Supplementary Figures 1 – 5 for

Complement regulator CD46 temporally regulates cytokine production by conventional and unconventional T cells

John Cardone, Gaelle Le Friec, Pierre Vantourout, Andrew Roberts, Anja Fuchs, Ian Jackson, Tesha Suddason, Graham Lord, John P. Atkinson, Andrew Cope, Adrian Hayday, and Claudia Kemper

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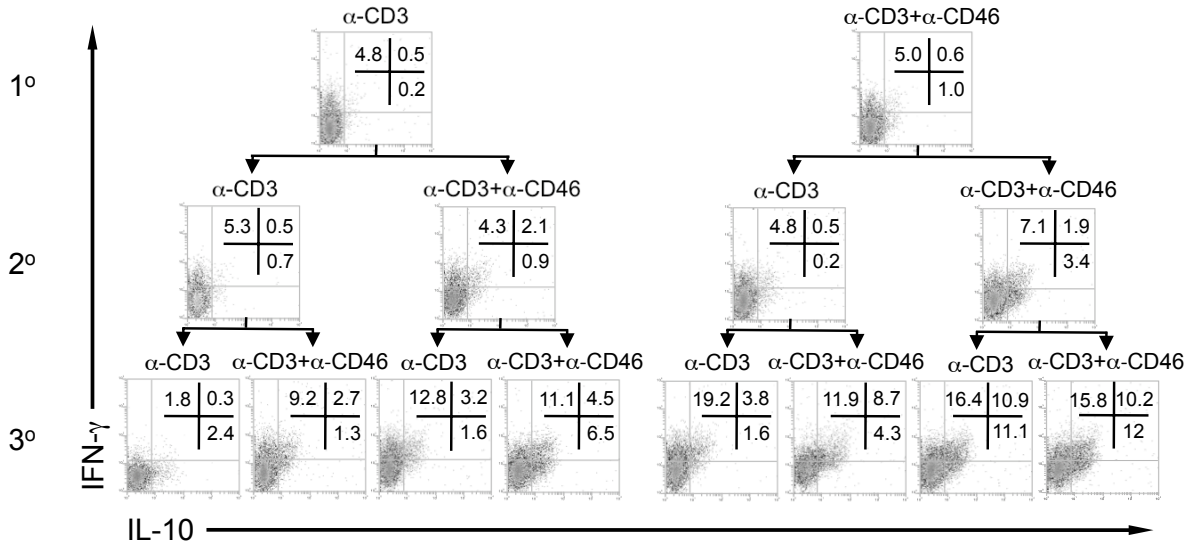
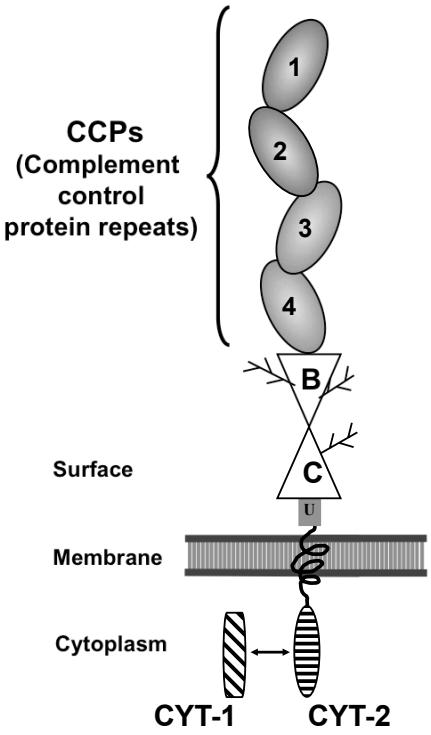


Supplementary Figure 1 Naïve CD4$^+$ T cells gradually acquire a Tr1-like phenotype. Purified naïve human CD4$^+$ T cells were activated for 36h with mAbs against CD3 +/- anti-CD46 in the presence of 50 U/ml IL-2 (1º), expanded for 5 days with 5 U/ml IL-2, and then restimulated with the indicated mAbs in the presence of 50 U/ml IL-2 (2º and 3º). After each round of activation, IFN-γ and IL-10 secretion was measured by CSA. Data are representative of two experiments.



Supplementary Figure 2 Schematic diagram of the CD46 protein structure. The four major isoforms of CD46 are generated by alternative splicing of the extracellular 'BC' region, and mutually exclusive usage of the CYT-1 or CYT-2 cytoplasmic domains.

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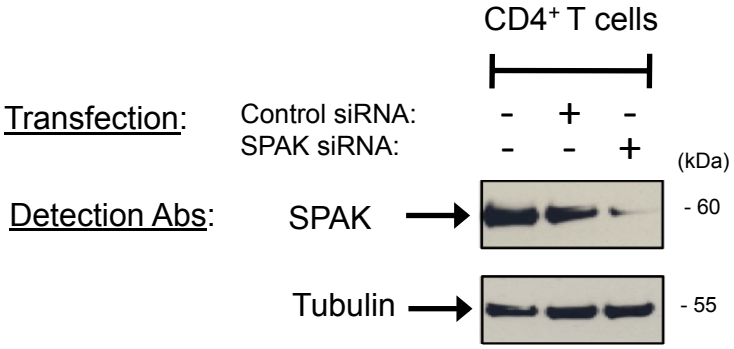
a



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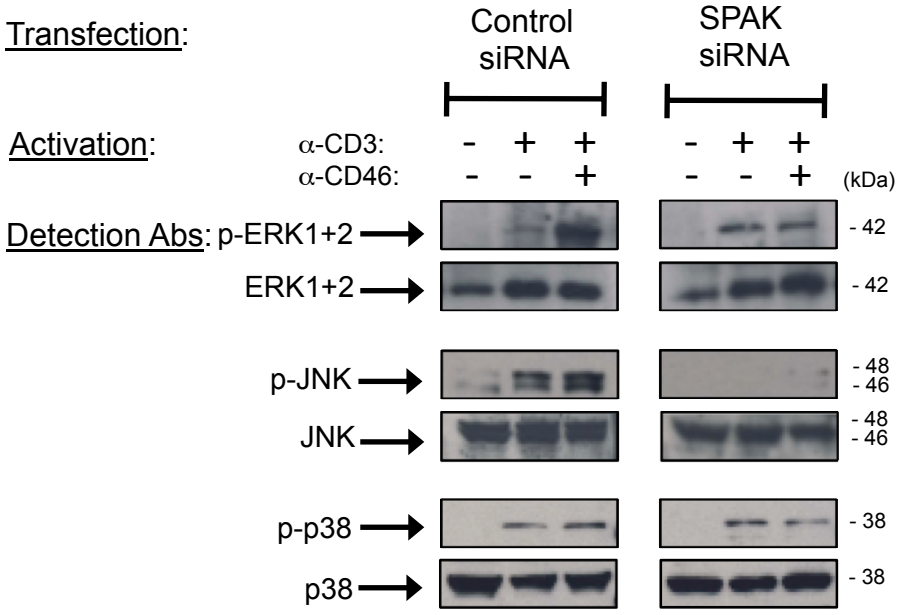
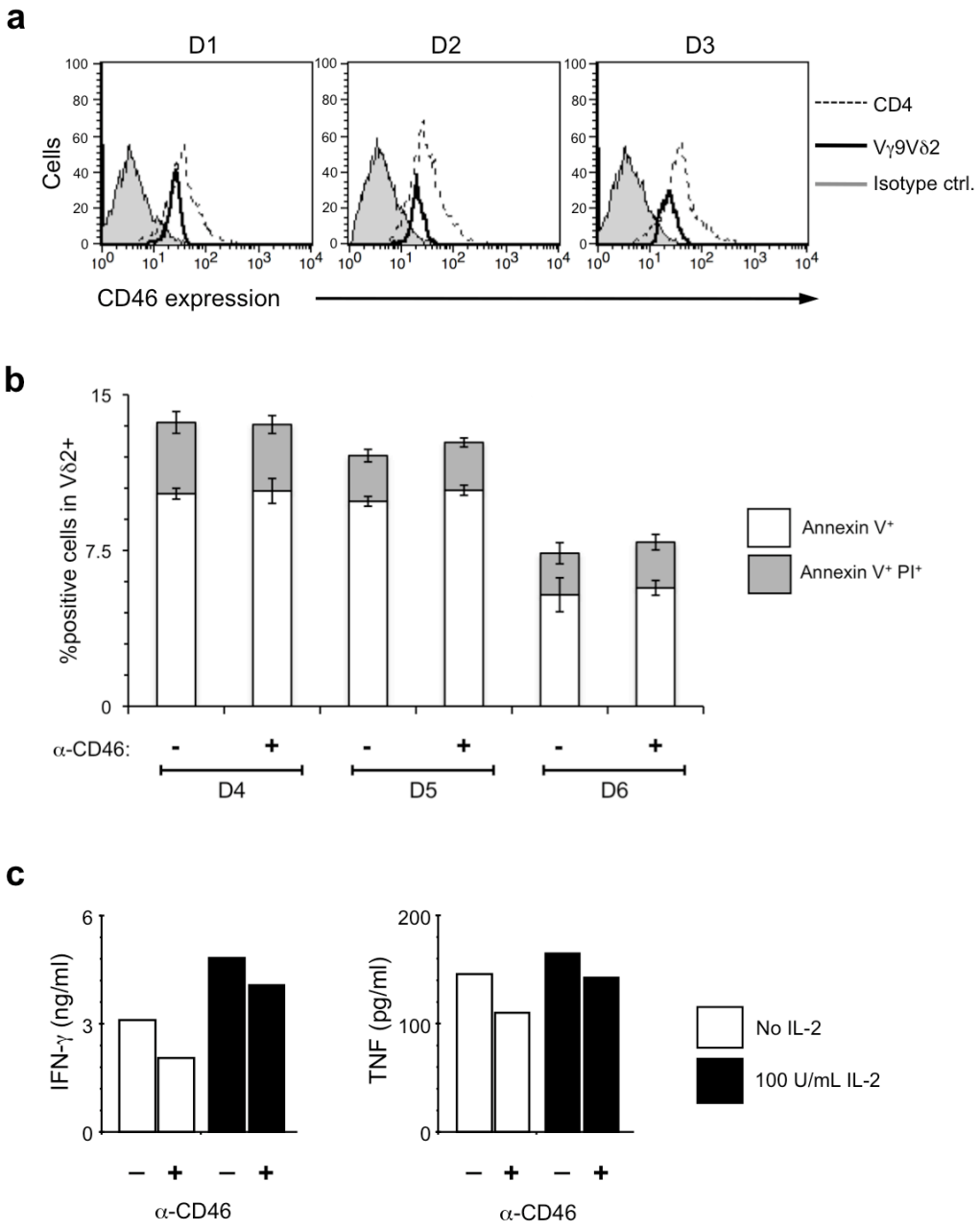
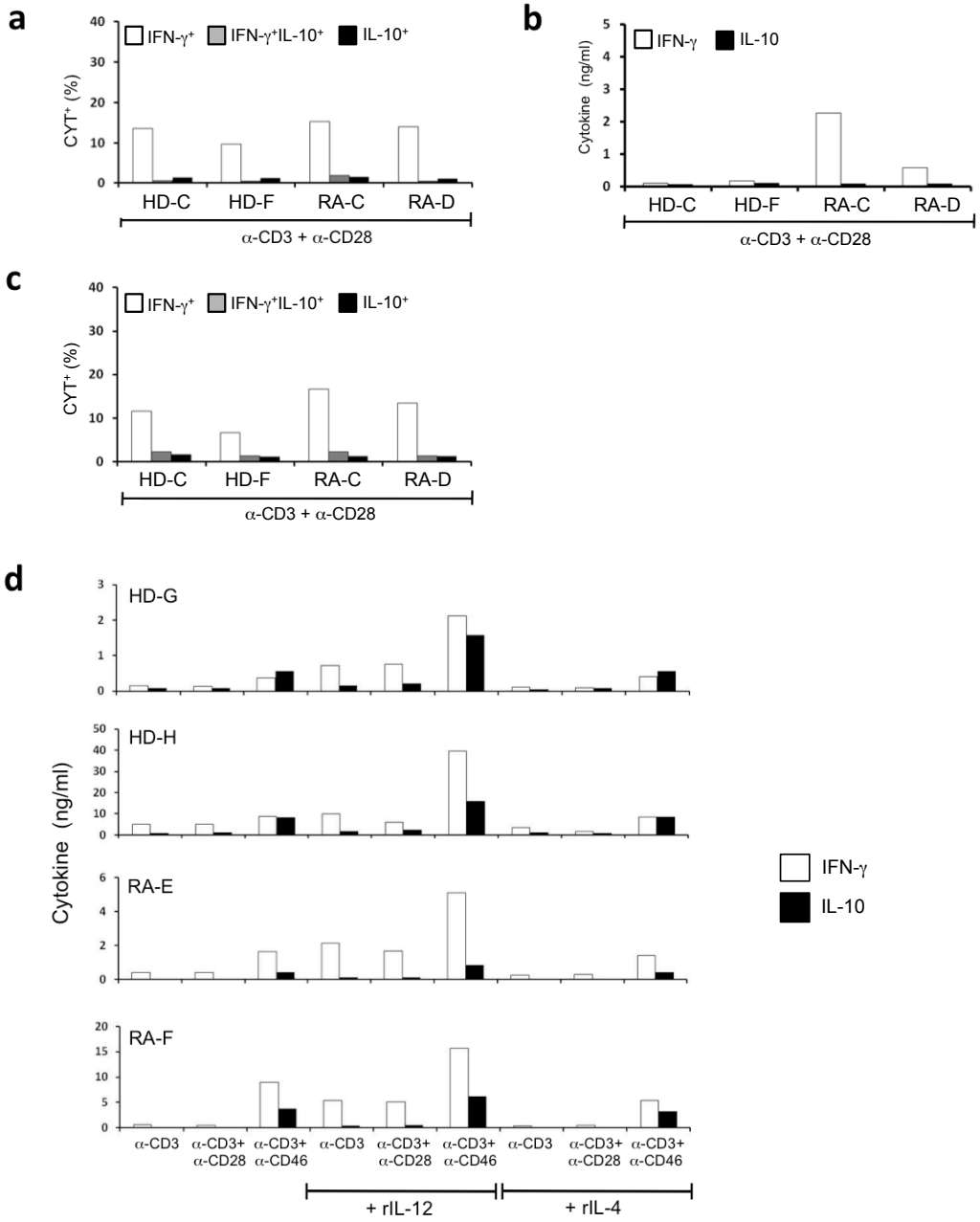


Supplementary Figure 3 SPAK participates in CD46-induced ERK1 and 2 and JNK phosphorylation. (**a**) Transfection of primary human CD4+ T cells with SPAK siRNA decreases SPAK protein expression. Purified T cells were transfected with either siRNA targeting SPAK, control siRNA and SPAK protein expression determined by immunoblotting 36h post-transfection. Cell viability was >75% (data not shown). Data are representative of two experiments. (**b**) SPAK knock-down inhibits CD46-mediated ERK1 and 2 and JNK phosphorylation. SPAK- or control siRNA-transfected primary T cells (36h) were activated as indicated for 10 min and nuclear cell lysates analyzed for their amounts of phosphorylated (p) vs. non-phosphorylated ERK1 and 2, JNK and p38 by immunoblotting. T cells, activated with anti-CD46 alone, did not induce ERK1 or 2, JNK or p38 phosphorylation (data not show). Data are representative of two experiments.



Supplementary Figure 4 γδ T cells express CD46 at the cell surface, but do not produce IL-10 following secondary stimulation with anti-CD46. (**a**) CD46 expression on the surface of $CD4^+$ and γδ T cells from three donors (D1-3) was measured by flow cytometry. Grey histograms represent isotype control staining. (**b**) PBMC (D4-6) were stimulated with HMBPP and IL-2 to induce the specific proliferation of Vγ9Vδ2 T cells as described in the Material and Methods section, in the absence or presence of the anti-CD46 antibody. 72h after the initiation of the culture (48h after addition of IL-2), cells were stained with AnnexinV-APC, PI, and anti-Vδ2-TCR-FITC. The percentage of apoptotic and necrotic Vγ9Vδ2 T cells was analysed by flow cytometry. Data are means of triplicate wells ± SD. (**c**) A polyclonal γδ T cell line (>75% pure) was generated by stimulating PBMC with HMBPP (10nM) and IL-2 (100 U/ml) for two weeks. Following restimulation with the indicated reagents, the levels of IFN-γ, TNF and IL-10 (not shown) in the culture supernatants were measured. Data in **a** and **b** are representative of experiments using PBMCs from three different donors and two independent γδ T cell lines derived from two different donors, respectively.



Supplementary Figure 5 CD4$^+$ T cells from rheumatoid arthritis (RA) patients respond normally to CD3-CD28 costimulation and T_H1 and T_H2-driving cytokines. (**a**) Blood-derived purified T cells from two healthy donors (HD-C and F) and two RA patients (RA-C and D) were CD3-CD28-activated (50 U/ml IL-2) for 36h and cytokine secretion assessed using the Cytokine Secretion Assay. Shown are middle values of percentages of each population measured in duplicate per activation condition. Data are representative of two experiments. (**b**) Purified T cells were activated as described for **a**, and then maintained in culture for 5 days with 50 U/ml IL-2. The levels of cytokines in the supernatant were assessed by CBA at day 5. (**c**) The cells were then re-stimulated with mAbs to CD3-CD28 (50 U/ml IL-2) for 18h, and the percentage of cytokine-secreting cells determined using the Cytokine Secretion Assay. Shown are middle values of percentages of each population. Data are representative of two experiments. (**d**) Blood-derived purified T cells from two healthy donors (HD-G and H) and two RA patients (RA-E and F) were activated with the indicated stimulating antibodies in the presence of hrIL-12 (10 ng/ml) or rhIL-4 (20 ng/ml) for 36h and IFN-γ and IL-10 content in cultures were measured. Cyt$^+$, cytokine-positive cells. Data are representative of two experiments.