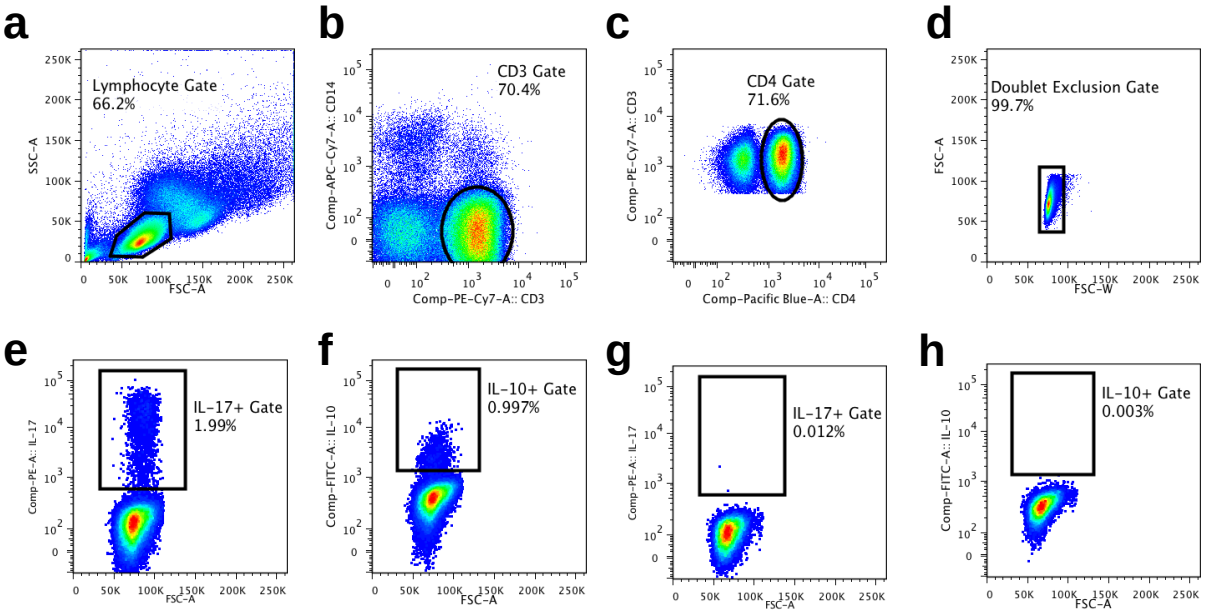
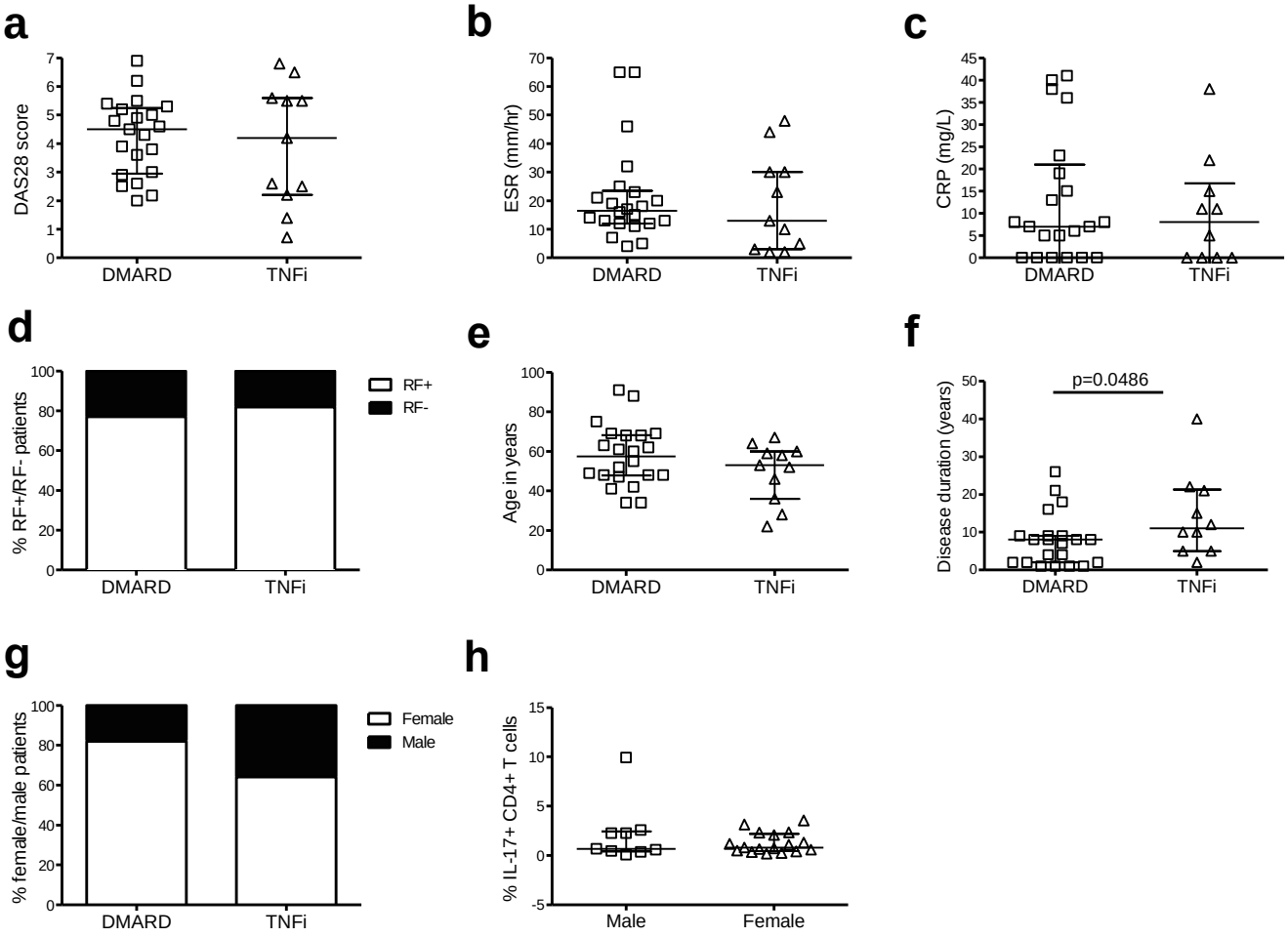


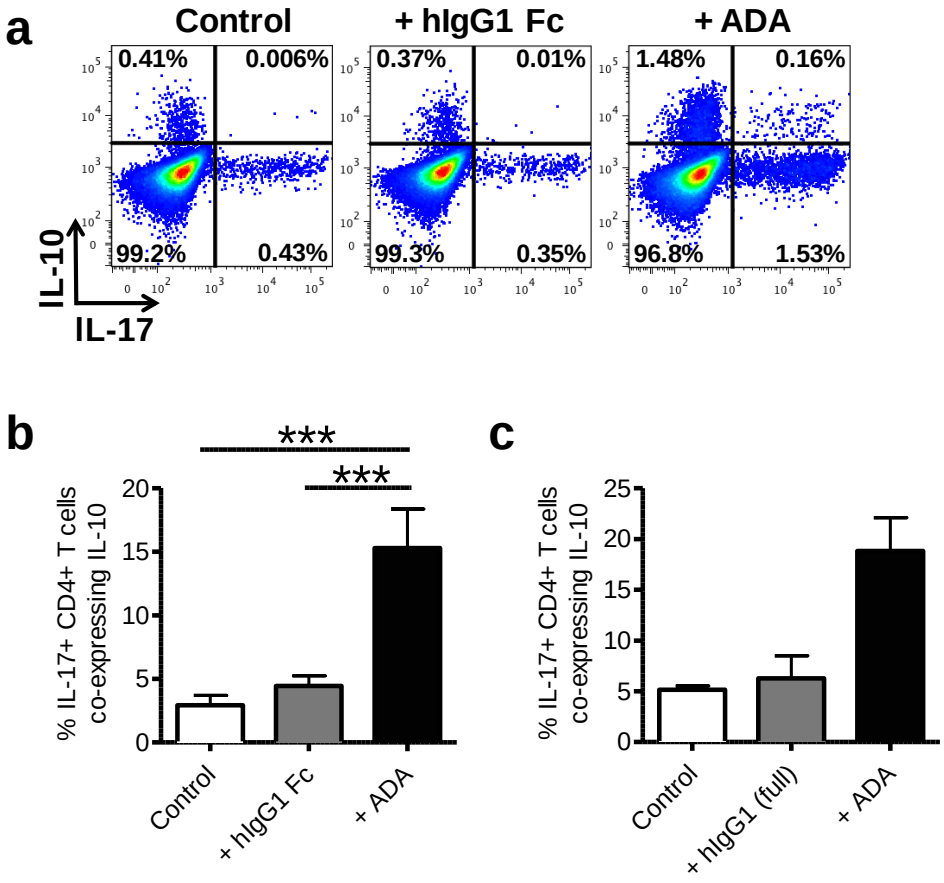
Supplementary Fig. 1. Gating strategy to detect IL-17+ and IL-10+ CD4+ T cells.

PBMCs were isolated and stimulated *ex vivo* with PMA (50ng/ml) and ionomycin (750ng/ml) for three hours in the presence of GolgiStop. Cells were stained for CD3 and CD14 followed by fixation and intracellular staining for CD4, IL-17 and IL-10. The following gating strategy was employed: first, the live lymphocyte population was gated (a), then CD14-CD3+ cells (b), then CD3+CD4+ (c), and then doublets were excluded based on FSC-W vs. FSC-A (d). Total IL-17+ or IL-10+ cells were gated using the cell gates shown (e, f), which were based on negative control stains (FMO) (g, h).



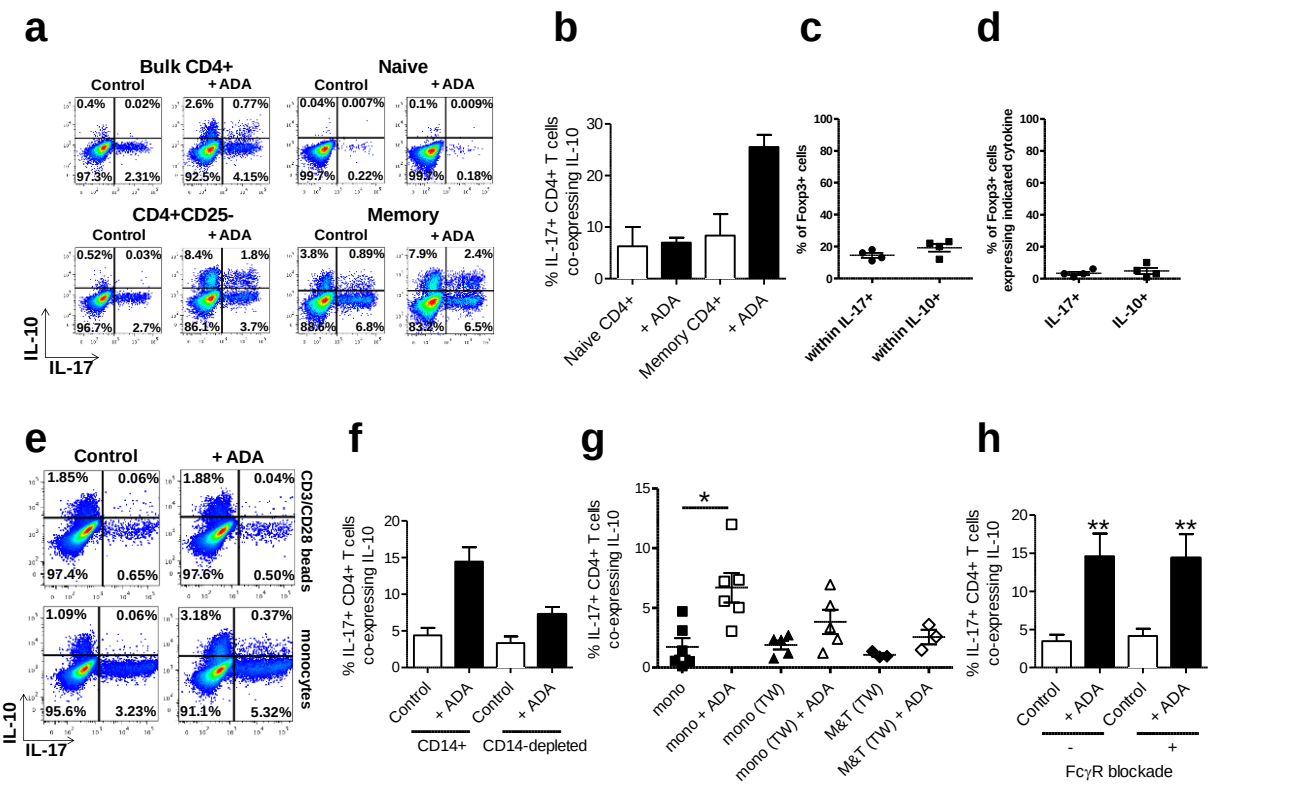
Supplementary Fig. 2. The increase in the percentage of IL-17+ CD4+ T cells in the blood of TNFi-treated patients is not due to differences in disease activity or patient characteristics.

Clinical parameters of disease and demographic data were collected from patients with RA receiving DMARD therapy (n=21) or TNFi therapy (n=11). Graphs show the median with interquartile range for disease activity score (DAS)28 (a); erythrocyte sedimentation rate (ESR, b); C-reactive protein (CRP, c); proportion of rheumatoid factor (RF) seropositive patients (d); age (e); disease duration (f) and gender distribution (g) of the patients in the two different treatment groups. The TNFi group was slightly enriched for males (18 vs. 36% male in DMARD vs. TNFi), however no significant differences were observed in the percentage Th17 cells between male and female patients (h). Data were normality tested followed by unpaired t-test or Mann-Whitney test. Patients receiving TNFi therapy had a slight increase in median disease duration relative to those on DMARD therapy (11 vs. 8 years, $p<0.05$), which probably reflects the fact that in the UK guidelines mandate that patients with RA must fail to respond to at least two different DMARDs before they qualify for TNFi therapy and are thus likely to have longer disease duration.



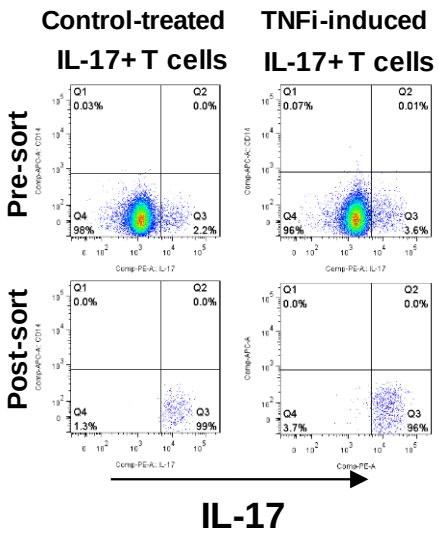
Supplementary Fig. 3. Addition of isotype control antibodies does not induce IL-10 expression in CD4+ T cells

Healthy control CD4+ T cells were cultured 1:1 with CD14+ monocytes and anti-CD3 mAb, in the absence (Control) or presence of 1 µg/ml hIgG1 Fc or adalimumab (ADA) for three days. Cells were stimulated for six hours with PMA/ionomycin with Golgistop present during the last three hours and then stained for IL-17 and IL-10. Representative dot plots for IL-17 vs. IL-10 staining are shown in (a) and the collective data showing IL-10 co-expression in IL-17+ CD4+ T cells (mean with SEM for n=8 independent donors) in (b). Data were analysed by repeated measures ANOVA followed by Bonferroni‘s Multiple Comparison Test, *** $p<0.0001$. The experiment was repeated with full length human IgG1 (n=3) using CD4+ T cells enriched for memory cells showing similar results (c).



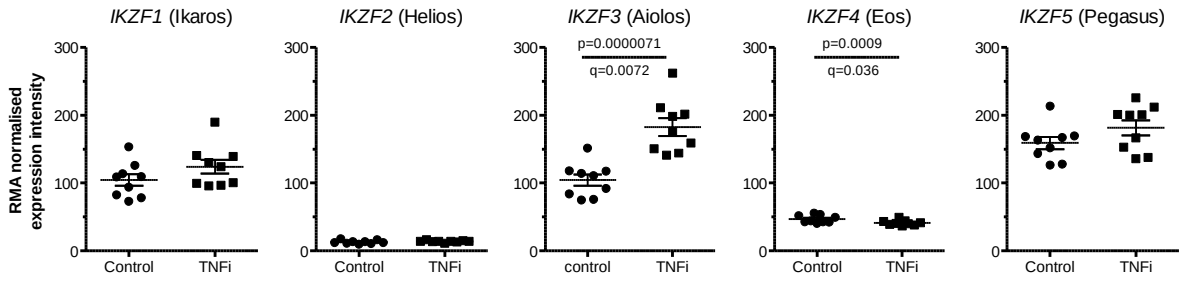
Supplementary Fig. 4. Role of Tregs and monocytes in TNFi-mediated induction of IL-10 co-expression in human IL-17+ CD4+ T cells.

Healthy control PBMC were separated into bulk CD4+ T cells, or Treg-depleted CD4+CD25- T cells (n=3), or enriched for CD45RO- (naive) or CD45RO+ (memory) CD4+ T cells (n=2). Cells were cultured 1:1 with CD14+ monocytes with anti-CD3 mAb, in the absence (Control) or presence of 1 µg/ml of adalimumab (ADA) for three days. Cells were stimulated for six hours with PMA/ionomycin with Golgistop present during the last three hours and then stained for IL-17 and IL-10. Representative dot plots for IL-17 vs. IL-10 staining are shown in (a) and the collective data for the naive and memory co-cultures in (b). (c, d) CD4+ T cells, monocytes and anti-CD3 mAb were cultured +/- ADA and at day 3 stained for IL-17, IL-10 and Foxp3. Scatter plots show the percentage of Foxp3+ cells within the IL-17+ or IL-10+ CD4+ T cells (c) or the percentage of Foxp3+ CD4+ T cells expressing IL-17 or IL-10 (d). (e) Representative dot plots of CD4+ T cells cultured +/-ADA in the presence of CD3/CD28 beads (upper panel) vs. CD14+ monocytes (lower panel), then stained for IL-17 and IL-10 expression. (f) CD4+ T cells were co-cultured with anti-CD3 mAb and CD14+ monocytes or with CD14-CD4- PBMC, in the absence/presence of ADA (n=5). (g) CD4+ T cells were cultured with anti-CD3 mAb +/- ADA in the presence of monocytes (squares, n=6), or cultured in a transwell system with beads in the bottom compartment, and monocytes (triangles, n=5) or monocytes and CD4+ T cells (diamonds, n=3) in the upper compartment. Data analysed by Wilcoxon matched-pairs signed rank test (* $p<0.05$). (h) CD4+ T cell/monocyte/anti-CD3 mAb co-cultures were cultured with or without TNFi drugs in the absence or presence of FcγR blocking reagent (n=8, paired t-test, ** $p<0.01$). All data shown as mean with SEM.



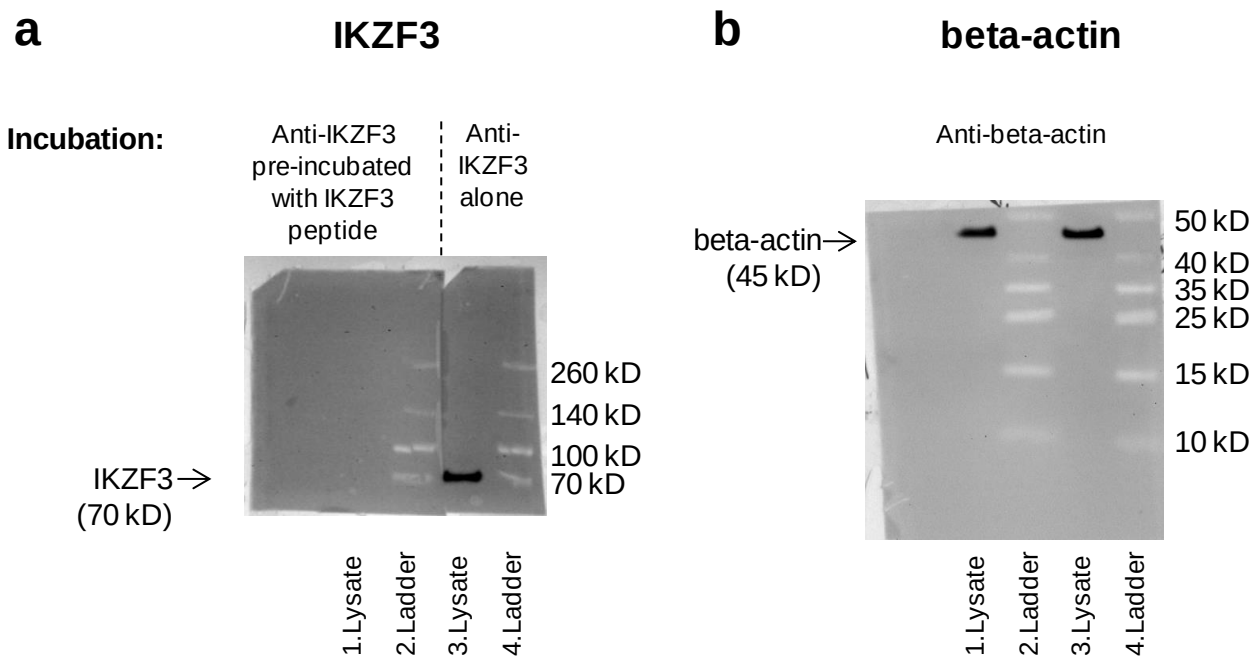
Supplementary Fig. 5. Purity of IL-17+ CD4+ T cells sorted by cytokine secretion assay.

CD4+ T cells and CD14+ monocytes were co-cultured with anti-CD3 mAb in the absence or presence of adalimumab. At day 3, cells were stimulated for 1.5 hours with PMA/ionomycin, followed by an IL-17 cytokine secretion assay and flow sorting. Representative example of IL-17+ cell purities before and after sorting using the IL-17 cytokine secretion assay.



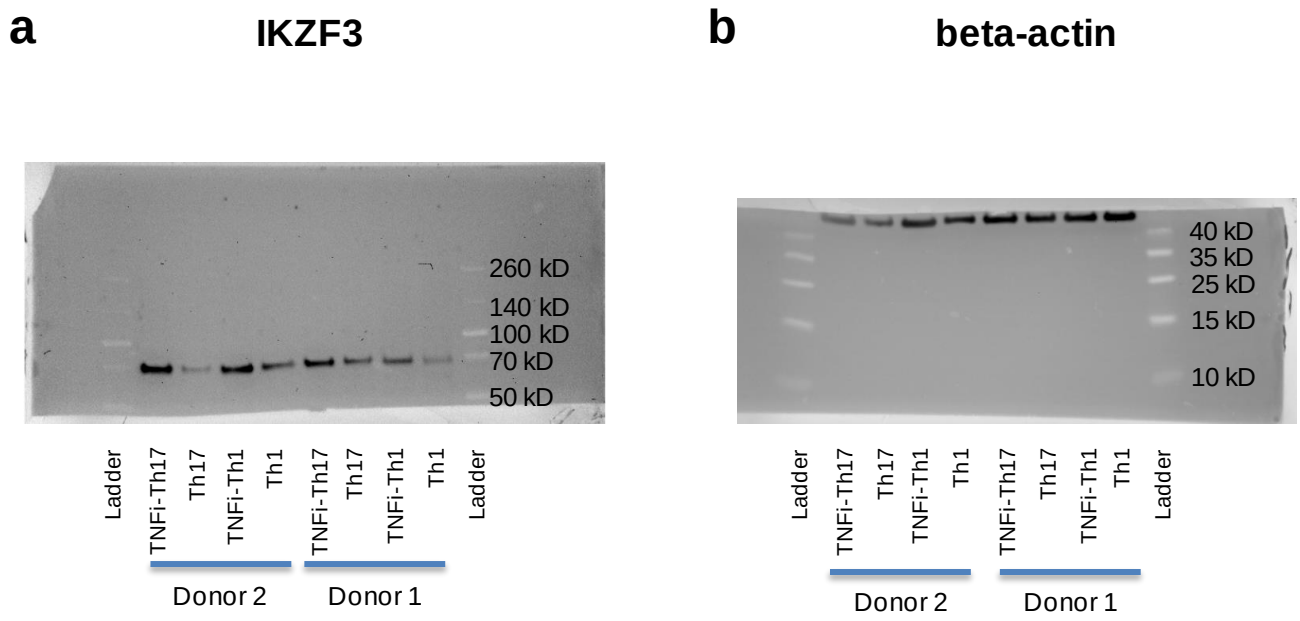
Supplementary Fig. 6. Expression of IKAROS family gene members in TNFi-exposed vs control Th17 cells.

CD4+ T cells and monocytes were co-cultured with anti-CD3 mAb in the absence (circles) or presence (squares) of adalimumab. IL-17+ T cells were sorted by cytokine secretion assay on day 3 for gene expression profiling. RMA normalised expression levels of the indicated genes (mean ± SEM, n=9 independent healthy donors) are shown (q-values indicate p-values corrected for multiple testing (Benjamini Hochberg method)).



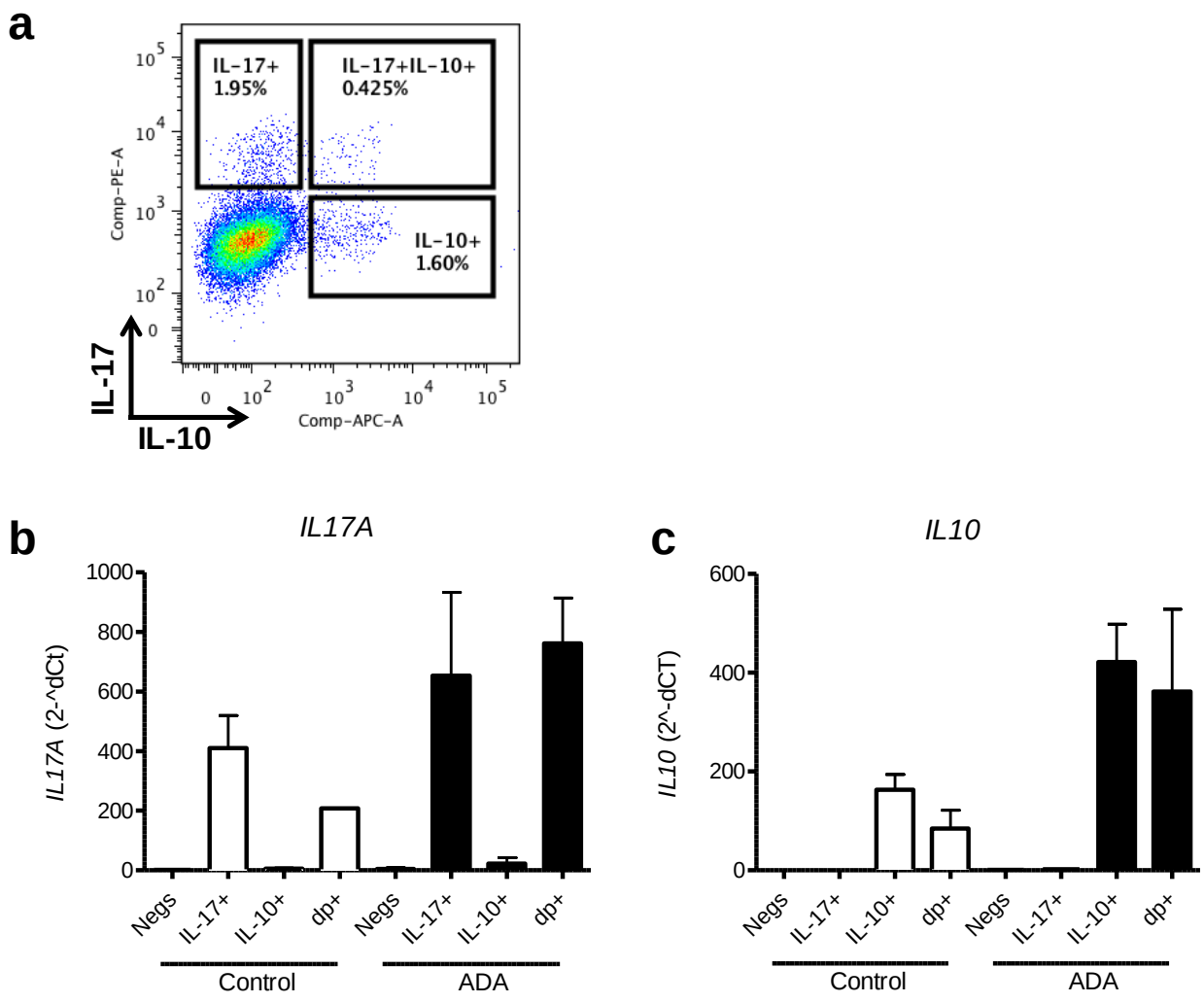
Supplementary Fig. 7. Specificity of anti-IKZF3 antibody demonstrated by peptide competition assay.

Lysates from IFN-γ- IL-17- CD4+ T cells (30 μl per lane) were run in parallel and blotted onto PVDF membrane. The blot was cut near the 50 kD marker to stain the >50 kD part with anti-IKZF3 and the <50 kD part with anti-beta-actin. The >50 kD blot was cut into two; one half was incubated with anti-IKZF3 (1:5,000) pre-incubated with IKZF3 peptide (40 μg/ml) and the other was incubated with anti-IKZF3 alone without pre-incubation with peptide (a). Equal loading was confirmed by beta-actin staining (b).



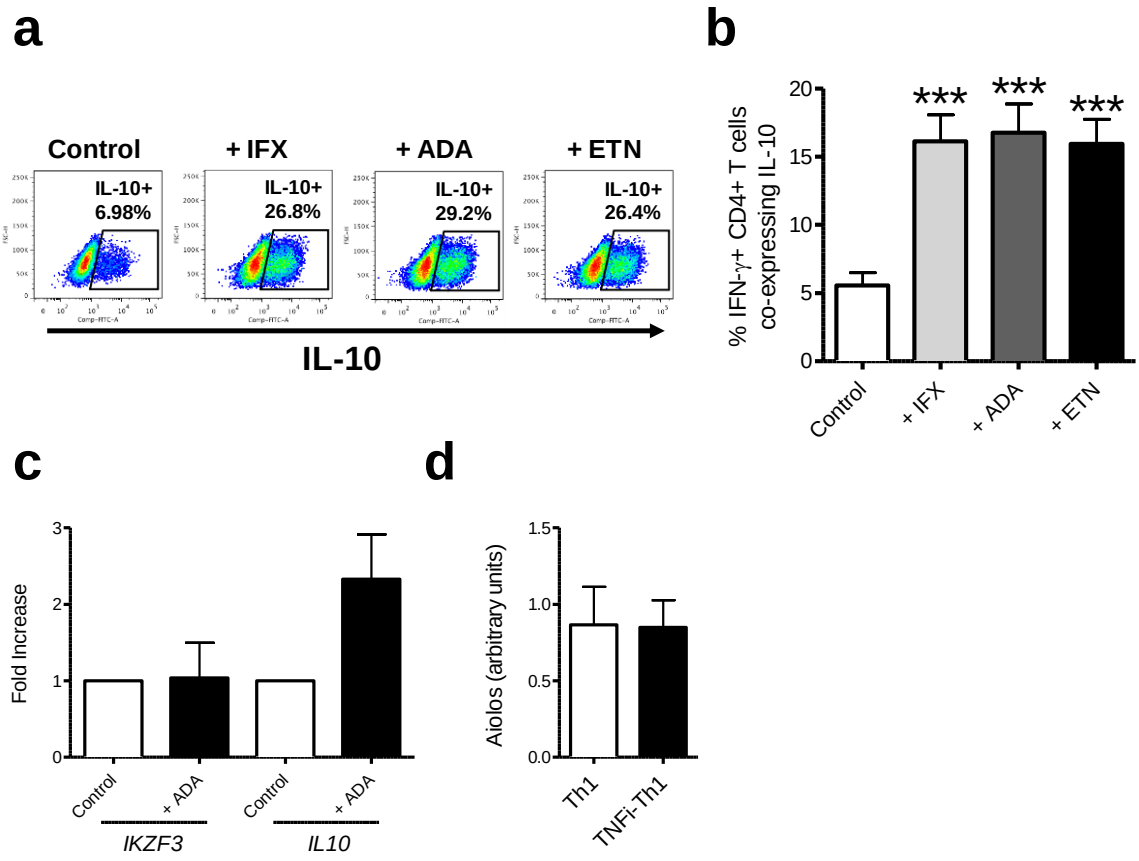
Supplementary Fig. 8. Western blot analysis of control and TNFi-exposed IL-17+ CD4+ or IFN-γ+ CD4+ T cells.

CD4+ T cells and CD14+ monocytes were co-cultured with anti-CD3 mAb in the absence or presence of adalimumab. At day 3, cells were stimulated for 1.5 hours with PMA/ionomycin, followed by an IL-17 (Th17) or IFN-γ (Th1) cytokine secretion assay and flow sorting. Cells were washed with cold PBS followed by direct lysis in Laemmli buffer. Lysates were boiled for 5 min at 95°C and resolved by electrophoresis on a 4–12% Bis-Tris NuPAGE gel. Proteins were transferred onto a PVDF membrane which was cut horizontally near the 50 kD marker and probed with either anti-IKZF3 (a) or anti-beta-actin (b), followed by horseradish peroxidase-conjugated polyclonal swine anti-rabbit immunoglobulins. The blot was developed with SuperSignal West Pico Substrate and signals were acquired with the ChemiDoc XRS+ System using Image Lab software.



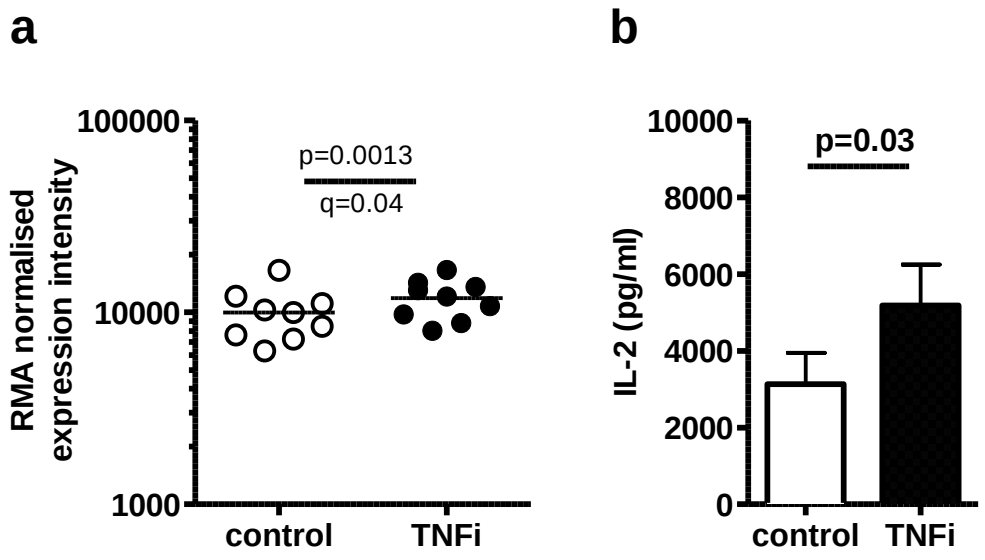
Supplementary Fig. 9. IL-17 and IL-10 mRNA expression correlates with T cell subsets sorted by cytokine secretion assay.

CD4+ T cells and CD14+ monocytes were co-cultured with anti-CD3 mAb in the absence or presence of adalimumab. At day 3, cells were stimulated for 1.5 hours with PMA/ionomycin, followed by an IL-17 and/or IL-10 cytokine secretion assay and flow sorting. CD4+ T cells were sorted into IL-17-IL-10- (Negs), IL-17+IL-10- (IL-17+), IL-17-IL-10+ cells (IL-10+) or IL-17+IL-10+ (dp+) (a), and the expression of *IL17A* (b) and *IL10* (c) determined in each of the four subsets by real-time PCR. Expression levels (mean ± SEM, n=3) were normalised to *PPIA*.



Supplementary Fig. 10. *In vitro* induction of IL-10 in Th1 cells is not consistently associated with increased expression of *IKZF3* or Aiolos.

CD4+ T cells and CD14+ monocytes from healthy controls were co-cultured with anti-CD3 mAb (Control) with or without the indicated drug for three days followed by PMA/ionomycin restimulation. (a, b) Representative flow cytometry plots (a) and cumulative data (n=15, mean ± SEM, *** $p<0.0001$) showing the percentages of IL-10+ cells within gated IFN-γ+ CD4+ T cells. Data analysed by repeated measures ANOVA followed by Bonferroni's Multiple Comparison test. (c, d) At day 3, control-treated *vs*. TNFi-exposed Th1 cells were sorted based on IFN-γ expression using a cytokine secretion assay and flow sorting. (c) The expression of *IKZF3* and *IL10* was determined in each subset by real-time PCR. Expression levels (mean ± SEM, n=3) were normalised to *PPIA*. (d) Cumulative data (mean with SEM) from Western blot analysis of Aiolos (normalised to actin by densitometry) of n=5 independent experiments.



Supplementary Fig. 11. IL-2 expression is slightly increased in TNFi-exposed IL-17+ CD4+ T cells.

CD4+ T cells and monocytes were co-cultured with anti-CD3 mAb in the absence (open circles) or presence (solid circles) of adalimumab. IL-17+ T cells were sorted by cytokine secretion assay on day 3 for gene expression profiling and cytokine secretion. (a) RMA normalised expression levels of *IL2* (mean ± SEM, n=9 independent healthy donors) (q-values indicate p-values corrected for multiple testing (Benjamini Hochberg method)). (b) IL-2 secretion of sorted cells (n=6, paired t-test) as measured by Luminex.

	Forward	Reverse
IL-17A	PrimerDesign Ltd	PrimerDesign Ltd
IL-10	PrimerDesign Ltd	PrimerDesign Ltd
IKZF3	AGCAGGCCAACCAGTGGAAAGA	TGGGCGTTCACCAGTATGGCT
GAPDH	PrimerDesign Ltd	PrimerDesign Ltd
PPIA	PrimerDesign Ltd	PrimerDesign Ltd
SDHA	ACTCAGCATGCAGAAGTCAATGC	ACCTTCTTGCAACACGCTTCCC
Motif 1	AAGGTGGTGGGGACTCAATA	GCTGAGCAGGTCATACCATC
Motif 2	TGGGGAGAGTGACAAAGGAA	TGAACCCCCAGGTGTATTTG
Motif 3	CCAACTGTGCGTGAACTTT	GCCCTGACCTACTATGAGAAAT
Motif 4	ATTCAATTCCTCTGGGAATGTTAC	AGACAAGAGTCAACTGACACCA
Motif 5	GACCTGACTCACTTCGGTTA	CCCCCTCTCCTTATCTTCTG
PROM	GTCAGTGTTCCTCCCAGTTACA	AAGAAGTCCTGATGTCACTGCCCCG
hFTTNA	CACACTTGCCTGGGGTAAAC	AGGCCAGTCTGTGGAGTAAC
hFTTNB	CCTCCCTCAACACCTGAAGT	GGTACCTCCGTCGTCTACTG

Supplementary Table 1. Primer list.

Sequences for the forward (F) and reverse (R) primers for genes used in this study are listed above. Those listed as PrimerDesign Ltd were bought directly from the company and sequences can be obtained via PrimerDesign Ltd.