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BTNL2 promotes colitis-associated tumorigenesis in mice by regulating IL-22 production

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Dr. Wang,

Thank you for the submission of your manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

The referees acknowledge that the findings are potentially interesting, and that the technical quality of the study is adequate for publication. However, as you will see, they also have a number of suggestions for the improvement of the study and the manuscript. Referee #1 recommends improving the clarity of the text, and both referees #1 and #2 provide suggestions for the revision of the discussion. Furthermore, referees #2 and #3 suggest a few experiments that would strengthen the data and the conclusions. I think that all referee comments are reasonable and should be addressed before your manuscript can be published in EMBO reports.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript. If you have any questions or comments, we can also discuss the revisions in a video chat, if you like.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (December 25th). Please discuss with me the revision progress ahead of this time if you require more time to complete the revisions.

*****
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4) a complete author checklist, which you can download from our author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

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- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) Please note that a Data Availability section at the end of Materials and Methods is now mandatory. In case you have no data that requires deposition in a public database, please state so instead of refereeing to the database.
See also <https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

8) We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the new policy (<https://www.embopress.org/competing-interests>) and update your competing interests statement if necessary. Please name this section 'Disclosure and Competing Interests Statement' and place it after the Acknowledgements section.

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- the name of the statistical test used to generate error bars and P values,
- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,
- the nature of the bars and error bars (s.d., s.e.m.)
- If the data are obtained from n {less than or equal to} 2, use scatter plots showing the individual data points.

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

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- Please also include scale bars in all microscopy images.

10) We would also encourage you to include the source data for figure panels that show essential data.

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11) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at <https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>.

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link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

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I look forward to seeing a revised form of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

You can use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Yours sincerely,

Ioannis Papaioannou, PhD
Editor
EMBO reports

Referee #1:

This manuscript uses genetic knockout, immune suppression and epitope engagement to weave a complex story of how the mucosal protein BTNL2, through effects on IL22 alters immune functions in the AOM/DSS model of inflammation associated colon cancer. Each experiment and resulting data seem clear, and the statements made seem to correctly describe the result. However, this reviewer found it very difficult to follow the complex web. If understood correctly, the broad conclusion is that the role of BTL2 dissociates what is usually considered the tight link between inflammation and its protumorigenic effects, with differential effects on the two overall phenotypes.
Significant clarification might be achieved in two ways: First, by including a carefully developed summary schematic showing the repressive and stimulatory links established among the multiple components experimentally dissected, and how these branch to repress and stimulate inflammation and tumorigenesis. And rather than including this as a summary, it might be more up-front to help guide the reader through the multiple experiments and data. Second, a brief discussion might be included to address what I think is an overall conclusion that there are disparate - and indeed opposite - effects on inflammation and tumorigenesis, which, if understood correctly, comes as a surprise to this reviewer.

Referee #2:

BTNL2 is a transmembrane protein highly expressed in intestinal tract. Although the potential receptor for BTNL2 is unknown, previous studies report that BTNL2-Fc recombinant protein suppresses CD4+ T cell activation in vitro. The authors recently report that BTNL2 inhibits anti-tumor immunity in the subcutaneous tumor model (Du et al Nat Commun 2022). In the manuscript, the authors showed the important role of BTNL2 in colon disease mouse models. They found that BTNL2 promoted IL-22 production and consequently promoted DSS+AOM-induced mouse colon tumor and protected against DSS-induced colitis and citrobacter infection. Importantly, the authors also showed clinical relevance of BTNL2 expression association with IL-22. As the authors discussed, it is still not clear how BTNL2 promotes IL-22 production.

Specific points:
1. The authors showed BTNL2-Fc induced STAT3 activation in splenocytes and all the inhibitors of STAT3, RORC, JAK1/2 and HIF1a inhibited BTNL2-Fc-induced IL-22 production in Fig. 4. It is better also check if BTNL2-Fc treatment activates the other signaling pathways (RORC, JAK1/2 and HIF1a). It is also helpful to discuss on this point for potential upstream signaling that drives the multiple pathways although the potential receptor is unknown.
2. The authors used Lpr, splenocyte and isolated cells (ILC3, CD4+ T, γδT) to check BTNL2-Fc-induced IL-22 production in Fig. 4. Dose BTNL2 promote differentiation of IL-22 producing cells (such as CD4+ T cells to Th17/Th22 cells), or directly act on the differentiated cells to enhance IL-22 production? Or maybe both?
3. In Fig. 5, it appears that the protocol used and the data presented indicate the preventive effects instead of therapeutic effects of BTNL2-Fc in the DSS colitis and Citrobacter models. If authors claims the therapeutic effects, they need to use a protocol with the treatment after the disease started. Otherwise, authors need to modify their claims.
4. In Fig. 6, it is better determine whether the tumors form before the ip Ab treatments. For the BTNL2 blocking antibody-mediated effects on the tumor model, is it mainly due to the reduced IL-22 production? The authors recently report the role of BTNL2 in suppression of antitumor immunity (Du et al Nat Commun 2022). Does this mechanism also works on the colon tumor

model? This point is better discussed.

Referee #3:

Peng, et al. describe the involvement of BTNL2 in the suppression of disease activity in inflammatory bowel disease and the exacerbation of colorectal cancer through the promotion of IL-22 production. The administration of an anti-BTNL2 monoclonal antibody (mAb) ameliorated the tumor growth of colorectal cancer. BTNL2-Fc recombinant protein showed the improvement in dextran sulfate sodium (DSS)-induced colitis. All experimental results are consistent with the hypothesized function of BTNL2 and previous reports. In particular, it is interesting that anti-BTNL2 mAb showed remarkable efficacy against colorectal tumors in mice. Anti-BTNL2 mAb is therefore expected to be a novel therapeutic agent as well as anti-PD-1 antibody. My comments are as follows.

1. Fig. 1F, Fig. 2E, Fig. 6F: Additional measurement for other anti-inflammatory cytokines, such as IL-10, is recommended to prove that BTNL2 produces IL-22 in a specific manner.
2. Fig. 4D: Experiments with other cell lineages, such as CD8-positive T cells, are recommended.
3. Fig. 4E: The lower panel shows IL-22 production of CD4+T cells and γδ T cells. The authors should explain the upper panel in detail.
4. Fig. 6B-E: Anti-BTNL2 mAb showed an anti-tumor effect. If the authors could show that α-BTNL2 mAb worsens DSS-induced colitis, the data would increase the reliability of their hypothesis. This experiment is also important to know adverse events when α-BTNL2 mAb is used for patients with colorectal cancer and colitis.
5. The overexpression of BTNL2 in human colon cancer was shown in Fig. 7B. It is recommended that the authors also show that the BTNL2 expression was increased in tumor lesions induced by DSS+ azomethan in comparison to normal lesions in mice. Obtaining the same data from mice and humans would give us more confidence in the anti-tumor efficacy of anti-BTNL2 mAb therapy in humans.

Point-by-point response

Referee #1:

This manuscript uses genetic knockout, immune suppression and epitope engagement to weave a complex story of how the mucosal protein BTNL2, through effects on IL-22 alters immune functions in the AOM/DSS model of inflammation associated colon cancer. Each experiment and resulting data seem clear, and the statements made seem to correctly describe the result. However, this reviewer found it very difficult to follow the complex web. If understood correctly, the broad conclusion is that the role of BTNL2 dissociates what is usually considered the tight link between inflammation and its protumorigenic effects, with differential effects on the two overall phenotypes.

Significant clarification might be achieved in two ways: First, by including a carefully developed summary schematic showing the repressive and stimulatory links established among the multiple components experimentally dissected, and how these branch to repress and stimulate inflammation and tumorigenesis. And rather than including this as a summary, it might be more up-front to help guide the reader through the multiple experiments and data.

We thank the reviewer's comments and suggestions. We drew a summary schematic and presented it in the Sup Figure 5D of the revised manuscript.

Second, a brief discussion might be included to address what I think is an overall conclusion that there are disparate - and indeed opposite - effects on inflammation and tumorigenesis, which, if understood correctly, comes as a surprise to this reviewer.

We are thankful for the reviewer's suggestions. We added a brief discussion in the Discussion section of the revised manuscript as: "In the present study, we showed that BTNL2 is a critical regulator of IL-22 production in the colonic tract. Interestingly, we found that BTNL2 promotes mouse colorectal tumorigenesis while protect mice from colitis or *Citrobacter rodentium* infection. The contradictory phenotypes of promoting tumorigenesis and protection from colitis and bacterial infection of BTNL2 are actually

consistent with the role of BTNL2 in the regulation of IL-22 in the intestinal system, as IL-22 was shown the same phenotype in the tumorigenesis and colitis/anti-bacterial infections". Page 13, lines 350-356.

Referee #2:

BTNL2 is a transmembrane protein highly expressed in intestinal tract. Although the potential receptor for BTNL2 is unknown, previous studies report that BTNL2-Fc recombinant protein suppresses CD4+ T cell activation in vitro. The authors recently report that BTNL2 inhibits anti-tumor immunity in the subcutaneous tumor model (Du et al Nat Commun 2022). In the manuscript, the authors showed the important role of BTNL2 in colon disease mouse models. They found that BTNL2 promoted IL-22 production and consequently promoted DSS+AOM-induced mouse colon tumor and protected against DSS-induced colitis and citrobacter infection. Importantly, the authors also showed clinical relevance of BTNL2 expression association with IL-22. As the authors discussed, it is still not clear how BTNL2 promotes IL-22 production.

Specific points:

1. The authors showed BTNL2-Fc induced STAT3 activation in splenocytes and all the inhibitors of STAT3, RORC, JAK1/2 and HIF1a inhibited BTNL2-Fc-induced IL-22 production in Fig. 4. It is better also check if BTNL2-Fc treatment activates the other signaling pathways (RORC, JAK1/2 and HIF1a).

We thank the reviewer's comments and suggestions. We examined phosphorylation level of JAK and RNA expression of RORC and HIF1α in splenocytes after treatment with BTNL2-Fc. The results show that BTNL2-Fc could induce JAK2 activation and upregulate the gene expression of *RORC and HIF-1α*. Please find the data in the Figure 4G and 4I of the revised manuscript.

It is also helpful to discuss on this point for potential upstream signaling that drives the multiple pathways although the potential receptor is unknown.

We thank the reviewer's comments and suggestions. We added some discussion as

"Although the exact signaling transduction induced by BTNL2 is still unknown, one possibility is that, after BTNL2 ligation, an unknown receptor on the cell membrane directly recruits Jak kinases to activate STAT3, and activated STAT3 upregulates RORC expression together with HIF1α. So, the receptors which activate Jak kinases may be good candidates for searching the receptor of BTNL2" in the revised manuscript (pages 13, lines 363-367).

2. The authors used Lpr, splenocyte and isolated cells (ILC3, CD4+ T, γδT) to check BTNL2-Fc-induced IL-22 production in Fig. 4. Dose BTNL2 promote differentiation of IL-22 producing cells (such as CD4+ T cells to Th17/Th22 cells), or directly act on the differentiated cells to enhance IL-22 production? Or maybe both?

We thank the reviewer's comments and questions. we isolated mouse naïve $CD4^+$ T cells and differentiated into Th17 and Th22 cells, then treated either naïve $CD4^+$ T cells or differentiated Th17 and Th22 cells with BTNL2-Fc. We found that BTNL2-Fc promoted differentiated Th17 and Th22 cells to produce IL-22, but not naïve $CD4^+$ T cells to produce IL-22. This data indicates that BTNL2 didn't promote the differentiation of IL-22 producing cells. Please find the data in the Figure 4E of the revised manuscript.

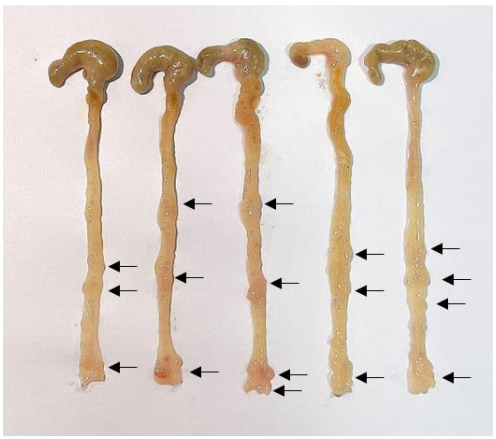
3. In Fig. 5, it appears that the protocol used and the data presented indicate the preventive effects instead of therapeutic effects of BTNL2-Fc in the DSS colitis and Citrobacter models. If authors claims the therapeutic effects, they need to use a protocol with the treatment after the disease started. Otherwise, authors need to modify their claims.

We thank the reviewer's comments and questions. We treated mice with BTNL2-Fc or Fc proteins 3 days after establishment of colitis or *Citrobacter rodentium* infection model. We found that BTNL2-Fc still have therapeutic effect on colitis or *Citrobacter* infection, and please find the data in the Sup Figure 4A-F of the revised manuscript.

4. In Fig. 6, it is better determine whether the tumors form before the ip Ab treatments.

We thank the reviewer's suggestion. We examined the tumor formation before BTNL2 mAb treatment, and found that there were already tumors formation at 58 days of AOM+DSS treatment (before the BTNL2 mAb treatment), and please find the data below. This data further illustrates the therapeutic effect of blocking BTNL2 on colon tumor.

AOM+DSS 58 days



For the BTNL2 blocking antibody-mediated effects on the tumor model, is it mainly due to the reduced IL-22 production?

We thank the reviewer's question. We believe that the therapeutic effect of BTNL2 blockages on colon tumors is major due to the blockage of IL-22 production in the gut. First, we found that IL-22 production was reduced after BTNL2-mAb treatment (Figure 6E-F), which was consistent with the phenotype observed in BTNL2 KO mice (Figure 1E-F); Second, IL-22-Fc recombinant protein could in some extent, rescue the tumor phenotype of BTNL2-KO mice in the colon tumor model (Figure 3A-E). So, we believe that the therapeutic effect of BTNL2 blockage is mainly due to decreased production of IL-22 in the gut, although we can't exclude that there may be other mechanism which may also accounts for the therapeutic effect of BTNL2 blockage.

The authors recently report the role of BTNL2 in suppression of antitumor immunity (Du et al Nat Commun 2022). Does this mechanism also works on the colon tumor model? This point is better discussed.

We thank the reviewer's question. We discussed the point in the Discussion section of

the revised manuscript (pages 12, lines 336-348).

Referee #3:

Peng, et al. describe the involvement of BTNL2 in the suppression of disease activity in inflammatory bowel disease and the exacerbation of colorectal cancer through the promotion of IL-22 production. The administration of an anti-BTNL2 monoclonal antibody (mAb) ameliorated the tumor growth of colorectal cancer. BTNL2-Fc recombinant protein showed the improvement in dextran sulfate sodium (DSS)-induced colitis. All experimental results are consistent with the hypothesized function of BTNL2 and previous reports. In particular, it is interesting that anti-BTNL2 mAb showed remarkable efficacy against colorectal tumors in mice. Anti-BTNL2 mAb is therefore expected to be a novel therapeutic agent as well as anti-PD-1 antibody. My comments are as follows.

1. Fig. 1F, Fig. 2E, Fig. 6F: Additional measurement for other anti-inflammatory cytokines, such as IL-10, is recommended to prove that BTNL2 produces IL-22 in a specific manner.

We thank the reviewer's comments and suggestions. We measured the mRNA level of IL-10, and we didn't find any significant changes of IL-10 in the experimental group compared to the control group. Please find the data in the Figure 1F, 2E, 6F of the revised manuscript.

2. Fig. 4D: Experiments with other cell lineages, such as CD8-positive T cells, are recommended.

We thank the reviewer's suggestion. We examined the IL-22 production by $CD8^+$ T cells after BTNL2-Fc treatment, and found that $CD8^+$ T cells was also able to produce small amounts of IL-22 after treatment with BTNL2-Fc recombinant proteins. Please find the data in the Figure 4D of the revised manuscript.

3. Fig. 4E: The lower panel shows IL-22 production of CD4+T cells and γδ T cells. The

authors should explain the upper panel in detail.

We thank the reviewer's suggestion, and we explained the upper panel as "Schematic diagram of $CD45^{Med}CD3^{-}CD90.2^{+}IL\text{-}22^{+}$ ILC3s, $CD45^{+}CD4^{+}$ $IL\text{-}22^{+}$ $CD4^{+}$ T cells and $CD45^{+}$ $\gamma\delta^{+}$ $IL\text{-}22^{+}$ γδ T cells in mouse colonic LPLs analyzed by flow cytometry" in the revised manuscript. We put the original Figure 4E to Figure EV 3B in the revised manuscript.

4. Fig. 6B-E: Anti-BTNL2 mAb showed an anti-tumor effect. If the authors could show that α-BTNL2 mAb worsens DSS-induced colitis, the data would increase the reliability of their hypothesis. This experiment is also important to know adverse events when α-BTNL2 mAb is used for patients with colorectal cancer and colitis.

We thank the reviewer's comments and suggestions. We performed the experiment suggested by the reviewer, and found that α-BTNL2 mAb aggravated DSS-induced colitis compared to control Ab treatment. Please find the data in the Figure EV 5A-C of the revised manuscript.

5. The overexpression of BTNL2 in human colon cancer was shown in Fig. 7B. It is recommended that the authors also show that the BTNL2 expression was increased in tumor lesions induced by DSS+ azomethan in comparison to normal lesions in mice. Obtaining the same data from mice and humans would give us more confidence in the anti-tumor efficacy of anti-BTNL2 mAb therapy in humans.

We thank the reviewer's comments and suggestions. We examined the expression of BTNL2 in colon tissues and AOM+DSS-induced tumors tissues, and found that RNA and protein level of BTNL2 were significantly increased in the tumor tissues compared to colon tissues. Please find the data in the Figure 5D-E of the revised manuscript.

Overall, we are really grateful for the reviewers' comments and suggestions, which helped us to further increase the quality of this manuscript.

Dear Dr. Wang,

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of reports from the three referees that were asked to re-evaluate your study. Please find their comments appended below.

As you will see, the referees find that their previous concerns were successfully addressed during revision, and they now recommend publication. The only remaining issue (also mentioned by referee 1) is that there are many grammar (and spelling) mistakes throughout the manuscript, which I need you to correct before I can accept the manuscript. I recommend that the text be revised by a native English speaker or a language editing service. Please make sure that all changes are highlighted (or "tracked") to be clearly visible in the revised manuscript file.

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your manuscript:

- Please consider revising the title to "BTNL2 promotes colitis-associated tumorigenesis in mice by regulating IL-22 production".

- Since Qianwen Peng and Ting Pan are equally contributing authors, the number "7" should be marked against their names in the author list on the title page.

- Please note that all corresponding authors are required to supply ORCID IDs for their names upon submission of the revised manuscript; please provide ORCID IDs for Du Cheng and Yanyun Du.

- "Methods" needs correcting to 'Materials and Methods'.

- Please update your data availability statement: since your study does not include datasets requiring deposition in public databases, please insert the following statement: "This study includes no data deposited in external repositories.".

- Please enter all relevant funding information in the online submission system when you submit your revised manuscript.

- Please update your competing interests statement: the heading should be "Disclosure and competing interests statement" and the statement "The authors declare that they have no conflict of interest.", since you have no competing interests to declare.

- The author contributions statement should be removed from the manuscript file. Instead, we now use CRediT to specify the contributions of each author in the journal submission system. Please use the free text box to provide more detailed descriptions. See also guide to authors:
<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>.

- Your Figure legends have been inspected by our data editors for completeness and accuracy. Please see the required changes in the attached Word file and address all comments in your revised manuscript (with tracked changes).

- All main and Expanded View (EV) Figures should be provided as separate files; please upload your EV Figures individually using the file type "Figure".

- The EV Figure legends should be included in the manuscript file following the main Figure legends, with the heading "Expanded View Figure Legends".

- The Sup. Table 1 should be uploaded individually as Table EV1 using the file type "Expanded View".

- Please re-organize your source data: one (zipped) folder should be provided per main Figure, and all source data that are relevant to EV Figures should be zipped together.

- Please transfer all information from the last column of your checklist to the appropriate sections of the manuscript (e.g. Materials and Methods, Figure legends etc.). In the last column of the corrected checklist, please indicate only in which section of the manuscript each piece of information is available. Please also provide information about ethics approval regarding samples from human participants. The completed author checklist will be part of the Review Process File (RPF); please see below for more information.

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A) a short (1-2 sentences) summary of the findings and their significance,
B) 2-4 bullet points highlighting the key results, and
C) a synopsis image that is exactly 550 pixels wide and 200-600 pixels high (the height is variable). You can either show a model or key data in the synopsis image. Please note that text needs to be readable at the final size.

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Please also note that as part of the EMBO publications' Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You can opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

We would also welcome the submission of cover suggestions or motifs to be used by our Graphics Illustrator in designing a cover.

We look forward to seeing a final version of your manuscript as soon as possible.

Yours sincerely,

Ioannis Papaioannou, PhD
Editor
EMBO reports

Referee #1:

The suggestions made have been addressed. The data seem clear and the authors have added a statement regarding similar effects of IL22 supporting the disparate effects on inflammation and tumorigenesis. However, it still strikes me odd that tumorigenesis is not affected while inflammation is repressed, since the point of the AOM/DSS model is that tumor development is dependent on inflammatory promotion of cells that are mutated by the carcinogen. It may be that a key aspect of the inflammatory response is still there, subtle but still effective in tumor promotion. In that sense, it can be considered that this makes the data provocative, although without a resolution of this issue.
The remaining issue is that there are numerous cases of incorrect grammar: thus, while meaning is clear, the manuscript needs detailed editing to correct this - this can be done by the journal if the manuscript is accepted or by the investigator's colleagues or an editing service.

Referee #2:

All my comments have been addressed. No further comments.

Referee #3:

The authors have appropriately responded to the reviewers' comments.

Point-by-point response

Referee #1:

The suggestions made have been addressed. The data seem clear and the authors have added a statement regarding similar effects of IL22 supporting the disparate effects on inflammation and tumorigenesis. However, it still strikes me odd that tumorigenesis is not affected while inflammation is repressed, since the point of the AOM/DSS model is that tumor development is dependent on inflammatory promotion of cells that are mutated by the carcinogen. It may be that a key aspect of the inflammatory response is still there, subtle but still effective in tumor promotion. In that sense, it can be considered that this makes the data provocative, although without a resolution of this issue.

We thank the reviewer's comment, and we agree with the reviewer that AOM/DSS model is that tumor development is dependent on inflammatory promotion of cells that are mutated by the carcinogen. While, we think that if the proliferative pathway in the cells is interrupted (such as IL-22 pathway), the tumor can't form even in the presence of inflammation.

The remaining issue is that there are numerous cases of incorrect grammar: thus, while meaning is clear, the manuscript needs detailed editing to correct this - this can be done by the journal if the manuscript is accepted or by the investigator's colleagues or an editing service.

We thank the reviewer's comments and suggestions, and we asked a language editing service to revise the text to correct the grammar and spelling mistakes.

Referee #2:

All my comments have been addressed. No further comments.

We thank the reviewer’s comment.

Referee #3:

The authors have appropriately responded to the reviewers' comments.

We thank the reviewer’s comment.

Overall, we are grateful for all of three reviewers’ efforts, which helped us to improve the quality of the manuscript.

Dr. Chenhui Wang
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The data shown in figures should satisfy the following conditions:

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