EMBO Molecular Medicine

FFAR2 activation induces IL-22-dependent intestinal immune responses to suppress CNS autoimmunity

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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.)

23rd Feb 2026

Dear Dr. Miljkovic,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received feedback from the three reviewers who agreed to evaluate your manuscript. All three referees recognize interest of the study but also raise serious concerns that should be addressed in a major revision. Focus of the revision should be on providing experimental evidence for the specificity of FFAR2 activation and subsequent IL-22 production in ameliorating EAE (referee #1), analyzing effects of Cpd1 on IL-4 expression (referee #2) and conducting a more in-depth analysis of human samples (referee #3). Considering that the revision will require extensive experimentation we think six months rather than three months would be more appropriate to provide the complete revision. If you would like to discuss further the points raised by the referees, I am available to do so via email or video. Let me know if you are interested in this option.

Further consideration of a revision that addresses reviewers' concerns in full will entail a second round of review. EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision.

We would welcome the submission of a revised version within six months for further consideration. Please let us know if you require longer to complete the revision.

I look forward to receiving your revised manuscript.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

*****

When preparing your revised manuscript, please refer to our guidelines: https://link.springer.com/journal/44321/submission-guidelines#cms-Revised-submissions. We perform an initial quality control of all revised manuscripts before re-review; failure to include requested items will delay the evaluation of your revision.

We require:

1) A .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) Individual production quality figure files as .eps, .tif, .jpg (one file per figure). For guidance, download the 'Figure Guide PDF': https://media.springernature.com/original/springer-cms/rest/v1/content/27825798/data/v1.

3) A .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) A complete author checklist, which you can download from our author guidelines. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript.

6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public.

In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.

7) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

8) At EMBO Press we ask authors to provide source data for the main manuscript figures. You will receive a separate email with instructions for providing source data with your revised manuscript, including how to upload and organize the files.

9) 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.

10) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc.

- 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.

11) The paper explained: EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting

- the medical issue you are addressing,

- the results obtained and

- their clinical impact.

This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.

12) Author contributions: You will be asked to provide CRediT (Contributor Role Taxonomy) terms in the submission system. These replace a narrative author contribution section in the manuscript.

13) A Conflict of Interest statement should be provided in the main text.

14) Every published paper includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one-sentences bullet points that summarizes the paper. Please write the bullet points to summarize the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.

Please also suggest a striking image or visual abstract to illustrate your article as a PNG file 550 px wide x 300-600 px high.

15) Include a Reagents and Tools Table as part of the Methods section, which can be downloaded from our author guidelines.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

The study relies heavily on pharmacological tools (Cpd1 and anti-IL-22 antibodies) without sufficient controls to rule out off-target effects.

Referee #1 (Remarks for Author):

Jevtić et al. investigate the potential of Cpd1, a FFAR2 agonist, to suppress autoimmune inflammation in the EAE model via an interleukin-22 (IL-22)-dependent mechanism. The authors report that Cpd1 treatment ameliorates EAE disease severity and is associated with the stimulation of regulatory T cells (Tregs) and Group 3 Innate Lymphoid Cells (ILC3s) within the intestine. Furthermore, they utilize anti-IL-22 neutralizing antibodies to suggest that the protective effect of Cpd1 is dependent on IL-22 signaling.
While the topic of targeting FFAR2 to modulate neuroinflammation is timely and potentially interesting, the current dataset is premature and does not fully support the central conclusions. Specifically, the study relies heavily on pharmacological tools (Cpd1 and anti-IL-22 antibodies) without sufficient controls to rule out off-target effects. Consequently, the claim that the observed amelioration of EAE is driven strictly by specific FFAR2 activation and subsequent IL-22 production remains unproven. Additionally, the manuscript lacks a detailed characterization of the immunological landscape and neuropathology required to substantiate the mechanism of action.
Main concerns:
1. Almost all small molecule agonists, including Cpd1, have off-target effects that can confound interpretation. The authors have not provided convincing evidence that Cpd1 ameliorates EAE severity specifically through FFAR2 activation.
2. The conclusion that the mechanism is IL-22-dependent rests on the use of anti-IL-22 antibodies. Antibody neutralization experiments can be prone to non-specific binding or incomplete neutralization, and the authors have not provided evidence that this antibody selectively and exclusively blocks IL-22 in this model.
3. The authors do not perform detailed analyses to characterize the immune response in the peripheral immune system and the CNS of EAE mice.
4. The authors do not perform detailed analyses to determine tissue damage in the CNS of EAE mice, such as demyelination, oligodendrocyte death, and axon degeneration.

Referee #2 (Remarks for Author):

The revision opinion
This research demonstrates that the FFAR2-specific agonist Cpd1, administered orally, effectively ameliorates central nervous system autoimmune symptoms during the effector phase of the EAE model. Its mechanism of action relies on IL-22-mediated signaling, Specifically, it stimulates the activation of intestinal Tregs and ILC3s, inhibits pathways associated with the proliferation and activation of intestinal CD4+ T cells, and reduces immune cell infiltration and inflammatory responses in the central nervous system. Furthermore, it inhibits inflammatory cytokine secretion in PBMCs from human MS patients, suggesting that FFAR2 agonists hold promise as potential therapeutic agents for autoimmune diseases such as multiple sclerosis. However, the article still has some issues that need to be addressed to improve its quality：
1． In the study, RNA sequencing results revealed that Cpd1 upregulates IL-4 expression in CD4+ T cells within the small intestinal lamina propria while simultaneously suppressing proinflammatory signaling pathways such as IFN-γ. As a core cytokine of the Th2 immune response, IL-4 directly inhibits the differentiation of naive CD4+ T cells into Th1/Th17 cells. The authors should supplement this information.
2． To investigate whether Cpd1 can affect human cells, peripheral blood mononuclear cells (PBMCs) were obtained from MS patients and healthy controls. The cells were activated using ConA. Why not activate the TCR using specific antigens?

Referee #3 (Comments on Novelty/Model System for Author):

The story is interesting and the quality of the mouse study is sufficiently high. However, for the paper to reach the higher level (suitable to EMOB MM), the part of human immunology should be strengthened. At this stage, they should show more data to make a good translational immunology paper.

Referee #3 (Remarks for Author):

Short chain fatty acid (SCFA) has immunoregulatory potentials and reduced intake of SCFA may have roles in human health and diseases. Cpd1 is a small molecule ligand which may mimic the effects of SCFA as it binds to free fatty acid receptor 2 (FFAR2), receptor for SCFA. The authors have examined the effects of Cpd1 in mouse EAE model and human MS, to clarify the immunomodulatory effects of Cpd1. The significant advance made was that they have revealed the role of IL-22 producing innate lymphoid cells type 3 (ILC3) and Treg cells in the effects of Cpd1. The therapeutic effects of Cpd1 is significant in the mouse EAE model, and this effect was abrogated by injection of anti-IL-22 antibody. Another important finding was that the main target of Cpd1are located within the gut and the therapeutic effects in EAE could be explained by Cpd1 effects within gut. Although only small intestine derived cells CD4+ T cells were analyzed, the mouse study is well designed and their message appears to be based on good experimental results. In contrast, analysis of human samples is not satisfactory. They showed simply that Cpd1 would suppress the Con A induced activation of human peripheral blood T cells, which is not supportive for their discussion on the role of ILC3 and Treg in Cpd1 effects.

Comments
1. Is Cpd1 not absorbed from the gut? If PK/PD data of Cpd1 is available, the authors may refer to it.
2. Cpd1 appears to modulate gut microbiota in mouse. Is there any reasonable consistency compared to their study in rats?
3. Study for the in vitro effects of Cpd1 on human T cells should be further sophisticated, if Cpd1 is not simply a immunosuppressive drug.

All three referees recognize interest of the study but also raise serious concerns that should be addressed in a major revision. Focus of the revision should be on providing experimental evidence for the specificity of FFAR2 activation and subsequent IL-22 production in ameliorating EAE (referee #1), analyzing effects of Cpd1 on IL-4 expression (referee #2) and conducting a more in-depth analysis of human samples (referee #3).

We have addressed all the comments raised by the referees and paid particular attention to the issues emphasized by the Editor. Specifically, novel data showing specificity of FFAR2 activation by Cpd1, data on the effect of Cpd1 on IL-4 expression in our experimental system, as well as deeper *in vitro* analysis of the effect of Cpd1 on human PBMC are incorporated in the revised version. Detailed point-by-point response to the reviewer's comments follows.

Referee #1 (Comments on Novelty/Model System for Author):

The study relies heavily on pharmacological tools (Cpd1 and anti-IL-22 antibodies) without sufficient controls to rule out off-target effects.

Referee #1 (Remarks for Author):

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While the topic of targeting FFAR2 to modulate neuroinflammation is timely and potentially interesting, the current dataset is premature and does not fully support the central conclusions. Specifically, the study relies heavily on pharmacological tools (Cpd1 and anti-IL-22 antibodies) without sufficient controls to rule out off-target effects. Consequently, the claim that the observed amelioration of EAE is driven strictly by specific FFAR2 activation and subsequent IL-22 production remains unproven. Additionally, the manuscript lacks a detailed characterization of the immunological landscape and neuropathology required to substantiate the mechanism of action.
Main concerns:
1. Almost all small molecule agonists, including Cpd1, have off-target effects that can confound interpretation. The authors have not provided convincing evidence that Cpd1 ameliorates EAE severity specifically through FFAR2 activation.

The receptor pharmacology of Cpd1 is presented in the revised manuscript (Fig S5 and corresponding text: Page 22, line 9). Cpd1 has the same maximal activity (%E_{max}) with 100-1000 fold superior potency to propionate in GTPγS and ERK signaling assays performed on CHO cells expressing the human FFAR2 receptor; CAPTB and GLPG0974 were also tested to demonstrate the absence of receptor activation for these FFAR2 reference antagonists in these assays. Receptor selectivity is demonstrated using assays conducted on human polymorphonuclear cells collected from healthy donors; the Ca^{2+} flux response to Cpd1 is completely blocked by the selective antagonists CAPTB and GLPG0974, respectively. Thus, we are confident that Cpd1 acts through FFAR2 in our study.

2. The conclusion that the mechanism is IL-22-dependent rests on the use of anti-IL-22 antibodies. Antibody neutralization experiments can be prone to non-specific binding or incomplete neutralization, and the authors have not provided evidence that this antibody selectively and exclusively blocks IL-22 in this model.

To respond to the reviewer's comment regarding the specificity of the anti-IL-22 neutralizing antibody (clone IL22JOP), we agree that selective neutralization is essential for interpreting pathway-dependent effects. Importantly, as we detail below, both established literature and

data already contained within our manuscript (including newly generated data) demonstrate unequivocally that IL22JOP selectively and exclusively blocks IL-22 in our experimental setting. Together, these sources provide a level of validation sufficient to avoid further ad-hoc experiments that would only replicate what is already firmly documented.

i. Validation and specificity of the anti-IL-22 antibody (IL22JOP)

The neutralizing anti-IL-22 antibody used in our study (rat monoclonal clone IL22JOP, Thermo Fisher Scientific) has been extensively validated in the literature as a selective IL-22–blocking antibody. As documented in the manufacturer's datasheet and multiple peer-reviewed articles (see below), IL22JOP reliably inhibits IL-22 bioactivity in both mouse and human systems. These data include functional neutralization assays demonstrating that IL22JOP blocks IL-22–dependent IL-10 induction (ND50 10–40 ng/mL). This antibody has been used repeatedly and successfully in published studies to define IL-22 function in immune and epithelial systems. Collectively, these independent validations establish the antibody's specificity and neutralizing activity. The following is the list of papers in which IL22JOP was demonstrated to neutralize IL-22.

Yeaman et al., Mechanisms of NDV-3 vaccine efficacy in MRSA skin versus invasive infection. Proc Natl Acad Sci U S A. 2014 Dec 23;111(51):E5555-63. doi: 10.1073/pnas.1415610111.

Peng et al., Interleukin-22 promotes T helper 1 (Th1)/Th17 immunity in chlamydial lung infection. Mol Med. 2014 Mar 20;20(1):109-19. doi: 10.2119/molmed.2013.00115.

Pinto et al., Joint production of IL-22 participates in the initial phase of antigen-induced arthritis through IL-1β production. Arthritis Res Ther. 2015 Sep 2;17(1):235. doi: 10.1186/s13075-015-0759-2.

Busbee et al., Indole-3-carbinol prevents colitis and associated microbial dysbiosis in an IL-22-dependent manner. JCI Insight. 2020 Jan 16;5(1):e127551. doi: 10.1172/jci.insight.127551.

Wu et al., Interleukin-1β secretion induced by mucosa-associated gut commensal bacteria promotes intestinal barrier repair. Gut Microbes. 2022 Jan-Dec;14(1):2014772. doi: 10.1080/19490976.2021.2014772.

Holloman et al., Aryl Hydrocarbon Receptor Activation Ameliorates Acute Respiratory Distress Syndrome through Regulation of Th17 and Th22 Cells in the Lungs. mBio. 2023 Apr 25;14(2):e0313722. doi: 10.1128/mbio.03137-22.

ii. Use of appropriate controls: isotype control adequately excludes non-specific effects

In accordance with accepted immunological practice, we included a matched rat IgG2a isotype control in all experiments involving IL22JOP. This control is the correct method to exclude non-specific Fc-mediated interactions, injection-related effects, or antibody-associated immune activation. Importantly, the isotype control did not affect any Cpd1-mediated phenotypes, whereas IL22JOP selectively reversed the effects attributed to IL-22. Thus, the specificity of the observed effects is supported by internal experimental controls, in line with standard practices for antibody-neutralization studies.

iii. "Irrelevant binding antibody" as negative control is not appropriate or necessary

Irrelevant antibody that binds an unrelated antigen but does not interfere with the IL-22 pathway might not be considered methodologically appropriate, as binding antibodies inherently introduce unpredictable biological effects by engaging their respective antigens and potentially triggering receptor signaling, crosslinking, or activation pathways unrelated to IL-22. This would confound interpretation and reduce—not increase—specificity of the experimental design.

In contrast, the isotype control used in our study is intentionally non-binding, thereby representing the correct and widely accepted control to rule out non-specific effects. Thus, the additional control suggested by the reviewer would not strengthen the conclusions and is not used in standard antibody-neutralization studies.

iv. Functional alignment with IL-22 biology supports pathway specificity (Fig 6)

Importantly, the observed effects after IL-22 neutralization closely match established IL-22 biology. In co-culture, IL22JOP selectively reversed the suppression of IL-17 (but not IFN-γ), and *in vivo*, anti-IL-22 completely abrogated the therapeutic benefit of Cpd1, whereas the isotype had no effect. This selective reversal is entirely consistent with the mechanistic role of IL-22 and cannot be explained by non-specific antibody activity. These observations constitute

explicit, internal demonstration of IL-22-exclusive neutralization, something that no external experiment could improve upon.
v. **To further support validity of IL22JOP as the neutralizing antibody, levels of IL-22 were measured in supernatants of cell cultures of SILP cells exposed to the antibody. As the result IL-22 levels in the cell cultures supernatants were annulled in the presence of IL22JOP. These data are incorporated in the revised version of the manuscript as Fig 6H.**

Together, the use of a literature-validated neutralizing antibody, the inclusion of a proper isotype control, and the IL-22–consistent functional outcomes robustly support our conclusion that IL-22 is a key mediator of Cpd1 activity. For these reasons, we respectfully maintain that IL22JOP's specificity is both scientifically established and experimentally demonstrated within our dataset and the conclusions drawn in our manuscript remain fully supported. We hope this detailed explanation satisfactorily addresses the reviewer's concern and confirms the appropriateness of our experimental design.

3. The authors do not perform detailed analyses to characterize the immune response in the peripheral immune system and the CNS of EAE mice.

Following the reviewer's comment additional data obtained with SILP cells (Fig 2 M-R) and lymph node cells (Fig S3). As for immune cells within the CNS, our aim was to explore if Cpd1 restricts inflammation within the CNS, and we think that we have provided sufficient evidence to show that Cpd1 is indeed efficient in limiting infiltration into the CNS. Also, effects of Cpd1 treatment on $CD4^+$ T cells as the major pathogenic drivers of EAE are presented in the manuscript. Thus we think that additional analysis of immune cells within the CNS will not add to the value of conclusions of the study. We have to emphasize that due to the profound limiting effect of Cpd1 on immune cell numbers within the CNS, relatively high number of mice is required to obtain samples that can be efficiently analysed by flow cytometry. This raises ethical issues about necessity of using additional mice for this purpose. Alternatively, we have performed additional analyses to determine tissue damage in the CNS of EAE mice as suggested by the reviewer (see the next point).

4. The authors do not perform detailed analyses to determine tissue damage in the CNS of EAE mice, such as demyelination, oligodendrocyte death, and axon degeneration.

Luxol fast blue staining of spinal cord sections for demyelination, as well as SMI32 staining for axonal damage were performed. Data are presented in Fig S2 of the revised manuscript and commented accordingly in the revised text (Page 16, line 1).

Referee #2 (Remarks for Author):

The revision opinion
This research demonstrates that the FFAR2-specific agonist Cpd1, administered orally, effectively ameliorates central nervous system autoimmune symptoms during the effector phase of the EAE model. Its mechanism of action relies on IL-22-mediated signaling, Specifically, it stimulates the activation of intestinal Tregs and ILC3s, inhibits pathways associated with the proliferation and activation of intestinal CD4+ T cells, and reduces immune cell infiltration and inflammatory responses in the central nervous system. Furthermore, it inhibits inflammatory cytokine secretion in PBMCs from human MS patients, suggesting that FFAR2 agonists hold promise as potential therapeutic agents for autoimmune diseases such as multiple sclerosis. However, the article still has some issues that need to be addressed to improve its quality :

1. In the study, RNA sequencing results revealed that Cpd1 upregulates IL-4 expression in CD4+ T cells within the small intestinal lamina propria while simultaneously suppressing proinflammatory signaling pathways such as IFN-γ. As a core cytokine of the Th2 immune response, IL-4 directly inhibits the differentiation of naive CD4+ T cells into Th1/Th17 cells. The authors should supplement this information.

Following the reviewer's suggestion we have analysed the effects of Cpd1 on purified CD4+ T cells. Cpd1 did not upregulate release of IL-4 from CD4+ T cells (Fig 4H). Also, neutralizing anti-IL-4 antibody was applied to co-cultivation of lamina propria cells and MOG_{35-55}-stimulated lymph node cells, yet without effect on IFN-gamma or IL-17 levels (Fig 6 D, E). These data suggest that IL-4 is not a mediator of Cpd1 effects in our system and are discussed in the revised version of the manuscript (Discussion, Page 38, line 23).

2. To investigate whether Cpd1 can affect human cells, peripheral blood mononuclear cells (PBMCs) were obtained from MS patients and healthy controls. The cells were activated using ConA. Why not activate the TCR using specific antigens?

PBMC obtained from healthy donors were challenged with PPD. Cpd1 efficiently reduced IFN-gamma release from PPD-stimulated human PBMC (Fig 5E).

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Comments
1. Is Cpd1 not absorbed from the gut? If PK/PD data of Cpd1 is available, the authors may refer to it.

Cpd1 is orally bioavailable. For example, the pharmacokinetic curve following oral dosing to non-fasted, female mice is presented below.

Figure for reviewers removed

Our composite dataset indicates that the gut is the primary target organ consistent with the findings presented in the manuscript that SILP ILC3 and Treg, as well as the microbiota, are altered in response to Cpd1 treatment. The oral bioavailability of Cpd1 leaves open the possibility that its gut pharmacology could be triggered by the presence of the compound in the lumen and/or circulating in the body.

2. Cpd1 appears to modulate gut microbiota in mouse. Is there any reasonable consistency compared to their study in rats?

Cpd1 has effects on microbiota in both mice and rats, however different bacterial strains were affected across species. We have compared data in mice from this study with data on rats from our previous study and we have provided additional comment on the comparison between species in the revised manuscript (Discussion, Page 40, line 7).

3. Study for the in vitro effects of Cpd1 on human T cells should be further sophisticated, if Cpd1 is not simply a immunosuppressive drug.

Effects of Cpd1 on human PBMC *in vitro* were further explored. PBMC were challenged with a specific antigen (PPD) in the absence or presence of Cpd1. As the results Cpd1 downregulated IFN-gamma release from PBMC in a dose-dependent fashion (Fig 5E). Further, RT-PCR was performed for multiple cytokines and it was shown that Cpd1 did not affect expression of IL-1beta, IL-6, TNF, IL-8, and IL-27, while it increased expression of IL-10 (Fig 5 F-K). Thus, with these additional data we can be confident that the effect of Cpd1 on human PBMC is not immunosuppressive, but rather immunomodulatory. The novel data are commented in the revised version of the manuscript (Page 32, line 23).

13th Aug 2026

Dear Dr. Miljkovic,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received feedback from the two reviewers who agreed to evaluate your manuscript. As you will see from their reports pasted below both referees support publication of your manuscript. Therefore, I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) Authors: Email address for natasa.radulovic@ibiss.bg.ac.rs no longer valid, please update.
2) Figures:
- Remove all figures from the manuscript file and move their legends to the end of the file.
- Please rename Figures S1 - S7 to Figures EV1 - EV7 and update their callout in the main manuscript text. EV figure legends should be placed in the manuscript file after main figure legends under the heading "Expanded View Figure Legends".
3) Source Data: Please upload source data for the main figures as one zipped file per figure. Fig 4I/J/K are mislabeled, please correct.
4) Title: Please consider revising the title to better emphasize the main findings, e.g. "FFAR2 activation induces IL-22-dependent intestinal immune responses to suppress CNS autoimmunity"
5) Abstract: I have gone through your text and revised it (see below). Please review it for accuracy and amend as you see fit:

Short-chain fatty acids (SCFAs) protect against central nervous system (CNS) autoimmunity, but the receptor-mediated mechanisms underlying these effects remain poorly understood, limiting their therapeutic exploitation. Here, we show that selective oral activation of free fatty acid receptor 2 (FFAR2) with the synthetic agonist Cpd1 effectively ameliorates established experimental autoimmune encephalomyelitis (EAE), demonstrating therapeutic efficacy after the onset of the autoimmune response. Cpd1 treatment promoted regulatory T cells and type 3 innate lymphoid cells in the intestine while reducing inflammatory infiltration in the CNS, indicating that modulation of intestinal immunity is sufficient to restrain neuroinflammation. Transcriptomic analysis of intestinal $CD4^+$ T cells revealed broad suppression of pathways controlling T-cell activation and proliferation. Mechanistically, the therapeutic effects of Cpd1 required interleukin-22 (IL-22), as antibody-mediated IL-22 neutralization abolished disease protection, identifying IL-22 as a key downstream effector of FFAR2 signaling. Together, these findings establish selective FFAR2 agonism as a tractable strategy to therapeutically modulate the gut-CNS immune axis and identify FFAR2 as a promising target for the treatment of multiple sclerosis and other autoimmune diseases.

6) In the main manuscript file, please do the following:
- Please address all comments suggested by our data editors listed below:
o Figure legends:
1. Please note that the legends for figure 5 are not provided in a sequential manner (legend for figure 5D is provided before legend of figure 5C). This needs to be rectified.
2. Please note that the exact p values are not provided in the legends of figures 1B-G,I-P; 2D-G,I-L,N,P; 4B,C,E,F,J; 5B,E,I; 6B,C,G,H,J.
- Remove "data not shown" (p. 38).
- Please rename "Background" to "Introduction".
- Please update the order and the headings of the manuscript sections to: Abstract / The Paper Explained / Introduction / Results / Discussion / Methods / Data Availability / Acknowledgements / Disclosure and Competing Interests Statement / References / Figure Legends / Tables / Expanded View Figure Legends
- Rename Suppl Tables 1 and 2 to Table 1 and Table 2.
- In Methods, provide the antibody dilutions that were used for each antibody.
- In Methods, provide the statement that informed consent was obtained from all human subjects and that in addition to the WMA Declaration of Helsinki the experiments also conformed to the principles set out in the Department of Health and Human Services Belmont Report.
- Indicate in legends exact n and exact p values, not a range, along with the statistical test used. To keep the figures "clear" some authors found providing an Appendix table Sx with all exact p-values preferable. You are welcome to do this if you want to.
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Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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***** Reviewer's comments *****

Referee #1 (Remarks for Author):

No further comments.

Referee #2 (Comments on Novelty/Model System for Author):

The mechanism by which FFAR2 agonists alleviate neuroinflammation via intestinal IL-22 is innovative; the authors have provided a comprehensive response to the questions raised.

The authors addressed the remaining editorial issues.

20th Aug 2026

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Yours sincerely,
Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
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Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
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- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
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Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Yes	Material and Methods, Human samples
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