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Article

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ABSTRACT

A computational platform, the Boolean network explorer (*BoNE*), has recently been developed to infuse AI-enhanced precision into drug discovery; it enables querying and navigating 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 two nuclear receptors, PPARα/γ. Balanced agonism of PPARα/γ was predicted to impact macrophage processes, ameliorate colitis in network-prioritized animal models, 'reset' the gene expression network from disease to health, and achieve a favorable therapeutic index that tracked other FDA-approved targets. Predictions were validated using a balanced and potent PPARα/γ-dual agonist (PAR5359) in two pre-clinical murine models, i.e., *Citrobacter rodentium*-induced infectious colitis and DSS-induced colitis. Mechanistically, we show that such balanced dual agonism promotes bacterial clearance more efficiently than individual agonists both *in vivo* and *in vitro*; PPARα is required and its agonism is 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 and induces the anti-inflammatory cytokine, IL10. Balanced agonism achieved controlled inflammation while protecting the gut barrier and 'reversal' of the transcriptomic network. Furthermore, dual agonism effectively 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 accelerate and enhance the precision of drug discovery.

INTRODUCTION

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 [1-3]. It is also heterogeneous with complex sub-disease phenotypes (i.e., strictures, fistula, abscesses and colitis-associated cancers) [4,5]. 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 [6,7]. 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[8]. 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 [8].

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 [9]; this network differs from other computational methods (e.g., Bayesian and Differential Expression Analyses) because gene clusters here are interconnected by directed edges that represent Boolean Implication Relationships 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. Treatment efficacy was confirmed in patient-derived organoids using multivariate analyses. This AI-assisted approach provided a first-in-class epithelial barrier-protective agent in IBD and predicted Phase-III success with higher accuracy over traditional approaches [9].

Here we use the same AI-guided drug discovery pipeline, this time to identify and validate a first-in-class macrophage modulator that is predicted to restore mucosal barrier and homeostasis in IBD. 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-

120 clinical murine models and in patient-derived PBMCs. We also reveal the mechanism(s) of
121 action that enable balanced agonists of this pair of nuclear receptors to reverse some of the
122 fundamental imbalances of the innate immune system in IBD, such that immunity can be
123 achieved without overzealous inflammation.

124
125 ## RESULTS
126

127 **Development of a web-based platform for generating a 'target report card'**
128 We first developed an interactive, user-friendly web-based platform that allows the querying
129 of our Boolean network-based-IBD map [9] with just a few intuitive clicks of the mouse, even
130 for biologists (**Supplementary Fig. 1**). With the goal of enabling researchers to pick high-
131 value targets, we programmed the platform to generate a comprehensive automated 'target
132 report card' that contains predictions on five components (**Fig. 1A**): (i) *Impact on the outcome*
133 *of IBD in response to treatment*, which shows how levels of expression of any proposed target
134 gene(s) relates to the likelihood of response to therapies across diverse cohorts; (ii) *Therapeutic*
135 *index*, a computationally generated index using Boolean implication statistics which provides
136 a likelihood score of indicate whether pharmacologic manipulation of the target gene(s) would
137 lead to success in Phase III clinical trials; (iii) *Appropriateness of mouse model*, a component
138 that indicates which murine models of colitis shows the most significant change in the target
139 genes (and hence, likely to be best models to test the efficacy of any manipulation of that
140 target); (iv) *Gender bias*, a component that indicates whether the gene is differentially
141 expressed in IBD-afflicted men *versus* women; and (v) *Target tissue/cell type specificity*, which
142 shows the likely cell type where the target is maximally expressed, and hence, the cell type of
143 desirable pharmacologic action. Details of how therapeutic index is computed are outlined in
144 *Methods* and in **Supplementary Fig. 2**; it is essentially a statistical score of how tightly any
145 proposed target gene(s) associates with FDA-approved targets *versus* those that failed.
146 Similarly, details of how cell type of action is computer are outlined in *Methods* and in
147 **Supplementary Fig. 3**.

148

149

150 **PPAR α/γ dual agonists are predicted to be effective barrier-protective agents in IBD**
151 Previous work had identified a little over 900 genes in 3 clusters (Clusters #1-2-3 within the
152 IBD map; **Supplementary Fig. 1A-B**) as potentially high-value targets, all of which were
153 invariably downregulated in IBD-afflicted colons[9]. Reactome analyses showed that epithelial

tight junctions (TJs), bioenergetics, and nuclear receptor pathway (PPAR signaling) related genes that are responsible for colon 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 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, PRKAB1, 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 [9]. Here, we prioritized two more of those 17 targets, PPARA and PPARG, which encode a pair of nuclear receptors, PPARα and PPARγ. These two stress/stimuli-responsive genes are equivalent to each other and to PRKAB1, and like PRKAB1, 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**)[9]. Together, these findings imply three things: (i) that PPARA/G 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/G with agonists may restore the gut barrier. The last point is particularly important because PPARα/γ agonists are known to augment the expression of PPARA/G, and depletion of either reduced the expression of the other [10].

Noteworthy, while the role of PPARγ in colitis has been investigated through numerous studies over the past 3 decades [11-13] (**Table 1**), the role of PPARα has been contradictory (**Table 2**), and their dual agonism has never been explored. PPARγ agonists undeniably ameliorate DSS-induced colitis [13-15]. 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 [16] [17] [18] [19]. Furthermore, Phase I and II clinical trials with PPARγ agonists either alone [20,21] or in combination with mesalamine [22] 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 [23,24]. Intriguingly, and of

187 relevance to this work, the addition of PPARα-agonistic activity to PPARγ or δ agonists have

188 led to a higher safety profile, leading to their development for use in many diseases, including

189 type 2 diabetes, dyslipidemia and non-alcoholic fatty liver disease [25].

190

An automated target 'report card' for PPARA/G in IBD

192 We next generated an automated target report card for PPARA/G. High levels of both PPARs

193 were sufficient to distinguish healthy from IBD samples, not just in the test cohort that was

194 used to build the IBD-map (ROC AUC of 0.74; **Fig 1B;** see also **Supplementary Fig. 2A-D**),

195 but also in four other independent cohorts with ROC AUC consistently above 0.88 (**Fig 1C**).

196 High levels of both PPARs also separated responders from non-responders receiving TNFα-

197 neutralizing mAbs, GSE16879, E-MTAB-7604 or Vedolizumab that block the α4β7 integrin

198 to prevent selective gut inflammatory, GSE73661 (ROC AUC 0.63-0.89, **Fig 1D**), inactive

199 disease from active disease (two independent cohorts ROC AUC above 0.93; **Fig 1D**), and

200 quiescent UC that progressed, or not to neoplasia (ROC AUC=1.00 for qUC *vs*. nUC; **Fig 1D**).

201 High level of PPARA/G was also able to distinguish healthy from diseased samples in diverse

202 murine models of colitis (**Fig 1E**); but such separation was most effectively noted in some

203 models (*Citrobacter* infection-induced colitis, adoptive T-cell transfer, TNBS and *IL10*^{-/-}), but

204 not in others (DSS, and *TNFR1/2*[-/-]). These findings imply that therapeutics targeting these two

205 genes are best evaluated in the murine models that show the most consistent decrease in the

206 gene expression, e.g., *Citrobacter* infection-induced colitis, adoptive T-cell transfer, TNBS,

207 etc. This was intriguing because the majority (~90%) of the published work on PPARA/G

208 agonists have been carried out in DSS models (**Table 1-2**).

209 The expression profile of the target genes in the gut mucosa revealed that PPARA/G

210 are co-expressed at the highest levels in the crypt top epithelial cells and macrophages (**Fig 1F;**

211 **Supplementary Fig. 3**), predicting that dual agonists are likely to preferentially act on these

212 two cell types. The therapeutic index was below 0.1 for both genes (0.06 for PPARA and 0.04

213 for PPARG; **Fig 1G; Supplementary Fig. 2E-F**), aligned well with two other FDA-approved

214 targets shown on the line graph (ITGB1, 0.046 and JAK2, 0.032). The index, which is a

215 statistical measure of the strength of association of PPARA/G with genes that are targets of

216 FDA-approved drugs that have successfully moved through the three phases of drug discovery

217 (i.e., proven efficacy, with acceptable toxicity). A low number is indicative of a high likelihood

218 of success in Phase-III trials. Finally, PPARA/G expression was downregulated to a similar

219   extent in both genders (**Fig 1H**), predicting that therapeutics targeting them are likely to be

220   effective in both men and women.

221

222   **Rationalization of PPARA/G as targets in IBD**

223   Because proteins, but not transcripts, are the targets of therapeutic agents, the impact of

224   therapeutics is translated to cellular processes *via* protein-protein interaction (PPI) networks,

225   a.k.a interactomes. We next asked how dual agonists of PPARα/γ might impact cellular

226   pathways and processes. A PPI network visualized using PPARα/γ as 'query/input' and the

227   interactive STRING v11.0 database (https://string-db.org/) as a web resource of known and

228   predicted protein–protein interactions curated from numerous sources, including experimental

229   data, computational prediction methods and public text collections. PGC1a (a product of the

230   gene PPARGC1A) was a common interactor between the two PPARs (**Fig 2A**). We noted that

231   PGC1a also happens to be a major component within the PPARα/γ functional network, serving

232   as a central hub for positive feedback loops between the PPARs and their biological function

233   (**Fig 2B**), i.e., mitochondrial biogenesis, DNA replication and energetics (electron transport

234   chain and oxidative phosphorylation). When we analyzed the functional role of the

235   interactomes of PPARα/γ we noted that indeed both interactomes converged on lipid

236   metabolism, mitochondrial bioenergetics and circadian processes (**Fig 2C**). These findings are

237   consistent with the finding that PPARA/G and PPARGC1A are located within clusters #1-2-3

238   and all of them are predicted to be progressively and simultaneously downregulated in IBD

239   samples (**Fig 2D;** based on the IBD map, **Supplementary Fig. 1**).

240

241   **PPARA/G is downregulated in Ulcerative colitis and Crohn's Disease**

242   Previous work demonstrated that both PPARα and PPARγ are highly expressed in the colon

243   [26]. They have also shown that both PPARα/γ protein and mRNA are downregulated (by ~60%)

244   in active UC and the expression of PPARγ was significantly associated with disease activity [27].

245   This impaired expression was found in both inflamed and noninflamed areas [28].

246   Polymorphisms have also been detected in PPARγ; while some studies found those to be

247   associated with an increased risk for CD [29,30], others found no evidence suggesting any form

248   of association with an increased disease risk [31]. We collected endoscopically obtained biopsies

249   from the colons of healthy (n = 7) and IBD (n = 14 and 14 of UC and CD, respectively) patients

250   and assessed the levels of transcripts for PPARA/G and PPARGC1A by qPCR (**Fig 2E**). We

251   confirmed that all three transcripts were significantly downregulated in UC and CD samples

compared to healthy; both PPARG and PPARGC1a were more significantly downregulated in CD compared to UC (**Fig 2F**). 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 Fig. 4-5**). While both PPARA and PPARG are in cluster #2 in the UC map, PPARG and PPARA are in separate clusters, clusters 2 and 6, respectively, in the CD map (**Supplementary Fig. 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 NFkB 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 both targets are downregulated in IBD and that they may regulate key pathophysiologic processes that are vital for cellular homeostasis.

Synthesis and validation of PAR5359, a potent and specific PPAR α/γ dual agonist

We noted that all commercially available PPAR α/γ dual agonists lack 'balanced' agonistic activities (**Table 3**) [32,33]. Drugs that have fallen aside due to safety concerns also lack balanced agonism; most of them are more potent on PPARγ than on PPARα by a log-fold (**Table 3**). All these PPAR α/γ dual agonists have been withdrawn due to safety concerns [25], 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 PPARγ [34]. 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, PAR5359 [35,36] (see **Table 4**). In the absence of commercial sources or well-defined methods on how to synthesize this molecule, we generated PAR5359 in 4 synthetic steps (see details in *Methods*) and confirmed its specificity and the comparable agonistic activities using pure single PPARα [GW7647 [37]] or PPARγ [Pioglitazone [38]] agonists as controls (**Supplementary Fig. 6**). 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 pre-clinical models. PPARα/γ's role (or the role of their agonists) in protecting the gut barrier has been evaluated primarily in DSS-induced colitis (**Table 1, 2**). However, *BoNE* prioritized other models over DSS, many of which accurately recapitulate the PPARα/γ–downregulation that is observed in the barrier-defect transcript signature in human IBD (**Fig. 1E**). 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 in IBD [39-41]. Furthermore, this model requires the balanced action of macrophages (a cell line predicted to be the preferred cell type target; **Fig. 1F**) to promote bacterial clearance and healing [42].

Colitis was induced by oral gavage of *C. rodentium* and mice were treated daily with the drugs *via* the intraperitoneal route (see workflow detailed in **Fig. 3A, Supplementary Fig. 7A**). The dose for each drug was chosen based on their EC_{50} on their respective targets so as to achieve equipotent agonistic activities (**Supplementary Fig. 6**). 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. 3B**). Compared against all other conditions, PAR5359-treated mice cleared the gut bacterial load significantly and rapidly (**Fig. 3B-D**). *Citrobacter* infection was associated with significant epithelial damage and profuse infiltration of inflammatory cells and edema by day 7 (**Supplementary Fig. 7B**) most of which resolved by day 18 (DMSO control; **Fig. 3E**). 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. 7B**). 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. 3E**), 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 infection and resolved inflammation. PAR5359 also reduced spleen inflammation as evidenced by a decreased spleen weight and length compared to vehicle control (**Supplementary Fig. 7C-F**). The spleens of mice treated with DMSO, PPARα-alone agonist, GW7647 and PPARγ-alone agonist, Pioglitazone showed black-discoloration,

318 presumably infarcts (arrows, **Supplementary Fig. 7C, 7E**). Notably, the spleens of mice
319 treated with PPARα-alone agonist, GW7647, showed a significant increase in spleen length
320 (**Supplementary Fig. 7D, 7F**).

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

325

326 **PAR5359 resists *C. rodentium*-induced gene expression changes in the colon**
327 We next sought to determine if pharmacologic augmentation of PPARA/G was sufficient to
328 influence the entire transcriptomic network *via* the invariant Boolean implication relationships
329 between the gene clusters. RNA sequencing (RNA-seq) studies were carried out on the *C.*
330 *rodentium*-infected colons in each treatment group (**Fig. 3A**). As expected, downregulation of
331 genes in clusters #1-2-3 of the IBD-map was significant in infected (DMSO) vs uninfected
332 controls, indicative of network shift from health towards disease (**Fig. 3F**); PAR5359-treatment
333 resisted such shift.

334   Pre-ranked gene set enrichment analyses (GSEA) based on pair-wise differential
335 expression analysis showed that when compared to DMSO control, dual PPARα/γ agonism
336 with PAR5359, but not individual agonists Pioglitazone or GW7647 was able to significantly
337 preserve epithelial junction signatures (both tight and adherens junctions) and balance
338 macrophage processes (compare **Fig. 3G** with **Supplementary Fig. 8A**). These findings are in
339 keeping with the predictions that epithelial cells and macrophages maybe the primary cell type
340 of action for dual PPARα/γ agonists. Comparison of all treatment cohorts against each other
341 revealed that although both PAR5359 and Pioglitazone were superior to GW7647 in
342 maintaining some epithelial processes (differentiation, tight junctions) and macrophage
343 processes (**Supplementary Fig. 8B-E**), PAR5359 emerged as the only group that maintained
344 homeostatic PPAR signaling in nature and extent as uninfected control (**Supplementary Fig.**
345 **8F**).

346   Taken together, these findings suggest that dual agonists of PPARα/γ are sufficient to
347 either resist network shift and/or reverse the disease network in the setting of colitis. They also
348 offer clues suggestive of epithelial and macrophage processes, two key cellular components of
349 innate immunity in the gut lining as major mechanisms. These transcriptome wide impacts

350   suggest that PPARα/γ dual agonist PAR5359 is superior in restoring colon homeostasis in *C.*

351   *rodentium*-induced colitis than either PPARα or PPARγ agonist used alone.

352

353   **PAR5359 ameliorates DSS-induced colitis**

354   It is well known that no single mouse model recapitulates *all* the multifaceted complexities of

355   IBD [43,44]. Because almost all studies evaluating PPARα/γ-modulators have been performed on

356   the DSS-induced colitis model (**Table 1-2**), we asked whether the PPARα/γ dual agonist

357   PAR5359 can ameliorate colitis in this model. Mice receive intrarectal DMSO vehicle control

358   or PAR5359 while receiving DSS in their drinking water (**Supplementary Fig. 9A**). Disease

359   severity parameters, i.e., weight loss, disease activity index, shortening of the colon and histology

360   score were significantly ameliorated in the PAR5359-treated group (**Supplementary Fig. 9A-E**).

361   These findings show that the PPARα/γ-dual agonist, PAR5359, is also effective in DSS-

362   induced colitis. It is noteworthy that the PAR5359 dual agonist offered protection in the DSS-

363   model, because prior studies using the same model have demonstrated that PPARα-agonists

364   worsen [45,46], and that the PPARγ-agonists ameliorate colitis [47-49] (see **Table 1-2**).

365

366   **PAR5359 promotes bacterial clearance with controlled production of ROS and**

367   **inflammation in peritoneal macrophages**

368   Because the intestinal macrophages play a crucial role in maintaining the integrity of the gut

369   epithelial barrier and in the control of pathogen invasion by triggering an appropriate immune

370   response, the fine-tuning macrophage is essential in maintaining homeostasis and, potentially,

371   the development of IBD. To explore the mechanism(s) by which dual, but not individual, PPAR

372   agonists may alter macrophage response to microbes, we incubated macrophages treated or not

373   with the drugs and challenged them with CD-associated adherent invasive *E. coli (AIEC)-*

374   *LF82;* this strain, originally isolated from a chronic ileal lesion from a CD patient [50]. As for the

375   source of macrophages, we isolated metabolically active primary murine peritoneal

376   macrophages using Brewer thioglycolate medium using established protocols [51,52]. These

377   macrophages are known to have high phagocytic activity [51] (**Fig. 4A**). Thioglycolate-induced

378   peritoneal macrophages (TG-PMs) were lysed, and viable intracellular bacteria were counted

379   after plating on an agar plate. Pre-treatment with 1 μM PAR5359 and an equipotent amount of

380   GW7647 (PPARα agonist) promoted bacterial clearance and reduced the bacterial burden

381   when compared to vehicle control (**Fig. 4B**). By contrast, pre-treatment with Pioglitazone

382   (PPARγ agonist) inhibited bacterial clearance; notably, bacterial burden was significantly

383 higher at both 3 h and 6 h after infection (**Fig. 4B**). Reduced clearance of microbes in the latter

384 was associated also with reduced cellular levels of reactive oxygen species (ROS) (**Fig. 4C**);

385 oxidative burst and induction of ROS is key component for effective bacterial killing [53,54].

386 PAR5359 did not interfere with the production of microbe-induced ROS, and the PPARα

387 agonist (GW7647) was permissive to ROS induction (in fact, even induces it over bacteria-

388 alone control) during initial time points after infection (**Fig. 4C**).

389 These patterns of microbial clearance and cellular ROS were associated also with the

390 expression of cytokines, as determined by qRT-PCR analyses (**Fig. 4D**). As expected, infection

391 of TG-PM with *AIEC*-LF82 induced *il1β, il6, tnfα* and *il10*. PAR5359 significantly and

392 selectively suppressed the expression of the pro-inflammatory cytokines *il1β, il6* and *tnfα* (but

393 not the anti-inflammatory cytokine, *il10*) (**Fig. 4D**). By contrast, the PPARγ-specific agonist

394 pioglitazone significantly and indiscriminately suppressed all the cytokines, while there was

395 no effect of the PPARα specific agonist GW7647 (**Fig. 4D**). ELISA studied on the supernatant

396 media further confirmed these findings (**Fig. 4E**), demonstrating that the effects in gene

397 expression were also translated to the levels of secreted cytokine protein released by the

398 macrophages in the supernatant.

399 It is noteworthy that for the most part, the qPCR (**Fig. 4D**) and ELISA (**Fig. 4E**) studies

400 matched, except *il10*; although pioglitazone appeared to suppress *il10* mRNA, it did not

401 suppress the levels of the *il10* protein, suggesting that PPARγ-agonist is sufficient for an overall

402 anti-inflammatory phenotype. Similarly, although GW7647 appeared to not affect *il10* mRNA,

403 it suppressed the levels of the *il10* protein, suggesting that PPARα agonist is sufficient for an

404 overall pro-inflammatory phenotype. Similar findings were also observed in the case of another

405 enteric pathogen, *S. enterica*, i.e., unlike the dual agonist, neither PPARα nor PPARγ agonist

406 could enhance bacterial clearance with a modest induction of pro-inflammatory cytokines

407 (significantly lower than control) and, yet, had no impact on anti-inflammatory IL10

408 production (**Supplementary Fig. 10**).

409 Taken together, these results show that- (i) PPARγ-agonism induces 'tolerance' by

410 suppressing inflammation, inhibiting ROS production and delaying bacterial clearance; (ii)

411 PPARα-agonism enhances the induction of inflammation and ROS, and promotes bacterial

412 clearance; and (iii) PPARα/γ-dual agonism strikes a somewhat balanced response. The latter

413 suppresses proinflammatory cytokines without suppressing anti-inflammatory cytokine *il10*,

414 and is permissive to inflammation and ROS induction that is optimal and sufficient to promote

415 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 (**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. 5A**). As expected, LPS induced the cellular levels of ROS (**Fig. 5B**) and inflammatory cytokines (**Fig. 5C-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. 5B-D**). By contrast, inhibition of PPARγ did not interfere with either response (**Fig. 5B-D**). Simultaneous inhibition of both PPARα and PPARγ mimicked the cellular phenotypes in the presence of PPARα-inhibitors (**Fig. 5B-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 PAR5359 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 (**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. 6A** to isolate PBMCs. Pre-treatment for 30 min with vehicle or PAR5359 was followed by infection for 1h. 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. 6B, 6E**), indicative of either defective clearance and/or increased permissiveness to bacterial replication within the cells is limited to the CD. *Second*, pre-treatment with PAR5359 could improve clearance significantly (**Fig. 6C-D, 6F-G**). These results indicate that bacterial clearance is delayed in PBMCs of patients with CD and that PPARα/γ dual agonism with PAR5359 can reverse that defect. The possibility that such reversal could be due to any direct bacteriostatic/-cidal effect of PAR5359 agonist was ruled

449 out (see bacterial viability assay in **Supplemental Fig. 11**). Our findings demonstrate that
450 bacterial clearance is delayed primarily in CD and not UC are in keeping with the fact that
451 delayed bacterial clearance from inflamed tissues (up to ~4-fold) is uniquely observed in CD
452 [55]. These findings are also in keeping with our own observation that the downregulation of
453 PPARG/PPARGC1A was more prominent in patients with CD (**Fig. 2E-F**). In fact, delayed
454 clearance is one of the major reasons for persistent inflammation and disease progression
455 among patients with CD [55,56].

456
457 ## DISCUSSION
458 Barrier-protection/restoration is the treatment endpoint for all clinical trials in IBD
459 therapeutics; however, despite much success in the development of anti-inflammatory
460 therapies [7,57], barrier-protective therapeutics in IBD have been slow to emerge [58]. Here we
461 report the discovery of an effective barrier-protective therapeutic strategy in IBD identified
462 using an AI-guided navigation framework (summarized in **Fig 7**). *First*, a network-based drug
463 discovery approach [9] was used to identify, rationalize and validate dual and balanced agonism
464 of PPARα/γ (but not one at a time) is necessary for therapeutic success. *Second*, we provided
465 evidence in the form of proof-of-concept studies (in two different pre-clinical murine models)
466 demonstrating that the simultaneous and balanced agonistic activation of the pair of PPARs as
467 an effective barrier protective strategy in IBD. *Third*, we demonstrate that macrophages are
468 one of the primary target cell type of this therapeutic strategy; dual agonist (but not single) was
469 permissive to the induction of macrophage responses expected for optimal immunity without
470 overzealous inflammation. There are <u>*three*</u> notable takeaways from this study, which are
471 unexpected observations and/or insights that fill key knowledge gaps in the fields of – (a)
472 network medicine, (b) IBD therapeutics and (c) macrophage biology.

473 *First*, with regard to network medicine, the AI-guided approach we used here differs
474 from the current practice in three fundamental ways: 1) Unlike most studies that prioritize
475 targets based on Differential Expression Analysis (DEA, or integrated DEA) or Bayesian
476 approaches, target identification and prediction, this work was guided by a Boolean implication
477 network of continuum states in *human* disease [9]; 2) Instead of conventional approaches of trial-
478 and-error, intuitive guess and/or knowledge-based prioritization of study models (animal or
479 cell-type of action), target validation in network-rationalized animal and cell-type models that
480 most accurately recapitulate the role of the target(s) during disease progression; 3) Inclusion of
481 human pre-clinical model (patient-derived PBMCs) for target validation, inspiring the concept
482 of *Phase '0' trials* that have the potential to personalize the choice of therapies. The combined

483 synergy of these approaches validates a first-in-class macrophage modulator in addressing the

484 broken gut barrier in IBD.

485   The impact of using such an approach is 4-fold: (i) Because the network approach used

486 here relies on the fundamental invariant Boolean implication relationships between genes, and

487 their patterns of changes in expression between healthy and IBD samples, such 'rule of

488 invariant' implies that any given relationship and/or change in expression pattern annotated

489 within the network *must* be fulfilled in every IBD patient. By that token, targets/drugs

490 prioritized based on this network is expected to retain efficacy beyond inbred laboratory mice,

491 into the heterogeneous patient cohorts in the clinic. (ii) This AI-guided approach not just helped

492 compute pre-test probabilities of success ("*Therapeutic Index*"), but also helped pick models

493 that are most insightful and appropriate to demonstrate therapeutic efficacy (e.g., *Citrobacter*

494 *rodentium* infection-induced colitis) and to pinpoint the cell type and mechanism of action

495 (microbial clearance by macrophages). This is noteworthy because the conventional approach

496 in studying PPARs has been limited to the use of DSS-induced colitis (see **Table 1-2**), which

497 has often given conflicting results (see **Table 2**). In fact, without the use of the *Citrobacter*

498 *rodentium* infectious colitis model, the deleterious effects of PPARγ agonists would have been

499 overlooked. (iii) Having a computational framework improves precision in target

500 identification; it is because of the emergence of the two PPARs (alongside their positive

501 feedback regulator, PGC1a) within our network, we rationalized their dual agonism as a

502 preferred strategy (over single) and our experiments validated that prediction both *in vivo* and

503 *in vitro*. This is noteworthy because conventional approaches have demonstrated a protective

504 role of PPARγ agonists and a conflicting (both protective and exacerbating) role of PPARα in

505 IBD [46,59-61]; the advantage of dual agonism has neither been rationalized nor tested. (iv) The

506 'target report card', like the one shown here, is a project navigation tool that is geared to

507 streamline decision-making (i.e., which genes, which animal models, which cell type/cellular

508 process, what is the likelihood of success, etc.), which in turn should reduce attrition rates,

509 waste and delays; the latter are well-recognized flaws in the current process of drug discovery.

510   *Second*, regarding IBD therapeutics, our studies demonstrate that single or unbalanced

511 combinations of PPAR agonists are inferior to dual/balanced agonists. Conventional and

512 reductionist approaches have inspired numerous studies with single PPAR agonists over the

513 past decade (**Tables 1-2**). However, given the devastating side effects of most single or

514 unbalanced PPARα/γ agonists (**Table 3**), translating to the clinic beyond a Phase II trial [20,62,63]

515 has not been realized. Because the therapeutic index for the dual PPARα/γ agonists matches

516   that of other FDA-approved targets/drugs, it is predicted that barring unexpected side effects,

517   dual PPAR agonists are likely to be effective as barrier-protective agents. As for side effects,

518   we noted is that balanced PPARγ/α agonists are rare; while all dual PPARα/γ agonists that have

519   been discontinued due to side effects happen to be either single (only PPARγ) or 'unbalanced'

520   (PPARγ >> PPARα agonistic activity), the newer generation formulations that are currently in

521   the clinical trial have a reversed agonistic potency (PPARα >> PPARγ agonistic activity) (see

522   **Table 3**). Because macrophage responses require finetuning (discussed below), our studies

523   show how unopposed agonism of either PPARγ or PPARα is harmful and can

524   impair/dysregulate the way macrophages respond when microbes breach past the gut barrier.

525   It is possible that many of the side effects of the discontinued thiazolidinediones are due to

526   their inability to achieve that 'optimal' spectrum of macrophage function.

527   *Third*, when it comes to macrophage biology, this work sheds some unexpected and

528   previously unforeseen insights into the role of the PPARs in the regulation of macrophage

529   processes. Extensively studied for over ~3 decades, PPARs are known to regulate macrophage

530   activation in health and disease [64]. Targeting PPARs as a host-directed treatment approach to

531   infectious/inflammatory diseases appears to be a sound strategy because they regulate

532   macrophage lipid metabolism, cholesterol efflux, inflammatory responses (ROS and cytokine

533   production), apoptosis, and production of antimicrobial byproducts [65]. We found that

534   unopposed PPARγ activation suppresses bacterial clearance and blunts the induction of

535   proinflammatory (but not anti-inflammatory, IL10) cytokines and ROS in response to infection

536   both *in vivo* and *in vitro*. In other words, and consistent with prior reports, PPAR γ activation

537   suppressed inflammation at the cost of impairing immunity. Our findings are in keeping with

538   the findings of a systematic review and meta-analysis of 13 long-term randomized controlled

539   trials that involved 17,627 participants (8,163 receiving PPARγ agonists and 9,464 receiving

540   control drugs)[66]. Long-term (~1–5.5 y) use of PPARγ agonists increases the risk of pneumonia

541   or lower respiratory tract infection significantly, some of which result in hospitalization,

542   disability, or death[66]. In the case of PPARα, unopposed activation-induced ROS and

543   proinflammatory cytokines and accelerated bacterial clearance. Inhibitor studies further

544   confirmed that PPARα was required for these responses (**Fig 5**). These findings are in keeping

545   with others' showing that PPARα, but not PPARγ is required for NADPH-induced ROS

546   formation both in human and murine macrophages [67]. PPARα agonists induce the expression

547   of NADPH oxidase subunits p47(phox), p67phox, and gp91phox, which are all essential

548   functional components of NADPH complex [67]. Dual and balanced PPARα/γ agonism enhanced

549 bacterial clearance with only a moderate induction of proinflammatory cytokines or ROS. Such
550 a response ensures that the macrophage functions within a 'goldilocks' zone, mounting
551 inflammation that is just sufficient for microbial clearance and immunity. In our analysis, the
552 only other PPAR-related gene within the IBD network, i.e., PGC1a, and its role within the
553 PPARα/γ axis suggests that the intricate network of forward feedback loops orchestrated by
554 PGC1a may be critical for achieving the critical balance between immunity and inflammation,
555 which is a key outcome of the dual PPARα/γ agonists.

556 Because previous studies using cell-specific gene depletion have indicated that the
557 barrier-protective role of PPARγ may be mediated *via* cells other than the macrophages [48],
558 namely, the T cells [68] and the epithelial cells [69], it is possible that the dual PPARα/γ agonists
559 also act on those cells, promoting bacterial clearance and balancing cellular bioenergetics, ROS
560 and cytokine production, in manners similar to that we observe in macrophages.

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

568
569 **AUTHOR CONTRIBUTIONS**

Role	Authors
Conceptualization; Supervision; Project administration • Conceptualization (PG and GDK) • Supervision (PG, SD, DS) • Project Administration (PG)	PG, DS, SD and GDK
Investigation; Methodology; Data curation, Formal Analysis • Animal studies (GDK, VC, MSA, IMS, FU) • Cell and tissue analysis (qPCR, ROS, bacterial clearance, ELISA)- (GDK, VC, MSA, IMS, DT, FU, SD) • Computational modeling (DS) • Computational analysis (DS)	GDK, VC, MSA, EV, IMS, DT, FU, JRS, SD
Funding acquisition	DS, SD, PG
Resources	JRS, GL, JY, WJS
Software	DS
Visualization; Writing – original draft; Writing – review & editing	GDK, DS, PG

586 **CODE AVAILABILITY**
587 The codes are publicly available at the following links: https://github.com/sahoo00/BoNE;
588 https://github.com/sahoo00/Hegemon

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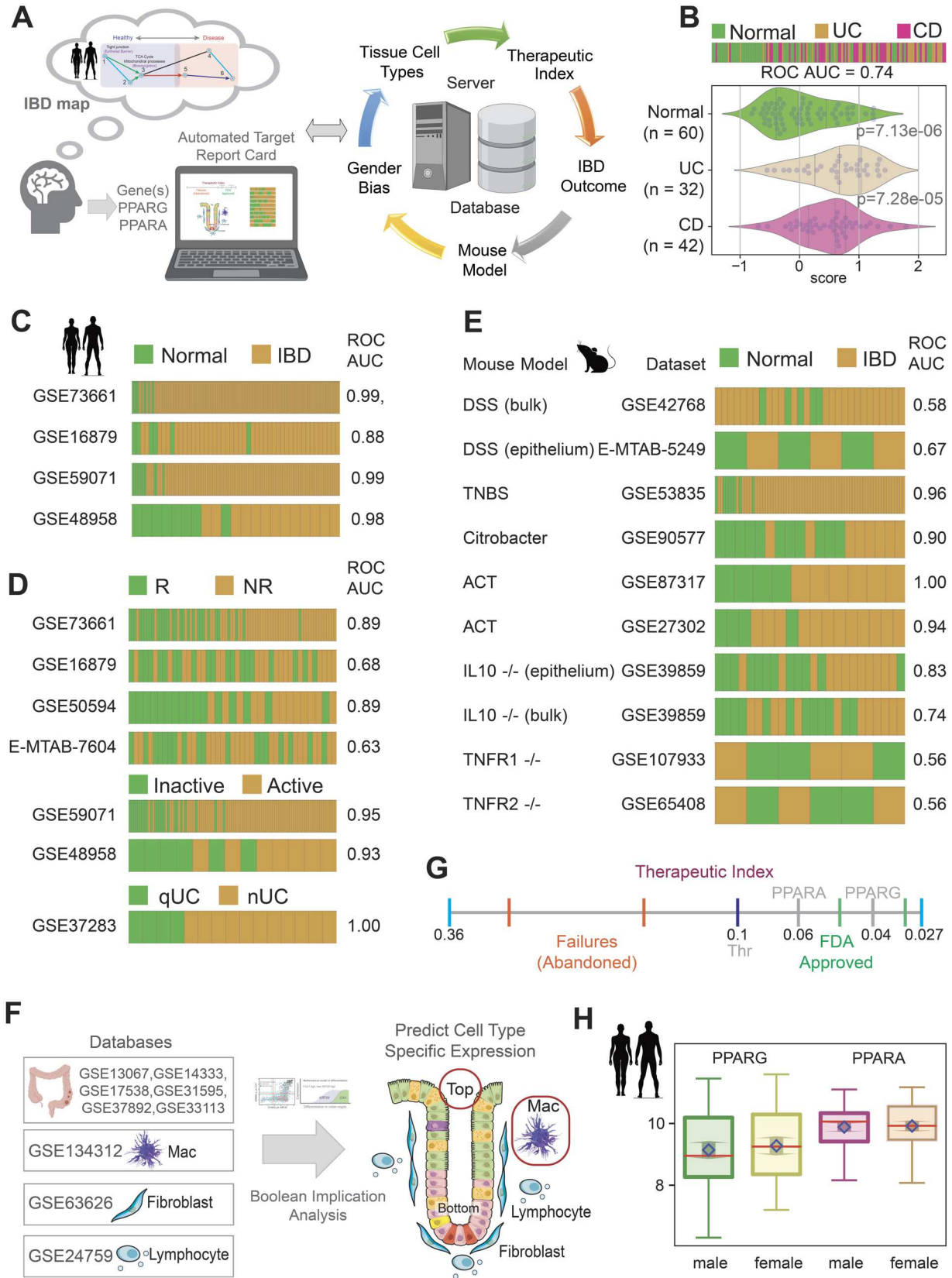
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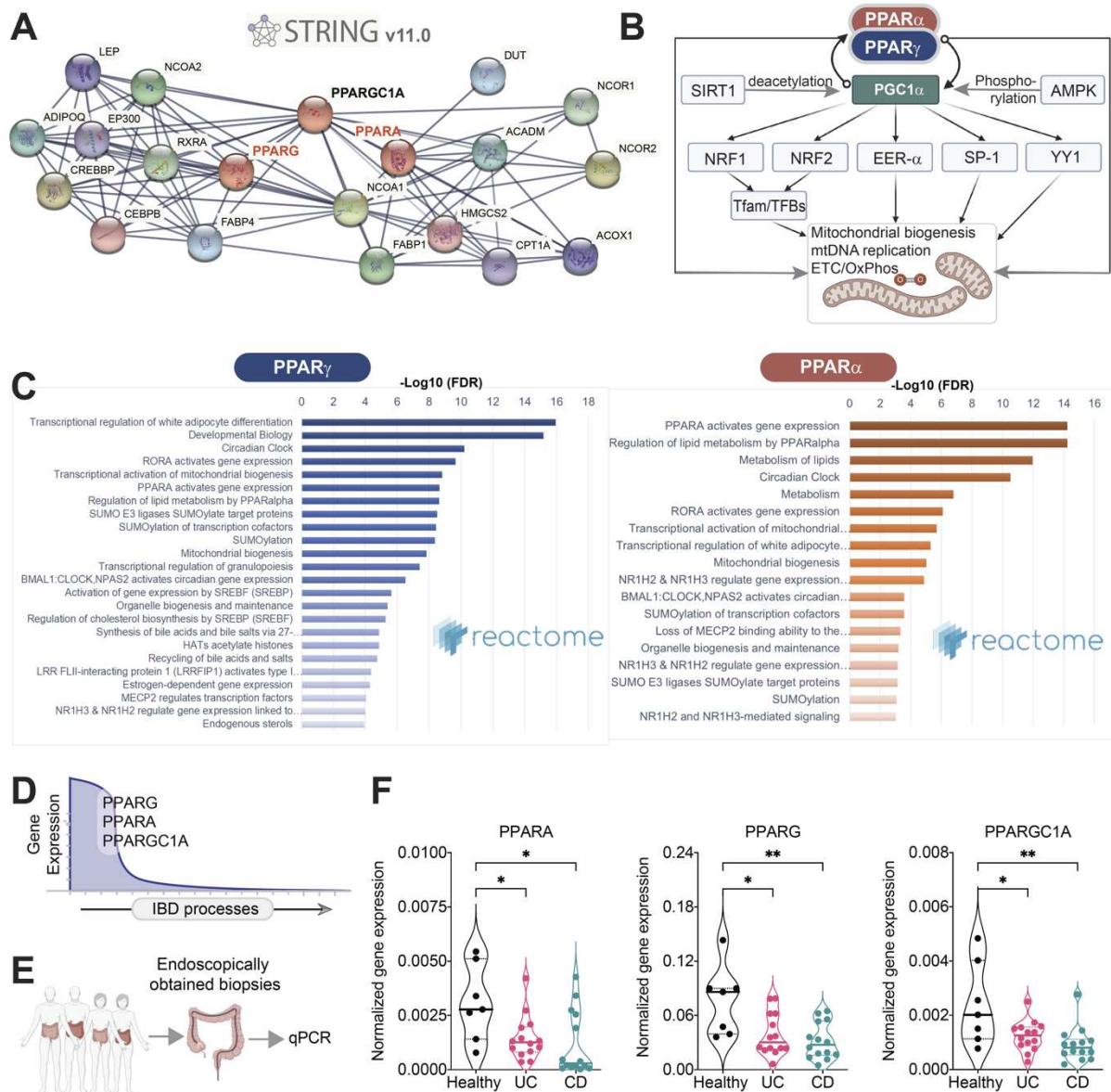
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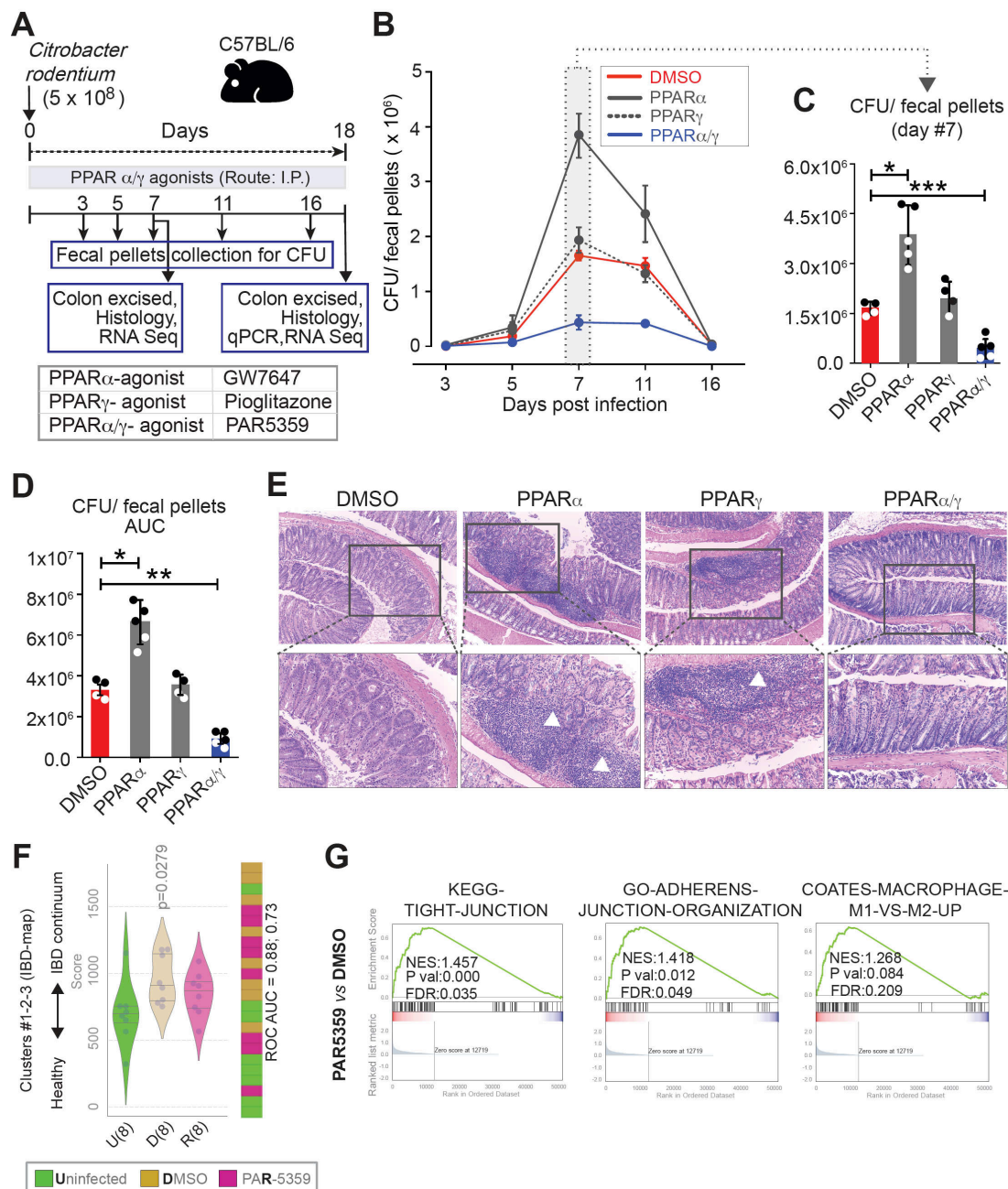793
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FIGURES AND LEGENDS

Figure 1: Network-rationalized target identification and study design. (A) Schematic displays the overall computationally guided study design. An interactive web-based platform allows the querying of paths of gene clusters in the IBD map [9; see **Supplementary Fig. 1**] to pick high-value targets with a few mouse clicks and

801 generate a comprehensive automated target 'report card'. The components of a 'target report card' is shown
802 (*right*): predicted 'therapeutic index' (likelihood of Phase III success), IBD outcome (prognostic potential in UC
803 and/or CD), network-prioritized mouse model, estimation of gender bias and predicted tissue cell type of action.
804 (**B-H**) Components of a target report card for PPARA and PPARG are displayed. Bar plot (B; top) displays the
805 rank ordering of normal vs ulcerative colitis (UC) /Crohn's Disease (CD) patient samples using the average gene
806 expression patterns of the two genes: PPARG/PPARA. ROC-AUC statistics were measured for determining the
807 classification strength of normal vs IBD. Bar plots (B; top) and violin plots (B; bottom) display the differences in
808 the average expression of the two genes in normal, UC and CD samples in the test cohort used to build the IBD-
809 map. Bar plots in panel C-D show the rank ordering of either normal vs IBD samples (C) or responder vs non-
810 responder (R *vs*. NR; D), or active vs inactive disease, or neoplastic progression in quiescent UC (qUC *vs*. nUC;
811 D) across numerous cohorts based on gene expression patterns of PPARG and PPARA, from high to low, left to
812 right. Classification strength within each cohort is measured using ROC-AUC analyses. Bar plots in panel E show
813 the rank ordering of either normal vs IBD samples across numerous published murine models of IBD based on
814 gene expression patterns of PPARG and PPARA as in D. ACT = adoptive T cell transfer. Classification strength
815 within each cohort is measured using ROC-AUC analyses. Bulk = whole distal colon; epithelium = sorted
816 epithelial cells. Schematic in F summarizes the computational prediction of the cell type of action for potential
817 PPARA/G targeted therapy, as determined using Boolean implication analysis. GSEID# of multiple publicly
818 available databases of the different cell types and colorectal datasets used to make sure predictions are cited. Red
819 boxes/circles denote that PPARA/G-targeted therapeutics are predicted to work on monocytes/macrophages and
820 crypt-top enterocytes. Computationally generated therapeutic index (see *Methods*) is represented as a line graph
821 in G. The annotated numbers represent Boolean implication statistics. PPARA and PPARG align with FDA
822 approved targets on the right of threshold (0.1). Two FDA approved targets (green; ITGB1, 0.046; JAK2, 0.032),
823 two abandoned targets (red; SMAD7, 0.33; IL11, 0.16), PPARA (grey, 0.064), PPARG (grey, 0.04), and the
824 threshold (black, 0.1) are shown in the scale. Box plot in panel H shows that the level of PPARA/G expression is
825 similar in the colons of both genders in health and in IBD, and hence, PPARα/γ-targeted therapeutics are predicted
826 to have little/no gender predilection.
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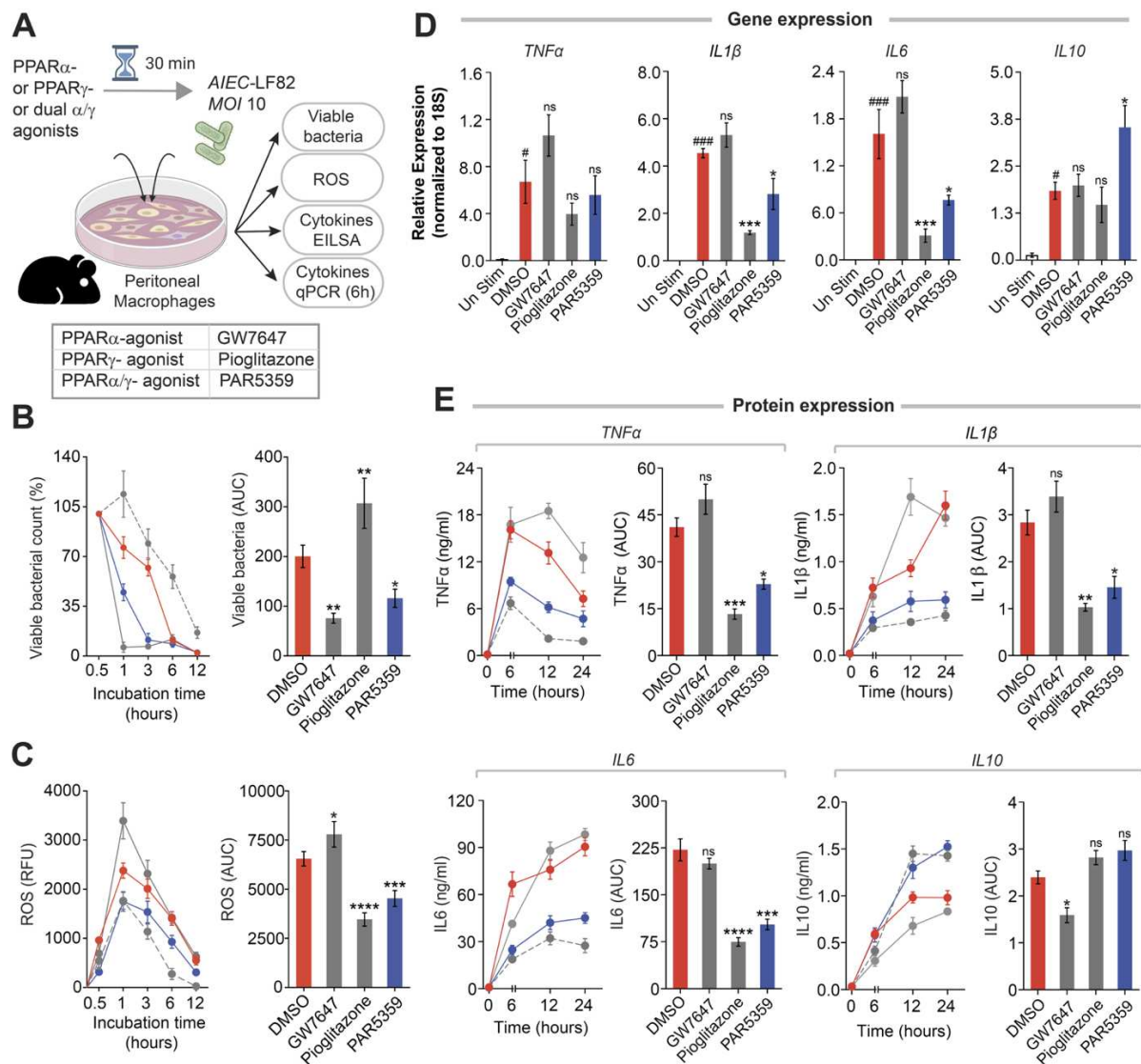
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857 **Figure 2: Rationalization of PPARα and PPARγ as targets in IBD.**
858 **(A)** A protein-protein interaction network (i.e., interactomes) for PPARα and PPARγ, generated using STRING
859 v.11 (https://string-db.org). **(B)** Schematic summarizing the roles of PPARα, PPARγ and PGC1α on mitochondria
860 biogenesis and function (based on). PGC1-α emerges as a critical hub for forward feedback loops. **(C)** Reactome
861 pathway analyses (www.reactome.org) on PPAR-α and PPAR-γ interactomes in A show convergence on
862 metabolism, mitochondria bioenergetics and the circadian clock. **(D)** Graphical visualization of the predicted
863 changes in the expression of PPARA (PPAR-α), PPARG (PPAR-γ) and PPARGC1A (PGC1-α) genes during the
864 progression of IBD processes (indicated with an arrow). **(E)** Schematic showing validation workflow; the
865 expression of PPARA, PPARG and PPARGC1A transcript levels were assessed in the ileum/colon biopsies of
866 IBD patients (UC=14 and CD= 14)) or healthy controls (n=7). **(F)** Violin plots display the qPCR results in E.
867 Results are displayed as mean ± SEM. Significance was tested using one-way ANOVA followed by Tukey's test
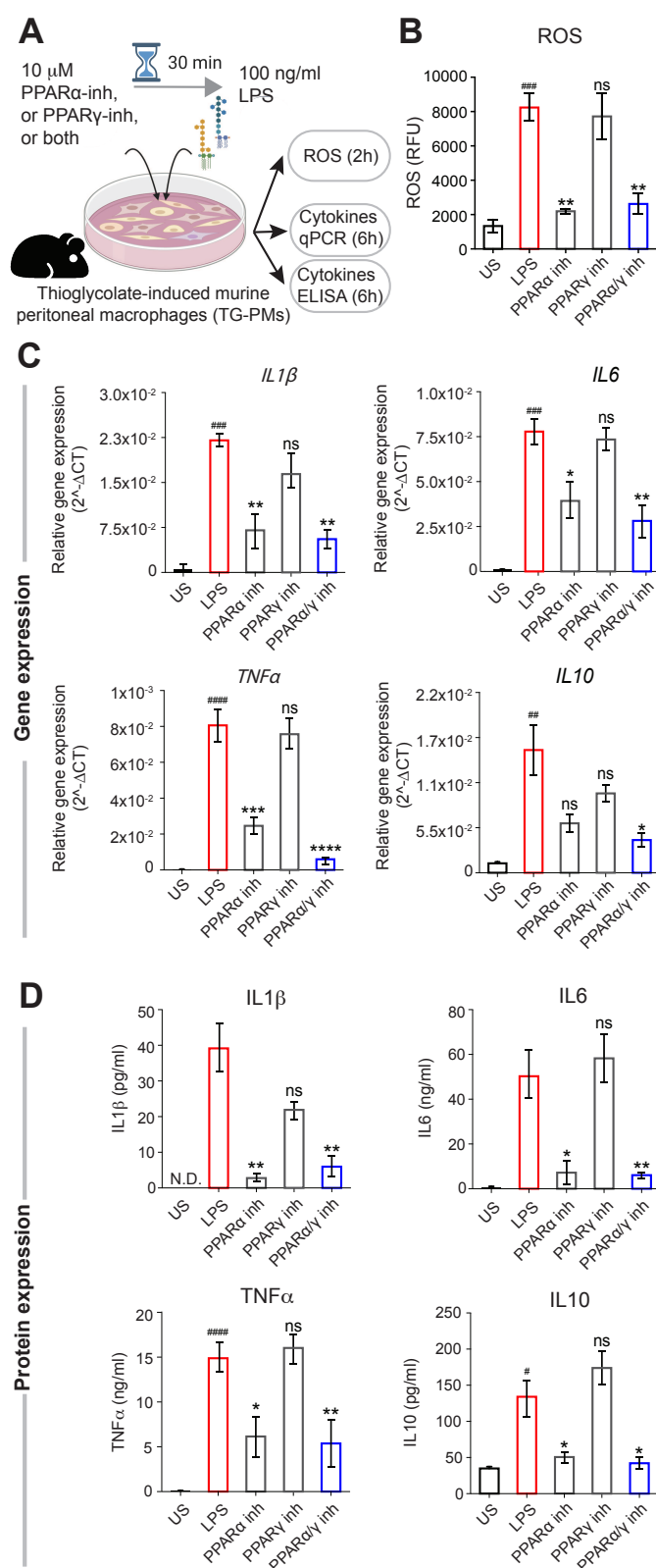868 for multiple comparisons. Significance: *, $p < 0.05$; **, $p < 0.01$.
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Figure 3: PPARα/γ dual agonists ameliorate *Citrobacter rodentium*-induced infectious colitis in mice.
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874 (**A**) Schematic summarizing the workflow for testing PPAR-targeted therapeutics in *C. rodentium*-induced colitis.
875 Mice were gavaged with *C. rodentium* on day 0 and subsequently treated daily with PPAR agonists. Fecal pellets
876 were collected to test viable bacterial burden, as determined by dilution plating and colony counting. Colons were
877 excised on day 7 and 18 and analyzed using the indicated readouts. (**B-D**) Line graphs in B display time series of
878 the burden of viable bacteria in feces. Scatter plots with bar graphs in C compare the peak burden of viable bacteria
879 in feces on day 7. Scatter plots with bar graphs in D display the area under the curve (AUC) for the line graph in
880 B. (**E**) Images display representative fields from H&E-stained colon tissues. Mag = 100x (top) and 200x (bottom).
881 White arrowheads point to immune cell infiltrates. Statistics: All results are displayed as mean ± SEM.
882 Significance was tested using two-way/one-way ANOVA followed by Tukey's test for multiple comparisons.
883 Significance: *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. (**F**) Violin plots (left) display the deviation of expression
884 of genes in Clusters #1-2-3 in the IBD network, as determined by RNA Seq on murine colons. Bar plot (right)
885 displays the rank ordering of the samples. (**G**) Pre-ranked GSEA based on pairwise differential expression
886 analyses (DMSO vs PAR5359 groups) are displayed as enrichment plots for epithelial tight (left) and adherens
887 (middle) junction signatures and balanced macrophage processes (right). See also Supplementary **Fig. 7** for the
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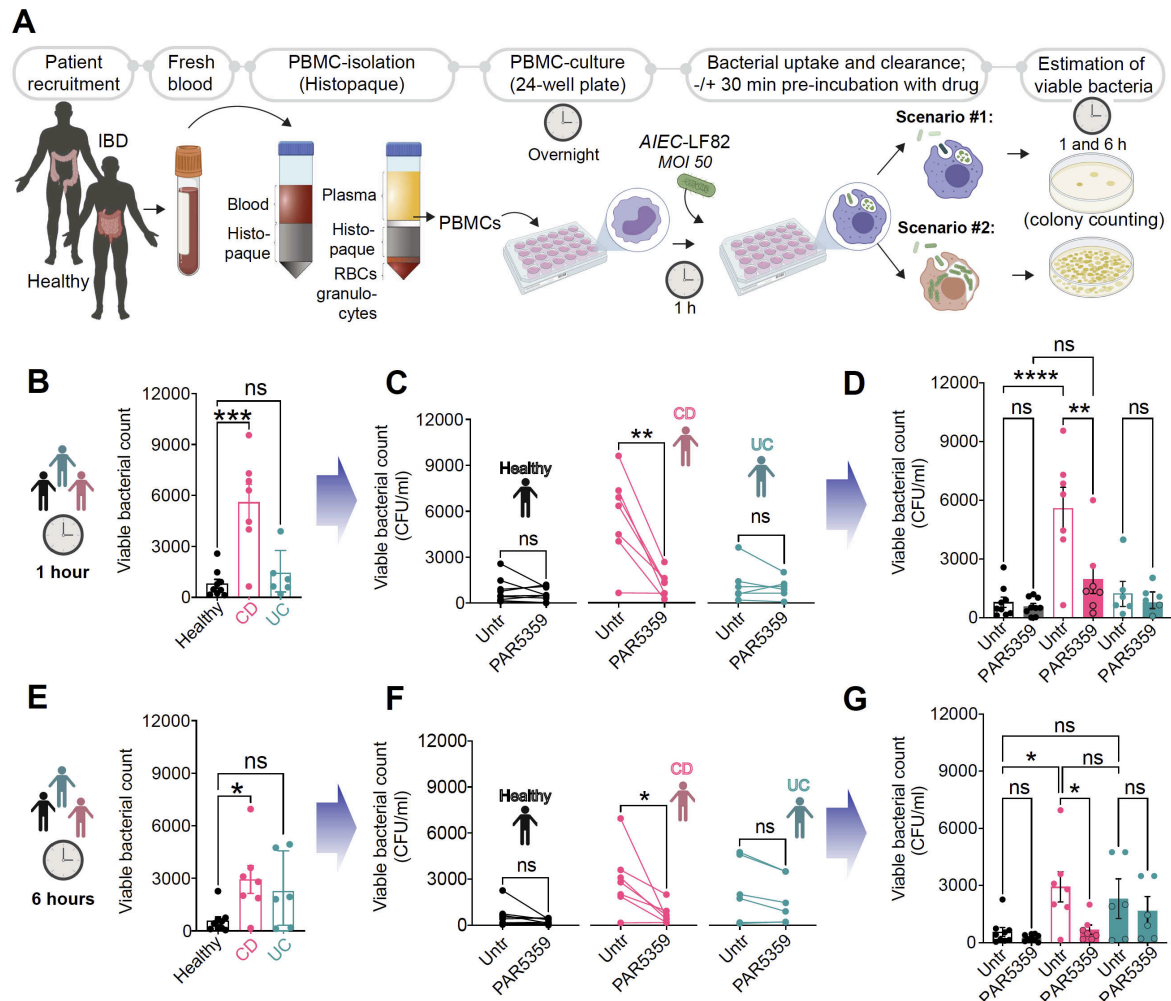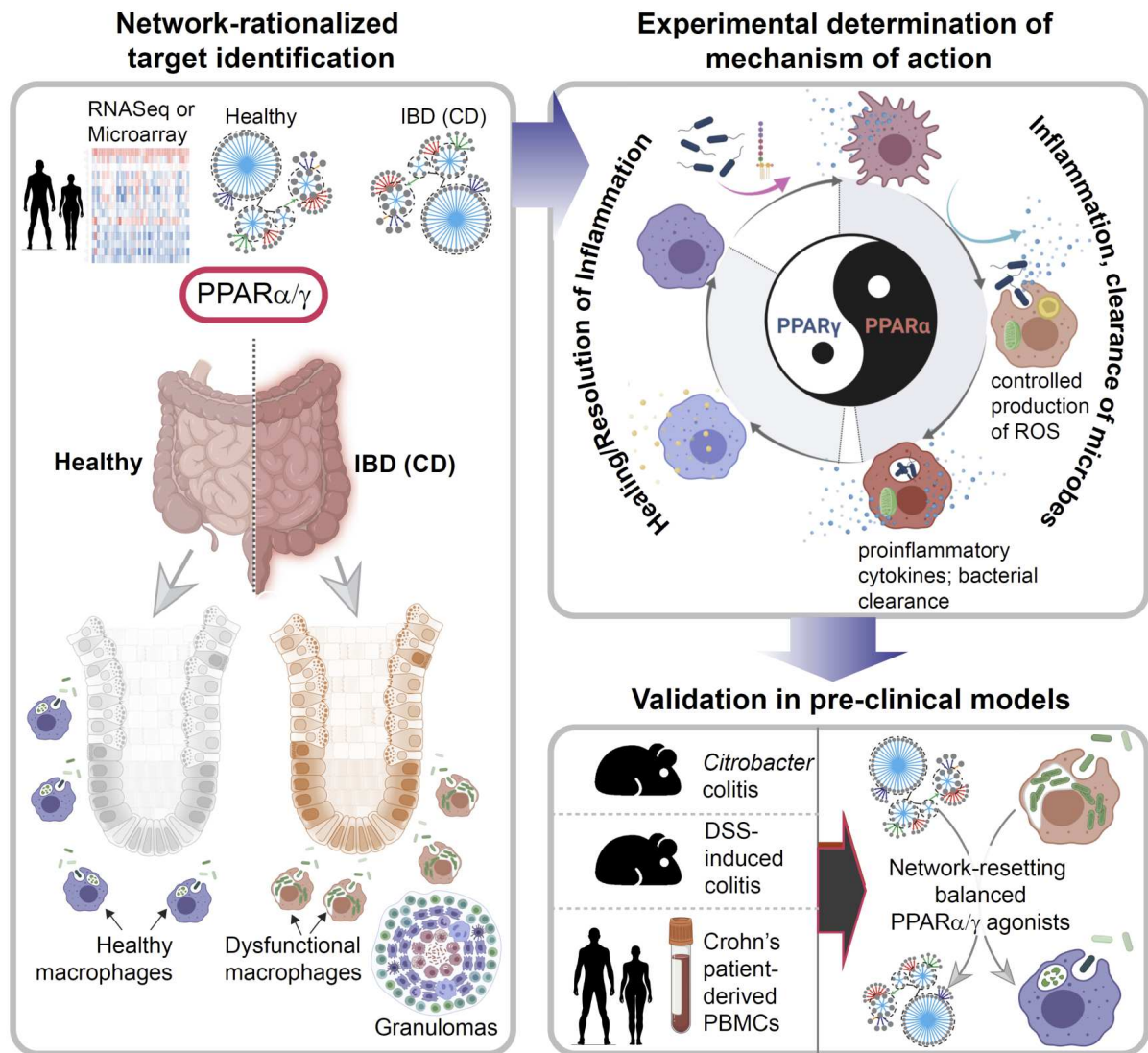889 and Supplementary **Fig. 9** for the effect of PAR5359 on DSS-induced colitis in mice.

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892 *LF82*) clearance.** (**A**) Schematic displays the experimental design and workflow. Thioglycolate-induced murine
893 peritoneal macrophages (TG-PM) pretreated with PPAR agonists (see box, below; 20 nM GW7647, 10 μM
894 Pioglitazone and 1 μM PAR5359) were infected with *AIEC*-LF82 (*MOI* 10) and subsequently analyzed for the
895 bacterial count (Gentamycin protection assay), generation of cellular ROS, secretion of inflammatory cytokines
896 (in supernatant media by ELISA) and the induction of cytokines (gene transcript analysis by qPCR). (**B**) Line
897 graphs (left) display percent viable bacterial counts at indicated times after infection. Bar graphs (right) display
898 the AUC. (**C**) Line graphs (left) and bar graphs (right) display the extent of ROS generation over time. (**D**) Bar
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900 graphs (left) and bar graphs (right) showing the levels of secreted cytokines in the media. Statistics: All results
901 are from at least three independent experiments and results displayed as means ± SEM. Significance was tested
902 using two-way/one-way ANOVA followed by Tukey's test for multiple comparisons. Significance: '#'
903 significance over uninfected TG-PMs and '*' shows significance over AIEC-LF82 infected cells. ns, non-
904 significant, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. See **Supplementary Fig. 10** for similar
905 bacterial clearance assays performed using *Salmonella enteritica*.
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915 **Figure 5: PPARα but not PPARγ is required for induction of cellular ROS and proinflammatory cytokines.**
916 **(A)** Schematic of experimental design. TG-PMs were pre-incubated with 10 μM PPARα or PPARγ inhibitors,
917 either alone or in combination for 30 min prior to stimulation with 100 ng/ml LPS. Cells were analyzed at 2 and
918 6 h to estimate cellular ROS and cytokine induction, respectively. **(B-D)** Bar graphs display the levels of cellular
919 ROS (B), relative levels of mRNA (C) and protein (D) expression of cytokines (IL1β, IL6, TNFα and IL10).
920 Statistics: Results are from three independent experiments and displayed as mean ± SEM. One-way ANOVA
921 followed by Tukey's test for multiple comparisons was performed to test significance. Significance: ns: non-
922 significant, *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$ and ****, $p < 0.0001$.

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924 **Figure 6: PPARα/γ dual agonist, PAR5359, promotes the clearance of *AIEC*-LF82 from CD patient-derived**
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929 to lysis and plating to determine the intracellular abundance of viable bacteria at t1h and t6h, as determined by
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931 of viable intracellular bacteria at 1h (**B**) and 6h (**E**) after infection. Paired line plots display the rate of clearance
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953 **Figure 7: Summary of findings and working model**
954 Schematic summarizes key approaches and findings of this study. First, network-rationalized target identification
955 (*Left*) was performed using web-based platform that queries > 1000 IBD datasets [9; see *Methods*] that served as
956 'input' to create a map of gene clusters that are progressively altered in the gut in the setting of IBD. Predictions
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958 potential for both PPARs), the choice of animal models of IBD, predict cell types of action (macrophage
959 processes), and finally, the subtype of IBD that could benefit most based on the cell type of action (i.e., CD).
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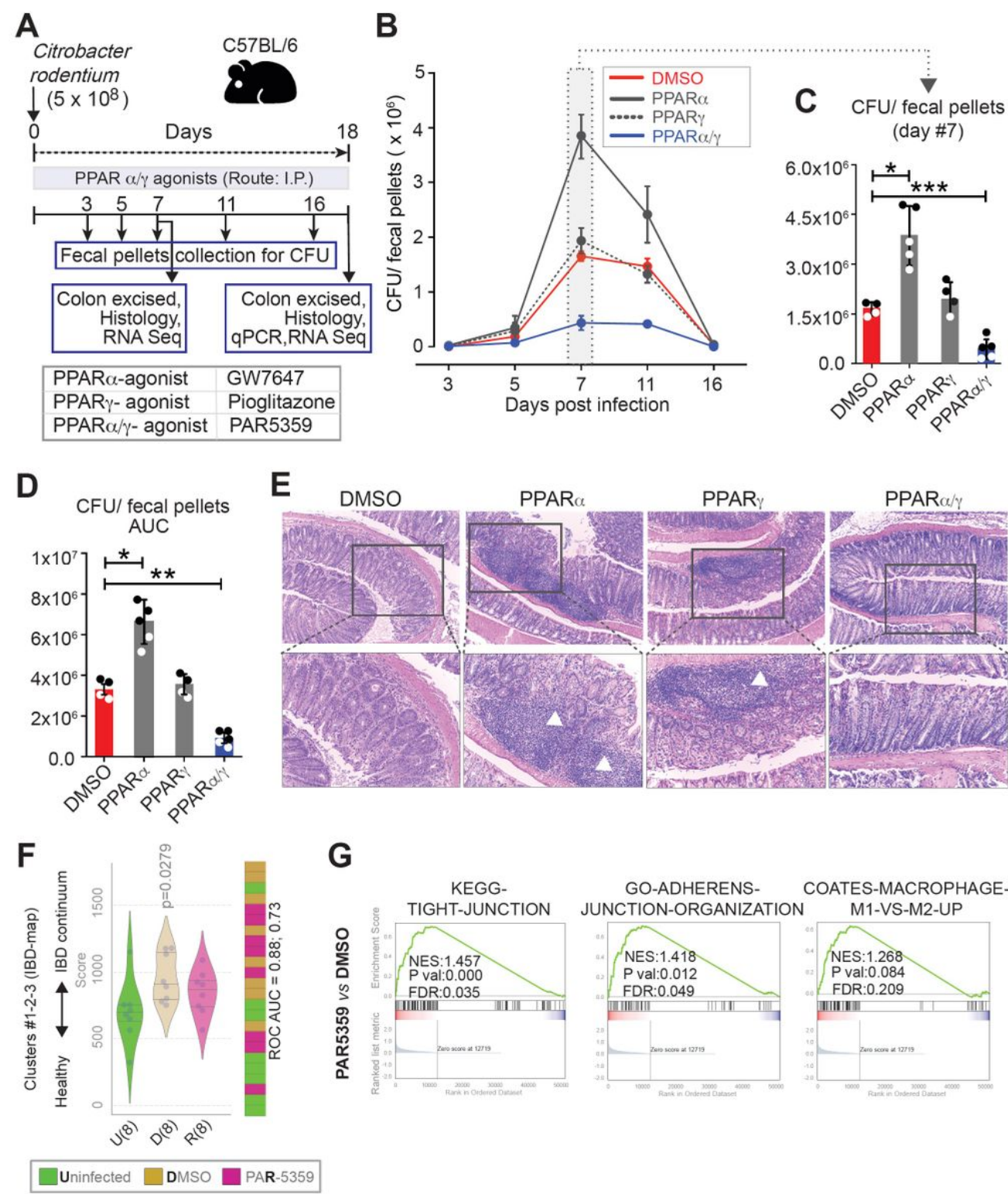967
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Figures

Figure 1

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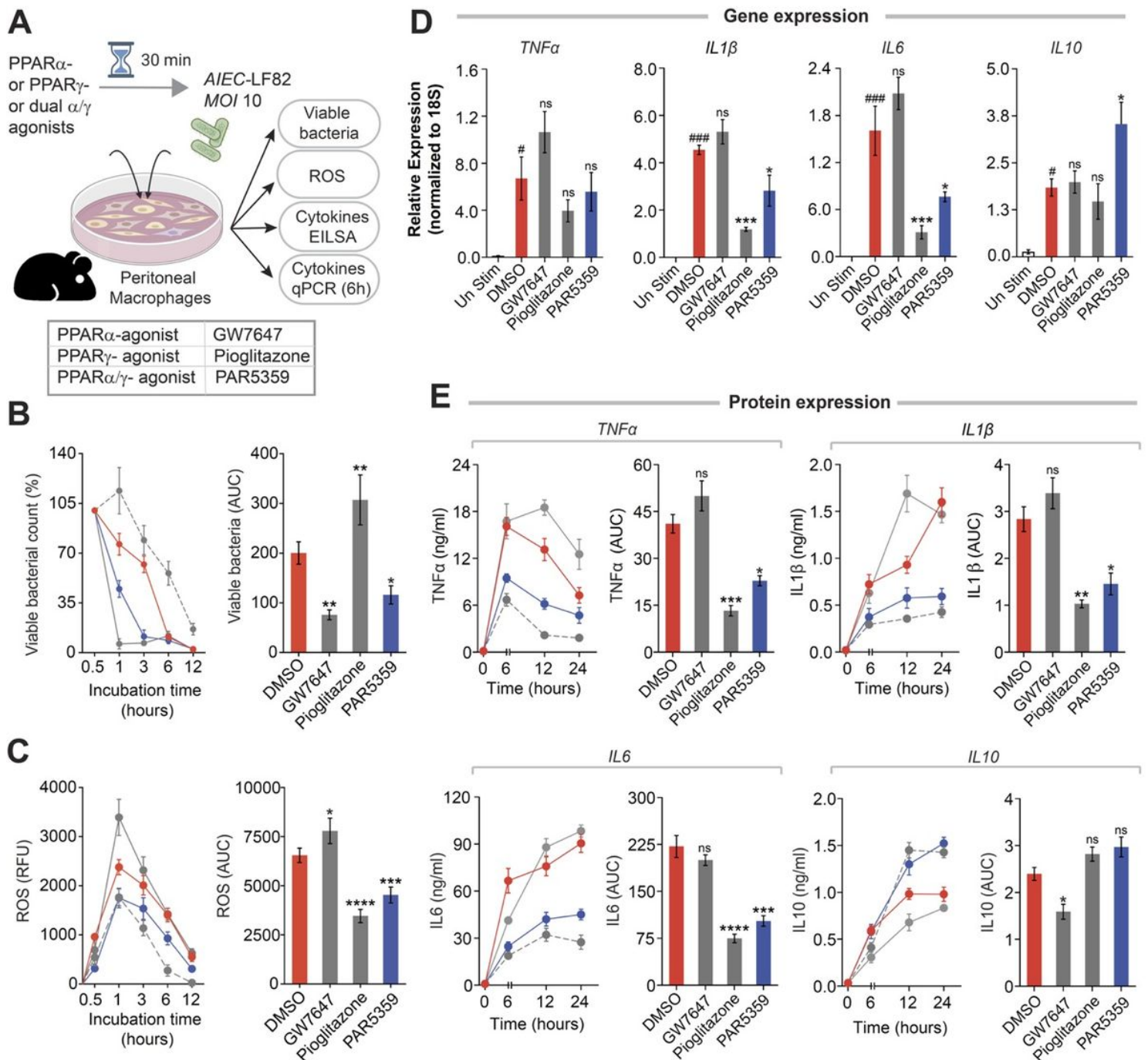
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PPARⲅ/ⲅ dual agonists ameliorate Citrobacter rodentium-induced infectious colitis in mice. (A) Schematic summarizing the workflow for testing PPAR-targeted therapeutics in C. rodentium-induced colitis. Mice were gavaged with C. rodentium on day 0 and subsequently treated daily with PPAR agonists. Fecal pellets were collected to test viable bacterial burden, as determined by dilution plating and colony counting. Colons were excised on day 7 and 18 and analyzed using the indicated readouts. (B-D) Line graphs in B display time series of the burden of viable bacteria in feces. Scatter plots with bar graphs in C compare the peak burden of viable bacteria in feces on day 7. Scatter plots with bar graphs in D display the area under the curve (AUC) for the line graph in B. (E) Images display representative fields from H&E-stained colon tissues. Mag = 100x (top) and 200x (bottom). White arrowheads point to immune cell infiltrates. Statistics: All results are displayed as mean ± SEM. Significance was tested using two-way/one-way ANOVA followed by Tukey's test for multiple comparisons. Significance: *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. (F) 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. (G) Pre-ranked GSEA based on pairwise differential expression analyses (DMSO vs PAR5359 groups) are displayed as enrichment plots for epithelial tight (left) and adherens (middle) junction signatures and balanced macrophage processes (right). See also Supplementary Fig. 7 for the Day #7 results in the C. rodentium-induced colitis model, Supplementary Fig. 8 for extended GSEA analyses, and Supplementary Fig. 9 for the effect of PAR5359 on DSS-induced colitis in mice.

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Supplementary Files

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