

The interleukin 22–oncostatin M axis promotes intestinal inflammation and tumorigenesis

Corresponding Author: Professor Ahmed Hegazy

Version 1:

Decision Letter:

12th Jul 2024

Dear Ahmed,

Thank you for providing your point-by-point response to the referees' comments on your manuscript entitled, "The interleukin 22–oncostatin M axis promotes intestinal inflammation and tumorigenesis". As noted previously, while they find your work of considerable potential interest, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the current manuscript for publication, but would be very interested in considering a revised version that addresses these concerns along the lines proposed in your point-by-point response.

We invite you to submit a substantially revised manuscript, however please bear in mind that we will be reluctant to approach the referees again in the absence of major revisions.

Specifically, the revision should include new experiments to address:

- (1) perform intracellular staining of CD3e to interrogate if any gd T cells may contribute as a source of IL-22
- (2) perform more extensive analysis of cytokine production by ILCs in your intestinal inflammation models
- (3) look at the IL-22-OSM-OSMR axis in the Citrobacter infection model
- (4) perform OSM/OSMR immunohistochemistry on human CAC tissue samples
- (5) measure OSMR protein measurements in the inflammation models

Please include the additional textual clarifications as indicated in your response letter.

When you revise your manuscript, please take into account all reviewer and editor comments, please highlight all changes in the manuscript text file in Microsoft Word format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

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* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

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Please do not hesitate to contact me if you have any questions or would like to discuss the required revisions further.

Thank you for the opportunity to review your work.

Kind regards,

Laurie

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Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

This manuscript investigates the role of IL-22 in activating intestinal epithelial cell transcription of STAT3 and responsiveness to oncostatin. Together this axis acts to drive sustained inflammation in models of large intestinal inflammation and tumorigenesis. This study assembles a number of interesting findings which are new to the field and shows that pharmacological blocking is feasible, at least in an experimental setting. The study is well performed and aims to discriminate the cell type driving the regulation of the oncostatin pathway. While OSM and OSMR are relatively restricted in their expression, this demarcation is not complete and not completely addressed experimentally.

To explore the contributions of the epithelium to this pathway, the group have generated multiple cell-specific knockout animals to elucidate the involvement of oncostatin and have performed RNAseq analysis. This identified that the OSM-OSMR signalling pathway appeared to be a non-redundant pathway in intestinal inflammation.

The authors found that IL-22 was expressed in ILC3 and gd T cells but have not examined gd T cells. These seem to be excluded by the utilization of Rag2^{-/-}. However, some gd T cells can remain but express T cell markers intracellularly and have previously been assumed to be ILC3. Intracellular staining for this would address this concern.

Oncostatin M appears to be mainly expressed in gd T cells, Treg and ILC2. What role do ILC2 play in this pathway? Are all effects mediated directly through ILC3 or could ILC3 be intermediate regulators of ILC2? Given this expression pattern at the mRNA level at least, it is unclear how as indicated by the author, targeting amongst immune cells would work and what effects might actually be driven by more complex interactions.

Organoids have been utilized for some of the experiments. While such approaches are innovative, it is unclear how closely these replicate the situation in vivo in dissecting the key cells involved in this pathway. This would seem important as small populations of immune cells also appear to express low levels of OSMR.

Cytokine production is measured following the strong stimulus PMA and ionomycin demonstrating what a cell is capable of rather than how it might respond constitutively to relevant stimuli. What is the response to IL-7, IL-2 and IL-23 as stimuli? Was IFN- γ measured across ILC populations?

Figures. Please provide scale bars for all histological sections. It is not clear how many sections were analysed and from how many independent animals these were examined.

Figure 7a examines OSMR expression and indicates that this is expressed in epithelial cells and stromal cells but it is not clear how the different cell types have been delineated as this is not evident in the sections provided.

Reviewer #2:

Remarks to the Author:

In this study, Cineus et al. provide novel and important insights into how the IL-22/OSM/OSMR axis can regulate intestinal inflammation and tumorigenesis. The authors first show an upregulation of OSM and OSMR in enterocytes during intestinal inflammation in mice and confirm the relevance of this finding by analyzing human IBD samples. The authors next perform functional experiments using the H. hepaticus + α -IL-10R model and identify a causal role of OSM/OSMR in regulating intestinal inflammation. Mechanistic experiments then revealed that OSMR in enterocytes is regulated by ILC-3-derived IL-22 via STAT3 activation. Finally, the authors describe an important role of OSM/OSMR in intestinal tumorigenesis using a murine CAC model.

The data appear to be clear and are presented in a very good way. The manuscript is written well, structured and easy to follow. Overall, the findings could be highly relevant for scientists working in the field of intestinal immunology and oncology.

However, despite the overall convincing findings by the authors, I believe that several points need to be addressed in order to further improve the manuscript and strengthen their findings.

I outline my individual concerns in detail below:

1. The authors show a crucial role of the IL-22-OSM-OSMR axis in intestinal inflammation using the H. hepaticus + α -IL-10R model. The authors have in detail analyzed the mechanism in this model, including the relevant cellular source of IL-22 and the signaling pathway. However, the cellular and molecular mechanisms described could be specific to this one specific murine colitis model. Thus, it would be important to study other colitis models, to confirm the role of the IL-22-OSM-OSMR axis as well as to analyze the respective cellular source of IL-22.

2. The authors should clarify, if there is a difference in colitis severity in the AOM/DSS model as inflammation is one key driver of tumors in this model

3. Related to the above point - the relevance of the IL-22-OSM-OSMR axis should also be tested in a second inflammation independent model of colon cancer. If data cannot be confirmed then a second inflammation driven colitis model would further strengthen the data.

4. The authors do show some limited human data, which point towards a possible relevance of the IL-22-OSM-OSMR axis in IBD. However, it remains unclear whether there is any molecular or clinical association in human CAC (or CRC) patients that would suggest a possible role of this pathway in human CRC. For example, is the expression of OSM/OSMR dysregulated in human CRC? Does it correlate with IL-22 levels? Is there an association with oncological outcome? Those questions could be answered using publicly available data sets and would provide important complementary information to the data shown.

5. The colitis severity assessment relies primarily on histopathological scoring. Since the results are based mainly on this method and proper histopathological assessment requires extensive experience and skill, it would be important to describe, if the scoring was performed by someone with a formal histopathological training in a blinded fashion? Of note, tumor development was assessed using a murine endoscopy system. I would encourage the authors to also use this system for colitis scoring.

6. The endoscopy procedure/method is not described in the methods section – this should be included.

7. The authors have analyzed the functionally relevant source of IL-22 in the H. hepaticus + aIL10R model and describe ILC-3-derived IL-22 as the functionally relevant source for regulating OSMR in enterocytes. However, the main and functionally relevant source was only studied in this one particular disease model. Are ILC-3 also the main and functionally relevant source of IL-22 in tumors and other inflammation models – thus, is the identified cellular cross-talk universal or is it limited to early colitis (or to this one particular colitis model)? I make this point since various cellular sources of IL-22 can play a pivotal role in different models and stages of colitis, and multiple sources of IL-22 have been attributed a key role in cancer development and progression.

8. To what extent are the effects of IL-22 dependent on the OSM-OSMR axis? For example, does pharmacological inhibition of IL-22 show anti-tumor effects in OSM(R) knock-out mice? The authors should discuss the other known mechanisms of how IL-22 can promote tumorigenesis.

Reviewer #3:

Remarks to the Author:

The authors comprehensively dissected a series of cytokine-mediated events that contribute to intestinal inflammation and tumorigenesis. Central to this study is the OSM-OSMR axis in epithelial cells, which upon activation upregulates transcripts involved in inflammation, proliferation, and recruitment of immune cells. Intriguingly, while OSMR is constitutively expressed in stromal cells, intestinal pathology is only evident following induced OSMR expression by epithelial cells, which requires ILC3-derived IL-22. Indeed, mice engineered to lack IL-22R or those administered neutralizing antibodies to prevent IL-22 signaling showed abolished epithelial OSMR expression levels and concurrent intestinal pathology. In agreement with these findings, neutralizing IL-23, a cytokine required for ILC3-mediated IL-22 expression, also reduced intestinal inflammation.

This study provides important insights into how a cellular network of epithelial and resident innate immune cells is tightly intertwined to orchestrate a protective response or, when dysregulated, drive severe immunopathology.

Several concerns remain that need to be addressed:

Main

1. A central point in this study is the OSM-OSMR axis, but strikingly, only gene expression data is presented without corresponding protein data. This is important because, for example, the presented ISH images for *Osmr* appear completely negative at steady-state, while scRNA-seq shows that endothelial, stromal, and smooth muscle cells abundantly express this gene at steady-state (up to 75% of the cells). Moreover, following inflammation, the *Osmr*-expressing epithelial fraction represents only 5% of the cells, and while those expression levels are 10-fold lower compared to endothelium, ISH images show clear expression for only those cells. How do the authors explain this discrepancy? Additionally, *Osmr* is only modestly expressed in a subset of epithelial cells (fig. 1i, suppl. Fig. 3) and is virtually absent at steady-state as stated several times throughout the manuscript. However, depicted expression levels are normalized to *Osmr* levels at steady state (e.g., fig. 1j, 3e, g, j, m, 5g). How is this possible if the *Osmr* gene is not expressed under these conditions?

2. The authors state that *Osmr* is clearly induced following infection and reaches levels comparable to *Il22ra1* (line 119). However, suppl. fig. 3 indicates that this cytokine is only very modestly expressed, and *Osmr* expression does not reach levels comparable to *Il22ra* at all. Instead, *Il22ra* expression levels seem to be rather downregulated in all depicted enterocyte subtypes following infection.

3. The authors state that *Il22* is mainly expressed in ILCs and to a lesser extent by $\gamma\delta$ T cells, hence they continue to test this as presented in figure 5. However, a gating strategy to select for ILCs is lacking, and prior to that, no gating is shown for the IL-22-expressing fraction of cells used for fig. 5a-d. This should all be shown. The data presented in fig. 5d is unclear and seems to be incorrect, with more than 75% of IL-22-expressing cells being $\alpha\beta$ T cells; the presented scRNAseq dataset (suppl. Fig. 3) shows differently. Indeed, the authors acknowledge that it is mainly $\gamma\delta$ T cells and ILCs that are the IL-22+ cells (line 231). So, either this line should be changed or fig. 5d needs to be reevaluated. To properly interpret the data, it is very important to present all the FACS scatter plots, including IL-22 expression.

4. Figure 6c, f: for a non-bioinformatician, it is difficult to understand the meaning or relevance of a “pathway activity score.” The legends here are not very helpful. Additionally, this figure is essentially a re-analysis of data presented in figure 1 and figure 2, and does not add much as a stand-alone figure.

5. Supplementary Fig. 11a is confusing as the authors claim that *Osmr* is largely absent under steady-state and is induced following IL-22 stimulation. In this figure, however, the authors show that the epithelium is already responsive to OSM without prior exposure to IL-22. How do the authors explain this? Moreover, the manuscript lacks a (time-course) experiment in which OSM is exposed to organoids that are either pre-treated with IL-22 or not. A readout could be pSTAT3 or induced genes as identified in fig. 2h.

6. Does neutralization of IL-23 also have an effect on tumor burden?

7. Line 152: no human data is shown in figure S6c.

Version 2:

Decision Letter:

Our ref: NI-A38071B

11th Mar 2025

Dear Admed,

Thank you for submitting your revised manuscript "The interleukin 22–oncostatin M axis promotes intestinal inflammation and tumorigenesis" (NI-A38071B). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Immunology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

We will now perform detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

If you had not uploaded a Word file for the current version of the manuscript, we will need one before beginning the editing process; please email that to immunology@us.nature.com at your earliest convenience.

Thank you again for your interest in Nature Immunology Please do not hesitate to contact me if you have any questions.

Kind regards,

Laurie

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Reviewer #1 (Remarks to the Author):

In general the authors have addressed my comments.

Scale bars: these have been added but are extremely small and difficult to read on the micrographs. Some do not seem to match the number quoted in the text eg. Fig. 3f appears to be 100um but is indicated to be 20 in text.

Reviewer #2 (Remarks to the Author):

The authors have addressed all my comments and concerns.

Reviewer #3 (Remarks to the Author):

The manuscript has improved substantially and the vast majority of the raised concerns have been sufficiently addressed. A few points still require further attention, as listed below.

1. The authors claim that ILC3s are the predominant IL-22 producing cells following infection. To support this claim, the authors now added supplemental figure 13c, and supplemental figure 16 in addition to the existing figures 5a-f. Furthermore, the material and methods section now describes the full procedure in which fresh isolated LPMCs were re-stimulated with IL-1b, IL-23, and PMA/ionomycin for 4 hrs in the presence of brefeldin A, followed by surface staining, fixation, and intracellular staining for IL-22. While these additions help to understand the exact workflow, for reasons of clarity I suggest the following:

- provide the FACS plot with total LPMC IL-22 expressing cells at steady-state and in disease upon which figure 5a is based. This would clarify the gating strategy used to generate figure 5b-d.
- Figure 5c aims to describe the cell populations within this IL-22 expressing fraction as summarized in figure 5a. However, it is not clear whether this is from the control or inflamed intestine. Please add this information.
- Figure 5d is a summary of the percentages of each cell type that express IL-22 (control and inflamed), but from figure 5c it seems that the fraction of gdT cells is very small (< 1%), while the pie-diagram seems to depict a much higher percentage. As no percentages are given in figure 5c, it makes it difficult to interpret the pie-diagram.

2. The study employs three colitis models to demonstrate the role of IL-22 to activate in activating the OSM-OSMR axis:
a. Helicobacter hepaticus + anti-IL10 Abs,

- b. Acute DSS (fig. S15f-h)
- c. *Citrobacter Rodentium*

The authors convincingly show that IL-22 drives epithelial OSMR upregulation in all three models, and that neutralizing IL-22 reduces colitis severity. However, previous studies have established that IL-22 as protective in *C. Rodentium* infection. Given that this study describes an opposite effect (new added data after revision), could the authors elaborate in the on how their findings reconcile with prior literature?

3. The authors demonstrate that blocking the OSM-OSMR axis ameliorates colitis by reducing epithelial STAT3 activation, which otherwise promotes immune cell recruitment and inflammation. The inflammatory response is typically aimed at controlling infection, yet their newly added supplemental data suggest that recruited immune cells do not impact bacterial dissemination or bacterial load (supplemental figure . Could the authors include a brief discussion—perhaps a single sentence—speculating on the physiological function of this pathway?

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Reviewer #1

(Remarks to the Author)

This manuscript investigates the role of IL-22 in activating intestinal epithelial cell transcription of STAT3 and responsiveness to oncostatin. Together this axis acts to drive sustained inflammation in models of large intestinal inflammation and tumorigenesis. This study assembles a number of interesting findings which are new to the field and shows that pharmacological blocking is feasible, at least in an experimental setting. The study is well performed and aims to discriminate the cell type driving the regulation of the oncostatin pathway. While OSM and OSMR are relatively restricted in their expression, this demarcation is not complete and not completely addressed experimentally.

To explore the contributions of the epithelium to this pathway, the group have generated multiple cell-specific knockout animals to elucidate the involvement of oncostatin and have performed RNAseq analysis. This identified that the OSM-OSMR signalling pathway appeared to be a non-redundant pathway in intestinal inflammation.

The authors found that IL-22 was expressed in ILC3 and gd T cells but have not examined gd T cells. These seem to be excluded by the utilization of Rag2^{-/-}. However, some gd T cells can remain but express T cell markers intracellularly and have previously been assumed to be ILC3. Intracellular staining for this would address this concern.

Response: We thank the reviewer for this thoughtful suggestion and agree that it is important to evaluate $\gamma\delta$ -T cells as a potential source of IL-22. In our original manuscript, we used flow cytometry to detect IL-22 protein expression in different cell subsets. Using 16-color flow cytometry, we excluded selected cell populations (CD19⁻, CD11c⁻, Ly6G/C⁻, F4/80⁻, FcER1 α ⁻) (**Fig. 5c**), then gated on lineage-negative, live cells, and divided the remaining cells into TCR α , TCR $\gamma\delta$, and ILCs. We stimulated the cells with PMA/ionomycin, IL-1 β , and IL-23 (a standard approach in the study of innate immune cells), to explore cytokine expression in both ILCs and T cells. After stimulation, we observed that ILCs increase the production of IL-22 in inflammation and identified ILC3 as the primary source of IL-22 expression within the broader ILC population (**Fig. 5d, f**). Please find below (**Figure for Reviewer 1**), where we now show a significant increase in IL-22 expression by colon ILCs during colitis, whereas there is a reduction in IL-22 expression by $\alpha\beta$ T cells and no significant change in IL-22 production by $\gamma\delta$ T cells. This depiction was not included in the initial submission and has now been added in **Fig. S13c** of our revised manuscript. To assess the role of ILC3 in the upregulation of Oncostatin M receptor (Osmr) in IECs during intestinal inflammation, we used Rag2^{-/-} and Rag2^{-/-} x Rorc^{-/-} mice to demonstrate that Osmr can be induced in the absence of T cells. Using Rag2^{-/-} x Rorc^{-/-} mice, which are deficient in ILC3s, we observed a strong reduction in IL-22 expression in the absence of ILC3s and a lack of OSMR upregulation in epithelial cells after colitis induction (**Fig. 5i**). Thus, our findings highlight an important role for ILCs, specifically ILC3, in the early induction of Osmr expression by IECs.

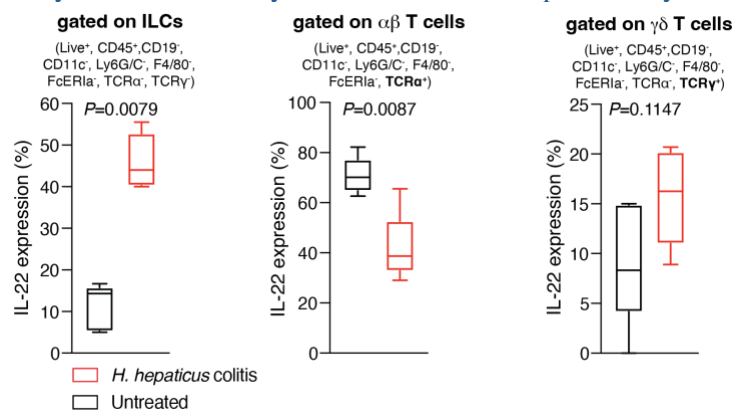


Figure for Reviewer 1. *H.h.* + α IL-10R colitis was induced in wild-type mice for 7 days. Flow cytometry was used to assess cytokine expression after stimulation with PMA/ionomycin, IL-23, and IL-1 β . IL-22 production

within the indicated populations is shown. Data are representative of 2 experiments, $n=12$. P -values are derived from Mann-Whitney U tests.

To specifically address the reviewer's concern, we additionally performed intracellular staining for CD3 ϵ , TCR $\alpha\beta$, and TCR $\gamma\delta$ to determine whether residual $\gamma\delta$ T cells contribute to IL-22 production in colitic Rag-deficient mice. We gated on IL-22⁺ CD45⁺ CD90⁺ CD19⁻ CD11c⁻ Ly6G/C⁻ F4/80⁻ Fc ϵ RI α ⁻ cells and subsequently analyzed the expression of CD3, CD3 ϵ , TCR $\alpha\beta$, and TCR $\gamma\delta$. B6 mice were included as controls to validate staining specificity.

Our analysis revealed that no TCR $\gamma\delta$ ⁺ cells were detected among the IL-22-producing population (**Figure 2 for Reviewer, Panel A, upper row**). While a small fraction of intracellular CD3⁺ cells were TCR $\alpha\beta$ ⁺, they accounted for only a minor subset of IL-22⁺ cells. Notably, more than 80% of IL-22-producing cells were intracellular CD3⁻ and ROR γ t⁺ (**Figure 2 for Reviewer, Panel B**), strongly supporting their identity as ILC3s.

It is important to note that intracellular expression of CD3, TCR $\alpha\beta$, CD4, and CD8 has been previously reported in innate lymphoid cells under certain conditions. However, these intracellularly localized CD3 and TCR molecules are functionally distinct, as they fail to mediate CD3/CD28-dependent activation due to their lack of surface expression¹⁻⁵.

Taken together, our data demonstrate that ROR γ t⁺ ILC3s are the primary source of IL-22 in colitic Rag-deficient mice. Furthermore, the absence of TCR $\gamma\delta$ ⁺ T cells within this population excludes their contribution to IL-22 production and OSMR upregulation in intestinal inflammation. However, we acknowledge that in different contexts and time points, CD4 T cells and $\gamma\delta$ T cells might also be capable of driving or sustaining Osmr expression during inflammation. Therefore, we have now included a paragraph in the discussion addressing the possible contribution of different cell types as a source of IL-22 (Main manuscript, page 20, line: 433-445).

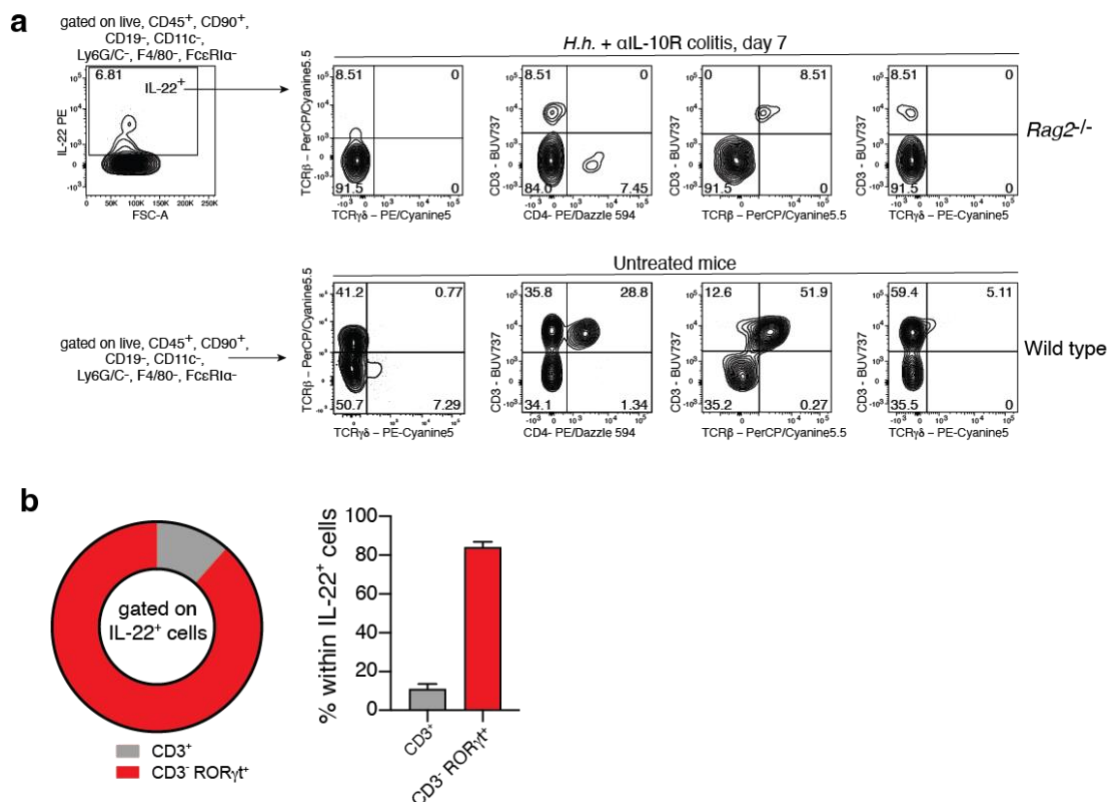


Figure for Reviewer 2. Identification of the cellular source of IL-22⁺ cells in *H. hepaticus* + aIL-10R colitis in Rag2^{-/-} mice. (a) Flow cytometric analysis of IL-22⁺ cells in *H. hepaticus* + aIL-10R colitis (day 7) in Rag2^{-/-} and wild-type mice. Cells were gated on live, CD45⁺, CD90⁺, CD19⁻, CD11c⁻, Ly6G/C⁻, F4/80⁻, and Fc ϵ RI α ⁻ populations. The leftmost panel shows the gating strategy for IL-22⁺ cells. Subsequent panels depict the characterization of IL-22⁺ cells based on the expression of the indicated markers. Rag2^{-/-} colitic mice (top row) and naïve wild-type mice (bottom row) are shown as staining controls. (b) Quantification of IL-22⁺ cell

composition. The left panel shows a donut chart representing the proportion of $CD3^+$ and $CD3^-ROR\gamma t^+$ cells within the $IL-22^+$ population. The right panel displays a bar graph quantifying the percentage of $CD3^+$ and $CD3^-ROR\gamma t^+$ cells among $IL-22^+$ cells.

Oncostatin M appears to be mainly expressed in gd T cells, Treg and ILC2. What role do ILC2 play in this pathway? Are all effects mediated directly through ILC3 or could ILC3 be intermediate regulators of ILC2? Given this expression pattern at the mRNA level at least, it is unclear how as indicated by the author, targeting amongst immune cells would work and what effects might actually be driven by more complex interactions.

Response: In our manuscript, we show that Oncostatin M (OSM) is broadly expressed by various immune cells in the steady state, including monocytes, neutrophils, dendritic cells (DCs), and to a lesser extent, ILC2 and ILC3 (**Fig. 1h**). However, during inflammation, we observe an increase in *Osm*-expressing inflammatory monocytes, neutrophils, and DCs, and a reduction in *Osm* expression in ILC2 (**Fig. 1h**). This increase in *Osm* expression is accompanied by a strong expansion and accumulation of inflammatory monocytes and neutrophils in colonic tissue, whereas the abundance of ILC2 is reduced. Additionally, we observe that $\gamma\delta$ T cells are not a significant source of OSM in either the steady state or during inflammation (**Fig. 1h**). Furthermore, our analysis of publicly available scRNA-seq datasets from humans confirms that the majority of OSM expression is driven by inflammatory monocytes and dendritic cells (**Fig. S6a, b**).

To support this point, please find below a figure specifically showing the frequency and abundance of different immune cells expressing *Osm* in the steady state and how this changes during inflammation (**Figure for Reviewer 3a, b**). This data supports our conclusion that myeloid-derived OSM is the main source of OSM in intestinal inflammation. Interestingly, ILC2 frequency and absolute counts within *Osm*-expressing cells are actually reduced upon colitis induction (**Figure for Reviewer 3a, b**).

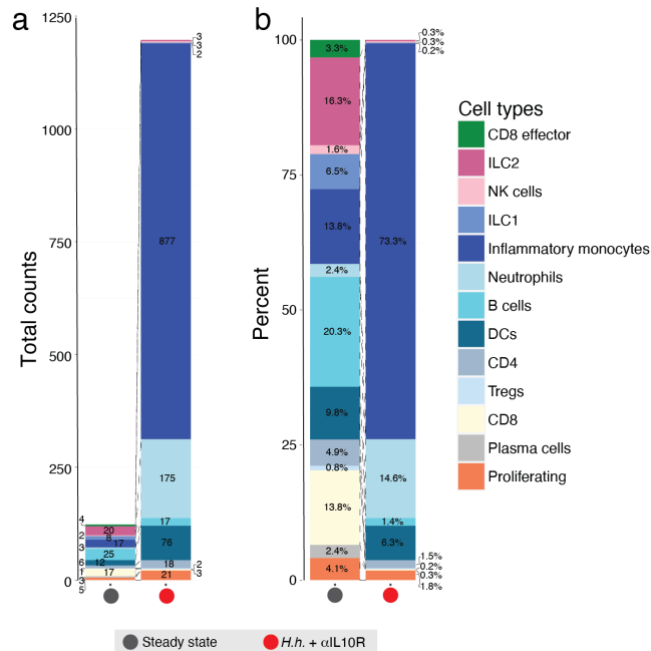


Figure for Reviewer 3. A reanalysis of scRNA sequencing data is shown in Figure 1g. Quantification of immune cell populations within the *Osm*-expressing cells in steady-state mice and after *H.h.* + $\alpha IL-10R$ colitis induction. (a) Shown are the absolute counts of the different immune cell populations within *Osm*-expressing cells. (b) Percentage of the different immune cell populations within *Osm*-expressing cells.

Our findings in the paper highlight that ILCs, specifically ILC3, can be the inducers of OSMR expression by epithelial cells in the early phase of colitis. This was supported by the IL-22 blockade experiments showing that blockade of IL-22 or deletion of IL22ra1 on epithelial cells leads to a

reduction in OSMR upregulation in IECs in inflammation, highlighting that IL-22 is a key driver and sustainer of OSMR expression in IECs. Based on our observation that IEC-intrinsic OSMR expression is a driver of colitis, these data identify a novel pathogenic facet of IL-22 biology.

Finally, we note that our paper does not focus on the source of OSM but primarily on the responder cell populations—stromal, endothelial, and epithelial cells—and their contribution to intestinal inflammation. We are aware that OSM expression is broad among immune cells, as previously shown, and therefore we focused on the impact of OSM signaling on various resident cell types⁵. We proposed targeting OSM or OSMR therapeutically *in vivo* using monoclonal antibodies, as shown in **Fig. 7d, e**, which would be expected to inhibit OSM activity regardless of the cell of origin. We have included the above figure as **Fig. S1f** to support our statement regarding the source of OSM in the lamina propria.

Organoids have been utilized for some of the experiments. While such approaches are innovative, it is unclear how closely these replicate the situation *in vivo* in dissecting the key cells involved in this pathway. This would seem important as small populations of immune cells also appear to express low levels of OSMR.

Response: In scRNA sequencing experiments and by qPCR and ISH, we identified that OSMR is surprisingly expressed in epithelial cells after colitis induction. We utilized the epithelial organoid system to screen and identify potential signals that promote *Osmr* expression in epithelial cells *in vitro*. We chose primary epithelial organoid systems to closely mimic *in vivo* conditions and avoid using established immortalized cell lines. This approach also allowed us to use primary human epithelial cells to validate our findings from the mouse system in humans. These organoids were well-suited to address this question, as they did not express OSMR when cultivated from steady-state mice. After screening 22 different stimuli, we found that epithelial cells upregulated the receptor after stimulation with IL-22 (**Fig. 3a**). This finding was also confirmed in human epithelial organoid cultures, highlighting the relevance of IL-22 in modulating OSMR expression in epithelial cells (**Fig. S10c**). To validate these *in vitro* findings, we conducted several *in vivo* studies in which we utilized IL-22 knockout mice, conditional epithelium-restricted *IL22RA1* knockout mice, and anti-IL-22 antibody blockade. These experiments confirmed that IL-22, the primary OSMR-inducing signal identified in the organoid system, is indeed a key modulator of epithelial *Osmr* expression *in vivo* (**Fig. 3e-m**). Thus, we believe that the organoid cultures were well-suited for our study and delivered key insights into the regulation of OSMR in epithelial cells in both mice and humans.

Furthermore, we would like to highlight that OSMR is not detected in murine CD45⁺ immune cells by scRNAseq, as shown in **Fig. S1h**, nor did we detect any OSMR expression in scRNA sequencing data of CD45⁺ cells from human IBD (**Fig. S6a, b**).

To further support our scRNA-seq findings, we validated murine OSMR expression by flow cytometry, using OSMR-deficient mice as controls to ensure specificity. Our analysis confirms that OSMR is constitutively expressed in stromal cells but is absent from epithelial and CD45⁺ immune cells in the lamina propria under homeostatic conditions. However, as demonstrated in our RNA analysis, this expression pattern changes following colitis induction. Consistently, flow cytometry now confirms that OSMR is induced in epithelial cells during inflammation (**Fig. S4d-e**). These findings support our observation that OSMR is constitutively expressed in stromal cells under both steady-state and inflammatory conditions, whereas its expression in epithelial cells is induced upon inflammation. Importantly, based on our scRNA-seq and flow cytometry data, we confirm that OSMR is not expressed in CD45⁺ immune cells, neither at steady state nor during inflammation.

Cytokine production is measured following the strong stimulus PMA and ionomycin demonstrating what a cell is capable of rather than how it might respond constitutively to relevant stimuli. What is the response to IL-7, IL-2 and IL-23 as stimuli? Was IFN- γ measured across ILC populations?

Response: To address this concern, we stimulated lamina propria mononuclear cells (LPMCs) isolated from the colon of naïve B6 mice and colitic mice (*H.h.*+ α IL-10R) with various cytokine signals, as suggested, or left them unstimulated with only brefeldin A to capture spontaneously produced cytokines (**Figure for Reviewer 4a**). We then performed intracellular staining for cytokines and transcription

factors, along with surface marker analysis to distinguish different innate lymphoid cell (ILC) populations (**Figure for Reviewer 4b**).

Our analysis confirmed that ILC3s isolated from colitic mice produce higher levels of IL-22 compared to those from non-inflamed mice (**Figure for Reviewer 4c**, upper left panel). Moreover, stimulation with IL-2, IL-7, IL-23, or a combination of IL-23, IL-1 β , and PMA/ionomycin, resulted in IL-22 expression levels comparable to those observed in ILC3s that were not further stimulated ex vivo. Additionally, IFN- γ expression was primarily detected in ILC1s, though at lower levels than IL-22 in ILC3s (**Figure for Reviewer 4c**, lower right panel).

Given that IL-1 β and IL-23 are upregulated during intestinal inflammation, our findings suggest that ex vivo stimulation with these cytokines, in combination with PMA/ionomycin, does not artificially alter the cytokine profile of ILCs. Instead, this approach allows us to assess how ILCs respond to relevant inflammatory stimuli in a physiologically meaningful manner. Furthermore, this combination enables the simultaneous analysis of cytokine expression in ILCs, TCR $\alpha\beta$ T cells, and TCR $\gamma\delta$ T cells, facilitating a direct comparison of their responses under identical stimulation conditions without biasing ILC cytokine production. To support the physiological relevance of our technical approach, we have now included these data in the revised manuscript (**Fig. S16** and main manuscript: page 55, line 1021-1035).

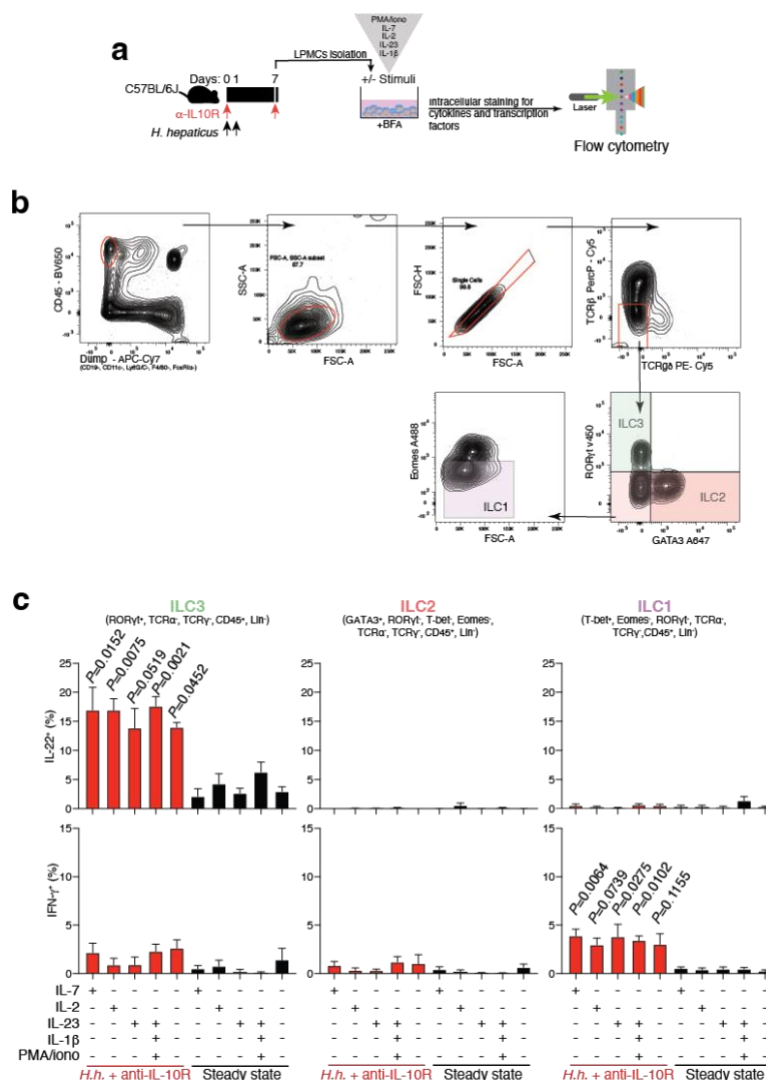


Figure for Reviewer 4. Optimisation of innate lymphoid cell activation and cytokine production. (a) Experimental design for the *H.h.* + anti-IL-10R colitis model and analysis of lamina propria innate lymphoid cells (ILCs). C57BL/6J mice were treated with anti-IL-10R and infected with *H.h.* for seven days before analysis. Lamina propria mononuclear cells (LPMCs) were isolated from inflamed (n = 6) and steady-state (n = 6) mice, stimulated ex vivo under the indicated conditions, and analyzed for cytokine and transcription factor expression by flow cytometry. (b) Gating strategy for identifying ILC subsets in colonic LPMCs. Sequential gating was

performed to exclude doublets, dead cells, and lineage-positive cells. ILCs were identified based on CD90.2 (*Thy1*) expression and further classified into ILC3 (*RORγt*⁺), ILC2 (*GATA3*⁺), and ILC1 (*T-bet*⁺) subsets. (c) Bar graphs show the percentage of cytokine-producing cells among ILC3 (left), ILC2 (middle), and ILC1 (right) subsets upon stimulation with the indicated conditions. Statistical analysis was performed using the Kruskal-Wallis test, with comparisons made to the steady-state group.

Figures. Please provide scale bars for all histological sections. It is not clear how many sections were analysed and from how many independent animals these were examined.

Response: We appreciate the reviewer's feedback and have made the following revisions to address these concerns:

1. **Scale Bars and magnifications.** We have now included clearly visible scale bars in all histological images. Each figure legend has been updated to specify both the magnification level and the corresponding scale bar length to enhance clarity and reproducibility.
2. **Number of sections and animals analyzed.** To ensure transparency and reproducibility, we have now clarified the number of sections analyzed per experiment and the number of independent animals used in the figure legends and Materials and Methods section. Additionally, we state that multiple sections per animal were analyzed to ensure a representative evaluation of the histological findings.

Updated Figure Legends

- Fig. 1k: *In situ* hybridization (ISH) for *Osmr* in colonic sections from mice under *H.h.* + αIL-10R treatment and steady-state conditions. *Osmr* signal appears as punctate purple dots. The upper image shows ISH combined with H&E staining, while the lower image presents the Ilastik-processed image. Acquired at 400× magnification. Scale bar: 20 μm. n = 6–8 per group.
- Fig. 1n: ISH detection of *Osmr* in mucosal samples from healthy individuals and patients with UC. The upper image shows ISH combined with H&E staining, while the lower image presents the Ilastik-processed image. Acquired at 400× magnification. Scale bar: 20 μm. n = 2–3 per group.
- Fig. 2d: Representative H&E-stained colon sections from steady-state and *H.h.* + αIL-10R-treated mice (day 21) of the indicated genotypes. Acquired at 100× magnification. Scale bar: 100 μm. n = 8–14 mice per genotype.
- Fig. 3d: ISH showing punctate brown signals representing Il22 expression in mucosal samples from steady-state and *H.h.* + αIL-10R-treated mice. Acquired at 400× magnification. Scale bar: 20 μm. n = 2 per group.
- Fig. 3f: Representative H&E-stained colon sections from steady-state and *H.h.* + αIL-10R-treated mice (day 7) of the indicated genotypes. Acquired at 100× magnification. Scale bar: 100 μm. n = 10 mice per genotype. Data presented as mean ± s.e.m. Statistical significance determined by Mann-Whitney U tests.
- Fig. 3i: ISH for *Osmr* expression (punctate brown signals) in colonic tissue from steady-state, anti-IL-22 (clone 8e11), or isotype-treated mice. The upper image shows ISH combined with H&E staining, while the lower image presents the Ilastik-processed image. Acquired at 400× magnification. Scale bar: 20 μm. n = 3–6 per group.
- Fig. 4b: Immunofluorescence staining for pSTAT1 and pSTAT3 in steady-state and *H.h.* + αIL-10R-treated (day 14) mouse samples, with subsequent quantification in the epithelium. Acquired at 400× magnification. Scale bar: 20 μm. Data pooled from two independent experiments (n = 7–10 mice per genotype). Statistical significance determined by Mann-Whitney U tests.
- Fig. S4e: Workflow schematic for the detection and quantification of *Osmr* expression in mouse colonic tissue by RNAscope ISH. ISH was performed to detect *Osmr* expression (punctate brown signal), with hematoxylin counterstaining. Image processing was conducted using Ilastik, and *Osmr* expression was quantified using ImageJ. Acquired at 400× magnification. Scale bar: 20 μm.
- Fig. S4g: Exemplary positive and negative control probes for mouse RNAscope ISH. Acquired at 400× magnification. Scale bar: 20 μm.
- Fig. S4h: Exemplary positive and negative control probes for human RNAscope ISH. Acquired at 400× magnification. Scale bar: 20 μm.

- Fig. S7d: ISH detection of *Il22* mRNA expression in colonic mucosal samples from inflamed *Il22*^{-/-} mice (punctate brown signals), confirming probe specificity. Acquired at 400× magnification. Scale bar: 20 μm.
- Fig. S7e: ISH analysis of *Osmr* (left) and *Il22* (right) mRNA expression in colonic tissue from sequential slides following 14 days of *H.h.* + αIL-10R treatment in wild-type mice. Acquired at 400× magnification. Scale bar: 20 μm. n = 3 per group.
- Fig. 11d: Representative H&E-stained colon sections from steady-state and *H.h.* + αIL-10R-treated mice (day 7) of the indicated genotypes. n=6 mice per genotype. Acquired at 100× magnification. Scale bar: 100 μm.
- For the *in situ* hybridization analysis shown in Fig. S5, samples were collected and analyzed at various time points after colitis induction, with the following numbers of mice and corresponding images: Day 0 (steady state), 6 mice with 130 images; Day 3, 2 mice with 50 images; Day 7, 8 mice with 100 images; Day 14, 3 mice with 80 images; and Day 21, 4 mice with 100 images. Image processing was performed using Ilastik, and *Osmr* expression was quantified with ImageJ.
- For histopathology scoring, all mice were evaluated via microscopy by two independent individuals. Three distinct sections of the colon—proximal, mid, and distal—were collected and assessed. For each mouse, 2–4 representative images were captured at different magnifications. The histopathology scores for individual mice are presented in the bar graphs within the corresponding figure sections, with each dot representing a single mouse. Additionally, one representative image is shown for each mouse group.

Summary:

- Scale bars added to all histological sections for clarity.
- Figure legends revised to specify scale bar lengths.
- Clarified the number of sections and animals used, ensuring transparency.
- Ensured representative sampling by analyzing multiple sections per animal.

Figure 7a examines OSMR expression and indicates that this is expressed in epithelial cells and stromal cells but it is not clear how the different cell types have been delineated as this is not evident in the sections provided.

Response: To address this question, we utilized platelet-derived growth factor receptor alpha (*Pdgfra*) *in situ* hybridization (red dots) to distinguish between stromal (*Pdgfra*⁺) and epithelial (*Pdgfra*⁻) cells (Fig. 7a). PDGFRA is a classical marker for distinguishing stromal cells⁶. Our observations revealed that in addition to *Pdgfra*⁺ (red dots) in the tumors and normal mucosa, *Osmr* (blue dots) was also expressed by *Pdgfra*-negative cells in the tumor, which were determined to be epithelial cells based on morphological assessment. To confirm this, we isolated tumor epithelial cells from AOM-DSS tumor-bearing mice and assessed the expression of *Osmr* by qPCR (Fig. S15f). This analysis confirmed the presence of high *Osmr* expression in tumor epithelial cells compared to steady-state epithelial cells. In the revised manuscript, we now mention that the distinction between stromal and epithelial cells is based on the expression of *Pdgfra* in the main text and figure legend (main manuscript, page 16, line:339-340 and page 76, line: 1426).

Reviewer #2

(Remarks to the Author)

In this study, Cineus et al. provide novel and important insights into how the IL-22/OSM/OSMR axis can regulate intestinal inflammation and tumorigenesis. The authors first show an upregulation of OSM and OSMR in enterocytes during intestinal inflammation in mice and confirm the relevance of this finding by analyzing human IBD samples. The authors next perform functional experiments using the *H. hepaticus* + a-IL-10R model and identify a causal role of OSM/OSMR in regulating intestinal inflammation. Mechanistic experiments then revealed that OSMR in enterocytes is regulated by ILC-3-derived IL-22 via STAT3 activation. Finally, the authors describe an important role of OSM/OSMR in intestinal tumorigenesis using a murine CAC model. The data appear to be clear and are presented in a very good way. The manuscript is written well, structured and easy to follow. Overall, the findings could be highly relevant for scientists working in the field of intestinal immunology and oncology.

However, despite the overall convincing findings by the authors, I believe that several points need to be addressed in order to further improve the manuscript and strengthen their findings.

I outline my individual concerns in detail below:

1. The authors show a crucial role of the IL-22-OSM-OSMR axis in intestinal inflammation using the *H. hepaticus* + a-IL-10R model. The authors have in detail analyzed the mechanism in this model, including the relevant cellular source of IL-22 and the signaling pathway. However, the cellular and molecular mechanisms described could be specific to this one specific murine colitis model. Thus, it would be important to study other colitis models, to confirm the role of the IL-22-OSM-OSMR axis as well as to analyze the respective cellular source of IL-22.

Response: We thank the reviewer for the encouraging and constructive feedback, including the suggestion above. We opted to use the *H. hepaticus* + anti-IL-10R model due to its unique characteristics. This model is induced in lymphoreplete wild-type mice through colonization with a pathobiont and blockade of IL-10 signaling, leading to chronic colitis that persists for over four weeks (**Fig. 1a–c**). Unlike chemically induced colitis models, this system closely resembles therapy-resistant human IBD associated with IL-10 pathway deficiency. Importantly, we have previously demonstrated the clinical relevance of this pathway in human IBD, showing that IL-10 pathway dysregulation predicts anti-TNF failure and correlates with upregulation of OSM and OSMR in patients with IBD⁷. A key advantage of this model is that it enables the study of immune-epithelial interactions in a chronic inflammatory context, without the acute epithelial injury and disruption typically observed in chemically induced models. Given the clinical significance of this pathway, a clinical trial for anti-OSMR therapy in ulcerative colitis is currently underway.

In this study, we provide a comprehensive characterization of the OSM-OSMR axis in intestinal inflammation, demonstrating significant upregulation of OSMR on epithelial cells in murine colitis models and in tissue samples from IBD patients. Our data reveal that IL-22 is essential for both the induction and sustained expression of OSMR in epithelial cells during colitis. Specifically, we show that the IL-22-OSMR axis is active in the *H. hepaticus* + anti-IL-10R model (**Fig. 3e–m**), the acute DSS model (**Fig. S15f–h**), and the AOM+DSS colitis-associated cancer model (**Fig. 7i, j, and Fig. S15j–m**). These findings confirm that the IL-22-OSMR axis is engaged across multiple inflammatory conditions in mice, including acute colitis and colitis-associated cancer.

To further validate our observations in human disease, we now include additional *in situ* hybridization (ISH) analysis of OSMR in an independent IBD cohort from Mayo clinic, USA (n = 10 UC, n = 5 controls, **Fig. S7a**). Moreover, we have now examined the expression and spatial distribution of OSM and OSMR in human colitis-associated cancer (CAC, n=10) and healthy controls (n = 3). These data show a significant upregulation of OSM and OSMR in CAC tissue, with OSMR expression specifically elevated in epithelial cells (**Fig. 7b, c and Fig. S15e**)

To expand the physiological relevance of our study, we now introduce an additional bacterially driven colitis model using *Citrobacter rodentium* infection, which allows us to investigate pathogen–

host interactions and the role of the OSM-OSMR axis in enteric bacterial infection and intestinal inflammation (**Fig. S9a, b**). Our data demonstrate that OSM and OSMR expression increase over time in *Citrobacter rodentium*-induced colitis, with OSMR upregulation occurring specifically in epithelial cells (**Fig. S9 c, d**). This was further confirmed by flow cytometry (**Fig. S9e**). Consistent with previous reports⁸, we also confirm IL-22 upregulation in this model (**Fig. S9i**).

To explore the functional relevance of epithelial OSMR expression in *Citrobacter*-induced colitis, we used *Villin^{CreERT2} x Osmr^{fl/fl}* mice to assess the role of epithelial-specific OSMR deletion in inflammation and bacterial control. Mice lacking epithelial OSMR exhibited reduced intestinal pathology compared to control mice, while bacterial burden remained unaffected (**Fig. S9g, h**). Similar to our findings in the *H. hepaticus* + anti-IL-10R model, immune cell recruitment was also reduced in *Citrobacter rodentium* colitis upon OSMR deletion (**Fig. S9m**).

Further mechanistic studies confirm that IL-22 is critical for OSMR upregulation in colon epithelial cells of *Citrobacter*-infected mice, as IL-22 blockade inhibited OSMR expression (**Fig. S9j**). This IL-22 induction in *Citrobacter rodentium*-induced colitis was found to be IL-23 dependent, as IL-23 blockade led to reduced IL-22 expression in colon tissue, which in turn diminished OSMR upregulation on epithelial cells (**Fig. S9l**). Finally, we confirm that ILC3s also induce IL-22 expression in this model, in line with previous findings **Fig. S9k**)⁹.

Taken together, these additional human IBD and colitis-associated cancer data reinforce the concept of OSMR upregulation by epithelial cells in human disease and provide further *in vivo* evidence supporting the role of the OSM-OSMR axis in intestinal inflammation and colitis-associated cancer. By introducing a bacteria-induced colitis model, we strengthen the translational relevance of our findings and further highlight the pathogenic role of epithelial OSMR signaling in chronic intestinal inflammation.

2. The authors should clarify, if there is a difference in colitis severity in the AOM/DSS model as inflammation is one key driver of tumors in this model.

Response: In the current manuscript, we used OSM-deficient mice to establish the relevance of the OSM pathway in the colitis-associated cancer (CAC) model. However, we acknowledged in the manuscript that differential inflammation might underlie these differences (Main manuscript, page 16, line: 351-352). Therefore, we opted to evaluate the role of OSM in CAC using two independent approaches. First, we blocked OSM with a neutralizing antibody in wild-type mice at a late timepoint, after tumor establishment and following recovery from three cycles of DSS, to avoid influencing inflammation (**Fig. 7e**). In the histology images provided in **Fig. 7a**, one can observe that at this late timepoint, tumors are established and the adjacent non-tumor mucosa has largely recovered from DSS challenge. Second, we employed a conditional deletion approach using *Villin^{CreERT2} Osmr^{fl/fl}* mice, where we induced OSMR deletion after three cycles of DSS and tumor establishment to evaluate the impact of OSM signaling in established tumors (**Fig. 7i, j**). In both cases, we observed a clear impact of OSM signaling on tumor progression. Thus, based on these experiments, we can exclude the possibility that differences in DSS-driven inflammation and tumor initiation were responsible for our observations. We now highlight these findings in the results section and discussion (Main manuscript, page 16, line: 352-355 and page:17, line: 359-360).

3. Related to the above point - the relevance of the IL-22-OSM-OSMR axis should also be tested in a second inflammation independent model of colon cancer. If data cannot be confirmed then a second inflammation driven colitis model would further strengthen the data.

Response: The DSS-AOM system is the most widely employed and well-characterized model of colitis-driven colon cancer. This model was specifically designed to mimic the relapsing-remitting nature of IBD and does not rely on specific genetic drivers, making it an appropriate choice for evaluating the role of epithelial OSM signaling in inflammation-induced CRC. While our findings demonstrate that OSM signaling in tumor epithelial cells promotes tumorigenesis in this model, we acknowledge that a second CAC model would further strengthen our conclusions.

To provide additional supporting data, we employed the *Apc^{min/+}* DSS model, which introduces a strong genetic predisposition to tumorigenesis. *Apc^{min/+}* mice typically develop spontaneous tumors in

the small intestine due to unrestrained β -catenin signaling, a pattern that differs from human CRC. However, treatment with DSS shifts tumorigenesis to the colon, making it a valuable complementary model¹⁰.

In this model, we treated $Apc^{min/+}$ mice with DSS, allowed them to recover for two weeks on regular drinking water, and subsequently treated them with anti-OSM blocking antibody for three weeks. At the study endpoint, mice receiving OSM blockade exhibited a significant reduction in colon tumor burden compared to those treated with an isotype control antibody (**Figure for Reviewer 5**). These findings indicate that OSM promotes CAC even in a setting of strong genetic predisposition.

As mentioned above, we now provide evidence that the OSM–OSMR axis is upregulated in human colitis-associated cancer, with OSMR expression clearly elevated in epithelial cells. Given these findings, we believe that the additional data from the $Apc^{min/+}$ mouse model are not essential for inclusion, as they introduce new avenues of research that extend beyond the scope of this manuscript and are better suited for future studies.

In conclusion, we now strengthen our findings with an additional pre-clinical model of colon cancer and supporting human data, further supporting a role for the OSM-OSMR axis in intestinal inflammation and tumorigenesis.

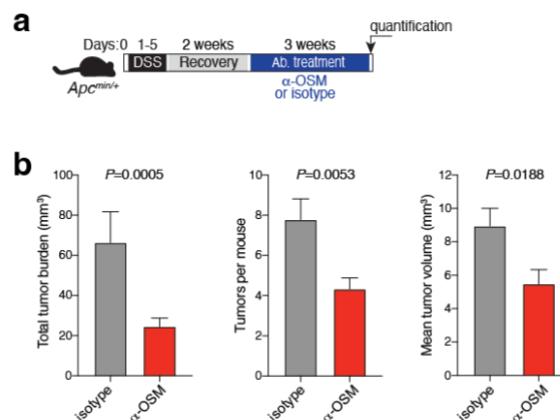


Figure for Reviewer 5. (a) $Apc^{min/+}$ mice were given 2% (w/v) DSS in drinking water for 1 week. Mice then received regular drinking water for 2 weeks and were subsequently treated with an anti-OSM blocking antibody (clone 24A8, Genentech) or the respective isotype control (anti-gp120, Genentech), administered twice per week for 3 weeks. (b) Tumor burden, multiplicity, and average volumes; $n=15-18$. Differences between groups were assessed with Mann-Whitney U tests.

4. The authors do show some limited human data, which point towards a possible relevance of the IL-22-OSM-OSMR axis in IBD. However, it remains unclear whether there is any molecular or clinical association in human CAC (or CRC) patients that would suggest a possible role of this pathway in human CRC. For example, is the expression of OSM/OSMR dysregulated in human CRC? Does it correlate with IL-22 levels? Is there an association with oncological outcome? Those questions could be answered using publicly available data sets and would provide important complementary information to the data shown.

Response: While the analysis proposed by the reviewer is theoretically possible, it presents significant limitations. Our study specifically models colitis-associated cancer (CAC), which is rare compared to sporadic colorectal cancer (CRC). Consequently, suitable datasets correlating gene expression with clinical outcomes in CAC are not readily available. Any attempt to analyze public datasets would necessitate the use of sporadic CRC data, despite its well-established differences in etiology and pathophysiology from CAC.

Another critical limitation is that OSM expression in CRC is likely enriched in tumors with high microsatellite instability-high (MSI-H), which are highly inflamed but generally associated with better prognosis due to a robust immune response. Similarly, OSMR expression in bulk tissue datasets is difficult to interpret, as a significant portion of the signal comes from stromal cells, which are not relevant to our focus on epithelial-intrinsic OSMR signaling. Given these constraints, an analysis of

publicly available CRC datasets would be difficult to interpret and could lead to misleading conclusions. Therefore, we believe it is most appropriate to focus our analysis on CAC to maintain clarity and avoid potential misinterpretation.

We have previously demonstrated that the OSM pathway is upregulated in human IBD, particularly in patients with difficult-to-treat disease who failed anti-TNF therapy⁷. Additionally, we now showed through PCR and ISH analysis that OSMR is upregulated in epithelial cells in human IBD (Fig. 1m-n). We also demonstrated that IL-22 induces OSMR expression in epithelial cells in both mouse models and human intestinal organoids (Fig. 3 a, and Fig. S10c).

To further support these findings, we now include additional ISH analysis of OSMR from an independent human IBD cohort (Mayo Clinic, USA), strengthening our observations in human IBD (Fig. S7a). To establish a link between IL-22 signaling and the OSM/OSMR pathway, we assessed the correlation between the IL-22 gene signature, previously identified by Pavlidis et al., and OSM/OSMR expression in four independent cohorts (Fig. S10f, g)¹¹. Our data now demonstrate that IL-22 strongly correlates with OSM and OSMR expression in IBD mucosal samples, reinforcing our hypothesis that the IL-22-OSM-OSMR axis is actively involved in intestinal inflammation (Fig. S10f, g).

Furthermore, we provide new data confirming the upregulation of OSM and OSMR in human colitis-associated cancer (CAC, n=10; HC, n=3, Fig. 7b, and S15e). In particular, we observe strong induction of OSMR in tumor epithelial cells from CAC, relative to normal mucosa. These additional findings further validate our initial observations and strengthen the translational relevance of our study by incorporating human data from both IBD and CAC patients.

5. The colitis severity assessment relies primarily on histopathological scoring. Since the results are based mainly on this method and proper histopathological assessment requires extensive experience and skill, it would be important to describe, if the scoring was performed by someone with a formal histopathological training in a blinded fashion? Of note, tumor development was assessed using a murine endoscopy system. I would encourage the authors to also use this system for colitis scoring.

Response: We would like to highlight that we collaborated with trained pathologists at both Charité and Genentech to thoroughly assess our inflammation and cancer models. The Department of Research Pathology and iPATH.Berlin, specialists in mouse pathology who run core facilities for mouse pathology, were responsible for the pathology assessment and staining. Furthermore, all our histological scoring was conducted in a blinded fashion by two independent trained scientists, and the results were averaged. This information is provided in the materials and methods section under "Histological scoring" (Main manuscript, page 46, line: 810-821).

6. The endoscopy procedure/method is not described in the methods section – this should be included.

Response: We apologize for not including this in our methods. This has now been added to the materials and methods section in the revised manuscript (Main manuscript, page 46, line: 805-806).

7. The authors have analyzed the functionally relevant source of IL-22 in the *H. hepaticus* + aIL10R model and describe ILC-3-derived IL-22 as the functionally relevant source for regulating OSMR in enterocytes. However, the main and functionally relevant source was only studied in this one particular disease model. Are ILC-3 also the main and functionally relevant source of IL-22 in tumors and other inflammation models – thus, is the identified cellular cross-talk universal or is it limited to early colitis (or to this one particular colitis model)? I make this point since various cellular sources of IL-22 can play a pivotal role in different models and stages of colitis, and multiple sources of IL-22 have been attributed a key role in cancer development and progression.

Response: We have demonstrated in our manuscript that OSMR upregulation on intestinal epithelial cells (IECs) is dependent on IL-22 across multiple models. Specifically, we show this in the *H. hepaticus* + anti-IL-10R model (Fig. 3) as well as in both acute and chronic DSS + AOM models (Fig. S15h and Fig. S15k). Additionally, our findings indicate that IL-22 promotes OSMR expression in human intestinal organoids, supporting the conservation of this pathway across species (Fig. S10c).

To further strengthen our conclusions, we now provide additional data (introduced above in response to point 1), demonstrating that OSMR is induced by IL-22 in the *Citrobacter rodentium* model, where ILC3-derived IL-22 production is IL-23 dependent (**Fig. S9j, k, l**). Furthermore, in response to question 4, we present additional human data showing a correlation between IL-22 expression and OSMR upregulation in both inflammation and colitis-associated cancer. Collectively, these data support our hypothesis that the IL-22-OSMR-OSM axis is active in intestinal inflammation and colitis-associated cancer.

However, we acknowledge that distinct cellular sources of IL-22 may play different roles depending on the inflammatory context and stage of tumorigenesis^{12–16}. While early studies identified IL-17⁺ CD4⁺ T cells (Th17 cells) as producers of IL-22, more recent work has demonstrated that several innate lymphoid cell (ILC) populations also contribute to IL-22 production. Additionally, natural killer (NK) cell subsets, lymphoid tissue inducer cells, and CD11c⁺ colonic cells stimulated through Toll-like receptor signaling have been shown to secrete IL-22. Studies using IL-23R reporter mice further revealed that $\gamma\delta^+$ T cells in the intestinal lamina propria, along with certain CD11b⁺ cells, express IL-23R and may contribute significantly to IL-22 production *in vivo*¹⁷. More recently, neutrophils have also been shown to produce IL-22 in acute DSS colitis¹⁵.

Given the versatility of IL-22-producing cell types, we have now expanded our discussion to acknowledge the potential contributions of other innate and adaptive immune cells, particularly in chronic intestinal inflammation and colitis-associated cancer. This is especially relevant in human IBD, where the disease course extends over many years, making it difficult to exclude additional IL-22 sources that may influence OSMR expression (Main manuscript, page: 20, line: 433-445).

8. To what extent are the effects of IL-22 dependent on the OSM-OSMR axis? For example, does pharmacological inhibition of IL-22 show anti-tumor effects in OSM(R) knock-out mice? The authors should discuss the other known mechanisms of how IL-22 can promote tumorigenesis.

Response: We have addressed the complex role of IL-22 in epithelial biology in the discussion section (Main manuscript, page: 19, line: 417-432). Additionally, we now further elaborate on this point, acknowledging that the diverse and context-dependent effects of IL-22 cannot be fully delineated within the scope of this study. However, this complexity highlights an important avenue for future research to better understand the nuanced functions of IL-22 in intestinal homeostasis, inflammation, and tumorigenesis.

Reviewer #3

(Remarks to the Author)

The authors comprehensively dissected a series of cytokine-mediated events that contribute to intestinal inflammation and tumorigenesis. Central to this study is the OSM-OSMR axis in epithelial cells, which upon activation upregulates transcripts involved in inflammation, proliferation, and recruitment of immune cells. Intriguingly, while OSMR is constitutively expressed in stromal cells, intestinal pathology is only evident following induced OSMR expression by epithelial cells, which requires ILC3-derived IL-22. Indeed, mice engineered to lack IL-22R or those administered neutralizing antibodies to prevent IL-22 signaling showed abolished epithelial OSMR expression levels and concurrent intestinal pathology. In agreement with these findings, neutralizing IL-23, a cytokine required for ILC3-mediated IL-22 expression, also reduced intestinal inflammation. This study provides important insights into how a cellular network of epithelial and resident innate immune cells is tightly intertwined to orchestrate a protective response or, when dysregulated, drive severe immunopathology.

Several concerns remain that need to be addressed:

Main

1. A central point in this study is the OSM-OSMR axis, but strikingly, only gene expression data is presented without corresponding protein data. This is important because, for example, the presented ISH images for *Osmr* appear completely negative at steady-state, while scRNA-seq shows that endothelial, stromal, and smooth muscle cells abundantly express this gene at steady-state (up to 75% of the cells). Moreover, following inflammation, the *Osmr*-expressing epithelial fraction represents only 5% of the cells, and while those expression levels are 10-fold lower compared to endothelium, ISH images show clear expression for only those cells. How do the authors explain this discrepancy? Additionally, *Osmr* is only modestly expressed in a subset of epithelial cells (fig. 1i, suppl. Fig. 3) and is virtually absent at steady-state as stated several times throughout the manuscript. However, depicted expression levels are normalized to *Osmr* levels at steady state (e.g., fig. 1j, 3e, g, j, m, 5g). How is this possible if the *Osmr* gene is not expressed under these conditions?

Response: We thank for the reviewer for providing constructive feedback. We have provided detailed responses to the respective questions raised on this first point:

A central point in this study is the OSM-OSMR axis, but strikingly, only gene expression data is presented without corresponding protein data.

Response: To further substantiate our findings, we established OSMR receptor staining to evaluate its expression on epithelial cells, confirming results obtained from scRNA-seq, ISH, and qPCR. The specificity of the antibody was validated using OSMR-deficient endothelial cells, which express OSMR under steady-state conditions (**Fig. S4e**).

Our analysis demonstrates that OSMR expression is elevated during inflammation in both *H. hepaticus* + anti-IL-10R-induced colitis and *Citrobacter rodentium* infection at days 7 and 14 (**Fig. S4d, and Fig. S9e**). Additionally, using our previously established staining protocol⁷, we confirm that OSMR is expressed on epithelial cells in human IBD samples and that its expression correlates with histological inflammation (**Fig. S7c**).

By quantifying OSMR protein expression, we provide strong evidence that OSMR upregulation observed at the RNA level corresponds to increased protein expression in epithelial cells during intestinal inflammation, reinforcing the robustness of our findings.

This is important because, for example, the presented ISH images for *Osmr* appear completely negative at steady-state, while scRNA-seq shows that endothelial, stromal, and smooth muscle cells abundantly express this gene at steady-state (up to 75% of the cells). Moreover, following inflammation, the *Osmr*-expressing epithelial fraction represents only 5% of the cells, and while those expression levels are 10-

fold lower compared to endothelium, ISH images show clear expression for only those cells. How do the authors explain this discrepancy?

Response: In our manuscript, we focus on epithelial OSMR expression and its unexpected regulation during inflammation, contrasting with its low abundance or absence in intestinal epithelial cells under homeostatic conditions and its functional relevance in disease. To confirm the OSMR upregulation shown in **Fig. 1**, we employed multiple complementary techniques, including single-cell RNA sequencing (scRNA-seq), *in situ* hybridization (ISH), and quantitative PCR (qPCR). Each of these techniques has different detection sensitivities and limitations, making direct comparisons challenging. For instance, scRNA-seq is limited in detecting low-abundance transcripts, such as cytokines and cytokine receptors. ISH, on the other hand, is biased toward the most abundant cell types in a tissue section, meaning that epithelial cells dominate our ISH data, while stromal cells, such as fibroblasts and endothelial cells, are comparatively underrepresented.

To overcome these limitations and further validate our findings, we incorporated additional methods to confirm and expand on our scRNA-seq data. ISH allowed us to determine the spatial localization of OSMR expression, while qPCR provided a reliable and sensitive approach for quantifying expression kinetics over time. Due to the inherent differences between these technologies, direct comparisons of OSMR expression levels across methods are not feasible.

To further support our findings, we have now included OSMR protein staining in epithelial cells, as mentioned above (**Fig. S4d, e**). Additionally, to investigate the differential regulation of OSMR in intestinal tissue, we provide an expanded spatial analysis of OSMR expression and distribution in intestinal tissue under steady-state conditions and during inflammation by using Ilastik and ImageJ (**Fig. S5a, b**). Ilastik is an interactive, machine-learning-based software for segmentation, classification, tracking, and quantification of objects in images, widely used in biological and microscopy analysis (see methods section). This analysis clearly highlights the differences in OSMR regulation between the epithelial and non-epithelial compartments of the lamina propria (**Fig. S5c-e**). Specifically, we observe a significant increase in both the abundance and intensity of OSMR expression in epithelial cells over time, while OSMR expression in the non-epithelial compartment of the lamina propria is less affected during colitis.

Additionally, *Osmr* is only modestly expressed in a subset of epithelial cells (fig. 1i, suppl. Fig. 3) and is virtually absent at steady-state as stated several times throughout the manuscript. However, depicted expression levels are normalized to *Osmr* levels at steady state (e.g., fig. 1j, 3e, g, j, m, 5g). How is this possible if the *Osmr* gene is not expressed under these conditions?

Response: Referring to the above argument, qPCR detection of OSMR expression in steady-state epithelial cells shows cycle thresholds between 32–40, indicating very low but detectable expression levels. Furthermore, when we generated organoids from steady-state mice and stimulated them with OSM, we observed minimal STAT3 phosphorylation, further supporting the absence of functional OSMR expression in steady-state epithelial cells (**Figure for Reviewer 6**). Similarly, by ISH, OSMR-expressing epithelial cells are rare but nevertheless detectable at steady state, providing a means to estimate fold changes (between pathology and steady state) in the epithelial OSMR expression level.

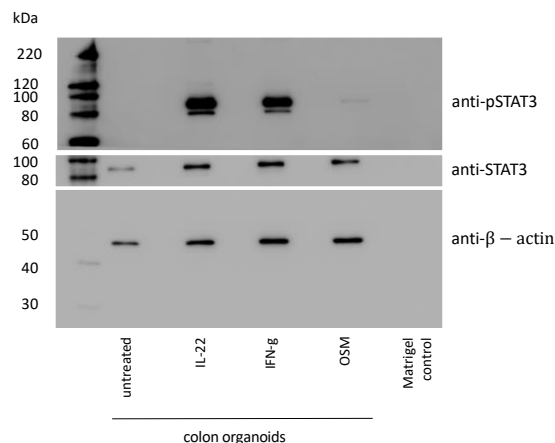


Figure for Reviewer 6. Analysis of STAT3 phosphorylation after treatment with different cytokines. Intestinal epithelial colon organoids generated from C57BL/6 mice were stimulated with either IL-22, IFN- γ or OSM (100 ng/mL) for 30 min after starvation for 4h. 30 μ g of total protein extract were separated on 10% SDS polyacrylamide gels and transferred to a PVDF membrane. Western Blot protein detection by using antibodies directed against the proteins. Protein detection of STAT3 (clone: D3Z2G), phospho-STAT3 (clone: D3A7) and anti- β actin (clone: 13E5) in total protein of cytokine-treated intestinal epithelial colon organoids.

With the additional data provided in response to the above questions, particularly the flow cytometry evaluation of OSMR expression in mouse and man, we now further substantiate our conclusion that OSMR expression is dynamic, expressed at low levels under steady-state conditions, and upregulated in intestinal inflammation and colitis-associated cancer in both mice and humans.

2. The authors state that *Osmr* is clearly induced following infection and reaches levels comparable to *Il22ra1* (line 119). However, suppl. fig. 3 indicates that this cytokine is only very modestly expressed, and *Osmr* expression does not reach levels comparable to *Il22ra1* at all. Instead, *Il22ra1* expression levels seem to be rather downregulated in all depicted enterocyte subtypes following infection.

Response: We acknowledge that the detected expression levels in the provided scRNA-seq data are relatively low. To further clarify this comparison, we now provide qPCR data to assess the dynamic modulation of *Osmr* and *Il22ra1* in total epithelial cells during inflammation. Our analysis reveals that *Osmr* is upregulated following inflammation; however, it does not reach the same expression levels as *Il22ra1*. In contrast, *Il22ra1* expression remains stable over time in colitis (**Figure for Reviewer 7**). Given these findings, we have removed the initial statement from the results section and no longer emphasize this comparison in our analysis.

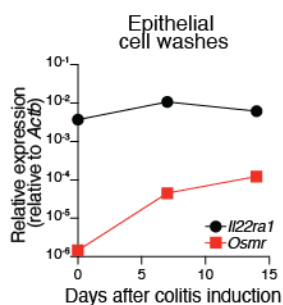


Figure for Reviewer 7. Time-course analysis of *Osmr* and *Il22ra1* mRNA expression in epithelial cells following colitis induction. Gene expression levels were measured at different time points and normalized to housekeeping gene, *Actb*. *Osmr* expression (red line) increased progressively over time, while *Il22ra1* expression (black line) remained stable.

3. The authors state that IL22 is mainly expressed in ILCs and to a lesser extent by $\gamma\delta$ T cells, hence they continue to test this as presented in figure 5. However, a gating strategy to select for ILCs is lacking, and prior to that, no gating is shown for the IL-22-expressing fraction of cells used for fig. 5a-d. This should all be shown. The data presented in fig. 5d is unclear and seems to be incorrect, with more than 75% of IL-22-expressing cells being $\alpha\beta$ T cells; the presented scRNAseq dataset (suppl. Fig. 3) shows differently. Indeed, the authors acknowledge that it is mainly $\gamma\delta$ T cells and ILCs that are the IL-22+ cells (line 231). So, either this line should be changed or fig. 5d needs to be reevaluated. To properly interpret the data, it is very important to present all the FACS scatter plots, including IL-22 expression.

Response: The reviewer noted differences in IL-22 expression, which can be attributed to the different technical approaches used in our experiments (scRNAseq vs flow cytometry). In **Fig. 5**, we employed *ex vivo* stimulation using IL-23, IL-1 β , PMA/Ionomycin, and brefeldin A to induce cytokine production and accumulation in both innate immune cells and $\alpha\beta$ T cells, followed by intracellular staining. This approach resulted in high IL-22 expression by $\alpha\beta$ T cells, as PMA/Ionomycin is a classical polyclonal stimulator of T cells.

In contrast, in **Fig. S3**, we directly isolated immune, stromal, and epithelial cells *ex vivo* and subjected them to single-cell sequencing without any *ex vivo* stimulation. This analysis revealed IL-22 expression primarily in innate immune cells, particularly ILC3s. Thus, the differences in IL-22 expression observed by Reviewer 3 are a consequence of the distinct technical approaches applied in these figures.

Regarding the FACS scatter plots, we would like to refer to our supplementary **Fig. S13a**, and **Fig. 5c, e**, which provide the ILC pre-gating strategy. Additionally, we have included all pre-gating steps for our FACS data in **Fig. S11a-b**, as well as our FACS sorting strategy in **Fig. S14**, ensuring full transparency of our gating approach.

4. Figure 6c, f: for a non-bioinformatician, it is difficult to understand the meaning or relevance of a “pathway activity score.” The legends here are not very helpful. Additionally, this figure is essentially a re-analysis of data presented in figure 1 and figure 2, and does not add much as a stand-alone figure.

Response: We have improved the description of **Fig. 6** in the legend and further clarified the approach used in **Fig. 6c** in the Materials and Methods section, where we apply the PROGENy package to analyze pathway activity (Main manuscript, page 61, line: 1185-1187 and page: 74, line: 1400-1403). PROGENy is a computational method that utilizes a large compendium of publicly available perturbation experiments to identify a core set of Pathway Responsive Genes (PRGs), enabling the inference of pathway activity from transcriptomic data¹⁸.

In this figure, we assess the transcriptional impact of OSMR signaling in primary epithelial cells during inflammation by integrating single-cell RNA sequencing and bulk RNA sequencing data to compare IEC ^{Δ OSMR} and wild-type epithelial cells. Our analysis demonstrates that OSM signaling induces a STAT3-dependent response in epithelial cells, leading to immune cell recruitment and enhanced chemotaxis.

To validate this finding, we directly measured STAT3 phosphorylation in IEC ^{Δ OSMR} compared to wild-type epithelial cells and observed reduced STAT3 phosphorylation, further supporting our computational prediction of decreased JAK/STAT activation in our transcriptomic data (**Fig. S14b**). We additionally confirmed the functional relevance of this altered STAT3 signaling by showing reduced immune cell recruitment in IEC ^{Δ OSMR} compared to wild-type mice following colitis induction, reinforcing the role of OSMR in shaping the epithelial inflammatory response (**Fig. 6j**).

We believe this figure provides key mechanistic insights into how OSM modulates epithelial cell function in intestinal inflammation. However, we are open to editorial suggestions on whether to retain this data in the main figures or move it to the supplementary section.

5. Supplementary Fig. 11a is confusing as the authors claim that Osmr is largely absent under steady-state and is induced following IL-22 stimulation. In this figure, however, the authors show that the epithelium is already responsive to OSM without prior exposure to IL-22. How do the authors explain this? Moreover, the manuscript lacks a (time-course) experiment in which OSM is exposed to organoids

that are either pre-treated with IL-22 or not. A readout could be pSTAT3 or induced genes as identified in fig. 2h.

Response: Previous studies have shown that cancerous and immortalized colonic and mammary epithelial cell lines express OSMR, unlike ex vivo primary colonic epithelial cells, as demonstrated in our manuscript for both mice and humans. Beigel et al. reported that several immortalized CRC cell lines, including Caco-2, DLD-1, SW480, HCT116, and HT-29, express OSMR- β mRNA and respond to OSM¹⁹.

In our current manuscript, we focused on the impact of OSM on primary epithelial cells using two complementary approaches: analyzing primary IECs by single-cell sequencing and comparing IEC ^{Δ OSMR} epithelial cells to wild-type IECs to assess the molecular impact of OSM on epithelial cells in inflammation, as shown in **Fig. 6**. The HCA7 cell line (**Fig. S14a, c, d**) was used exclusively to generate an OSM gene signature, serving as a reference to validate our *in vivo* findings and confirm that the observed transcriptional changes *in vivo* are directly related to OSM's effects on IECs.

We appreciate the reviewer's suggestion to investigate the combined effect of IL-22 and OSM on organoids by evaluating pSTAT3 levels or OSM-induced gene signatures. However, our manuscript already establishes that OSMR expression depends on continuous IL-22 exposure, as demonstrated by the withdrawal experiments in organoids (**Fig. S11e**) and anti-IL-22 treatment in colitic mice (**Fig. 3m**). Upon IL-22 withdrawal, OSMR expression was rapidly downregulated, both *in vitro* and *in vivo*, highlighting the requirement of IL-22 for sustained OSMR expression in epithelial cells.

Given this dependence, performing the suggested co-treatment experiments would be technically challenging, as distinguishing the individual contributions of IL-22 and OSM would be difficult. Both pathways activate STAT3, making it challenging to isolate OSM-specific effects from the broader IL-22-induced signaling.

6. Does neutralization of IL-23 also have an effect on tumor burden?

Response: We have not specifically investigated the role of IL-23 in the AOM-DSS model, as its pathogenic involvement in colitis-associated cancer has been well established in previous studies, including those by Grivennikov et al. (2012) and Richter et al. (2017)^{20,21}. Instead, our study focuses on the novel aspects of the IL-22-OSM axis in intestinal inflammation and colitis-associated cancer pathogenesis.

To provide broader clinical context, we have now included in the discussion that future observational studies in IBD patients, particularly those with UC, would be valuable to assess the incidence of colitis-associated cancer in patients who achieved remission under anti-IL-23p19 treatment (Main manuscript, page: 19, line: 409-416). Additionally, we now mention the recently initiated Phase II trial on Vixareimab (anti-OSMR) in ulcerative colitis, highlighting the potential translational relevance of our findings (Main manuscript, page: 21, line: 461-464).

7. Line 152: no human data is shown in figure S6c.

Response: In this sentence, we refer to our data in **Fig. 6c** and data published in West et al. 2017⁷. We have improved the text accordingly to enhance readability.

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1. Bekiaris, V. *et al.* Human CD4⁺CD3⁻ Innate-Like T Cells Provide a Source of TNF and Lymphotoxin- $\alpha\beta$ and Are Elevated in Rheumatoid Arthritis. *J. Immunol.* **191**, 4611–4618 (2013).
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7. West, N. R. *et al.* Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. *Nat Med* **23**, 579–589 (2017).
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13. Artis, D. & Spits, H. The biology of innate lymphoid cells. *Nature* **517**, 293–301 (2015).
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15. Zindl, C. L. *et al.* IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. *Proc. Natl. Acad. Sci.* **110**, 12768–12773 (2013).
16. Sonnenberg, G. F., Fouser, L. A. & Artis, D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat. Immunol.* **12**, 383–390 (2011).
17. Awasthi, A. *et al.* Cutting Edge: IL-23 Receptor GFP Reporter Mice Reveal Distinct Populations of IL-17-Producing Cells. *J. Immunol.* **182**, 5904–5908 (2009).
18. Holland, C. H. *et al.* Robustness and applicability of transcription factor and pathway analysis tools on single-cell RNA-seq data. *Genome Biol.* **21**, 36 (2020).
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20. Grivennikov, S. I. *et al.* Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* **491**, 254–258 (2012).
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Reviewer #1:

Remarks to the Author:

In general the authors have addressed my comments.

Scale bars: These have been added but are extremely small and difficult to read on the micrographs. Some do not seem to match the number quoted in the text, e.g., Fig. 3f appears to be 100um but is indicated to be 20 in the text.

Response: We have corrected this error and reviewed all text referring to scale bars across the figures to ensure accuracy in the respective figure captions.

Reviewer #2:

Remarks to the Author:

The authors have addressed all my comments and concerns.

Response: We thank the reviewer for their positive feedback and appreciate their thoughtful comments throughout the review process.

Reviewer #3:

Remarks to the Author:

The manuscript has improved substantially and the vast majority of the raised concerns have been sufficiently addressed. A few points still require further attention, as listed below.

1. The authors claim that ILC3s are the predominant IL-22 producing cells following infection. To support this claim, the authors now added supplemental figure 13c, and supplemental figure 16 in addition to the existing figures 5a-f. Furthermore, the material and methods section now describes the full procedure in which fresh isolated LPMCs were re-stimulated with IL-1b, IL-23, and PMA/ionomycin for 4 hrs in the presence of brefeldin A, followed by surface staining, fixation, and intracellular staining for IL-22. While these additions help to understand the exact workflow, for reasons of clarity I suggest the following:

- provide the FACS plot with total LPMC IL-22 expressing cells at steady-state and in disease upon which figure 5a is based. This would clarify the gating strategy used to generate figure 5b-d.

Response: As requested, we have added a FACS plot showing IL-22 and Ki67 expression in untreated and colitic mice, gated on CD45⁺ Lin⁻ cells, in Figure 5a (Page 36).

- Figure 5c aims to describe the cell populations within this IL-22 expressing fraction as summarized in figure 5a. However, it is not clear whether this is from the control or inflamed intestine. Please add this information.

Response: As requested, this information has been added to the caption of Figure 5c (Page 36).

- Figure 5d is a summary of the percentages of each cell type that express IL-22 (control and inflamed), but from figure 5c it seems that the fraction of gdT cells is very small (< 1%), while the pie-diagram seems to depict a much higher percentage. As no percentages are given in figure 5c, it makes it difficult to interpret the pie-diagram.

Response: We would like to clarify that Figure 5d displays the percentages of $\alpha\beta^+$ T cells, $\gamma\delta^+$ T cells, and ILCs within the IL-22⁺ CD45⁺ cells. We have now revised the figure caption for Figure 5d to improve clarity (Page 36).

2. The study employs three colitis models to demonstrate the role of IL-22 to activate in activating the OSM-OSMR axis:

a. Helicobacter hepaticus + anti-IL10 Abs,

b. Acute DSS (fig. S15f-h)

c. Citrobacter Rodentium

The authors convincingly show that IL-22 drives epithelial OSMR upregulation in all three models, and that neutralizing IL-22 reduces colitis severity. However, previous studies have established that IL-22 as protective in C. Rodentium infection. Given that this study describes an opposite effect (new added data after revision), could the authors elaborate in the on how their findings reconcile with prior literature?

Response: Indeed, multiple studies have firmly established IL-22 as a critical protective cytokine during *Citrobacter rodentium* infection^{1,2}. In this context, IL-22 is rapidly produced by innate lymphoid cells and CD4⁺ T cells in response to pathogen recognition and plays a key role in preserving mucosal barrier integrity and limiting bacterial burden. IL-22 signaling induces antimicrobial peptides and strengthens tight junction integrity, thereby containing the infection at the mucosal surface. However, emerging evidence indicates that IL-22 can also exert pathogenic effects, particularly under conditions of chronic or dysregulated inflammation^{3,4}. These observations underscore that, in contrast to acute infection, IL-22 can amplify inflammation in settings where its production is excessive or sustained.

The seemingly opposing roles of IL-22 can be reconciled by considering the immunological context and tissue state in which IL-22 acts. During acute infection or transient injury, IL-22 promotes antimicrobial responses and epithelial repair to restore homeostasis. Conversely, in chronic, non-resolving inflammation such as IBD, prolonged or dysregulated IL-22 exposure may drive maladaptive epithelial remodeling and contribute to ongoing inflammation.

Our data suggest that oncostatin M (OSM) may be a critical modifier of IL-22 biology in chronic intestinal inflammation. In the *H. hepaticus* + anti-IL-10R colitis model, we observed that OSM expression was not only strongly induced but also chronically maintained for at least 30 days (Figure 1c). In contrast, during *C. rodentium* infection, OSM expression was transient Extended Data Figure 5c). This suggests that chronic OSM expression in the context of inflammation may shift the IL-22–driven response from a protective to a pathogenic one.

Taken together, our findings support a model in which the IL-22–OSMR–OSM circuit acts as a context-dependent switch between protective and pathogenic responses. This mechanistic insight may help explain the dichotomy of IL-22 function in intestinal immunity and highlights the broader relevance of our findings for understanding IL-22 and OSM biology in mucosal inflammation.

3. The authors demonstrate that blocking the OSM-OSMR axis ameliorates colitis by reducing epithelial STAT3 activation, which otherwise promotes immune cell recruitment and inflammation. The inflammatory response is typically aimed at controlling infection, yet their newly added supplemental data suggest that recruited immune cells do not impact bacterial dissemination or bacterial load (supplemental figure . Could the authors include a brief discussion—perhaps a single sentence—speculating on the physiological function of this pathway?

Response: We added few sentences on the physiological relevance of OSM pathway in homeostasis in the discussion section (Page 19, second paragraph).

References:

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