

Supplementary Information

Pivotal role of CD103 in the development of psoriasiform dermatitis

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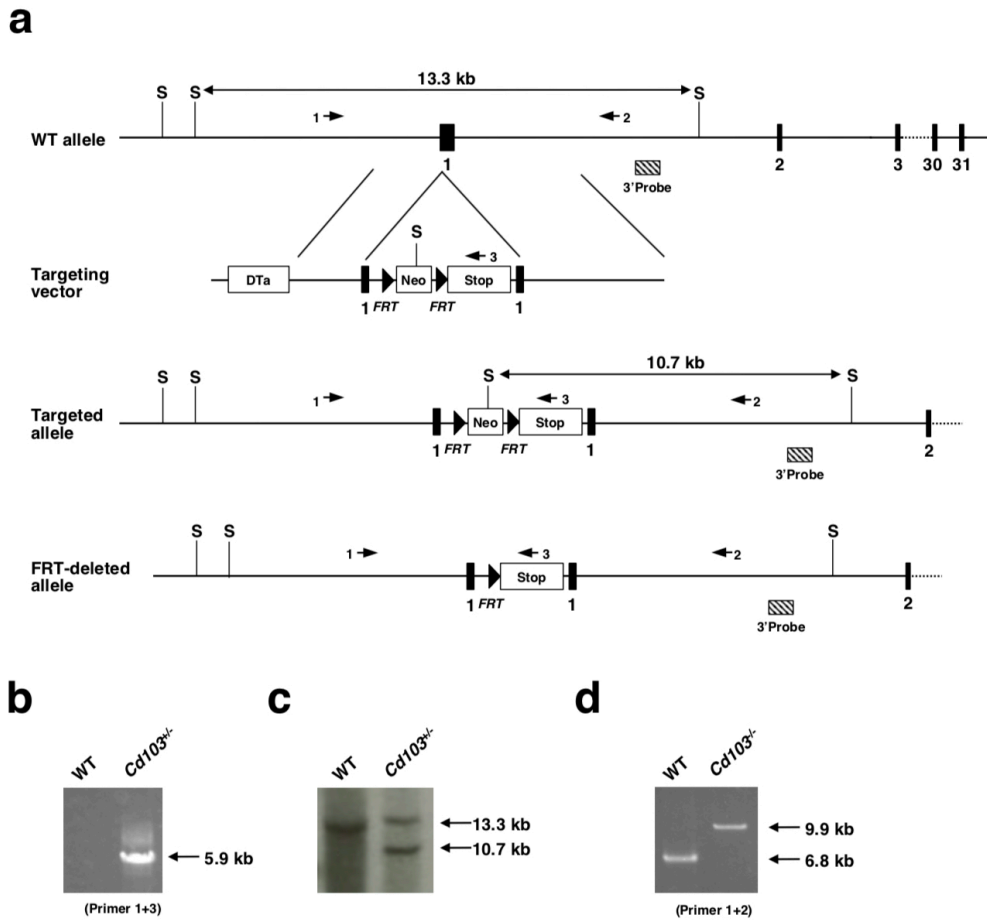
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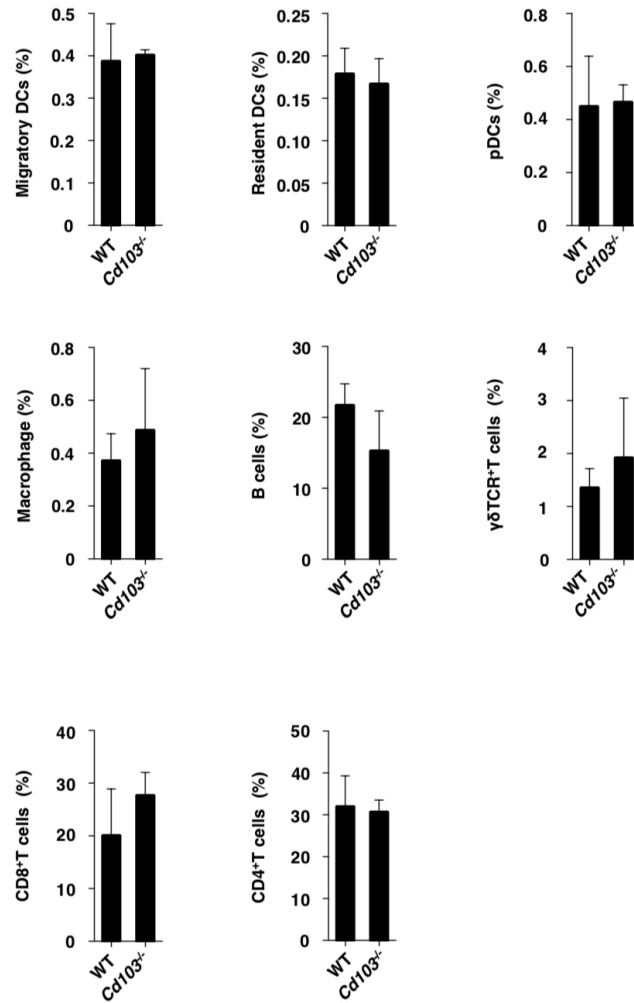
Supplementary Table 1

RT-PCR primer Sequence

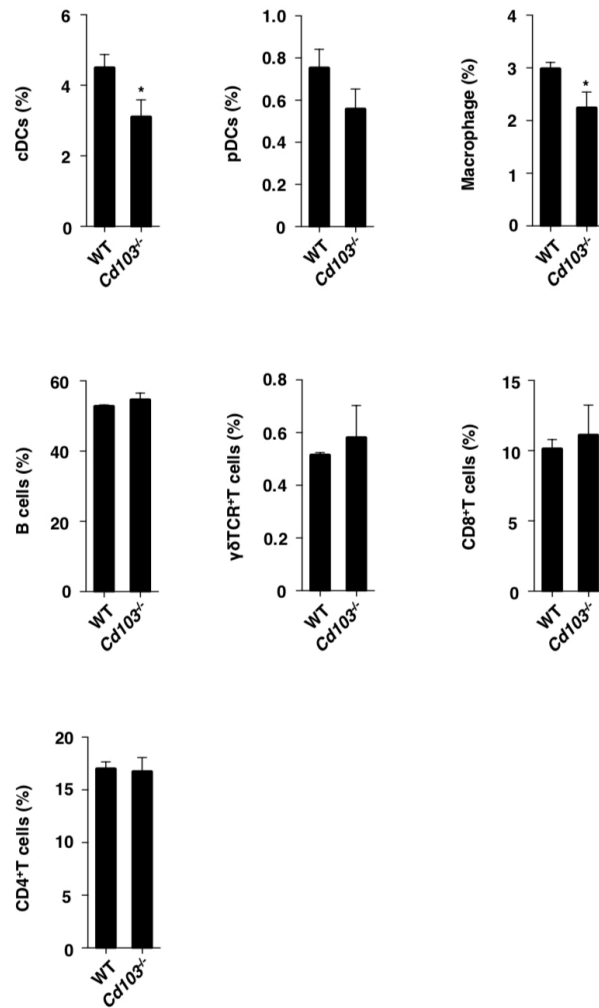
<i>Gapdh</i>	F-5'-AAATTC AACGGCACAGTCAAG-3' R-5'-TGGTGGTGAAGACACCAGTAG-3'
<i>Il1a</i>	F-5'-TCCAGGGCAGAGAGGGAGT-3' R-5'-GGAAC TTTGGCCATCTTGATTT-3'
<i>Il1b</i>	F-5'-GAAGAAGAGCCCATCCTCTG-3' R-5'-TCATCTCGGAGCCTGTAGTG-3'
<i>Il6</i>	F-5'-GACTGATGCTGGTGACAACC-3' R-5'-CCTCCGACTTGTGAAGTGG-3'
<i>Il10</i>	F-5'-TGCAGCAGCTCAGAGGGT-3' R-5'-TGGCCACAGTTTTTCAGGGAT-3'
<i>Il12a</i>	F-5'-AAACCAGACCCGCCCAAGAAC-3' R-5'-AAAAAGCCAACCAAGCAGAAGACAG-3'
<i>Il17a</i>	F-5'-CTGCTGAGCCTGGCGGCTAC-3' R-5'-CATTGCGGTGGAGAGTCCAGGG-3'
<i>Il19</i>	F-5'-GCCAACTCTTTCCTCTGCGT-3' R-5'-GGTGGCTTCCTGACTGCAGT-3'
<i>Il20</i>	F-5'-GACCCCTGACCACCATACCC-3' R-5'-CCATTGCTTCTTCCCCACAA-3'
<i>Il22</i>	F-5'-CAGCTCCTGTCACATCAGCGGT-3' R-5'-AGGTCCAGTTCCCAATCGCCT-3'
<i>Il23a</i>	F-5'-TCCGTTCCAAGATCCTTCG-3' R-5'-GAACCTGGGCATCCTTAAGC-3'
<i>Tnf</i>	F-5'-GCCACGTCGTAGCAAACCAC-3' R-5'-GCAGGGGCTCTTGACGGCAG-3'
<i>Cxcl1</i>	F-5'-GCCTATCGCCAATGAGCTG-3' R-5'-ATTCTTGAGTGTGGCTATGA-3'
<i>Cxcl2</i>	F-5'-ATGCCTGAAGACCCTGCCAAG-3' R-5'-GGTCAGTTAGCCTTGCCTTTG-3'
<i>S100a7</i>	F-5'-GGGCAGCTGACAAAAACAAG-3' R-5'-TGGAAC TGGAGATGGTAGTCC-3'
<i>S100a8</i>	F-5'-CCATGCCCTCTACAAGAATG-3' R-5'-ATCACCATCGCAAGGA ACTC-3'



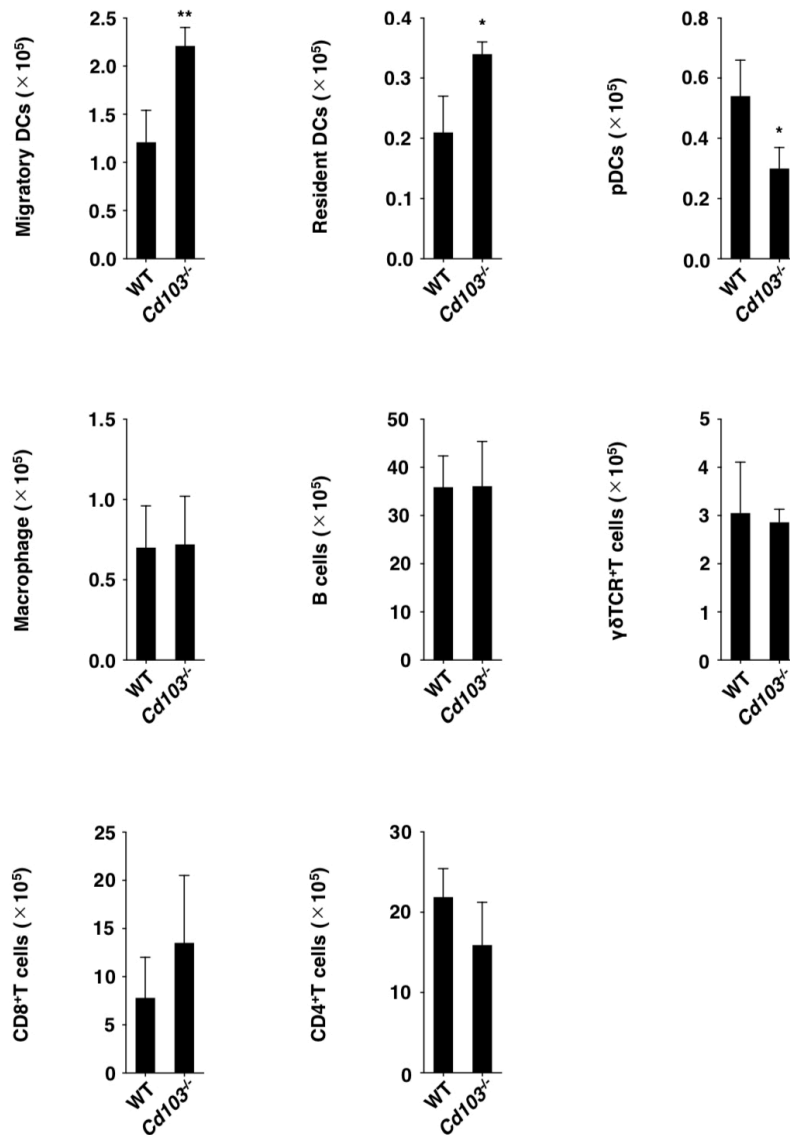
Supplementary Figure 1. Generation and identification of *Cd103*^{-/-} mice. **(a)** Strategy used to produce the *Cd103*^{-/-} mice. (1) Partial restriction map of the WT *Cd103* gene. Exons are depicted as black boxes. The restriction site indicated is S: *SpeI* (2) Targeting vector used to introduce the mutations in the *Cd103* gene. A *SalI* site engineered in place of the start codon in exon 1 of the *Cd103* gene was used to clone the *FRT-PGK-gb2-Neo-FRT-Stop* cassette. DTa: diphtheria toxin a expression cassette, Stop: Stop cassette, Neo: Neo cassette. The Neo cassette is shown bracketed by FRT sites (filled triangles). (3) Structure of the targeted allele following homologous ESC clones. (4) Structure of the *Cd103* allele following expression of FLP recombinase and excision of the Neocassette in mutant mice. The 3' external single-copy probe (a hatched box) and the PCR primers at the 5' end (blackarrows) used to verify proper homologous recombination events are shown. **(b,c)** DNA-PCR **(b)** and Southern blot **(c)** analysis of WT and recombinant ESC clones. **(d)** Genotyping of tail DNA from WT mice and homozygous mice for the *Cd103* allele by DNA-PCR. The results are representative of at least three independent experiments.



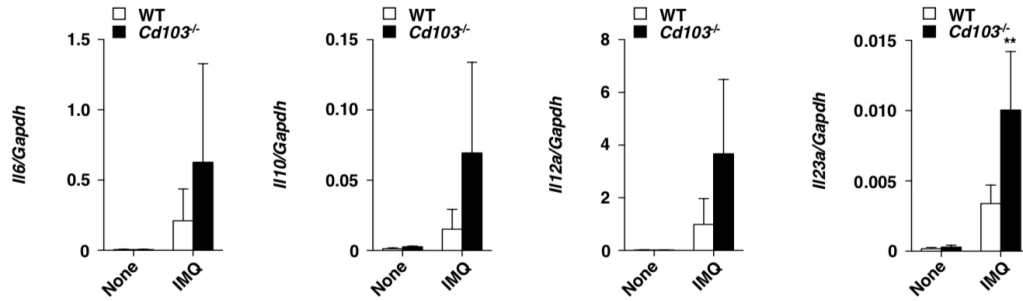
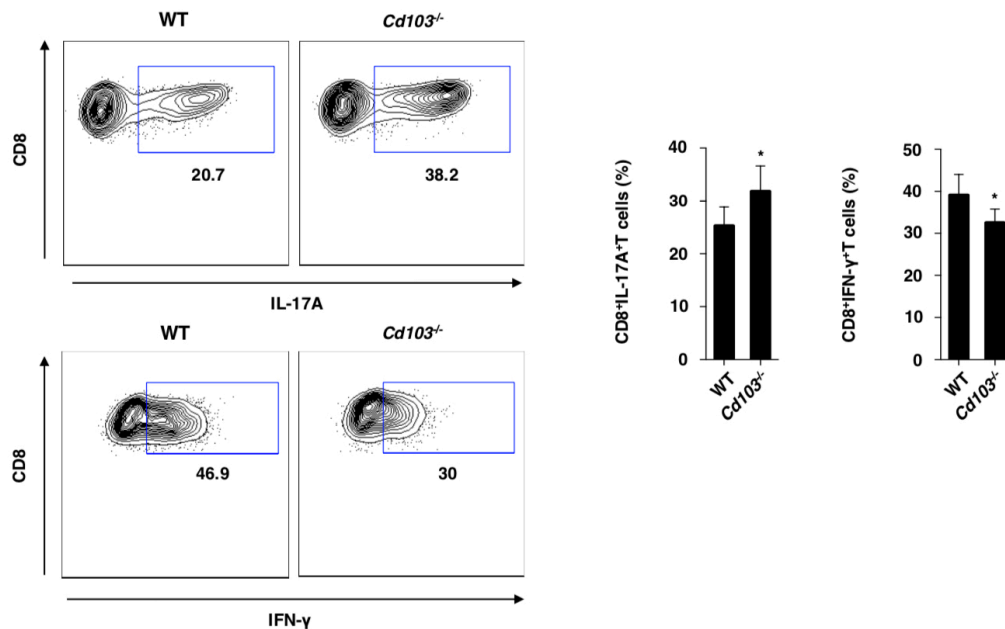
Supplementary Figure 2. Constituencies of leukocytes in PLNs in *Cd103*^{-/-} mice. The frequency of the indicated leukocytes in skin-draining PLNs was analyzed by flow cytometry in WT mice (n=3) and *Cd103*^{-/-} mice (n=3). Data are the mean \pm s.d. from three individual samples in a single experiment. The results are representative of at least three independent experiments.



Supplementary Figure 3. Constituencies of splenic leukocytes in *Cd103^{-/-}* mice. The frequency of the indicated leukocytes in Spl was analyzed by flow cytometry in WT mice (n=3) and *Cd103^{-/-}* mice (n=3). Data are the mean \pm s.d. from three individual samples in a single experiment. * $P < .05$ compared with WT mice. The results are representative of at least three independent experiments.

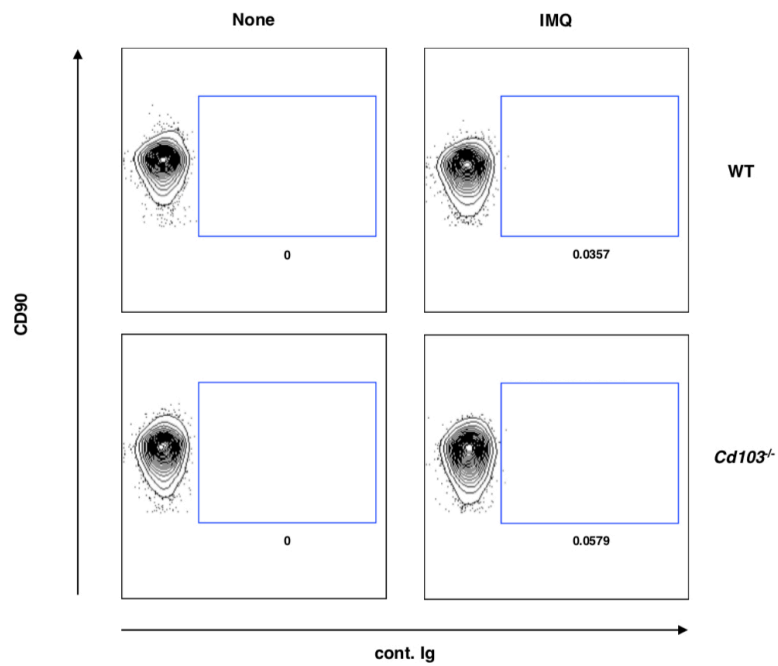


Supplementary Figure 4. Absence of CD103 enhances the accumulation of inflammatory leukocytes in skin-draining PLNs. The absolute cell numbers of leukocytes in the skin-draining PLNs at day 6 after topical application of IMQ on the left ear skin every day for 6 days in WT mice (n=5) and *Cd103*^{-/-} mice (n=5). Data are the mean ± s.d. in three to four individual samples in a single experiment. **P* < .05, ***P* < .01 compared with WT mice. The results are representative of at least three independent experiments.

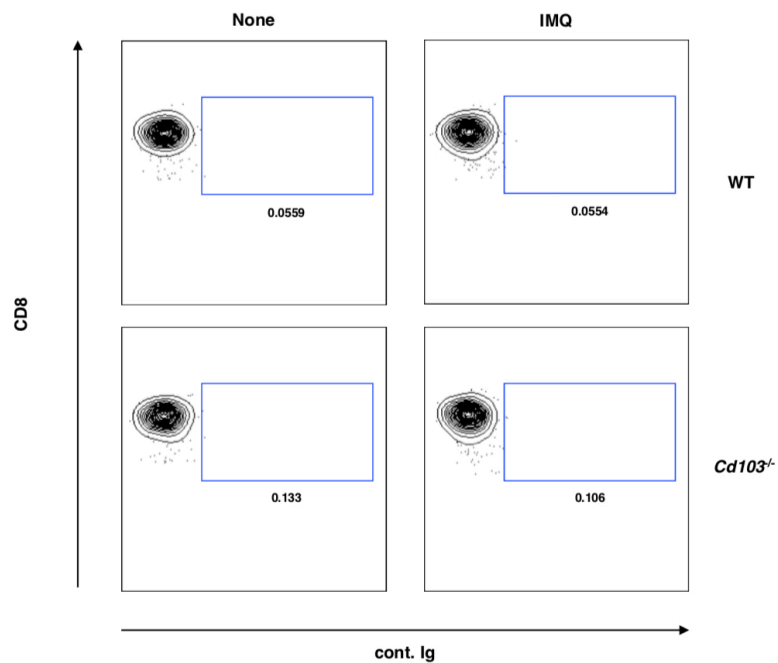
a**b**

Supplementary Figure 5. CD103 deficiency enhances the TLR7-mediated activation of cDCs. **(a)** Splenic cDCs derived from WT mice and *Cd103*^{-/-} mice were not stimulated (None) or stimulated with IMQ (IMQ). The transcriptional expressions of cytokines in cDCs. Data are the mean \pm s.d. from three to six individual samples in a single experiment. ** $P < .01$ compared with WT mice. **(b)** CD8⁺ T cells were cultured with cDCs obtained from the skin-draining PLNs in WT mice and *Cd103*^{-/-} mice for 5 days under T_c17-polarized culture conditions. The frequency of IL-17A- and IFN- γ -producing cells among CD8⁺ T cells. Data are presented as a contour plot, and numbers mean the proportion of the indicated cell populations in each gate (left panel). Data are the mean \pm s.d. in three individual samples in a single experiment (right panel). * $P < .05$ compared with WT mice. The results are representative of at least three independent experiments.

a

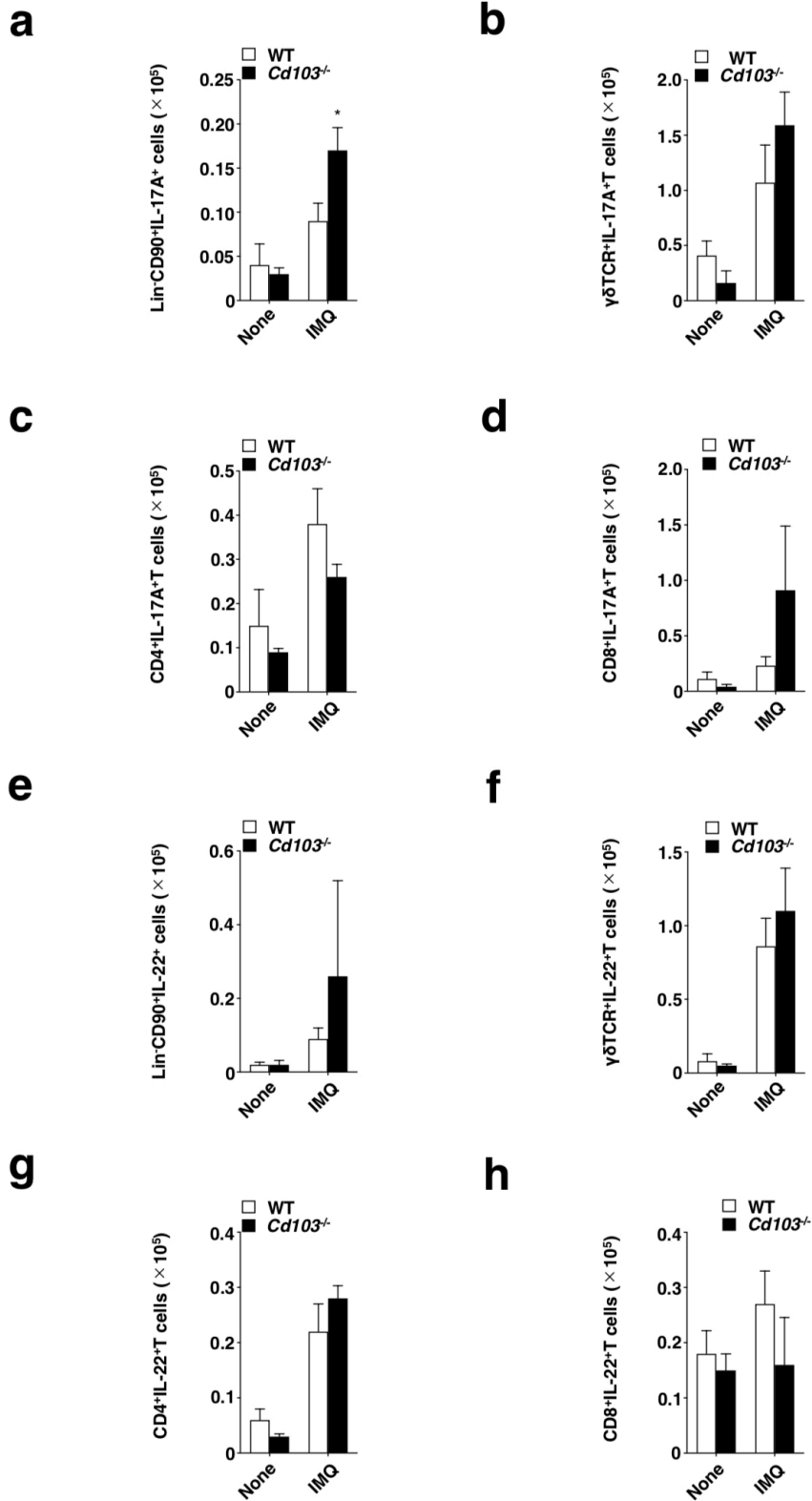


b



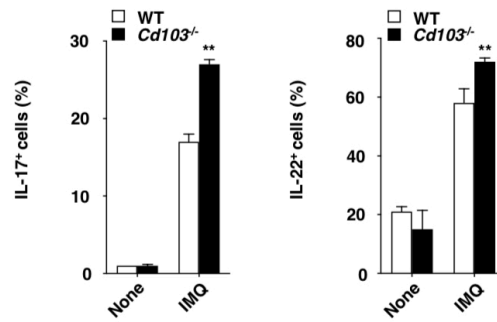
Supplementary Figure 6. Staining of IL-17A-producing lymphocytes with isotype control mAb. WT mice (n=3) and *Cd103*^{-/-} mice (n=3) were treated topically with IMQ on the left ear skin every day for 6 days. Data are presented as a contour plot for isotype control staining (cont. Ig) of innate lymphocytes (a) and CD8⁺ T cells (b) in the skin-

draining PLNs. The results are representative of at least three independent experiments.

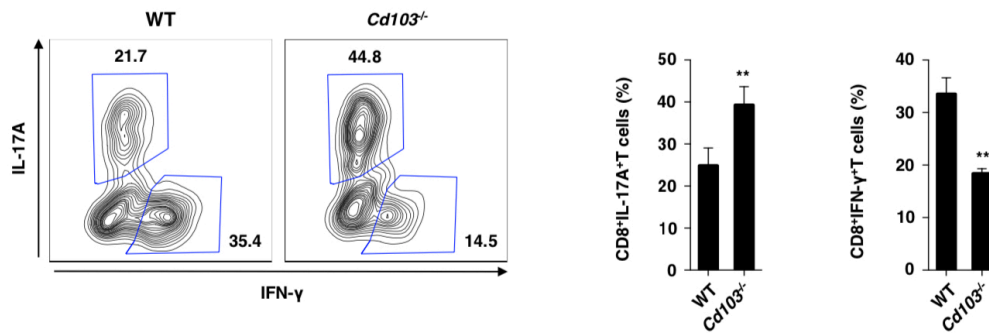


Supplementary Figure 7. CD103 deficiency enhances the generation of IL-17A-producing lymphocytes in the skin-draining PLNs in the progression of psoriasiform skin

inflammation. WT mice (n=3) and *Cd103*^{-/-} mice (n=3) were treated topically with IMQ on the left ear skin every day for 6 days. The absolute cell numbers of IL-17A-producing cells (**a-d**) and IL-22-producing cells (**e-h**) among innate lymphocytes (**a,e**), $\gamma\delta$ TCR⁺ T cells (**b,f**), CD4⁺ T cells (**c,g**), and CD8⁺ T cells (**d,h**) in the skin-draining PLNs at days 0 and 6. Data are the mean \pm s.d. in three individual samples in a single experiment. **P* < .05, ***P* < .01 compared with WT mice. The results are representative of at least three independent experiments.



Supplementary Figure 8. CD103 deficiency promotes the generation of IL-17A-producing lymphocytes in the dermal tissues in the development of psoriasiform skin inflammation. WT mice (n=3) and *Cd103*^{-/-} mice (n=3) were treated topically with IMQ on the left ear skin every day for 6 days. The frequencies of IL-17A-producing cells and IL-22-producing cells CD45.2⁺ leukocytes in the dermal tissues at days 0 and 6. Data are the mean \pm s.d. in three individual samples in a single experiment. * $P < .05$, ** $P < .01$ compared with WT mice. The results are representative of at least three independent experiments.



Supplementary Figure 9. Influence of CD103 deficiency on the generation of T_c17 cells. CD8⁺ T cells derived from WT mice and *Cd103*^{-/-} mice were cultured for 5 days under T_c17-polarized culture conditions. The frequency of IL-17A- and IFN- γ -producing cells among CD8⁺ T cells. Data are presented as a contour plot, and numbers mean the proportion of the indicated cell populations in each gate (left panel). Data are the mean \pm s.d. in three to five individual samples in a single experiment (right panel). ** $P < .01$ compared with WT mice. The results are representative of at least three independent experiments.

Figure S1b

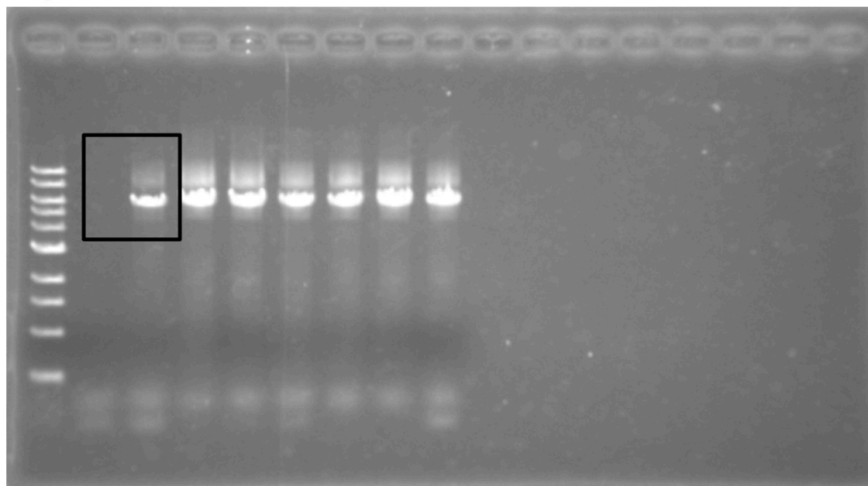


Figure S1c

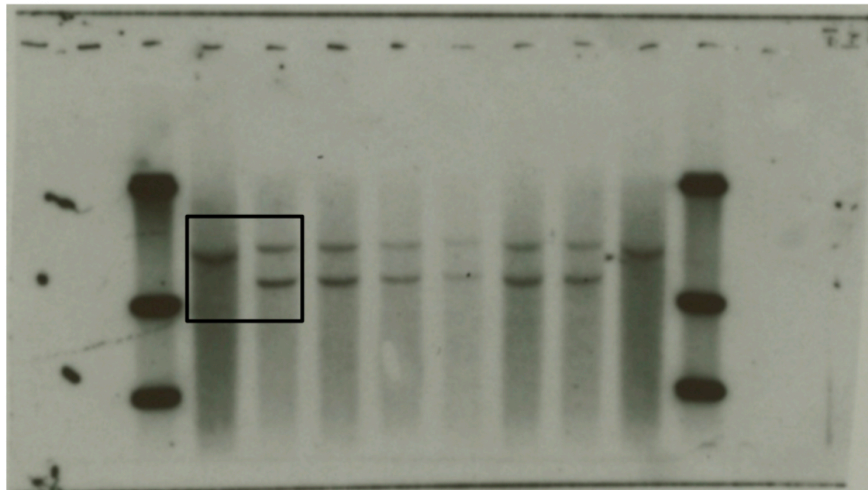
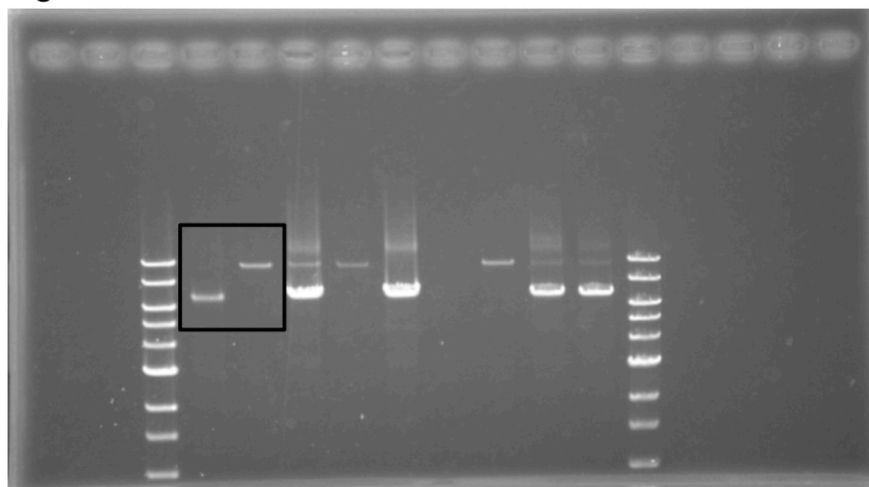


Figure S1d



Supplementary Figure 10. Full-length gels and blots of Supplementary Figure 1b-d.