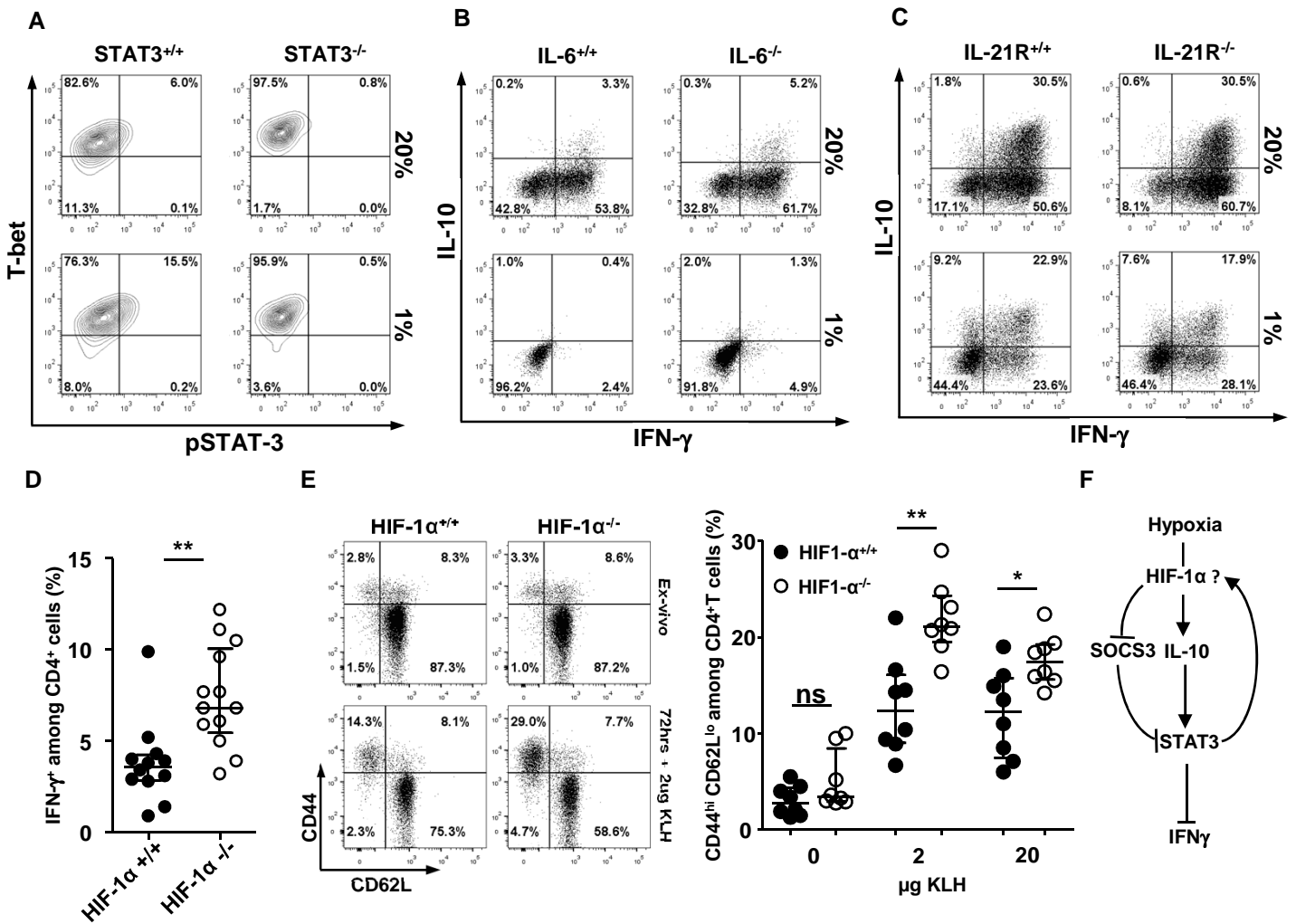


Supplemental Fig 1. Differential effect of hypoxia on Th1, Th17 and Treg cells.

CD4⁺ T cells from CD4 Cre HIF-1 $\alpha^{fl/fl}$ (HIF-1 $\alpha^{-/-}$) or CD4 HIF-1 $\alpha^{fl/fl}$ (HIF-1 $\alpha^{+/+}$) mice were activated for 72 h with anti-CD3 and anti-CD28 mAbs under Th1, Th17 or Treg polarizing conditions, rested for 24 h and cultured in 20% or 1% pO₂ for additional 72 h in the same conditions. (A) Cells were shortly restimulated with PMA/ionomycin in the presence of Brefeldin A and analyzed by flow cytometry for TCR-β, CD4, IFN-γ, IL-17 and Foxp3 expression. (B) HIF-1 α and GLUT1 mRNA expression in Th1 cells was analyzed by qPCR. Histograms show the means of duplicate wells \pm SDs. (C) Pre-activated Th1 cells were stained with CFSE after the rest period and cultured in 20% or 1% pO₂ as described above. Left panel: percentage of proliferating cells (CFSE^{lo}). Right panel: the total cell counts were measured at the end of the culture (starting number 10⁶). Histograms show the means of 4 independent experiments \pm SDs. (A-C). Data are representative of at least 4 independent experiments. (D) Clones of antibodies used. (E) Concentrations of recombinant cytokine and antibodies used for T cell differentiation. (F) Primer sequences used for cloning.



Supplemental Fig. 2.

(A) CD4⁺ T cells were isolated from CD4 Cre STAT3^{fl/fl} (STAT3^{-/-}) or CD4 STAT3^{fl/fl} (STAT3^{+/+}) mice, cultured as described in Figure 1A and analyzed for T-bet and pSTAT3 expression by flow cytometry. Data are representative of 3 independent experiments. (B-C) CD4⁺ T cells from WT, IL-6^{-/-} or IL-21R^{-/-} mice were cultured as described in Figure 1 and shortly restimulated with PMA/ionomycin in the presence of Brefeldin A. Cells were stained for IFN-γ and IL-10 and analyzed by flow cytometry. KLH-pulsed DCs from C57BL/6 mice were injected into the footpads of CD4 Cre HIF-1α^{fl/fl} or CD4 HIF-1α^{fl/fl} mice. Draining LNs were harvested 5 d later and analyzed by flow cytometry. (D) Data are expressed as percentage of IFN-γ expressing CD4⁺ TCR-β⁺ gated cells (following 3 h incubation with PMA/ionomycin and Brefeldin A). Each symbol represents the production of cytokine by individual mice. (E) Data are expressed as the percentage of LN CD4⁺ TCR-β⁺ (gated) cells expressing CD44 and/or CD62L *ex vivo* (upper panel) or after stimulation with 2μg/ml of KLH (lower panel). Data from 2 pooled experiments showing the % of cells expressing CD44 and/or CD62L among CD4⁺ T cells after stimulation with graded doses of KLH (mean ± SD). (F) A model for the role of HIF-1 in modulating Th1 function in hypoxia. Hypoxia (probably via HIF-1α stabilization) increases IL-10 expression and STAT3 signaling, which in turn inhibits SOCS3 transcription and enhances HIF-1α. This positive feedback loop results in impaired Th1 activation.