nature portfolio

Peer Review File

TREM1 signaling amplifies neutrophil-mediated inflammation in inflammatory bowel disease

Corresponding Author: Dr Saiyu Hang

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Interesting and noteworthy results, significant for the field and related areas. The conclusions are well supported by in vitro, in vivo, and importantly ex vivo evidence in IBD patients. Congratulations on a beautiful study. My comments are as follows:

Extended Figure 1b
Neutrophils and monocytes generally respond to LPS because they express TLR4 and co-receptors, but this is not generally the case for lymphocytes. Therefore, LPS as a stimulus for lymphocytes might not be the best choice to specifically demonstrate that TREM1 is not induced. Of course, indirect effects could occur, as LPS would activate myeloid cells, which could then stimulate lymphocytes through cytokine release. Additionally, in addition to an unstimulated control, an isotype control should be shown, as monocytes and neutrophils often have higher background levels.

Lines 132–134
“Unlike neutrophils, monocytes do not exhibit significant self-amplification through PGLYRP1 release upon TREM1 activation (Extended Data Fig. 2a).”
It is not entirely clear how Extended Data Fig. 2a supports this statement, as the figure only shows a difference between monocytes and neutrophils. Moreover, all three monocyte data points are above fold change 1, indicating that levels do increase upon stimulation with TREM1, even if the increase is less pronounced than in stimulated neutrophils. Please revise the interpretation accordingly.

Figure 2c
Several IBD-related cytokines are measured in monocytes, but it should be noted that the main source of IL-23 in colon biopsies of treatment-naïve pediatric patients were the neutrophils themselves. This suggests another level of complexity regarding neutrophil recruitment, or a possible “loop 2”, in addition to the described mechanism where monocyte-derived IL-23 induces IL-17, which further promotes neutrophil recruitment. Please consider discussing this point.

Figure 2
What type of monocytes were used in these experiments? Classical, intermediate, non-classical, or a mixture of all three? As these subsets likely have different effects and functions, it would be valuable to either specify the subset used, investigate further, or—if a mixture was used (likely because the kit was used for isolation, not sorting)—discuss this as a potential limitation in the discussion.

Figure 3i, j
Is this effect myeloid cell-specific? If you included other non-myeloid cell types as a control, would you also observe TEER changes?

Figure 5 and Discussion
The authors use multiple datasets and perspectives to demonstrate the role of TREM1 and its pathway in IBD pathogenesis, including correlations with clinical scores, which is very helpful.
However, based on the evidence presented, I suggest making the discussion more nuanced, particularly when discussing

“TREM1 as a biomarker in clinical settings”. The differences reported likely do not point to TREM1 as a strong biomarker candidate. My impression is that it may not show excellent diagnostic or predictive performance (e.g., ROC curves, sensitivity, specificity). Therefore, I recommend slightly toning down the discussion in this section.

Reviewer #2

(Remarks to the Author)
The manuscript by Andrade, et al. titled “TREM1-driven feedback loops amplify neutrophil mediated inflammation in Inflammatory Bowel Disease” addresses an important topic, specifically the high failure rates of novel biologic anti-inflammatory agents in IBD. The well written manuscript employs standard techniques for in vitro and in vivo assays and demonstrates plans for appropriate sharing of the proteomic and RNA-sequencing datasets upon publication. An additional strength of the manuscript is the well-conceived assays evaluating the differences between human and murine TREM1, thus highlighting again, the need for cautious interpretation of studies conducted in preclinical models. The confirmatory studies evaluating B. faecis, a known commensal microbe implicated in IBD with the mechanistic TREM1-dependent innate immune response represents another strength.
However, the descriptions of the two mechanistic feedback loops involving neutrophils (PGN:PGLYRP1/TREM1, PGLYRP1 secretion during degranulation) and monocytes (PGN:PGLYRP1 cytokine induction leading to direct neutrophil activation (TNF-a, IL-8) and Th17-dependent neutrophil activation through IL-17) seem iterative given extensive descriptions of these TREM1-dependent innate immune responses in many disease processes, including ROS generation, degranulation, and NET formation. The application of these pathways to IBD adds some novelty, but here as well, TREM1 already represents a therapeutic target in IBD (TREM1-KO and pharmacologic inhibition in IBD preclinical models) and is considered a major mechanistic player in the hyperinflammatory phenotype of patients with ulcerative colitis and Crohn’s disease. Thus, while the techniques and approach are solid, the impact of this report on the field may be minimal. In addition to the concern regarding novelty and impact, the following critiques should be addressed by the authors:
Major:
1. The presentation of NET formation related findings in Figure 1 i-n lack rigor. While the Incucyte assay images and quantification represent some attempt to quantify NET formation in response to PMA and the TREM1 agonist, NET formation in response to PMA occurs much earlier than 9 hours after stimulation, and the PMA control curve should be shown as well to aid in interpretation of these data. Also, the Isotype Ab control image in the immunofluorescence study (n) shows significant citrullinated histone H3 levels although no NET-like extensions are seen in the MPO channel. The authors should account for that. Finally, the addition of quantitative MPO-DNA complex levels in the supernatants of these in vitro experiments would be expected as standard in the field of NET formation.
2. The very unexpected finding of decreased phagocytosis as determined using the pHRODO-red Phagocytosis assay (ED Fig. 1e) should be interpreted with caution given the ability of ROS generation to increase the pH of the phagolysosome. As the authors clearly show that neutrophil ROS generation is dramatically increased by the agonists studied, including TREM1-agonist, perhaps a more standard immunofluorescence-based assessment of fluorochrome-labelled bioparticles, would be helpful.
Minor:
1. Figure 5 has both human ulcerative colitis and Crohn’s disease data labeled in orange or green in separate graphs across the figure (UC-green: a, g; CD-green, f).

Reviewer #3

(Remarks to the Author)
In this study, the authors propose a two-loop amplification model where TREM1 drives both autocrine neutrophil hyperactivation and paracrine recruitment of additional neutrophils, establishing self-sustaining inflammation in IBD.
Strengths are listed below:
Technical execution:
1. Comprehensive multi-omics approach: The authors integrate proteomics (TMTPro-MS), transcriptomics (bulk RNA-seq), functional immunology assays, and large-scale clinical dataset analysis. The proteomics work is particularly rigorous, using state-of-the-art Orbitrap Eclipse MS3 with real-time database searching and appropriate statistical methods (MSstatsTMT with FDR correction).
2. Orthogonal validation strategy: Key findings are validated with multiple independent approaches. For example, TREM1 activation is tested with both agonist antibodies and the PGLYRP1/PGN ligand complex, and functional outputs are measured by flow cytometry, ELISA, live-cell imaging, and confocal microscopy.
3. Large clinical dataset integration: Analysis of 911 patients across 7 clinical trials with appropriate batch correction and statistical methods represents substantial bioinformatic work.
4. Appropriate statistical rigor in proteomics and transcriptomics: Use of proper multiple testing correction, linear mixed models, and empirical Bayes approaches.
Novel observations:
1. PGLYRP1 as neutrophil-derived amplification factor: This is the first demonstration that neutrophil activation releases PGLYRP1, which then participates in TREM1 activation. Previous work (Read et al. 2015) identified PGLYRP1 as a TREM1 ligand component but did not show it is released upon neutrophil activation. This creates a conceptual advance: the product of TREM1 activation (degranulated PGLYRP1) becomes part of the ligand for further activation, establishing a self-amplification mechanism.
2. Integrated two-loop amplification model: While individual components are known, the integration of autocrine neutrophil amplification (Loop 1) with paracrine monocyte-mediated recruitment (Loop 2) into a unified framework is novel. This model

provides a mechanistic explanation for sustained neutrophilic inflammation and offers multiple potential therapeutic intervention points.
3. Blautia faecis as TREM1 activator: Among 15 gut commensal species tested, only B. faecis activated human TREM1. This is the first identification of a gut commensal with TREM1-activating capacity, linking microbiome composition to TREM1 pathway activity.
4. TREM1 as pan-therapy resistance biomarker: The finding that elevated TREM1 pathway signature predicts non-response across mechanistically distinct therapies (anti-TNF, anti-IL-23, anti-integrin) in ulcerative colitis is novel and clinically significant. This suggests a common mechanism of treatment resistance.
5. Monocyte-neutrophil cross-talk via TREM1: Systematic demonstration that TREM1-activated monocytes produce factors that functionally activate and recruit neutrophils, with validation of specific mediators (TNF for activation, IL-8 for chemotaxis) using blocking antibodies.
Important clinical problem:
1. Treatment resistance in IBD: With only 20-30% remission rates for standard biologics, identifying mechanisms of resistance is clinically urgent.
2. Neutrophil-driven pathology: Multiple studies link neutrophil infiltration to treatment failure. Understanding neutrophil activation mechanisms could enable targeted interventions.
3. Convergence point hypothesis: Positioning TREM1 upstream of multiple validated therapeutic targets (TNF, IL-23, TL1A) provides a molecular explanation for why single-cytokine blockade has limited efficacy.
4. Biomarker potential: Both the gene signature and serum sTREM1 could potentially stratify patients for clinical trials or treatment selection.

Specific comments pertaining to weaknesses in the study
Fig. 1 – Extended Data 1c- the sample size appears to be two biological replicates with technical triplicates- statistically questionable, add a third sample or replicate with different samples.

Fig. 2A. Correlation between PGLYRP-PGN and agonist antibody only 0.26- that is not that high. Would argue that PGLYRP is not that specific to TREM1, is it possible it binds other targets, and if so how does this affect the interpretation of in vitro experiments.
Fig 2E. ROS in neutrophils- unclear whether the PGN/PGLYRP complex in the medium is transferred from monos to neutrophils; clarify whether this is the case; if yes, repeat after washing monos.

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Extended data Fig 4. Nice SPR comparison btw different putative ligands to mouse TREM1. Interesting that eCIRP did not work as it is claimed to be a ligand for TREM1. SPR also shows lack of response to mouse PGLYRP, which is surprising- does TREM1 behave differently in the mouse and not bind PGLYRP/PGN? Was the mouse PGLYRP dimerized? Does this finding undermine the relevance of using the mouse DSS model? The workaround of using the multivalent agonist antibody to mouse TREM1 helps to show that TREM1 activation worsens colitis, but the species differences regarding PGLYRP-TREM1 interaction undermines the translation relevance of the mouse model.

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Fig. 5. Correlation analysis of 911 patients across 7 clinical trials with appropriate batch correction and statistical methods represents substantial bioinformatic work.
Correlation of increased PGLYRP RNA with UC and IBD - is this in exosomes?.
Abstract: Modify lines 37-40 state the TREM1 signature "underscores its potential as a biomarker for disease progression and therapeutic efficacy." However, the study shows association with baseline non-responsiveness to other therapies and not progression over time.
Overall impression: Interesting study, but several points described in Specific Comments above need to be further understood to establish importance/translational relevance of B. faesis and TREM1 in human IBD.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Additional experiments performed by authors answered all my comments. I have no further comments.

Reviewer #2

(Remarks to the Author)
The authors have responded in a substantive manner to my critiques on initial review. I have no further significant critiques for this manuscript.

Reviewer #3

(Remarks to the Author)

Reviewer #4

(Remarks to the Author)
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Review

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Overall impression: Interesting study, but several points described in Specific Comments above need to be further understood to establish importance/translational relevance of B. faesis and TREM1 in human IBD.

We thank the reviews for their detailed and constructive feedback, which has helped us improve the quality and clarity of our manuscript. Below, we provide a point-by-point response to each comment, outlining the changes made to the manuscript. All revisions have been highlighted in the manuscript.

Reviewer #1

Interesting and noteworthy results, significant for the field and related areas. The conclusions are well supported by in vitro, in vivo, and importantly ex vivo evidence in IBD patients. Congratulations on a beautiful study. My comments are as follows:

Extended Figure 1b
Neutrophils and monocytes generally respond to LPS because they express TLR4 and co-receptors, but this is not generally the case for lymphocytes. Therefore, LPS as a stimulus for lymphocytes might not be the best choice to specifically demonstrate that TREM1 is not induced. Of course, indirect effects could occur, as LPS would activate myeloid cells, which could then stimulate lymphocytes through cytokine release. Additionally, in addition to an unstimulated control, an isotype control should be shown, as monocytes and neutrophils often have higher background levels.

Response:

We agree with the reviewer's concern and thank the reviewer for this thoughtful comment. To address this, we repeated the experiment to include an isotype control and additional stimulation using anti-CD3/CD28 for lymphocyte activation. Activation of lymphocytes with anti-CD3/CD28 also failed to induce TREM1 expression (**Figure 1 below**). Consistent with our original findings, LPS stimulation increased TREM1 expression in neutrophils and monocytes, whereas lymphocyte activation did not.

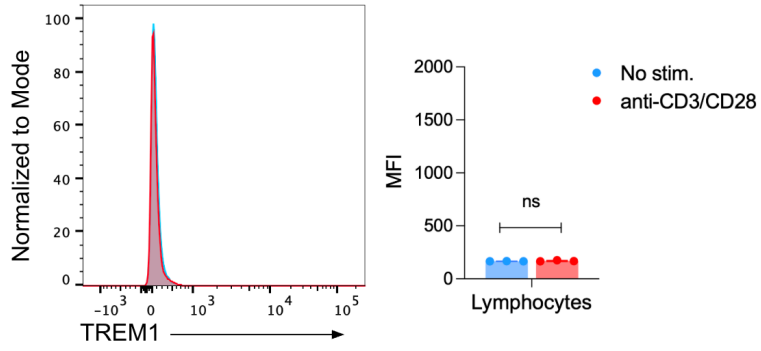
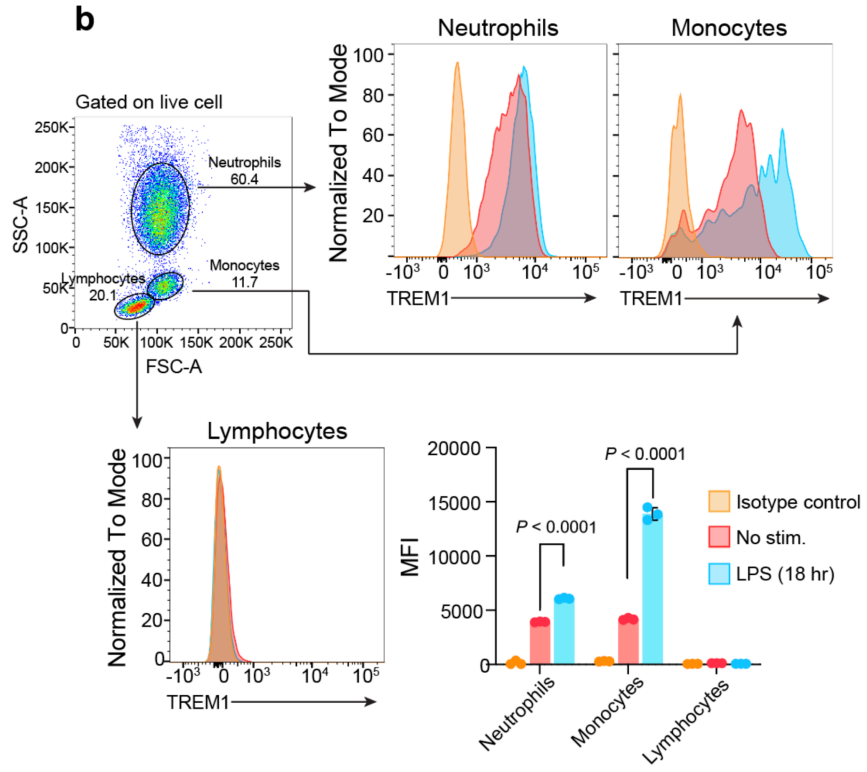


Figure 1.

Human peripheral blood pan-T cells were activated with anti-CD3/CD28 for 48 hours, and TREM1 surface expression was assessed by flow cytometry.

Extended Figure 1b has been updated to include isotype control for the anti-TREM1 antibody (**Revised Extended Data Fig. 1b**).



Revised Extended Data Fig. 1b.

Cells from whole blood were purified from healthy donors and treated with or without LPS for 18 hours. The cell surface expression of TREM1 was assessed by flow cytometry using an anti-TREM1 antibody or isotype control.

Lines 132–134
"Unlike neutrophils, monocytes do not exhibit significant self-amplification through PGLYRP1 release upon TREM1 activation (Extended Data Fig. 2a)."
It is not entirely clear how Extended Data Fig. 2a supports this statement, as the figure only shows a difference between monocytes and neutrophils. Moreover, all three monocyte data points are above fold change 1, indicating that levels do increase upon stimulation with TREM1, even if the increase is less pronounced than in stimulated neutrophils. Please revise the interpretation accordingly.

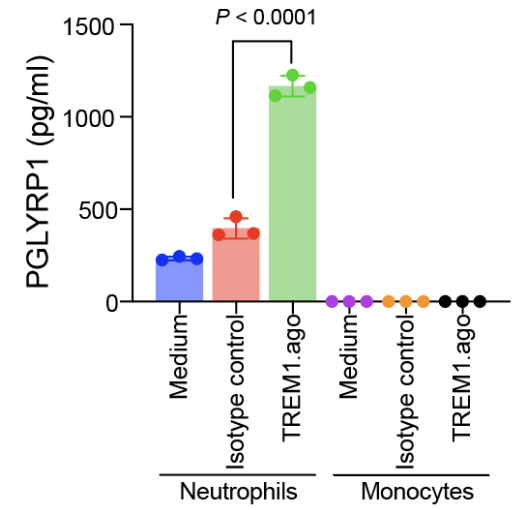
Response:

We appreciate the reviewer's observation and agree that the original data showed a slight increase of PGLYRP1 above baseline in monocyte samples upon TREM1 activation. We believe this is likely due to the contamination of neutrophils during the monocyte isolation process using the StemCell negative selection kit.

To directly address this, we repeated the experiment using a two-step purification process. Neutrophils and monocytes were first isolated using the StemCell kit, followed by a secondary FACS sorting step to ensure high purity. Monocytes were sorted as CD14 single-positive cells, and neutrophils were sorted as CD16 single-positive cells. With these purified populations, we observed robust PGLYRP1 release exclusively from neutrophils following TREM1 activation,

whereas sorted monocytes showed no detectable PGLYRP1 production. This new data strongly supports the conclusion that PGLYRP1-mediated self-amplification downstream of TREM1 activation is a neutrophil-specific mechanism.

We have updated the Extended Data Fig.2a with the new data, shown below, and revised the figure legends (**Revised Extended Data Fig.2a**).



Revised Extended Data Fig. 2a

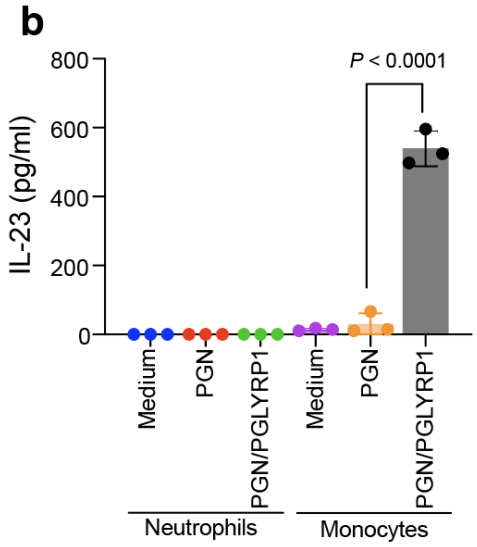
Neutrophils and monocytes were first isolated from donor blood using the StemCell kit and subsequently purified by FACS sorting to ensure high purity. Neutrophils (CD16+) and monocytes (CD14+) were stimulated with isotype control or TREM1.ago antibodies for 18 hours, and PGLYRP1 levels in the culture supernatants were measured by ELISA

Figure 2c
Several IBD-related cytokines are measured in monocytes, but it should be noted that the main source of IL-23 in colon biopsies of treatment-naïve pediatric patients were the neutrophils themselves. This suggests another level of complexity regarding neutrophil recruitment, or a possible “loop 2”, in addition to the described mechanism where monocyte-derived IL-23 induces IL-17, which further promotes neutrophil recruitment. Please consider discussing this point.

Response:

We thank the reviewer for raising this important point regarding the source of IL-23. We agree that neutrophils have been reported as a significant contributor to IL-23 in colon biopsies from treatment-naïve pediatric IBD patients. To investigate this possibility in our system, we conducted follow-up experiments using purified neutrophils and monocytes stimulated in vitro with the PGN/PGLYRP1 complex, measuring IL-23 production. Under these conditions, IL-23 production was only detected in monocytes, while neutrophils did not produce IL-23, indicating that TREM1 activation alone is not sufficient to induce IL-23 release from neutrophils in vitro.

These results suggest that the IL-23 production observed in vivo by neutrophils may rely on additional cues or context not fully recapitulated in our system. Based on this finding, we have updated the main text (lines 150-155) to include the following statement: "*Although IL-23 production in our system was restricted to monocytes (Extended Data Fig. 2b), neutrophils have also been reported as a significant source of IL-23 in colon biopsies from pediatric IBD patients*[36]*. This suggests that IL-23 production by neutrophils may depend on additional inflammatory cues or in vivo conditions not fully recapitulated in our in vitro TREM1 activation system*"



Revised Extended Data Fig. 2b

Purified neutrophils and monocytes were stimulated with PGN alone or PGN/PGLYRP1 for 18 hours. IL-23 levels in the culture supernatants were measured by ELISA.

Figure 2
What type of monocytes were used in these experiments? Classical, intermediate, non-classical, or a mixture of all three? As these subsets likely have different effects and functions, it would be valuable to either specify the subset used, investigate further, or—if a mixture was used (likely because the kit was used for isolation, not sorting)—discuss this as a potential limitation in the discussion.

Response:

We thank the reviewer for this insightful comment. All experiments involving monocytes in the current study used total monocytes, without further separation into subsets. Monocytes were isolated using a StemCell negative selection kit (Stemcell #19669), which does not distinguish between classical, intermediate, or non-classical subsets.

To address this question, we performed additional experiments investigating the response of classical and non-classical monocytes to TREM1 activation. We found that non-classical monocytes exhibited a more robust response to TREM1 activation, producing higher levels of TNF and IL-23 compared to classical monocytes, even though TREM1 expression levels were

similar between the two subsets (**Figure 2, below**). These findings are intriguing and suggest that the underlying mechanism driving the differential response requires further investigation in future studies.

For the current study, we clarify that all experiments were performed using total monocytes in the Methods section, and this limitation has been discussed in the revised manuscript (lines 338-343) to highlight the need for future exploration of monocyte subset-specific role in TREM1-driven inflammation.

Lines 366-371:
"All experiments in this study were conducted using total monocytes, without further subdivision into classical, intermediate, or non-classical subsets. While this approach captures the collective response of monocytes, it does not account for potential functional differences between subsets. Future studies investigating the specific contributions of monocyte subsets to TREM1-driven inflammation could provide additional mechanistic insights and refine our understanding of their roles in IBD pathogenesis."

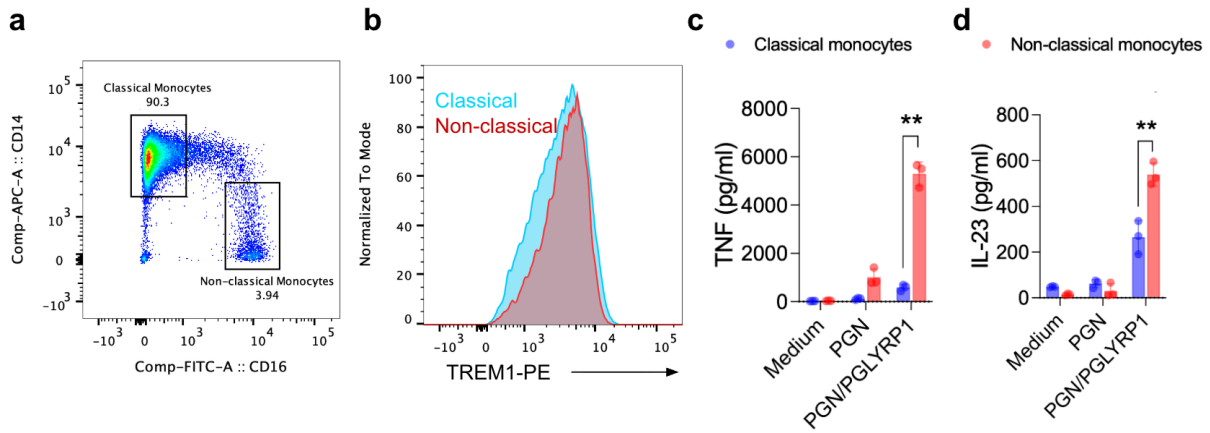


Figure 2. Differential responses of classical vs non-classical monocytes to TREM1 activation **a**-**b**, Monocytes were sorted into classical ($CD14^{+}$) and non-classical ($CD14^{+}CD16^{++}$) populations (**a**). TREM1 cell surface expression was evaluated by flow cytometry and quantified by Geometric mean (MFI) (**b**). c-d, Cells were then stimulated with PGN alone or PGN/PGLYRP1 for 18 hours. Levels of TNF (**c**) and IL-23 (**d**) in the culture supernatants were measured by ELISA.

Figure 3i, j
Is this effect myeloid cell-specific? If you included other non-myeloid cell types as a control, would you also observe TEER changes?

Response:

We Thank the reviewer for raising this question. As we demonstrated in Extended Data Fig. 1b, TREM1 expression is restricted to the myeloid cells, thus non-myeloid cells do not respond to TREM1 activation or mediate TREM1-dependent barrier disruption. To address this directly, we repeated the TEER assay using purified lymphocytes as a non-myeloid control. Consistent with the data in the manuscript, stimulation of monocytes and neutrophils resulted in a significant reduction in TEER. By contrast, activation of purified lymphocytes produced no detectable change in TEER (**Figure 3. below**), confirming that the barrier disruption mediated by TREM1 activation is myeloid cell-specific.

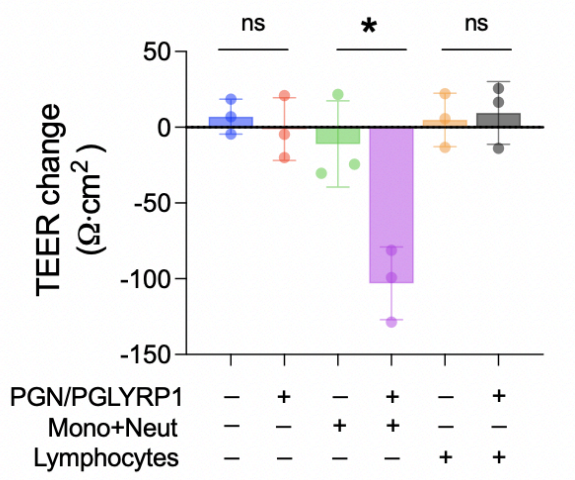


Figure 3.

Gut epithelial barrier integrity was assessed with TEER assay. A Caco-2 cell monolayer was seeded in the upper chamber of the transwell, while freshly purified monocytes and neutrophils or lymphocytes were added to the lower chamber and stimulated with PGN/PGLYRP1.TEER values were recorded immediately before stimulation (baseline) and 24 hours post-stimulation.

Reviewer #2 [neutrophils, innate immunity](Remarks to the Author):

The manuscript by Andrade, et al. titled “TREM1-driven feedback loops amplify neutrophil mediated inflammation in Inflammatory Bowel Disease” addresses an important topic, specifically the high failure rates of novel biologic anti-inflammatory agents in IBD. The well written manuscript employs standard techniques for in vitro and in vivo assays and demonstrates plans for appropriate sharing of the proteomic and RNA-sequencing datasets upon publication. An additional strength of the manuscript is the well-conceived assays evaluating the differences between human and murine TREM1, thus highlighting again, the need for cautious interpretation of studies conducted in preclinical models. The confirmatory studies evaluating B. faecis, a known commensal microbe implicated in IBD with the mechanistic TREM1-dependent innate immune response represents another strength. However, the descriptions of the two mechanistic feedback loops involving neutrophils (PGN:PGLYRP1/TREM1, PGLYRP1 secretion during degranulation) and monocytes (PGN:PGLYRP1 cytokine induction leading to direct neutrophil activation (TNF-a, IL-8) and Th17-dependent neutrophil activation through IL-17) seem iterative given extensive descriptions of these TREM1-dependent innate immune responses in many disease processes, including ROS generation, degranulation, and NET formation. The application of these pathways to IBD adds some novelty, but here as well, TREM1 already represents a therapeutic target in IBD (TREM1-KO and pharmacologic inhibition in IBD preclinical models) and is considered a major mechanistic player in the hyperinflammatory phenotype of patients with ulcerative colitis and Crohn’s disease. Thus, while the techniques and approach are solid, the impact of this report on the field may be minimal. In addition to the concern regarding novelty and impact, the following critiques should be addressed by the authors:

Response:

We thank the reviewer for their thoughtful comments and recognition of the strengths of our study. While TREM1 is an established target in IBD, our study provides novel insights into its role through the following findings: 1) two amplification loops involving both neutrophils and monocytes, where an autocrine loop drives PGLYRP1-mediated neutrophil activation and a paracrine loop involves TREM1-activated monocytes secreting cytokines and chemokines to recruit and activate neutrophils, sustaining inflammation; 2) species-specific differences showing that human TREM1 responds to ligands like PGLYRP1-PGN, whereas murine TREM1 does not, highlighting upstream divergence but conserved downstream amplification mechanisms; 3) identification of *Blautia faecis* as a commensal activator of human TREM1, linking microbiome composition to TREM1 activation; and 4) the development of a defined TREM1 pathway gene signature that correlates with disease severity and treatment resistance, offering value for clinical use in patient stratification, biomarker development, and predicting treatment response. Together, these findings refine our understanding of TREM1 biology, its translational relevance, and its potential utility for therapeutic development and clinical application in IBD.

Major:

1. The presentation of NET formation related findings in Figure 1 i-n lack rigor. While the

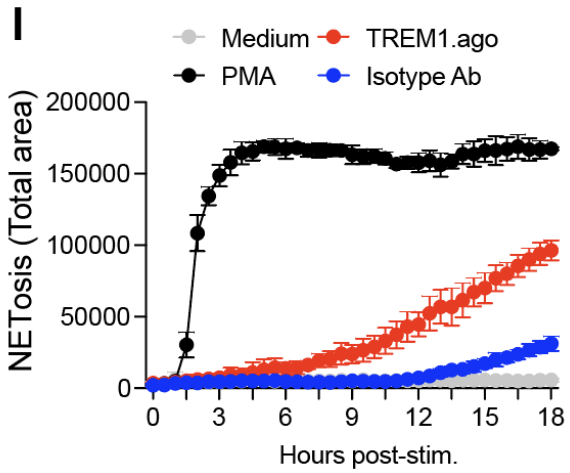
Incucyte assay images and quantification represent some attempt to quantify NET formation in response to PMA and the TREM1 agonist, NET formation in response to PMA occurs much earlier than 9 hours after stimulation, and the PMA control curve should be shown as well to aid in interpretation of these data. Also, the Isotype Ab control image in the immunofluorescence study (n) shows significant citrullinated histone H3 levels although no NET-like extensions are seen in the MPO channel. The authors should account for that. Finally, the addition of quantitative MPO-DNA complex levels in the supernatants of these in vitro experiments would be expected as standard in the field of NET formation.

Response:

We thank the reviewer for this careful and constructive critique. These are important technical points for interpreting NETosis, and we agree that greater rigor and clarity are needed. We have therefore repeated and expanded the NETosis analyses and revised the figures, legends, and text accordingly.

1) PMA time-course control and Incucyte images

We added the PMA control curve to the Incucyte NETosis kinetics data so that the effect of PMA is directly visible alongside the TREM1 agonist data (**Revised Fig.1l**). To address concerns about the timing discrepancy, we removed the original Incucyte image panel (original Fig. 1m), as the representative PMA image shown at 1 hour did not align with the later time points used for the TREM1 agonist data.



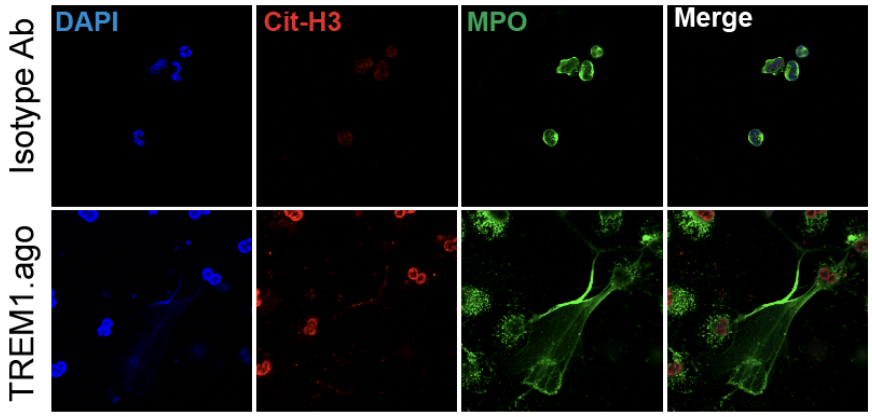
Revised Fig.1l

Neutrophils were purified from health donors and activated with isotype control or TREM1.ago antibody, and PMA was used as a positive control. NETosis was quantified in real-time using the Incucyte instrument.

2) Immunofluorescence (Cit-H3/MPO) repeated with different antibodies:

The original anti-Cit-H3 antibody (Abcam, ab5103) and its isotype control appeared to produce high background levels. We repeated the immunofluorescence experiment using a new anti-citrullinated histone H3 antibody (Thermo Fisher, 630-180ABBOMAX) and new isotype control to reduce background signal. The new images clearly show robust Cit-H3 staining and NET-like MPO/DNA co-localized extensions in the TREM1-activated condition compared with

the isotype control, which now shows only minimal background staining (**Revised Fig.1m**). These new results remove the ambiguity in the original panel.

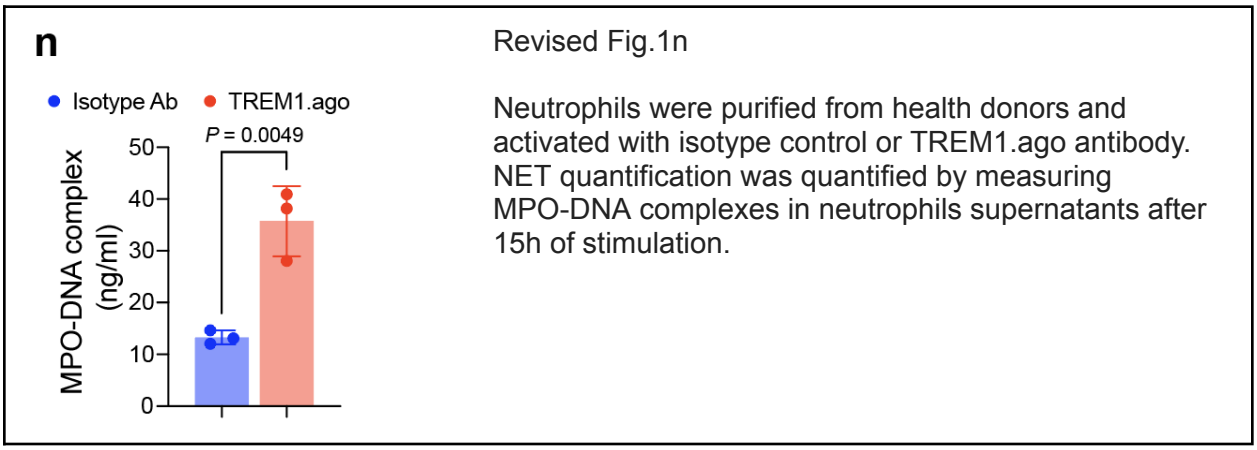


Revised Fig.1m

Neutrophils were purified from health donors and activated with isotype control or TREM1.ago antibody for 15 hours. Representative confocal images of NET release. Cells were stained with DAPI for nuclei (blue), a new Citrullinated Histone H3, Cit-H3 (red), and MPO (green).

3) Quantitative MPO-DNA complexes

Following the established methodology described previously (Veras FP et al. J Exp Med. 2020, PMID: 32926098), we quantified MPO-DNA complexes in supernatants as a biochemical measurement of NETs. TREM1 activation significantly increased MPO-DNA complexes, confirming NET formation upon TREM1 stimulation. These data are now included in the revised manuscript as a new panel (**Revised Fig. 1n**).



Revised Fig.1n

Neutrophils were purified from health donors and activated with isotype control or TREM1.ago antibody. NET quantification was quantified by measuring MPO-DNA complexes in neutrophils supernatants after 15h of stimulation.

We updated the text in the manuscript to reflect these changes in line 123-128:

*"TREM1 activation also induced NETosis, as demonstrated by the Incucyte kinetic analysis (**Fig. 1l**), immunofluorescence staining of neutrophil extracellular traps (NETs) components, including extracellular DNA, citrullinated histone H3 (Cit-H3), and MPO (**Fig. 1m**), and quantification of MPO-DNA complexes in the supernatants as a biochemical measurement of NETs (**Fig. 1n**).*

These functional changes highlight the role of TREM1 in amplifying neutrophil-driven inflammation."

We updated the Methods section for the MPO-DNA complex measurement in line 709-717:

"Quantification of MPO-DNA Complex for NET Measurement: The quantification of NETs was performed by measuring MPO-DNA complexes using an ELISA-based method. Briefly, an ELISA plate was coated with an anti-MPO antibody (Thermo Fisher Scientific, # PA5-16672) to capture MPO-DNA complexes from plasma or neutrophil supernatants. After incubation, bound DNA within the complexes was detected using the Quant-iT PicoGreen dsDNA reagent (Invitrogen, Cat# P11496), and fluorescence was measured with a microplate reader. The fluorescence intensity, proportional to the concentration of MPO-DNA complexes, was compared to a standard curve to determine NET levels in the samples."

2. The very unexpected finding of decreased phagocytosis as determined using the pHRODO-red Phagocytosis assay (ED Fig. 1e) should be interpreted with caution given the ability of ROS generation to increase the pH of the phagolysosome. As the authors clearly show that neutrophil ROS generation is dramatically increased by the agonists studied, including TREM1-agonist, perhaps a more standard immunofluorescence-based assessment of fluorochrome-labelled bioparticles would be helpful.

Response:

We thank the reviewer for this important and technical comment. We fully agree that interpretation of phagocytosis using pH-sensitive probes such as pHrodo must be made with caution, particularly in settings where increased ROS generation could alter phagosomal pH independently of particle uptake.

To address this concern, we performed additional experiments to rigorously reassess neutrophil phagocytic capacity following TREM1 activation (**Figure 1, below**). First, we repeated the Incucyte-based phagocytosis assay using alternative pHrodo-conjugated targets, including pHrodo–zymosan particles and pHrodo–Staphylococcus aureus. Surprisingly, unlike the pHrodo–E. coli assay, these reagents did not show a reproducible reduction in phagocytosis following TREM1 activation (**Figure 1A-C**). Second, we performed a direct bacterial phagocytosis and killing assay, where neutrophils were infected with live E. coli following TREM1 activation and bacterial uptake and killing were quantified by CFU plating. Using this approach, we observed no significant difference between TREM1-activated and isotype control-treated neutrophils (**Figure 1D**).

These results indicate that the apparent reduction in phagocytosis observed with pHrodo–E. coli is likely an assay-specific artifact and not reflective of a true impairment in neutrophil phagocytic capacity. Given the lack of concordance across multiple orthogonal assays, we have decided to remove the original phagocytosis data (Extended Data Fig. 1e) from the revised manuscript. Importantly, this change does not affect the central conclusions of the study, which focus on

TREM1-driven neutrophil hyperactivation, ROS production, degranulation, NET formation, and inflammatory amplification. We believe this revision strengthens the overall clarity of the manuscript.

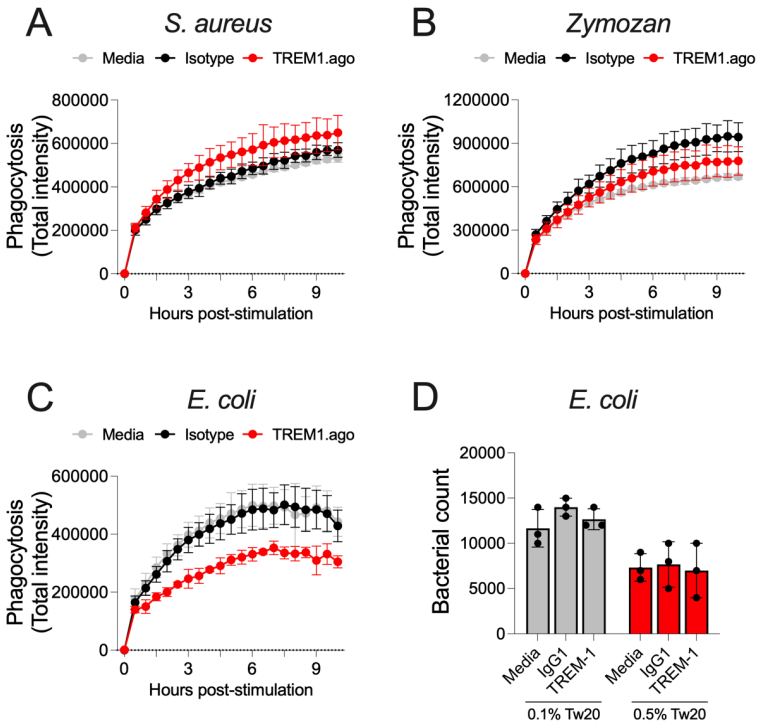


Figure 1. A-B, Neutrophils were purified from health donors and activated using TREM1.ago or isotype control for 30 minutes and infected with pHrodo Red *Staphylococcus aureus* (A), pHrodo Red zymosan particles (B), or pHrodo Red *Escherichia coli* (C). Phagocytosis was quantified in real-time using the Incucyte system. D, Neutrophils were purified and activated using TREM1.ago or isotype control for 30 minutes and infected with *E. coli* for 6h. Cells were treated with gentamicin to kill extracellular bacteria, lysed with Tween 20 (0.1 or 0.5%) and colony-forming unit (CFU) assessed on LB agar plates.

Reviewer #3 [trem1]:

In this study, the authors propose a two-loop amplification model where TREM1 drives both autocrine neutrophil hyperactivation and paracrine recruitment of additional neutrophils, establishing self-sustaining inflammation in IBD. Strengths are listed below:
Technical execution:
1. Comprehensive multi-omics approach: The authors integrate proteomics (TMTPro-MS), transcriptomics (bulk RNA-seq), functional immunology assays, and large-scale clinical dataset analysis. The proteomics work is particularly rigorous, using state-of-the-art Orbitrap Eclipse MS3 with real-time database searching and appropriate statistical methods (MSstatsTMT with FDR correction).
2. Orthogonal validation strategy: Key findings are validated with multiple independent approaches. For example, TREM1 activation is tested with both agonist antibodies and the PGLYRP1/PGN ligand complex, and functional outputs are measured by flow cytometry, ELISA, live-cell imaging, and confocal microscopy.
3. Large clinical dataset integration: Analysis of 911 patients across 7 clinical trials with appropriate batch correction and statistical methods represents substantial bioinformatic work.
4. Appropriate statistical rigor in proteomics and transcriptomics: Use of proper multiple testing correction, linear mixed models, and empirical Bayes approaches.
Novel observations:
1. PGLYRP1 as neutrophil-derived amplification factor: This is the first demonstration that neutrophil activation releases PGLYRP1, which then participates in TREM1 activation. Previous work (Read et al. 2015) identified PGLYRP1 as a TREM1 ligand component but did not show it is released upon neutrophil activation. This creates a conceptual advance: the product of TREM1 activation (degranulated PGLYRP1) becomes part of the ligand for further activation, establishing a self-amplification mechanism.
2. Integrated two-loop amplification model: While individual components are known, the integration of autocrine neutrophil amplification (Loop 1) with paracrine monocyte-mediated recruitment (Loop 2) into a unified framework is novel. This model provides a mechanistic explanation for sustained neutrophilic inflammation and offers multiple potential therapeutic intervention points.
3. Blautia faecis as TREM1 activator: Among 15 gut commensal species tested, only B. faecis activated human TREM1. This is the first identification of a gut commensal with TREM1-activating capacity, linking microbiome composition to TREM1 pathway activity.
4. TREM1 as pan-therapy resistance biomarker: The finding that elevated TREM1 pathway signature predicts non-response across mechanistically distinct therapies (anti-TNF, anti-IL-23, anti-integrin) in ulcerative colitis is novel and clinically significant. This suggests a common mechanism of treatment resistance.
5. Monocyte-neutrophil cross-talk via TREM1: Systematic demonstration that TREM1-activated monocytes produce factors that functionally activate and recruit neutrophils, with validation of specific mediators (TNF for activation, IL-8 for chemotaxis) using blocking antibodies.
Important clinical problem:
1. Treatment resistance in IBD: With only 20-30% remission rates for standard biologics, identifying mechanisms of resistance is clinically urgent.

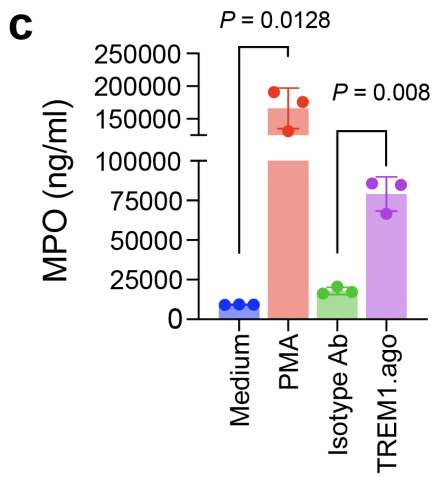
2. Neutrophil-driven pathology: Multiple studies link neutrophil infiltration to treatment failure. Understanding neutrophil activation mechanisms could enable targeted interventions.
3. Convergence point hypothesis: Positioning TREM1 upstream of multiple validated therapeutic targets (TNF, IL-23, TL1A) provides a molecular explanation for why single-cytokine blockade has limited efficacy.
4. Biomarker potential: Both the gene signature and serum sTREM1 could potentially stratify patients for clinical trials or treatment selection.

Response:

We sincerely thank this reviewer for their thoughtful and encouraging feedback on our manuscript. We appreciate the recognition of the strengths of our study, including its methodological rigor, conceptual advances, and clinical relevance. This reviewer's positive assessment reinforces the importance of our findings, and we are confident that our revisions will further enhance the manuscript.

Specific comments pertaining to weaknesses in the study
Fig. 1 – Extended Data 1c- the sample size appears to be two biological replicates with technical triplicates- statistically questionable, add a third sample or replicate with different samples.
Response:

We thank the reviewer for raising this important point regarding sample size and statistical robustness. To address this concern, we repeated the experiment using neutrophils isolated from three independent healthy donors, reflecting true biological replication. PMA stimulation was included as a positive control, and as expected, TREM1 activation induced robust MPO production, while PMA showed stronger activation compared to TREM1 agonist since both were stimulated overnight. The revised figure and legend have been updated to clarify this point (Revised Extended Data Fig.1c).



Revised Extended Data Fig.1c

Neutrophils were activated with overnight stimulation using PMA, plate-coated TREM1 agonistic antibody (TREM1.ago), or isotype control, and MPO levels were measured by ELISA. Each data point represents one biological replicate (n = 3 donors per group).

Fig. 2A. Correlation between PGLYRP-PGN and agonist antibody only 0.26- that is not that high. Would argue that PGLYRP is not that specific to TREM1, is it possible it binds other targets, and if so how does this affect the interpretation of in vitro experiments.

Response:

We thank the reviewer for raising this important point. The observed correlation coefficient of 0.26 is indeed expected because the PGN/PGLYRP1 preparation used in our experiments is not a pure ligand-receptor complex; it also contains free peptidoglycan (PGN) in addition to the PGN/PGLYRP1 complex. Free PGN is a well-known activator of multiple innate immune sensors, including NOD1, NOD2, and TLR2. These additional pathways likely contribute to the transcriptional responses observed, reducing the correlation between PGN/PGLYRP1 and the TREM1 agonist antibody.

This is why we performed the correlation analysis to identify which genes are TREM1-dependent and which are influenced by other innate immune sensors. By generating a TREM1 pathway gene signature (as shown in Extended Data Fig. 5c), we were able to distinguish TREM1-specific transcriptional changes from those driven by other pathways. Additionally, throughout our experiments, we used PGN alone or TREM1 blocking antibody as a control alongside PGN/PGLYRP1, to consistently demonstrate TREM1 pathway-specific activity.

We have clarified this point in the main text with the following statement in line 139-143:

"The moderate correlation coefficient between PGN/PGLYRP1 and the TREM1 agonistic antibody is expected, as the PGN/PGLYRP1 preparation contains not only the ligand complex but also free PGN. PGN is a known activator of multiple innate immune sensors, including NOD1, NOD2, and TLR2, which likely contribute to the observed transcriptional responses."

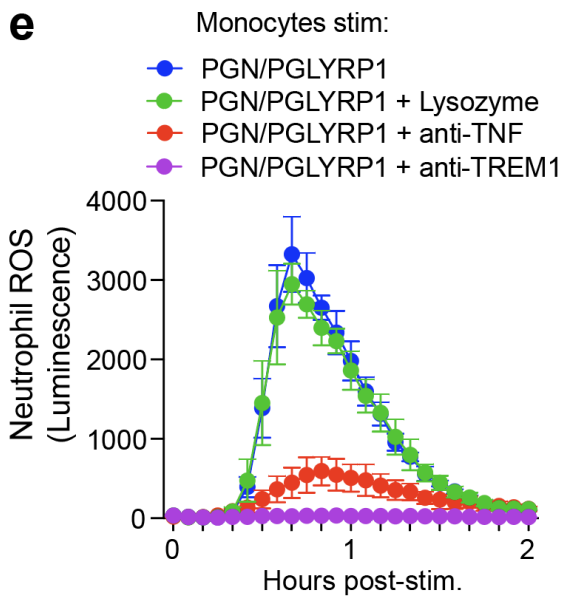
Fig 2E. ROS in neutrophils- unclear whether the PGN/PGLYRP complex in the medium is transferred from monos to neutrophils; clarify whether this is the case; if yes, repeat after washing monos.

Response:

We thank the reviewer for raising this important question regarding the potential carryover of the PGN/PGLYRP1 complex from monocyte culture supernatants into neutrophil stimulation assays. To address this, we treated the monocyte supernatant with lysozyme to degrade PGN prior to adding it to neutrophils. Lysozyme treatment did not affect the ability of monocyte supernatant to induce neutrophil ROS production (**Revised Fig.2e**). Instead, ROS induction was predominantly suppressed by anti-TNF treatment, indicating that the effect is due to TNF present in the monocyte supernatant and not due to carryover of the PGN/PGLYRP1 complex.

We have updated Fig. 2e to include the lysozyme-treated condition as a control and revised the main text to reflect these findings in line 161-165:

"To rule out the possibility of PGN/PGLYRP1 complex carryover from monocyte supernatants, we treated the supernatants with lysozyme to degrade PGN. Lysozyme treatment did not affect neutrophil ROS production, further confirming that the effect is mediated by monocyte-derived TNF rather than carryover of PGN/PGLYRP1 (Fig. 2e)."



Revised Fig. 2e

Freshly purified monocytes were stimulated with PGN/PGLYRP1 for 18 hours, with or without TREM1 blocking antibody (anti-TREM1). The culture supernatant was collected, left untreated or treated with lysozyme or ant-TNFα antibody. Supernatant was used to treat neutrophils to assess ROS production in real time.

Fig. 3- shows activation of TREM1 only in the presence of gram neg PGN, but Blautia faecis is gram positive, so this is confusing. Would test effect of PGN derived from B faecis to show specificity. Important as this would indicate that TREM1 will only be activated in the context of specific PGN/PGLYRP complexes, ie TREM1 is activated in the context of bacterial cell wall structure. How is the PGN of Blautia Faecis different from other gram positives and similar to that of e coli? Also confusing is that B faesis appears to be commensal, with potential probiotic properties- how does this relate then to IBD which presumably is associated with pathogenic bacteria?

Response:

We thank the reviewer for raising these important points. We hypothesize that TREM1 activation in this system is not solely determined by Gram status (Gram+ or Gram-), but rather by whether a given PGN fragment can engage PGLYRP1 to form a productive PGN/PGLYRP1 complex that supports TREM1 multimerization and activation. PGNs are highly diverse across bacterial species due to differences in glycan modifications, peptide composition, crosslinking, and the fragments generated by hydrolases. Recent work (Chen et al. Nat Commun 2025, PMID: 39984444) suggests that PGLYRP1 selectively binds disaccharide-containing GMTriP-K motifs

in the PGN, with downstream signaling depending on specific structural features of the PGN fragment.

We detected direct binding of PGLYRP1 to live *B. faecis* cells using immunofluorescence staining (**Figure 1, below**). We propose that PGN fragments shed from dividing *B. faecis* and *E. coli* cells may share a PGLYRP1-permissive GMTriP-K motif, whereas other Gram+ species tested may lack such structural motifs necessary to form the appropriate PGN/PGLYRP1 complex for TREM1 activation. These repeating motifs on the PGN serve as binding sites for PGLYPR1 to form oligomers, which in turn directly bind to TREM1, leading to receptor clustering and downstream signaling.

We agree that directly testing purified or defined PGN fragments from *B. faecis* would provide more precise insights into the structural basis of TREM1 activation. However, such an analysis, including PGN purification, structural characterization, or synthetic muropeptide approaches, is beyond the scope of this study.

Regarding the classification of *B. faecis* as a commensal, it is important to clarify that being labeled a “commensal” does not equate to being non-inflammatory or anti-inflammatory. Commensals are defined as microbes that coexist with the host without causing overt harm under homeostatic conditions. However, this does not preclude their ability to stimulate the immune system when exposed to mucosal immune cells, particularly under conditions where barrier integrity is compromised. For instance, *Enterococcus faecalis,* a gut commensal, has been shown to drive inflammation and exacerbate colitis under conditions of epithelial barrier dysfunction in IL10-/- or TNFΔARE mice, but not in wild-type mice (Steck et al. Gastroenterology 2011, PMID: 21699778). Similarly, *B. faecis* (or its PGN fragments) could translocate across the compromised epithelial barrier in IBD and form complexes with PGLYRP1, leading to TREM1 activation and subsequent inflammation.

We have clarified these points in the revised manuscript to emphasize the structural diversity of PGN and potential for specific motifs to drive TREM1 activation, as well as the context-dependent role of commensals in inflammation. These updates are included in the discussion (line 308-314 and line 323-331):

Line 313-319:
“While commensals are generally defined as microbes that coexist with the host under homeostatic conditions, this does not preclude their ability to stimulate the immune system in certain contexts. For example, B. faecis (or its PGN fragments) could translocate across a damaged epithelial barrier, activating TREM1 and contributing to inflammation. Similarly, Enterococcus faecalis, another gut commensal, has been shown to exacerbate inflammation under conditions of epithelial barrier dysfunction [51].”

Line 327-334:
“Furthermore, our findings suggest that TREM1 activation by B. faecis is not determined by Gram status alone but is likely driven by specific PGN fragments that engage PGLYRP1 to form

a productive complex. PGNs vary widely across bacterial species in glycan modifications, peptide composition, and fragment generation. Recent studies show that PGLYRP1 selectively binds disaccharide-containing PGN motifs[54], which are critical for downstream signaling. We hypothesize that B. faecis and E. coli share PGLYRP1-permissive motifs, enabling TREM1 activation, while other Gram-positive species lack these features. Future studies isolating and characterizing B. faecis PGN fragments will clarify the structural basis of its TREM1 activation."

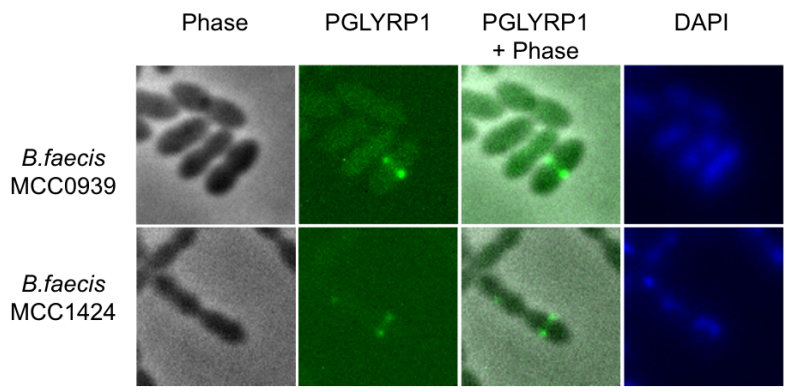


Figure 1. Direct binding of PGLYRP1 to live *B. faecis* cells.
Live *B. faecis* strains MCC0939 and MCC1424 were incubated with recombinant human PGLYRP1 (60 ug/mL), followed by staining with biotinylated anti-PGLYRP1 (1:100, R&D systems) and Streptavidin-AF488 (1:1000). Fluorescent Images show specific binding of PGLYRP1, particularly at cell division sites where PGN is exposed during bacterial replication.

Extended data Fig 4. Nice SPR comparison btw different putative ligands to mouse TREM1. Interesting that eCIRP did not work as it is claimed to be a ligand for TREM1. SPR also shows lack of response to mouse PGLYRP, which is surprising- does TREM1 behave differently in the mouse and not bind PGLYRP/PGN? Was the mouse PGLYRP dimerized? Does this finding undermine the relevance of using the mouse DSS model? The workaround of using the multivalent agonist antibody to mouse TREM1 helps to show that TREM1 activation worsens colitis, but the species differences regarding PGLYRP-TREM1 interaction undermines the translation relevance of the mouse model.

Response:

We thank the reviewer for highlighting these important points and for their thoughtful interpretation of our findings.

1) eCIRP as a TREM1 ligand:

Our data demonstrate that eCIRP is not a ligand for mouse TREM1. In addition to the lack of SPR binding between eCIRP and mouse TREM1 (Extended Data Fig. 4), our functional assay

showed that eCIRP induced MyD88-dependent signaling in mouse bone marrow-derived macrophages (BMDMs) (**Figure 2, below**), indicating that eCIRP signals through TLR pathways rather than TREM1. Together, these results confirm that eCIRP is not a bona fide ligand for mouse TREM1.

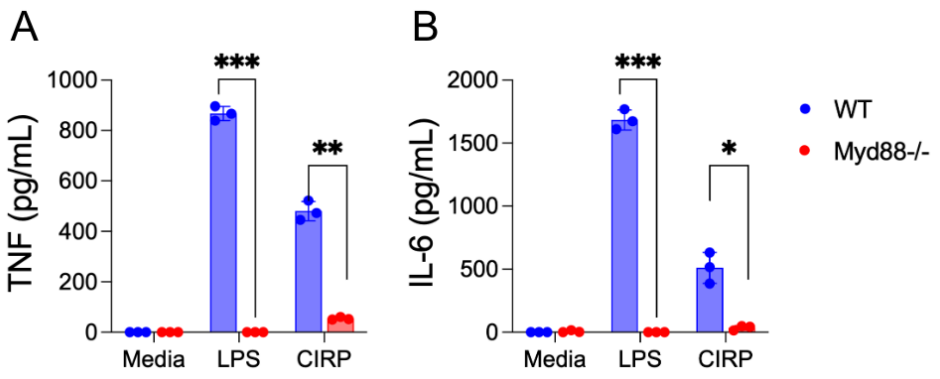


Figure 2. CIRP activity is Myd88-dependent

BMDM were differentiated from bone marrow from wild-type or Myd88-deficient mice, cultured in a medium containing 30% (v/v) conditioned medium from L929 mouse fibroblasts, and 20% FBS. Cells were treated for 18h with LPS (100ng/ml) or eCIRP (1µ/ml) and (A) and IL-6 (B) levels were measured by ELISA.

2) Mouse PGLYRP1 and TREM1 binding:

We acknowledge the surprising lack of binding between mouse TREM1 and mouse PGLYRP1 in our SPR data (Extended Data Fig. 4a). In addition, our results from a Protein-protein interaction (Sanwitch) ELISA results demonstrated that human PGLYRP1 directly binds to human TREM1 but not mouse TREM1, and that mouse PGLYRP1 does not bind to mouse TREM1 (**Figure 3, below**). These results confirm that the binding between PGLYRP1 and TREM1 is species-specific.

Regarding the dimerization, size-exclusion chromatography and SDS-PAGE analysis revealed that human PGLYRP1 forms a disulfide-linked dimer (**Figure 4, below**). In contrast, mouse PGLYRP1 does not dimerize during purification, which may explain its inability to form a functional ligand complex with mouse TREM1.

Furthermore, using structural modeling based on the crystal structure of human PGLYRP3 bound to muramyl tripeptide (*Guan et al. PNAS, PMID: 15572450*), we analyzed the PGN-binding interface of mouse PGYRP1 (**Figure 5, below**). Of the 19 residues in contact with the PGN analog, 12 are conserved between mouse and human PGLYRP1. However, mouse PGLYRP1 D103 (noted with an asterisk in the sequence alignment) may sterically occlude PGN

binding, further supporting the lack of functional interaction between mouse PGLYRP1 and TREM1.

These findings provide preliminary insights into the structural and biochemical differences between human and mouse PGLYRP1-TREM1 interaction. Future work of further structural and functional studies are needed to draw conclusive mechanistic insights. As such, these data are not incorporated into the revised manuscript but are highlighted here to address the reviewer's questions.

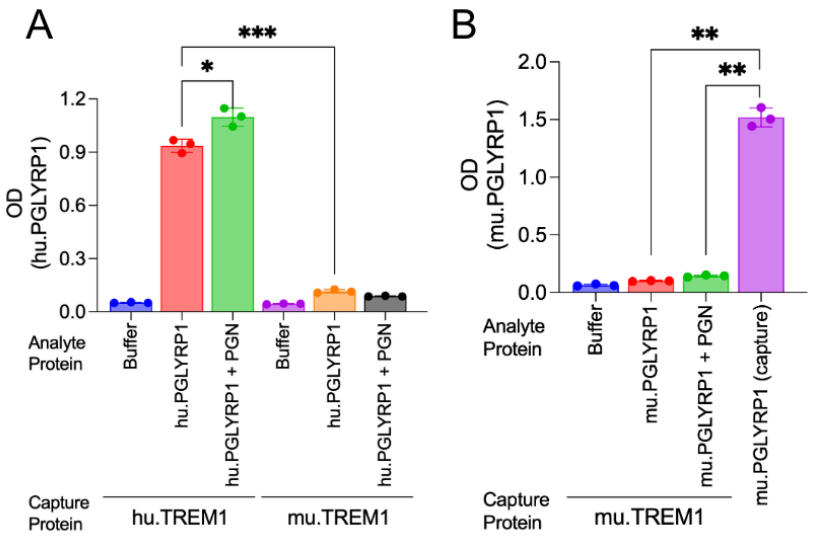


Figure 3. Sandwich ELISA to detect binding between TREM1 and PGLYRP1

(A) Human or mouse TREM1 in Fc format was coated onto the ELISA plate as the capture protein. Human PGLYRP1, with or without PGN, was added as the analyte protein. After washing, anti-human PGLYRP1 detection antibody was used to quantify the binding between human PGLYRP1 and human or mouse TREM1.
(B) Mouse TREM1 in Fc format was coated on the ELISA plate as the capture protein. Mouse PGLYRP1, with or without PGN, was added as the analyte protein. After washing, anti-mouse PGLYRP1 was used as the detection antibody to quantify binding between mouse PGLYRP1 and mouse TREM1. Mouse PGLYRP1 coated directly onto the plate was used as positive control for the detection antibody.

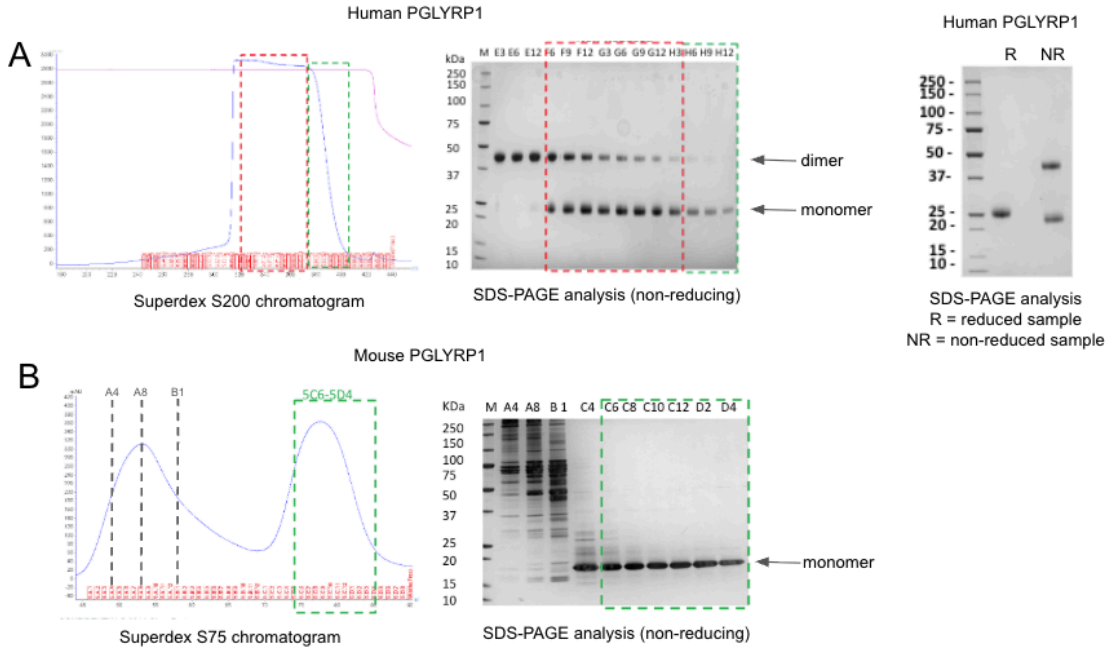


Figure 4. Biochemical properties of human and mouse PGLYRP1.

Size-exclusion chromatogram analysis of human PGLYRP1 (A) and mouse PGLYRP1 (B), demonstrating distinct elution profiles. Human PGLYRP1 predominantly forms a disulfide-linked dimer as evidenced by non-reducing SDS-PAGE analysis (A, middle panel). In contrast, mouse PGLYRP1 does not exhibit dimerization under similar conditions. SDS-PAGE analysis under reducing (R) and non-reducing (NR) conditions confirms that human PGLYRP1 forms a disulfide-linked dimer (dimer band present under non-reducing condition) (A, right panel).

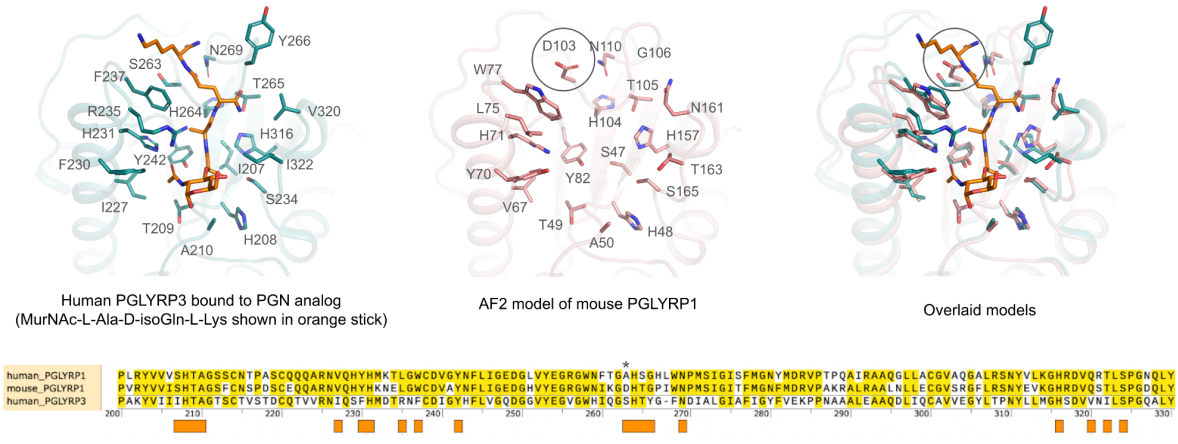


Figure 5. Structural comparison of human and mouse PGLYRP1.

(A) Structural alignment of human PGLYRP3 bound to a peptidoglycan (PGN) analog, MurNAc-L-Ala-D-isoGln-L-Lys (orange stick representation), and AlphaFold2 (AF2) model of mouse PGLYRP1. The crystal structure of human PGLYRP3 reveals 19 residues in contact with the PGN analog. (B) Sequence alignment highlights residues involved in PGN binding. Twelve of the 19 residues are conserved between human and mouse PGLYRP1. However, residue D103 in mouse PGLYRP1 (indicated with an asterisk) may sterically hinder binding to the PGN analog, suggesting potential differences in ligand interaction between the two species.

3) Translational relevance of the mouse DSS model:

We appreciate the reviewer's concern regarding the translatability of the mouse DSS model due to species-specific differences in TREM1 ligand interactions. However, while the upstream ligand-receptor mechanisms are not conserved, our data demonstrate that TREM1's downstream signaling and functional outputs are conserved between humans and mice. Specifically, Similar to human TREM1, mouse TREM1 activation by plate-coated antibody agonists demonstrated the same ROS production in mouse primary neutrophils and TNF secretion in mouse monocyte/macrophage cell line (RAW264.7) engineered to express mouse TREM1 and DAP12 (**Figure 6, below**). This conserved downstream effect of TREM1 signaling supports the relevance of using the mouse model to study TREM1-mediated inflammatory processes in IBD. Nonetheless, we acknowledge the nuances of translatability and have added language to the Discussion to highlight these limitations and contextualize the interpretation of findings in line 377-382:

"Despite these upstream differences, our data demonstrate that downstream TREM1 signaling outputs, including neutrophil hyperactivation and cytokine production, are conserved. The use of a multivalent agonistic antibody in the DSS model bypasses species-specific ligand differences and allows for the study of conserved inflammatory consequences of TREM1 activation in vivo. These findings underscore the challenges and nuances of using mouse models to study TREM1-mediated pathways and highlight the need for validation in humanized mouse models or in vitro human systems to ensure translatability of therapeutic strategies."

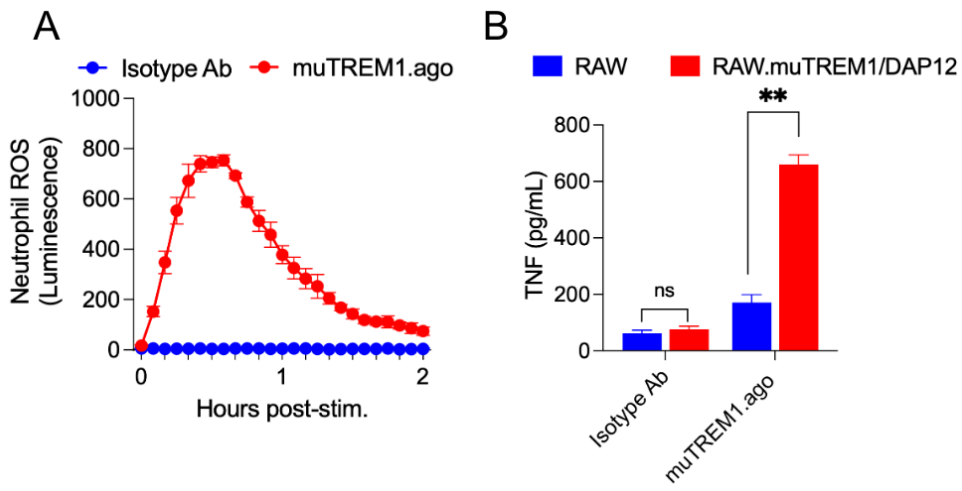


Figure 6. Mouse TREM1 activation leads to ROS production in neutrophils and TNF production in RAW cells.

(A) ROS production in mouse neutrophils was measured using Lucigenin in a kinetic assay. Neutrophils were isolated from mouse bone marrow activated with plate-coated anti-mouse TREM1(muTREM1.ago) or isotype control;
(B) TNF production was measured in parental RAW264.7 mouse monocyte/macrophage cells, or RAW264.7 cells engineered to express mouse TREM1 and mouse DAP12 (RAW.muTREM1/DAP12). Cells were stimulated with plate-coated anti-mouse TREM1 (muTREM1.ago) or isotype control for 18 hours, and TNF levels in the culture supernatants were quantified by ELISA.

It would be important then to validate the human in vitro TREM1 findings in human IBD biopsy samples. The proposed mechanism is not explored in IBD tissues, for example with immunostaining showing TREM1 positive neutrophils, macrophages and PGLYRP localization; spatial mapping of neutrophils relative to bacteria and damaged epithelium, or quantification of TREM1 in inflamed versus non inflamed regions. Relevance of B. faecis to IBD- for example localization, amounts of B faecis in IBD samples- is not explored. Also, it is not clear how initial set of 15 bacterial species was selected from IBD patients.

Response:

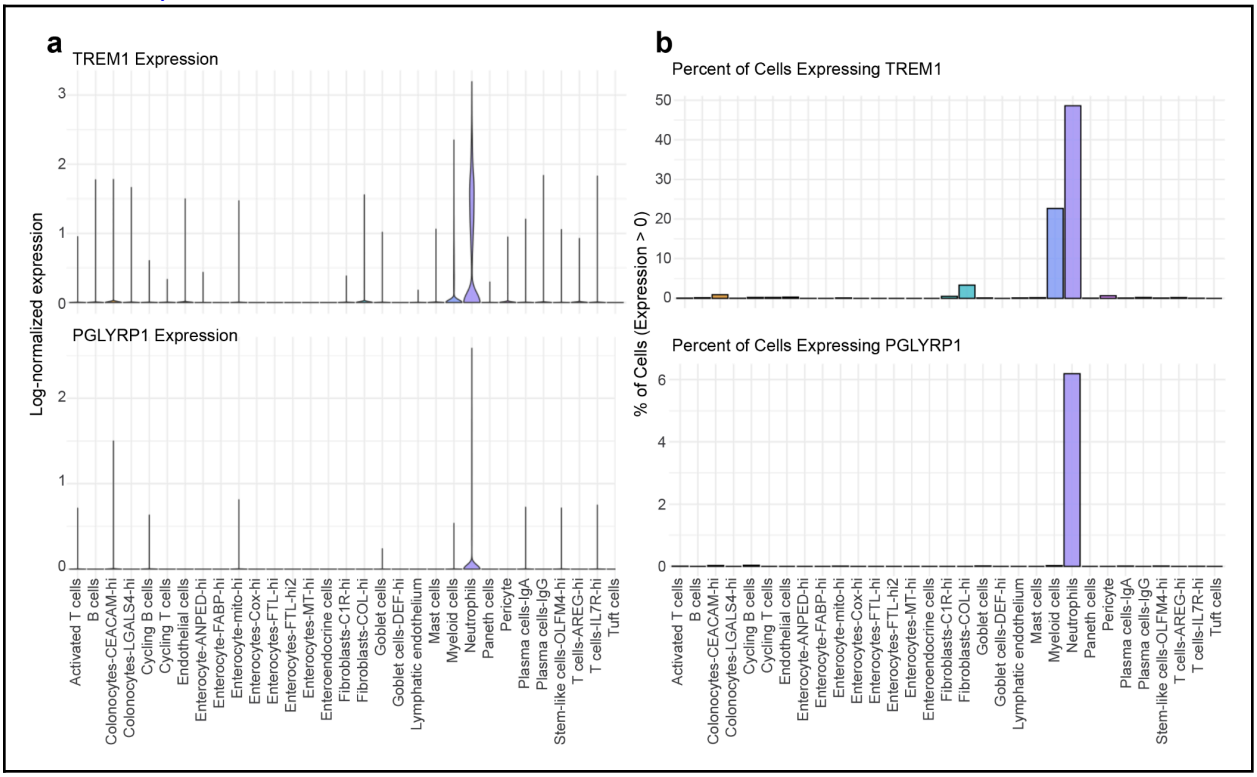
We thank the reviewer for raising these valid points.

1) Validation of TREM1 findings in human IBD biopsies:

To validate our in vitro findings, We first analyzed scRNAseq dataset from Crohn's disease patient biopsies (dataset from *Kong et al. Nat Genet. 2025, PMID: 40562913*). These data showed that both TREM1 and PGLYRP1 are predominantly expressed in neutrophils in the inflamed gut, with additional lower expression of TREM1 in other myeloid cells. These data have been incorporated into the revised manuscript (**Revised Extended Data Fig.5a-b**).

Next, we examined the localization of TREM1-expressing cells in human IBD biopsy samples compared to normal colonic mucosa from non-IBD donor using in situ hybridization (ISH) of TREM1 transcript (**Figure 7, below**). In biopsies from IBD patients, TREM1-expressing cells were predominantly localized at ulcerated regions with damaged epithelium. Quantification of TREM1 expression in IBD vs healthy mucosa revealed significantly higher TREM1 levels in IBD biopsies. In normal colonic mucosa, TREM1 transcript is detected at very low level (1-2 transcripts/cell) in most epithelial cells, consistent with scRNAseq data showing background expression in colonocytes. In contrast, biopsies from IBD patients showed a marked increase in TREM1 transcript inflammatory cells (up to 8 transcripts/cell), particularly in ulcerated regions or areas of resolving inflammation where wound-associated epithelium overlies granulation tissue. These findings are consistent with the elevated TREM1 pathway signature in inflamed regions, compared to non-inflamed regions in IBD biopsies (Fig. 5b).

These findings demonstrate that TREM1-expressing cells are localized at the damaged barrier surface in IBD, where they are positioned to interact with bacterial-derived PGN. This spatial relationship supports the proposed mechanism of TREM1 activation in the context of epithelial barrier disruption and microbial invasion.



Revised Extended Data Fig.5a-b. Expression of TREM1 and PGLYRP1 in human IBD biopsies.
The cell-type-specific expression patterns of TREM1 and PGLYPR1 in human IBD biopsies were examined using a public IBD dataset (*PMID: 40562913*). Gene expression per cell (a) and the percentage of cells expressing these genes (b) were plotted to illustrate their distribution across cell types captured in this dataset.

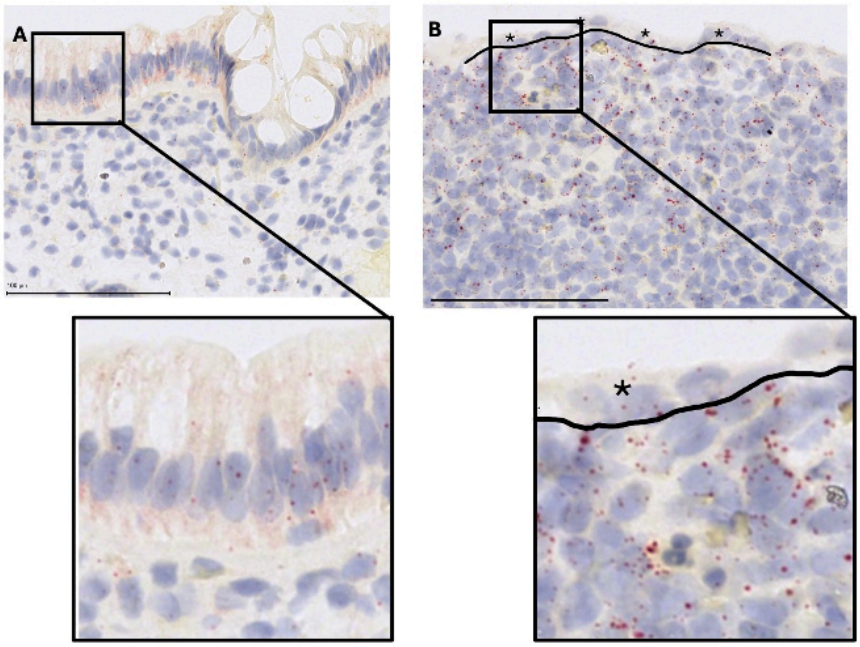


Figure 7. Localization of TREM1 expressing cells in the IBD biopsy.

TREM1 ISH on human colonic mucosal biopsies. A: Normal colonic mucosa, 1-3 transcripts per cell in healthy surface epithelium and scattered lamina propria immune cells. B: Biopsy from ulcerative colitis patient with severely active disease, with high expression in granulation tissue underlying wound-associated epithelium (asterisks). Scale bar=100 microns.

2) Localization and abundance of *B. faecis* in IBD tissues:

We agree that localizing and quantification of *B. faecis* in IBD tissues is important. However, this is challenging due to the lack of established probes for in situ hybridization and the small abundance of *B. faecis* in available bacterial genomic datasets. Using both our internal datasets and public data from the curatedMetagenomicData package, we estimate *B. faecis* abundance at a median value of 0.7%, which is too low to generate meaningful comparisons between IBD and non-IBD samples with current marker gene-based profiling methods. Future studies will require improved methodologies to address this limitation.

3) Selection of Bacterial Species:

The initial set of 15 bacterial species was selected based on our ability to isolate and culture bacteria under anaerobic conditions from IBD stool samples in our laboratory. While this is a limited number of species, it reflects practical constraints during initial screening efforts. This selection was intended as a first step to identify potential TREM1 activators among gut commensals, and our identification of *B. faecis* as a TREM1 activator highlights the utility of this approach.

Fig. 5. Correlation analysis of 911 patients across 7 clinical trials with appropriate batch correction and statistical methods represents substantial bioinformatic work.
Correlation of increased PGLYRP RNA with UC and IBD - is this in exosomes?.
Abstract: Modify lines 37-40 state the TREM1 signature "underscores its potential as a biomarker for disease progression and therapeutic efficacy." However, the study shows association with baseline non-responsiveness to other therapies and not progression over time.
Overall impression: Interesting study, but several points described in Specific Comments above need to be further understood to establish importance/translational relevance of B. faesis and TREM1 in human IBD.

Response:

We thank the reviewer for their positive comments on the bioinformatics work and for highlighting areas for clarification.

PGLYRP1 RNA and Exosomes: Whether PGLYRP1 RNA is found in exosomes is currently unknown. However, our analysis measures PGLYRP1 RNA in total blood cells, which reflects either an increase in neutrophil cell number or an upregulation of PGLYRP1 expression in IBD patients. We have clarified this point in the Methods.

TREM1 Signature and Abstract Update: Our data demonstrate that the TREM1 pathway signature is associated with inflamed versus non-inflamed tissue, disease severity, and response to therapy in IBD patients. We acknowledge that the study focuses on baseline non-responsiveness to therapy rather than disease progression over time. Accordingly, we have updated the abstract to accurately reflect these findings, in line 37-39:

“An elevated TREM1 pathway signature is associated with inflamed versus non-inflamed tissue, disease severity, and baseline nonresponsiveness to therapy in IBD patients, underscoring its potential as a biomarker for therapeutic response and disease activity.”

We appreciate the reviewer’s comments and agree that further studies are needed to establish the translational relevance of *B. faecis* and TREM1 in human IBD, particularly the structural basis of *B. faecis*-mediated TREM1 activation and its role in disease pathology.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response:

We thank the reviewer for their time and thoughtful participation in the review process. Your feedback, along with the comments from the co-reviewer, has been invaluable in strengthening our manuscript.

Reviewer #1

Additional experiments performed by authors answered all my comments. I have no further comments.

Response:
We thank the reviewer for the positive assessment. No additional changes were required in response to this comment.

Reviewer #2

The authors have responded in a substantive manner to my critiques on initial review. I have no further significant critiques for this manuscript.

Response:
We appreciate the reviewer's careful evaluation and are grateful that our previous revisions addressed the concerns raised. No additional changes were required in response to this comment.

Reviewer #3/#4

Point-by-point scrutiny: Revised TREM1/IBD manuscript (NC 650066)

Context

The manuscript by Andrade et al. proposes a two-loop TREM1 amplification model driving neutrophil-mediated inflammation in IBD. Below is a point-by-point audit of each of our concerns: what the authors claimed in the rebuttal, what they actually incorporated into the revised manuscript, and where gaps remain.

Summary table

#	Concern	Rebuttal claim	In manuscript?	Verdict
1	ED Fig. 1c: n=2 biological replicates is too low	Repeated with n=3 donors; added PMA positive control	Yes – ED Fig. 1c now shows three donors per group with PMA, Isotype, TREM1.ago	**Fully addressed**
2	Fig. 2a: correlation 0.26 between PGN/PGLYRP1 and agonist Ab; PGLYRP1 specificity	Explained as consequence of free PGN co-activating NOD1/2/TLR2; used correlation to extract TREM1-specific genes	Yes – text added at lines 139–143; no new experiment	**Adequate (text only)**
3	Fig. 2e: possible PGN/PGLYRP1 carryover from monocyte supernatant to neutrophils	Added lysozyme treatment of supernatant; confirmed TNF-dependent	Yes – revised Fig. 2e now contains lysozyme, anti-TNF, anti-TREM1 conditions; text lines 161–165	**Fully addressed**
4	Fig. 3: Gram-negative PGN activates TREM1 but B. faecis is Gram-positive; test B. faecis-derived PGN; commensal-vs-pathogen paradox	Propose GMTriP-K motif hypothesis; IF showed PGLYRP1 binds live B. faecis; structural testing of B. faecis PGN is "beyond scope"	Partially – Discussion expanded (lines 313–319 and 327–334); IF binding data NOT added to manuscript; no direct B. faecis PGN experiment	**Partially addressed**
5	ED Fig. 4: species differences (no mouse PGLYRP1/TREM1 binding, eCIRP failure); DSS model translatability	Provided sandwich ELISA, SEC, AlphaFold structural modeling, and RAW/muTREM1 ROS-TNF data in rebuttal only; added discussion	Partially – Discussion added (lines 377–382). Supporting mechanistic data kept in rebuttal, not added to manuscript	**Partially addressed**
6	Need validation in human IBD biopsies (TREM1/PGLYRP1 localization, B. faecis in tissue, 15-species selection rationale)	Added scRNAseq from Kong 2025; TREM1 ISH performed; B. faecis ISH not feasible; species chosen by anaerobic culturability	Partial – ED Fig. 5a–b added (scRNAseq). TREM1 ISH only acknowledged, NOT shown as a figure panel. Species rationale only in rebuttal, not Methods/Results	**Partially addressed**

7	Fig. 5: is PGLYRP1 RNA in exosomes?	No – measured in total blood cells; clarified in Methods	Need to verify Methods language; not visible as new manuscript text	**Adequate**
8	Abstract: "biomarker for disease progression" overstates findings	Abstract to be rewritten to "therapeutic response and disease activity"	Incomplete – first clause updated ("baseline nonresponsiveness"), but final clause of abstract (lines 39–40) still reads "biomarker for disease progression and therapeutic efficacy." Same phrasing retained in Discussion line 356	**NOT fully addressed**

Detailed analysis

Point 1. ED Fig. 1c sample size (n=2 biological replicates) [Fully addressed]

Our concern: n=2 biological replicates with technical triplicates is statistically weak.

Rebuttal: Repeated with n=3 independent donors, added PMA as positive control.

Revised manuscript: ED Fig. 1c now plots MPO ELISA for Medium, PMA, Isotype Ab, and TREM1.ago with three data points per group representing biological donors. PMA produces the strongest MPO release (p=0.0128) and TREM1.ago is significantly above isotype (p=0.008). The PMA control also helps calibrate the relative magnitude of TREM1-driven degranulation.

Verdict: Fully addressed. This is now a properly powered experiment.

Point 2. Fig. 2a correlation 0.26 and PGLYRP1 specificity [Adequate (text only)]

Our concern: The 0.26 correlation between PGN/PGLYRP1 and the agonist antibody is weak, raising the possibility that the complex preparation activates more than just TREM1.

Rebuttal: The low correlation is expected because the PGN/PGLYRP1 preparation contains free PGN that activates NOD1, NOD2, and TLR2. They argue the correlation-based selection is itself a way to isolate TREM1-specific genes, and they use PGN-only and anti-TREM1 blockade controls throughout.

Revised manuscript: Lines 139–143 now explicitly state the free-PGN/NOD/TLR2 caveat.

Verdict: Adequate. The explanation is defensible and the use of anti-TREM1 blockade across figures provides some specificity control. However, the authors did NOT run a parallel experiment with purified recombinant TREM1-ligand-complex free of unbound PGN, which would have been the definitive way to demonstrate pathway-specific activation. The textual concession is acceptable but not fully reassuring on its own.

Point 3. Fig. 2e PGN/PGLYRP1 carryover in monocyte supernatant [Fully addressed]

Our concern: The neutrophil ROS induced by monocyte supernatant could be due to PGN/PGLYRP1 carried over from monocyte culture, not monocyte-derived mediators.

Rebuttal: Added lysozyme pretreatment of the monocyte supernatant before transfer to neutrophils. Lysozyme did not abolish ROS induction, while anti-TNF did.

Revised manuscript: Fig. 2e now shows four stim conditions (PGN/PGLYRP1, +Lysozyme, +anti-TNF, +anti-TREM1). The PGN/PGLYRP1 and +Lysozyme curves overlap; anti-TNF and anti-TREM1 both strongly suppress ROS. Text at lines 161–165 describes this control.

Verdict: Fully addressed. This is a clean, direct control. The ROS induction is clearly driven by a soluble monocyte product (TNF), not residual ligand.

Point 4. Gram-positive B. faecis activating TREM1 when only Gram-negative PGN worked [Partially addressed]

Our concern: Fig. 3a shows only E. coli PGN (Gram-negative), not S. aureus or B. subtilis PGN (Gram-positive), activates TREM1. Yet B. faecis is Gram-positive and activates TREM1. Three requests followed: (i) test purified B. faecis PGN directly; (ii) explain how B. faecis PGN differs from other Gram-positives; (iii) reconcile its "commensal/probiotic" status with a role in IBD.

Rebuttal:

- Proposed that activation depends on whether a given PGN fragment contains a PGLYRP1-permissive GMTriP-K motif (Chen et al. 2025), not on Gram status per se.
- Showed direct IF binding of recombinant PGLYRP1 to live B. faecis cells (rebuttal Figure 1).
- Acknowledged that purifying and structurally characterizing B. faecis PGN is beyond the scope of this study.
- Argued that "commensal" does not mean "non-inflammatory," invoking E. faecalis as a precedent: harmless in healthy hosts, inflammatory once barrier integrity is lost.

Revised manuscript: Discussion expanded at lines 313–319 (E. faecalis analogy and context-dependent commensal behavior) and lines 327–334 (GMTriP-K motif hypothesis). The IF binding data showing PGLYRP1 association with live B. faecis is NOT in the manuscript, only in the rebuttal.

Verdict: Partially addressed.

- **Strengths:** The motif-based hypothesis is biologically plausible and well-referenced; the commensal-in-a-damaged-barrier framing is reasonable and well supported by the E. faecalis analogy.
- **Remaining gap:** The central claim – that B. faecis activates TREM1 via its PGN – is still not directly demonstrated. Purifying B. faecis PGN and showing it recapitulates the bacterial supernatant activity (as the authors did for E. coli PGN in Fig. 3a) is the experiment that would close this loop. The hypothesis about PGLYRP1-permissive motifs is speculation without it. At minimum, the IF binding data (rebuttal Figure 1) should have been added as an extended-data panel – it's a useful, straightforward piece of evidence that's currently hidden in the response letter.

Point 5. ED Fig. 4 species differences and mouse model translatability [Partially addressed]

Our concern: SPR showed mouse PGLYRP1 doesn't bind mouse TREM1, and eCIRP (a published ligand) also doesn't. These undermine the translational relevance of the DSS model, because the upstream arm of the pathway is not conserved.

Rebuttal: Extensive supplementary evidence was provided:

- Sandwich ELISA confirming that the PGLYRP1/TREM1 interaction is species-specific (rebuttal Figure 3).
- SEC + SDS-PAGE showing human PGLYRP1 forms a disulfide-linked dimer while mouse PGLYRP1 does not (rebuttal Figure 4) — potentially explaining the functional difference.
- AlphaFold2 modeling highlighting a non-conserved residue (D103) in mouse PGLYRP1 that may sterically block PGN engagement (rebuttal Figure 5).
- eCIRP was tested functionally and found to signal via MyD88 (not TREM1) in BMDMs (rebuttal Figure 2).
- Mouse TREM1 activation by plate-coated agonist (the DSS experimental tool) does reproduce the expected downstream outputs (ROS in mouse neutrophils; TNF in mTREM1/DAP12 RAW cells; rebuttal Figure 6).

Revised manuscript: Added language at Discussion lines 377–382 acknowledging upstream divergence, defending the downstream conservation argument, and calling for validation in humanized mouse models. Crucially, the authors

wrote: "these data are not incorporated into the revised manuscript." So none of rebuttal Figures 2–6 made it into the paper.

Verdict: Partially addressed.

- **Scientifically defensible:** The argument that downstream TREM1 signaling (neutrophil activation, cytokine production) is conserved even when upstream ligand recognition is not, and that a multivalent agonist bypasses the ligand problem, is a legitimate workaround. The added discussion acknowledges this nuance honestly.
- **Missed opportunity:** The sandwich ELISA, SEC/SDS-PAGE of PGLYRP1 dimerization, and the AlphaFold-based structural argument are exactly the kind of mechanistic story that strengthens the species-difference claim. They should be an extended-data figure. Burying them in the rebuttal deprives future readers of a clean reference. The eCIRP MyD88-dependence result is also worth adding.
- **Remaining concern:** The DSS model still speaks only to downstream effector biology. Any claim that a TREM1-targeting therapy would block B. faecis-driven or PGLYRP1-driven inflammation in humans is, in practice, unvalidated in vivo. This limitation should be spelled out more forcefully in the abstract/introduction, not only in the Discussion.

Point 6. Validation in human IBD biopsies; B. faecis in tissue; rationale for 15-species panel [Partially addressed]

Our concern: The entire mechanism is proposed to operate in human IBD tissue but was never demonstrated there. We requested:

- Immunostaining for TREM1+ cells and PGLYRP1 in IBD biopsies.
- Spatial mapping relative to bacteria and damaged epithelium.
- Quantification of TREM1 in inflamed vs non-inflamed regions.
- Localization/abundance of B. faecis in IBD tissue.
- A clear rationale for how the initial 15-species panel was chosen.

Rebuttal:

- scRNAseq re-analysis of Kong et al. 2025 IBD data confirms TREM1 is strongly enriched in neutrophils (49% of cells) and PGLYRP1 is almost exclusively neutrophil-restricted.
- TREM1 ISH on healthy vs UC biopsies was performed: elevated TREM1 transcripts in ulcerated regions, up to ~8 transcripts/cell, co-localized with wound-associated epithelium and granulation tissue (rebuttal Figure 7).
- B. faecis spatial localization: acknowledged as infeasible with current probes; quantification estimates ~0.7% median abundance, too low for IBD-vs-healthy comparison.
- 15-species panel rationale: selected by what could be anaerobically cultured from in-house IBD stool samples.

Revised manuscript:

- **Added:** ED Fig. 5a–b (scRNAseq expression and % positive cells across gut cell types; TREM1 in neutrophils and other myeloid cells, PGLYRP1 almost exclusively in neutrophils). Main-text mention at lines 251–255.
- **Not added:** The TREM1 ISH dataset. This is striking. The Acknowledgements explicitly thank "Serena Y. Lee for the generation of the TREM1 ISH data," so the experiment exists and was performed – but no ISH panel appears in Fig. 5 or any Extended Data figure. Of all the data in the rebuttal, this is the single most directly relevant to our concern, and it should have been incorporated.
- **Not added:** The 15-species selection rationale. A brief note in Methods (or the Fig. 3 legend) would have resolved this.

Verdict: Partially addressed. The scRNAseq data (ED Fig. 5a–b) is a solid addition and directly addresses the "where is TREM1 expressed in IBD tissue" question at the cell-type level. However, the ISH data — which was actually generated — is inexplicably absent from the figures. This omission is the single most fixable gap in the revision. B. faecis tissue localization being infeasible is acceptable with the honest explanation they gave, provided it's stated in the Discussion rather than only in the rebuttal.

Point 7. PGLYRP1 RNA – measured where? Exosomes? [Adequate]

Our concern: Fig. 5 reports blood PGLYRP1 RNA increase; we asked whether this reflects exosomal PGLYRP1 or whole-blood transcript.

Rebuttal: Measured in total blood cells, reflecting either more neutrophils or PGLYRP1 upregulation. Not known whether PGLYRP1 RNA is in exosomes.

Revised manuscript: Authors state this was clarified in Methods. The main-text narrative around Fig. 5f–i (lines 277–282) now says "RNA level of PGLYRP1 ... in blood samples," which is clearer than the original but still does not disambiguate cell-of-origin in the text.

Verdict: Adequate. The whole-blood interpretation is reasonable. If the exact source matters for the biomarker claim (e.g., if regulation vs. cellular composition changes), the authors could state that it is not disentangled here — a sentence in the Discussion would suffice.

Point 8. Abstract wording: "biomarker for disease progression" [NOT fully addressed]

Our concern: The abstract's claim that the TREM1 signature underscores its potential as a biomarker for "disease progression and therapeutic efficacy" overstates the results. The data show association with baseline non-response and disease activity/severity, not with progression over time.

Rebuttal: Authors committed to changing the abstract to: "...underscoring its potential as a biomarker for therapeutic response and disease activity."

Revised manuscript (lines 37–40, as extracted from the PDF):

> *"An elevated TREM1 pathway signature is associated with inflamed versus non-inflamed tissue, disease severity, and baseline nonresponsiveness to therapy in IBD patients, underscoring its potential as a biomarker for disease progression and therapeutic efficacy."*

The first clause was updated (it now correctly says "baseline nonresponsiveness"), but the terminal phrase — the precise wording we flagged — is unchanged. "Disease progression and therapeutic efficacy" is still there. The Discussion (line 356) also still contains "biomarker for disease progression and therapeutic response."

Verdict: NOT fully addressed. This is the clearest incomplete correction in the revision. The authors explicitly told the reviewer they would change this wording, and they changed half of it but left the wording we objected to intact. This should be flagged to the editor; an easy fix, but it demonstrates the response letter is not fully reconciled with the revised file.

Overall impression

What the revision does well

- Real new experiments where they mattered most: proper n=3 biology in ED Fig. 1c; lysozyme carryover control in Fig. 2e; purified monocyte/neutrophil sorting in ED Fig. 2a confirming neutrophil-restricted PGLYRP1 release; MPO-DNA complex ELISA in Fig. 1n; improved Cit-H3 immunofluorescence with a better antibody (Fig. 1m); PMA kinetic control in Fig. 1l; isotype control in ED Fig. 1b; scRNAseq reanalysis across cell types (ED Fig. 5a–b).
- Honest textual engagement with the Gram-status / commensal paradox and with the human-vs-mouse species differences.
- Rewritten abstract correctly moves away from "therapy response" in favor of "baseline nonresponsiveness."

What is incompletely addressed

- Abstract and Discussion still retain the phrase "biomarker for disease progression," contradicting both our critique and the rebuttal's own promise. Easy fix; must be corrected before acceptance.
- TREM1 ISH data on IBD biopsies was generated (acknowledged to Serena Y. Lee) but never appears as a figure panel. This is arguably the single most important translational validation and its absence is difficult to justify.

- Direct B. faecis PGN experiment was declined as "beyond scope," and the IF binding of PGLYRP1 to live B. faecis (rebuttal Figure 1) was not incorporated into the manuscript. Without either, the Gram-positive / Gram-negative puzzle is resolved only at the level of a hypothesis.
- The species-specificity follow-up data (sandwich ELISA, SEC, structural modeling, eCIRP MyD88-dependence, mouse TREM1 ROS/TNF validation) are solid and belong in an extended-data figure; they should not live only in the rebuttal.
- 15-species selection criterion is still invisible to the reader; a one-line methodological note would close this.

Recommendation

Conditional acceptance after minor-to-moderate revision. Specifically, we would ask the authors to:

- Fix the abstract and Discussion wording about "disease progression" to match what the rebuttal promised.
- Add the TREM1 ISH data as an Extended Data panel (this is the highest-value change, given the data already exist).
- Add the species-specificity mechanistic data (at minimum the sandwich ELISA + dimerization result) as an Extended Data panel; it is the cleanest explanation of why mouse TREM1 doesn't bind mouse PGLYRP1.
- Add a methods sentence stating how the 15 commensal species were selected.
- Either add the PGLYRP1-on-B. faecis IF panel or explicitly cite it in the text as supporting evidence for the GMTriP-K motif hypothesis.

Scientifically, the core story remains interesting and well-supported. The in vitro mechanism for the two-loop amplification is convincingly demonstrated, the clinical signature in 911 patients is robust, and the new controls genuinely tighten several panels. The outstanding issues are mostly communicative rather than foundational — but one of them (the abstract wording) is a direct compliance failure with the rebuttal and needs to be fixed.

Response:
We appreciate the detailed comments. As noted in the “Overall impression” section:

- Points 1–3 (ED Fig. 1c, Fig. 2a, Fig. 2e) were already considered fully or adequately addressed, and we did not make further changes beyond what is described in the prior rebuttal.
- Point 7 (PGLYRP1 RNA source) was considered adequate; in the current revision we further clarified in the Methods and in the Results/Discussion that PGLYRP1 RNA was measured in total whole-blood RNA and that we cannot distinguish changes in cell composition from per-cell expression.
- We have implemented the requested additional changes for Points 4–6 and 8 as described below in response to the reviewer’s recommendation (B. faecis IF, species-specific PGLYRP1–TREM1 data, TREM1 ISH, 15-species selection rationale, and biomarker wording).

We are grateful that the reviewer regards the core mechanism and clinical signature as robust and agree that the outstanding issues were primarily communicative. We have therefore focused this revision on making these aspects as transparent and accessible as possible.

Recommendation
Conditional acceptance after minor-to-moderate revision. Specifically, we would ask the authors to:

• Fix the abstract and Discussion wording about "disease progression" to match what the rebuttal promised.

Response:
We have revised the wording in both the abstract and Discussion about “biomarker” and “disease progression”.

• Add the TREM1 ISH data as an Extended Data panel (this is the highest-value change, given the data already exist).

Response:

We have now incorporated the TREM1 in situ hybridization (ISH) data into the main manuscript:

- We added the ISH results as Supplementary Fig. 5c, showing TREM1 transcript signal in human colon biopsies.
- In the Results section, we now state (lines 276-278):
  “In situ hybridization on colon biopsies shows increased TREM1 transcript signal in ulcerated regions of UC compared to non-inflamed normal tissue (Supplementary Fig. 5c).”

- We have also added a dedicated Methods subsection describing the ISH procedure (sample source, platform/probe, staining and imaging).

• Add the species-specificity mechanistic data (at minimum the sandwich ELISA + dimerization result) as an Extended Data panel; it is the cleanest explanation of why mouse TREM1 doesn't bind mouse PGLYRP1.

Response:

We agree that these data are important for clarifying species specificity and have now incorporated them into the manuscript:

- We added the protein-protein interaction (sandwich) ELISA demonstrating that human PGLYRP1 binds human TREM1 but not mouse TREM1, and that mouse PGLYRP1 does not bind mouse TREM1, as Supplementary Fig. 4b-c.
- We added the size-exclusion chromatography and SDS-PAGE dimerization analysis showing that human PGLYRP1 forms a disulfide-linked dimer, whereas mouse PGLYRP1 remains monomeric during purification, as Supplementary Fig. 4d-e.
- The Results section has been updated accordingly (lines 220-228) to describe these species-specific binding and dimerization findings and to relate them to the lack of functional PGLYRP1-TREM1 coupling in mice.
- We also added the corresponding Methods description for these assays (lines 659-670).

• Add a methods sentence stating how the 15 commensal species were selected.

Response:
We added a sentence at the beginning of the “Bacteria strains” subsection in Methods (lines 477-480).

• Either add the PGLYRP1-on-B. faecis IF panel or explicitly cite it in the text as supporting evidence for the GMTriP-K motif hypothesis.

Response:
We have added the PGLYRP1-on-*B. faecis* immunofluorescence data as a new panel in Supplementary Fig. 3 (panel d), and we include a brief description of the IF method in the Methods section (lines 717-733), and cited in the Discussion below (lines 343-347):

“Recent studies show that PGLYRP1 selectively binds disaccharide-containing PGN motifs, such as GMTriP-K-like structure, which are critical for downstream signaling. Consistent with this idea, recombinant PGLYRP1 shows clear surface binding to live B. faecis cells by

immunofluorescence (Supplementary Fig. 3d), suggesting that B. faecis presents PGLYRP1-permissive PGN motifs."