

European Journal of Immunology

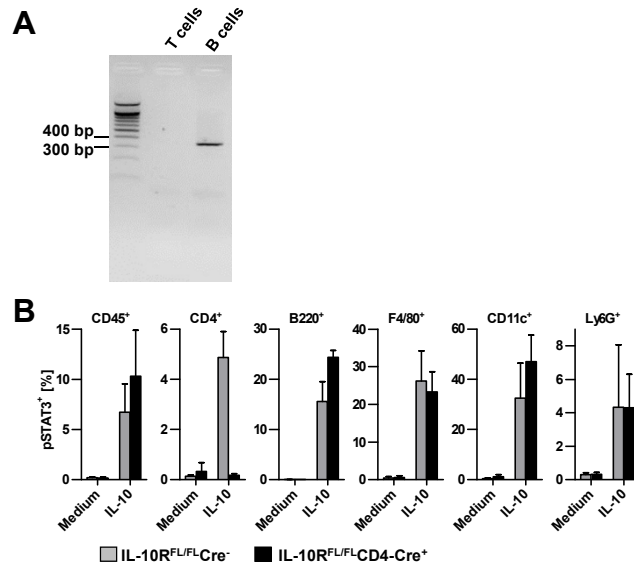
Supporting Information

for

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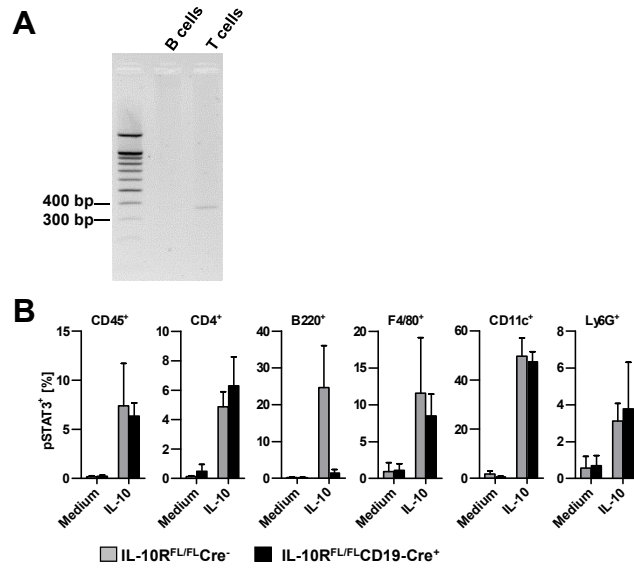
**IL-10 signaling in dendritic cells is required for tolerance induction in a murine
model of allergic airway inflammation**



Suppl. Figure 1:
T cell specific deletion of IL-10R α and IL-10 signaling in IL-10R^{FL/FL}CD4-Cre⁺ mice

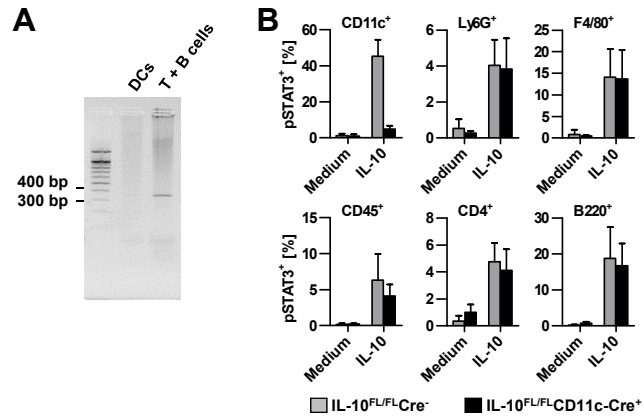
(A) PCR analysis for IL-10R α from DNA of sorted T cells (CD3⁺) and B cells (CD19⁺) of IL-10R^{FL/FL}CD4-Cre⁺ mice. Intact IL-10R α results in a PCR product of 373 bp length. One exemplary experiment of four with 1-2 mice is shown. (B) Flow cytometric analysis of STAT3 phosphorylation upon *in vitro* IL-10 stimulation in CD45⁺ cells, T cells (CD4⁺), B cells (B220⁺), macrophages (F4/80⁺), DCs (CD11c⁺) and neutrophils (Ly6G⁺) from spleens of IL-10R^{FL/FL}CD4-Cre⁺ mice and Cre⁻ littermates. Data of 6 mice per group are shown as mean+SD.

Suppl. Figure 1



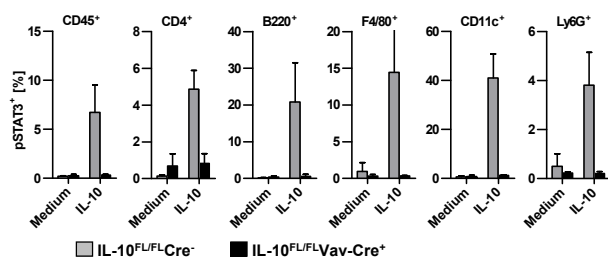
Suppl. Figure 2:
B cell specific deletion of IL-10R α and IL-10 signaling in IL-10R^{FL/FL}CD19-Cre⁺ mice
(A) PCR analysis for IL-10R α from DNA of sorted T cells (CD3⁺) and B cells (CD19⁺) of IL-10R^{FL/FL}CD19-Cre⁺ mice. Intact IL-10R α results in a PCR product of 373 bp length. One exemplary experiment of four with 1-2 mice is shown. **(B)** Flow cytometric analysis of STAT3 phosphorylation upon *in vitro* IL-10 stimulation in CD45⁺ cells, T cells (CD4⁺), B cells (B220⁺), macrophages (F4/80⁺), DCs (CD11c⁺) and neutrophils (Ly6G⁺) from spleens of IL-10R^{FL/FL}CD19-Cre⁺ mice and Cre⁻ littermates. Data of 4-6 mice per group are shown as mean+SD.

Suppl. Figure 2



Suppl. Figure 3:
IL10R α -deficiency and IL-10 signaling in IL10R^{FL/FL}-CD11c-Cre⁺ mice
 (A) PCR analysis for IL-10R α from DNA of sorted DCs (CD11c⁺MHCII⁺) and T and B cells (CD3⁺ and CD19⁺ cells) of IL-10R^{FL/FL}-CD11c-Cre⁺ mice. Intact IL-10R α results in a PCR product of 373 bp length. One exemplary experiment of two with 3 mice is shown (B) Flow cytometric analysis of STAT3 phosphorylation upon *in vitro* IL-10 stimulation in DCs (CD11c⁺), neutrophils (Ly6G⁺), macrophages (F4/80⁺), CD45⁺ cells, T cells (CD4⁺) and B cells (B220⁺) from spleens of IL-10R^{FL/FL}-CD11c-Cre⁺ mice and Cre⁻ littermates. Data of n=6-13 mice/group are shown as mean+SD.

Suppl. Figure 3

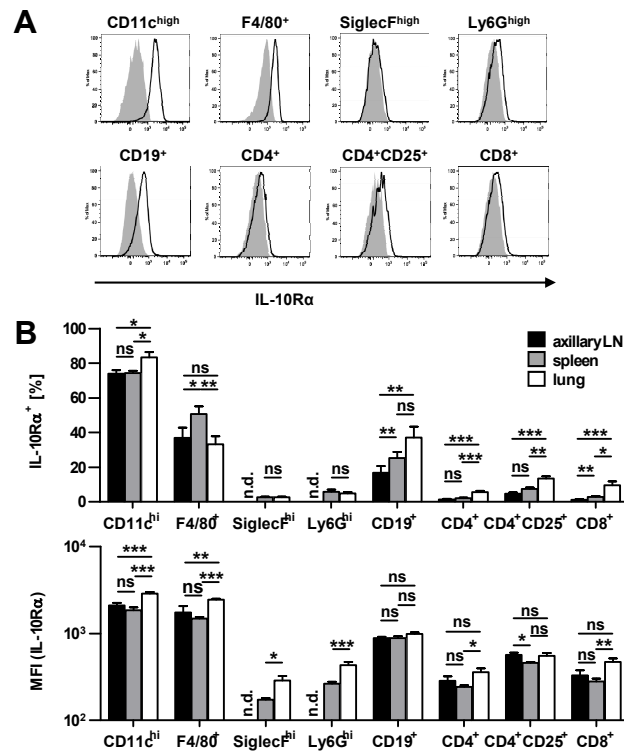


Suppl. Figure 4:

Lack of IL-10 signaling in IL-10^{FL/FL}Vav-Cre⁺ mice

Flow cytometric analysis of STAT3 phosphorylation upon *in vitro* IL-10 stimulation in CD45⁺ cells, T cells (CD4⁺), B cells (B220⁺), macrophages (F4/80⁺), DCs (CD11c⁺) and neutrophils (Ly6G⁺) from spleens of IL-10^{FL/FL}Vav-Cre⁺ mice and Cre⁻ littermates. Data of 4-7 mice per group are shown as mean±SD.

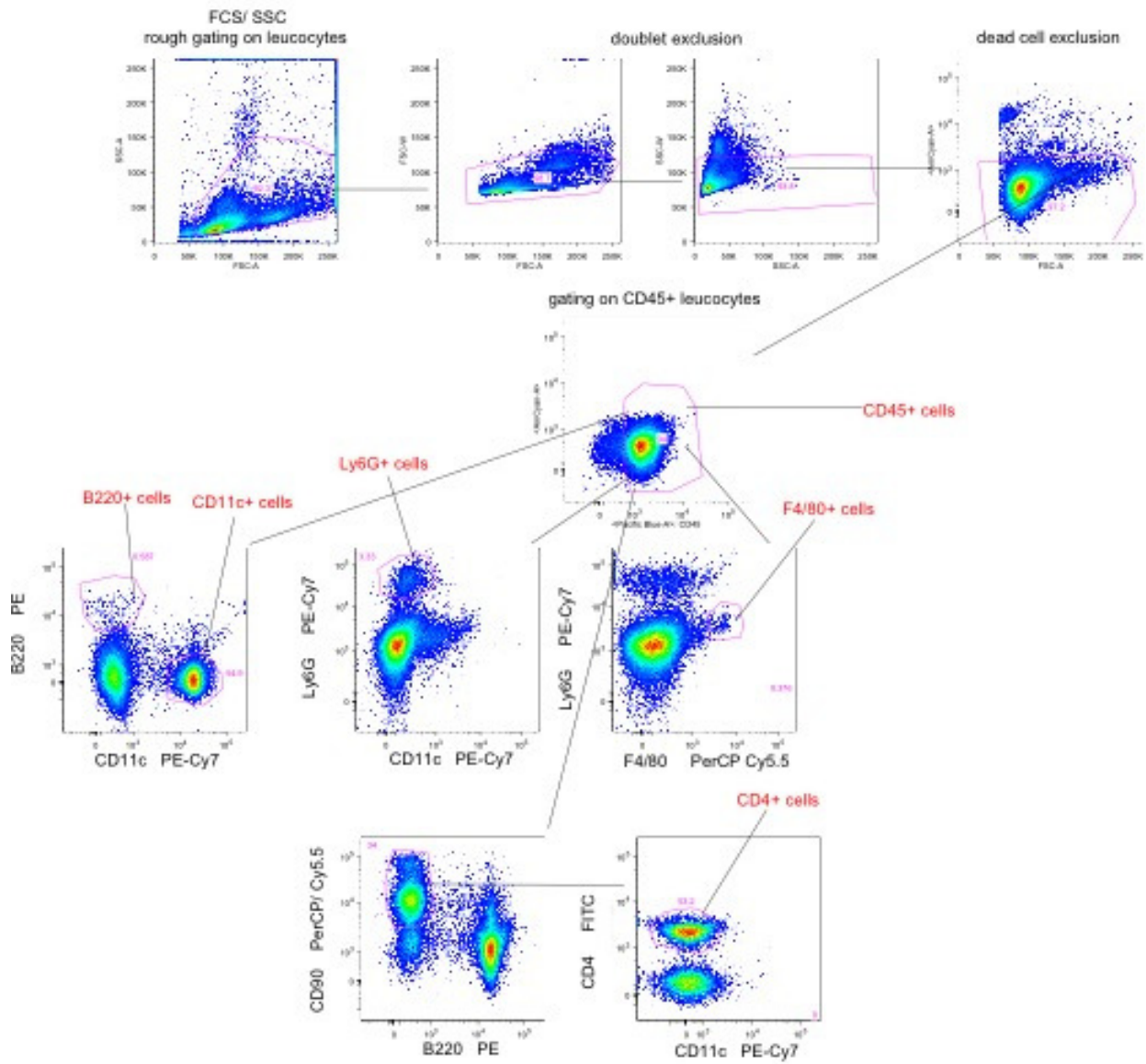
Suppl. Figure 4



Suppl. Figure 5:
IL-10Rα expression of immune cells
 IL-10Rα expression on different immune cells from lung, lymph node and spleen of naïve mice was analyzed by flow cytometry. (A) Representative histograms of IL-10Rα staining (black line) compared to isotype control (grey) in DCs (CD11c^{high}), macrophages (F4/80⁺), eosinophils (SiglecF^{high}), neutrophils (Ly6G^{high}), B cells (CD19⁺), CD4⁺ T cells, CD4⁺CD25⁺ T cells and CD8⁺ T cells from lung tissue. (B) Percentage of IL-10Rα⁺ cells of the indicated populations from axillary lymph nodes (LN), spleen and lung (upper panel) and corresponding mean fluorescence intensity (MFI) of IL-10Rα staining (lower panel). Pooled data (n=6 mice/group and experiment; mean±SEM, Mann-Whitney U test) of 3 independent experiments are shown. * p<0.05, ** p<0.01, *** p<0.001

Suppl. Figure 5

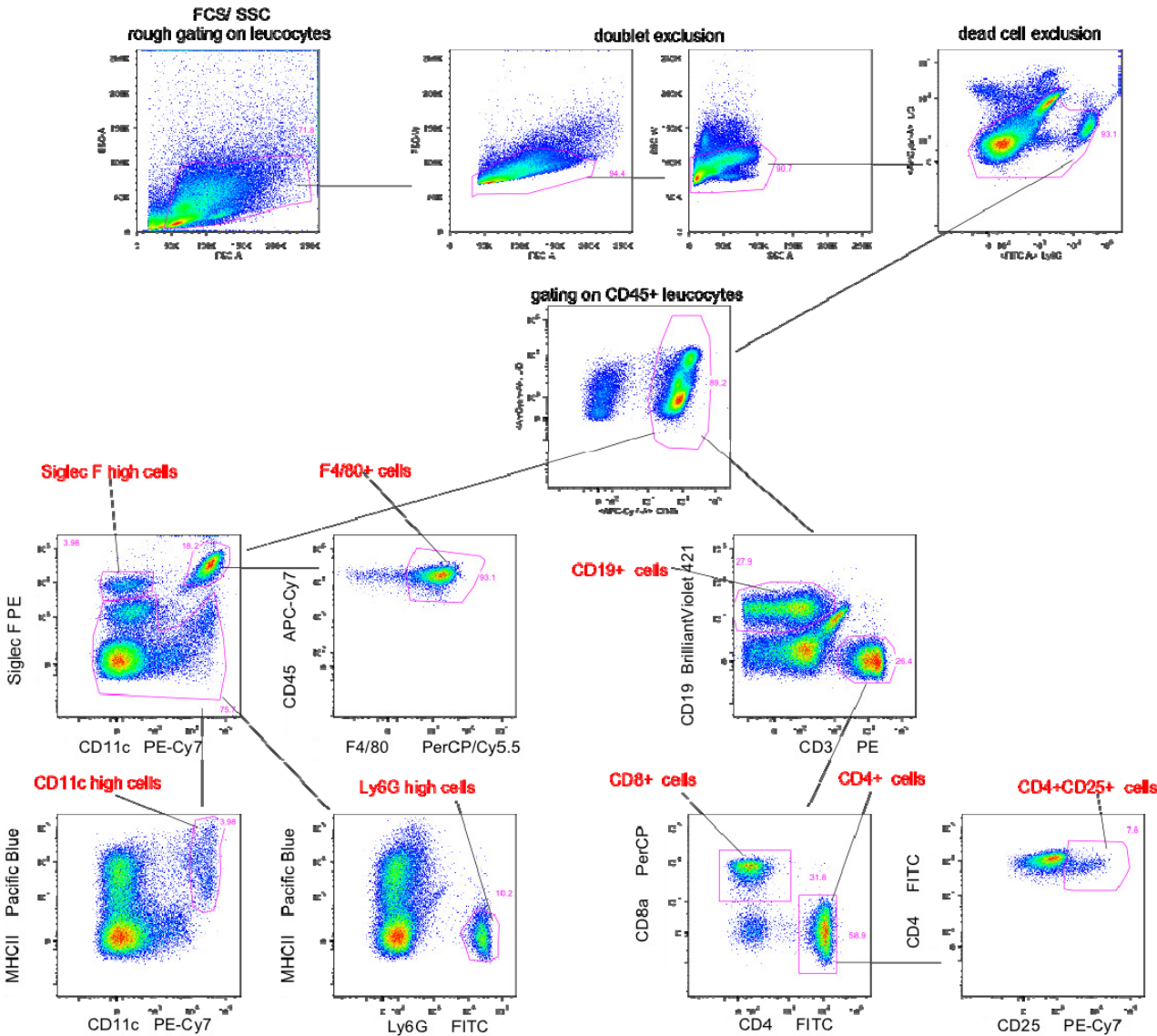
Gating Strategy pStat3 Staining



Suppl. Figure 6:
Gating strategy for pStat3 staining
 Gating strategy used for flow cytometric analysis of STAT3 phosphorylation upon in vitro IL-10 stimulation of spleen cells

Suppl. Figure 6

Gating Strategy IL-10R Staining Example Lung



Suppl. Figure 7:
Gating strategy for IL-10R staining
Gating strategy used for flow cytometric analysis of IL-10R α expression on different immune cells. One representative lung cell sample is shown.

Suppl. Figure 7