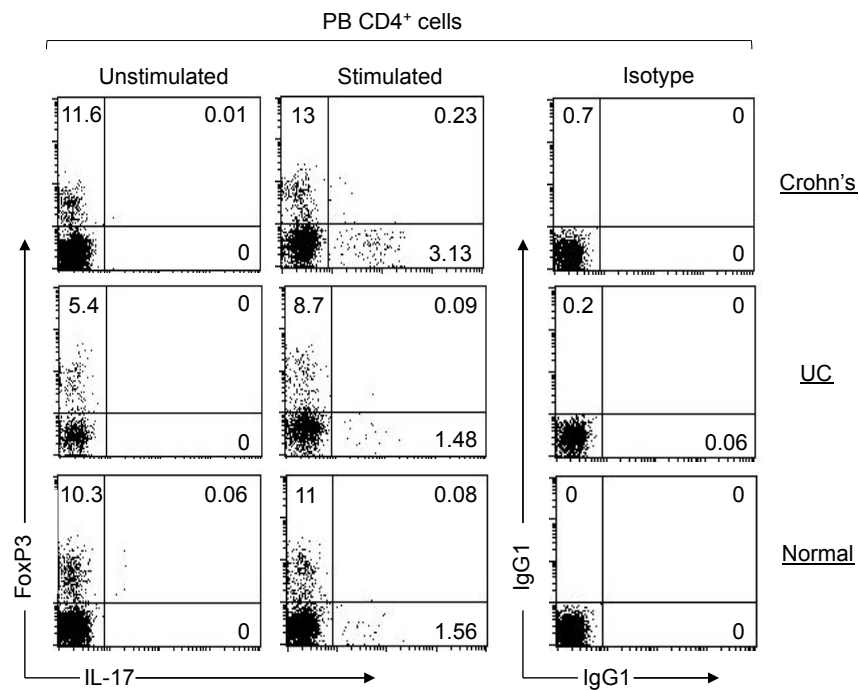
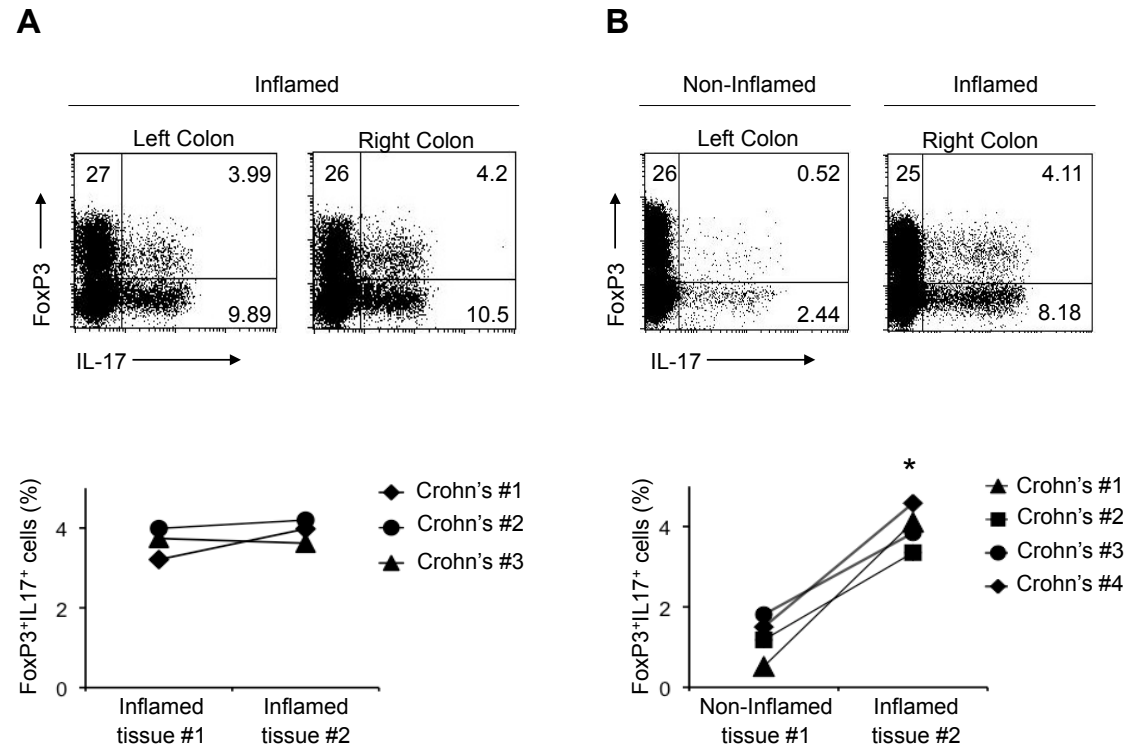


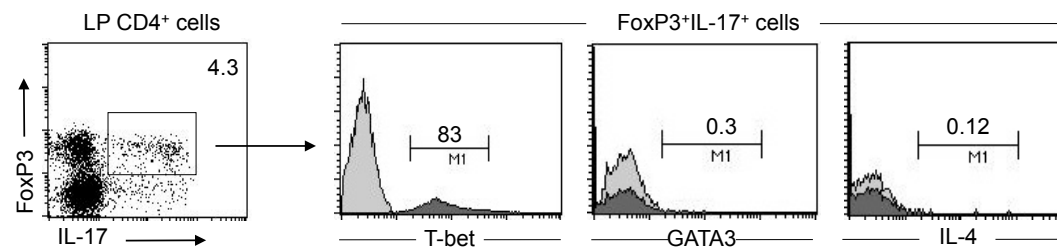
Supplementary Figure 1. The expression of IL-17 and FoxP3 by LP CD4⁺ T cells derived from CD patients. Flow cytometry of (a) IL-17 production and (b) FoxP3 expression by LP CD4⁺ T lymphocytes freshly derived from disease-affected and apparently healthy gut areas of CD patients and healthy individuals, respectively, assessed 4h post stimulation with PMA/ionomycin. Data represent the mean \pm s.d. of ten independent donors (a, b) **, P<0.005.



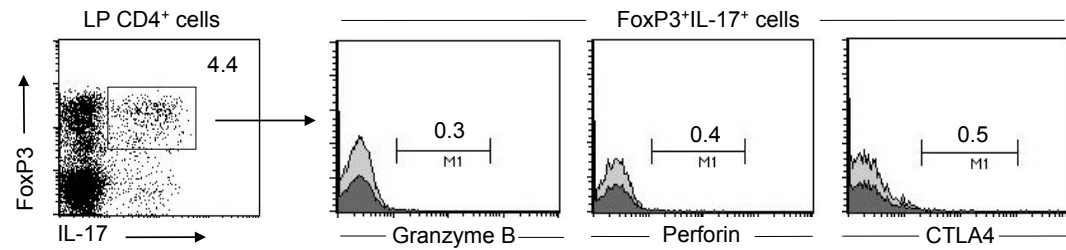
Supplementary Figure 2. FoxP3⁺ IL-17-producing CD4⁺ T cells are virtually undetectable in the periphery of IBD patients and healthy controls. Flow cytometry analyzing the expression of FoxP3 and IL-17 by peripheral blood (PB) CD4⁺ T lymphocytes derived from IBD patients and healthy individuals, assessed 4h post stimulation with PMA/ionomycin. Numbers in the quadrants indicate the percent of cells in each. Data are representative of at least ten independent experiments.



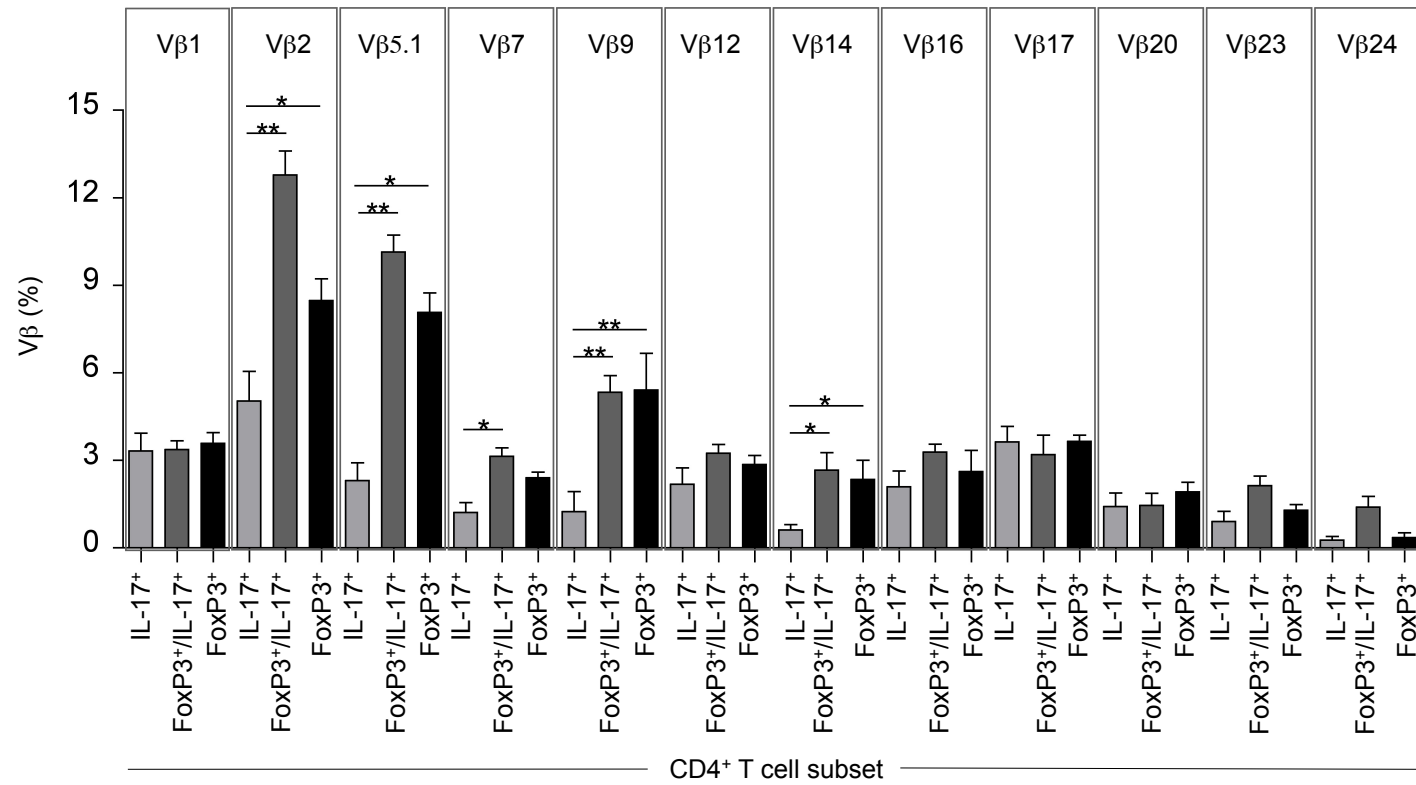
Supplementary Figure 3. FoxP3⁺ IL-17-producing LP CD4⁺ T cells are found in increased numbers when derived from inflamed CD tissues. Flow cytometry analyzing the expression of FoxP3 and IL-17 by LP CD4⁺ T lymphocytes derived from independent (a) inflamed and (b) inflamed and non-inflamed intestinal compartments of the same CD patient, respectively. Numbers in the quadrants indicate the percent of cells in each. Data are from (a) three and (b) four different CD tissues, and lines connecting data points represent the same donor. *, P<0.05.



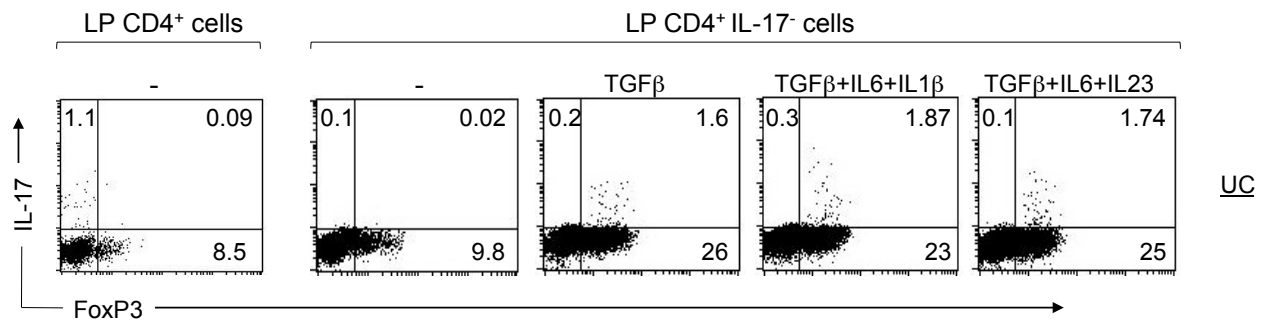
Supplementary Figure 4. CD-derived FoxP3⁺ IL-17-producing CD4⁺ T cells express high levels of T-bet, but not GATA3 or IL-4. Intracellular staining for the expression of IL-17, FoxP3, T-bet, Gata3 and IL-4 by the LP CD4⁺ T cells derived from CD patients, assessed 4h post stimulation with PMA/ionomycin. Gating on FoxP3⁺IL-17⁺ cells: filled histograms, specific staining (antibodies to markers shown below the plots); open histograms, isotype-matched control antibodies. Numbers adjacent to outlined areas and above the lines indicate the percent of cells in the gate. Data are representative of four independent experiments.



Supplementary Figure 5. CD-derived IL-17-producing Tregs do not express granzyme B, perforin or CTLA4. Surface and intracellular staining for the expression of IL-17, FoxP3, granzyme B, perforin and CTLA4 by the LP CD4⁺ T cells derived from CD patients, assessed 4h post stimulation with PMA/ionomycin. Gating on FoxP3⁺IL-17⁺ cells: filled histograms, specific staining (antibodies to markers below plots); open histograms, isotype-matched control antibodies. Numbers adjacent to outlined areas and above the lines indicate the percent of cells in the gate. Data are representative of four independent experiments.



Supplementary Figure 6. TCR β-chain variable region usage by IL-17⁺, FoxP3⁺IL-17⁺ and FoxP3⁺ CD4⁺ T cells derived from lamina propria of CD patients. Sorted LP CD4⁺ T cells were stained with anti-IL-17 and anti-FoxP3 4 h post stimulation with PMA/ionomycin. The TCR β-chain usage was defined by flow cytometry using specific anti-Vβ TCR antibodies. Data represent the mean±s.d. of five different CD donors. *, P<0.05. **, P<0.01.



Supplementary Figure 7. FoxP3⁺IL-17⁺ cells develop from FoxP3⁺ precursor cells in the presence of TGF-β. Flow cytometry analyzing the expression of FoxP3 and IL-17 by UC-derived LP CD4⁺ cells depleted of IL-17-producing cells (CD4⁺ IL-17⁻ fraction) primed with αCD2/αCD3/αCD28 coated beads in the absence or presence of indicated cytokines and TGF-β, analyzed on day 6. Numbers in the quadrants indicate the percent of cells in each. Data are representative of three independent experiments.