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Supplemental Information

Differential Roles of the mTOR-STAT3 Signaling in Dermal γδ T Cell Effector Function in Skin Inflammation

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Supplementary Figures

Figure S1

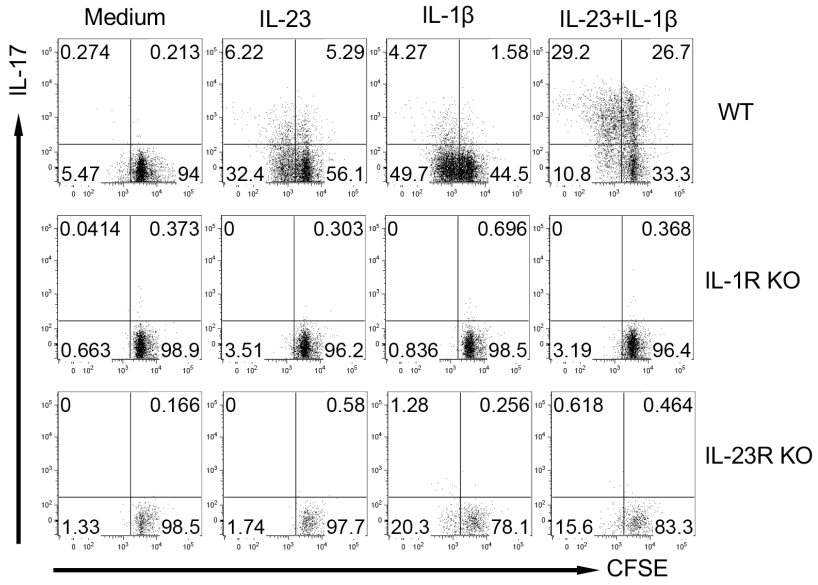

Figure S1. IL-1R and IL-23R signaling pathways regulate dermal γδT cell expansion and IL-17 production. Whole skin cell suspensions from WT, IL-1R KO and IL-23R KO mice were labeled with CSFE and then stimulated with IL-23, IL-1β, or IL-23 plus IL-1β for 3 days. Cell proliferation and intracellular IL-17 were analyzed by flow cytometry. Flow plots gated on $CD3^{+}\gamma\delta TCR^{int}$ cells are representative of at least two independent experiments with similar results. Each experiment includes at least three mice from WT, IL-1R KO or IL-23R KO strains. Related to Figure 1.

Figure S2

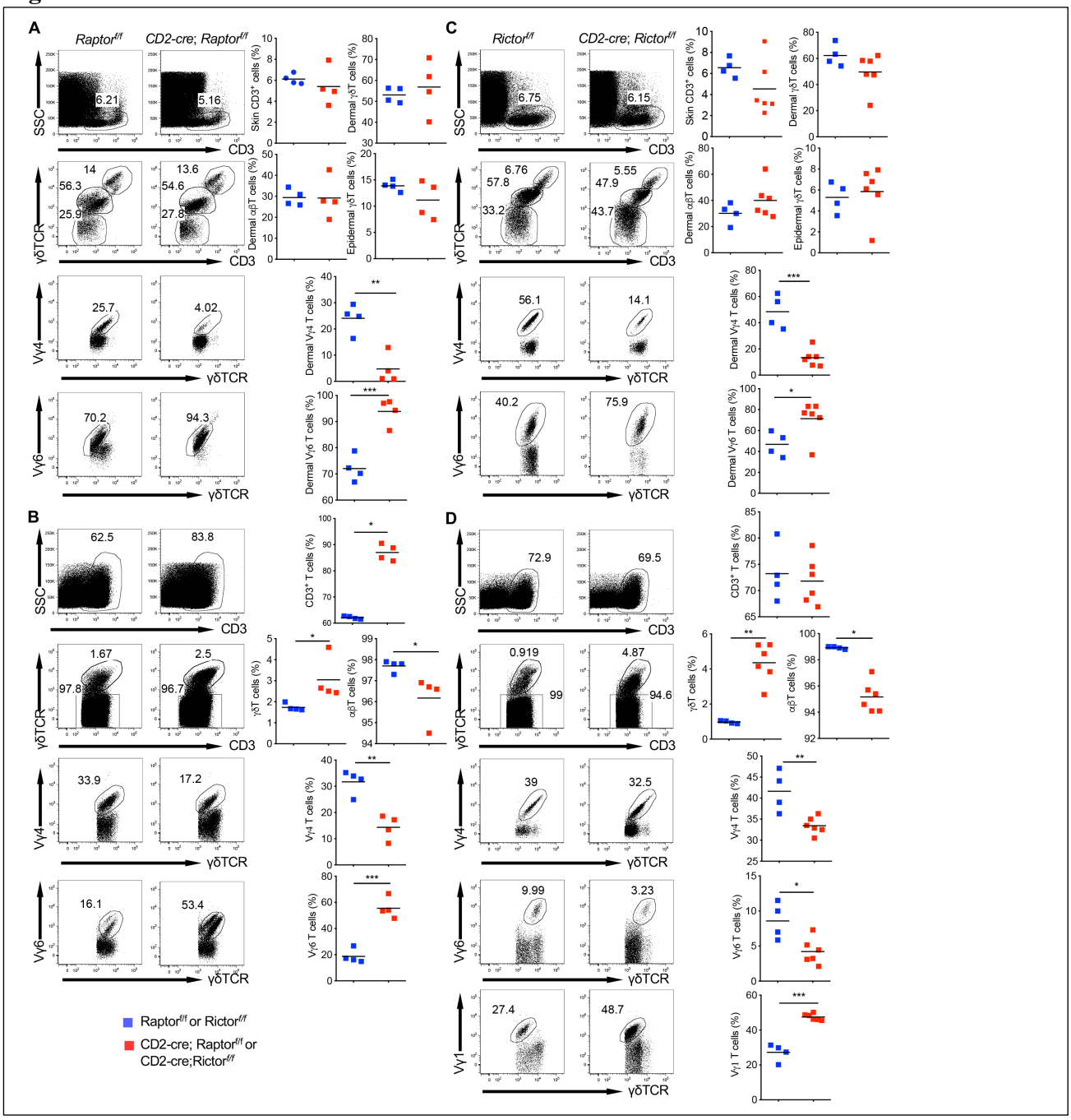

Figure S2. mTOR signaling is critical in γδT cell homeostasis in the periphery. (A, B) Skin tissues (A) and lymph nodes (B) from control Raptor$^{f/f}$ mice and CD2-cre;Raptor$^{f/f}$ mice were stained with CD3, αβTCR, γδTCR, Vγ4, and Vγ6. Percentages of CD3+ T cells, αβT cells, dermal γδT cells (CD3^{+}γδTCR^{int}) and epidermal γδT cells (CD3^{+}γδTCR^{hi}) and different subsets of γδT cells are shown. Data are are representative of at least two independent experiments with similar results. Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 (unpaired Student's t test). (C, D) Skin tissues (C) and lymph nodes (D) from control Rictor$^{f/f}$ mice and CD2-cre;Rictor$^{f/f}$ mice were stained with CD3, αβTCR, γδTCR, Vγ4, and Vγ6. Percentages of CD3+ T cells, αβT cells, dermal γδT cells (CD3^{+}γδTCR^{int}) and epidermal γδT cells (CD3^{+}γδTCR^{hi}) and different subsets of γδT cells are shown. Data are are representative of at least two independent experiments with similar results. Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 (unpaired Student's t test). Related to Figure 3.

Figure S3

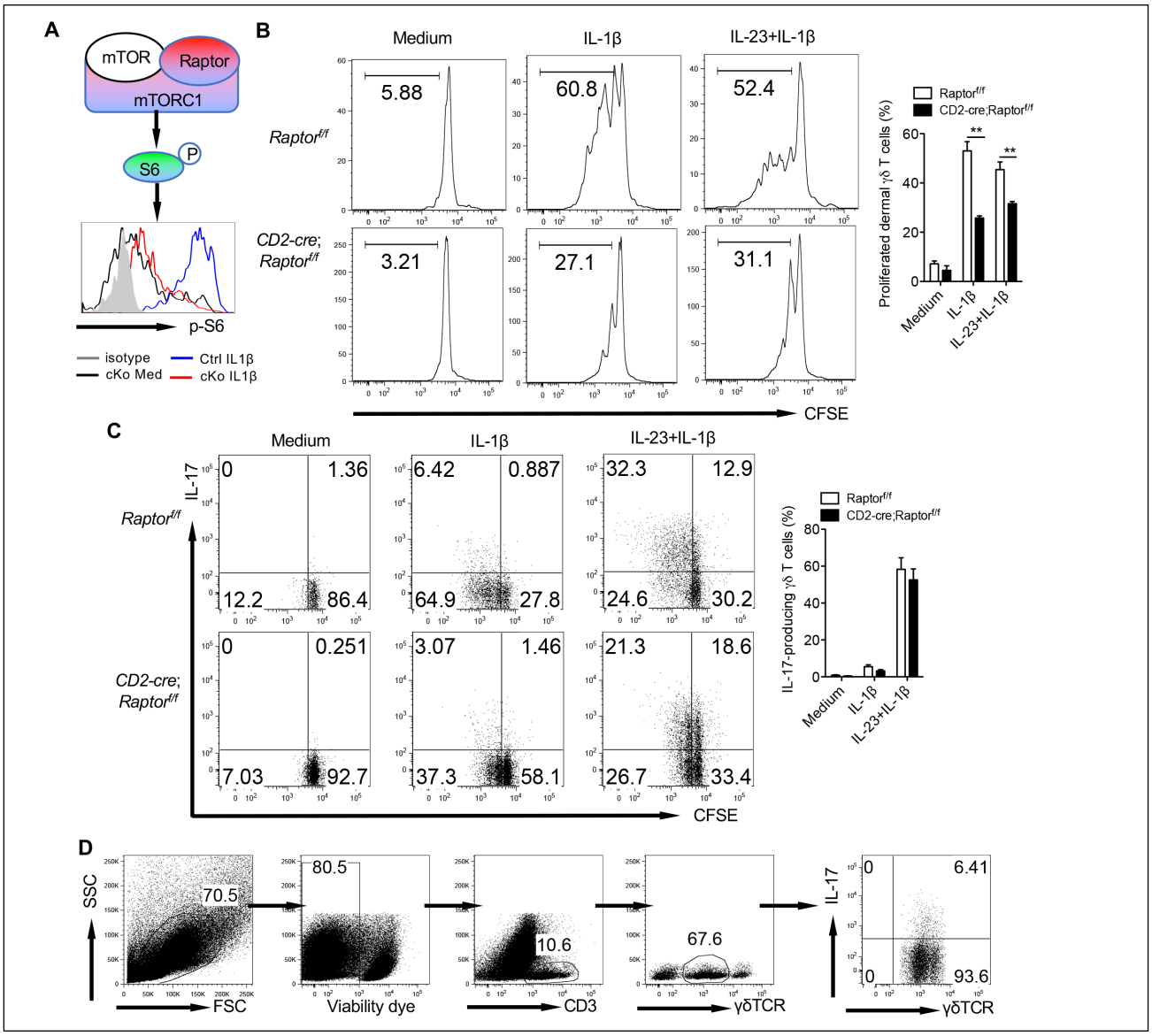

Figure S3. Roles of mTORC1 signaling in dermal γδT cell proliferation and IL-17 production. (A) Schematic of mTORC1 with representative histogram showing abolished mTORC1 activity (p-S6) in Raptor-deficient dermal γδ T cells upon IL-1β stimulation. (B, C) Whole skin cell suspensions from CD2-cre;Raptor$^{f/f}$ or control Raptor$^{f/f}$ mice (n=3-4) were labeled with CFSE and then stimulated with IL-1β or IL-23 plus IL-1β for 3 days. CFSE dilution (B) and intracellular IL-17 production (C) by dermal γδ T cells were determined by flow cytometry. Flow plots gated on CD3^{+}γδTCR^{Int} cells are representative of at least three independent experiments with similar results. Data are shown as mean ± SEM. **p < 0.01 (unpaired Student's t test). (D) Gating strategy for dermal γδ T cells and IL-17 production upon stimulation shown in Figure 3E. Related to Figure 3.

Figure S4

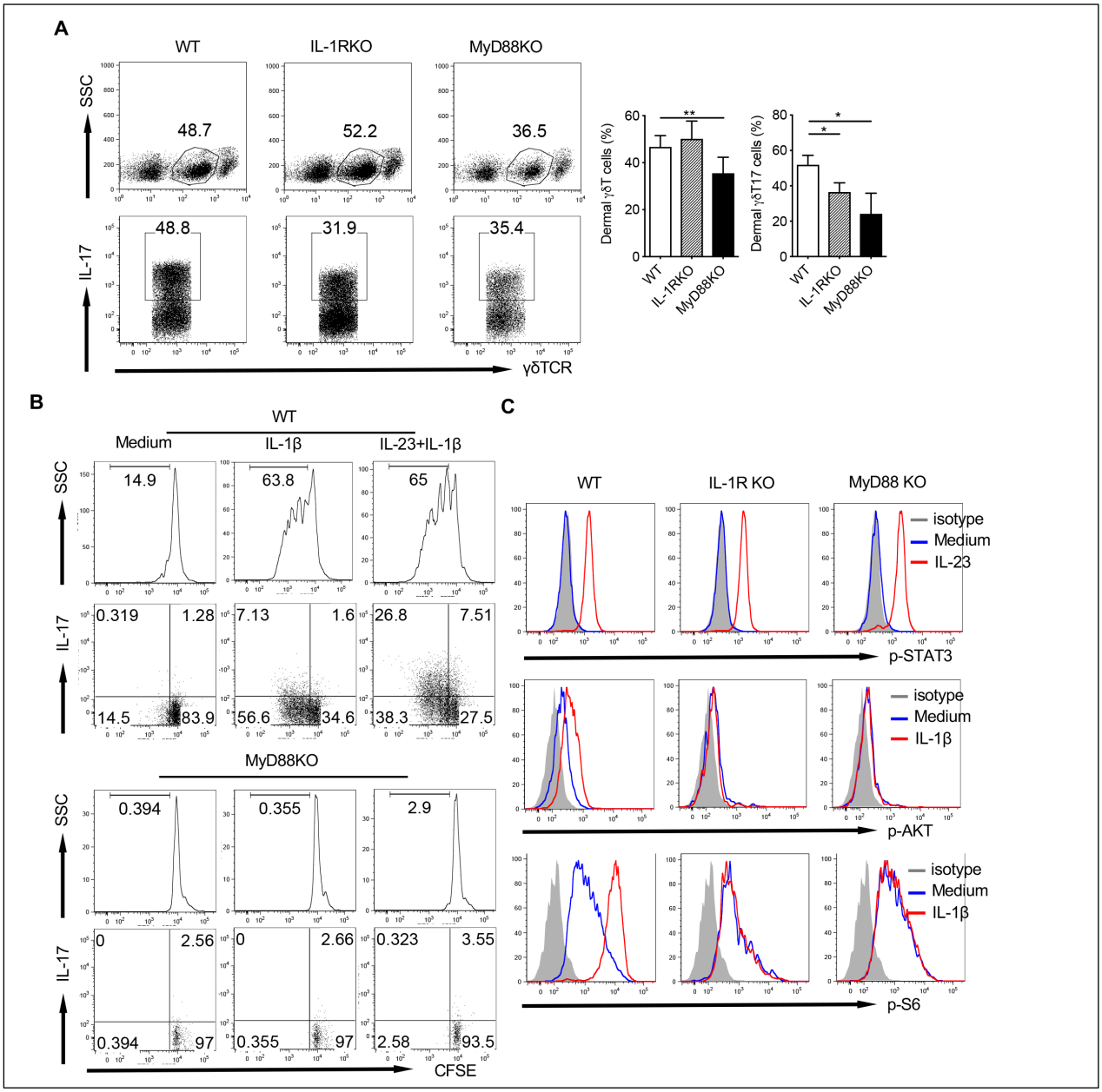

Figure S4. MyD88-mediated signaling pathway is essential in IL-1β-induced dermal γδT cell activation. (A) The frequency of dermal γδT cells (n=6) and the percentage of IL-17-producing γδT cells (n=3) after PMA plus ionomycin stimulation in C57BL/6 WT, IL-1R KO and MyD88 KO mice are shown. Flow plots gated on $CD3^+$ cells (top) or $CD3^+\gamma\delta TCR^{int}$ cells (bottom) are representative of two independent experiments with similar results. Data are shown as mean ± SD. *$p < 0.05$, **$p < 0.01$ (one-way ANOVA). (B) Whole skin cell suspensions from C57BL/6 WT or MyD88 KO mice were labeled with CFSE and then stimulated with IL-1β or IL-23 plus IL-1β for 3 days. CFSE dilution and intracellular IL-17 production by dermal γδ T cells were determined by flow cytometry. Flow plots gated on $CD3^+$ $\gamma\delta TCR^{int}$ cells are representative of at least three independent experiments with similar results. (C) Cultured skin γδ T cell lines from C57BL/6 WT, IL-1RKO and MyD88KO mice were stimulated with IL-23 or IL-1β for 30 minutes. p-Stat3, p-AKT and p-S6 were examined by flow cytometry. Flow plots gated on $CD3^+$ $\gamma\delta TCR^+$ cells are representative of at least two independent experiments with similar results. Plots from WT mice were the same shown in Figure 3B. Related to Figures 2 & 3.

Figure S5

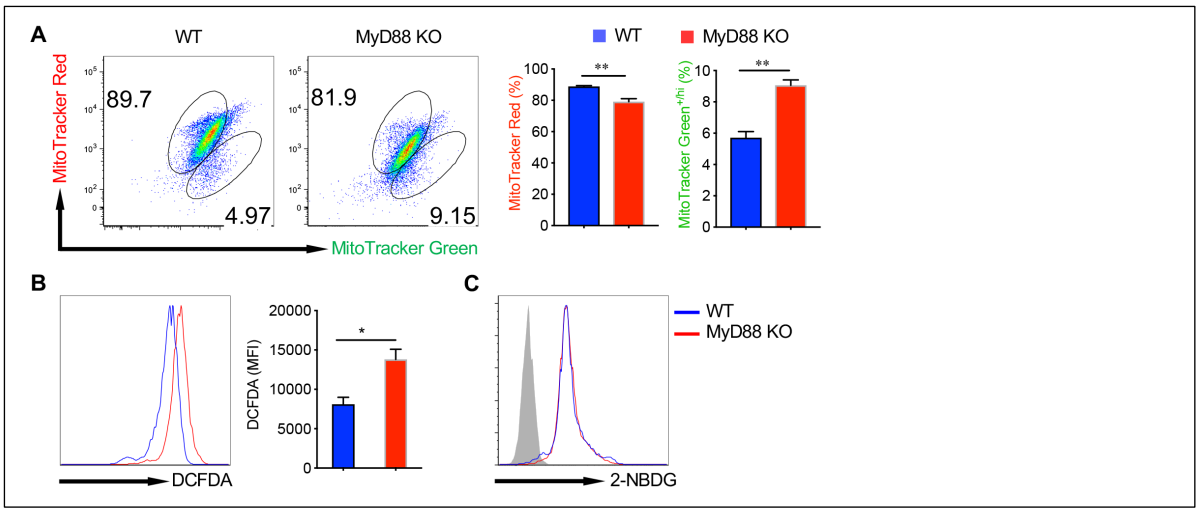

Figure S5. Reduced respiring mitochondria with enhanced ROS production in MyD88 KO dermal γδT cells. (A) Whole skin cell suspensions from WT and MyD88 KO mice (n=3) were stained with MitoTracker Green and MitoTracker Red. Percentages of MitoTracker Green$^{+/hi}$ and MitoTracker Red were analyzed by flow cytometry. Flow plots gated on CD3^{+}γδTCR^{int} cells are representative of two independent experiments with similar results. Percentages of MitoTracker Green$^{+/hi}$ and MitoTracker Red^{+} dermal γδT cells are shown as mean ± SEM. **p< 0.01 (unpaired Student's t test). (B, C) Whole skin cell suspensions from WT or MyD88 KO mice were stained with DCFDA (B) or 2-NBDG (C). Expressions of DCFDA and 2-NBDG were analyzed by flow cytometry. Flow histograms gated on CD3^{+}γδTCR^{int} cells are representative of two independent experiments with similar results. Summarized DCFDA MFI data are shown as mean ± SEM. *p<0.05, **p< 0.01(unpaired Student's t test). MFI: mean fluorescent intensity. Related to Figure 4.

Figure S6

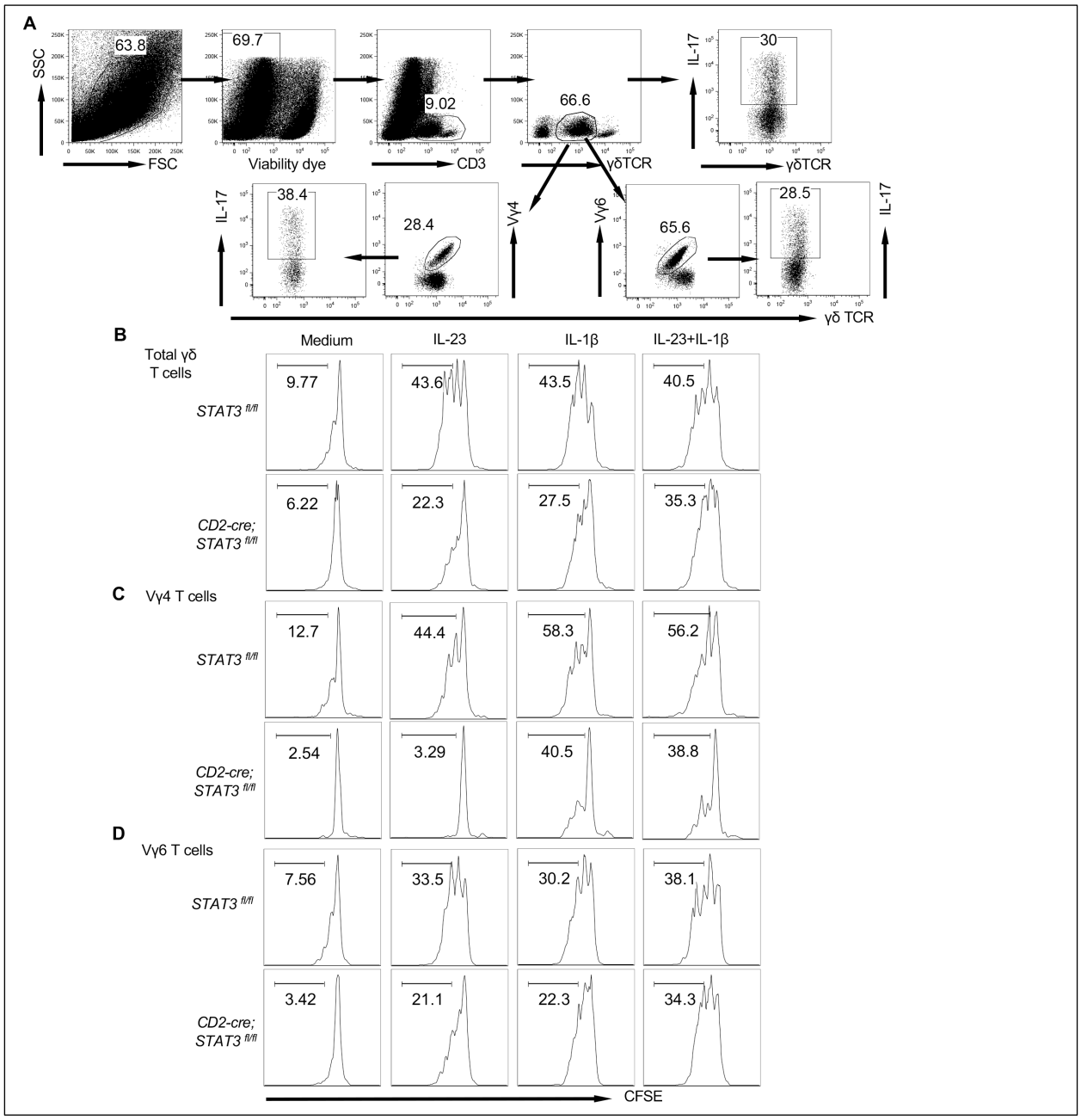

Figure S6. Roles of STAT3 signaling in dermal γδT cell proliferation. (A, B, C, D) Whole skin cell suspensions from CD2-cre;Stat3$^{f/f}$ or control Stat3$^{f/f}$ mice were labeled with CFSE and then stimulated with IL-1β, IL-23 or IL-23 plus IL-1β for 3 days. Gating strategy is shown (A). Dermal γδT cell proliferation (CFSE dilution) was determined by flow cytometry. Flow plots gated on CD3^{+}γδTCR^{int} cells (B), on CD3^{+}γδTCR^{int} Vγ4 (C), or Vγ6 (D) cells are representative of at least three independent experiments with similar results. Related to Figure 5.

Figure S7

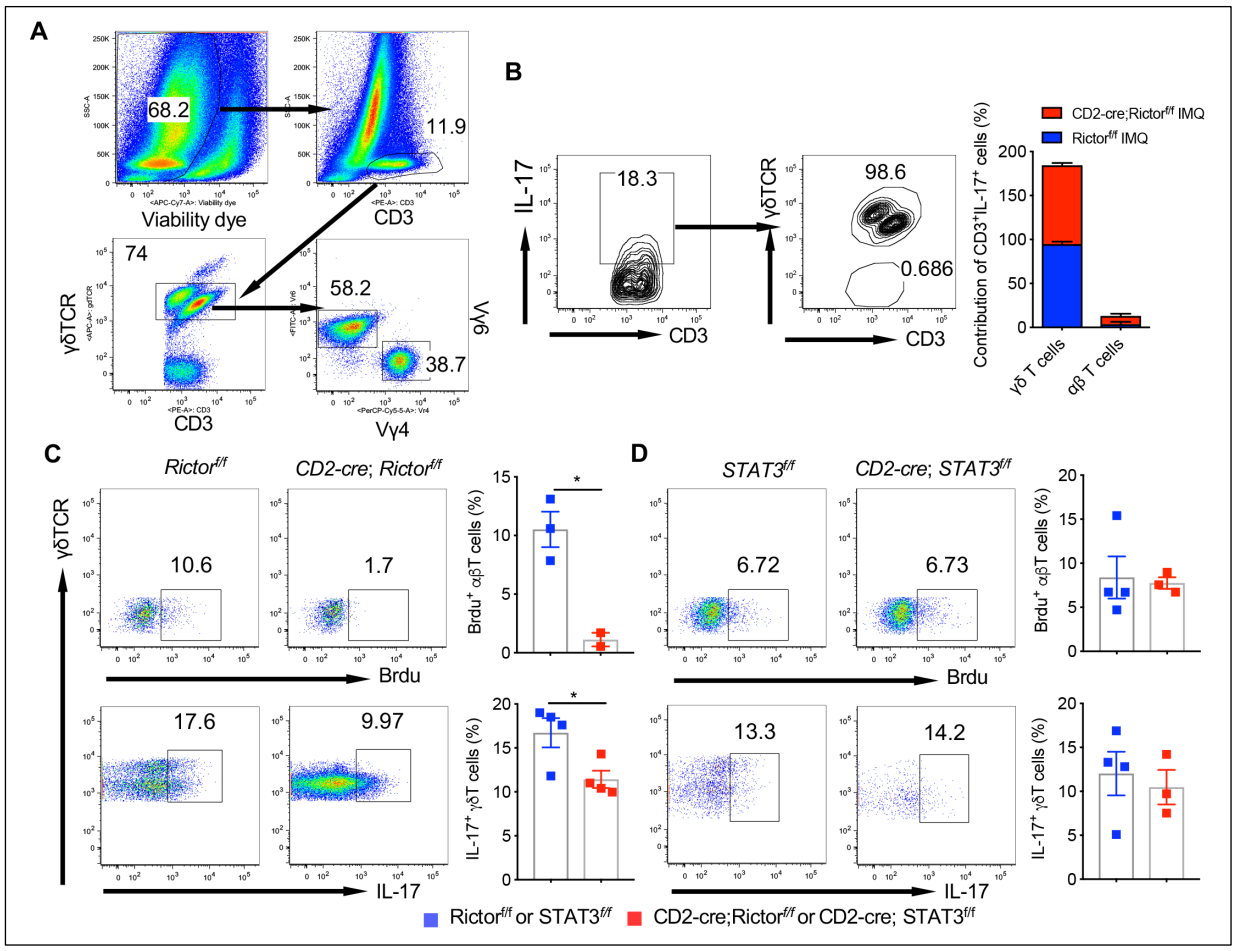

Figure S7. Dermal γδT cells are the major cellular source of IL-17 in the inflamed skin. (A) Gating strategy for dermal Vγ4 and Vγ6 T cells in control and Rictor cKO or STAT3 cKO mice treated with IMQ. (B) CD2-cre;Rictor$^{f/f}$ or control Rictor$^{f/f}$ mice (n=3) were treated daily for 5 days with IMQ or vehicle control. Single cells were stained for intracellular IL-17 without further stimulation. Flow plots were gated from CD3$^+$IL-17$^+$ cells first and percentages of CD3$^+$γδTCR$^-$ cells (αβ T cells) or CD3$^+$γδTCR^{int} (γδ T cells) were examined. Summarized data combined from three independent experiments are shown as mean ± SEM. (C, D) CD2-cre;Rictor$^{f/f}$ or control Rictor$^{f/f}$ mice (C) and CD2-cre;Stat3$^{f/f}$ or control Stat3$^{f/f}$ mice (D) were applied topically with IMQ for 5 days. Brdu were injected one day before mice were sacrificed. Skin single cell suspensions were stained for Brdu (gated on CD3$^+$γδTCR$^-$) and spontaneous IL-17 production without stimulation (gated on CD3$^+$γδTCR^{int}). Flow plots are representative of two independent experiments with similar results. Data are shown as mean ± SEM. *p<0.05, **p<0.01 (unpaired Student's t test). Related to Figure 7.