and frozen in liquid N₂. Frozen section (5 µm) was fixed with cold acetone, and blocked with PBS containing 5% of normal rat serum and Avidin/Biotin Blocking Kit (Vector laboratories). Subsequently, slide of Spl was stained with Alexa Fluor 488-conjugated anti-B220/CD45R mAb, phycoerythrin (PE)-eFluor610-conjugated anti-CD11c mAb (eBiosciences), allophycocyanin (APC)-conjugated anti-CD3ε mAb (BioLegend), and mounted with ProLong Diamond (Thermo Fisher Scientific). Alternatively, slide of skin was stained with PE-conjugated anti-CD207 mAb (BioLegend) followed by horseradish peroxidase (HRP)-labeled goat anti-rat IgG. HRP-sites were visualized by fluorescein isothiocyanate (FITC)-tyramide conjugates and counterstained with DAPI (Invitrogen). The stained slides were analyzed with BZ-X710 fluorescence microscope (KEYENCE, Osaka, Japan).

**Bacterial Culture**

To measure CFU of *S. aureus* (16), the homogenates of ear skin were placed into 1 ml of PBS, and serial dilutions were prepared. 100 µl of each dilution was transferred on Mannitol salt agar with egg yolk plates (BD), and colonies were counted after 2-day incubation. CFUs were calculated by dividing the number of colonies per plate by the dilution factor.
### TABLE S1. mAbs for flow cytometry

| Name      | Supplier       | clone     | conjugate                  |
|-----------|----------------|-----------|----------------------------|
| CD3ε      | BD             | 145-2C11  | BV421,BV510                |
| CD3ε      | Biologend      | 145-2C11  | PerCP-Cy5.5,APC,PE-Cy7     |
| CD4       | BD             | RM4-5     | PE,PE-Cy7                  |
| CD8α      | BD             | 53-6.7    | FITC,PE,APC                |
| CD8α      | Biologend      | 53-6.7    | APC-Cy7                    |
| CD11b     | BD             | M1/70     | PE-Cy7,BV510               |
| CD11b     | Biologend      | M1/70     | FITC                       |
| CD11c     | BD             | HL3       | FITC,APC,BV421             |
| CD19      | BD             | 1D3       | FITC                       |
| CD19      | Biologend      | 6D5       | BV510                      |
| CD44      | BD             | IM7       | BV510                      |
| CD45      | Biologend      | 30-F11    | FITC,APC-Cy7               |
| CD45R/B220| BD             | RA3-6B2   | Alexa-488                  |
| CD45R/B220| Biologend      | RA3-6B2   | PE-Cy7,APC-Cy7             |
| CD45.1    | BD             | A20       | FITC                       |
| CD45.2    | Biologend      | 104       | APC-Cy7                    |
| CD45.2    | Tonbo Biosciences | 104     | violetFluor 450            |
| CD49b     | BD             | DX5       | FITC,APC                   |
| CD90.2    | Biologend      | 6D5       | BV510                      |
| CD103     | BD             | M290      | APC                        |
| CD117     | BD             | 2B8       | PE-Cy7                     |
| CD127     | Biologend      | A7R34     | PE                         |
| CD138     | Miltenyi       | REA104    | PE                         |
| CD207     | Biologend      | 4C7       | PE                         |
| CD335     | Biologend      | 29A1.4    | PE-Cy7                     |
| CXCR5     | Biologend      | L138D7    | APC                        |
| Fas       | BD             | Jo2       | PE                         |
| FcεRIα     | eBioscience    | MAR-1     | PE,APC                     |
| Foxp3     | eBioscience    | FJK-16s   | APC                        |
| Gata-3     | eBioscience    | TWAJ      | PerCP-eFluor710            |
| GL7       | Biologend      | GL7       | Pacific Blue               |
| Gr-1       | Biologend      | RB6-8C5   | FITC                       |
| IFN-γ      | eBioscience    | XMG1.2    | APC                        |
| IgE       | Biologend      | RME-1     | FITC                       |
| IgG1      | BD             | A85-1     | APC                        |
| IgM       | Biologend      | RMM-1     | APC                        |
| IL-4       | Biologend      | 11B11     | Alexa-488                  |
| IL-5       | Biologend      | TRFK5     | BV421                      |
| IL-13      | eBioscience    | eBio13A   | PE                         |
| IL-17A     | BD             | TC11-18H10| PE                         |
| I-A/I-E    | Biologend      | M5/114.15.2| APC-Cy7,BV510             |
| Ly6G       | Biologend      | IA8       | FITC                       |
| NK1.1      | BD             | PK136     | FITC                       |
| PD-1       | BD             | J43       | PE                         |
| RORγt      | BD             | Q31-378   | BV421                      |
| Siglec-F   | Miltenyi       | REA798    | PE                         |
| Siglec-H   | Biologend      | 551       | PE                         |
| ST2        | BD             | U29-93    | BV421                      |
| T-bet      | Biologend      | 4B10      | PE                         |
| TCRβ       | Biologend      | GL3       | PE                         |
| TCR γδ     | Biologend      | GL3       | APC,BV421                  |
| TER-119    | Biologend      | TER-119   | FITC                       |
| RT-PCR primer | Sequence                      |
|--------------|-------------------------------|
| bactin       | F-5’-ATCATTTGCTCCTCCTGAGCG-3’ |
|              | R-5’-GCTGATCCATGCCTGCTGGAA-3’ |
| Filaggrin    | F-5’-GCTGAAAGGAACCTCCTGAAAG-3’|   |
|              | R-5’-GCCAACTTTGAATACCATGGAAG-3’|   |
| Il4          | F-5’-GGCATTGGAACGAGTGCAC-3’   |
|              | R-5’-AAATATCGAAGCACTCTTG-3’   |
| Il5          | F-5’-AGCACAGTTGGAAGAGAGACTT-3’|
|              | R-5’-TCCAATGCAAGCTGTTGATT-3’  |
| Il13         | F-5’-GGAGCTAGCAACATCACCACA-3’ |
|              | R-5’-GGTTCCTGATGAGATGCACTG-3’ |
| Il17a        | F-5’-CTGCTGAGCCTGCGGCTAC-3’   |
|              | R-5’-CATTGGCGGAGAGACGCTGG-3’  |
| Il22         | F-5’-CAGCTCTGCTAGCTACGCCT-3’  |
|              | R-5’-AGGTCCAGTCTCCCAATCGCT-3’ |
| Il25         | F-5’-ACAGGAGCTTGAATCGGGTC-3’  |
|              | R-5’-TGGTAAAGTGGGACGGAGTTG-3’ |
| Il31         | F-5’-ACAAGGGACTTGAATCGGGTC-3’ |
|              | R-5’-GATTCTCAGTATTTCCAGGCA-3’ |
| Il33         | F-5’-GCTGCTGATCCTGACACATT-3’  |
|              | R-5’-CACCTGGCTTGGGCTTGGGT-3’  |
| Loricrin     | F-5’-CACATCACGCACCTCTCTCC-3’  |
|              | R-5’-CTCTCTCCACAGGGATCTCC-3’  |
| Reg3g        | F-5’-TTCCTGCTCCTCCTGATCAG-3’  |
|              | R-5’-CATCCACCTCCTGTTGGGTC-3’  |
| Saa1         | F-5’-GGTCACGAGCTTCCTCCAAG-3’  |
|              | R-5’-CCCGAGCAAGGAAATATTT-3’   |
| Saa2         | F-5’-TGCAGAGAGAGTTCAGGG-3’    |
|              | R-5’-TCAGTTAATGCGAGGCAAGTC-3’ |
| S100a7       | F-5’-GGGCAGCTGACAAAAACAAAG-3’ |
|              | R-5’-TGGAACTGGAGATGAGTCC-3’   |
| S100a8       | F-5’-CCATGCTCTCTAAAGAATG-3’   |
|              | R-5’-ATCACCATCGCAAGGAACTC-3’  |
| S100a9       | F-5’-CAGCATAACCCACCATCTC-3’   |
|              | R-5’-AAGTTGCAACTGTGCTTC-3’    |
| Tarc         | F-5’-AGTGGAGTGTTCCAGGGGAT-3’  |
|              | R-5’-GTCACAGGCGCTTTATGTT-3’   |
| Tslp         | F-5’-ACGGATGGGGCTAATTACAAC-3’ |
|              | R-5’-AGTCCTCGATTTGCTCAGACT-3’|
FIGURE S1. Constituencies of leukocytes in ΔCD11c<sup>hi</sup>cDC mice in the homeostatic conditions. (A,B) Absolute cell numbers of leukocytes in Spl (A) and EDLNs (B) obtained from WT mice and ΔCD11c<sup>hi</sup>cDC mice (n = 7 per group). (C,D) The frequency of leukocytes in Spl (C) and EDLNs (D) obtained from WT mice and ΔCD11c<sup>hi</sup>cDC mice (n = 4 per group). Data are obtained from two to seven individual samples in a single experiment. *P < .05, **P < .01 compared with WT mice. All data are representative of at least 3 independent experiments.
FIGURE S2. Lack of CD11c<sup>hi</sup> cDCs in ΔCD11c<sup>hi</sup> cDC mice under steady-state conditions. (A) Cell surface expression profile of CD11c<sup>+</sup>I-A<sup>-</sup>-<wbr/>E<sup>-</sup> cDCs in Spl and EDLN<sub>s</sub> obtained from WT mice and ΔCD11c<sup>hi</sup> cDC mice (n = 4 per group). (B) Frozen sections obtained from Spl obtained from WT mice and ΔCD11c<sup>hi</sup> cDC mice (n = 2 per group) were stained for B220 (green), CD11c (red) and CD3ε (blue). Bars indicate 100 μm. (C,D) Cell surface expression profile (C) and proportion (D) of CD207<sup>+</sup>I-A<sup>-</sup>-<wbr/>E<sup>-</sup> LCs in epidermis obtained from WT mice and ΔCD11c<sup>hi</sup> cDC mice (n = 3 per group). (E) Frozen sections obtained from epidermis obtained from WT mice and ΔCD11c<sup>hi</sup> cDC mice (n = 3 per group) were stained for CD207 (green) and DAPI (blue). Bars indicate 50 μm. Data are obtained from two to four individual samples in a single experiment. *P < .05 compared with WT mice. All data are representative of at least 3 independent experiments.
FIGURE S3. Inflammatory status of ΔCD11c\textsuperscript{hi}cDC mice in the homeostatic conditions. (A) Serum production of cytokines in WT mice and ΔCD11c\textsuperscript{hi}cDC mice (n = 4 per group). n.d.; not determined. (B) Transcriptional expression of inflammation- and epithelium-related molecules in ear skin obtained from WT mice and ΔCD11c\textsuperscript{hi}cDC mice (n = 6 per group). Data are obtained from three to six individual samples in a single experiment. *P < .05, **P < .01 compared with WT mice. All data are representative of at least 3 independent experiments.
FIGURE S4. Ag-specific responses of T cells in ΔCD11c\textsuperscript{hi} cDC mice in the homeostatic conditions. WT mice and ΔCD11c\textsuperscript{hi} cDC mice (n = 3 per group) were adoptively transferred with eFluor\textsuperscript{TM} 670-labelled CD45.1\textsuperscript{+}OT-II CD4\textsuperscript{+} T cells (A,C) or CD45.1\textsuperscript{+}OT-I CD8\textsuperscript{+} T cells (B,D), and then mice were systemically administered with OVA protein. Cell dividing profile (left panel) and proportion (right panel) of CD45.1\textsuperscript{+}OT-II CD4\textsuperscript{+} T cells (A,C) or CD45.1\textsuperscript{+}OT-I CD8\textsuperscript{+} T cells (B,D) in Spl (A,B) and EDLNs (C,D) at 3 days after the administration. Data are obtained from two to three individual samples in a single experiment. **P < .01 compared with WT mice. All data are representative of at least 3 independent experiments.
**FIGURE S5.** Composition of CD4+ T_{eff} cells and ILCs in ΔCD11c^{hi} cDC mice in the homeostatic conditions. The frequency of the subsets of T cells (A,C) and ILCs (B,D) in Spl (A,B) and EDLNs (C,D) obtained from WT mice and ΔCD11c^{hi} cDC mice (n = 6 per group). Data are obtained from three to six individual samples in a single experiment. *P < .05, **P < .01 compared with WT mice. All data are representative of at least 3 independent experiments.
**FIGURE S6.** B-cell responses in ΔCD11c<sup>hi</sup>cDC mice in the homeostatic conditions. (A) Serum production of IgG and IgE in WT mice and ΔCD11c<sup>hi</sup>cDC mice. (B-E) The frequency of the subsets of IgM<sup>+</sup> B cells, IgG<sub>1</sub><sup>+</sup> B cells, and IgE<sup>+</sup> B cells in whole (B,C) and GC (D,E) of Spl (B,D) and EDLNs (C,E) obtained from WT mice and ΔCD11c<sup>hi</sup>cDC mice (n = 8 per group). (F) The frequency of the subsets of IgM<sup>+</sup> plasma cells, IgG<sub>1</sub><sup>+</sup> plasma cells, and IgE<sup>+</sup> plasma cells of BM obtained from WT mice and ΔCD11c<sup>hi</sup>cDC mice (n = 8 per group). Data are obtained from three to eight individual samples in a single experiment. *P < .05, **P < .01 compared with WT mice. All data are representative of at least 3 independent experiments.