Supplemental Figure 1. FOXP3 induction unaffected by anti-CD3mAb clone or method of stimulation.

(A) RA PBMC were unstimulated (Nil) or cultured with 1 µg/ml OKT3, HIT3a, UCHT1 or Otelixizumab for 24 h, then analyzed via flow cytometry for FOXP3 expression by CD8⁺ T cells. FACS plots are representative of 6 RA patients. Isotype = Ig control for FOXP3. (B) RA PBMC were stimulated with plate-bound (pCD3) or soluble (sCD3) anti-CD3 mAb at 0.5, 1.0 and 3.0 µg/ml for 24 h, then analyzed via flow cytometry. FACS plots and cumulative data showing mean ± SEM are representative of 6 RA patients. One-way repeated measures ANOVA used for statistical analysis.

Supplemental Figure 2. Induced CD8⁺FOXP3⁺ Treg have a regulatory phenotype.

(A) CTLA-4 and TGF-β expression on anti-CD3-induced CD8⁺FOXP3⁺ (FOXP3⁺) or CD8⁺FOXP3⁻ (FOXP3⁻) T cells. Tinted line represents the isotype control, dotted line represents CD8⁺FOXP3⁻ T cells, black line represents CD8⁺FOXP3⁺ Treg.(B) IFN-γ, IL-10, IL-17, IL-2, TNF-α, and their isotype control (iso) and (C) perforins and granzyme B staining on anti-CD3-induced CD8⁺FOXP3⁺ (FOXP3⁺) or CD8⁺FOXP3⁻ (FOXP3⁻) T cells. Representative flow cytometry plots shown (n=6). Cumulative data shows mean ± SEM MFI of CTLA-4 (n=4) and TGF-β (n=4). Two-tailed t-test used for statistical analysis.
Supplemental Figure 3. Naïve CD8\(^+\) T cells are the predominant FOXP3 inducing population.

RA PBMC were depleted of CD8\(^-\) T cells and the CD8\(^+\) T cells divided into CD8\(^+\)CD45RA\(^+\) T cells and CD8\(^+\)CD45RO\(^+\) T cells. These cells were checked for their expression of FOXP3, then reintroduced to PBMC depleted of CD8\(^-\) T cells at a ratio of 1 CD8\(^+\) T cell to 10 PBMC and cultured with OKT3 (aCD3) for 24 h and FOXP3 expression analyzed. (A) FACS plots and cumulative data showing mean ± SEM are representative of 6 RA patients. (B) Responder (R) and non-responder (NR) RA individuals were identified by their ability to induce FOXP3 in response to anti-CD3 mAb. Their CD8\(^+\)CD45RA\(^+\) T cells and CD8\(^+\)CD45RO\(^+\) T cells were analyzed for FOXP3 induction by OKT3 after 24 h, after reintroduction to PBMC depleted of CD8\(^+\) T cells. FACS plots and cumulative data showing mean ± SEM are representative of 6 responder and 6 non-responder RA patients. (C) Ex vivo %CD45RA and %CD45RO by CD8\(^+\) T cells was plotted against %FOXP3 expression by CD8\(^+\) T cells after culture of RA PBMC with 1 \(\mu\)g/ml OKT3 for 24 h. Correlation coefficients and their significance were calculated by two-tailed Spearman’s rank correlation.

Supplemental Figure 4. CD4\(^+\)FOXP3\(^+\) Treg are inferior suppressors compared to induced CD8\(^+\)FOXP3\(^+\) Treg.

RA PBMC were stimulated with OKT3 for 3 d, washed and rested for 2 d. CD8\(^+\) T cells were isolated from cultures and further divided into CD8\(^+\)CD25\(^-\), CD4\(^+\)CD25\(^-\) and CD4\(^+\)CD25\(^+\) T cells. (A and B) CFSE labeled CD8 depleted PBMC were co-cultured with CD8\(^-\)CD25\(^+\), CD4\(^+\)CD25\(^-\) T cells or CD4\(^+\)CD25\(^+\) T cells for 3 d at ratio of 1 CD8\(^+\) T cell, 1 CD4\(^+\)CD25\(^+\) or 1 CD4\(^+\)CD25\(^-\) T cell to
5 CD4\(^+\) T cells, in the presence of 1 \(\mu\)g/ml staphylococcal enterotoxin B (SEB). (A) FACS plots and cumulative data are representative of 6 RA patients. One-way repeated measures ANOVA was used for statistical analysis. (B) Supernatants from 3 d cultures were analyzed by ELISA for IL-17 and IFN-\(\gamma\). Cumulative data shows mean ± SEM of cytokine production (n=6). One-way repeated measures ANOVA was used for statistical analysis.

**Supplemental Figure 5. Naïve responder CD8\(^+\) T cells do not phosphorylate p38.**

Responder and non-responder RA PBMC were stimulated with OKT3 for 0, 3, 5 and 15 min. (A) CD8\(^+\) T cells were analyzed by FACS for expression of CD45RA and CD45RO. (B, C and D) Responder and non-responder RA PBMC were analyzed for expression of phosphorylated p38 (pp38). (B and C) Number in FACS plots indicates pp38 MFI. Dotted line represents the isotype control and black line represents pp38. FACS plots and (D) cumulative data showing shows mean ± SEM are representative of 6 responder and non-responder RA patients. One-way repeated measures ANOVA was used for statistical analysis.

**Supplemental Table 1. Baseline characteristics of RA patients and their prescribed therapies.**

The mean age and range of patients, the mean C-reactive protein (CRP) and range, the ratio of positive to negative patients for rheumatoid factor (RF) and anti-cyclic citrullinated protein antibodies (CCP) for all patients, those who were untreated and those who were on methotrexate only (Mtx), sulfasalazine
only (Ssz) or combined methotrexate and sulfasalazine therapy (Mtx+ssz) is shown for all patients whose PBMC have been used for experiments.