Supplemental Methods

Microarray Analysis

Gene expression pattern were investigated by Chip Selection Human Gene 1.0 ST microarray according to manufacturer's instructions. In brief, the quality of the total RNA was checked by the Agilent 2100 Bioanalyzer. For each sample, the Ambion WT Expression Kit (Life Technologies, Carlsbad, CA) synthesizes cDNA target from 50 nanograms of total RNA. The GeneChip WT -Terminal Labeling Kit (Affymetrix, Santa Clara, CA), was used to both chemically fragment and biotin – label the cDNA target. The arrays were scanned with the Affymetrix GeneChip Scanner 3000 7G. The Affymetrix Expression Console Program was used to examine the Affymetrix Gene Array quality control factors. Data was normalized using the Robust Multi-Array Analysis algorithm.

Immunohistochemical (IHC) Staining

FOXP3 protein expression was evaluated by Immunohistochemistry. Briefly, colon biopsy samples were fixed in 10% buffered formalin, dehydrated in ethanol, and embedded in paraffin. Slides (4 μm thickness) were treated with anti-FOXP3 antibody (PCH101, 1:200; eBiosciences, San Diego, CA) using Benchmark XT IHC/ISH Staining module (ROCH-Ventana Medical Systems, Tucson, AZ) as previously described.[20] Ten high power fields (1HPF= 0.237 mm^2) were analyzed for $FOXP3^+$ cell infiltration in HIV-infected colon tissue and matched normal controls. Scan scope software was used for digital image scanning and the analysis was performed blindly by two investigators both manually and automatically using NIH Image J software, version 1.46r for Windows (http://rsb.info.nih.gov/ij/). Color split channel plug in was used to isolate DAP stain, which represent FOXP3 positive areas from FOXP3 negative

Hematoxlyin stained areas. [21]The mean numbers and percent of FOXP3+ Treg cells were calculated per analyzed field in controls and patients (Appendix 2).

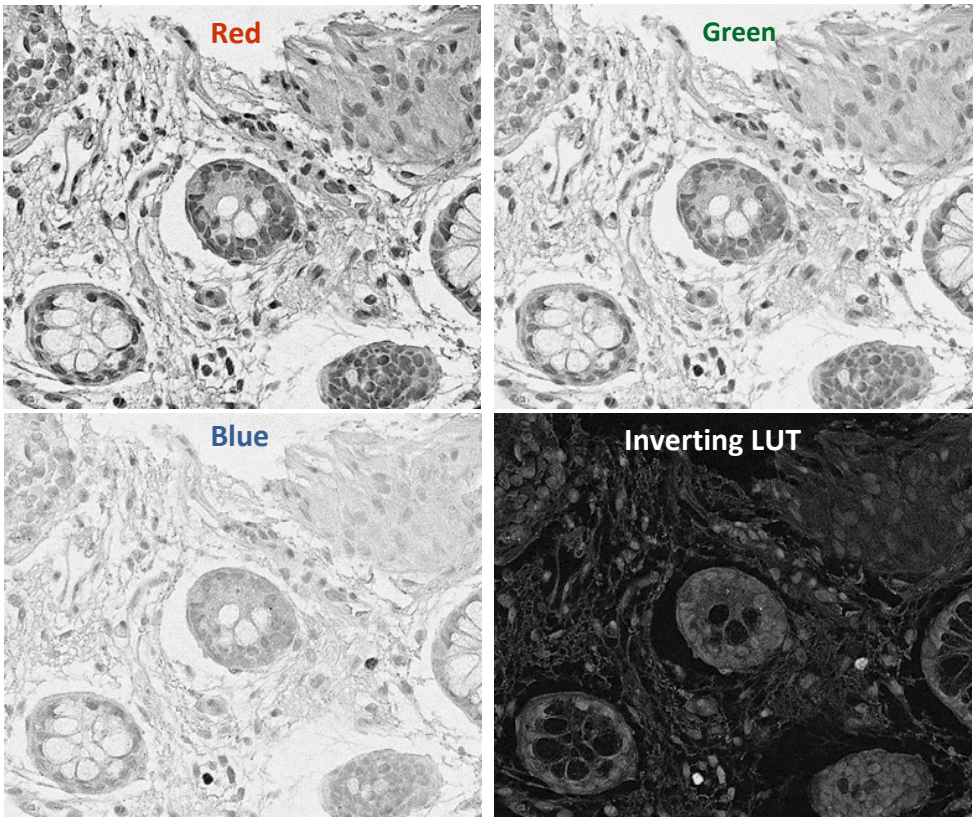

FIGURE S1a. FOXP3 Immunostaining in normal colon tissue. Image J software color split channel plug in was used to isolate DAP stain, which represent FOXP3 positive areas from *FOXP3* negative hematoxlyin stained areas by isolation of the color information from histological red, green and blue. Each image was processed into binary (black: 255 and white: 0) Inverting look up table (LUT). The mean score ± SD of FOXP3+ T_{reg} cells is 2 ± 0.85 cells/HPF.

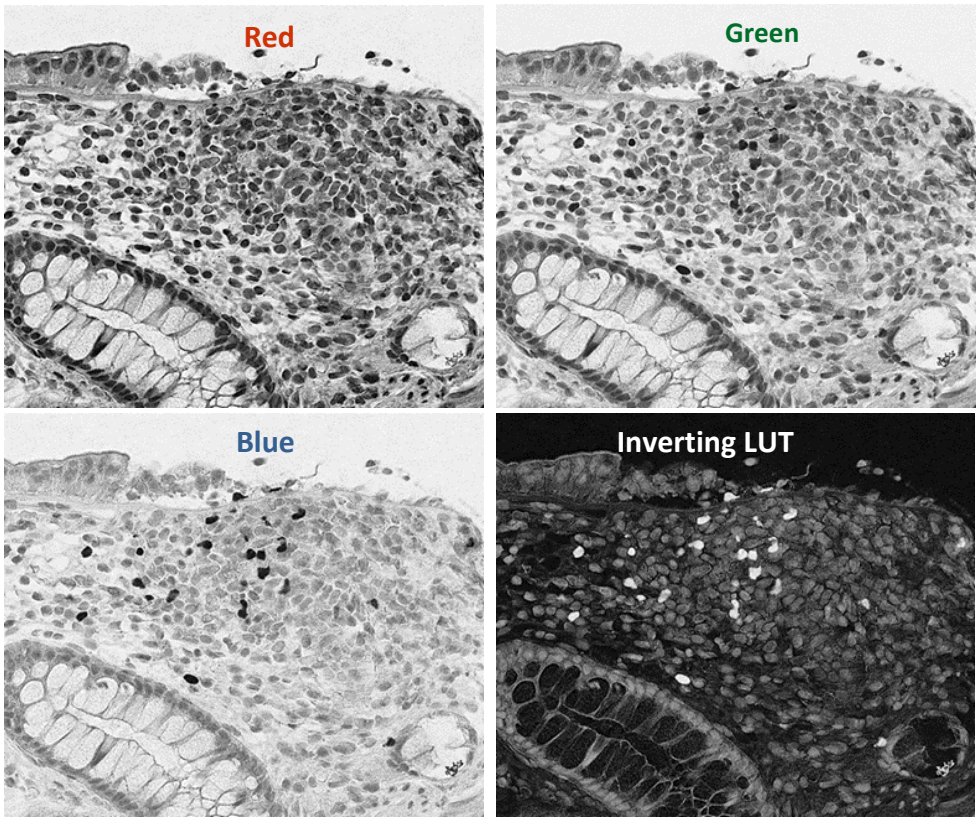

FIGURE S1b. FOXP3 Immunostaining in HIV colon tissue. Image J software color split channel plug in was used to isolate DAP stain, which represent FOXP3 positive areas from *FOXP3* negative hematoxlyin stained areas by isolation of the color information from histological red, green and blue. Each image was processed into binary (black: 255 and white: 0) Inverting look up table (LUT). The mean score ± SD of FOXP3+ T_{reg} cells is 20 ± 1.7 cells /HPF in HIV.

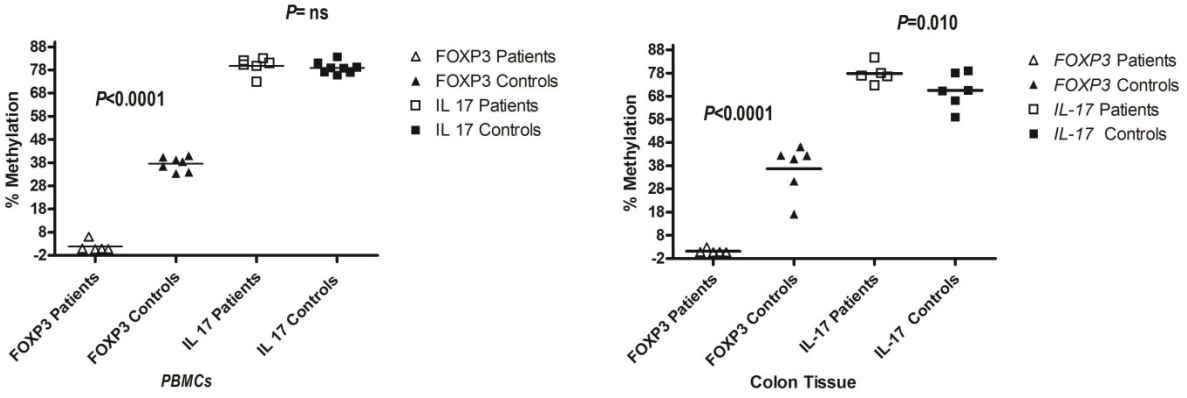

FIGURE S2: **Comparison of promoter methylation of *IL-17* and *FOXP3* in colon tissue and PBMCs.** Compared to *IL-17*, *FOXP3* was significantly less methylated in HIV infected patients and control in both blood and colon tissue. In the left panel PBMCs Mean ± SD for samples tested were: *FOXP3* patients PBMCs (1.7 ± 0.87), *FOXP3* control PBMCs (37.8 ± 1.04), *IL-17* patient PBMCs (79.9 ±1.47), *IL-17* control PBMCs (78.9 ± 0.89) respectively. In the right panel colon tissue Mean ± SD for samples tested were: *FOXP3* patient colon (1.12 ± 0.34), *FOXP3* control colon (37.1 ± 3.26), *IL-17* patient colon (79.0 ±2.00), *IL-17* control colon (69.4 ± 2.37) respectively.