Multi-omics analyses of the ulcerative colitis gut microbiome link *Bacteroides vulgatus* proteases with disease severity

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Ulcerative colitis (UC) is driven by disruptions in host-microbiota homeostasis, but current treatments exclusively target host inflammatory pathways. To understand how host-microbiota interactions become disrupted in UC, we collected and analysed six faecal- or serum-based omic datasets (metaproteomic, metabolomic, metagenomic, metapeptidomic and amplicon sequencing profiles of faecal samples and proteomic profiles of serum samples) from 40 UC patients at a single inflammatory bowel disease centre, as well as various clinical, endoscopic and histologic measures of disease activity. A validation cohort of 210 samples (73 UC, 117 Crohn’s disease, 20 healthy controls) was collected and analysed separately and independently. Data integration across both cohorts showed that a subset of the clinically active UC patients had an overabundance of proteases that originated from the bacterium *Bacteroides vulgatus*. To test whether *B. vulgatus* proteases contribute to UC disease activity, we first profiled *B. vulgatus* proteases found in patients and bacterial cultures. Use of a broad-spectrum protease inhibitor improved *B. vulgatus*-induced barrier dysfunction in vitro, and prevented colitis in *B. vulgatus* monocolonized, IL10-deficient mice. Furthermore, transplantation of faeces from UC patients with a high abundance of *B. vulgatus* proteases into germfree mice induced colitis dependent on protease activity. These results, stemming from a multi-omics approach, improve understanding of functional microbiota alterations that drive UC and provide a resource for identifying other pathways that could be inhibited as a strategy to treat this disease.

Ulcerative colitis (UC), an inflammatory bowel disease (IBD), is characterized by chronic inflammation of the colon, with severity and persistence of mucosal inflammation being associated with morbidity and mortality¹. Non-specific immunosuppressive agents targeting the host, such as steroids, thiopurines and/or biologics, are used to offset the natural history of disease in patients with moderate to severe inflammation. These therapies are, however, associated with significant risks and often ineffective in adequately managing the disease². There are numerous microbiome studies using genomic techniques that have identified associations with UC, highlighting microbial dysbiosis and temporal shifts in composition related to UC status³⁻⁵. While recent efforts extended profiling of microbiota in UC beyond genomics⁶⁻⁸, it remains poorly understood whether these shifts are causal or associative in nature⁹,¹⁰. Our group has previously investigated the integration of metagenomics and metaproteomics¹¹ to help elucidate host-microbiota interactions by contrasting gene profiles with community-level proteomes through mass spectrometry (MS)¹². Adoption of metaproteomics has historically lagged behind other omic technologies¹³, although our efforts have indicated that utilizing developing methods in the field, such as sample multiplexing and deep fractionation approaches¹⁴, hold potential for uncovering important findings¹¹,¹³. Here we investigated our metagenomic-metaproteomic approach alongside conventional 16S ribosomal RNA (16S) gene amplicon sequencing, faecal metabolomics and serum proteomics methods in a large cross-sectional cohort of IBD patients, seeking host-microbiota interactions that could be exploited for therapy⁵,¹⁶⁻¹⁸.

After broadly analysing our data, we focus on one finding with multi-omics evidence—that proteases from some *Bacteroides* spp. could be involved in UC pathogenesis. In particular, our meta-omics data highlighted *Bacteroides vulgatus* proteases as potential targets...
for treating UC. Proteases have been previously postulated as therapeutic targets in IBD, although a clear understanding of the contribution and identity of the microbial proteases contributing to the disease has been less investigated\(^2\). Our multi-omics results expand upon and bring more clarity to the recent report that *B. vulgatus* was correlated with stool protease activity in a small population of patients that were later diagnosed with UC\(^3\). Together, our results provide evidence that *B. vulgatus* protease inhibition may be a therapeutic approach for preventing or treating UC.

**Results**

**Study design.** To initiate our study, patient samples from a convenience biobank at a single academic IBD centre (University of California, San Diego) that underwent extensive phenotyping with clinical disease activity indices and blinded assessments of endoscopic and histologic severity were collected and analysed using a multi-omics approach\(^1\) (Supplementary Table 1). Our resulting data represent one of the most extensive multi-omic resources on IBD patients to date, utilizing patient-matched serum and faecal samples for metagenomic and 16S rRNA gene amplicon sequencing, metabolomic, metaproteomic, serum proteomic and metaproteomic analyses (Extended Data Fig. 1). An initial discovery group of 40 UC serum and faecal samples was collected and followed-up by a second group of 210 faecal samples, which included 73 UC, 117 Crohn’s disease (CD; roughly split by ileal, ileocolonic and colonic subtypes) and samples from 20 volunteers without IBD. Our previously established integrated metagenomic–metaproteomic approach of shared database assembly and quantification was used for direct comparisons between microbial genes and proteins\(^1\). Application of our multiplexing metaproteomic methods provided increased protein identifications and a greater than 10-fold increase in proteins quantified per sample compared with conventional label-free metaproteomic methodology. We demonstrate this important technical advantage by comparison with data downloaded from the Human Microbiome Project's IBD multi-omics database, which notably represents a smaller patient population than the cohorts of this study (Extended Data Fig. 2).

**Meta-omic associations to IBD severity.** Despite our cohort representing a diverse group of patients (Supplementary Table 1), many clinical severity metrics showed a high degree of correlation (Fig. 1a). Given the overlap of severity metrics, a representative metric including patient symptoms, partial Mayo for UC, alongside patient-reported outcomes (stool frequency, abdominal pain, general well-being) from the Crohn's Disease Activity Index (CDAI) for CD\(^3\) was chosen. Disease severity was significantly correlated with both alpha diversity and beta diversity in all meta-omics collected (Fig. 1b,c, Supplementary Table 2 and Fig. 1a–f). CD subtypes and the two separately processed UC cohorts displayed unique microbiota compositions distinct from healthy controls (Fig. 2a and Supplementary Fig. 2). We observed stronger correlations between the distributions of data in the faecal-based omics than the serum proteome (Fig. 2b and Supplementary Fig. 1g,h), and that the metaproteome allowed for the strongest prediction of UC activity, closely followed by the combined data and the metabolome (Fig. 2c and Supplementary Table 3). Unlike UC, an influential feature in CD patient microbiomes was the dominance of a member of the Enterobacteriaceae family (Extended Data Fig. 3). In patients with active UC, we also observed an increase in human proteins, and classes of metabolites such as phosphocholines correlated with activity (Fig. 2d,e).

Utilizing direct comparison of genes and proteins of the microbiota\(^1\), linear regression identified the most correlated features with clinical disease severity \((r>0.3)\). Comparing genera annotations of positive and negative associations identified that *Bacteroides* proteins represented 40–60% of proteins positively correlated with UC disease activity (Fig. 3a and Extended Data Fig. 4). This association between disease activity and *Bacteroides* was confirmed across both UC cohorts, and identified as unique to UC as CD subtypes each presented unique profiles of disease-correlated proteins (Extended Data Fig. 5). The metagenome largely reflected the direction and magnitude of the genera level bias of the associations identified in the metaproteome; however, *Bacteroides* genes showed a weaker relationship to high disease severity in UC relative to the metaproteome (Fig. 3b and Extended Data Fig. 4). A functional analysis of proteins from *Bacteroides* that are associated with disease activity displayed an increased representation of enzyme families, and more specifically, proteases (Fig. 3c). *B. vulgatus* and *B. dorei*, two closely related species prevalent among healthy adults\(^2\), contributed ~40% of all *Bacteroides* reads in the metagenome of UC patients (Fig. 3d). We next analysed the correlation with UC severity of the 119 distinct enzymes and proteases derived from 59 species of *Bacteroides*. Serine proteases, including six dipeptidases, were among the proteases commonly correlated with UC activity from prevalent *Bacteroides* species (Fig. 3e). Applying an outlier approach comparing metagenomic and metaproteomic data, we identified patient samples with over- or underproduction of *B. vulgatus* and *B. dorei* proteases, and observed that patients containing increased proteases had significantly higher clinical severity and endoscopic activity compared with the decreased proteases group and the typical UC patient sample (Fig. 3f and Extended Data Fig. 6a). From a histological perspective, only 18.8% of patients categorized as ‘overproducers’ were in histological remission, while 38.5% of patients categorized as ‘underproducers’ and 45% of all other patients were in histological remission (Extended Data Fig. 6b). As some of the correlated proteases included serine and metalloproteases—classes of proteases that largely function in the extracellular space\(^3\), we hypothesized that these proteins may play roles in extracellular proteolysis and exacerbation of disease activity.

**Assessing proteolysis in UC patient omics and *Bacteroides* supernatant.** Metabolomic and metaproteidomic analyses corroborated the importance of proteolysis in UC patients. This was initially observed through the identification of dipeptides as the metabolite class with the second highest correlation with UC disease activity (Figs. 2h and 4a). Dipeptides and oligopeptides were the two most common chemical classes among the metabolites positively correlated with disease activity \((r>0.3)\), accounting for 44% and 5.8% of the total positive correlations. To further analyse oligopeptides, a de novo sequencing approach was taken to analyse the metaproteidome (the peptides from complex multi-species samples). Results identified more peptide fragments within high severity UC faecal samples and patients with overproduction of *Bacteroides* proteases (Fig. 4b and Extended Data Fig. 7). The data also revealed the identity of peptide fragments from human proteins, including structural proteins from collagens and mucins (Fig. 4c). These human proteins represent potential targets of proteases in UC. The known cleavage patterns of Neutrophil elastase and Proteinase-3\(^3\) were not strong signals among termini of identified peptides (Extended Data Fig. 7b), indicating that neutrophil proteases were probably not primary contributors to the proteolysis in patients. Network analysis of host proteins correlated with disease activity from the faecal and serum of UC patients highlighted regulation of proteolysis as a common function (Supplementary Fig. 3).

To characterize the protease activity present in the *Bacteroides* species we identified as related to UC disease activity, bacterial cultures were grown and the supernatant was analysed through proteomics and protease activity assays. Inhibition of serine proteases was the most effective method of disrupting proteolysis from *B. vulgatus* supernatant (Fig. 4d). Proteomic analysis identified that serine-type activity was the most common class of enzymatic function from proteins in the supernatant of *B. vulgatus*, *B. dorei*...
and B. thetaiotaomicron (B. theta) (Supplementary Fig. 4a). Identified proteases were next ranked by increased abundance in the supernatant of B. vulgatus compared with B. theta (Supplementary Fig. 4b) and then ranked by the summed correlation values in UC cohorts (Fig. 4e). Further, a comparison of the identities of Bacteroides proteases correlated with UC patients and those found in the Bacteroides supernatant was conducted (Supplementary Fig. 4c and Table 4).

Protease inhibition prevents B. vulgatus-induced colonic epithelial damage in vitro and in vivo. We next tested the six most abundant Bacteroides species in UC for effects on the intestinal barrier using Caco-2 epithelial monolayers. Our results showed a significant decrease in trans-epithelial electrical resistance (TEER) after 38 h of incubation with the two most abundant Bacteroides species, B. vulgatus and B. dorei, while the other species increased TEER (Extended Data Fig. 8a). Although both B. vulgatus and
**Fig. 2 | Multi-omic analysis of IBD disease activity.** a, 16S phyla composition by disease activity states. The average phyla compositions of groups of patient samples are shown in barplots. Barplots represent sample sizes of \(n = 18\), 12, 10 for UC Cohort 1; \(n = 34\), 9, 13 for UC Cohort 2; \(n = 19\), 8, 1 for colonic CD; \(n = 22\), 7, 3 for ileocolonic CD; \(n = 19\), 4, 2 for ileal CD (each ordered low, moderate and high activity, respectively); \(n = 15\) samples for healthy controls. b, Data type correlations. Pearson correlations between data types are displayed in a heatmap. The Bray-Curtis distance metric was used for all data types and correlations were performed on distance matrices using Mantel’s test. c, Evaluating meta-omic performance in predicting UC disease activity. The mean squared error from 100 iterations of random forest analyses on each UC cohort trained to predict the partial Mayo disease activity diagnosis. The mean squared error from different between the UC gut microbiota and the healthy gut microbiota. We next assessed the contribution of protease activity to the disruption of epithelial permeability by adding a protease-inhibitor cocktail specific to serine and cysteine proteases. We found that protease inhibition significantly increased TEER at both 22h and 38h post inoculation with *B. vulgatus* (adjusted \(P\) value < 0.0001, \(\eta^2 = 0.64\), Fig. 5a,b). The phenotype was not due to effects on bacterial growth or viability, as colony-forming units (c.f.u.s) were not significantly different between the *B. vulgatus* wells treated with or without the...
Protease-inhibitor cocktail (adjusted $P = 0.98$, Fig. 5c and Extended Data Fig. 8b). We further tested whether the supernatant from *B. vulgatus* in log-phase growth had a similar impact on TEER (Extended Data Fig. 8c). No significant effect was found, indicating that either the proteases of interest are membrane-bound or that a stressor (for example, host–microbe interaction or nutrient deprivation) is necessary for protease secretion.

Confocal microscopy of the intestinal monolayers revealed dramatic impact on the *B. vulgatus*-treated epithelial cells, with apparent alteration of tight-junction proteins, Zo-1 and Occludin (Fig. 5d and Supplementary Fig. 5). Imaging studies also demonstrated potential impacts on cell morphology and actin networks of the Caco-2 cells treated with *B. vulgatus* (Supplementary Fig. 5). Analysis of the cell shape within monolayers showed a significant decrease in the circularity of the cells ($P = 0.0043$), which could be restored through protease inhibition (Fig. 5e).

To investigate the effect of *B. vulgatus* proteases in vivo, we performed monocolonization with *B. vulgatus* in an IL10−/− germfree mouse model, supplementing the drinking water of half the mice with our selected protease-inhibitor cocktail (Fig. 5f). After 10 weeks of colonization, protease inhibition had a protective effect on the colonic epithelia, decreasing inflammatory cell infiltration of the crypts (Fig. 5g). Histological colitis severity was significantly improved by protease-inhibitor treatment (adjusted $P = 0.0061$, Fig. 5h), along with significantly reduced *B. vulgatus*-induced crypt hyperplasia (adjusted $P = 0.0028$, Fig. 5i). Macroscopic features, such as the colon length of mice, were not significantly different (Supplementary Fig. 6a–h). Further,
immune cell profiles of mesenteric lymph nodes revealed no significant differences between the groups for CD4+, Th1, Th17 and Treg cell populations (Supplementary Figs. 6i–l and 7). In this study, we were not able to evaluate the extent to which the protease treatment reflected a state similar to that in mice colonized with non-proteolytic Bacteroides as this control group was not included.

**B. vulgatus** proteases present in UC patient’s faeces drive colitis severity upon transplantation into germfree mice. Next we sought to evaluate the extent to which the presence of high levels of *B. vulgatus* proteases in UC patients impacted development of gut inflammation. To this end, we performed a transplant of faeces from UC patients into colitis-prone IL10-deficient germfree mice (Fig. 5j). We selected patient faecal samples with or without high levels of *B. vulgatus* proteases (n = 3 UC patients per condition) for transplant into groups of colitis-prone IL10-deficient germfree mice. Half of these mice were administered a protease-inhibitor cocktail via their drinking water (n = 9 mice per condition). Mice administered protease-abundant faecal samples displayed overt colitis based on both gross indicators of disease (colon shortening and splenomegaly, Fig. 5k,l) and histopathologic analysis (Fig. 5m,n). These phenotypes were not evident in mice receiving faeces from UC patients that lacked an abundance of *B. vulgatus* proteases. Significant differences were not observed on other organs (Extended Data Fig. 9a–f). The protease-inhibitor cocktail did not significantly impact these parameters in mice administered the low-protease-containing faecal samples but markedly attenuated the colitis exhibited by mice that had received the protease-abundant faecal samples. Assessment of colonic inflammation via measurement of faecal lipocalin abundance and splenic bacterial load showed similar trends but did not reach statistical significance (Extended Data Fig. 9g,h). These studies reveal that the microbiomes of UC patients with increased *Bacteroides* proteases have high colitogenic potential and suggest protease inhibition as a therapeutic intervention in severe UC.

Finally, to confirm the presence of *B. vulgatus* proteases in the faecal transplantation study, metaproteomic analysis of mouse faecal material was performed. Comparing the faecal material of mice transplanted with samples from one patient with overabundant *B. vulgatus* proteases and one low-protease control patient, we were able to detect an increased abundance of *Bacteroides* proteins and *B. vulgatus* proteases from the overabundant transplantation irrespective of the presence of the protease-inhibitor cocktail (Extended Data Fig. 9i and Fig. 5n). Common functions among the proteases identified from *B. vulgatus* in these mice included serine-type peptidase activity and dipeptidase activity (Extended Data Fig. 9j). To guide future studies into *B. vulgatus* proteases, comparisons were performed between the identity of proteases highlighted in UC patients, the in vitro studies and the in vivo studies (Extended Data Table 4). Of note, several dipeptidyl peptidases (for example, DPPIV, DPPVII) were consistently identified throughout the study. These peptidases have known roles in amino acid metabolism in nutrient limited areas and virulence in *Porphyromonas gingivalis*, a bacterium linked to periodontal disease. Interestingly, human DPPIV is the target of numerous therapeutics...
for the treatment of diabetes19. DPPIV inhibitors were also shown to have a protective effect in a colitis model (attributed to preventing Glucagon-like peptide-2 degradation)20, therefore we speculate that *B. vulgatus* DPPIV may be of interest as a potential therapeutic target.

**Discussion**

Here we effectively collect and translate an extensive meta-omic profile of IBD patients into a hypothesis of biological and therapeutic value. By integrating faecal metaproteomics, metabolomics, 16S gene amplicon sequencing, shotgun metagenomic sequencing, metaproteomics and serum proteomics, in addition to in vitro and in vivo validation, we demonstrate that certain members of the microbiome, such as *B. vulgatus*, may contribute to exacerbating UC disease activity through protease activity. Further, given the promise of our in vitro and in vivo experiments, this study sets the stage for further investigation of *Bacteroides* protease inhibition as a therapeutic approach in UC.

To generate our hypothesis, we utilized several innovative omics advances that may be of broad interest, such as our integrated approach for comparing metagenomic and metaproteomic data42, and the analysis of peptide fragments. Given that previous high-profile IBD datasets that included metaproteomic data used methods that generated an order of magnitude more missing values, we had interest in further investigating findings unique to our metaproteome data. One striking observation uniquely highlighted by these data was that ∼50% of microbial proteins correlated with UC disease activity were derived from *Bacteroides*. While metaproteomic data are rarely collected in microbiome studies, this data type provided an important complementary tool for identifying that proteolysis, potentially derived from *Bacteroides* proteases, was correlated with UC activity. By integrating metagenomic data, we provided a genomic context to our findings and identified *Bacteroides* species of interest for in vitro studies. Other omic profiles (serum proteomics, metabolomics and 16S) further corroborated and contextualized the core hypothesis of *Bacteroides*-derived proteolysis as a contributing factor to UC severity.

Our study advances what is currently known about *B. vulgatus* and UC. *Bacteroides* spp. are among the most abundant species of the gut, residing in the outer mucosal layer of the colon32. Interestingly, the human version of one of our most promising bacterial proteases, DPPIV, has already been considered as a potential target for therapeutic development32. By our estimates, ∼40% of UC patients may have overexpression of *B. vulgatus* serine proteases, which are known molecular events in IBD, the role of bacterial proteases in IBD has been primarily a source of speculation19,25,43–46. Work in this area has mostly focused on the contribution of host proteases, such as trypsin, which is decreased in IBD patients44, or matrix metalloproteases, which can degrade commonly used therapeutics33,45. Some authors estimate that ∼27% of proteolysis in UC patients is from bacterial proteases43. Further evidence of the importance of bacterial protease activity in the gastrointestinal tract was seen when antibiotic exposure in mice reduced the activity of microbiome-derived serine proteases36. Here we were able to directly identify the species and identities of bacterial proteases that may contribute to IBD. Given that current treatments for UC are focused on targeting host inflammatory pathways, our findings represent an alternative approach for therapeutic development37. Interestingly, the human version of our most promising bacterial proteases, DPPIV, has already been considered as a potential target for therapeutic development32. By our estimates, ∼40% of UC patients may have overexpression of *B. vulgatus* serine proteases, which are known molecular events in IBD, the role of bacterial proteases in IBD has been primarily a source of speculation19,25,43–46.
which represents a substantial subset of patients that might benefit from bacterial-protease inhibition.

The role of proteases in *Bacteroides* remains an underexplored research area. Studies indicated that their proteases may have effects on host digestive enzymes, with *B. vulgatus* having higher protease activity than other *Bacteroides* species. However, studies of the roles of *Bacteroides* proteases in general are limited beyond the characterization of a metalloprotease enterotoxin from *B. fragilis*. Increased protease abundance could be related to extracellular membrane vesicles, which in *Bacteroides* are abundant in proteases. Interestingly, extracellular vesicles were linked to IBD from a recent metaproteomic study, and *Bacteroides* proteins were reported as a major contributor to bacterial extracellular proteins.

Our working hypothesis is that nutrient availability or host–microbe interactions in the UC gut may trigger increased production of *B. vulgatus* proteases relevant to UC activity (Extended Data Fig. 10). One or a combination of these proteases appears capable of disrupting the colonic epithelium, which may allow the influx of innate immune cells, such as neutrophils, which further exacerbate colitis. An alternative hypothesis would be that there are *B. vulgatus*...
strains carrying unique proteases, although we do not think this is likely given that our in vitro and in vivo work was performed using a strain of B. vulgatus isolated from healthy stool.

We note several limitations of our study. One limitation is that we utilized non-specific protease inhibitors in our experiments and therefore were not able to distinguish the specific protease or proteases that were most important to our phenotypes. Second, our monoclonization study did not include an additional control group to compare the extent to which protease inhibition treatment reflected a healthy phenotype. Finally, our experiments using the supernatant of B. vulgatus on the colonic epithelial barrier did not disrupt membrane integrity as observed in co-culture, emphasizing that more work is needed to determine the conditions and mechanisms underlying our observed phenotypes.

The multidimensional meta-omic integration shown here not only represents an important resource for future multi-omic investigation of IBD, but also serves as an example demonstrating the development of hypotheses from multi-omic data integration. Starting with broad-scale analysis of hundreds of IBD patients, and further refining our analyses according to an interest led to compounding evidence of our hypothesis within each dataset. We have further narrowed and validated our primary hypothesis with numerous in vitro and in vivo studies that demonstrate the efficacy of protease inhibition to prevent B. vulgatus-induced colitis. In total, our study highlights promising areas of investigation regarding the role of proteolysis in Bacteroides, and demonstrates that proteolysis from B. vulgatus may be relevant to UC pathogenesis and treatment.

Methods

Monoclonization experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California San Diego (UCSD). Faecal transplantation experiments were done in accordance with institutional approval from Georgia State University (Atlanta, Georgia, USA) and Cochin Institute (Paris, France) under institutionally approved protocols (IACUC A18006 and APAFIS 24788-201902806256593 v8). All studies were conducted in accordance with NIH guidelines for the Care and Use of Laboratory Animals. Human demographics, relevant medical information, disease activity, stool, serum and mucosal biopsies were collected with informed consent and following regulations at the Biobank at UCSD.

Patient population and clinical diagnostics. Ulcerative colitis and Crohn’s disease patients were selected from a convenience sampling biobank at UCSD (PI Dulai). Longitudinal data on patient demographics (age, gender, ethnicity), disease characteristics (prior surgeries, disease-related complications, phenotype classification according to Montreal sub-classifications), current and previous treatments (corticosteroids, immunomodulators, biologics), clinical disease activity (patient-reported outcomes using the partial Mayo score and CDAI), and endoscopic and histologic disease activity were collected. Patients also agreed to stool, serum and mucosal biopsy collection. When endoscopy was performed as part of routine practice, stool was collected within 24 h before endoscopy and serum was collected on the day of endoscopy. A detailed endoscopic disease activity assessment using the Mayo endoscopic sub-score and the Ulcerative Colitis Endoscopic Index of Severity (UCEIS), was conducted by a physician without knowledge of the clinical disease activity score or biomarker data. Routine standard of care biopsies were scored using the Geboes score by a pathologist, who was blinded to clinical, biomarker, and endoscopic data and scores. Further information regarding clinical, endoscopic and histologic activity scoring have been previously discussed23. All serum and stool samples were aliquoted within 3,220 μl vacuum, steriflip (Milipore) filters were desalted with C18 Sep-Paks (Waters) and eluted with a 40% and 80% acetonitrile mixture. Total protein content. After depletion, protein was processed as described below, with the exception of a Trichloroacetic acid precipitation being used in place of the protocol. Total protein content was measured using a PicoGreen dsDNA assay kit. Cells were pelleted by centrifugation at 10,000 × g for 10 min at 4 °C. Cells were lysed in 2 ml buffer containing 50 mM NaCl (Sigma), 3% sodium dodecyl sulfate (SDS, Fisher), 1 mM NaF (Sigma), 1 mM beta-glycerophosphate (Sigma), 1 mM sodium orthovanadate (Sigma), 10 mM sodium pyrophosphate (Sigma), 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) and 1× Complete Mini EDTA-free protease inhibitors (Roche) in 50 mM HEPES (pH 8.5) (Sigma). An equal volume of 8 M urea in 50 mM HEPES (pH 8.5) was added to each sample. Cell lysis was achieved by two 15 s intervals of probe sonication at 25% amplitude. Proteins were then precipitated with dithiothreitol (Sigma), alkylated with iodoacetamide (Sigma), and quenched as previously described23. Proteins were next precipitated via chlorof orm–methanol precipitation and pellets were dried23. Pellets were resuspended in 1 M urea in 50 mM HEPES (pH 8.5) and digested overnight at room temperature with LysC (Wako). A second 6 h digestion using trypsin at 37 °C was performed and the reaction was stopped by addition of 10% trifluoroacetic acid (TFA, Pierce). Samples were then desalted with C18 Sep-Paks (Waters) and eluted with a 40% and 80% acetonitrile
solution containing 0.5% acetic acid. The concentration of desalted peptides was determined, and 50 μg aliquots of each sample were dried in a speed-vac. Bridge channels consisting of 25 μg from each sample were created and 50 μg aliquots of this solution were used in either one or two channels (dependent on the experiment and listed in supplementary files) per Tandem Mass Tag (TMT, ThermoFisher) 10-plex MS experiment as previously described. These bridge channels were used to control for labelling efficiency, inter-run variation, mixing errors and the heterogeneity present in each sample. Mass defects for each TMT set were accounted for in the database search. Peptide access to the manufacturer’s report per lot number. The lot numbers for TMT reagents were SF253324 for metaproteomics and SG253268 for serum proteomics of the first cohort of UC patients, TG271363 for the second cohort of IBD patients, and lot number VA296083 for both the bacterial supernatant and metaproteomics of the mouse faecal transplant experiments. Each sample or bridge channel was resuspended in 30 μl of the appropriate TMT reagent78. Reagents 126 and/or 131 (ThermoFisher) were used to bridge between MS runs. Remaining reagents were used to label samples in random order. Labelling was performed at room temperature for 1 h, and quenched with 8 μl 5% hydroxylamine (Sigma). Labelled samples were acidified by adding 50 μl 1% TFA. After TMT labelling, each 10-plex experiment was combined, desalted (C18 Sep-Paks) and dried in a speed-vac.

Generation and processing of liquid chromatography (LC)–LC–MS/MS proteomic data. Basic pH reverse-phase LC, followed by data acquisition through LC–MS/MS was performed as previously described. Briefly, gradient elutions of acetonitrile were performed on C18 columns using an Ultimate 3000 HPLC (ThermoFisher). Subsequently, 96 fractions were combined as previously described, and further separation of fractions was performed with an in-line Easy-nLC 1000 (ThermoFisher) and a chilled autosampler. LC–MS/MS data were collected on an Orbitrap Fusion (ThermoFisher) mass spectrometer, with acquisition parameters were used according to PEAKS default qTOF settings, 0.1 Da parent mass tolerance and a 6 min retention time window. Peptides were searched against the appropriate TMT reagent78. Reagents 126 and/or 131 (ThermoFisher) were used to bridge between MS runs. Remaining reagents were used to label samples in random order. Labelling was performed at room temperature for 1 h, and quenched with 8 μl 5% hydroxylamine (Sigma). Labelled samples were acidified by adding 50 μl 1% TFA. After TMT labelling, each 10-plex experiment was combined, desalted (C18 Sep-Paks) and dried in a speed-vac.

Data were processed using Proteome Discoverer 2.1 (ThermoFisher). MS/MS data were searched against the shared metagenomic database and the uniprot human protein database (uniprot.org, accessed 11 May 2017). The Sequest searching algorithm94 was used to align spectra to database peptides. A precursor mass tolerance of 0.05 ppm95 and a 0.0 Da tolerance was specified for MS/MS fragments were specified. Included in the search parameters was static modification (M) and variable oxidation (M) and ubiquitination. The maximum variable post-translational modifications for each protein were taken as previously described96 to reduce false assignments for metaproteome datasets. The standardized methods in Proteome Discoverer (Version 2.1) preferentially assign peptides to proteins that previously had peptides reported. If this does not resolve an ambiguous assignment, the peptide is assigned to the longest protein. After the first search, all proteins reported in forward or reverse datasets were filtered into a smaller database for a second search97. This method effectively decreased the database search space in cohort 1 from 766 Mb to 22 Mb and from 1 Gb to 42 Mb in cohort 2. Additionally, a duplicate peptide filter was performed according to the Proteome Discoverer report. All signals from PSMs assigned to the same protein group were summed to represent protein abundances.

Protein relative abundances were normalized first to the pooled standards for each protein and then to the median signal across the pooled standard. An average of these normalizations was used for the next step. To account for slight differences in amounts of protein labelled, these values were then normalized to the median of the entire dataset. The dataset was filtered for the most representative ratios per protein per sample. Proteomic datasets generated from IBD patient samples resulted in final data tables containing 1,005 proteins for cohort 1 serum samples, 46,398 proteins for cohort 1 faecal samples, and 86,451 proteins for cohort 2 faecal samples.

Metabolite extraction and LC–MS. Metabolites were extracted by adding a 1:5 weight to volume solution of 70% methanol infused with a 5 μM internal standard sulfamethoxine. The samples were briefly vortexed and stored at 4 °C overnight. Extracts were then centrifuged at 1,500 x g for 5 min to pellet particulate matter and the supernatant was removed for MS analysis. The extracts were diluted 1:4 in a 96-well plate in 10% methanol before injection.

LC–MS/MS was performed on a Bruker Daltonics Maxis QTOF mass spectrometer (Bruker) with UltraMate 3000 Dionex UPLC (ThermoFisher). Metabolites were separated using a Kinex 2.6 μm C18 (30 x 2.0 mm) UPLC column with a guard column for cohort 1, and a Kinexet C18 1.7 μm C18 column for cohort 2. Mobile phases were A H2O and B 2.98% water to acetonitrile containing 0.1% formic acid, and a linear gradient from 0 to 100% for a total run time of 840 s at a flow rate of 0.5 ml min⁻¹ was used. The mass spectrometer was calibrated daily using Tuning Mix ES-TOF (Agilent Technologies) at a 3 ml min⁻¹ flow rate. For accurate mass measurements, lock mass internal calibration used a wink saturated with hexakis (1H,1H,3H-tetrafluoropropoxy) phosphazene ions (Synquest Laboratories, m/z 922.0098) located within the source. Full scan MS spectra (m/z 50–2,000) were acquired in the qTOF and the top-10 most intense ions in a scan were fragmented using collision-induced dissociation at 35 eV for +1 ions and 25 eV for +2 ions in the collision cell. Data-dependent automatic exclusion protocol was used so that an ion was fragmented when it was first detected, then twice more, but not again unless its intensity was 2.5x the first fragmentation. This exclusion method was cyclical, being restarted after every 30 s.

Metabolite annotation. Data were converted to the mZXML format using the Bruker Data Analysis software and uploaded to GNPS98 through the MassIVE server under ID MSV000082457 for cohort 1 and MSV000084988 for cohort 2. Molecular networking was performed as follows: precursor and fragment ion mass tolerance 0.03 Da, minimum cosine score 0.65, minimum matched fragment ions 4, and minimum cluster size 2. GNPS library searching was performed with the same minimum matched peaks and cosine score. All library hits were inspected for quality with the mirror plot feature on GNPS. Under area the curve feature abundances were calculated to produce a metabolome bucket table with the mZMine software99. Parameters were as follows: mass detection (MS/MS noise level of 3), Tolerance P (noise level of 3), Tolerance R (noise level of 3), Tion ratio less than 10. As metaproteome data represent a complex group of proteins that may contain homologues of similar sequence identity, several steps were taken to filter moderate-confidence peptide spectral matches. Including no added restriction enzymes, variable dehydration, acetylation (N-term), oxidation (M) and ubiquitination. The maximum variable post-translational modifications per peptide was set to 3. De novo sequences were filtered to keep only those with an average local confidence above 85%, resulting in 651 PSMS for cohort 1 and 369 PSMS for cohort 2.

For human protein features, label-free quantification was run through PEAKS Studio 8.5 (ref. 100). A 1% FDR cutoff was used, integrating peaks with a 20 ppm mass error tolerance and a 6 min retention time window. Peptides were searched against the human protein database (uniprot.org, accessed 11 May 2017) for identification. Quantification was normalized to the total ion chromatogram.

Comparison of metaproteonomic approaches. Raw mass spectra data were downloaded from faecal proteomic data generated from UC patient samples (N = 25, n = 102) in the IBD multi-omics database7. Data were searched using Proteome Discoverer with the settings described above using a two-step database approach101 and utilizing a generalized human gut metagenome database102. Data from each cohort were analyzed using false discovery rate (FDR) correction and included no added restriction enzymes, variable dehydration, acetylation (N-term), oxidation (M) and ubiquitination. The maximum variable post-translational modifications per peptide was set to 3. De novo sequences were filtered to keep only those with an average local confidence above 85%, resulting in 651 PSMS for cohort 1 and 369 PSMS for cohort 2.

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Meta-omic data analysis. Data analysis was performed in Python (version 3.5), and records of the code are available in corresponding Jupyter Notebooks for this project (https://github.com/knightlab-analytics/sev-ecrity-multomics). Clinical data correlations were performed on UC cohort 1 using the clustermap function of the Seaborn package (https://seaborn.pydata.org). The Implot function of Seaborn was used to display linear relationships between alpha-diversity measurements and disease activity.

16S fastq were split, demultiplexed, trimmed to 150 bp and processed through deblur using QIITA103 (Study ID 11549). A de novo phylogenetic tree was formed for 16S data using the reference hits through QIIME2 (ref. 104) (version 2018.4) commands ‘qiime alignment mafft’ , ‘qiime alignment mask’ , ‘qiime phylogeny build’ and ‘qiime phylogeny build’
fasttree’ and ‘qmc phylogeny midpoint-root’. 16S alpha diversity was generated using QIIME 2 (ref. 7) (version 2019.7) through the command ‘qmc diversity core-metrics-phylogenetic’. Statistical association between disease activity and alpha diversity was conducted using the one-way permutational ANOSIM of the package statsmodule (https://www.statsmodule.org/), accounting for diagnosis and the interaction between patient diagnosis and disease activity. For correlations of each clinical variable with alpha diversity, Kruskal–Wallis tests were performed on categorical variables using the ‘alpha-group-significance’ command in QIIME 2 (ref. 7) (version 2019.7). Quantitative variables were correlated with alpha-diversity measurements using the Linneegreg command from the Python package scipy (https://www.scipy.org/). All alpha-diversity associations were based on 16S data.

Community diversity analysis was performed using the ‘qmc diversity core-metrics’ command in QIIME 2. Statistical analysis of beta-diversity association with disease activity while accounting for the diagnosis of the patients was performed using the `adonis` module in QIIME 2. To facilitate faster analysis of the association of beta diversity and numerous patient variables, QIIME’s `compare_categories.py` function was used for single-variable associations with beta diversity, using ADONIS for quantitative measures and PERMANOVA for categorical measures.

16S taxonomic barplots grouped patients into three categories on the basis of either the partial Mayo activity score for UC patients or the CDIA for CD patients. Patients in the bottom 30% of activity scores were categorized as ‘Low’, patients in a range between 30% and 50% of the highest activity score were categorized as ‘Moderate’ and patients above 50% were categorized as ‘High’. All composition plots for omics data were plotted using the package matplotlib (https://matplotlib.org/).

For omics data were plotted using the package matplotlib (https://matplotlib.org/). Correlations between omics data types were performed using scikit-bio’s Mantel test (http://scikit-bio.org/) and visualized using a seaborn heatmap.

Random forest regressions were performed using QIIME 2 (ref. 7) (version 2018.11) using the sample-classifier regress-sample command. The test size was set to 0.1. Statistics and importance scores for each feature within the 100 independent analyses were used to facilitate comparisons between mass spectrometry datasets, where exact metabolic or protein matches are unfeasible, importance scores were summarized by annotation information about each metabolite or protein. For metaproteomics studies, importance scores were combined when exact protein name and species were found. For metabolomics studies, the importance scores were combined when exact annotations were found by the two annotation methods used and described above (the name of the GNPS spectral library match and the direct parent annotations provided by ClassyFire).

Linear regressions of metagenome, metabolome and metaproteome data on disease activity scores were performed, calculating the Pearson correlation coefficient using the linregress function in the Python package, scipy (https://www.scipy.org/). To identify classes of metabolites correlated with disease activity, the abundance of each metabolite with a direct parent annotation from ClassyFire was averaged, and regression was performed on the average values of metabolite classes. Given the nature of TMT-labelled proteomic data, where protein abundances are frequently normalized to account for differences in the number of missing values in an identical manner to metaproteomic data where missing values are frequently accounted for differences in the number of missing values in an identical manner to metaproteomic data where missing values were ignored and the percentage of missing values in each protein was calculated. When comparing metagenome and metaproteome data, metagenomic data were analysed, accounting for missing values in an identical manner to metaproteomic data where missing values were ignored and the percentage of missing values was filtered out. When performing specific analyses, such as measuring the Pearson r value for the protein names identified in Bacteroides species were plotted in a heatmap showing the summed r value of each protein name within a particular species of Bacteroides. The gene ontology (GO) molecular functions of these proteins were then analysed to group the proteases by activity type.

To identify patient samples containing an overabundance of B. vulgaris proteases, an outlier approach was taken using R studio (v. 1.1.383), utilizing the bagplot function from the aplpack package. After applying a BLASTp analysis (https://www.ncbi.nlm.nih.gov/BLAST/) to the protein sequences of the most significant metaproteomic studies that were assigned to proteins being correlated with disease activity and derived from B. vulgaris or B. dorei proteases, we determined that we could not specify the origin of these proteases beyond being derived from either B. vulgaris or B. dorei. Hence, outlier analysis and later analyses of B. vulgaris proteases in UC patients were performed using both B. dorei and B. vulgaris proteases. For the outlier approach, summed metagenomic abundances of all correlated (>0.3) proteases from B. vulgaris and B. dorei were compared with the summed abundance of metagenomic reads assigned to B. vulgaris and B. dorei. Outliers identified above the best-fit line were classified as Bacteroides protease ‘overprodubers’ while outliers identified below the best-fit line were classified as ‘underproducers’. All other patient samples were categorized as ‘others’. Statistical comparisons of patient endoscopic and disease activity scores between these groups of patients were performed using independent t-tests of unequal variance through the package scypy.

Host protein networks were compiled from serum and facel probe proteomics data from UC cohort 1. Linneegreg correlation values (r) between proteins and disease activity (partial Mayo scores) were used to rank associations. Top-ranked proteins were uploaded to STRING-db18, with associations between proteins determined through default settings, accounting for textmining, experiments, databases, co-expression, fusion/neighbouring and co-occurrence. Networks were next visualized using Cytoscape (version 3.5.1).

The programme iceLogo’s web application100 was used for consensus sequence analysis of de novo peptides identified in UC patients metabolome data. The first and last amino acids from peptides with an average local confidence over 85% were analysed for a background using the percentage scoring correction. For metaproteome consensus sequences, all residues from peptides with over 85% average local confidence were used as background. For human consensus sequences, the precompiled Homo sapiens Swiss-Prot database was used. Peptide fragment origins analysis was performed from the results of PEAKS studio database search described above, summarizing all PSMs assigned to each protein.

Bacterial supernatant protease activity studies. B. vulgaris (ATCC 8482) were grown anaerobically in brain-heart-infusion (BHI, BD) broth supplemented with 5 μg/ml hemin (Sigma) and 0.5 μg/ml vitamin K (Sigma). Overnight supernatant was collected by pelleting cells at 8,000 × g. Supernatant was then 8-fold concentrated at 1,300 × g for 15 min using 10kDa Amicon Ultra 15 filters (Millipore). Concentrated supernatant protease activity was tested using the EnzChek protease activity assay (Invitrogen) after incubation for 24 h at 37°C, measuring fluorescence at 485 nm for excitation and 530 nm for emission. Protease inhibitors were administered at 10% total volume and inhibition was measured on control wells. Proteins were stored and included water-solubilized 4-(2-Aminophenyl)benzenesulfonyl Fluoride (AEBSF, MP Biomedicals), water-solubilized E-64 (Sigma), Dimethylsulfoxide-solubilized GM6001 (EMD Millipore) and Dimethylsulfoxide-solubilized Pepstatin A (MP Biomedicals). After analysis of a preliminary dilution series, maximum inhibition was found for each protease inhibitor at the highest concentration allowed by the solubility of each compound, and these concentrations were used for subsequent studies.

Bacterial supernatant proteomics. B. vulgaris (ATCC 8482) and B. thetaiaotaomicron (B. theta, ATCC 29148), alongside Human Microbiome Project strain 717 B. dorei CLO20T001C5, were grown anaerobically in technical triplicate in BHI broth supplemented with 5 μg/ml hemin (Sigma) and 0.5 μg/ml vitamin K (Sigma). Supernatant was concentrated using 10kDa Amicon Ultra 15 filters (Millipore) and prepared for TMT-mediated LC–LC–MS2/MS3 analysis as described above, with samples compiled into one TMT 10-plex experiment. MS analysis resulted in 219,087 MS/MS spectra that were searched in Proteome Discoverer as described above using uniprot reference proteomes for each strain (www.uniprot.org; proteome identifiers UP000005974, UP000001414 and UP000002861; accessed 24 August 2020). Data processing resulted in a final table of 2,574 quantified protein groups that were analysed as described below.

Stacked barplots of proteome enzyme activity type were generated on the basis of the average relative abundance of each species, subsetting proteins annotated with a KEGG functional category annotation of ‘Enzyme families’ and proteins containing the terms ‘protease’ or ‘peptidase’ in their name, and then summing protein abundances by GO molecular functions. Seaborn barplots were used to display the proteomes most associated with B. vulgaris as determined by subsetting abundance values in the cases and comparing the average signal of each protein in samples from B. vulgaris and B. theta. Venn diagrams of the protein names identified in the reference proteomes for each Bacteroides species were generated using the matplotlib_venn function. Code for the normalization and analysis of the bacterial supernatant proteomics data can be found in the github repository for this project (https://github.com/knightlab-analyses/uc-severity-multimics).

Caco-2 transwell studies. Caco-2 cell transwell studies were performed, as previously described19. Briefly, Caco-2 cells (passage number 13–30; ATCC) were plated into collagen coated 6.5 mm inserts with 0.4 μm pores (Corning). Cells were cultured for 2.5 weeks before bacterial inoculation, changing media every 2 d. A 5% CO2 incubator was changed to 2% O2 and when indicated, Roche cOmplete EDTA-free protease-inhibitor cocktail (Sigma) was dissolved at 1× concentration. TEER was measured before inoculation of bacteria, and measurements at each subsequent timepoint referenced the original TEER measurement before inoculation. Transwell plates were incubated at 37 °C between measurements and allowed to equilibrate to room temperature for 20 min before each TEER measurement. Colony-forming unit estimates were performed through serial dilution of 10 μl media from inside of the transwell insert. Mammalian cell culture media consisted of DMEM with l-glutamine (Corning) with 10% heat-inactivated foetal bovine serum (FBS), 100 μM sodium pyruvate (Corning), 0.75% sodium bicarbonate, 1× insulin-transferrin-selenium (Gibco), 2% human serum albumin (HSA), 1× HEPES, and 1× penicillin/streptomycin (Thermofishic-free media were used during bacterial inoculation, with the same contents except for 2× heat-inactivated FBS.

Bacteroides species used were B. vulgaris (ATCC 8482), B. fragilis (ATCC 25285), B. thetaiaotaomicron (ATCC 29148), B. uniformis (ATCC 8492) and B. ovatus (ATCC 8483). B. dorei was derived from the Human Microbiome Project.
Bacteroides supernatant from B. vulgatus Caco-2 cells were fixed on the transwell membrane at 37 °C for 10 min in bacterial inoculation, cells were fixed and prepared for immunofluorescence. The laser powers used were 1.5%, 2%, 1.5% and 1.5% for the 405 nm, 488 nm, 561 nm and 640 nm lasers, respectively. Image stacks were acquired with the galvo operating in virtual bandpass mode. Image stacks were acquired with the galvo operating in virtual bandpass mode. Image stacks were acquired with the galvo operating in virtual bandpass mode. Image stacks were acquired with the galvo operating in virtual bandpass mode.

The impact of Bacteroides supernatant on TEER was assessed by collecting supernatant from B. vulgatus or B. thetaiotaomicron at mid-log-phase growth, and concentrating and filtering the supernatant using 10kDa Amicon Ultra-15 spin filters (Millipore) and a 0.22 µm filter. The supernatants were concentrated 10-fold and 50-fold, in a supernatant without a filter containing any bacteria was used as an additional control. A protease-inhibitor cocktail (Roche Complete EDTA-free) was added to the media of selected wells a day before the experiment as described above. For these studies, Caco-2 cells were cultured in DMEM (ATCC) with 10% heat-inactivated FBS, 1X MEM non-essential amino acids (Gibco) and 1X penicillin–streptomycin. When non-essential amino acids (Gibco) and 1

10 mM EGTA (Oakwood Chemical), 2 mM MgCl2·6H2O102). Cells were permeabilized for 5 min in PHEM with 0.5% Triton X-100 (Fisher) at room temperature, followed by 3x 5 min washes performed in PHEM containing 0.1% Triton X-100 at room temperature. Blocking was performed for 30 min in 1 ml AbDil (150 mM NaCl+, 20 mM Tris-HCl (pH 7.4) (JT Baker), 0.1% Triton X-100, 2% bovine serum albumin (Gemini BioProducts)) at room temperature. Primary antibodies for Occludin (ThermoFisher, 33–1500, 0.5 µg/mL−1) and ZO-1 (ThermoFisher, 61–7300, 1.5 µg/mL−1) were added into AbDil and left in a humidified chamber overnight at 4 °C. Cells were washed 4x in PHEM containing 0.1% Triton X-100 for 5 min at room temperature. Secondary antibodies, rhodamine red donkey anti-rabbit (Jackson ImmunoResearch, 711–295–152) and AlexaFluor 488 donkey anti-mouse (Jackson ImmunoResearch, 715–545–150) were diluted to 3 µg/mL−1 in AbDil containing a 1:1,000 dilution of Phallodin-iFluor 647 (Abcam, ab167769) and 1 µg/mL−1 DAPI (ThermoFisher). Secondary antibodies were incubated for 1 h at room temperature in a humidified chamber, followed by 3 washes in PHEM containing 0.1% Triton X-100 for 5 min at room temperature. Finally, cells were rinsed in PHEM, removed from the transwell insert and fixed onto microscope slides for imaging.

Cells were imaged using a Nikon A1R HD confocal microscope with a four-line (405 nm, 488 nm, 561 nm and 640 nm) LUM-N laser system and the D4 UU detector operating in virtual bandpass mode. Image stacks were acquired with the galvo scanning mode on both confocals, and Z-steps of 0.2 µm. To avoid cross-talk between channels, Z-stacks of the DAPI and rhodamine red channels were acquired first, followed by the AlexaFluor 488 and Phallodin-iFluor 647 channels. The laser powers used were 1.5%, 2%, 1.5% and 1.5% for the 405 nm, 488 nm, 561 nm and 640 nm lasers, respectively.

Cell morphology was analysed in representative images using protocols outlined previously105. Images were processed in ImageJ (https://imagej.nih.gov/ij/) using the Morpholplugin105. In brief, images were converted to binary, image borders were extended and morphological segmentation was performed. Images were outlined, dilated and analysed for circularity. Circularity values were plotted and significance of differences between groups was assessed using independent t-tests through the package scipy.

Monoclonalization studies. Germfree IL10−/− mice (7 male, 4 female) (B6.129P2 Il10−/−); Jackson Laboratory) were bred and housed in flexible film isolators until 6–8 weeks of age, and transferred to micro-isolator cages and maintained with autoclaved food (Lab Diet), bedding and water supplemented with gentamicin at 100 µg/mL−1. Mice were mono-associated with gentamicin-resistant B. vulgatus. Bacterial cells were washed twice and resuspended in sterile PBS before oral gavage. Both groups of IL10−/− mice were orally gavaged with B. vulgatus. In select experiments, drinking water was supplemented with a 1X concentration of Roche Complete EDTA-free protease-inhibitor cocktail (Sigma). Mice were monoclonized for 10 weeks. Statistical significance of differences in measurements in monoclonization studies was determined using unpaired t-tests conducted in GraphPad Prism (Version 7.0b). All procedures were performed in accordance with the approved protocols using IACUC guidelines of UCSF.

Histological procedure and scoring of monoclonized mouse studies. Colonies were removed, flushed with cold PBS, cut longitudinally and prepared as swiss rolls. Formalin-fixed 5 µm paraffin-embedded tissue sections were hematoxylin and eosin (H&E) stained and slides were scanned with a NanoZoomer slide scanner (Hamamatsu). Tissue sections were investigated using IBDview software (Hamamatsu) in a blinded fashion. Colitis scores were assessed using a semi-quantitative score as previously described105.

Flow cytometry and intracellular cytokine staining. Mesenteric lymph nodes of IL10−/− mice were processed by dissociating tissues through a 100 µm cell strainer (BD Falcon). Single-cell suspensions were stained for 30 min at 4 °C with AbDil, 1× LIVE/DEAD Fixable Aqua (1:5000), 1× DAPI 450-conjugated anti-mouse CD4 (eBioscience, 48–0042-82, clone RM4-5, 1:400 dilution) and APC-conjugated anti-mouse CD25 (eBioscience, 17–0251-82, clone PC61.5, 1:400 dilution) was used as a dump channel. Cells were blocked for non-specific binding to Fc receptors with a combination of TrueStain S100 (eBioscience, D19, 115554, clone 6D5, 1:400 dilution), APC-eFluor780-conjugated anti-mouse IFNγ (eBioscience, 47–7311-82, clone XMG1.2, 1:400 dilution), PE-Cy5.7-conjugated anti-mouse IL-17A (eBioscience, 25–7177-82, clone eBio17B7, 1:400 dilution) and PE-conjugated anti-mouse IL10 as control (eBioscience, 12–7101-82, clone JES5-16E3, 1:400 dilution). Cells were acquired on the Attune NxT flow cytometer (ThermoFisher) and data were analysed on FlowJo (version 10.6.2).

Faečal microbiota transplantation. Germfree C57BL/6 Il10−/− male mice (C57BL/6NTac-Il10−/−); Taconic model GF-16096) were maintained in isolated ventilated isolocages (Techniplast)106. At 6–8 weeks of age, mice were orally administered (by gavage) of faecal suspension from three patients with overabundant B. vulgatus (Bacteroides) (sample identifiers H5, H7 and H9) and three patients without overabundant B. vulgatus (Bacteroides) (sample identifiers L3, L15 and L19). Transplanted mice were group-housed (n = 3) in isolated ventilated cages, and fed autocultured Purina Rodent Chow 5021. Each study was conducted with mice isolated from the same patient group and one without a 1X concentration of Roche Complete EDTA-free protease-inhibitor cocktail (Sigma) administered in the drinking water of the mice throughout the colonization. Mice were housed at Georgia State University (Atlanta, Georgia, USA) or at Cochin Institute (Paris, France) in a controlled environment (12 h day/night cycle, lights off at 19:00, 20 ± 2 °C, 48 ± 6% humidity). At the terminal timepoint, mice were weighed and euthanized, and tissue was collected for further analysis. Statistical strength of measurements taken from transplanted mice were assessed by ordinary one-way ANOVA using GraphPad Prism (version 7.0b), with adjusted P values reported accounting for multiple comparisons.

H&E staining of faecal transplantation colonic tissue and histopathologic analysis. Mouse colons were fixed in Carnoy solution and then embedded in paraffin. Tissues were sectioned at 5 µm thickness and stained with H&E using a NanoZoomer slide scanner (Hamamatsu). Tissue sections were investigated using NDP .2 viewer scanner (Hamamatsu). Tissue sections were investigated using NDP .2 viewer scanner (Hamamatsu). Tissue sections were investigated using NDP .2 viewer scanner (Hamamatsu).

Statistical analysis. Statistical analysis was performed using RStudio (version 3.6.0). The level of significance was set at P < 0.05. Normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene’s tests, respectively. Data are expressed as mean ± SD or median (IQR). Statistical tests were selected based on the data distribution and included the Student’s t-test, Mann-Whitney test, Wilcoxon rank-sum test, Kruskal-Wallis test and linear regression analysis. Analyses were performed using GraphPad Prism (version 7.0b).
Quantification of faecal lipocalin-2 (Lcn-2) by ELISA. For quantification of faecal Lcn-2 by ELISA, frozen faecal samples were reconstituted in PBS to a final concentration of 100 mg ml^{-1} and vortexed for 20 min to get a homogeneous faecal suspension. These samples were then centrifuged for 10 min at 14,000 × g at 4 °C. Clear supernatants were collected and stored at −20°C until analysis. Lcn-2 levels were estimated in the supernatants using Duoset murine Lcn-2 ELISA kit (R&D Systems) utilizing the colorimetric peroxidase substrate tetramethylbenzidine, and optical density (OD) was read at 450 nm (SpectraMax ABS Plus microplate reader, Molecular Device).

Metaproteomic analysis of mouse faecal samples in transplant study. At the end of the 8-week colonization, faecal samples were collected and snap-frozen for further analysis. Metaproteomic sample preparation and acquisition were performed as described above for TMT-mediated LC–LC–MS/MS analysis of faecal samples from mice within cages associated with patient samples H19 and L3. For database search of mass spectra, a custom database was generated using the metagenomic database generation workflow described above on sequencing data from the UC patient donors. Here, to track the origin of protein sequences, H19 and L3 reads were assembled and searched for coding regions separately. Open-reading frames from each patient sample were combined and annotated as described above, resulting in a 70 mb fasta file containing 225,056 open-reading frames. MS analysis resulted in 500,120 spectra that were subsequently searched in Proteome Discoverer against the custom database and the uniprot mouse reference database (uniprot.org, accessed 3 September 2020). After data processing, a final table of 12,603 quantified protein groups was analysed as described below.

Stacked barplots were created as described above, plotting the proportion of the metaproteogenome signal of each sample dedicated to each genus. Data were further subset to contain only enzymes and proteases, and stacked barplots were created, stacking each sample by species-level annotations. Further subsetting the enzymes and proteases to only those from B. vulgatus, the abundance of each sample was plotted in stacked barplots stacked by GO molecular function. Student’s t-tests comparing protein abundances of samples from mice receiving patient samples H19 and L3 were performed in scipy using unequal variance. t-test P values were then combined with the fold-change differences to rank associated proteins via the Pi score statistic, as previously described. Top proteins associated with the H19 samples were then presented in a barplot using the seaborn Python package.

Statistics and reproducibility. Multi-omic data were collected and analysed in two independent experiments to increase the likelihood of reproducibility. Sample sizes from each cohort were largely driven by technical and financial constraints as opposed to power analysis, but our sample sizes are similar to those reported in previous publications. Several samples were removed after the identification of bacterial blooms or red blood cell contamination as indicated in the metadata. Experiments were randomized during sample processing.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Metabolomic data, proteomic data and additional supplementary files for reanalyzing the data collected here are available online at https://massive.ucsd.edu (Cohort 1 proteomics and metabolomics study ID MSV000082094, Cohort 2 study ID MSV000086509, Cohort 2 metabolomics study ID MSV000084908). Proteomic data and supplementary files for reanalyzing data collected from the faecal transplant study and Bacteroides supernatant are under MassIVE identifiers MSV000065510 and MSV000065611, respectively. Genomic data has been uploaded through EBI https://www.ebi.ac.uk/ena under the study identifiers PRJEB42151 for Cohort 1 and PRJEB42155 for Cohort 2. Comparisons with data generated in this study were also made with proteomics data downloaded from the IBD multi-omics database (https://ibdmdb.org/tunnel/public/HMP2/Proteomics/1633/rawfiles). Databases used in this study include UniRef50 (https://www.uniprot.org/downloads), the human proteome (https://www.uniprot.org/proteomes/UP000005640), mouse proteome (https://www.uniprot.org/proteomes/UP000005898), B. vulgatus proteome (https://www.uniprot.org/proteomes/UP000002861), B. theta proteome (https://www.uniprot.org/proteomes/UP000001411), B. rossiae proteome (https://www.uniprot.org/proteomes/UP000005974), a microbial genome database (https://biocore.github.io/wol/) and a human gut microbiome database (https://db.cngb.org/microbiome/genericatolog/genericatolog-human/). Source data are available for in vitro and in vivo experiments. Source data are provided with this paper.

Code availability
The code used in the analysis and visualization of data is available at https://github.com/knightlab-analyses/ac-severity-multimics.
Extended Data Fig. 1 | Study Design and Database Generation. Paired faecal and serum samples were collected from 40 patients with varying severity of Ulcerative Colitis. A separately analyzed cohort of faecal samples was also collected on 210 samples with 73 UC, 117 CD and 20 healthy controls. Samples were processed for proteomics using a Tandem Mass Tag multiplexing workflow. Faecal samples were also subjected to both 16S and shotgun metagenomic analyses for microbial composition and gene quantification respectively. In parallel, a metabolomics workflow was performed on faecal samples where collected MS2 spectra were analyzed for both metabolites and peptides in two separate computational pipelines. A custom database was compiled from the metagenome of faecal samples to mediate a comparative analysis between shotgun metagenomic and metaproteomic data sets. This eliminated database dependent bias and the shared reference was used for estimating copy number.
Extended Data Fig. 2 | A multiplexing approach improves the depth and sparsity of metaproteomics data. a, Multiplexed metaproteomic methods increase the total number of proteins quantified. Shown is a bar graph showing the total number of proteins identified when using identical database methodology between the 102 UC samples from the IBD multiomics database, the 40 UC samples from cohort 1 of this study, and the 205 samples from cohort 2 of this study. b, Multiplexed metaproteomic methods improve the number of proteins quantified per sample. Displayed are the mean +/− SD of the proteins identified per sample from studies shown in (a). Data derived from n = 102, 40, 205 biologically independent samples as described for (a). One-way ANOVA p-values adjusted for multiple comparisons are shown (P < 0.0001). c, Multiplexed metaproteomic methods decrease the sparsity of metaproteomic studies. The percentage of missing quantification values for proteins in each data set is shown.
Extended Data Fig. 3 | Characterizing uneven samples. a, Alpha diversity (using Pielou’s evenness metric) by disease activity as shown in Fig. 1b, but highlighting classification of samples as uneven when below Pielou Evenness of 0.5. Best-fit linear regression lines with 95% confidence intervals are shown and an R² statistic is reported from an ordinary least-squares regression using the formula (Disease Activity + Diagnosis + Disease Activity:Diagnosis). b, 16S beta-diversity is strongly influenced by community evenness and whether the most abundant 16S feature was from the family Enterobacteriaceae. c, Characterizing the most abundant 16S features. Each sample was classified as either “Uneven” (Pielou Evenness < 0.5) or “Other” as shown in (a). Abundances of each amplicon sequence variant were summed by their highest resolution taxonomic annotation and the most abundant feature of samples are represented in a donut plot. The inside ring represents the fractional composition of each patient subgroup and the outside rings represents the number of patients within each subgroup whom share a similar most abundant feature. Less common features for each patient subgroup are counted as “Other”.
Extended Data Fig. 4 | Comparison of genera annotations from genes and proteins correlated to disease severity. The genus composition of genes and proteins correlated to disease activity were compared with different levels of sparsity as a requirement for being deemed “correlated”. Stacked bar charts summarize the number of genes or proteins from the 10 most common genus assignments when correlated to either partial Mayo severity in UC cohorts or CDAI in CD patients. Only genes or proteins with |r| > 0.3 from linear regression were included. a, Genus composition of significant positively and negatively correlated genes from the MG with no sparsity requirement. b, Genus composition of significantly positively and negatively correlated proteins from the MP with no sparsity requirement. c, Genus composition of associated proteins as in Fig. 3a, but without removing host proteins (genus Homo). d, Genes correlated to disease activity from the MG when filtering out genes appearing in less than 40% of patients within each category. e, Summary of comparing the portions of positively and negatively correlated genes and proteins from each patient cohort when examining the top 10 genera identified in the MG. This analysis is analogous to Fig. 3b, but displaying the top MG genera.
Extended Data Fig. 5 | Comparison of genera and functional annotations from genes and proteins correlated to disease severity in CD subtypes. 

**a.** Genus level barcharts of significantly correlated genes or proteins stratified by CD subtype. The genus composition of genes and proteins from either the MG or MP were correlated to CDAI and shown in stacked bar charts. Only genes or proteins with $|r| > 0.3$ from linear regression were included, and the top 10 genera are displayed with other genera compiled into an “Others” category.

**b.** CD subtypes genus level association comparison. The portion of genes or proteins correlated with disease activity from (a) are plotted by a Log10 comparison between the proportion of positive to negative correlations.

**c.** CD subtypes functional association comparison. This analysis is analogous to (b) but summarizing the associations to KEGG functional category annotations in the MP.
Extended Data Fig. 6 | Patients with overproduction of *Bacteroides vulgatus* proteases have increased endoscopic and histological severity. a, Bacteroides protease production corresponds to increased endoscopic severity. The disease activity of overproducers, underproducers, and other patients are individually plotted over boxplots. Two-tailed, t-test p-values are displayed above the boxplots. Sample sizes include $n=16$, $n=14$ and $n=71$ for overproducers, underproducers and others respectively. Boxplots are defined by the median, quartiles and 1.5x inter-quartile range. b, *Bacteroides* protease production corresponds to a patient population with a decreased proportion of patients in histological remission. Each UC patient sample was categorized by *Bacteroides vulgatus* protease production category and the percent of patients in histological remission is shown in a bargraph with the number of samples in each category displayed above each bar. Histological remission is defined here as Geboes Grade $3=0$. 
Extended Data Fig. 7 | Peptide fragments are increased in active UC patients and Bacteroides protease enriched patients. a, Comparison of peptide fragments identified in patients with varying abundance of Bacteroides proteases. Overproducers from UC cohort 1 had increased peptide fragments in comparison to other patients (Two-tailed t-test $P = 3.5E-2$). Data was derived from $n = 8, 9, 23$ UC cohort 1 samples and $n = 6, 6, 49$ UC cohort 2 samples from patients classified as underproducer, overproducer and other respectively. b, Peptide termini indicate unique proteolysis of human and microbial proteins. The frequency of each amino acid within the N and C terminus of human and de-novo peptides was compared to either the human proteome or the total amino acid content of de novo peptides. The Y-axis represents the percent difference of each residue and the letter indicates the amino acid associated with the difference. The N and C terminus are shown separately and each residue is colored by chemical property (Green = polar, Black = Hydrophobic, Red = Acidic, Blue = Basic, Purple = Neutral). c, Peptide fragment identification comparison by disease activity in UC cohort 1. Boxplots with a two-tailed t-test p-value is shown ($P = 4.7E-3$). Data was derived from $n = 18, 12, 10$ patient samples with low moderate or high disease activity respectively. d, Peptide fragment identification comparison by disease and disease activity state for cohort 2 samples. Boxplots are shown with overlaid two-tailed t-test p-values. Data was derived from $n = 19$ healthy controls, $n = 39, 30, 12$ UC samples, and $n = 64, 30, 8$ CD samples from patients of low, moderate and high activity respectively. Boxplots in (a,c,d) are defined by the median, quartiles and 1.5x inter-quartile range.
Extended Data Fig. 8 | Determining the impact of Bacteroides species on TEER using co-culture, supernatants and protease inhibitors. 

**a.** Bacteroides vulgatus and Bacteroides dorei, but not other Bacteroides species disrupt Caco-2 epithelial barriers. Barplots are showing the mean and standard deviation of the change in TEER at different time points. Data was derived from $n=3$ independent cultures collected over $n=2$ independent experiments.

**b.** Growth curves of Bacteroides vulgatus with protease inhibitors under different growth conditions. OD600 was measured at indicated time points and a non-linear fit is shown. Data was derived from $n=3$ independent cultures collected over $n=1$ independent experiments.

**c.** Supernatants from Bacteroides in mid-log phase growth do not significantly impact TEER. B. vulgatus and B. theta were grown to mid-log phase, and their supernatants were concentrated and added to Caco-2 monolayers. TEER was measured at the initial time-point and compared to TEER measured after 1, 4, and 8 h of incubation. Plotted are the mean and SEM from $n=3$ independent experiments each representing the mean of $n=3$ independent wells/experiment ($n=4$ wells/experiment for B. vulgatus group). No significant differences were found at any timepoint.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Additional measurements from faecal transplant experiments. a-f Barplots showing the mean +/− SD of macroscopic organ measurements from faecal transplant of UC patients samples in IL10−/− mice with or without administration of a protease inhibitor. Dots represent one mouse, with each group representing results from 3 UC patient faecal samples with each sample given to 3 co-housed mice. Measurements include final weight of the mice (a), colon weight (b), ratios of the colon weight to length (c), caecum weight (d), fat pad weight (e), liver weight (f). g-h Barplots showing the mean +/− SEM for the concentration of an intestinal inflammatory marker, fecal lipocalin2 (g), and amount of 16S rRNA in the spleen of mice for an estimate of the splenic bacterial load (h). Each dot in g-h represents the mean of n = 3 mice transplanted with the same UC faecal sample (with the exception of a mean from n = 2 mice for one patient sample in the Abundant Proteases + Inhibitor Cocktail group) from n = 2 independent experiments. i, Metaproteome genera composition of mice transplanted with UC faecal samples. Fecal samples taken at 8-weeks from mice transplanted with one high protease containing sample (H19) and one control patient sample (L3) were analyzed by mass spectrometry based metaproteomics. Stacked barplots are shown for each mouse displaying the proportion of protein signal derived from the most common genera. j, Molecular function of B. vulgatus proteases identified in mice receiving UC faecal samples. The relative abundance of each B. vulgatus protease is shown in stacked barplots grouped by the Gene Ontology molecular function associated with each protein. k, Top B. vulgatus or B. dorei proteases associated with the faecal samples of mice receiving the H19 sample. Each protein is ranked by pi-score, which combines two-sided t-test p-values and the fold-change difference between all H19 and L3 samples. l, Cumulative protease comparisons. A venndiagram is shown comparing the protein names of B. vulgatus or B. dorei proteases from four independent proteomics experiments performed in this study. A full list of the Bacteroides proteases identified in this analysis can be found in Supplementary Table 4.
Extended Data Fig. 10 | Working hypothesis. The results of our study may indicate that certain species from the genus Bacteroides, particularly those recently reclassified under the genus Phocaeicola (for example Bacteroides vulgatus & Bacteroides dorei), may be implicated in the transition from remission to active disease in UC. We hypothesize that a stressor in the UC gut such as nutrient deprivation or cell-to-cell competition may increase protease production, and a switch in the utilization of carbohydrates to proteins as a nutrient source. Some of these proteases may be involved in the disruption of the epithelial barrier, allowing an influx of innate immune cells which further exacerbate disease.
Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
Primary data sets were collected for this study and not published elsewhere. Comparative studies were made with data downloaded from https://ibdmdb.org/tunnel/public/HMP2/Proteomics/1633/rawfiles. Flow cytometry data was collected with FlowJo (Version 10.6.2)

Data analysis
Custom code was generated to analyze the data located at https://github.com/knightlab-analyses/uc-severity-multiomics. Data tables required for reproduction of the analyses are available through repositories with access instructions provided on github.

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Metabolomic data, proteomic data and additional supplementary files for reanalyzing the data collected here are available online at https://massive.ucsd.edu. (Cohort 1 proteomics & metabolomics study ID MSV000082094, Cohort 2 study ID MSV000086509, Cohort 2 metabolomics study ID MSV000084908). Proteomic data and supplementary files for reanalyzing data collected from the fecal transplant study and Bacteroides supernatant are under MassIVE identifiers MSV000086510 and MSV000086511 respectively. Genomic data has been uploaded through EBI https://www.ebi.ac.uk/ena under the study identifiers PRJEB42151 for Cohort 1 and PRJEB42155 for Cohort 2. Comparisons with data generated from this study were also made with proteomics data downloaded from the IBD multi-
Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size: Sample sizes from each cohort were largely driven by technical and financial constraints as opposed to power analysis, but our sample sizes are similar to those reported in previous publications.

Data exclusions: There were no samples removed from metaproteomic, metagenomic, or metabolomic analyses. For PCoA of patient serum, 7 serum samples with contaminating blood were removed as described in the manuscript's methods. For 16S analyses from the second cohort, several samples were excluded after finding bacterial blooms. The code on github details the exclusion process.

Replication: Validation of -omic data was confirmed using two separately collected cohorts. Hypotheses from omics data were validated using in vitro and in vivo follow-up studies. For in vitro studies three independent experiments were performed with 3-4 technical replicates (detailed in manuscript). In vivo studies were performed in two separate studies. Replication of in vivo studies was successful. Replication of in vitro studies was largely successful, though protease inhibition treatment was less significant in restoring the Bacteroides vulgatus phenotype when using Caco-2 cells passaged beyond what was listed in our methods (passages > 30).

Randomization: Our cohorts of patients were selected from a variety of factors, largely based on endoscopic scores, to create a distribution of severity scores. Randomization was used throughout -omic data collection to remove run to run variation. In vitro studies utilized randomization in the experimental outline so that resistance measurements for each group were being taken from different plates and in a randomly distributed order.

Blinding: We used blinding whenever possible. For sample processing and data collection, we were blind to variables associated with each sample. When performing data analysis of multiomics data to identify correlations to severity, it would not be possible to find correlations without knowing patient information. For in vitro and in vivo studies, the researchers were not blind to the study conditions they were evaluating as this was infeasible and would require additional trained staff to separately setup and evaluate the results.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a                              | n/a     |
| ☐ ☒ Antibodies                   | ☒ ChiP-seq |
| ☐ ☒ Eukaryotic cell lines        | ☒ Flow cytometry |
| ☒ Palaeontology and archaeology  | ☒ MRI-based neuroimaging |
| ☒ Animals and other organisms    |         |
| ☐ Human research participants    |         |
| ☒ Clinical data                  |         |
| ☐ ☒ Dual use research of concern |         |

Antibodies

Antibodies used: Microscopy: Occludin (Thermo, catalog number 33-1500, 0.5 ug/mL) and ZO-1 (Thermo, catalog number 61-7300, 1.5 ug/mL), Rhodamine Red Donkey Anti-Rabbit (Jackson ImmunoResearch, Code Number 711-295-152), and Alexa Fluor 488 Donkey Anti-Mouse (Jackson ImmunoResearch, Code Number 715-545-150), 1:1000 dilution of Phalloidin-iFluor 647 (abcam, ab176759).

Flow cytometry: eFluor 450-conjugated anti-mouse CD4 (eBioscience, 48-0042-82, clone R4-5, 1:400 dilution), APC-conjugated anti-mouse CD25 (eBioscience, 17-0251-82, clone PC61.5, 1:400 dilution), BV510-conjugated anti-mouse CD19 (BioLegend, 115545, clone 6D5, 1:400 dilution), TruStain FcX anti-mouse CD16/CD32 (BioLegend, 101319, clone 93, 1:400 dilution), FITC-conjugated anti-mouse Foxp3 (eBioscience, 11-5773-82, clone FJK-16s, 1:400 dilution), APC-eF780-conjugated anti-mouse IFNγ (eBioscience,
Validation

Antibodies were validated for microscopy as showed in Supplementary Figure 5. Antibodies for flow cytometry were not validated in house but the manufacturer verified the antibodies bind to each antigen using cell treatment.

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | ATCC |
|---------------------|------|
| Authentication      | Cells were not authenticated beyond ATCC. |
| Mycoplasma contamination | Manufacturer confirmed the cell line was free of mycoplasma detection. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used in this study. |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | For fecal transplant studies; Germ-free C57BL/6 IL10−/− male mice (C57BL/6NTac-Ili10em8Tac; Taconic model GF-16006), 5-6 weeks of age, maintained in isolated ventilated cages (IsoCages (Techniplast, West Chester, PA, USA). For monocolonization studies; Germ-free IL10−/− (7 male, 4 female) mice (B6.129P2-Ili10tm1Cgn/J; Jackson Laboratory), 6-8 weeks of age, maintained in micro-isolator cages. |
| Wild animals       | No wild animals were used in this study |
| Field-collected samples | No field-collected samples were used in this study |
| Ethics oversight   | Monocolonization experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California San Diego. Fecal transplantation experiments were done in accordance with institutional approval from Georgia State University (Atlanta, Georgia, USA) and Cochin Institute (Paris, France) under institutionally approved protocols (IACUC # A18006 and APAFIS#24788-2019102806256593 v8). All studies were conducted in accordance with NIH guidelines for the Care and Use of Laboratory Animals. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

| Population characteristics | Biobanked patient samples from a cohort with consent to longitudinal data collection on patient demographics (age, gender, ethnicity), disease characteristics (prior surgeries, disease-related complications, phenotype classification according to Montreal sub-classifications), current and prior treatments (corticosteroids, immunomodulators, biologics), and clinical disease activity (patient reported outcomes using the partial Mayo score and endoscopic scores). Cohort 1 consisted of 40 Ulcerative Colitis patients with a median age of 38.5, 11 were female, 30 were caucasian. Cohort 2 consisted of 73 Ulcerative Colitis patients, 117 Crohn’s disease patients and 20 healthy volunteers. UC patients from cohort 2 were a median age of 47, with 52 females, 48 were caucasian. CD patients had a median age of 35, 53 were female and 94 were caucasian. |
| Recruitment        | UC patients were selected from a convenience sampling biobank at the University of California at San Diego (UCSD: PI Dulai). |
| Ethics oversight   | UCSD Human Subjects protocol or exemption number(s) that covers this project: 150675 |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.
### Methodology

| Sample preparation | Mesenteric lymph nodes from mice were processed by dissociating tissues through a 100 um strainer. |
|--------------------|--------------------------------------------------------------------------------------------------|
| Instrument         | Attune NxT Flow Cytometer (Thermo Fisher, 4-Laser V4, 14 color system)                           |
| Software           | FlowJo (version 10.6.2).                                                                         |
| Cell population abundance | This does not apply because cells weren’t sorted.                                             |
| Gating strategy    | In the starting cell population, lymphocytes were identified by gating on cells with low forward scatter (FSC) and low side scatter (SSC). Height and area parameters for both FSC and SSC were used to exclude doublets. Live cells were identified within the singlet population. B cells were excluded by including a CD19 dump channel in the same channel as the viability dye. CD4+ cells were identified within the live cell population. Within the CD4+ population, Th1, Th17 and Treg populations were identified using IFNg+, IL-17A+, and Foxp3+ gates, respectively. Boundaries between "positive" and "negative" staining cell populations were defined based on single-stain controls. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.