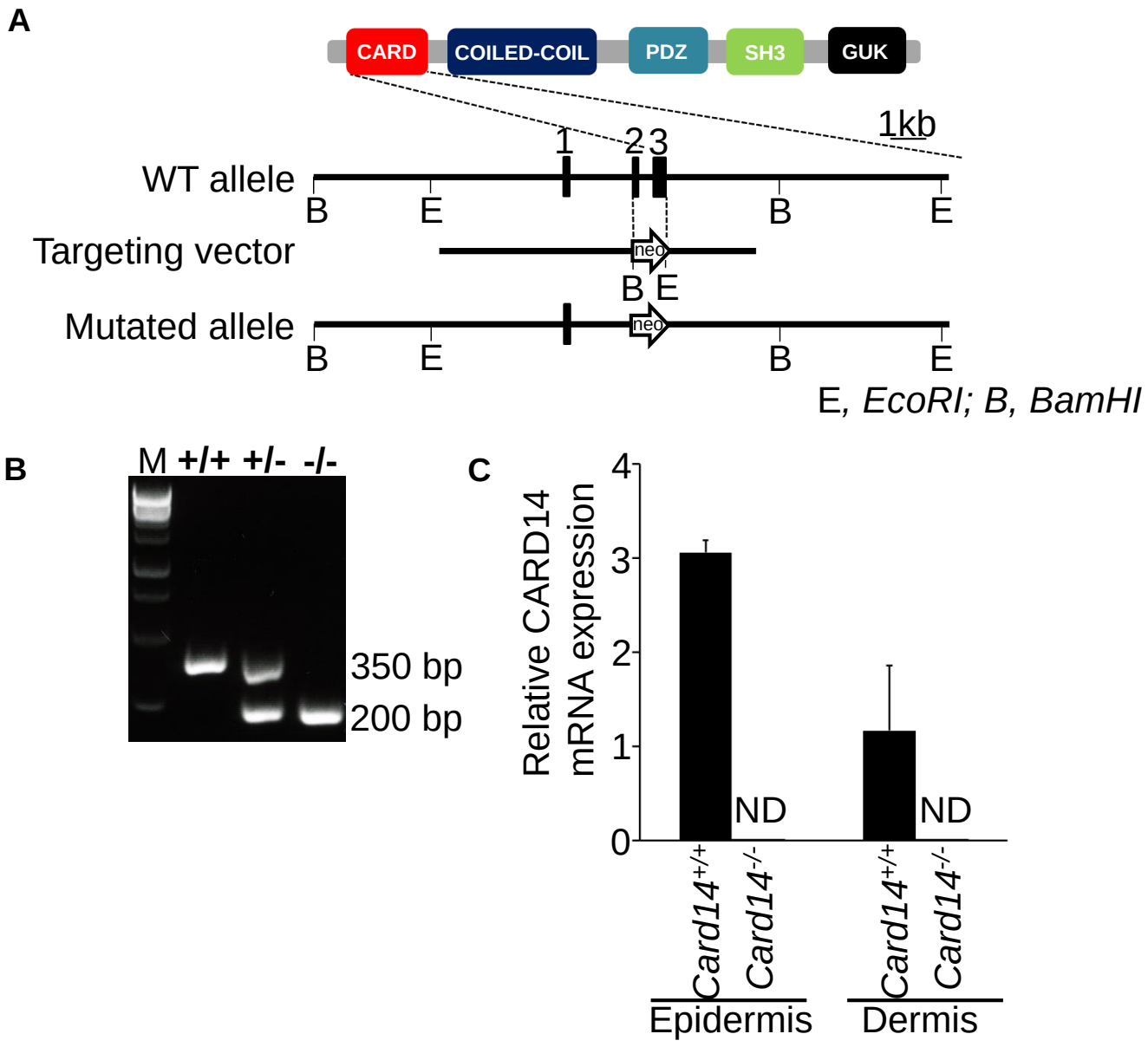
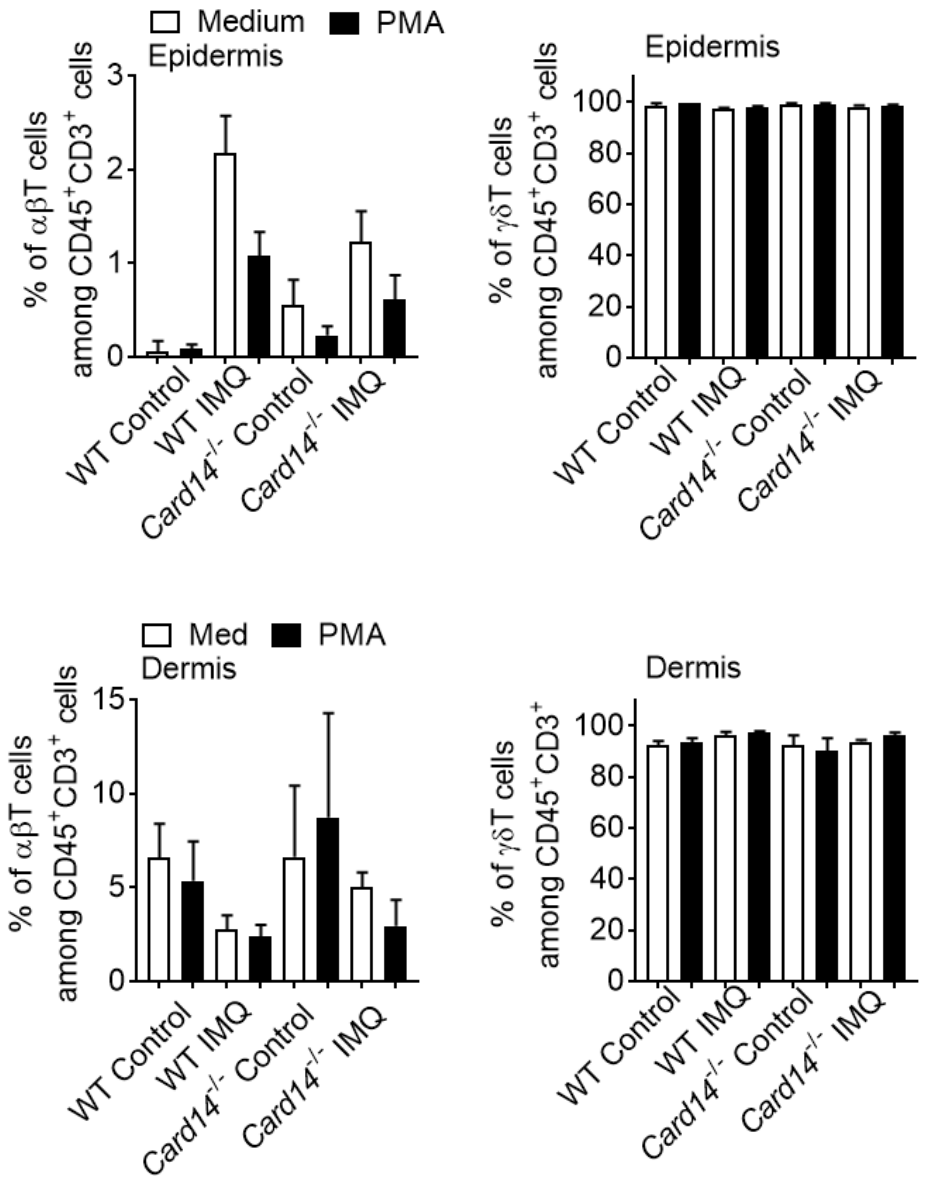
SUPPLEMENTAL FIGURE 1



Supplemental Figure 1. Generation of *Card14*$^{-/-}$ mice.
Card14$^{-/-}$ mice were generated by replacing exons 2 and 3 of the *Card14* gene with the neomycin (neo)-resistance gene. (A) Structure of the mouse *Card14* locus (WT allele), the *Card14*-targeting vector (targeting vector), and the predicted mutant *Card14* gene (mutated allele). Exons 2 and 3 of the *Card14* gene were replaced with the neo-resistance gene. Restriction enzymes: E, *Eco*RI; B, *Bam*HI. (B) *Card14* PCR genotyping of WT and mutant alleles using primers P1, P2, and P3. Genomic DNA was extracted from mouse tails. The WT product was 350 bp, the knockout product was 250 bp. Lane M contains base pair markers. (C) *Card14* mRNA levels in epidermal and dermal cells from WT and *Card14*$^{-/-}$ mice. Data were analyzed with real-time qPCR, and *Card14* expression is shown relative to *Gapdh* expression (n = 4 mice/group).

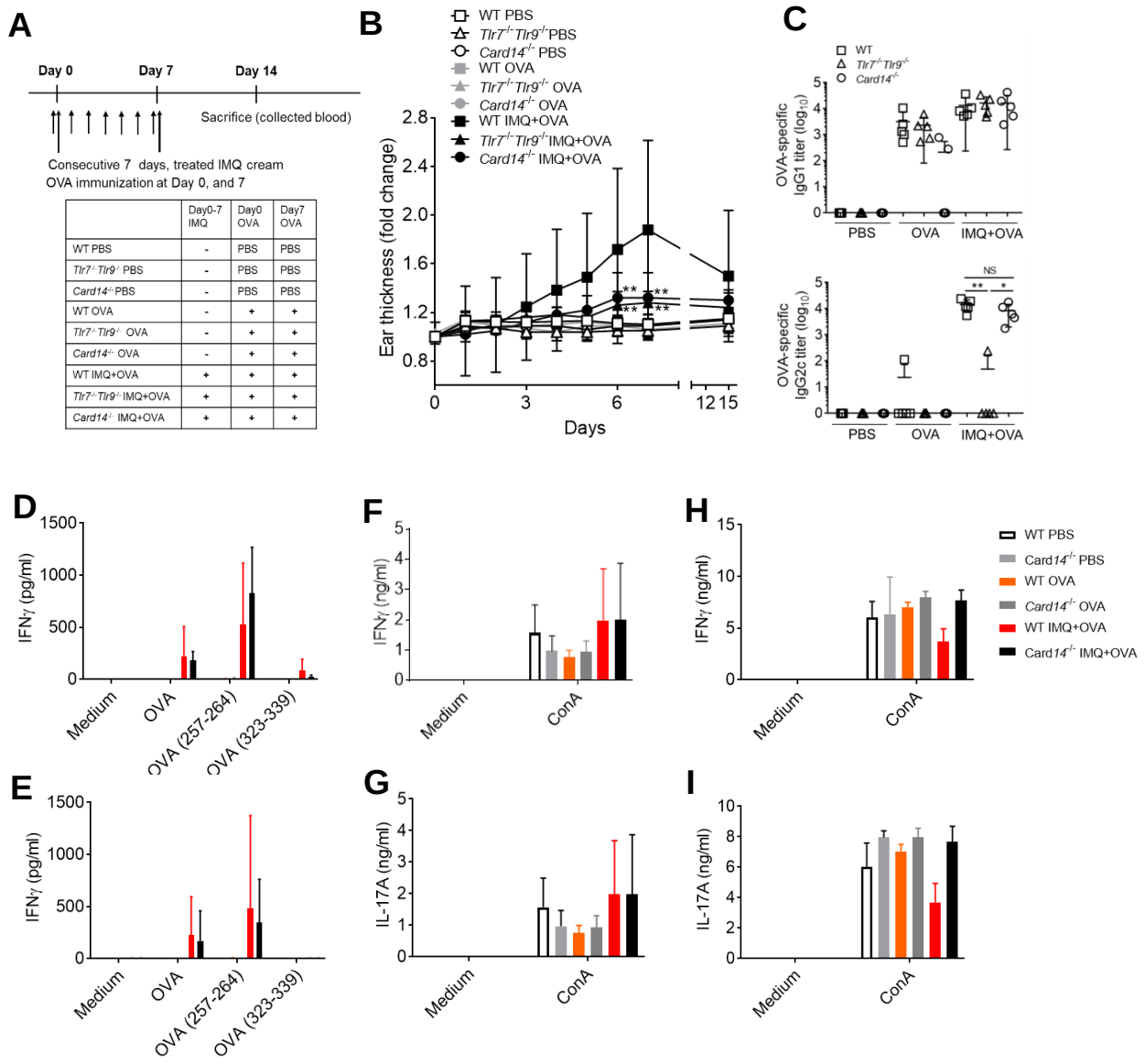
SUPPLEMENTAL FIGURE 2



Supplemental Figure 2. Skin cell populations

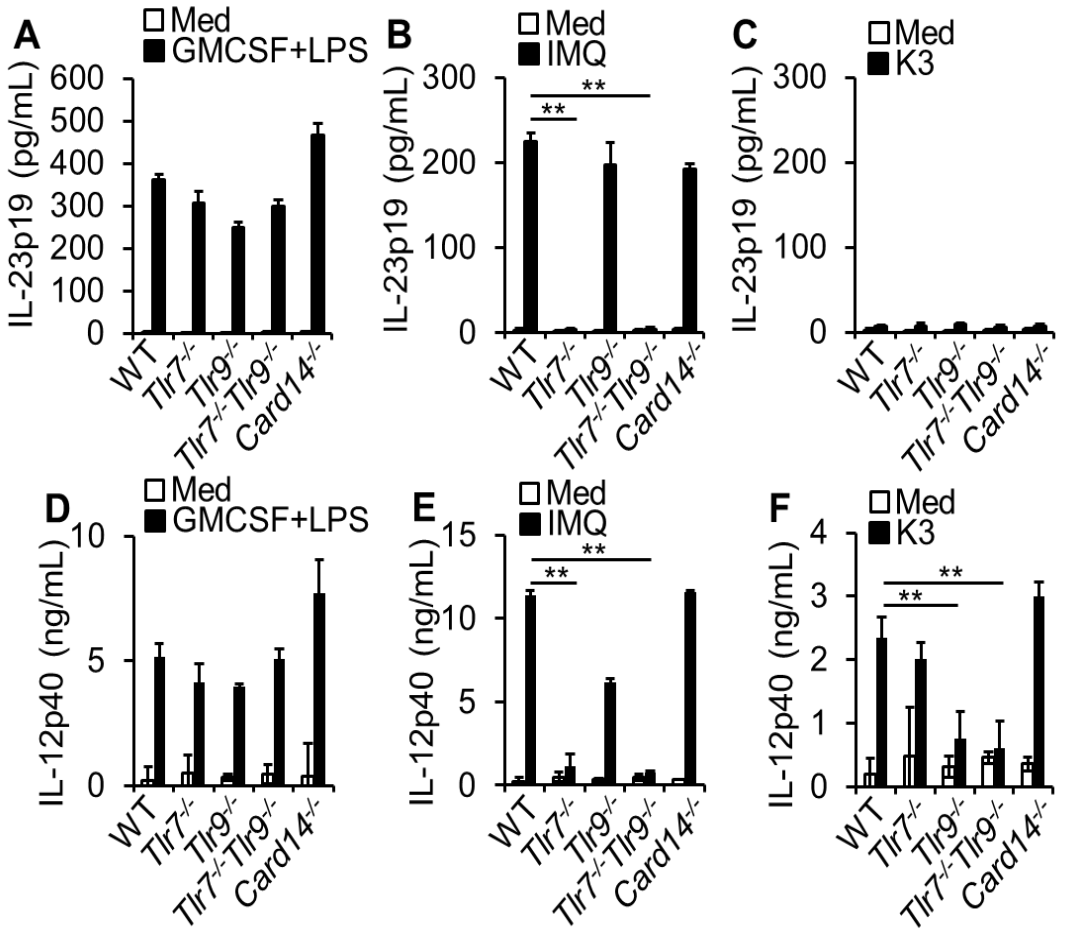
Ears of WT and *Card14*$^{-/-}$ mice (n = 4 per group) were treated with IMQ cream for 6 consecutive days. Epidermal and dermal cell suspensions from the WT and *Card14*$^{-/-}$ mouse ears were stimulated with or without PMA plus ionomycin and analyzed for αβ T-cell and γδ T-cell. Results are representative of at least two independent experiments.

SUPPLEMENTAL FIGURE 3



Supplemental Figure 3. CARD14 does not interfere with the adjuvant effect of IMQ on co-administered antigen. (A) Experiment scheme. For OVA immunization, WT, *Tlr7* $^{-/-}$ *Tlr9*$^{-/-}$ and *Card14*$^{-/-}$ mice (n = 5 mice/group) were intradermally injected in the ear (20 µL/ear) with 100-µg OVA protein at day 0 and 7. At day 14, blood was collected and antibody titer was measured. For induction of psoriasiform dermatitis, mouse ears were treated for 7 consecutive days with IMQ cream, as described previously. (B) Ear thickness was measured daily before cream treatment and injection. Data represent two independent experiments; results are reported as the mean ± s.d. ***P*<0.01 (WT IMQ+OVA vs. *Tlr7* $^{-/-}$ *Tlr9*$^{-/-}$ IMQ+OVA or *Card14*$^{-/-}$ IMQ+OVA). (C) The OVA-specific IgG_1 and IgG_{2c} in serum were measured using ELISA. (D-I) Cytokine secretion by OVA or IMQ + OVA immunized SP and dLN cells from WT and *Card14*$^{-/-}$ mice (n = 4 to 5 mice/group). (D) IFNγ production by SP cultured with OVA or OVA peptide ($OVA_{257-264}$ and $OVA_{323-339}$) for 48 h was measured by ELISA. (E) IFNγ production by dLN cells cultured with OVA or OVA peptide ($OVA_{257-264}$ and $OVA_{323-339}$) for 48 h was measured by ELISA. (F and G) IL-17A and IFNγ production by SP cultured with ConA for 48 h was measured by ELISA. (H and I) IL-17A and IFNγ production by dLN cells cultured with ConA for 48 h was measured by ELISA. ; results are reported as the mean ± s.d. **P*<0.05, ***P*<0.01.

SUPPLEMENTAL FIGURE 4



Supplemental Figure 4. IL-23p19 and IL-12p40 production was dependent on TLR7 and TLR9 single signaling *in vitro*. Bone marrow cells were isolated from WT, *Tlr7* $^{-/-}$, *Tlr9*$^{-/-}$, *Tlr7* $^{-/-}$ *Tlr9*$^{-/-}$, and *Card14*$^{-/-}$ femurs and hemolyzed. The hematopoietic progenitor cells were cultured in RPMI1640 supplemented with 20% FBS and 20 ng/ml mouse GM-CSF at a concentration of 5 × 10^5 cells/ml in 10 cm dish. On day 3, 10 ml of 20% FBS in RPMI including 20 ng/ml mouse GM-CSF was added in the dish. After generation of bone marrow-derived DCs on day 7, cells were harvested at a concentration of 1 × 10^6 cells/well. (A)- (F) ELISA of IL-12p40 and IL-23p19. Cytokines in culture supernatants of WT, *Tlr7* $^{-/-}$, *Tlr9*$^{-/-}$, *Tlr7* $^{-/-}$ *Tlr9*$^{-/-}$, and *Card14*$^{-/-}$ BMDCs were stimulated with GM-CSF plus LPS, IMQ, and K3 CpG-ODN for 24 h, and quantified using ELISA. Data are representative of three independent experiments, and the results are reported as the mean ± s.d., $^{**}P < 0.01$.