Supplemental Methods

Generation of humanized mice

NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). To generate NSG*H2*$^{dlAb1\text{-}Ea}$Tg(HLA-DQA1,HLA-DQB1) mice (NSGIIDQ8), we backcrossed the B6.129S2-*H2*$^{dlAb1\text{-}Ea}$/J strain with NOD.Cg-*Prkdc*^{scid}*H2-Ab1*^{tm1Gru}*Il2rg*^{tm1Wjl}Tg(HLA-DQA1,HLA-DQB1)1Dv/SzJ mice for 10 generations replacing *H2-Ab1*^{tm1Gru} in favor of *H2*$^{dlAb1\text{-}Ea}$ to prevent interspecies paring of MHCII. Whereas NSG mice were reconstituted with $2.0x10^7$ human PBMCs by intravenous injections, one-day old NSGIIDQ8 pups were pre-conditioned by γ-radiation (100 rads, ^{137}Cs source) and injected 5 hours later with $5x10^4$ human CD34$^+$ hematopoietic stem cells (HSC) via the intrahepatic route. LD IL-2 (Proleukin, Prometheus) or PBS (Gibco) were injected intraperitoneally, as indicated in Fig. 1A and Fig 2A. All mice were maintained in the specific-pathogen free facilities and animal experiments were approved by the institutional guidelines at Boston Children's Hospital.

Human PBMC and HSC isolation

PBMC were isolated from leukocyte collars obtained from healthy donors (HD) using Lympholyte® Cell Separation density gradient centrifugation media (Cedarlane, Burlington, NC, USA). Human CD34$^+$ HSC from HD cord blood were purified by positive selection using a CD34 MicroBead Kit following manufacturer's protocol (Miltenyi Biotec, San Diego, CA, USA).

Antibodies and immunophenotyping

Immune cells were isolated from blood, spleen, and lamina propria of humanized mice or peripheral blood and lamina propria of UC patients. Single cell suspensions of mouse spleens and mesenteric lymph nodes were prepared by passing through 70 μm cell strainer (BD Biosciences, BD). Red blood cells in isolated murine tissues were lysed using a commercial ACK lysis buffer (Gibco). To isolate lamina propria immune cells, colonic epithelial cells were separated by agitation in 2.0 mM EDTA (FisherScientific), 2% FCS, and

0.0016 % DTT (Sigma-Aldrich) in RPMI media for 15 minutes at 37°C. Tissue was digested using collagenase VIII (Sigma), 2% FCS in RPMI media for 30 minutes at 37°C with gentle agitation. Cells were washed twice with PBS and passed through a 70 μm nylon strainer prior to staining.

For immunophenotypic characterization of T and NK cells, samples were stained with the following monoclonal antibodies: CD3, clone SK7; CD127, clone A019D5; human CD45, clone HI30; CD56 clone 5.1H11; CD16, clone 3G8; mouse CD45, clone 30-F11; CD45RO, clone UCHL1 were purchased from Biolegend (San Diego, CA, USA). CD4, clone RPA-T4; CD25, clone M-A251; HLADR, clone G46-6; CCR7, clone 150503 were purchased from BD Bioscience (San Jose, CA, USA). FOXP3, clone 206D; CD8, clone RPA-T8; were purchased from eBioscience (Waltham, MA, USA). Live/Dead fixable aqua dead cell stain kit was purchased from Thermo Fisher (Waltham, MA, USA). For cell surface staining, cells were blocked in 10% rat serum followed by incubation with fluorochrome-conjugated antibodies for 20 minutes at 4^{o}C and washes with FACS buffer. Intracellular FOXP3 staining was performed by employing the eBioscience™ FOXP3/Transcription Factor Staining Buffer Set according to the manufacturer's protocol (eBioscience). The samples were acquired using a LSRFortessa or FACSCantoII Flow Cytometer (BD Biosciences) and data were analyzed using FlowJo Software (TreeStar).

DNBS/TNBS-induced colitis

In the PBMC reconstitution model, NSG mice were anesthetized using 3% isoflurane and a lubricated 3.5F soft silicon catheter was inserted into the colon to a distance of 3–4 cm to inject a 50 μL enema containing 2.5 mg 2,4-dinitrobenzenesulfonic acid (DNBS) (Sigma-Aldrich, St. Louis, MO) in a 50% ethanol solution. Mice were held inverted for 30 seconds before returning to cage. For HSC-reconstituted mice, 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Sigma-Aldrich) sensitization was performed by injecting 100 μL of a 1.0% TNBS solution in PBS subcutaneously one week prior to administering a 50 μL rectal enema containing 2.5 mg TNBS in 50% EtOH, as described above. Mice were weighed daily and sacrificed three days post-enema for histological and immunological analysis. Paraffin-embedded colonic sections were

hematoxylin/eosin stained and analyzed blinded for inflammation, crypt hyperplasia, ulcerations, bowel wall thickening, and edema. Each histologic parameter was scored in increments of 0.25 from 0 to 1 resulting in a possible maximum score of 5.

RNA In Situ Hybridization (RNAScope).

In situ hybridization was performed on formalin-fixed paraffin-embedded colonic tissue. The RNAScope 2.5 Duplex Detection Kit (chromogenic) was used according to the manufacturer's instructions (Advanced Cell Diagnostics, Newark, CA). FOXP3 was used in C1 and CD3 probe was used in C2.

CyTOF analysis

Briefly, $2x10^6$ cells were stimulated using 20 ng/ml PMA and 1 μM ionomycin for 4 hours (37°C/5% CO_2) or analyzed unstimulated for markers of activation. Cells were washed in staining buffer and blocked with 5μL Fc-Block for 10 minutes at RT. The metal-coupled surface antibody cocktail was added and incubated at RT for 30 minutes. Cells were washed twice and fixed using the eBioscience FOXP3/transcription factor staining buffer set (ThermoFisher). Metal-coupled intracellular antibody cocktail was added and incubated for 45 minutes at RT. Cells were washed twice and 125 μM Intercalator-Ir in staining buffer was added for 20 minutes at RT. Cells were spun and washed twice with staining buffer followed by two washes using MilliQ water (low barium). Cells were resuspended in 250-500 μL of MilliQ water containing 1:10 dilution of EQ beads (for normalization), acquired using Helios (Fluidigm, San Francisco, CA, USA), and analyzed using Cytobank (Cytobank, Inc., Santa Clara, CA, USA).

Phospho-flow Analysis

For *in vitro* stimulation assays, $2.5x10^5$ PBMCs from HD or patients with ulcerative colitis were starved in serum-free RPMI 1640 (Gibco, Waltham, MA, USA) for 4 hours and subsequently stimulated with IL-2 (10^{-1}-10^4 IU/mL) for 15 minutes. To analyze *in vivo* phosphorylation of STAT5, $1x10^6$ splenocytes from

humanized mice were isolated upon LD IL-2 treatment. Next, cells were stained for human surface antigens (CD3, CD4, CD25, CD127), followed by fixation with a BD Cytofix^{TM} Fixation Buffer, permeabilization with a Perm/Wash^{TM} Buffer (BD Bioscience), and staining with anti-human/mouse phospho-STAT5 (clone 47, Tyr694, BD Bioscience) according to manufacturer's protocols.

Statistical analysis

Statistical analysis were performed using an unpaired t-test (Figs. 1*B*, 2*B*,*C*,*H*) or One-way ANOVA with Tukey's multiple comparisons test (Figs. 1*C,E,* 2*E*,*F*). Error bars represent the SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.