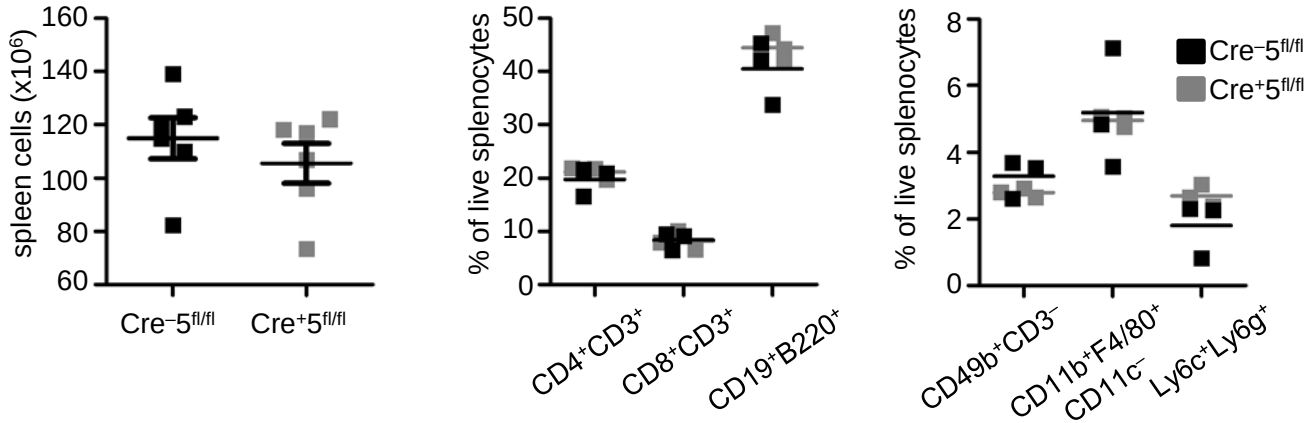
Supp. Figure 1.

a



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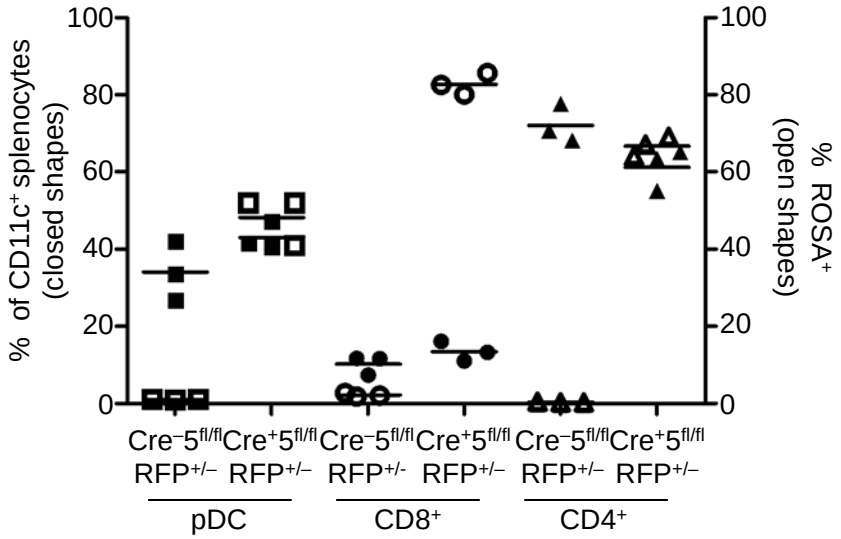

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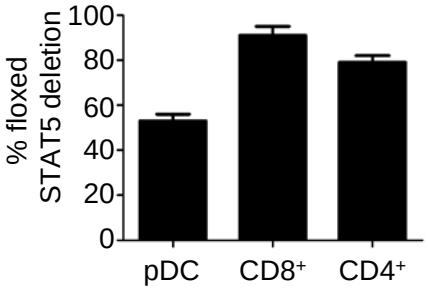
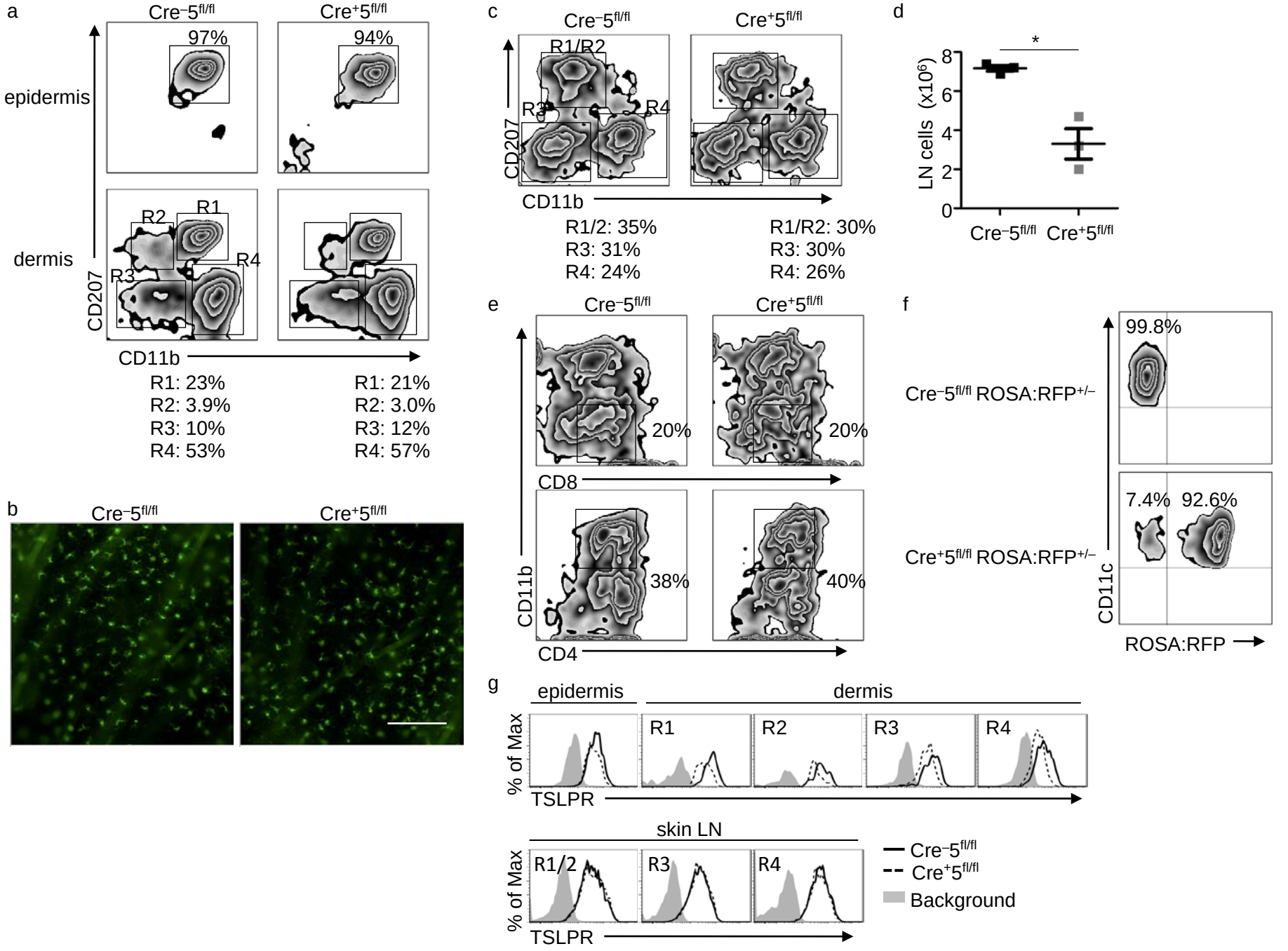


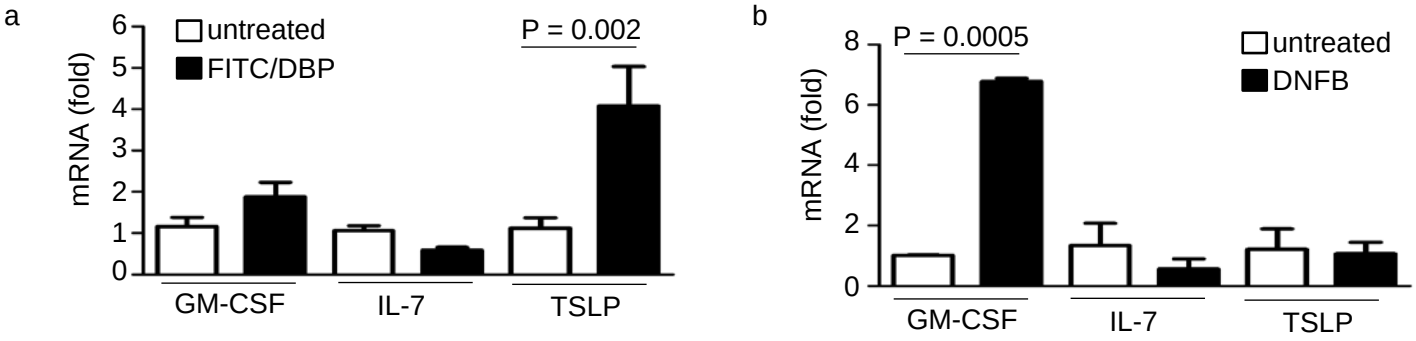
Supplemental Figure 1, STAT5 deficiency in splenic DCs has no effect on hematopoietic cell numbers. A, Total cellularity and percentages of hematopoietic cells in $Cre^{-}5^{fl/fl}$ and $Cre^{+}5^{fl/fl}$ mice. $CD49b^{+}CD3^{-}$ (NK cells), $CD11b^{+}F4/80^{+}$ (macrophages), $Ly6c^{+}Ly6g^{+}$ (granulocytes) Representative of three independent experiments. B, Percentages of pDC ($PCDA1^{+}CD11c^{+}$), $CD8^{+}$ DCs ($PDCA1^{-}CD11c^{+}CD11b^{-}CD8^{+}MHCII^{+}$), $CD4^{+}$ DCs ($PDCA1^{-}$ $CD11c^{+}CD11b^{+}CD4^{+}MHCII^{+}$) of total $CD11c^{+}$ splenocytes (closed shapes) and percentages of FLOX:STOP:RFP^{+} DCs within each gated DC population. Representative of three independent experiments. C, Deletion efficiency of STAT5 flox locus in sorted $Cre^{+}5^{fl/fl}$ splenic DCs. Graphed as percent of STAT5 flox allele deleted in the germline, in duplicate, representative of two experiments.

Supp. Figure 2.



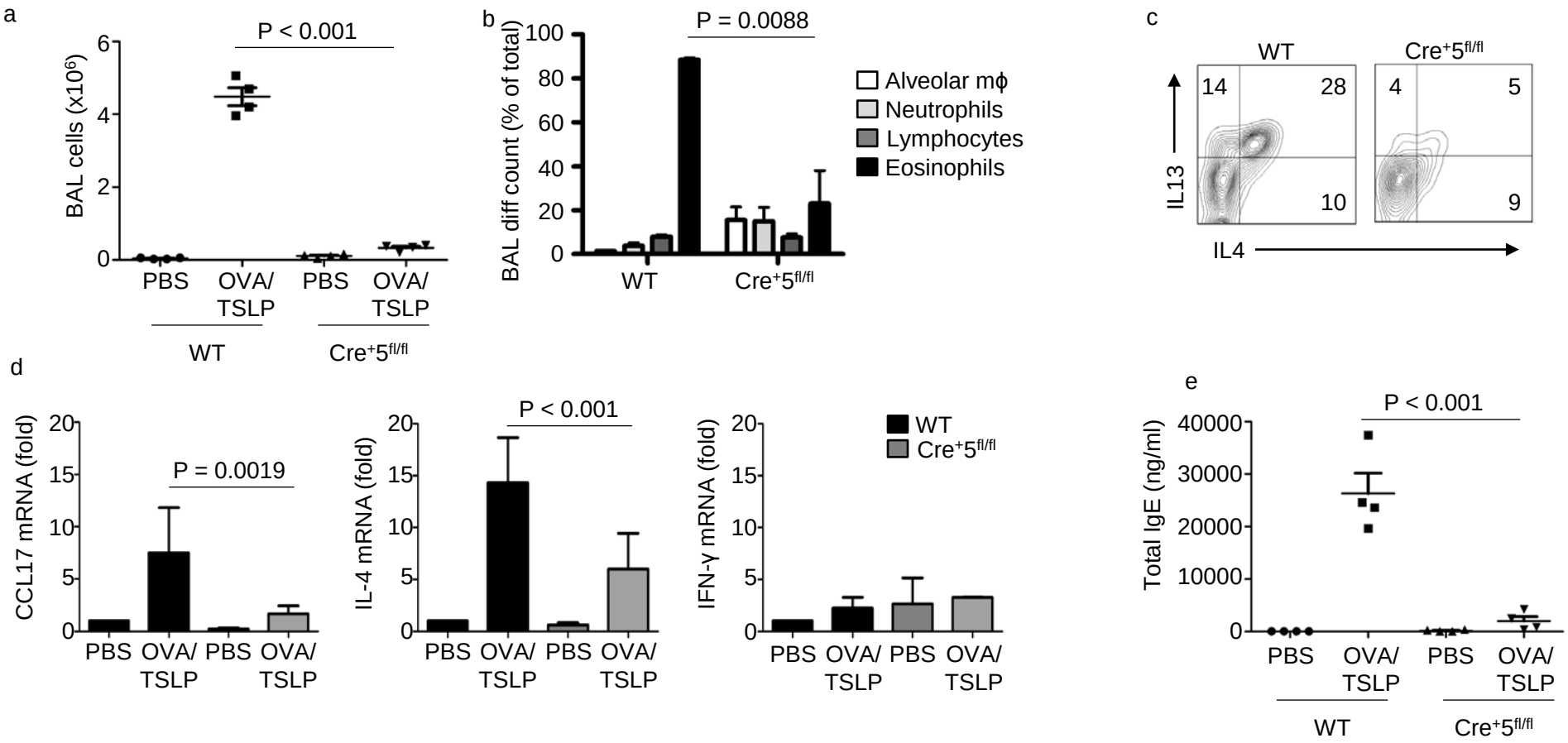
Supplemental Figure 2, Normal skin DC development in $Cre^{-}5^{fl/fl}$ and $Cre^{+}5^{fl/fl}$ mice. A, $MHCII^{+}$ cells were analyzed for the expression of CD207 (langerin) and CD11b in the epidermis and dermis of $Cre^{-}5^{fl/fl}$ and $Cre^{+}5^{fl/fl}$ mice. Graphs and percentages are representative of three independent experiments. B, Fluorescent microscopy for MHCII in epidermal sheets. Scale bar is 100μm. C, Pooled axillary and inguinal skin dLNs from $Cre^{-}5^{fl/fl}$ and $Cre^{+}5^{fl/fl}$ mice were analyzed as in (A). D, Total cell numbers from pooled axillary and inguinal LNs from $Cre^{-}5^{fl/fl}$ and $Cre^{+}5^{fl/fl}$ mice. E, FACs plots gated on $CD11c^{+}$ cells. The percentages of $CD4^{+}$ and $CD8^{+}$ DCs from pooled skin dLNs of $Cre^{-}5^{fl/fl}$ and $Cre^{+}5^{fl/fl}$ mice, representative of three independent experiments. F, Percent of $CD11c^{+}$ DCs which are RFP^{+} from pooled axillary and inguinal LNs from $Cre^{-}5^{fl/fl}$ ROSA:$RFP^{+/-}$ and $Cre^{+}5^{fl/fl}$ ROSA:$RFP^{+/-}$ mice. G, TSLPR expression on epidermal, dermal, and skin-draining LN DCs of $Cre^{-}5^{fl/fl}$ and $Cre^{+}5^{fl/fl}$ mice are analyzed as in (A) and (C). Histograms are representative of three independent experiments. *p>0.01

Supp. Figure 3.



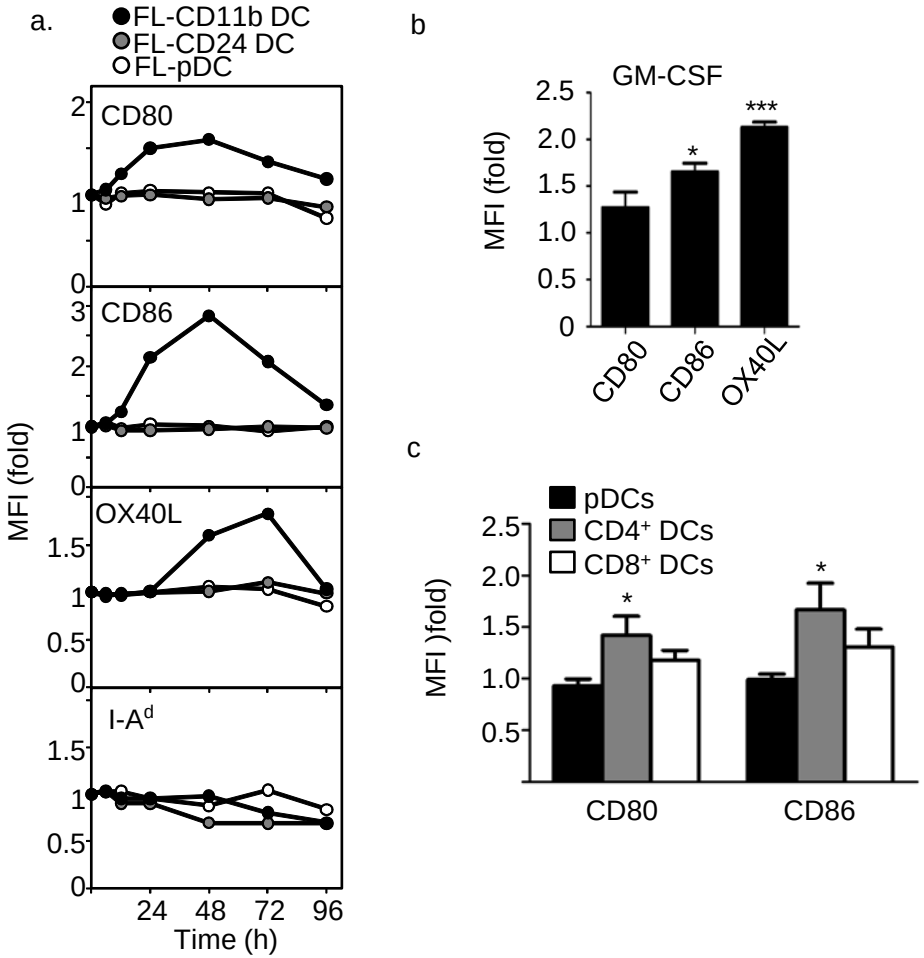
Supplemental Figure 3, Expression levels of STAT5 activating cytokines following CHS sensitization. A, WT mice were left untreated or sensitized on the ear with FITC/DBP for 24 hours, followed by qPCR for indicated STAT5 activating cytokines, normalized to HPRT and fold induction is relative to untreated ears. B, WT mice were left untreated or sensitized on the ear with DNFB for 24 hours, followed by qPCR for indicated STAT5 activating cytokines, normalized to HPRT and fold induction is relative to untreated ears. Minimum 3 mice per group, 3 independent experiments.

Supp. Figure 4.



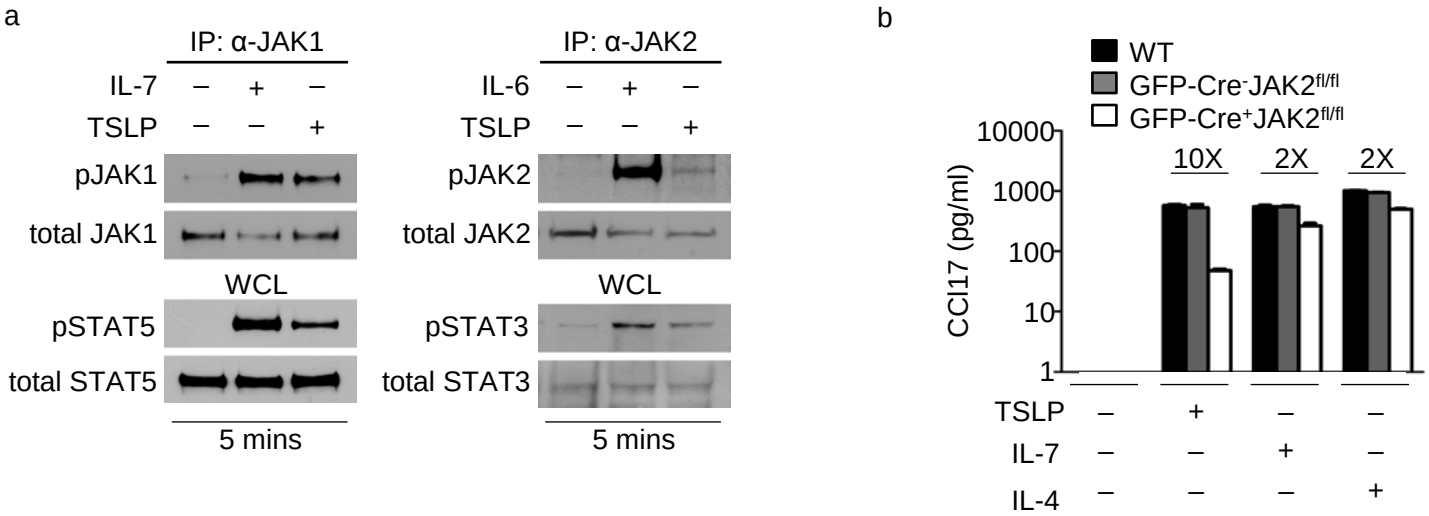
Supplemental Figure 4, Absence of STAT5 in DCs blocks TSLP-induced lung inflammation. A, Mice were treated with TSLP+OVA every other day for 7 treatments, followed by analysis 24 hrs after last treatment. BAL cell count (A) and diff count (B) were average of 4 mice per group. C, Intracellular staining of IL-4 and IL-13 following 4 hour restimulation. D, Expression levels of lung mRNA, relative to WT PBS controls. E, Serum total IgE in mice following last treatment. Two independent experiments with 3-5 mice per group.

Supp. Figure 5.



Supplemental Figure 5, TSLP activates specific DC subsets through upregulation of costimulatory molecules. A, Costimulatory molecule expression was determined by flow cytometry on FL-DC cultures over 96 hours following treatment with 50ng/ml TSLP or left untreated. B, Fold change in MFI of GM-CSF DCs following 48hr treatment with TSLP in triplicate. C, Fold change in MFI of the three splenic DC populations following TSLP treatment in triplicate. Representative of 3 independent experiments.

Supp. Figure 6.



Supplemental Figure 6, TSLP activation of JAK proteins in FL-CD11b-DCs. A, CD11b-DCs were sorted from Flt3-L cultures and treated with 50ng/ml of indicated cytokines for 5 minutes. Whole cell lysates (WCL) were split into two, half were immunoprecipitated (IP) for indicated JAK, half were used as WCL control. Immunoblot analysis of indicated JAK and STAT phospho- and total-antibodies. Representative of 5 independent experiments. B, $Jak2^{fl/fl}$ FL-CD11b-DCs were infected with GFP-Cre retrovirus. 72 hours post TSLP treatment, CCL17 ELISA was performed on WT, GFP^{+} $Jak2^{fl/fl}$ and GFP^{-} $Jak2^{fl/fl}$ CD11b-DCs. Representative of two independent experiments.