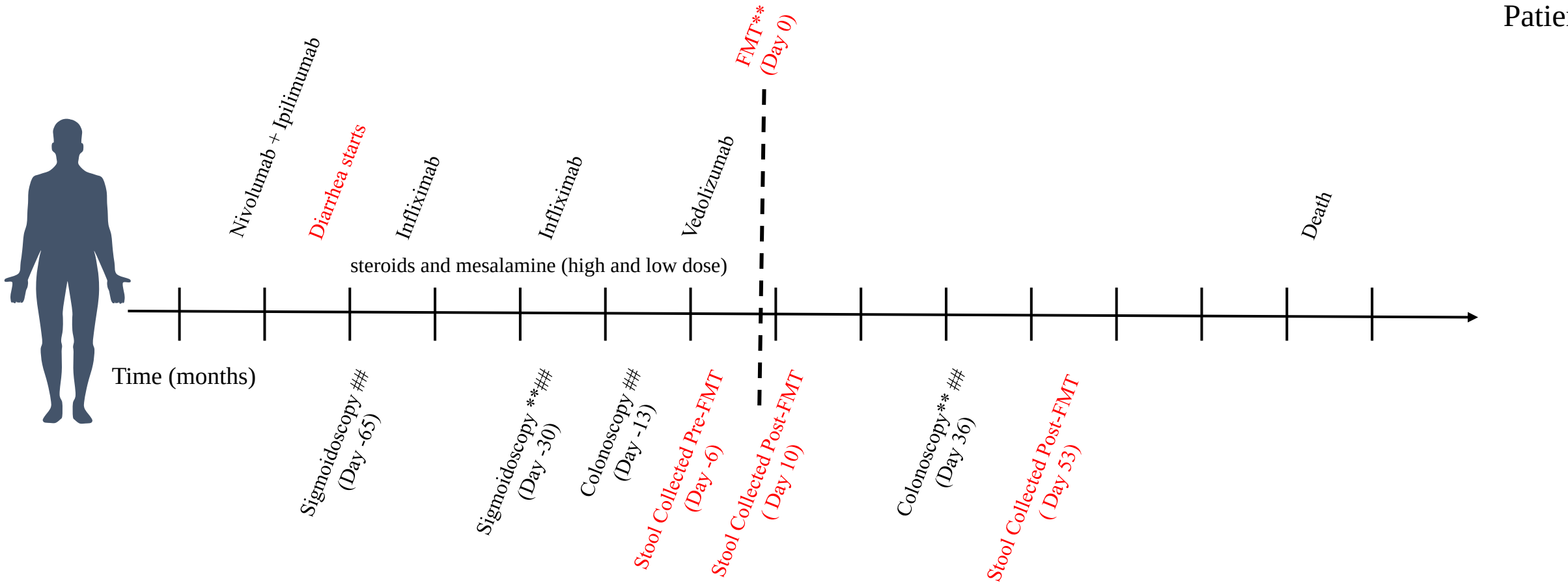
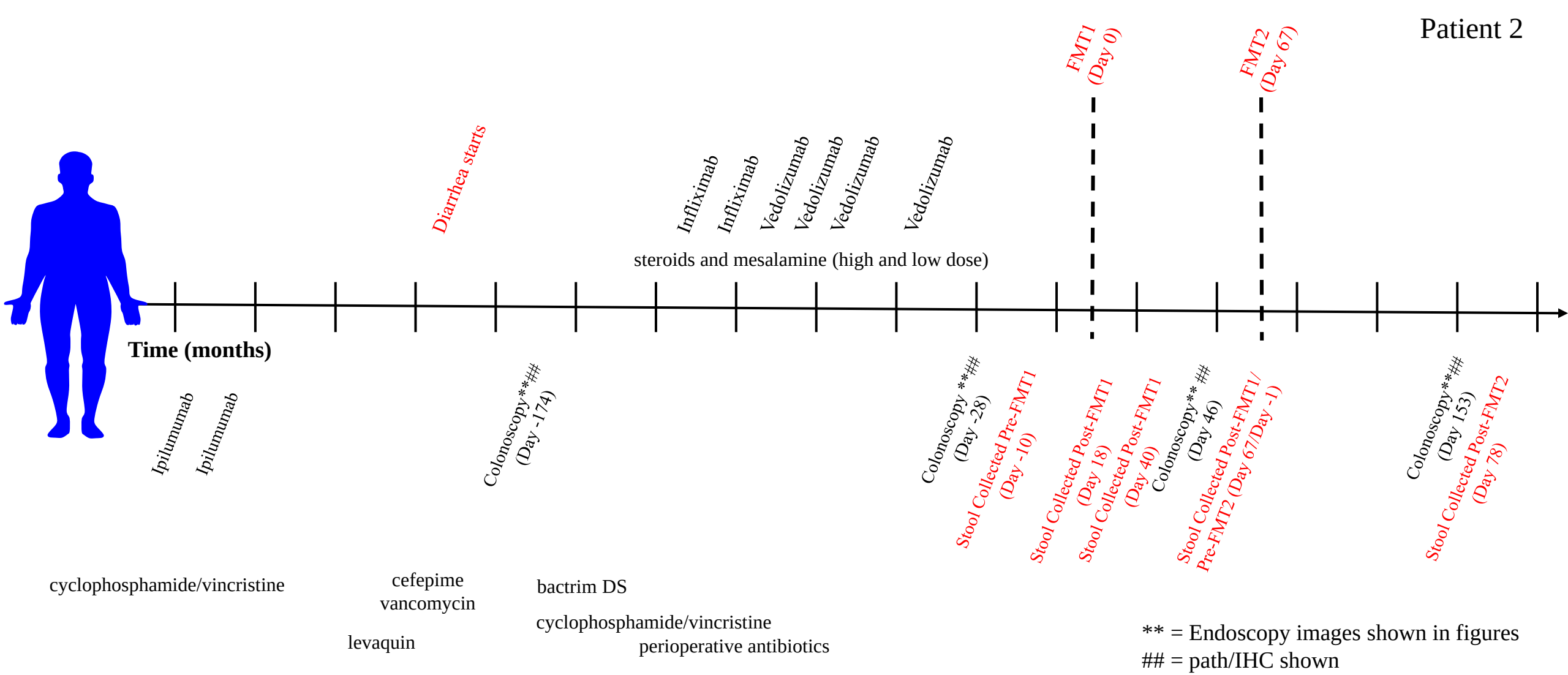


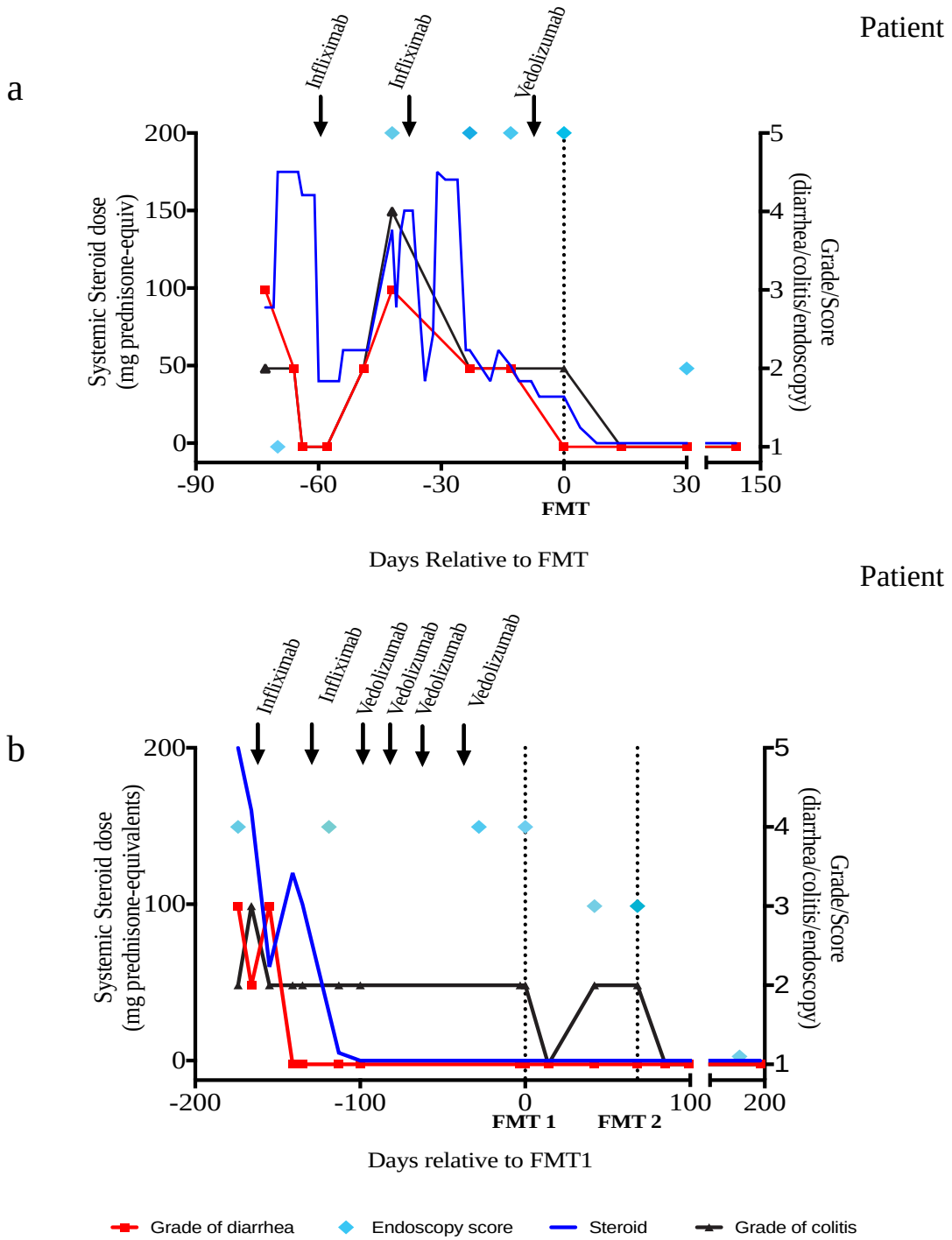
Timeline representing the clinical course of Patient 1. Key timepoints include timing of immunotherapy, diagnosis of colitis, length of time treated with traditional agents including steroids (initially dosed at 2 mg/kg IV and subsequently weaned slowly over the course of months), and other immunosuppressive agent including infliximab and vedolizumab, time of FMT. We also denote times during which endoscopy was performed and images are shown (**). Biopsies were taken for immunohistochemical analysis at designated times (##). We also denote timepoints at which time fecal material was collected for analysis of the gut microbiome. Below the timeline, are the approximate dates and duration of different antibiotic therapies the patients received for various clinical indications throughout this time course.

Supplemental Figure 1



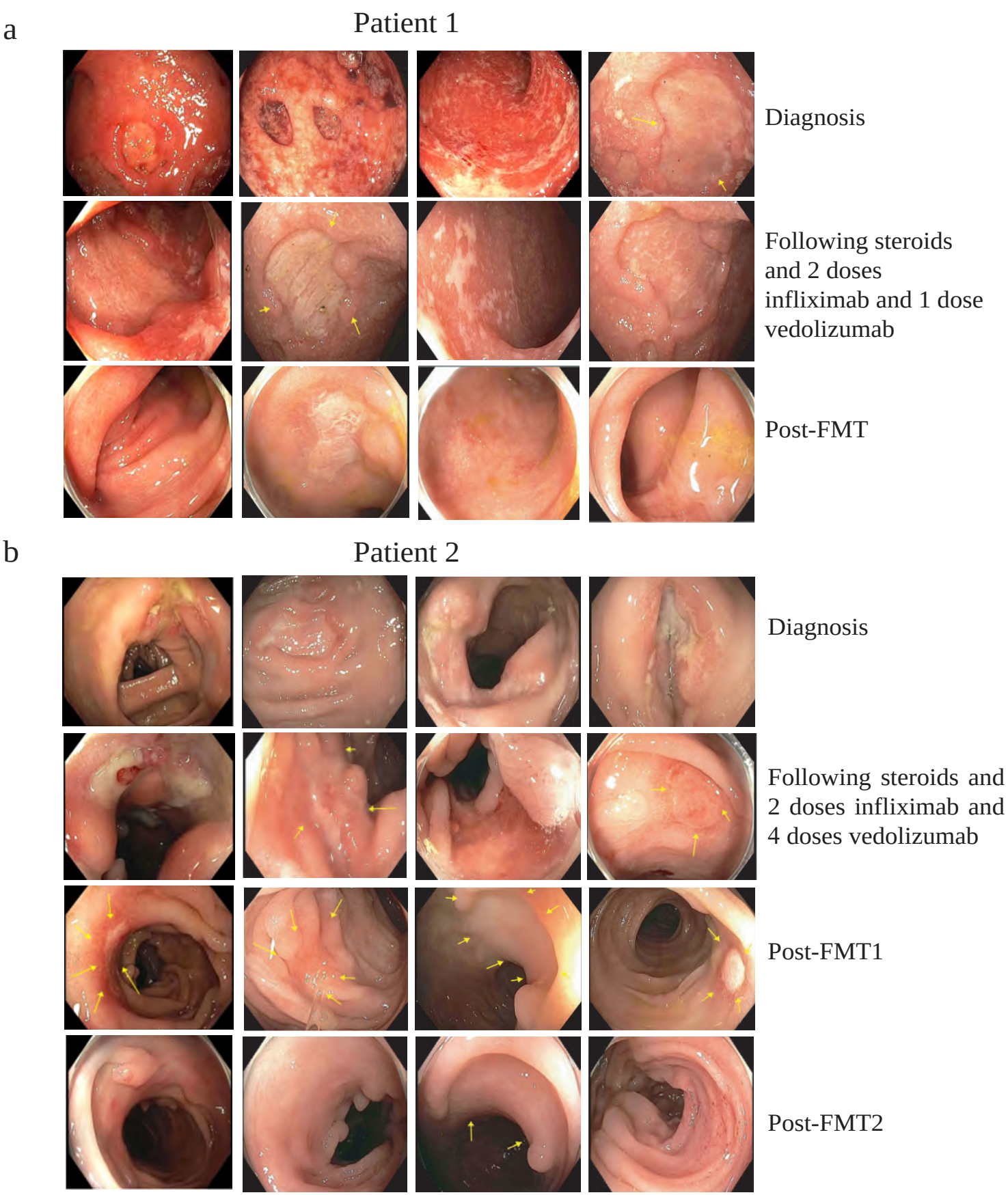
Timeline representing the clinical course of Patient 2. Key timepoints include timing of immunotherapy, diagnosis of colitis, length of time treated with traditional agents including steroids (initially dosed at 2 mg/kg IV and subsequently weaned slowly over the course of months), and other immunosuppressive agent including infliximab and vedolizumab, and timing of first and second FMT. We establish the time of first FMT as Day 0 and report other dates relative to this key time point. We denote times during which endoscopy was performed and images shown (**). Biopsies were taken for immunohistochemical analysis at times marked by ##. We also denote timepoints at which time fecal material was collected for analysis of the gut microbiome. Below the timeline, are the approximate dates and duration of different antibiotic therapies the patients received for various clinical indications throughout this time course. Additionally, we denote timing of other chemotherapeutic agents administered during this time period.

Supplemental Figure 2



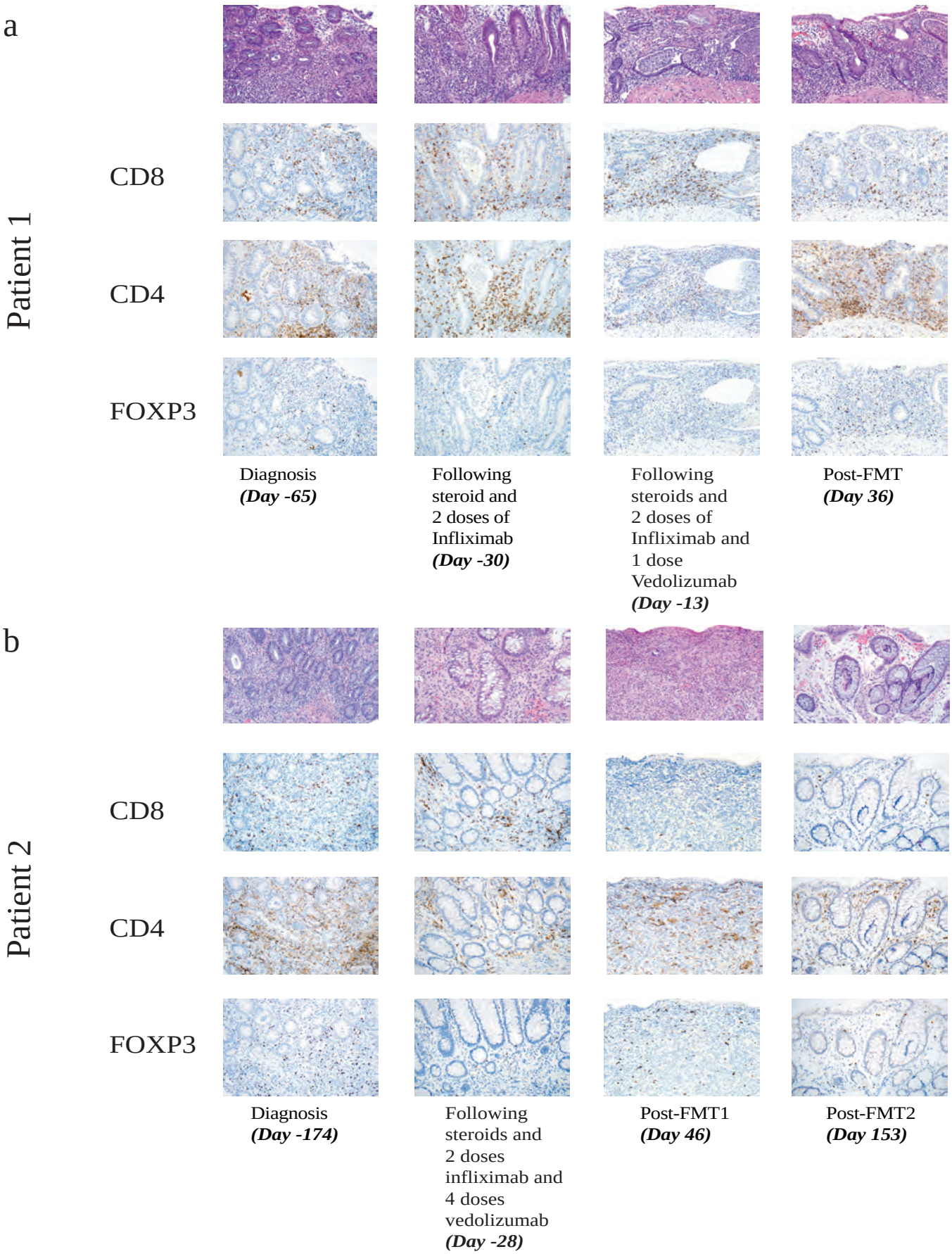
Severity of colitis. Various measures of disease severity are plotted throughout clinical course for (a) Patient 1 and (b) Patient 2, including daily dosage of systemic steroids (blue line), grade of diarrhea (red squares) and colitis (black triangles) as assessed by CTCAE Version 4 and endoscopic severity score (light blue diamonds) which incorporates presence of erythema/ erosions, presence of ulcer, and number (≥ 2), size (≥1cm) and depth (≥2mm) of mucosal ulcerations (each feature counts one point). Vertical dotted line indicates date of FMT. The endoscopic scoring criteria was created based on institutional expertise. Timing of doses of immunosuppressive agents is also noted.

Supplemental Figure 3



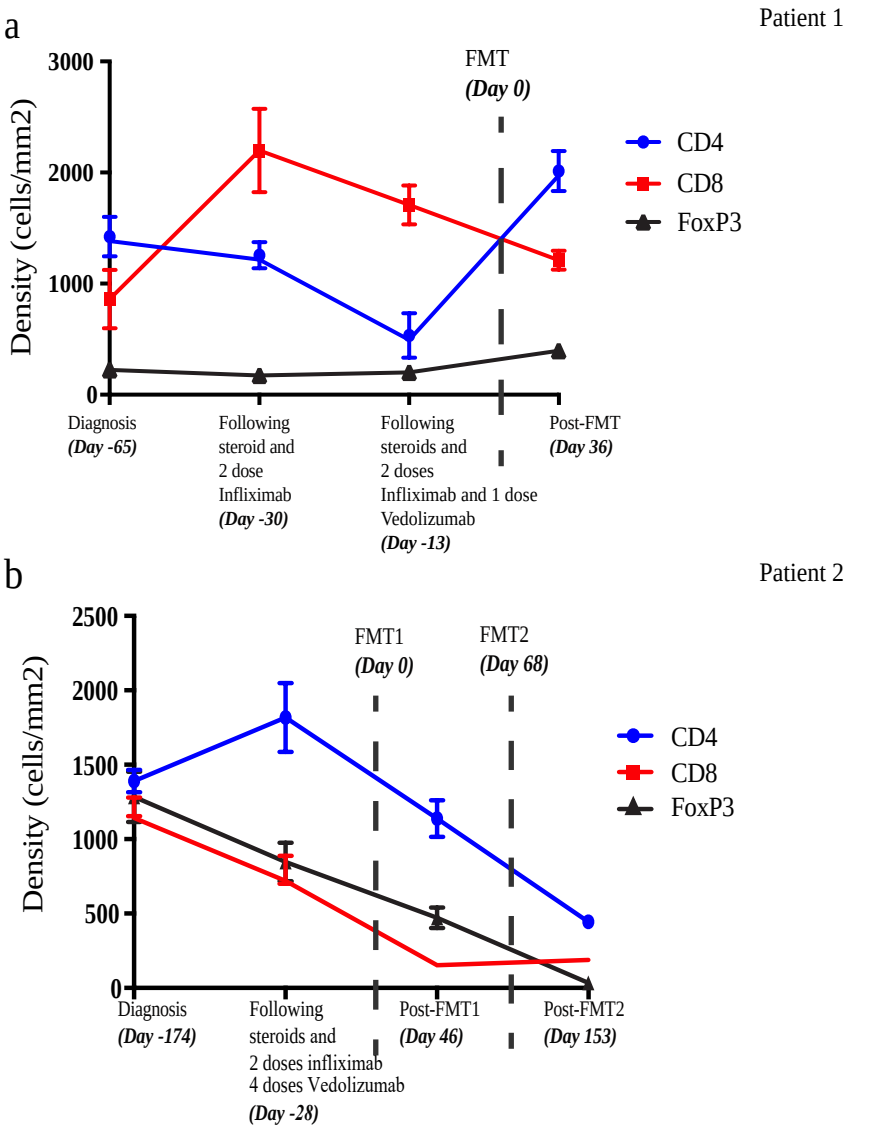
Additional endoscopic images from colonoscopy. (a) For Patient 1, images of colon and rectum near the time of diagnosis (row 1), after unsuccessful treatment with steroids and biologic immunosuppressive agents (steroid + 2 doses infliximab + 1 dose vedolizumab) (row 2) and approximately one month after FMT (row 3). The ulcers and inflammation were diffusely distributed in the distal 40 cm of the colon. The rest of the proximal colon appeared normal based on full colon endoscopy exam. (b) For Patient 2, images of colon and rectum near the time of diagnosis (row 1), after unsuccessful treatment with steroids and biologic immunosuppressive agents (steroid + 2 doses infliximab + 4 doses vedolizumab) (row 2), approximately 5 weeks following the first FMT (row 3), and approximately three months after the second FMT (row 4). Yellow arrows point to ulcerative lesions. The ulcers and inflammation were throughout the entire colon with patchy distribution pattern. Each photo represents a unique portion of the colon and rectum. It is important to note that the photos directly beneath one another in subsequent rows do not necessarily correlate to the exact same location within the colon or rectum. A single endoscopic exam was performed at each time point.

Supplemental Figure 4



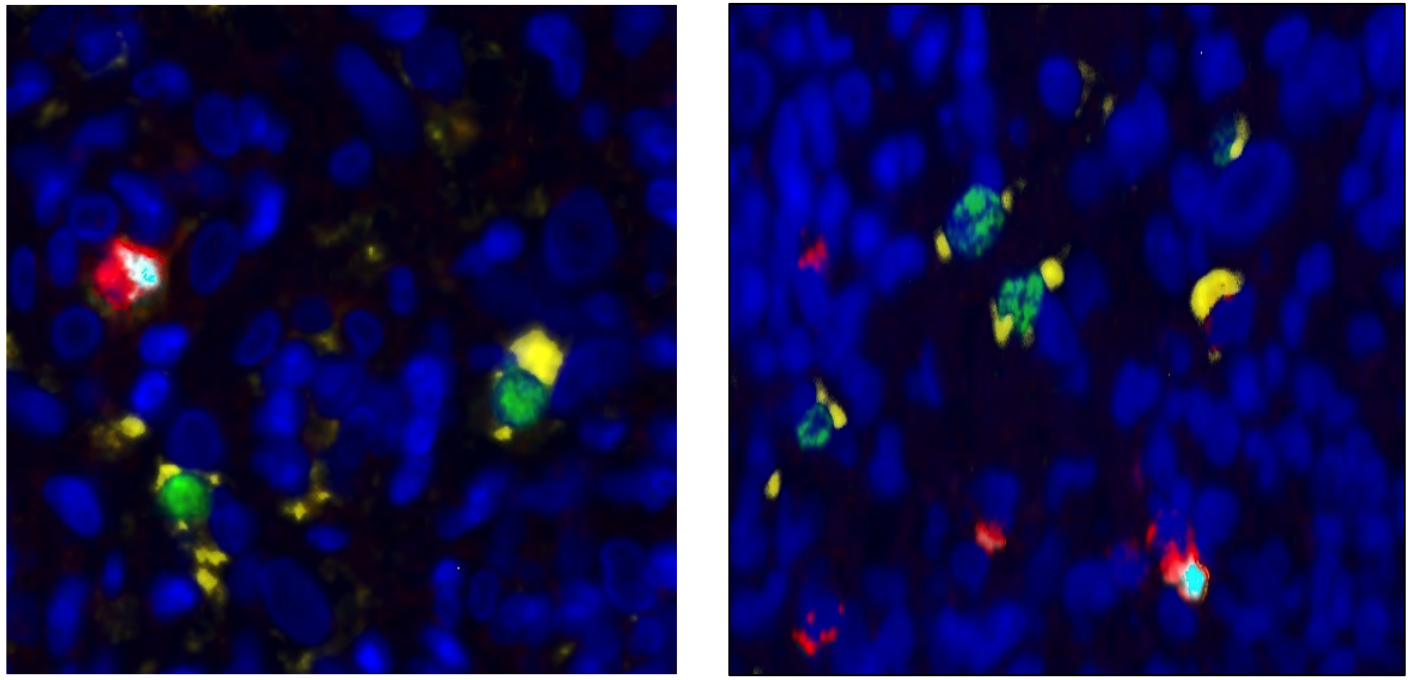
Immunohistochemical analysis of colonic mucosa. (a) For Patient 1, representative slides from multiple endoscopic biopsies taken at each of clinically relevant time points including at time of diagnosis, following unsuccessful treatment with steroids and 2 doses of infliximab, following additional one dose of vedolizumab, and following FMT (b) For Patient 2, representative slides from multiple biopsies taken at diagnosis, following unsuccessful treatment with steroids and biologic immunosuppression, and following first FMT and following second FMT. Shown is H&E staining for each as well as staining for individual markers common to T lymphocytes: CD8, CD4 and FoxP3. Date of FMT1 is considered as Day 0. For both patients, a single representative slide of the endoscopic biopsy specimen as a whole was stained per time point. For each sample, we analyzed 4 regions of interest (ROI). Each ROI measured 0.5 mm x 0.5 mm (for a total of ~1.00 mm2).

Supplemental Figure 5



Quantification of immunohistochemical analysis of colonic mucosa (a) For Patient 1, absolute densities of different immune cells (cells/mm2) at time of diagnosis, during initial treatment with steroids and 2 doses of infliximab, following additional one dose of vedolizumab treatment, and following FMT. Vertical dotted line represents timing of the FMT (Day 0). (b) For Patient 2, absolute densities of different immune cells (cells/mm^2) at time of diagnosis, following unsuccessful treatment with steroids and biologic immunosuppression, following FMT 1 and following the second FMT. For both patients, a single slide representative of the endoscopic biopsy specimen as a whole was stained per time point. These data represent the average (+/- standard deviation) cell density from 4 regions of interest per sample with each ROI measuring 500 µm x 500 µm. Vertical dotted line represents timing of FMT for each patient. Date of FMT1 is considered as Day 0.

Supplemental Figure 6



Blue: Dapi FoxP3: Green CD4: Yellow CD8: Red Granzyme B: Cyan

Colocalization of CD4 and FoxP3. Representative multiplex IHC demonstrating distinct CD8+ (red) and CD4+ (yellow) lymphocyte populations as well as co-localization of CD4+ and FoxP3 (green) in multiple cells which likely represent T regulatory lymphocytes. DAPI staining of nuclei in blue. This represents biopsy from Patient 2 following FMT1. Multiple replicates (2 or more) of at least two time points for each patient demonstrated similar results.

Supplemental Figure 7

Supplemental Table 1: Donor Screening Tests

Agent	Material	Acceptance Criteria
Hepatitis B Core Antibody	Blood	Negative
Hepatitis B Surface Antigen	Blood	Negative
Hepatitis C Virus Antibody	Blood	Negative
Hepatitis A Virus IgM	Blood	Negative
HIV-1 and HIV-2 Antibody	Blood	Negative
Anti-HTLV I / II	Blood	Negative
Serologic Test for Syphilis	Blood	Negative
Clostridium difficile toxin A/B	Stool	Negative
Shigella spp.	Stool	Negative
Salmonella spp.	Stool	Negative
Campylobacter spp.	Stool	Negative
Shiga-toxin producing Escherichia coli	Stool	Negative
Methicillin Resistant Staphylococcus aureus	Stool	Negative
Vancomycin Resistant Enterococcus spp.	Stool	Negative
Carbapenem Resistant Enterobacteriacea	Stool	Negative
Extended Spectrum b-lactanase Producing E. coli	Stool	Negative
Aeromonas spp.	Stool	Negative
Plesiomonas spp.	Stool	Negative
Yersinia spp.	Stool	Negative
Vibrio spp.	Stool	Negative
Cryptosporidum	Stool	Negative
Entamoeba histolytica	Stool	Negative
Cyclospora	Stool	Negative
Isospora	Stool	Negative
Rotavirus	Stool	Negative
Adenovirus	Stool	Negative
Norovirus	Stool	Negative
Giardia lamblia, EIA	Stool	Negative
H. Pylori EIA	Stool	Negative

Supplemental Table 2: Donor Screening Tests

Inclusion	Exclusion
1. Must be ≥ 18 years of age 2. Able to provide and sign informed consent 3. Able to complete and sign the donor questionnaire 4. Able to adhere to fecal transplantation stool collection requirements	1. Tested positive for any of variables mentioned below 2. History of autoimmune or atopic illness or active cancer or ongoing immune modulating therapy 3. First degree relative with intestinal carcinoma 4. History of risk factors for acquisition of HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the donor questionnaire 5 History of gastrointestinal disorder, e.g., inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), chronic constipation or diarrhea, gastrointestinal malignancies 6. New sexual contacts during past 6 months 7. Tattoos, body piercing or incarceration within 6 months 8 Major gastrointestinal surgical procedures 9. Antibiotic use during the preceding 3 months of donation 10. Drug or alcohol abuse 11. Fever > 100.4°F (38°C) for the past 3 months 12. Signs or any symptoms, including persistent symptoms of communicable infection, including cold 13. A history of chronic pain syndromes (fibromyalgia, chronic fatigue) or neurologic, neurodevelopmental disorders 14. Receipt of any type of live vaccine within 3 months prior to stool donation 15. Current or previous medical or psychosocial condition 16. Metabolic syndrome, body mass index over 30 or moderate-to-severe undernutrition (Malnutrition) 17. Hospitalization during the preceding 3 months of donation 18. Regular attendance at outpatient medical or surgical clinics 19. International travel or recent medical tourism within 3 months period