Artificial intelligence-rationalized balanced PPARα/γ dual agonism resets dysregulated macrophage processes in inflammatory bowel disease

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A computational platform, Boolean network explorer (BoNE), has recently been developed to infuse AI-enhanced precision into drug discovery; it enables invariant Boolean Implication Networks of disease maps for prioritizing high-value targets. Here we used BoNE to query an Inflammatory Bowel Disease (IBD)-map and prioritize a therapeutic strategy that involves dual agonism of two nuclear receptors, PPARα/γ. Balanced agonism of PPARα/γ was predicted to modulate macrophage processes, ameliorate colitis, ‘reset’ the gene expression network from disease to health. Predictions were validated using a balanced and potent PPARα/γ-dual-agonist (PAR5359) in Citrobacter rodentium- and DSS-induced murine colitis models. Using inhibitors and agonists, we show that balanced-dual agonism promotes bacterial clearance efficiently than individual agonists, both in vivo and in vitro. PPARα is required and sufficient to induce the pro-inflammatory cytokines and cellular ROS, which are essential for bacterial clearance and immunity, whereas PPARγ-agonism blunts these responses, delays microbial clearance; balanced dual agonism achieved controlled inflammation while protecting the gut barrier and ‘reversal’ of the transcriptomic network. Furthermore, dual agonism reversed the defective bacterial clearance observed in PBMCs derived from IBD patients. These findings not only deliver a macrophage modulator for use as barrier-protective therapy in IBD, but also highlight the potential of BoNE to rationalize combination therapy.
Inflammatory bowel disease (IBD) is an autoimmune disorder of the gut in which diverse components including microbes, genetics, environment, and immune cells interact in elusive ways to culminate in overt diseases. It is also heterogeneous with complex sub-disease phenotypes (i.e., strictures, fistula, abscesses, and colitis-associated cancers). Currently, patients are offered anti-inflammatory agents that have a ~30–40% response rate, and 40% of responders become refractory to treatment within one year. Little is known to fundamentally tackle the most widely recognized indicator/predictor of disease relapse i.e., a compromised mucosal barrier. Homeostasis within this mucosal barrier is maintained by our innate immune system, and either too little or too much reactivity to invasive commensal or pathogenic bacteria, is associated with IBD. Although defects in the resolution of intestinal inflammation have been attributed to altered monocyte–macrophage processes in IBD, macrophage modulators are yet to emerge as treatment modalities in IBD.

We recently developed and validated an AI-guided drug discovery pipeline that uses large transcriptomic datasets (of the human colon) to build a Boolean network of gene clusters that represent Boolean implication relationships (BIRs) that invariably hold true in every dataset within the cohort. Once built, the network is queried using machine learning approaches to identify in an unbiased manner which clusters most effectively distinguish healthy from diseased samples and do so reproducibly across multiple other cohorts (906 human samples, 234 mouse samples). Gene-clusters that maintain the integrity of the mucosal barrier emerged as the genes that are invariably downregulated in IBD, whose pharmacologic augmentation/induction was predicted to reset the network. These insights were exploited to prioritize one target, choose appropriate pre-clinical murine models for target validation, and design patient-derived organoid models (Fig. 1; Step 0). Treatment efficacy was confirmed in patient-derived organoids using multivariate analyses. This AI-assisted approach provided an epithelial barrier-protective agent in IBD and predicted Phase-III success with higher accuracy over traditional approaches.

Here we use the same AI-guided drug discovery pipeline to identify and validate a macrophage modulator that is predicted to restore mucosal barrier and homeostasis in IBD (Fig. 1; Steps 1). Using primary peritoneal macrophages and specific agonists and antagonists, we reveal the mechanism(s) of action that enable balanced agonists of this pair of nuclear receptors, PPARα/γ, to reverse some of the fundamental imbalances of the innate immune system in IBD, such that immunity can be achieved without overzealous inflammation (Fig. 1; Step 2). We demonstrate the accuracy and predictive power of this network-rationalized approach and reveal the efficacy of balanced-dual agonists of PPARα/γ in two pre-clinical murine models (Fig. 1; Step 3) and in patient-derived PBMCs (Fig. 1; Step 4).

**Results**

**Development of a web-based platform for generating a ‘target report card’**. We first developed an interactive, user-friendly web-based platform that allows the querying of our Boolean network-based-IBD map with the goal of enabling researchers to pick high-value targets (Fig. 1; Step 0; Supplementary Fig. 1). The platform generates a comprehensive automated report containing actionable information for target validation, a ‘target report card’, which contains predictions on five components (Fig. 2a): (i) Impact on the outcome of IBD in response to treatment, which shows how levels of expression of any proposed target gene(s) relates to the likelihood of response to therapies across diverse cohorts; (ii) Therapeutic index, a computationally generated index using Boolean implication statistics which provides a likelihood score of indicate whether pharmacologic manipulation of the target gene(s) would lead to success in Phase III clinical trials; (iii) Appropriateness of preclinical mouse models, a component that indicates which murine models of colitis show the most significant change in the target genes (and hence, likely to be best models to test the efficacy of any manipulation of that target); (iv) Gender bias, a component that indicates whether the gene is differentially expressed in IBD-afflicted men versus women; and (v) Target tissue/cell type specificity, which shows the likely cell type where the target is maximally expressed, and hence, the cell type of desirable pharmacologic action. Details of how therapeutic index is computed are outlined in Methods and in Supplementary Fig. 2; it is essentially a statistical score of how tightly any proposed target gene(s) associates with FDA-approved targets versus those that failed and serve as an indicator of the likelihood of success. Similarly, details of how cell type of action is computer are outlined in Methods and in Supplementary Fig. 3.

**PPARα/γ dual agonists are predicted to be effective barrier-protective agents in IBD**. Previous work had identified a little over 900 genes in 3 clusters (Clusters #1-2-3 within the IBD map; Fig. 1; Step 0; Supplementary Fig. 1a, b) as potentially high-value targets, all of which were invariably downregulated in IBD-affected colons. Reactome analyses showed that epithelial tight junctions (TJs), bioenergetics, and nuclear receptor pathway (PPAR signaling) related genes that are responsible for colonic homeostasis are the major cellular processes regulated by these genes (Supplementary Fig. 1b). Downregulation of genes in clusters #1-3 was invariably associated also with an upregulation of genes in clusters #4-5-6; reactome analyses of the latter showed cellular processes that concern immune cell activation, inflammation, and fibrosis, which are hallmarks of IBD (Supplementary Fig. 1b). Of the druggable candidates within C#1-2-3, 17 targets were identified as associated with GO biological function of ‘response to stress’/‘response to stimuli’. Targeting one of the 17 targets, PRRKAB1, the subunit of the heterotrimeric AMP-kinase engaged in cellular bioenergetics and stress response successfully restored the gut barrier function and also protected it from collapse in response to microbial challenge. Here, we prioritized two more of those 17 targets, PPARA and PPARG, which encode a pair of nuclear receptors, PPARα and PPARγ, respectively. These two stress/stimuli-responsive genes are equivalent to each other and to PRRKAB1, and like PRRKAB1, are invariably downregulated in all IBD samples (Supplementary Fig. 1b–d). PPARA is in cluster #2 and PPARG is in cluster #3 (Supplementary Fig. 1b). They both were located on the two major Boolean paths associated with epithelial barrier and inflammation/fibrosis (Supplementary Fig. 1b). Together, these findings imply three things: (i) that PPARA and PPARG are simultaneously downregulated in IBD; (ii) that such downregulation is invariably associated with inflammation, fibrosis, and disruption of the epithelial barrier, and (iii) that simultaneous upregulation of PPARA and PPARG with agonists may restore the gut barrier. The last point is particularly important because Ppara/γ agonists are known to augment the expression of PPARA and PPARG, and depletion of either reduced the expression of the other.

Noteworthy, while the role of PPARγ in colitis has been investigated through numerous studies over the past 3 decades (Supplementary Table 1), the role of PPARα has been contradictory (Supplementary Table 2), and their dual agonism in IBD has never been explored. The studies on PPARα are equally split on whether it is pro- or anti-inflammatory in...
By contrast, all studies on PPARγ agree that its agonism ameliorates DSS-induced colitis. Although claimed to be effective on diverse cell types in the gut (epithelium, T-cells, and macrophages), the most notable target cells of PPARγ agonists are macrophages and dendritic cells. Phase I and II clinical trials with PPARγ agonists either alone or in combination with mesalamine show barrier protective effects in UC patients. Despite these insights, the biopharmaceutical industry has not been able to harness the beneficial impact of this major target within emergent therapeutic strategies largely due to a trail of withdrawals after devastating long-term side effects including heart failure, bone fracture, bladder cancer, fluid retention, and weight gain. Intriguingly, and of relevance to this work, the addition of PPARα agonistic activity to PPARγ, balanced dual agonists of PPARα/g (not single), most optimally balance the pro- and anti-inflammatory signaling and favors microbe clearance.

Inhibitors and Agonists of PPARα PPARγ PPARα/g dual

Balanced dual agonists of PPARα/g (not single) enhances microbe clearance, resets IBD network.

Chemical (DSS)-induced colitis (Model of epi barrier disruption)

Histology qPCR/ELISA

Citrobacter colitis (Model of dysbiosis)

RNAseq

Defect rectified with dual PPARα/g agonist

Efficacy of bacterial clearance in peripheral blood derived monocytes

PBMCs from UC, CD and healthy subjects

Defective clearance in CD (not UC)

Inflammation

Balanced PPARα/g dual agonism

A new method for target prioritization and validation in IBD

Target(s) and therapeutic strategy:

Step 0: CREATION AND VALIDATION OF AN INFLAMMATORY BOWEL DISEASE (IBD) MAP

Artificial Intelligence-guided Discovery of a Barrier-Protective Therapy in Inflammatory Bowel Disease

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Major finding(s):

Step 1: GENERATION OF AN AUTOMATED TARGET REPORT CARD

Al-enabled prediction of the likelihood of success in Phase III trials and other key aspects of target biology; these guide choice of model systems.

Step 2: MECHANISM OF ACTION STUDIES IN VITRO

Balanced dual agonists of PPARα/g (not single) most optimally balance the pro- and anti-inflammatory signaling and favors microbe clearance.

Step 3: VALIDATION IN ANIMAL PRECLINICAL MODELS OF COLITIS

Dual agonists (not single) enhances microbe clearance, resets IBD network.

Step 4: VALIDATION IN HUMAN SUBJECTS

Efficacy of bacterial clearance in peripheral blood derived monocytes.
PPARγ, to PPARδ agonists has led to a higher safety profile, leading to their development for use in many diseases, including type 2 diabetes, dyslipidemia, and non-alcoholic fatty liver disease.

An automated target ‘report card’ for PPARα and PPARγ in IBD. We next generated an automated target report card for PPARα and PPARγ. A high level of both PPARs, determined using a composite score for the abundance of both transcripts, was sufficient to distinguish healthy from IBD samples, not just in the test cohort that was used to build the IBD-map (ROC AUC of 0.74; Fig. 2b; see also Supplementary Fig. 2a–d), but also in four other independent cohorts with ROC AUC consistently above 0.88 (Fig. 2c). High levels of both PPARα and PPARγ were also better at differentiating healthy from diseased murine models of disease 

Rationalization of PPARα/G and PPARγC1α as targets in IBD. Because proteins, but not transcripts, are the targets of therapeutic agents, the impact of therapeutics is translated to cellular processes via protein–protein interaction (PPI) networks, a.k.a. interactomes. We next asked how dual agonists of PPARα/γ might impact cellular pathways and processes. A PPI network is visualized using PPARα and PPARδ as ‘query/input’ and the interactive STRING v11.0 database (https://string-db.org/) as a web resource of known and predicted protein–protein interactions curated from numerous sources, including experimental data, computational prediction methods, and public text collections. Pgc1a (a product of the gene PPARC1A) was a common interactors between the two PPARs (Fig. 3a). We noted that Pgc1a also happens to be a major component within the PPARα/δ functional network, serving as a central hub for positive feedback loops between the PPARs and their biological function (Fig. 3b), i.e., mitochondrial biogenesis, DNA replication and energetics (electron transport chain and oxidative phosphorylation). When we analyzed the functional role of the interactomes of PPARα/γ we noted that indeed both interactomes converged on lipid metabolism, mitochondrial bioenergetics and circadian processes (Fig. 3c, d), all representing major cellular processes that are known to be dysregulated in IBD.

PPARα, PPARδ, and PPARγ are downregulated in ulcerative colitis and Crohn’s disease. Previous work demonstrated that both PPARα and PPARδ are highly expressed in the colon and that their expression (proteins and mRNA) is downregulated (by ~60%) in active UC, but not in noninflamed areas. Moreover, the expression of PPARδ was significantly associated with disease activity. Polymorphisms have also been detected in PPARδ; while some studies found those to be associated with an increased risk for CD, others found no evidence suggesting any form of association with an increased disease risk. We collected endoscopically obtained biopsies from the colons of healthy (n = 7) and IBD (n = 14 and 14 of UC and CD, respectively) patients and assessed the levels of transcripts for PPARα, PPARδ, and PPARγC1α by qPCR (Fig. 3f). We confirmed that all three transcripts were significantly downregulated in UC and CD samples compared to healthy; both PPARδ and PPARγC1α were more significantly downregulated in CD compared to UC (Fig. 3g). These findings are in keeping with the network-based predictions that these genes should be downregulated invariably in all IBD samples, regardless of disease subtype (see individual disease maps; Supplementary Figs. 4, 5). While both PPARα and PPARγ are in cluster #2 in the UC map, PPARδ and PPARγ are in separate clusters, clusters 2 and 6, respectively, in the CD map (Supplementary Figs. 4, 5). Reactome pathway analyses implied that in the case of UC, the two nuclear receptors may co-regulate similar cellular homeostatic processes associated with cluster #2, i.e., mitochondrial biogenesis and translation initiation.
infectious disease, and detoxification of ROS (see Supplementary Fig. 4). By contrast, in the case of CD, they may independently regulate diverse cellular processes that maintain cellular homeostasis; while PPARG is associated with cellular metabolism (TCA cycle) and inhibition of NFκB signaling, PPARA is associated with transcriptional activity of nuclear receptors, cholesterol biosynthesis and Met/Ras signaling (see Supplementary Fig. 5). Taken together, these findings demonstrate that PPARA/G and PPARGC1A are downregulated in IBD and that they may regulate key pathophysiologic processes that are vital for cellular homeostasis. Findings support our AI-guided hypothesis that restoration of the expression of these genes will increase the expression of genes in C#1-2-3 and suppress the expression of, and that such increase.
Fig. 2 Network-guided rationalization of PPARα/PPARG as targets in IBD. a An interactive web-based platform allows the querying of paths of gene clusters in the IBD map [ref. 5; see Supplementary Fig. 1] to pick high-value targets with a few mouse clicks and generate a comprehensive automated target 'report card'. The components of a 'target report card' is shown (right); predicted 'therapeutic index' (likelihood of Phase III success), IBD outcome (prognostic potential in UC and/or CD), network-prioritized mouse model, estimation of gender bias, and predicted tissue cell type of action. b-h Components of a target report card for PPARα and PPARG are displayed. Bar plot (b) displays the rank ordering of normal vs. ulcerative colitis (UC) /Crohn's Disease (CD) patient samples using the average gene expression patterns of the two genes: PPARG/PPARA. Samples are arranged from highest (left) to lowest (right) levels. ROC-AUC statistics were measured for determining the classification strength of normal vs IBD. Violin plots (b) display the differences in the average expression of the two genes, in normal and CD samples in the test cohort that was used to build the IBD-map in7. Bar plots in panel c-d show the rank ordering of either normal vs. IBD samples (c) or responder vs. non-responder (R vs. NR; d), or active vs. inactive disease, or neoplastic progression in quiescent UC (qUC vs. nUC; c) across numerous cohorts based on gene expression patterns of PPARG and PPARG, from highest (left) to lowest (right) levels. Classification strength within each cohort is measured using ROC-AUC analyses. Bar plots in panel (e) show the rank ordering of either normal vs IBD samples across numerous published murine models of IBD based on gene expression patterns of PPARG and PPARG as in (d). ACT adoptive T cell transfer. Classification strength within each cohort is measured using ROC-AUC analyses. Bulk = whole distal colon; epithelium = sorted epithelial cells. Schematic in (f) summarizes the computational prediction of the cell type of action for potential PPARG/G-targeted therapy, as determined using Boolean implication analysis. GSEID# of multiple publicly available databases of the different cell types and colorectal datasets used to make sure predictions are cited. Red boxes/circles denote that PPARA/G-targeted therapeutics are predicted to work on monocytes/macrophages and crypt-top enterocytes. Computationally generated therapeutic index (see Methods) is represented as a line graph in (g). The annotated numbers represent Boolean implication statistics. PPARG and PPARG align with other targets of FDA-approved drugs on the right of threshold (O.1). Two FDA-approved targets (green; JG81, 0.046; JAK2, 0.032), two abandoned targets (red; SMAD7, 0.33; IL11, 0.16), PPARG (gray, 0.064), PPARG (gray, 0.04), and the threshold (black, 0.1) are shown in the scale. Box plot in panel h shows that the level of PPARG/G expression is similar in the colon of both genders in health and in IBD, and hence, PPARα/γ-targeted therapeutics are predicted to have little/no gender predilection. The diamond square is the mean, and the arrows around it are 95% confidence interval.

Synthesis and validation of PAR5359, a potent and specific PPARα/γ dual agonist. We next sought to identify appropriate pharmacologic tools to test our hypothesis. Direct agonism of PPARG/PGC1α was deemed as not feasible because the only known agonist, ZLN005, non-specifically and potently also activates AMPK43, a target that is known to independently improve barrier integrity in IBD9. Because PGC1α is intricately regulated by feedback loops by PPARα/γ (Fig. 3b), we strategized targeting PGC1α indirectly via PPARα/γ instead. As for PPARα/γ dual agonists, we noted that all commercially available compounds lack 'balanced' agonistic activities (Supplementary Table 3)44,45. Drugs that have fallen aside due to safety concerns also lack balanced agonism; most of them are less potent on PPARγ than on PPARα by a log-fold (Supplementary Table 3). All these PPARα/γ dual agonists have been withdrawn due to safety concerns29, but the cause of the ‘unsafe’ profile remains poorly understood. Saroglitazar, the drug that is the only active ongoing Phase-III trial (NCT03061721) in this class, has ~3 log-fold more potency on PPARα than PPARγ46. Because our AI-guided approach suggested the use of simultaneous and balanced agonism, we favored the use of the only balanced and yet, specific PPARα/γ agonist described to date, PAR535947,48 (see Supplementary Table 4). In the absence of commercial sources or well-defined methods on how to synthesize this molecule, we generated PAR5359 in four synthetic steps (see details in Methods, Supplementary Fig. 6) and confirmed its specificity and the comparable agonistic activities using pure single PPARα [GW764749] or PPARγ [Pioglitazone50] agonists as controls (Supplementary Fig. 7). With these potent and specific compounds as tools, and their doses adjusted to achieve the same potency, we set out to validate the network-based predictions using pre-clinical models.

PAR5359 ameliorates C. rodentium-induced colitis, enhances bacterial clearance. We next sought to assess the efficacy of individual and dual agonists of our compounds in murine preclinical models. PPARα/γ’s role (or the role of their agonists) in protecting the gut barrier has been evaluated primarily in DSS-induced colitis (Supplementary Tables 1, 2). This model is more related to the UC patient pathology. However, BoNE prioritized other models over DSS, many of which accurately recapitulate the PPARα and PPARγ-downregulation that is observed in the barrier-defect transcript signature in human IBD (Fig. 2c). Among those, we chose C. rodentium-induced infectious colitis, a robust model to study mucosal immune responses in the gut and understand derailed host-pathogen interaction and dysbiosis, which is closely related with IBD, and more specifically, CD pathophysiology51–53. This model is also known to emulate the bioenergetic dysbalance and mitochondrial dysfunction54, both key cellular processes represented in G1–2–3 within the IBD map (Supplementary Fig. 1). Furthermore, this model requires the balanced action of macrophages (a cell line predicted to be the preferred cell type target; Fig. 2f) to promote bacterial clearance and healing55.

Colitis was induced by oral gavage of C. rodentium and mice were treated daily with the drugs via the intraperitoneal route (see Fig. 4a for workflow; Supplementary Fig. 8a). The dose for each drug was chosen based on their EC50 on their respective targets so as to achieve equipotent agonistic activities (Supplementary Fig. 7). Fecal pellets of individual mice were collected to determine the number of live bacteria present in the stool. As anticipated, the bacterial burden in all mice increased from day 5, reaching a peak on day 7, forming a plateau until day 11 before returning to pre-infection baseline by day 18 (Fig. 4b). Compared against all other conditions, PAR5359-treated mice cleared the gut bacterial load significantly and rapidly (Fig. 4b–d). Citrobacter infection was associated with significant epithelial damage and profuse infiltration of inflammatory cells and edema by day 7 (Supplementary Fig. 8b) most of which resolved by day 18 (DMSO control; Fig. 4e). Colons collected on day 7 showed that treatment with PAR5359 significantly reduced these findings when compared to vehicle (DMSO), PPARα and PPARγ agonists alone (Supplementary Fig. 8b). Unexpectedly, when we analyzed the colons on day 18, we noted persistent immune infiltrates in tissues in two treatment arms, pioglitazone and GW7647 (arrowheads; Fig. 4e–j), but not in the vehicle control group, or those treated with PAR5359. These findings indicate that individual PPARα or PPARγ agonists may either retard bacterial clearance and/or induce an overzealous amount of inflammation, but the balanced-dual agonist (PAR5359) may have effectively cleared the infection and resolved inflammation. PAR5359 also reduced spleen inflammation as evidenced by a decreased spleen
weight and length compared to vehicle control (Supplementary Fig. 8c–f). The spleens of mice treated with DMSO, PPARα-alone agonist, GW7647, and PPARγ-alone agonist, Pioglitazone showed black-discoloration, presumably infarcts (arrows, Supplementary Fig. 8c, e). Notably, the spleens of mice treated with PPARα-alone agonist, GW7647, showed a significant increase in spleen length (Supplementary Fig. 8d, f).

Taken together, these findings indicate that PPARα/γ dual agonist PAR5359 is superior in ameliorating *C. rodentium-*induced colitis than either PPARα or PPARγ agonist used alone. Treatment with the dual, but not the single agonists hastened bacterial clearance, resolved inflammation, and induced healing.

PAR5359 resets the colonic gene expression changes induced by *C. rodentium* infection. Pharmacologic augmentation of PPARα and PPARγ was hypothesized to be sufficient to upregulate genes in C#1-2-3, and restore the entire transcriptomic
network to 'healthy' state via the invariant BIRs between the genes/clusters. We asked if that was achieved. RNA sequencing (RNA-seq) studies were carried out on the C. rodentium-infected colons in each treatment group (Fig. 4a). As expected, down-regulation of genes in clusters #1-2-3 of the IBD-map was significant in infected untreated (DMSO control) vs. uninfected controls, indicative of network shift from health towards disease, and treatment with PAR5359 resisted such shift (Fig. 4k).

Pre-ranked gene set enrichment analyses (GSEA) based on pair-wise differential expression analysis showed that when compared to DMSO control, dual PPARα/γ agonism with PAR5359, but not individual agonists Pioglitazone or GW7647 was able to significantly preserve epithelial junction signatures...
PAR5359 ameliorates DSS-induced colitis in mice. a Schematic summarizing the workflow for testing PPARα/γ-targeted therapeutics in C. rodentium-induced colitis. Mice were gavaged with C. rodentium on day 0 and subsequently treated daily with PPAR agonists. Fecal pellets were collected to test viable bacterial burden, as determined by dilution plating and colony counting. Colonos were excised on day 7 and 18 and analyzed using the indicated readouts. b-d Line graphs (b) display time series of the burden of viable bacteria in feces. Scatter plots with bar graphs (c) compare the peak burden of viable bacteria in feces on day 7. Scatter plots with bar graphs (d) display the area under the curve (AUC) for the line graph in b. CFU, colony forming units. Data points were plotted as black or white simply to improve visibility. e Images display representative fields from H&E-stained colon tissues of 4-5 mice in each group. Mag = 100x (top) and 200x (bottom); Scale bars, 100 μm. White arrowheads point to immune cell infiltrates. f-j Bar graphs display four parameters of inflammation that were quantified in H&E stained colon tissues in (e). Statistics: All results are displayed as mean ± SEM. Significance was tested on the cumulative histological score using one-way ANOVA followed by Tukey’s test for multiple comparisons. Significance: n.s., not significant, p-value < 0.05 was considered significant. k Violin plots (left) display the deviation of expression of genes in Clusters #1-2-3 in the IBD network, as determined by RNA Seq on murine colons. Bar plot (right) displays the rank ordering of the samples. Welch’s t-test was used to determine statistical significance. Significance: n.s., not significant, p-value < 0.05 was considered significant. l Pre-ranked GSEA based on pairwise differential expression analyses (DMSO vs PAR5359 groups) are displayed as enrichment plots for epithelial tight (top) and adherens (middle) junction signatures and balanced macrophage processes (bottom). See also Supplementary Fig. 8 for the day #7 results in the C. rodentium-induced colitis model, Supplementary Fig. 9 for extended GSEA analyses, and Supplementary Fig. 10 for the effect of PAR5359 on DSS-induced colitis in mice.

(both tight and adherens junctions) while maintaining balanced macrophage processes (compare Fig. 4l with Supplementary Fig. 9a). These enrichment analyses confirmed that, compared to DMSO-treated infected colons, the epithelial junction-related genes were significantly enriched in dual PPARα/γ-agonist treated samples (Fig. 4l, top and middle) without significant changes in macrophage polarization (Fig. 4l, bottom). These findings are in keeping with the predictions that epithelial cells and macrophages may be the primary cell type of action for dual PPARα/γ agonists. Comparison of all treatment cohorts against each other revealed that although both PAR5359 and Pioglitazone were superior to GW7647 in maintaining some epithelial integrity and extent as uninfected control (Supplementary Fig. 9f). Taken together, these findings suggest that dual agonists of PPARα/γ are sufficient to either resist network shift and/or reverse the disease network in the setting of colitis. They also offer clues suggestive of epithelial and macrophage processes, two key cellular components of innate immunity in the gut lining as major mechanisms. These transcriptome-wide impacts suggest that PPARα/γ dual agonist PAR5359 is superior in restoring colon homeostasis in C. rodentium-induced colitis than either PPARα or PPARγ agonist used alone.

PAR5359 ameliorates DSS-induced colitis. It is well known that no single mouse model recapitulates all the multifaceted complexities of IBD.66,67. Because almost all studies evaluating PPARα/γ-modulators have been performed on the DSS-induced colitis model (Supplementary Tables 1–2), we asked whether the PPARα/γ dual agonist PAR5359 can ameliorate colitis in this model. Mice receive intrarectal DMSO vehicle control or PAR5359 while receiving DSS in their drinking water (Supplementary Fig. 10a). Disease severity parameters, i.e., weight loss, disease activity index, shortening of the colon, and histology score were significantly ameliorated in the PARS5395-treated group (Supplementary Fig. 10a–e). These findings show that the PPARα/γ-dual agonist, PAR5359, is also effective in DSS-induced colitis. It is noteworthy that the PAR5359 dual agonist offered protection in the DSS-model, because prior studies using the same model have demonstrated that PPARα agonists worsen66,67 and that the PPARγ agonists ameliorate colitis58–60 (see Supplementary Tables 1, 2).

PAR5359 promotes bacterial clearance with controlled production of ROS and inflammation in peritoneal macrophages. Next, we sought to study the mechanism of action of PAR5359, and the target cell type responsible for the superiority of dual agonism over single agonism. Our AI-guided approach predicted crypt top epithelium and macrophages as a site of action (Fig. 2f). Based on prior studies with single agonists in cell-specific KO mice (Supplementary Table 1) and the phenotypes observed in our animal models (Fig. 4; Supplementary Figs. 8–10), single PPAR agonism appears sufficient to protect the epithelium in chemical-induced colitis (dual agonism did not offer additional advantage). The advantage of dual agonism is apparent in the Citrobacter-colitis model, which most robustly recapitulates the paradoxical immune suppression in the setting of dysbiosis that is seen in IBD, and most prominently in CD.51,61,62. Because the intestinal macrophages are alternatively polarized in this model63,64, we hypothesized that balanced agonism may alter macrophage response to dysbiosis. To test this hypothesis, we incubated macrophages treated or not with the drugs and challenged them with CD-associated adherent invasive E. coli (AIEC)-LF82; this strain, originally isolated from a chronic ileal lesion from a CD patient.65. As for the source of macrophages, we isolated metabolically active primary murine peritoneal macrophages using Brewer thioglycolate medium using established protocols66,67 (Fig. 5a). These macrophages are known to have high phagocytic activity.66. Thioglycolate-induced peritoneal macrophages (TG-PMs) were lysed, and viable intracellular bacteria were counted after plating on an agar plate. Pre-treatment with 1 μM PAR5359 and an equipotent amount of GW7647 (PPARα agonist) promoted bacterial clearance and reduced the bacterial burden when compared to vehicle control (Fig. 5b). By contrast, pre-treatment with Pioglitazone (PPARγ agonist) inhibited bacterial clearance; notably, the bacterial burden was significantly higher at both 3 and 6 h after infection (Fig. 5b). Reduced clearance of microbes in the latter was associated also with significant reductions in the cellular levels of reactive oxygen species (ROS) (Fig. 5c), which is believed to be a key component for effective bacterial killing.68. By contrast, GW7647 did not reduce ROS induction, and PAR5359 treatment permitted an intermediate amount of induction of ROS (significantly lower than GW7647; Fig. 5c). Thus, the dual agonist PAR5359 allowed effective bacterial clearance (like GW7647; Fig. 5b) with minimal ROS induction (like Pioglitazone; Fig. 5c).

These patterns of microbial clearance and cellular ROS were associated also with the expression of cytokines, as determined by qRT-PCR analyses (Fig. 5d). As expected, infection of TG-PM with AIEC-LF82 induced Il1β, Il6, Tnfα, and Il10. PAR5359 significantly and selectively suppressed the expression of the pro-inflammatory cytokines Il1β, Il6, and Tnfα (but not the anti-inflammatory cytokine, Il10) (Fig. 5d). By contrast, the
Fig. 5 PPAR\(\alpha\) and PPAR\(\alpha/\gamma\)-dual agonists enhance, whereas PPAR\(\gamma\) agonist delay bacterial (AIEC-LF82) clearance. a Schematic displays the experimental design and workflow. Thiglycylate-induced murine peritoneal macrophages (TG-PMs) pretreated with PPAR agonists (see box, below; 20 nM GW7647, 10 \(\mu\)M Pioglitazone and 1 \(\mu\)M PAR5359) were infected with AIEC-LF82 (MOI 10) and subsequently analyzed for the bacterial count (Gentamicin protection assay), generation of cellular ROS, secretion of inflammatory cytokines (in supernatant media by ELISA) and the induction of cytokines (gene transcript analysis by qPCR). The cycle threshold (CT) of target genes was normalized to 18S rRNA gene and the fold change in the mRNA expression was displayed in line graphs (left) and bar graphs (right). b Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. c Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. d Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. e Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. f Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. g Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. h Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. i Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. j Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. k Line graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) show the fold change in bacterial count over time. l Line graphs (left) display percent viable bacterial counts at indicated times after infection. 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PPAR\(\gamma\) specific agonist pioglitazone significantly suppressed the cytokines IL1\(\beta\) and IL6, and there was no effect of the PPAR\(\alpha\) specific agonist GW7647 (Fig. 5d). ELISA studies on the supernatant media further confirmed these findings (Fig. 5e), demonstrating that the effects in gene expression were also translated to the abundance of secreted cytokines released by the macrophages in the supernatant. Thus, both mRNA and protein estimations agreed that PPAR\(\alpha/\gamma\) dual agonism inhibits pro-inflammatory IL1\(\beta\) and IL6 cytokines while permitting the induction of the major anti-inflammatory cytokine IL10. Similar findings were also observed in the case of another enteric pathogen, Salmonella (S. enterica); it is noteworthy that unlike
AIEC-LF82, *S. enteritica* is more efficient in surviving and multiplying inside the macrophage. The dual PPARγ agonist enhances bacterial clearance (Supplementary Fig. 11a, b) with limited induction of pro-inflammatory cytokines (significantly lower than control) and full augmentation of anti-inflammatory *Il10* cytokine (similar to control) (Supplementary Fig. 11c). Neither PPARα-, nor PPARγ- single agonists achieved this desirable profile, i.e., effective bacterial clearance with controlled inflammation. PPARα-agonist improved clearance, but failed to reduce proinflammatory cytokines. By contrast, PPARγ-agonist failed to promote clearance, but significantly reduced all cytokines (Supplementary Fig. 11).

Taken together, these studies on enteric pathogens show that (i) PPARγ agonism induces 'tolerance' by suppressing inflammation, inhibiting ROS production and delaying bacterial clearance; (ii) PPARα agonism induces 'reactivity' by promoting bacterial clearance, permitting the full extent of ROS production and the induction of proinflammatory cytokines, but suppressing the anti-inflammatory *Il10* cytokine; and (iii) PPARα/γ dual agonism achieves a more balanced response; it suppresses proinflammatory cytokines without suppressing anti-inflammatory cytokine *Il10*, and thereby, permits the extent of inflammation and ROS induction that is optimal and sufficient to promote bacterial clearance.

PPARα, but not PPARγ is required for the induction of inflammatory cytokines and ROS. To further dissect which nuclear receptors are responsible for the balanced actions of the dual agonist, we next used a set of highly specific and potent PPARα/γ inhibitors (Supplementary Table 4). We pre-treated TG-PMs with PPARα and PPARγ inhibitors, either alone, or in combination, followed by stimulation with bacterial cell wall component LPS (Fig. 6a). As expected, LPS induced the cellular levels of ROS (Fig. 6b) and inflammatory cytokines (Fig. 6c, d) in TG-PMs significantly higher than in untreated control cells. Inhibition of PPARα suppressed the induction of cellular ROS and inflammatory cytokines, both at the level of gene and protein levels (Fig. 6b–d). By contrast, inhibition of PPARγ did not interfere with either response (Fig. 6b–d). Simultaneous inhibition of both PPARα and PPARγ mimicked the cellular phenotypes in the presence of PPARα inhibitors (Fig. 6b–d), indicating that inhibition of PPARα is sufficient to recapitulate the phenotype of dual inhibition. Taken together, these findings indicate that PPARα is required for the proinflammatory response of macrophages.

PPARα/γ dual agonist PPAR5359 promotes bacterial clearance in patient-derived PBMCs. In search of a pre-clinical human model for testing drug efficacy, we next assessed microbial handling by PBMCs derived from patients with IBD and compared them with that in age-matched healthy volunteers. We enrolled both male and female patients and both CD and UC (Supplementary Table 5). Consecutive patients presenting for routine care to the UC San Diego IBD clinic were enrolled into the study; the only exclusion criteria were failure to obtain informed consent for the study or active infections and/or disease flare. Peripheral blood collected in the clinic was freshly processed as outlined in Fig. 7a to isolate PBMCs. Pre-treatment for 30 min with vehicle or PPAR5359 was followed by infection for 1 h. Subsequently, the cells were treated with gentamicin for 60 min to kill extracellular bacteria to assess intracellular bacterial burden at 1 and 6 h after the gentamicin wash.

Two observations were made: First, CD but not UC patient-derived PBMCs when infected with AIEC-LF82 showed an increased number of internalized viable bacteria when compared to healthy PBMCs (Fig. 7b, e), indicative of either defective clearance and/or increased permissiveness to bacterial replication within the cells is limited to the CD. Second, pre-treatment with PPAR5359 could improve clearance significantly (Figs. 7c, d, 7f, g). These results indicate that bacterial clearance is delayed in PBMCs of patients with CD and that PPARα/γ dual agonism with PPAR5359 can reverse that defect. The possibility that such reversal could be due to any direct bacteriostatic/cidal effect of PPAR5359 agonist was ruled out (see bacterial viability assay in Supplementary Fig. 12). Our findings demonstrate that bacterial clearance is delayed primarily in CD and not UC are in keeping with the fact that delayed bacterial clearance from inflamed tissues (up to ~ 4-fold) is uniquely observed in CD90. These findings are also in keeping with our own observation that the downregulation of *PPARG/PPARGC1A* was more prominent in patients with CD (Fig. 3f, g). In fact, delayed clearance is one of the major reasons for persistent inflammation and disease progression among patients with CD90,91.

**Discussion**

Barrier-protection/restoration is the treatment endpoint for all clinical trials in IBD therapeutics; however, despite much success in the development of anti-inflammatory therapies,92, barrier-protective therapeutics in IBD have been slow to emerge92. Here we report the discovery of an effective barrier-protective therapeutic strategy in IBD identified using an AI-guided navigation framework (summarized in Fig. 8). First, a network-based drug discovery approach9 was used to identify, rationalize and validate dual and balanced agonism of PPARα/γ (but not one at a time) is necessary for therapeutic success. Second, we provided evidence in the form of proof-of-concept studies (in two different pre-clinical murine models) demonstrating that the simultaneous and balanced agonistic activation of the pair of PPARs as an effective barrier protective strategy in IBD. Third, we demonstrate that macrophages are one of the primary target cell types of this therapeutic strategy; dual agonist (but not single) was permissive to the induction of macrophage responses expected for optimal immunity without overzealous inflammation. There are three notable takeaways from this study, which are unexpected observations and/or insights that filled key knowledge gaps in the fields of —(a) network medicine, (b) IBD therapeutics, and (c) macrophage biology.

First, with regard to network medicine, the AI-guided approach we used here differs from the current practice in three fundamental ways: (1) Unlike most studies that prioritize targets based on Differential Expression Analysis (DEA, or integrated DEA) or Bayesian approaches, target identification and prediction, this work was guided by a Boolean implication network (BIN) of continuum states in human disease9; (2) Instead of conventional approaches of trial-and-error, intuitive guess and/or knowledge-based prioritization of study models (animal or cell-type of action), target validation in network-rationialized animal and cell-type models that most accurately recapitulate the role of the target(s) during disease progression; (3) Inclusion of human pre-clinical model (patient-derived PBMCs) for target validation, inspiring the concept of Phase ‘0’ trials that have the potential to personalize the choice of therapies. The combined synergy of these approaches validates a macrophage modulator in addressing the broken gut barrier in IBD.

The impact of using such an approach is fourfold: (i) Because the network approach used here relies on the fundamental invariant BIRs between genes, and their patterns of changes in expression between healthy and IBD samples, such ‘rule of invariant’ implies that any given relationship and/or change in expression pattern annotated within the network must be fulfilled...
in every IBD patient. By that token, targets/drugs prioritized based on this network is expected to retain efficacy beyond inbred laboratory mice, into the heterogeneous patient cohorts in the clinic. (ii) This AI-guided approach not just helped compute pre-test probabilities of success (“Therapeutic Index”), but also helped pick models that are most insightful and appropriate to demonstrate therapeutic efficacy (e.g., *Citrobacter rodentium* infection-induced colitis) and to pinpoint the cell type and mechanism of action (microbial clearance by macrophages). This is noteworthy because the conventional approach in studying PPARs has been limited to the use of DSS-induced colitis (see Supplementary Tables 1, 2), which has often given conflicting results (see Supplementary Table 2). PPARγ agonists work best for the UC patients, perhaps because it is a potent inhibitor of proinflammatory cytokines and, as shown before, protects the intestinal epithelium38. Our findings in the *Citrobacter* model imply that such single PPARγ agonism may worsen the macrophage dysfunction that is observed in the setting of CD, which is characterized by ineffective microbial clearance, insufficient proinflammatory response in the setting of luminal dysbiosis35. In fact, without the use of the *Citrobacter rodentium* infectious colitis model, the deleterious effects of PPARγ agonists would have been overlooked. (iii) Having a computational framework improves precision in target identification; it is because of the emergence of the two PPARs (alongside their positive feedback advantage of dual agonism has neither been rationalized nor tested. (iv) The ‘target report card’, like the one shown here, is a project navigation tool that is geared to streamline decision-making (i.e., which genes, which animal models, which cell type/cellular process, what is the likelihood of success, etc.), which in

![Figure 6](https://example.com/figure6.png)

**Fig. 6 PPARα but not PPARγ is required for induction of cellular ROS and proinflammatory cytokines.** a Schematic of experimental design. TG-PMs were pre-incubated with 10 μM PPARα or PPARγ inhibitors (illustrated on the right), either alone or in combination for 30 min prior to stimulation with 100 ng/ml LPS. Cells were analyzed at 2 and 6 h to estimate cellular ROS and cytokine induction, respectively. b–d Bar graphs display the levels of cellular ROS (b), relative levels of mRnA (the target genes were normalized to 18S rRNA gene and the fold change in the mRNA expression was determined using the 2−ΔΔCt method) (c) and protein (d) expression of cytokines (IL1β, IL6, TNFα, and IL10). Statistics: Results are from three independent experiments and displayed as mean ± SEM. One-way ANOVA followed by Tukey’s test for multiple comparisons was performed to test significance. Significance: n.s, not significant, p-value < 0.05 was considered significant.
turn should reduce attrition rates, waste, and delays; the latter are well-recognized flaws in the current process of drug discovery.

Second, regarding IBD therapeutics, our studies demonstrate that single or unbalanced combinations of Ppar agonists are inferior to dual/balanced agonists. Conventional and reductionist approaches have inspired numerous studies with single Ppar agonists over the past decade (Supplementary Tables 1, 2). However, given the devastating side effects of most single or unbalanced PPARα/γ agonists (Supplementary Table 3), translating to the clinic beyond a Phase II trial has not been realized. Because the therapeutic index for the dual PPARα/γ agonists matches that of other FDA-approved targets/drugs, it is predicted that barring unexpected side effects, dual PPAR agonists are likely to be effective as barrier-protective agents. As for side effects, we noted is that balanced PPARα/γ agonists are rare; while all dual PPARα/γ agonists that have been discontinued due to side effects happen to be either single (only PPARγ) or ‘unbalanced’ (PPARγ > PPARα agonistic activity), the newer generation formulations that are currently in the clinical trial have a reversed agonistic potency (PPARα > PPARγ agonistic activity) (see Supplementary Table 3). Because macrophage responses require finetuning (discussed below), our studies show how unopposed agonism of either PPARγ or PPARα is harmful and can impair/dysregulate the way macrophages respond when microbes breach past the gut barrier. It is possible that many of the side effects of the discontinued thiazolidinediones are due to their inability to achieve that ‘optimal’ spectrum of macrophage function.

Fig. 7 PPARα/γ dual agonist, PAR5359, promotes the clearance of AIEC-LF82 from CD patient-derived PBMCs. a Schematic displays the overall experimental design using human subjects (see Supplementary Table 5 for patient demographics). Peripheral blood collected from healthy, CD, and UC patients was used as a source of PBMCs. PBMCs were pre-treated for 30 min with 1 μM PPARα/γ agonists prior to infection with AIEC-LF82 (MOI 50) for 1h. PBMCs were subsequently treated with gentamicin to kill extracellular microbes for 60 min prior to lysis and plating to determine the intracellular abundance of viable bacteria at 1h and 6h, as determined by dilution plating and colony counts (see Methods for details). b-g Bar graphs with scatter plots display the abundance of viable intracellular bacteria at 1h (b) and 6h (e) after infection. Paired line plots display the rate of clearance of bacteria in individual subjects at 1h (c) and 6h (f) after infection. Data in (b, e) of 1h infection is combined in (d) and data in (e, f) of 6h infection is combined in (g) with statistics: Results are displayed as mean ± SEM (CD patient n = 7, UC patients n = 6, and healthy n = 9). Paired t-test or one-way ANOVA followed by Tukey’s test for multiple comparisons was performed to test significance. Significance: n.s., not significant, p-value < 0.05 was considered significant.
Third, when it comes to macrophage biology, this work sheds some unexpected and previously unforeseen insights into the role of the PPARs in the regulation of macrophage processes. Extensively studied for over ~3 decades, PPARs are known to regulate macrophage activation in health and disease. Targeting PPARs as a host-directed treatment approach to infectious/inflammatory diseases appears to be a sound strategy because they regulate macrophage lipid metabolism, cholesterol efflux, inflammatory responses (ROS and cytokine production), apoptosis, and production of antimicrobial byproducts. We found that unopposed PPARγ activation suppresses bacterial clearance and blunts the induction of proinflammatory (but not anti-inflammatory, IL10) cytokines and ROS in response to infection both in vivo and in vitro. In other words, and consistent with prior reports, PPARγ activation suppressed inflammation at the cost of impairing immunity. Our findings are in keeping with the findings of a systematic review and meta-analysis of 13 long-term randomized controlled trials that involved 17,627 participants (8,163 receiving PPARγ agonists and 9464 receiving control drugs). Long-term (~1–5.5 y) use of PPARγ agonists increases the risk of pneumonia or lower respiratory tract infection significantly, some of which result in hospitalization, disability, or death. In the case of PPARα, unopposed activation-induced ROS and proinflammatory cytokines and accelerated bacterial clearance. Inhibitor studies further confirmed that PPARα was required for these responses (Fig. 6). These findings are in keeping with others showing that PPARα, but not PPARγ, is required for NADPH-induced ROS formation both in human and murine macrophages. PPARα agonists induce the expression of NADPH oxidase subunits p47(phox), p67phox, and gp91phox, which are all essential functional components of NADPH complex. Dual and balanced PPARα/γ agonism enhanced bacterial clearance with only a moderate induction of proinflammatory cytokines or ROS. Such a response ensures that the...
macrophage functions within a 'goldilocks' zone, mounting inflammation that is just sufficient for microbial clearance and immunity\(^8\). In our analysis, the only other PPAR-related gene within the IBD network, i.e., Pgc1a, and its role within the PPARα/γ axis suggests that the intricate network of forward feedback loops orchestrated by Pgc1a may be critical for achieving the critical balance between immunity and inflammation, which is a key outcome of the dual PPAR/α/γ agonists.

Because previous studies using cell-specific gene depletion have indicated that the barrier-protective role of PPARγ may be mediated via cells other than the macrophages\(^9\), namely, the T cells\(^8\) and the epithelial cells\(^8\), it is possible that the dual PPARα/γ agonists also act on those cells, promoting bacterial clearance and balancing cellular bioenergetics, ROS and cytokine production, in manners similar to that we observe in macrophages.

Taken together, our study uses an unconventional approach to rationalize and validate the use of PPARα/γ dual agonists as barrier-protective macrophage modulators in the management of IBD. The approach is powerful because it leverages the precision of mathematics (Boolean algebra of logic) and the fundamental invariant patterns in gene expression (Boolean Implications). The AI-navigated drug discovery approach defined here could serve as a blueprint for future studies not just in IBD, but in any other such complex chronic diseases.

**Method**

The identity and source of all resources (reagents, computational and other software and equipment) used for this study is cataloged in Supplementary Table 6.

**Computational**

* A Boolean network map of IBD. A BIN was created earlier [ref.\(^5^*\); Supplementary Fig. 1a], and this network is comprised of clusters of genes, interconnected by BIRs. The concepts, mathematical, statistical, datasets that went into building this map is detailed in ref.\(^9^*\), and briefly mentioned here.

Gene expression databases. Publicly available human colon tissue gene expression databases were downloaded from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus website (GEO)\(^82\)-\(^84\). If the dataset is not normalized, RMA (Robust Multichip Average)\(^85\),\(^86\) is used for microarrays and TPM (Transcripts Per Millions)\(^87\),\(^88\) is used for RNASeq data for normalization. We used log2(TPM - 1) to compute the final log-reduced expression values for RNASeq data. Accession numbers for these crowdsourced datasets are provided in the figures and manuscript. All of the above datasets were processed using the Hegenom data analysis framework\(^89\)-\(^91\).

Boolean analysis. Boolean logic is a simple mathematical relationship of two values, i.e., high/low, 1/0, or positive/negative. The Boolean analysis of gene expression data requires first the conversion of expression levels into two possible values. The StepMiner algorithm is reused to perform Boolean analysis of gene expression data\(^92\). The Boolean analysis is a statistical approach that creates binary logical inferences that explain the relationships between phenomena. The Boolean analysis is performed to determine the relationship between the expression levels of pairs of genes. The StepMiner algorithm is applied to gene expression levels to convert them into Boolean values (high and low). In this algorithm, first, the expression values are sorted from low to high and a rising step function is fitted to the series to identify the threshold. The middle of the step is used as the StepMiner threshold. This threshold is used to convert gene expression values into Boolean values. A noise margin of 2-fold change is applied around the threshold to determine intermediate values, and these values are ignored during Boolean analysis. In a scatter plot, there are four possible quadrants based on Boolean values (Supplementary Fig. 2a): (low, low), (low, high), (high, low), (high, high).

Invariant Boolean implication relationships. A BIR is observed if any one of the four possible quadrants or two diagonally opposite quadrants are sparsely populated. Following this rule, there are six different kinds of BIRs. Two of them are symmetric equivalent (corresponding to the highly positively correlated genes), opposite (corresponding to the highly negatively correlated genes). Four of the Boolean relationships are asymmetric, and each corresponds to one sparse quadrant: (low => low), (high => low), (low => high), (high => high). BooleanNet statistics (Equations listed below) is used to assess the sparsity of a quadrant and the significance of the BIRs\(^92\),\(^93\). Given a pair of genes A and B, four quadrants are identified by using the StepMiner thresholds on A and B by ignoring the intermediate values defined by the noise margin of twofold change (±0.5 around StepMiner threshold). The number of samples in each quadrant is defined as \(n_{A00}\), \(n_{A10}\), \(n_{A01}\), and \(n_{A11}\) (Supplementary Fig. 2a). The total number of samples where gene expression values for A and B are low is computed using the following equations.

\[
n_{A00} = (a_{00} + a_{10})
\]

\[
n_{A10} = (a_{00} + a_{01})
\]

\[
n_{A01} = (a_{00} + a_{10})
\]

\[
n_{A11} = (a_{00} + a_{01})
\]

The total number of samples considered is computed using the following equation.

\[
total = a_{00} + a_{01} + a_{10} + a_{11}
\]

The expected number of samples in each quadrant is computed by assuming independence between A and B. For example, the expected number of samples in the bottom left quadrant \(a_{00} = \frac{n}{total}\) is computed as probability of A low \((a_{00} + a_{01})/total\) multiplied by the probability of B low \((a_{00} + a_{10})/total\) multiplied by the total number of samples. The following equation is used to compute the expected number of samples.

\[
n = a_{00} \times \frac{n}{total} = n_{A00}/total \times n/total \times total
\]

To check whether a quadrant is sparse, a statistical test for \((a_{00} > a_{01})\) or \((n > n)\) is performed by computing \(S_{00}\) and \(p_{00}\) using the following equations.

\[
S\_{00} = \frac{n}{total} \times n/total \times total
\]

\[
p_{00} = \frac{1}{n} \times \frac{a_{00}}{a_{00} + a_{01}} \times \frac{a_{00}}{a_{00} + a_{10}}
\]

A threshold of \(S_{00} > sthr\) and \(p_{00} < pthr\) to check sparse quadrant. A BIR is identified when a sparse quadrant is discovered using the following equation.

\[
Equivalent = \left(\frac{S_{00} > sthr}{P_{00} < pthr}\right)
\]

\[
Opposite = \left(\frac{S_{00} < sthr}{P_{00} > pthr}\right)
\]

Boolean equivalent and opposite are symmetric relationship because the relationship from A to B is same as from B to A. A symmetric relationship forms if there is only one quadrant present in the scatter plot. For each gene and computing each gene and computing the ratio of the number of BIR discovered in the randomized dataset and original dataset. For IBD dataset the false discovery rate was <0.001.

Boolean Implication analysis looks for invariant relationship across all the different types of samples regardless of the conditions and treatment protocols. Therefore, it does not distinguish the sample types when discovering BIRs (Supplementary Fig. 2c). We assume that there are fundamental invariant Boolean implication formula that is satisfied by every sample regardless of their type (in this context it is limited to healthy and IBD colonic biopsies including both UC and CD). This means normal, UC, and CD samples share the same fundamental relationships.

**IBD datasets.** Both Peters-2017 GSE36867 and Arijis-2018 GSE73661 dataset were independently prepared for Boolean analysis by filtering genes that have reasonable dynamic range of expression values by analyzing the fraction of high and low values identified by the StepMiner algorithm\(^94\). Any probe sets or genes that contain <5% of high or low values or do not have a big dynamic range are dropped from the analyses (for Peters-2017 dataset 7559/25228 genes dropped—33%). To check if pairwise BIRs are consistent between two datasets, every gene in Peters-2017 dataset is mapped to the best probe set (identified by the biggest dynamic range)
the Arijs-2018 dataset, and genes/probes that do not match are dropped from the analysis (4841/23228 genes dropped—21%). Finally, 44% (10232/23228) of genes were not used in the Boolean Implication Network because their expression did not have a sufficient range. Since RNA-Seq expression values have slightly different characteristics than microarray expression values, the consistency of BIR was determined by using BooleanNet statistics in both datasets and a Pearson’s correlation coefficient in the Arijs-2018 dataset. A Pearson’s correlation coefficient > 0.5 was considered compatible with Equivalent, High ⇒ High, and Low ⇒ Low BIRs. Similarly, a Pearson’s correlation coefficient < −0.25 was considered compatible with Opposite, High ⇒ Low, and Low ⇒ High BIRs. The Boolean model is tested in several human datasets, each comprised of a heterogeneous collection of samples to demonstrate reproducibility (GSE16879, GSE59071, GSE48958, GSE50594, GSE37283, E-MTAB-7604). We have collected publicly available gene expression datasets derived from mouse models of IBD (DSS bulk GSE42768, DSS epithelium E-MTAB-5249, TNBS GSE53835, Citrobacter GSE90577, adoptive T-cell transfer ACT GSE78317, ACT GSE27302, IL10 -/- GSE39859, TNFR1 -/- GSE107933, TNFR2 -/- GSE65408) to test whether human to mouse is performed well in mice. The gene expression conversion from human to mouse is performed using human genome GRCh38.95 ensemble IDs and mapping data exported from ensemble BioMart web-interface.

Generation of target report card. A target report card is generated for one target or multiple targets to predict the efficacy of a potential drug. The target report card contains five different sections as described below. Therapeutic index1, BIR outcome3, Network-prioritized mouse model4, estimation of gender bias5, Predicted tissue cell type of action. Target report card – Therapeutic index. Therapeutic index is a number (lower the better) used for multi-target analysis to predict the efficacy of a potential drug. The therapeutic index is computed by measuring the strength of BIR with PRKAB1 (Supplementary Fig. 2e). Since PRKAB1 is an agonist, if $S_{11} > 0$ then gene A is an Antagonist because top-right quadrant will have fewer samples than expected and PRKAB1 high will be associated with gene A low. For antagonist, top-right and bottom-left quadrants are expected to be sparse. Therefore, $T_{index}$ for antagonist is computed as follows:

$$T_{index} = \frac{1}{4} \left( \frac{0.3}{\text{low}} + \frac{0.3}{\text{high}} + p_{00} + p_{11} \right)$$

Similarly, if $S_{11} < 0$ then gene A is an Agonist because top-right quadrant will have more samples than expected and PRKAB1 high will be associated with gene A high. For agonist, top-left and bottom-right quadrants are expected to be sparse. Therefore, $T_{index}$ for agonist is computed as follows:

$$T_{index} = \frac{1}{4} \left( \frac{0.3}{\text{low}} + \frac{0.3}{\text{high}} + p_{00} + p_{11} \right)$$

Therapeutic indices range from 0.36 to 0.027 where the most effective drug targets will be close to 0.027 and abandoned drug targets will be close to 0.36. Since all the currently known FDA-approved drug targets have therapeutic indices <0.1, we set $S_{11} = 0.01$ (from drug target prioritization) as a lower limit for therapeutic drug targets. Lower therapeutic indices mean a stronger BIR with PRKAB1 which predicted phase III successes for many drugs in IBD5. Only four out of 16 targets have therapeutic indices <0.1. For effectiveness, we also check EMT and Inflammation scores in addition to the therapeutic index. Effective targets are observed to have better scores for both EMT and Inflammation, and they are likely to be present in both EMT and Inflammation Boolean paths. See the section "Identification of Epithelial-Mesenchymal and Inflammation-Fibrosis continuum".

Target report card – BIR outcome. Several datasets with annotations of IBD (normal vs IBD, GSE73661, GSE16879, GSE9071, GSE48958) as well as the aggressiveness of IBD such as active from inactive disease (GSE9071, GSE48958), responders from non-responders receiving two different biologicals, Infliximab or Vedolizumab (GSE73661, GSE16879, GSE50594, E-MTAB-7604), and even distinguished those with the quiescent disease with or without remote neoplasia (GSE37283) were used to assess the strength of association of drug targets with IBD outcome. See the section "Generation of heat maps and drug targets score" of how drug targets score is computed and samples, ordered and association with disease outcome is measured. A list of barplots is used to visualize the sample ordering and the association with disease outcome.

Target report card – Network-prioritized mouse model. Drug targets score is computed for the mouse model IBD datasets (DSS bulk GSE42768, DSS epithelium E-MTAB-5249, TNBS GSE53835, Citrobacter GSE90577, adoptive T-cell transfer ACT GSE78317, ACT GSE27302, IL10 -/- GSE39859, TNFR1 -/- GSE107933, TNFR2 -/- GSE65408) to test whether combined gene expression values of the drug targets are associated with disease annotation. See the section "Generation of heat maps and drug targets score" of how drug targets score is computed and ordered and association with disease outcome is measured. A list of barplots is used to visualize the sample ordering and the association with disease outcome.

Target report card – estimation of gender bias. A box plot of the gene expression values of the individual target gene is computed in the Peters-2017 GSE36867 dataset to see if there are any gender differences. If there are no gender differences in gene expression of the target, then the box plots of individual genes for both males and females are plotted side-by-side to visualize the differences.

Target report card – Predicted tissue cell type of action. It is important to know which cell types are relevant for the optimal action of drug targets. We assume that the drug action is dictated by cell type-specific expression patterns of the drug targets. We predict cell type-specific expression patterns using various features including correlation, standard deviation, and previously published MiDREG algorithm35. We assembled several gene expression databases for this task. A large human colon tissue database (n = 1911) was assembled by recently published MiDREG algorithm, adenoma, and colorectal cancer datasets from NCBI GEO (Supplementary Fig. 3a). All the samples in this database were analyzed using bulk tissue in Affymetrix U133 Plus 2.0 microarray platform. A large human colorectal cancer cell line database (n = 264) was prepared to identify genes expressed in epithelium because these are likely homogeneous and devoid of stromal tissue such as fibroblasts and immune cells. Microarray datasets of FACS purified macrophages, FACS purified GI fibroblasts, and FACS purified lymphocytes were downloaded using GSE134312, GSE63626, and GSE24759, respectively (Supplementary Fig. 3a). The algorithm that predicts whether a gene is expressed in top, bottom, lymphocytes, macrophages, and fibroblasts is described in a flow chart (Supplementary Fig. 3b). MiDREG algorithm is performed on the human colon tissue dataset (n = 1911) to predict top/bottom of the crypt markers93,94. Boolean implication "KRT20 low ⇒ X low" is used to predict the expression of gene X at the top of crypt95. Boolean implication "KRT20 low ⇒ X high" is used to predict the expression of gene X at the bottom of the crypt. Since the human colon tissue database (n = 1911) contains bulk tissue samples, the expression of gene X is restricted to the epithelium by filtering the gene expression in the human colorectal cell cancer database (n = 264). LGR5 correlation > 0.8 is performed in the FACS purified colon crypt dataset (GSE31235) to predict the bottom of the crypt markers independently. Standard deviation > 0.5 in human B cells and T cells (GSE24759) is used to predict the lymphocyte-specific expression. Genes expressed in human macrophages and fibroblast are predicted by computing the StepMiner threshold on the bulk datasets GSE134312 and GSE63626, respectively, which is compared to the StepMiner threshold obtained in the original human global tissue database (GSE19087, n = 1915). While both PAQR4 and PAQR5 are predicted to be expressed in top of the crypt and macrophages, PAQR5 is predicted to be expressed in fibroblasts (Supplementary Fig. 3c).

Construction of a Network of Boolean Implications. A Boolean implication network (BIN) is created by identifying all significant pairwise BIRs that are consistent in both Peters-2017 GSE36867, Table S1, and a large colorectal cancer cell line database GSE1911 (n = 1911) to predict top/bottom of the crypt marker89,93. The Boolean implication "KRT20 low ⇒ X low" is used to predict the expression of gene X at the top of crypt89. The Boolean implication "KRT20 low ⇒ X high" is used to predict the expression of gene X at the bottom of the crypt. Since the human colon tissue database (n = 1911) contains bulk tissue samples, the expression of gene X is restricted to the epithelium by filtering the gene expression in the human colorectal cell cancer database (n = 264). LGR5 correlation > 0.8 is performed in the FACS purified colon crypt dataset (GSE31235) to predict the bottom of the crypt markers independently. Standard deviation > 0.5 in human B cells and T cells (GSE24759) is used to predict the lymphocyte-specific expression. Genes expressed in human macrophages and fibroblast are predicted by computing the StepMiner threshold on the bulk datasets GSE134312 and GSE63626, respectively, which is compared to the StepMiner threshold obtained in the original human global tissue database (GSE19087, n = 1915). While both PAQR4 and PAQR5 are predicted to be expressed in top of the crypt and macrophages, PAQR5 is predicted to be expressed in fibroblasts (Supplementary Fig. 3c).

Generation of clustered Boolean implication network. Clustering was performed in the BIN to dramatically reduce the number of nodes in the network (Supplementary Fig. 1b). A clustered Boolean implication network (CRIN) was created by clustering nodes in the original BIN by following the equivalent BIRs. One approach is to build connected components in a undirected graph of Boolean equivalences. However, because of noise, the connected components become internally inconsistent. We use two genes opposite each other to identify the same connected component. In addition, the size of clusters became unusually big with almost everything in one cluster. To avoid such a situation, we need to break the component by removing the weak links. To identify the weakest links, we first computed a minimum spanning tree for the graph and computed Jaccard similarity coefficient for every edge in this tree. Ideally, if two members are part of the same
cluster they should share as many connections as possible. If they share less than half of their total individual connections (Jaccard similarity coefficient < 0.5) the edges between these clusters are deleted. The robustness of the clustering is confirmed on this network to identify the Boolean path that can distinguish normal vs IBD samples in the cohort GSE6731 (4 N, 5 UC, 7 CD). Pathway analysis of gene lists was carried out via the Reactome database and gene set enrichment analysis (GSEA). Reactome identifies signaling and metabolic molecules and organizes their relations into biological pathways and processes. Kaplan–Meier analysis is performed using lifelines python package version 0.22.8. Violin, Swarm, and Bubble plots are created using python seaborn package version 0.10.1.

**Experimental**

Reagents. All reagents were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise indicated. Goat anti-rabbit and goat anti-mouse Alexa Fluor 680 and IRDye 800 (Fab’2) were purchased from LI-COR Biosciences (Lincoln, NE). Pigeonitazone was purchased from Selleck Chemicals (Houston, TX). GW7647, GW6647, and GW6962 were purchased from Tocris Biosciences (Bristol, UK). PAR5359 was synthesized by the Yang lab, Department of Chemistry and Biochemistry, University of California San Diego.

**Synthesis of PAR5359.** Ethyl (S)-2-ethoxy-3-(4-(2-hydroxyethoxy) phenyl)propanoate (compound 1, Supplementary Fig. 6a): Ethylene carbonate (663 mg, 5.04 mmol, 3 equiv.) was added to the solution of ethyl (S)-2-ethoxy-3-(4-hydroxyphenyl) propanoate (400 mg, 1.68 mmol) and potassium carbonate (K2CO3) (695 mg, 4.95 mmol, 3 equiv.) in dry DCM (3 mL). The reaction mixture was extracted three times with EtOAc (~50 mL), the combined organics were washed with brine (~50 mL), dried over MgSO4, filtered, and concentrated in vacuo, to give the title compound 1 as a colorless oil (335 mg, 70% yield).

1H NMR (300 MHz, CDCl3) δ ppm = 7.16 (d, 2H), 6.84 (d, 2H), 4.04 (t, 2H), 4.04 (t, 2H), 3.98-3.91 (m, 3H), 3.64-3.54 (m, 1H), 3.38-3.28 (m, 1H), 2.95 (d, 2H), 2.23 (s, 1H), 1.21 (t, 3H), 1.15 (t, 3H).

**ethyl (S)-2-ethoxy-3-(4-(2-((methylsulfonyl)oxy)ethoxy)phenyl)propanoate (compound 2, Supplementary Fig. 6b):** Methanesulfonyl chloride (MsCl, 237 mg, 0.16 mL, 2.02 mmol, 1.7 equiv.) was added dropwise to an ice-cold solution of compound 1 (335 mg, 1.19 mmol) and triethylamine (TEA) (240 mg, 0.331 mL, 2.23 mmol, 2 equiv.) in dry dichloromethane (DCM) (7 mL). The reaction mixture was stirred at room temperature for 2.5 h, then diluted with ~50 mL of 1 M HCl aq solution. The aqueous layer was then extracted with DCM (50 mL x 2), the combined organic layers were washed with sequence of ~50 mL of saturated NaHCO3 aq solution, ~50 mL of water, and ~50 mL of brine. The organic layer was then dried over MgSO4, filtered, and concentrated in vacuo, to give the title compound 2 as a brown oil, which was purified by an additional chromatography (using a gradient of EtOAc in hexanes as eluent) to give the title compound 3 as a clear oil (363 mg, 75% yield). 1H NMR (300 MHz, CDCl3) δ ppm = 7.19 (d, 2H), 6.83 (d, 2H), 4.57 (t, 2H), 4.22 (t, 2H), 4.16 (t, 2H). 3.97 (q, 1H), 3.66-3.55 (m, 1H), 3.40-3.30 (m, 1H), 3.09 (s, 3H), 2.97 (d, 2H), 1.24 (t, 3H), 1.18 (t, 3H).

**ethyl (S)-3-(4-(2-(4-(4-chlorophenyl)-3,6-dihydropyridin-1(2H)-yl)ethoxy)ethyl (S)-2-ethoxy-3-(4-(2-hydroxyethoxy)phenyl)propanoate (compound 3, Supplementary Fig. 6c):** Ethylene carbonate (663 mg, 5.04 mmol, 3 equiv.) was added to the solution of ethyl (S)-2-ethoxy-3-(4-hydroxyphenyl)propanoate (400 mg, 1.68 mmol) and potassium carbonate (K2CO3) (695 mg, 4.95 mmol, 3 equiv.) in dry DCM (3 mL). The reaction mixture was extracted three times with EtOAc (~50 mL), the combined organics were washed with brine (~50 mL), dried over MgSO4, filtered, and concentrated in vacuo, to give the title compound 3 as a clear oil (189 mg, 36% yield). 1H NMR (300 MHz, CDCl3) δ ppm = 7.33 (d, 2H), 7.28 (d, 2H), 7.17 (d, 2H), 6.86 (d, 2H), 6.06 (s, br, 1H), 4.20-
4.08 (m, 3H), 3.97 (t, 1H), 3.65-3.55 (m, 1H), 3.40-3.32 (m, 1H), 3.31 (d, 2H), 2.94 (t, 4H), 2.86 (t, 2H), 2.57 (s, br, 1H), 1.23 (t, 3H), 1.16 (t, 3H). 

(S)-2-(4-ethylphenyl)-3,6-dilhydropropyridin-2(2H)-yl(ethoxy)ethyl-2-ethoxypropanoic acid (PAR3559, Supplementary Fig. 6d): The reaction was then evaporated to dryness in vacuo. The resultant solids were purified via SiO2 column chromatography (using a gradient of 4% to 6% to 10% MeOH in DCM as eluent) to give the title compound PAR3559 as a white solid (109 mg, 71% yield) H NMR (500 MHz, CD3OD-d4) δ ppm = 7.25-7.23 (d, 2H), 7.13-7.11 (d, 2H), 6.98-6.96 (d, 2H), 6.70-6.68 (d, 2H), 5.94 (t, J = 2.2 Hz, 1H), 4.15-4.13 (m, 2H), 3.74-3.73 (m, 2H), 3.60-3.59 (m, 2H), 3.38-3.32 (m, 5H), 3.05-2.98 (m, 1H), 2.74-2.71 (m, 1H), 2.63-2.55 (m, 3H), 0.84 (t, J = 7.0 Hz, 3H); 13 C NMR (126 MHz, CD3OD-d4) δ ppm = 151.8, 138.7, 139.6, 134.6, 135.7, 134.2, 132.5, 132.0, 130.6, 130.5, 128.7, 128.8, 119.0, 118.9, 116.2, 116.2, 84.6, 67.4, 64.7, 61.7, 53.3, 51.7, 40.6, 26.5, 16.3; ESI-MS: 402.1 [M+H+].

Bacteria and bacterial culture. For bacterial culture adherent Invasive Escherichia coli strain LF82 (AIEC-LF82), Citrobacter rodentium (strain DBS100), and Salmonella enterica serovar typhimurium (strain SL3144), a single colony was inoculated into LB broth and grown for 6-8 h on shaking incubator, followed by overnight culture under oxygen-limiting conditions, but without shaking, to maintain their pathogenicity as done previously88–90. Cells were infected with bacteria with indicated MOI in one of the following: 0.01, 0.1, 1, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10000-fold. In one experiment, cells were infected with bacteria at MOI = 10000-fold. DSS-exposed mice were infected with 10^8 CFU/ml of AIEC-LF82 or 10^7 CFU/ml of Citrobacter rodentium in drinking water.

DNA isolation. DNA was isolated as per standard protocols. Histological scores were scored in a blinded manner using H&E stained colonic tissue105. Briefly, paraffin-embedded tissue sections were deparaffinized and rehydrated, and endogenous peroxidase activity was blocked by incubating with 3% H2O2 for 10 min. Sections were washed with PBS and incubated for 1 h with the primary antibody. After washing with PBS, sections were incubated with biotinylated secondary antibody for 1 h. After washing with PBS, sections were incubated with ABC reagent for 30 min. After washing with PBS, reaction was visualized with DAB and counterstained with hematoxylin. Sections were dehydrated, cleared, and mounted with cover slips.

RNA extraction and real-time quantitative PCR (RT-qPCR). Total RNA was isolated using TRIzol reagent. 

RNA-seq library preparation. Sequencing libraries were prepared using the Illumina TruSeq Stranded Total RNA Library Prep Gold with TruSeq Unique Dual Indexes (Illumina, San Diego, CA)110. Libraries were amplified and sequenced on an Illumina NovaSeq 6000 by the Institute of Genomic Medicine (IGM) at the University of California San Diego. 

Cytokine assays. Cytokines including TNFa, IL6, IL1b, and IL10 were measured in cell supernatant using ELISA MAX Deluxe kits from Biologic.

Statistics and reproducibility. Experimental values are presented as the means of replicate experiments ±SEM. Statistical analyses were performed using GraphPad Prism software version 8.0 (GraphPad Software). Differences between the two groups were evaluated using Student’s t-test (parametric) or Mann–Whitney U-test (non-parametric). To compare more than three groups, one-way analysis of variance was used. Post hoc test was used. For multiple comparisons at P<0.05 were considered significant. Please see Supporting Information for details regarding sample size calculation and power analysis.

Study approval. Human subjects. Blood samples were obtained from either healthy volunteers or from IBD patients undergoing colonoscopies as part of their routine care and follow-up at UC San Diego’s Inflammatory Bowel Disease (IBD) Center. Patients were recruited and consented using a study proposal approved by the Institutional Review Board of the University of California, San Diego. Isolation of blood monocytes was carried out using an approved human research protocol (IRB# 160246) that covers human subject research at the UC San Diego HUMANOID Center of Research Excellence (CORE). The clinical phenotype and information were curated based on histopathology reports from Clinical Pathology and Chart check, followed by consultation with a specialist at UC San Diego’s IBD Center.

Animals. Animals were used for the study of UC San Diego Institutional Animal Care and Use Committee (IACUC). Adult C57BL/6 mice were acquired from Jackson Laboratories. All animals were maintained in institutional animal care. Provided with standard light–dark cycle, fed with standard laboratory chow and clean drinking water.

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

Data availability. The findings of this study are available in Supplemental Data 1. RNA sequencing data that support the findings of this study have been deposited in NCBI GEO with the accession number GSE171057. Publicly available datasets used: GSE83687, GSE73661, GSE16879, GSE59071, GSE48958, GSE16889.
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Code availability
The codes are publicly available at the following links: https://github.com/ahalago/BoNe; https://github.com/ahalago/Hegemon.
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Author contributions
P.G., D.S., S.D., and G.D.K. conceptualized, supervised, administered the project, and acquired funding to support it. G.D.K., I.M.S., M.S.A., E.V., F.U., and J.R.S. were involved in data curation and formal analysis. D.S. developed computational modeling and software for analysis and performed all computational analysis. G.D.K., I.M.S., M.S.A., E.V., and F.U. conducted animal studies for colitis. G.D.K., I.M.S., M.S.A., E.V., F.U., and S.D. performed cell and tissue analysis including qPCR, ROS, bacterial clearance, ELISA. D.T. assisted G.D.K. for qPCR and analysis. J.R.S., G.L., and J.Y. prepared PAR5359 compound. W.J.S. provided key resources for human subjects. D.S., G.D.K., and P.G. prepared figures for data visualization, wrote the original draft, reviewed and edited the manuscript. All co-authors approved the final version of the manuscript.

Competing interests
The authors declare the following competing interests: S.D., D.S., and P.G. have a patent on the methodology. Barring this, the authors have declared that no conflict of interest exists.