

Supplementary Material

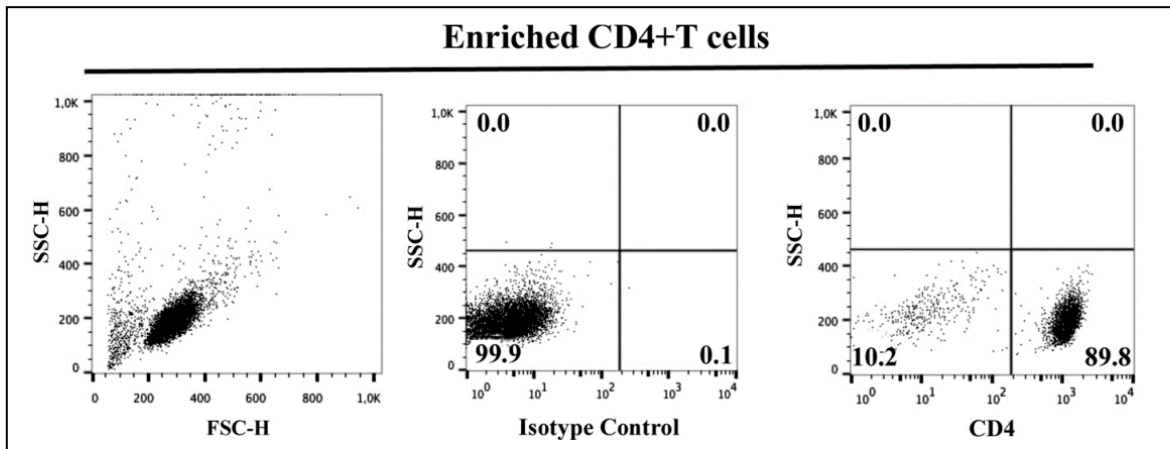


Figure S1. Gating strategy to assess the isolation of peripheral blood CD4⁺ T helper lymphocytes by flow cytometry. CD4⁺ T cells were enriched from PBMCs by negative selection using a RosetteSep™ kit (Stem Cell Technologies). The representative dot plots of the forward (FSC-H) and side (SSC-H)-scatter properties of enriched CD4⁺ T cells are from a healthy donor. Cell purity was detected with an anti-CD4 mAb. Antibody specificity was confirmed with an isotype control.

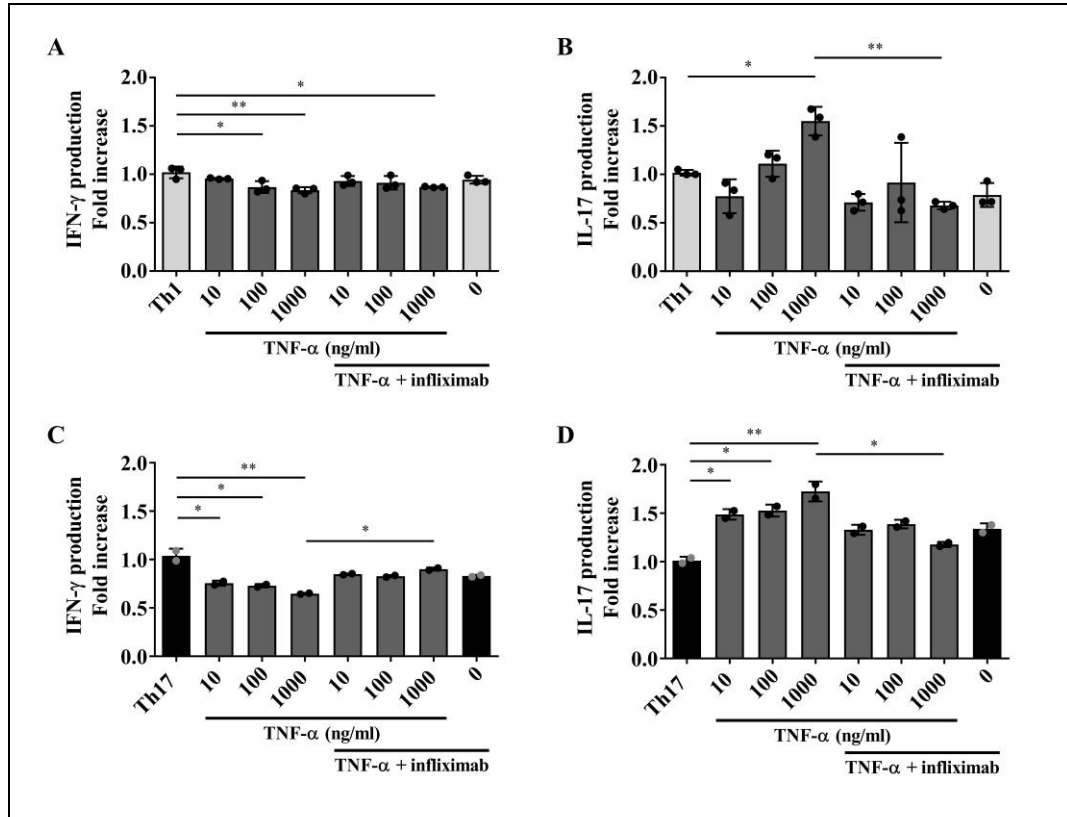


Figure S2. Effect of TNF- α on IFN- γ and IL-17 production by Th1 and Th17 lymphocytes. Sorted T lymphocyte subsets were cultured for 4 days with increasing concentrations of recombinant human TNF- α (0, 10, 100, or 1000 ng/ml) alone or in the presence of an anti-TNF- α blocking mAb (infliximab). Cells were stimulated with PMA and ionomycin plus brefeldin A for 5 h and analyzed for intracellular cytokine production by flow cytometry. IFN- γ (A) and IL-17 (B) production was measured on Th1 cells from three healthy donors; IFN- γ (C) and IL-17 (D) were detected on Th17 cells from two healthy controls. Bars represent the mean values $\pm$ SD. Data were normalized against cells that did not receive treatment with TNF- α or TNF- α plus infliximab. Statistical analyses were performed with Kruskal-Wallis followed by Dunn's multiple comparison tests. * $p < 0.05$, ** $p < 0.01$.

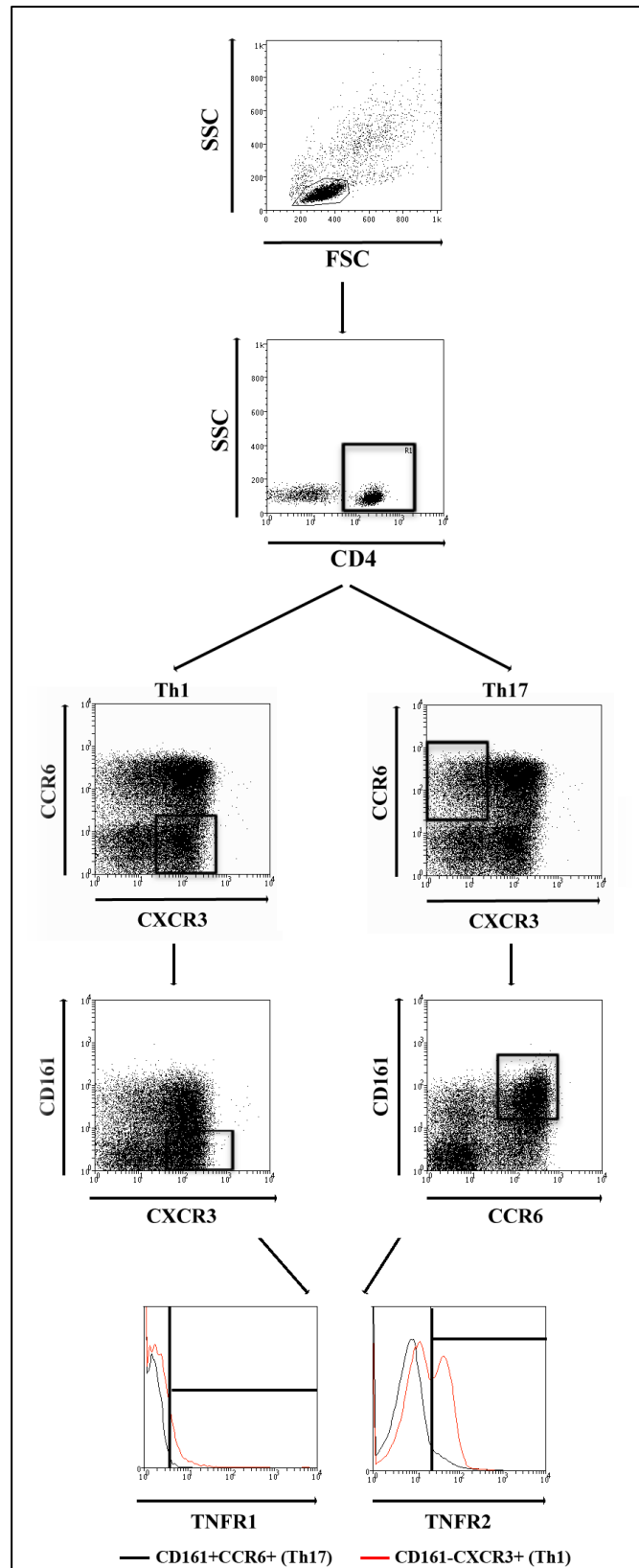


Figure S3. Gating strategy to analyze the expression of TNF- α receptors on peripheral blood Th1 and Th17 cells by flow cytometry. The dot plots and histograms represent staining of PBMCs from a healthy control. TNFR1 and TNFR2 expression was evaluated on CD4⁺CCR6⁻CXCR3⁺CD161⁻ (Th1) cells and CD4⁺CCR6⁺CXCR3⁻CD161⁺ (Th17) cells.