

Cytokine stimulation (30 min, 10 ng/ml):

Stattic-V (50 ng/ml):

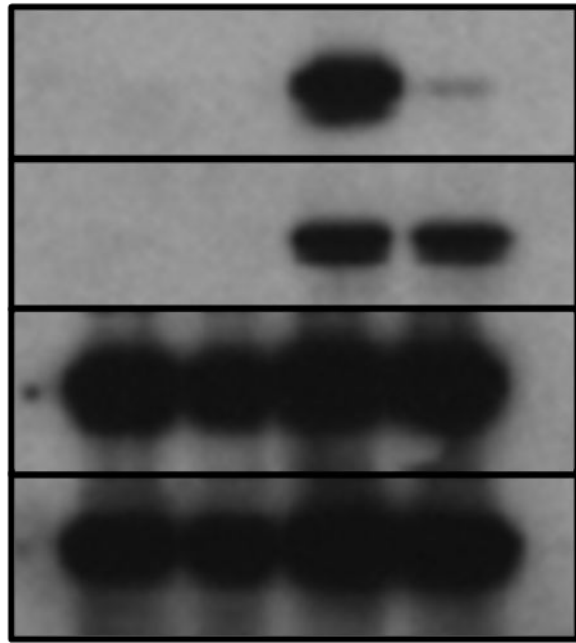
(-)		rhIL-6	
-	+	-	+

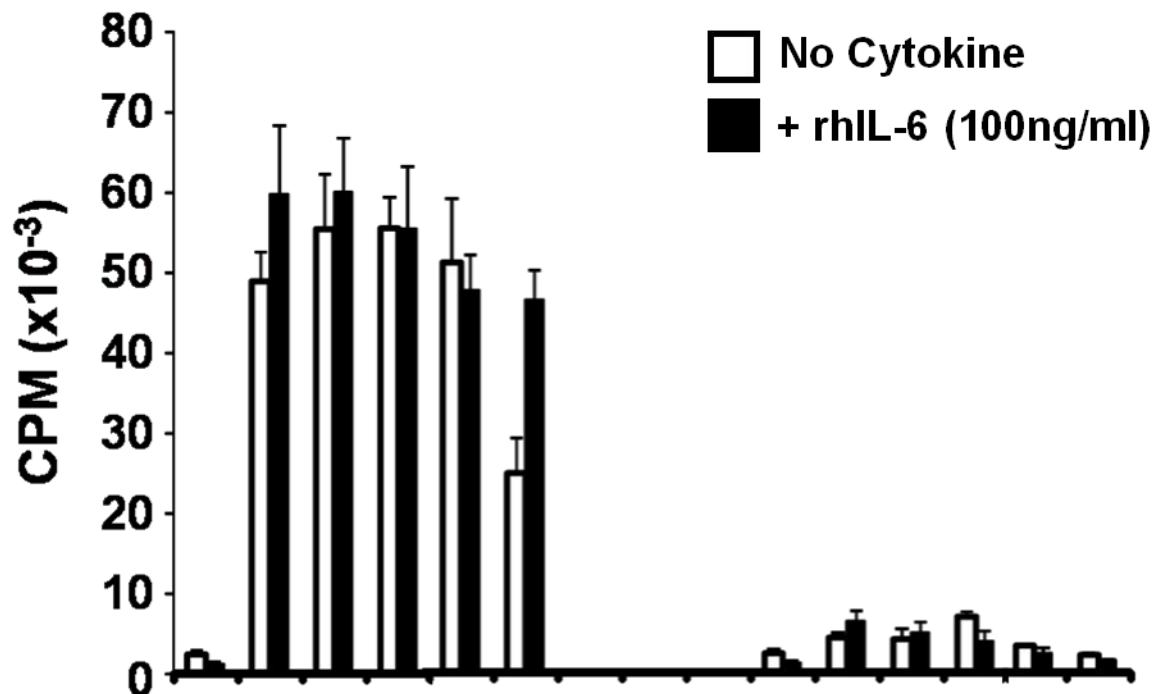
pStat3 →

pStat1 →

Total Stat3 →

Total Stat1 →





APC	+	+	+	+	+	+	+	+	+	+	+	+
CD4 ⁺ CD25 ⁻ T cell	-	+	+	+	+	+	-	-	-	-	-	-
CD4 ⁺ CD25 ^{high} Treg	-	-	-	-	-	-	+	+	+	+	+	+
Stattic-V	-	-	+	-	-	-	-	-	+	-	-	-
Peptide inhibitor	-	-	-	+	-	-	-	-	-	+	-	-
Inactive peptide	-	-	-	-	+	-	-	-	-	-	+	-
Stat Inhibitor VII	-	-	-	-	-	+	-	-	-	-	-	+

**CD4⁺CD25⁻
T cell**

IL-6 (ng/ml):

0 2.5 5 10 20 40 80 100

pStat3 →

pStat1 →

Total Stat3 →

Total Stat1 →

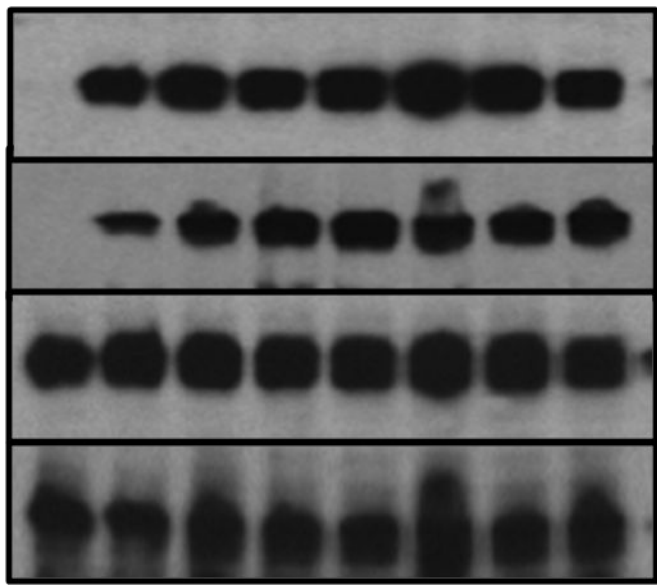


Figure S1: Stattic-V decreases level of Stat3, but not Stat1, phosphorylation in IL-6-stimulated CD4⁺CD25⁻ T cells. CD4⁺CD25⁻ T cells were isolated from healthy donors by flow cytometry sorting. Cells were stimulated with or without rhIL-6 (10 ng/ml) and Stattic V (10 ng/ml) for 30 min. Protein lysates were prepared and immunoblotted using antibodies specific for pStat3, total Stat3, pStat1, and total Stat1. Immunoblot shown is representative of six separate experiments.

Figure S2: Inhibition of Stat3 phosphorylation does not alter proliferation of CD4⁺CD25⁻ T cells or CD4⁺CD25^{high} Treg cells. CD4⁺CD25⁻ T cells and CD4⁺CD25^{high} Treg cells were isolated from the peripheral blood of healthy volunteers by flow cytometry sorting, and cultured with allo-APC and indicated inhibitors of Stat3 phosphorylation. Data shown is representative of three separate experiments \pm SEM.

Figure S3: Primary T cells phosphorylate Stat1 and Stat3 in response to very low concentrations of rhIL-6. CD4⁺CD25⁻ T cells were isolated from peripheral blood of healthy donors. Cells were stimulated for 30 min with indicated concentrations of rhIL-6. Blot shown is representative of three separate experiments.

Table SI. Antigen presenting cells express minimal levels of IL-6R α and gp130.

	Normal APC non-irradiated^a	Normal APC irradiated 30Gy
IL-6R α	0.2 \pm 0.1	0.7 \pm 0.3
gp130	8.7 \pm 1.8	12.3 \pm 1.4

^a n=3; measured by flow cytometric analysis and expressed as percent of cells positive for indicated surface protein.