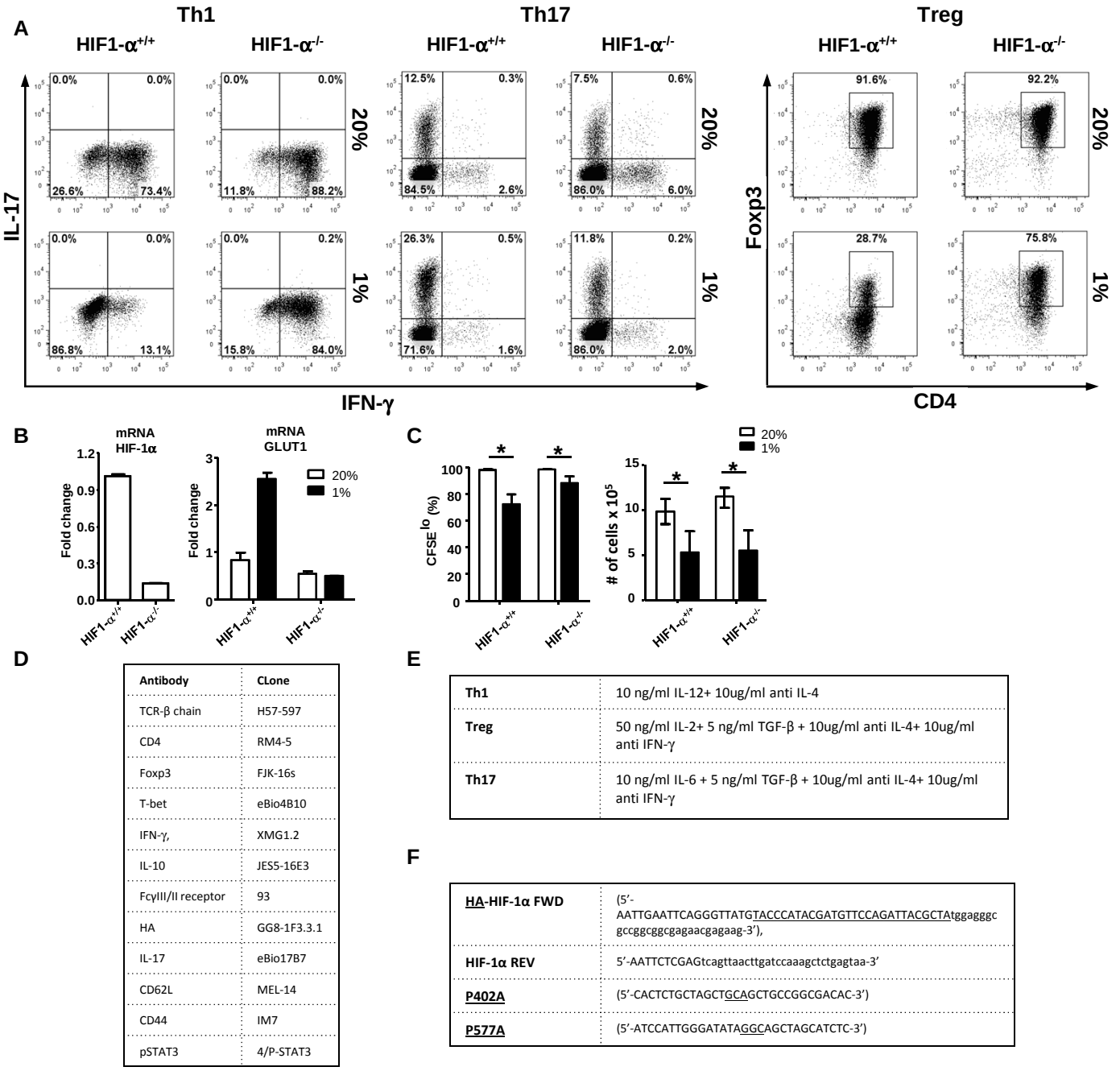


D

Antibody	CLone
TCR-β chain	H57-597
CD4	RM4-5
Foxp3	FJK-16s
T-bet	eBio4B10
IFN-γ,	XMG1.2
IL-10	JES5-16E3
FcγIII/II receptor	93
HA	GG8-1F3.3.1
IL-17	eBio17B7
CD62L	MEL-14
CD44	IM7
pSTAT3	4/P-STAT3

E

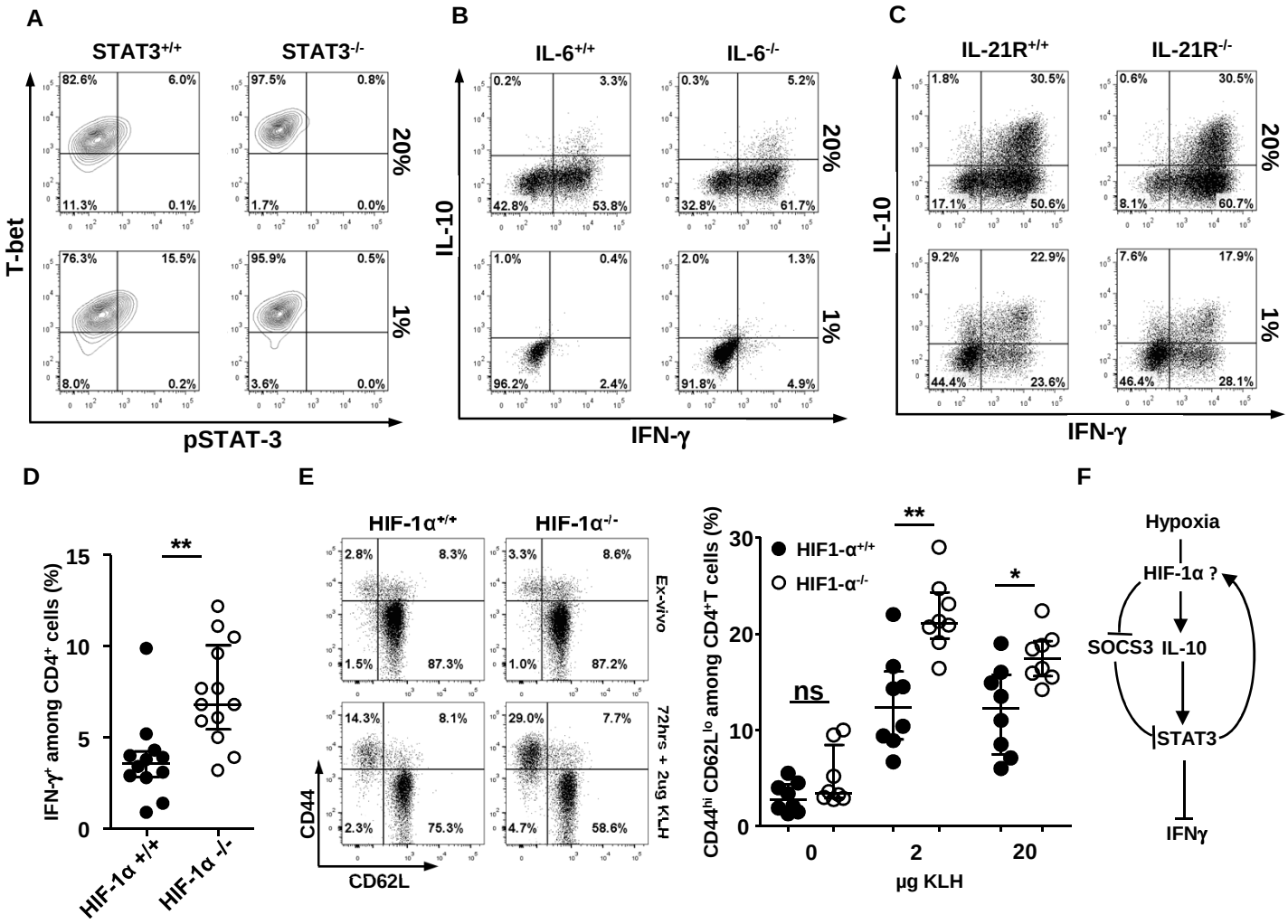
Th1	10 ng/ml IL-12+ 10ug/ml anti IL-4
Treg	50 ng/ml IL-2+ 5 ng/ml TGF-β + 10ug/ml anti IL-4+ 10ug/ml anti IFN-γ
Th17	10 ng/ml IL-6 + 5 ng/ml TGF-β + 10ug/ml anti IL-4+ 10ug/ml anti IFN-γ

F

HA-HIF-1α FWD	(5′-AATTGAATTCAGGGTTATGTACCCATACGATGTTCCAGATTACGCTAtggagggcgccggcggcgagaacgagaag-3′),
HIF-1α REV	5′-AATTCTCGAGtcagttaacttgatccaaagctctgagtaa-3′
P402A	(5′-CACTCTGCTAGCTGCAGCTGCCGGCGACAC-3′)
P577A	(5′-ATCCATTGGGATATAGGCAGCTAGCATCTC-3′)

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

CD4$^+$ T cells from CD4 Cre HIF-1α$^{fl/fl}$ (HIF-1α$^{-/-}$) or CD4 HIF-1α$^{fl/fl}$ (HIF-1α$^{+/+}$) 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% pO_2 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-1α and GLUT1 mRNA expression in Th1 cells was analyzed by qPCR. Histograms show the means of duplicate wells ± 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 10^6). Histograms show the means of 4 independent experiments ± 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 STAT3fl/fl (STAT3$^{-/-}$) or CD4 STAT3fl/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-1a$^{fl/fl}$ or CD4 HIF-1a$^{fl/fl}$ 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.