Figure S1

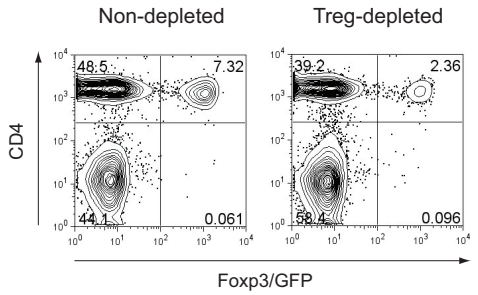

Figure S1: Treatment with a monoclonal antibody to CD25 depletes more than 50% of Foxp3⁺ T-reg. *Il6*$^{-/-}$×*Foxp3gfp*.KI mice were treated with rat IgG1 (non-depleted) or anti-CD25 antibody (PC61) by intraperitoneal injection of 500 µg on days –5 and –3 prior to immunisation with MOG_{35-55} in complete Freund's adjuvant. Seven days after immunisation, lymph node cells were isolated, stained for CD4 and analysed by flow cytometry for the presence of CD4⁺Foxp3/GFP⁺ T-cells. The numbers in the quadrants represent percentages.

Figure S2

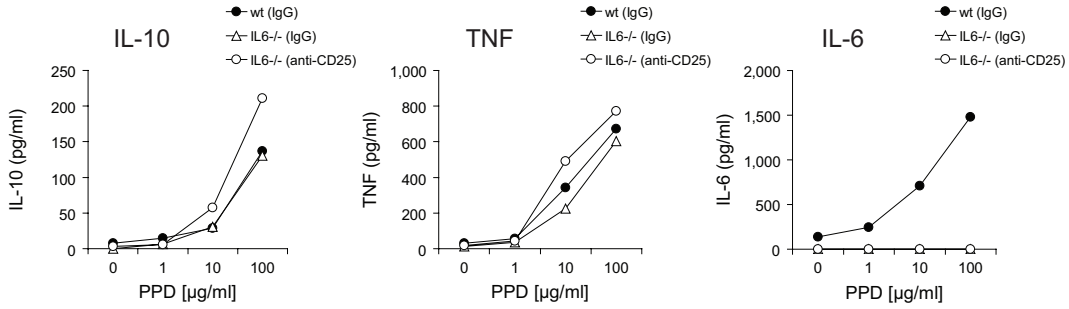

Figure S2: Il6$^{-/-}$ mice do not exhibit residual IL-6 production. Wildtype, *Il6*$^{-/-}$, or T-reg-depleted (anti-CD25) *Il6*$^{-/-}$ mice were immunised with MOG_{35-55} emulsified in complete Freund's adjuvant. Six days after immunisation, splenocytes were isolated and re-stimulated in vitro with increasing concentrations of purified protein derivative from M. tuberculosis (PPD). Since M. tuberculosis is the major constituent of the immunising emulsion, we re-activated the splenocyte cultures with PPD to induce a maximum response and to determine whether under these conditions *Il6*$^{-/-}$ mice would show any indication of residual IL-6 production. After 48 h supernatants were collected and assayed for IL-10, TNF, and IL-6 by cytometric bead array. Splenocytes from *Il6*$^{-/-}$ mice continued to produce IL-10 and TNF, but failed to produce any IL-6. Furthermore, prior depletion of T-reg did not result in IL-6 production from splenocytes of *Il6*$^{-/-}$ mice either.

Figure S3

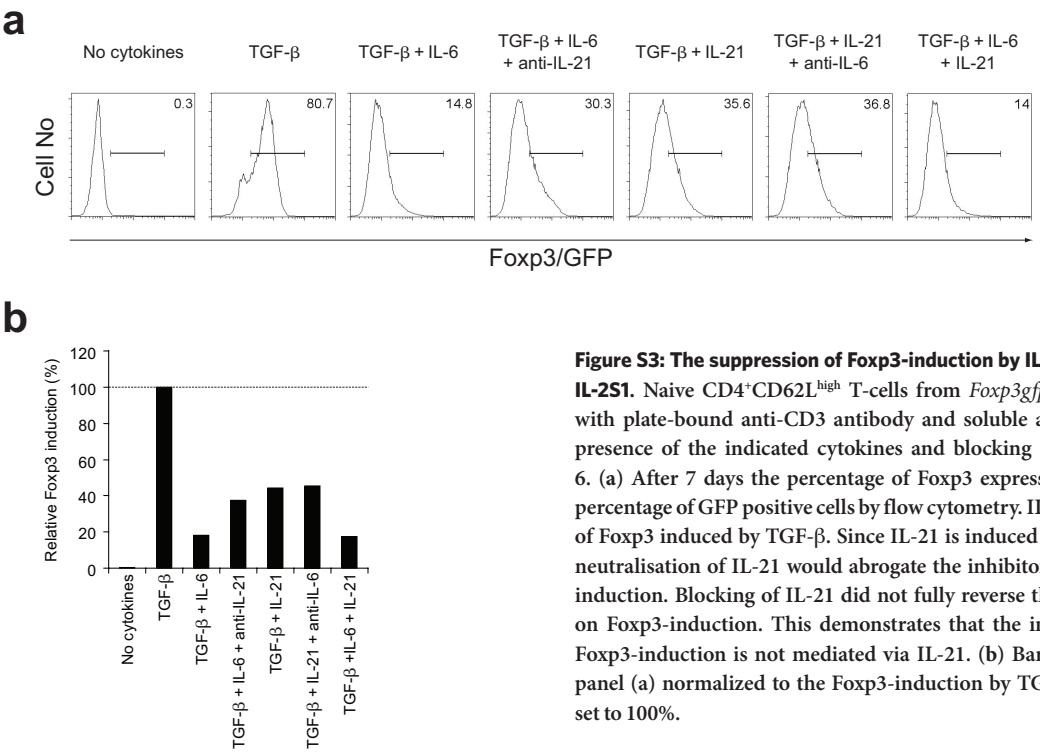

Figure S3: The suppression of Foxp3-induction by IL-6 is not mediated through IL-2S1. Naive CD4⁺CD62L^{high} T-cells from *Foxp3gfp*.KI mice were stimulated with plate-bound anti-CD3 antibody and soluble anti-CD28 antibody in the presence of the indicated cytokines and blocking antibodies to IL-21 or IL-6. (a) After 7 days the percentage of Foxp3 expressing cells was measured as percentage of GFP positive cells by flow cytometry. IL-6 inhibited the expression of Foxp3 induced by TGF-β. Since IL-21 is induced by IL-6, we tested whether neutralisation of IL-21 would abrogate the inhibitory effect of IL-6 on Foxp3-induction. Blocking of IL-21 did not fully reverse the inhibitory effect of IL-6 on Foxp3-induction. This demonstrates that the inhibitory effect of IL-6 on Foxp3-induction is not mediated via IL-21. (b) Bar graph of the results from panel (a) normalized to the Foxp3-induction by TGF-β, which was arbitrarily set to 100%.

Figure S4

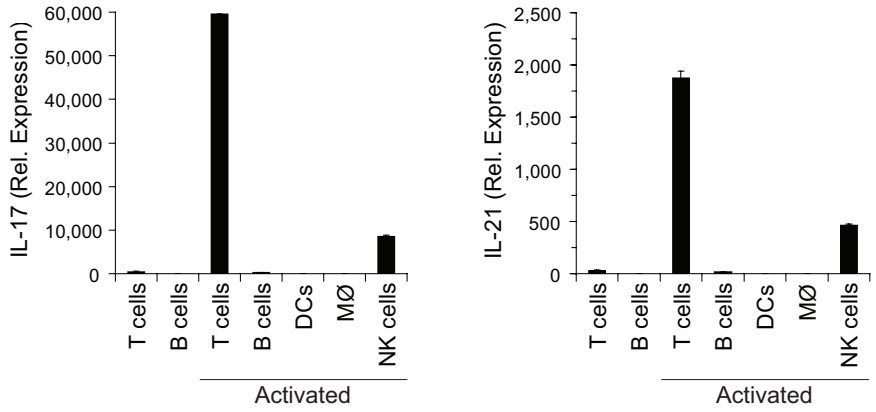

Figure S4: T-cells are the major source of IL-21 in T-reg-depleted Il6$^{-/-}$ mice. *Il6*$^{-/-}$ mice were depleted of T-reg by injection of anti-CD25 antibody followed by immunisation with MOG_{35-55} emulsified in CFA (as described in Figure 2). Seven days after immunisation, different cell types were purified from pooled draining lymph node cells and splenocytes by flow cytometry sorting. T-cells (Thy1.2$^+$) were activated with PMA/ionomycin and B cells (CD19$^+$), dendritic cells (CD11c$^+$, DCs), macrophages (CD11b$^+$, MØ), and NK cells (NK1.1$^+$) were activated with LPS before isolation of RNA for quantitative RT-PCR to determine the cell type-specific expression of IL-17 and IL-21. For comparison, T-cells and B cells were also analysed without *in vitro*-activation.

Figure S5

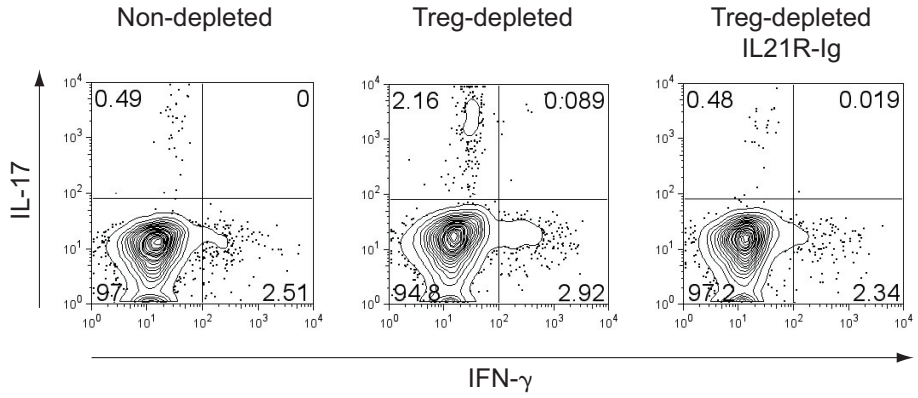

Figure S5: *In vivo*-blocking of IL-21 inhibits the generation of Th17 cells in T-reg-depleted Il6$^{-/-}$ mice. *Il6*$^{-/-}$ mice were treated with control rat IgG1 (non-depleted) or with anti-CD25 antibody to deplete T-reg (T-reg-depleted) followed by immunisation with MOG_{35-55} emulsified in complete Freund's adjuvant. The T-reg depleted groups were either treated with PBS (control) or with IL-21 receptor-Ig (4 times 200 μg intraperitoneally every other day, starting on the day of immunisation) in order to block IL-21-activity *in vivo*. On day 7 after immunisation, draining lymph node cells were isolated, stimulated with PMA/ionomycin, stained for intracellular IL-17 and IFN-γ, and analysed by flow cytometry. The gate is set on CD4$^+$Foxp3/GFP$^-$ T-cells. Numbers indicate percentages.