EMBO reports

Myeloid cell-specific topoisomerase 1 inhibition using DNA origami mitigates neuroinflammation

Keying Zhu, Yang Wang, Heela Sarlus, Keyi Geng, Erik Nutma, Jingxian Sun, Shin-Yu Kung, Cindy Bay, Jinming Han, Jin-Hong Min, Irene Benito-Cuesta, Harald Lund, Sandra Amor, Jun Wang, Xingmei Zhang, Claudia Kutter, André Guerreiro Cacais, Björn Högberg, and Robert Harris
DOI: 10.15252/embr.202154499

Corresponding author(s): Keying Zhu (keying.zhu@ki.se) , Robert Harris (robert.harris@ki.se)

Review Timeline:

Submission Date:	16th Dec 21
Editorial Decision:	18th Jan 22
Revision Received:	29th Mar 22
Editorial Decision:	25th Apr 22
Revision Received:	1st May 22
Accepted:	4th May 22

Editor: Achim Breiling

Transaction Report:

(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. Zhu,

Thank you for the submission of your research manuscript to EMBO reports. We have now received the reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, the referees think that these findings are of interest. However, all referees have several comments, concerns and suggestions, indicating that a major revision of the manuscript is necessary to allow publication of the study in EMBO reports. As the reports are below, and all their points need to be addressed, I will not detail them here.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript or in the detailed point-by-point response. Acceptance of your 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 of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision. Please contact me to discuss the revision should you need additional time.

When submitting your revised manuscript, please also carefully review the instructions that follow below.

PLEASE NOTE THAT upon resubmission revised manuscripts are subjected to an initial quality control prior to exposition to re-review. Upon failure in the initial quality control, the manuscripts are sent back to the authors, which may lead to delays. Frequent reasons for such a failure are the lack of the data availability section (please see below) and the presence of statistics based on n=2 (the authors are then asked to present scatter plots or provide more data points).

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the final manuscript text (including legends for main figures, EV figures and tables), but without the figures included. Please make sure that changes are highlighted to be clearly visible. Figure legends should be compiled at the end of the manuscript text.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission.

The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf file labeled Appendix. The Appendix should have page numbers and needs to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.

For more details, please refer to our guide to authors:
http://www.embopress.org/page/journal/14693178/authorguide#manuscriptpreparation

Please consult our guide for figure preparation:
http://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf

See also the guidelines for figure legend preparation:
https://www.embopress.org/page/journal/14693178/authorguide#figureformat

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 (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.

Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines:
http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq, structural and array data) are deposited in an appropriate public database. If no primary datasets have been deposited, please also state this a dedicated section (e.g. 'No primary datasets have been generated and deposited'), see below.

See also: http://embor.embopress.org/authorguide#datadeposition

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Methods) that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

Moreover, I have these editorial requests:

6) We strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. If you want to provide source data, please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

7) 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: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

8) Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many independent experiments were performed, if these were biological or technical replicates, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable, and also add a paragraph detailing this to the methods section. See:
http://www.embopress.org/page/journal/14693178/authorguide#statisticalanalysis

9) Please also note our reference format:
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

10) For microscopic images, please add scale bars of similar style and thickness to all the microscopic images, using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend.

11) Please include the funding information into the Acknowledgements and order the manuscript sections like this:
Title page - Abstract - Introduction - Results - Discussion - Materials and Methods -DAS - Ethics statement - Acknowledgements - Author contributions - Conflict of interest statement - References - Figure legends - Expanded View Figure legends.

Finally, please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please find instructions on how to link the ORCID ID to the account in our manuscript tracking system in our Author guidelines: http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

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

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

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

Referee #1:

Zhu et al., submitted a manuscript entitled Myeloid cell-specific inhibition of TOP1 mitigates neuroinflammation - a novel drug repurposing therapy for consideration in EMBO Report. The authors report that the CPT/TPT drug, alone or loaded into a DNA-glycan backbone (Topo-Gami), can mitigate inflammation in myeloid cells specifically in microglia cells. This conclusion is well supported by their experiments. First, they choose CPT/TPT because these drugs score the highest in their drugs screening analysis to modulate of M1-M2 microglia phenotypes. They went on to show that in vitro, CPT/TPT dampen the expression of many inflammatory cytokines of LPS stimulated microglia cells. Then they successfully alleviate the pathological symptoms observed in two different mouse models of neuroinflammation (LPS injection and EAE). They also demonstrate that the expression of CPT/TPT target gene topoisomerase 1 was increased in neurological cell population/tissues. Finally, they use a delivery system made of a backbone of DNA coated with glycan to load their TPT (TopoGami). They showed that TopoGami was preferentially uptake in vitro by myeloid cells and has similar effect than TPT alone in the EAE model.

Overall, the use of two different model of neuroinflammation besides their in vitro work with primary microglial cells is a major strength of this manuscript. In addition, they analysed many databases and used tissues from MS patient to strengthen their conclusion, which added biological significance to their findings.

My major concern is the novelty aspect of the work. In fact, Rialdi et al in 2016 (DOI: 10.1126/science.aad7993) nicely explained how CPT/TPT was able to reduce the expression of inflammatory genes. While this paper was cited, a discussion on the mechanistic involved could have been done earlier in the introduction section. In addition, in He et al., Front. Immunol., 01 April 2021 | https://doi.org/10.3389/fimmu.2021.619761, they demonstrate similar results using a LPS-PD model of neuroinflammation and also focus on the phenotypical modulation of microglia by CPT. Although they have extensive data to support their claim, these reports limit the novelty of their conclusion mainly to the delivery system that preferentially targets myeloid cells. However, this is the weakest aspect of their manuscript. Indeed, the discrepancies between the in vitro results and the in vivo findings warrant further experiments to support this important claim (see major points section).

Major points:
1- In figure 8 A-B-C-D, myloGami treatment shows a very similar effect then TopoGami in terms of inhibiting the expression of inflammatory genes. However, in MyloGami, there are no TPT loaded. Is this a non-specific effect or a glycan specific effect since glycan can bind to Dectin-1 receptor to induce phagocytosis and signal transduction. An experiment to investigate this point would nicely support their conclusion even though I agree with the author that the effect is slightly stronger when TPT is added.

2- In the same figure 8 A-B-C-D the authors conclude that HR-TPT might not be stable due to lack of glycan. If this is the case, should not the TPT become freely available to the microglia cells and thus have similar effect then TPT alone ?

3-In figure 8G and F, the difference between MyloGami and TopoGami seems higher than in the previous figure. Could that be due to the instability of their delivery system in vivo and consequently a better release of TPT compare to the short time in vitro experiment where the glycan would have a preferred response? It would add to the discussion if the authors could speculate on that or even design an experiment to address that.

4- An analysis of the uptake of TopoGami after in vivo delivery rather than using the cells isolated in vitro would greatly strengthen their conclusion on the preferential uptake of TopoGami by myeloid cells.

Minor points:

1-CPT/TPT induce DNA damage and as such could potentially cause mutations during long term experiments. DNA damage could easily be analysed by microscopy to determine if at the concentration they used, cells display increase markers of DNA damage (like p-yH2AX). This marker could also be used to determine if TPT/CPT is indeed release in their in vitro experiments using TopoGami.

2-In figure 2, fold change is used for the Y axis of the different ELISA graph. To better appreciate the variation, concentration (pg/ml) is usually preferred.

3- The conclusion on the role of Izkf1 and Hdac2 would benefit from experimental data. As such, the data do not support the conclusion even though the authors mitigated their claim on this subject.

Referee #2:

The manuscript by the Harris lab and collaborators tackles an important biological question related to myeloid biology and neuroinflammation, and, at large, how to ameliorate disease associated with hyperinflammation by suppressing immune response/activation.

Their study is timely, novel and extensive, and point toward a potential use of inhibitors of Topoisomerase 1 to suppress microglia activation/ hyperinflammatory response. The experimental plan and execution have been conducted successfully and two most notable results (the suppression of microglia inflammation by TPT and the use of novel technology to ameliorate targeting and toxicity of TPT) are important for both the immunology and neurobiology community).

The part that is less impressive or better, less developed, is the work on the level of Top1 in cells and patients (MS and ALS), which could be placed in Supplementary Figures. Same goes for some of the control of the Topogomi (example: electropheresis).

The Discussion is well constructed, and fair with respect to the challenges ahead like make Topogami traverse the BBB. The part about TF and especially the discussion about Ikaros is speculative and do not add much, in my opinion.

As the authors mentioned, there are challenges ahead but their study is very extensive and important. This reviewers thinks that this manuscript might have been a manuscript that underwent review in another journal as the amount of experiments and quality is very high. Regardless of whether that is true, I do not think the authors should do more for this manuscript aside from potential edits suggested and I recommend publication.

Referee #3:

The authors used Connectivity Map to screen for drugs that can regulate microglial inflammation. They found that TOP1 inhibitor CPT could modulate microglial activation. TOP1 expression increased in M1 microglia. TOP1 inhibitor CPT reduced proinflammatory cytokine expression and increased IL-10 expression in LPS/INFg stimulated microglia, and therefore dampened inflammation in microglia. TPT, a FDA-approved analog of CPT, was protective in a LPS induced neuroinflammation model and the authors provided evidence that microglia from TPT treated mice were less inflammatory. In addition, the authors showed that TPT protected mice from the EAE model and TOP1 expression was elevated in the nerve system in mice or human with neuroinflammatory conditions. Last, the authors designed TopoGami that could target myeloid cells and dampen their inflammation. The study is based on a screening of immunoregulatory drugs of microglia and translates into a bioengineered TOP1 inhibitor. It is of interest to people in the neuroinflammation field.

There are a few questions that need to be clarified.
1. In the two neuroinflammatory models the authors used, monocytes are recruited. Recruited monocytes are usually more inflammatory than resident microglia. Did TPT mainly reduce monocyte recruitment or reduce recruited monocyte/microglia inflammation? Can M1/M2 macrophages or their markers be measured?
2. Can the author comment on how topoisomerase up-regulation enhances inflammation?
3. In recent years, the Netea group has found that B-glucan can prime macrophages. I wonder why MyloGami is anti-inflammatory in this study.

Rebuttal Letter

Zhu et al. *Myeloid cell-specific inhibition of TOP1 mitigates neuroinflammation - a novel drug repurposing therapy*

Referee #1:

General comments:

Zhu et al., submitted a manuscript entitled Myeloid cell-specific inhibition of TOP1 mitigates neuroinflammation - a novel drug repurposing therapy for consideration in EMBO Report. The authors report that the CPT/TPT drug, alone or loaded into a DNA-glycan backbone (Topo-Gami), can mitigate inflammation in myeloid cells specifically in microglia cells. This conclusion is well supported by their experiments. First, they choose CPT/TPT because these drugs score the highest in their drugs screening analysis to modulate of M1-M2 microglia phenotypes. They went on to show that in vitro, CPT/TPT dampen the expression of many inflammatory cytokines of LPS stimulated microglia cells. Then they successfully alleviate the pathological symptoms observed in two different mouse models of neuroinflammation (LPS injection and EAE). They also demonstrate that the expression of CPT/TPT target gene topoisomerase 1 was increased in neurological cell population/tissues. Finally, they use a delivery system made of a backbone of DNA coated with glycan to load their TPT (TopoGami). They showed that TopoGami was preferentially uptake in vitro by myeloid cells and has similar effect than TPT alone in the EAE model.

Overall, the use of two different model of neuroinflammation besides their in vitro work with primary microglial cells is a major strength of this manuscript. In addition, they analysed many databases and used tissues from MS patient to strengthen their conclusion, which added biological significance to their findings.

My major concern is the novelty aspect of the work. In fact, Rialdi et al in 2016 (DOI: 10.1126/science.aad7993) nicely explained how CPT/TPT was able to reduce the expression of inflammatory genes. While this paper was cited, a discussion on the mechanistic involved could have been done earlier in the introduction section. In addition, in He et al., Front. Immunol., 01 April 2021 | https://doi.org/10.3389/fimmu.2021.619761, they demonstrate similar results using a LPS-PD model of neuroinflammation and also focus on the phenotypical modulation of microglia by CPT. Although they have extensive data to support their claim, these reports limit the novelty of their conclusion mainly to the delivery system that preferentially targets myeloid cells. However, this is the weakest aspect of their manuscript. Indeed, the discrepancies between the in vitro results and the in vivo findings warrant further experiments to support this important claim (see major points section).

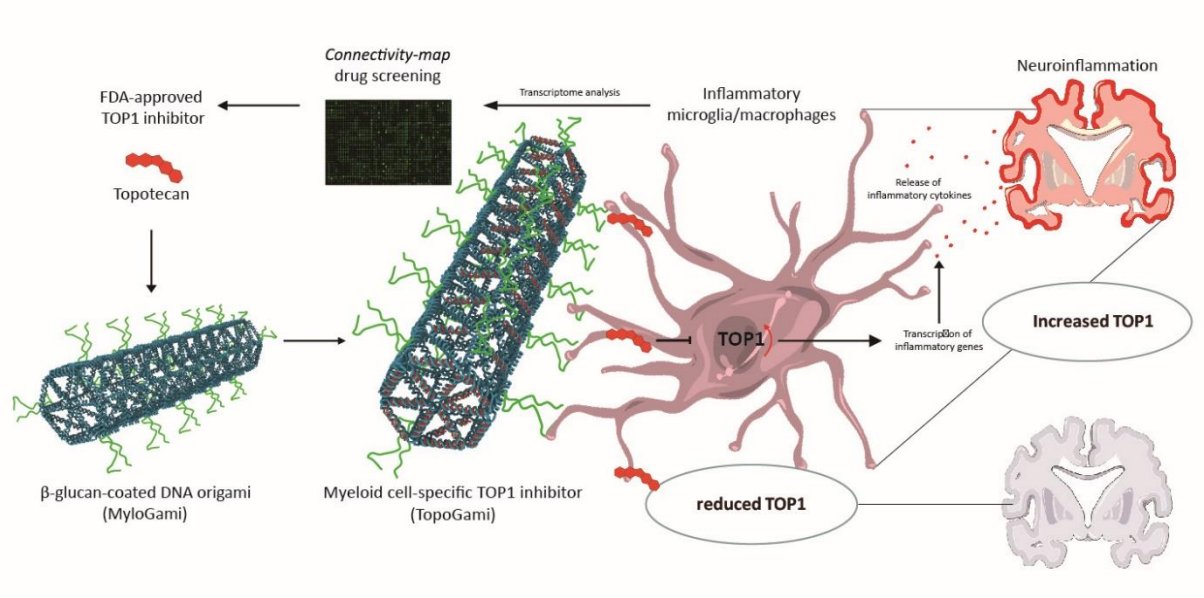
Response to the general comment:

We are grateful for all the reviewer's comments, which prompted us to perform additional experiments and significant revision of the manuscript.

The current study is an interdisciplinary project with novel information and new tools provided. As depicted in Rebuttal Figure. 1, the project sets out from an unbiased drug screening process (in March 2018), during which we determined a list of drugs that could provide insights for drug development of neuroinflammatory diseases featuring dysfunctional microglia (Table EV1). This

list is informative and after we uploaded our manuscript as a preprint in *BioRxiv*, we were contacted by another research group because they were very excited to see that coincidently, a drug they were investigating was predicted in this drug list.

We were invited to present our work regarding the finding of TOP1 inhibition in regulating



Rebuttal Figure. 1: Synopsis of the current study.

microglial activation at the 2019 International Congress of Immunology (abstracted in https://doi.org/10.1002/eji.201970400: P1619 Topoisomerase 1 inhibition mitigates microglia-mediated neuroinflammation), and the study by He *et al.* (Front. Immunol., 01 April 2021) also showed a similar finding, which further confirmed our results. The study by He et al. is quite different from ours in the following key aspects:

1) They used a BV2 microglial cell line throughout the whole study, whereas we cultured primary microglia from adult mice. A microglial cell line is not biologically comparable to primary microglia (Horvath *et al*, 2008), and primary embryonic or neonatal microglia are not imprinted with age-related properties, and therefore behave differently compared to adult microglia (Sierra *et al*, 2007; Moussaud & Draheim, 2010). Use of primary adult microglia is thus a more valuable approach to most closely recapitulating the properties of adult human microglia.

2) Instead of using the experimental drug Camptothecin (CPT), we characterized the effect of the FDA-approved and more soluble version of Topotecan (TPT) *in vivo* using a low, clinically relevant dose, which endows the current project a high translational value. In addition, the animal models of neuroinflammation we employed are also different from those used in the He *et al.* study.

3) We performed RNA-seq analysis on sorted microglia isolated from TPT treated mice (not previously conducted in any study), and this novel information we obtained increased our understanding of the effective mechanism of action of TPT on microglia.

4) We also described the novel finding of an increased TOP1 expression in both activated microglia *in vitro* and during neuroinflammatory disease states, which substantiates our suggestion that TOP1 is a viable treatment target.

5) Most importantly, with the targeted delivery of TPT to myeloid cells using the novel DNA origami-based drug delivery tool MyloGami, we show that targeting myeloid TOP1 is effective in

ameliorating EAE, which further highlights the importance of innate immune regulation in the context of EAE/MS.

In conclusion, we consider that our results not only confirm certain previous findings, which is an important principle in science, but also significantly extend them, providing sufficient novelty to warrant publication.

As the reviewer requested "While this paper was cited, a discussion on the mechanistic involved could have been done earlier in the introduction section.", we have elaborated on the mechanism in the *Introduction* (Lines 68-77). In addition, we have reformatted the introduction with more emphasis on the novelty of the manuscript (Lines 78-92).

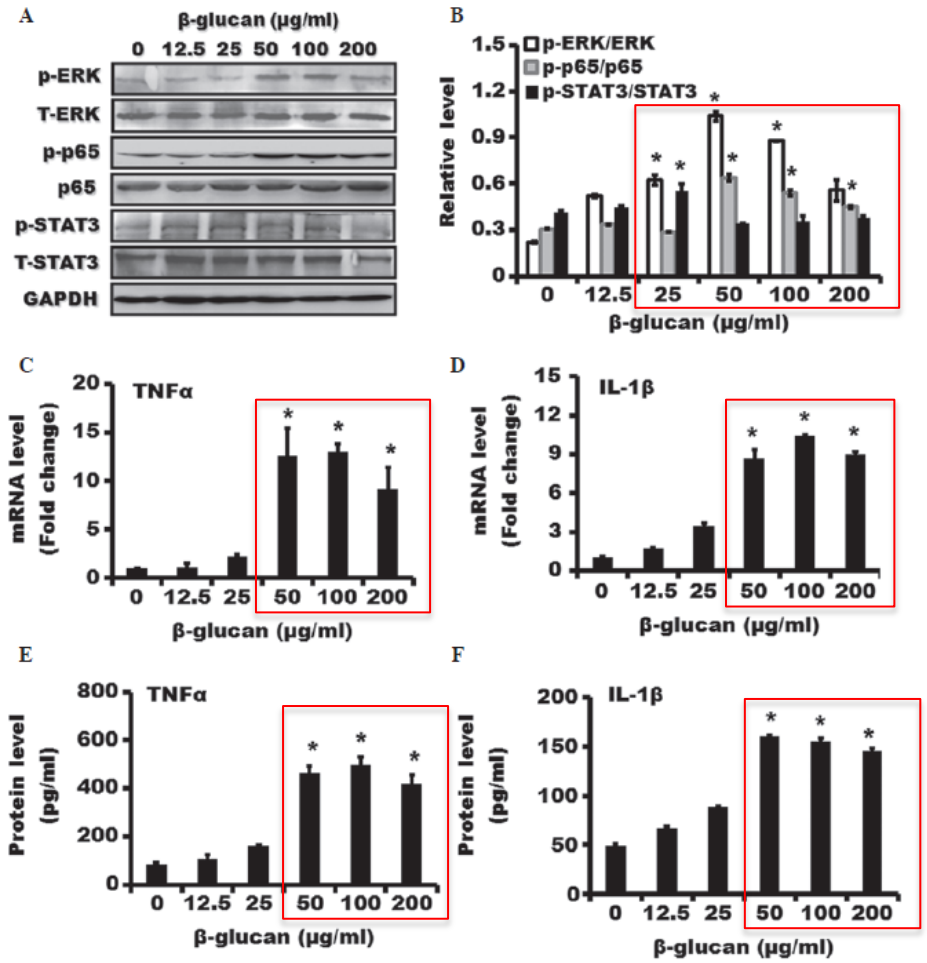
Major points:

Major Point 1- In figure 8 A-B-C-D, myloGami treatment shows a very similar effect then TopoGami in terms of inhibiting the expression of inflammatory genes. However, in MyloGami, there are no TPT loaded. Is this a non-specific effect or a glycan specific effect since glycan can bind to Dectin-1 receptor to induce phagocytosis and signal transduction. An experiment to investigate this point would nicely support their conclusion even though I agree with the author that the effect is slightly stronger when TPT is added.

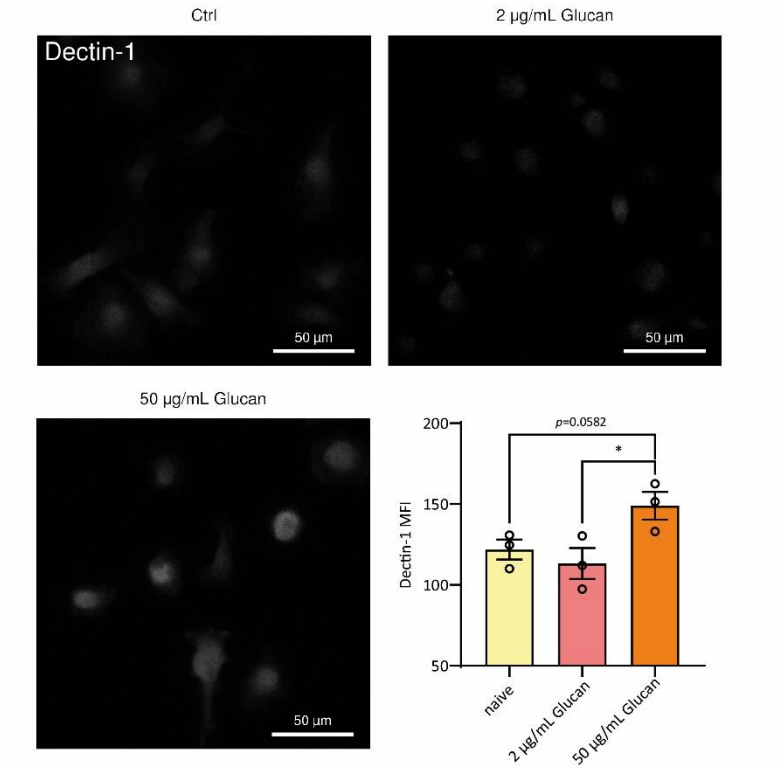
Reply to Major Point 1:

This is a very important question that we have been discussing a lot internally. We have conducted new experiments as suggested by the reviewer, and these new results, together with additional information mined from previous publications, led us to conclude that the effect of MyloGami is not mainly due to a β-glucan-specific effect.

The concentration of β-glucan used to modify the DNA origami is relatively low. We used a final concentration of around 1μM of TPT for macrophages/microglia, which contains around 0.4nM HR and 2μg/mL of β-glucan. This is a rather low concentration that may not induce a significant inflammatory response in cultured macrophages; a previous study reported that only at a concentration higher than 25μg/mL does β-glucan induce the phosphorylation of NF-κB, ERK, and STAT3 signaling pathways and augments TNFα and IL-1β expression in THP-1 monocyte/macrophage cell line (Rebuttal Figure. 2)(Xu *et al*, 2016).



Rebuttal Figure. 2: Xu *et al.* report the dose-dependent effect of β-glucan in inducing THP-1 inflammatory response.

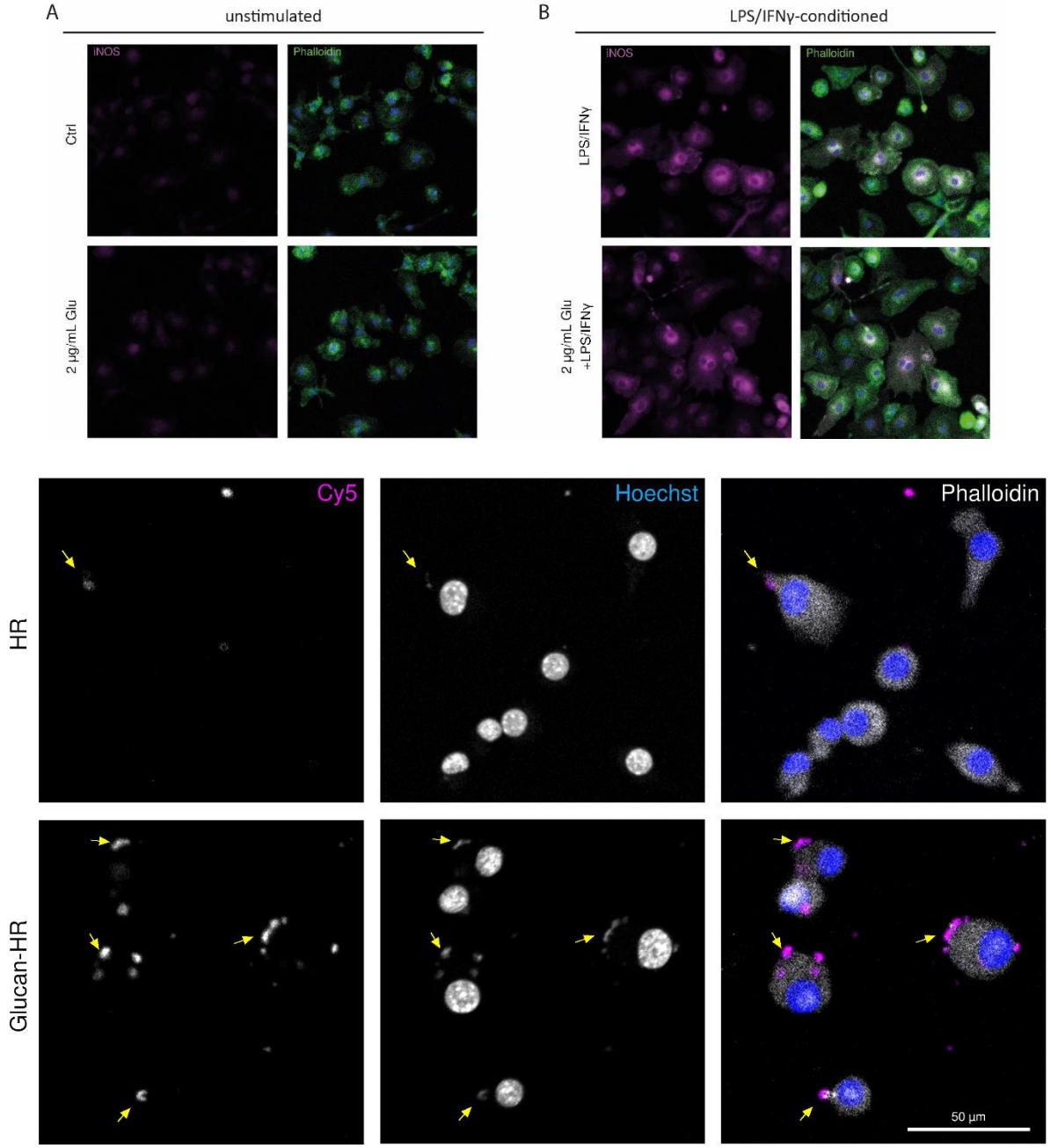


Rebuttal Figure. 3: Dectin-1 expression in macrophages after 12 hr incubation.

Expression of the Dectin-1 receptor, which is the main mediator of β-glucan uptake in macrophages, was upregulated in macrophages when incubated with β-glucan at a concentration of 50μg/mL, but **not** at a concentration of 2μg/mL (Rebuttal Figure. 3).

In addition, as expected, at a concentration of 50μg/mL, but not 2μg/mL, β-glucan also significantly increased the expression of iNOS, which is a proinflammatory effector molecule released by macrophage activated by LPS (Rebuttal Figure. 4 A, C). However, when macrophages were preincubated with 50μg/mL β-glucan before being challenged with LPS/IFN-ɣ stimulation, the expression of iNOS was completely inhibited (Rebuttal Figure. 4 B, C). This counteractive effect of β-glucan on secondary inflammatory stimulation has also been documented in several other studies (Xu *et al*, 2012; Camilli *et al*, 2020; Stothers *et al*, 2021). This effect could be ascribed to epigenetic remodelling and functional reprogramming of macrophages, which may induce innate immune tolerance with manifestations of a suppressed immune response to a secondary challenge (Moorlag *et al*, 2020; Stothers *et al*, 2021; Heng *et al*, 2021; Divangahi *et al*, 2021). Nevertheless, the innate immune response reshaped by β-glucan could be dose-dependent and context-dependent.

The DNA origami/nanostructure *per se* could modulate the macrophage inflammatory



Rebuttal Figure. 5 The microglial uptake of Cy5-conjugated hexagonal rod (HR) DNA origami with or without β-glucan coating.

response. In a previous study using tetrahedral DNA nanostructures (TDNs), the authors determined that TDNs inhibit the expression of nitric oxide, IL-1β, IL-6, and TNF-α in LPS-stimulated RAW264.7 macrophages by inhibiting MAPK phosphorylation (Zhang *et al*, 2018). This also supports our finding in Figure 8 as our hexagonal rod DNA origami (HR) also has an anti-inflammatory effect, and when the surface is coated with β-glucan, this effect is further enhanced. We speculate that this is due to the enhanced uptake of DNA origami when surface-coated with β-glucan (cf: MyloGami/TopoGami). As clearly depicted in Figure 7F and Rebuttal Figure. 5, one could appreciate that the glucan-coated HRs are much more efficiently associated with microglia compared to the uncoated HR. A greater number of DNA nanoparticles are therefore taken up by the cells via β-glucan-mediated binding to myeloid cells. DNA origami with high DNA density is more prone to phagocytosis by macrophages (Maezawa *et al*, 2020). In our study, the HR DNA origami has low DNA packaging density, and this may explain why they are not efficiently ingested by myeloid cells in the absence of β-glucan.

To conclude, the potential effect of MyloGami in attenuating inflammatory responses in macrophages may be mainly ascribed to the effect of DNA nanostructure; β-glucan may also prime macrophages and induce immune tolerance to LPS/IFNɣ stimulation, but the effect may be limited in our case due to the low concentration. However, the coating of the nanostructure with β-glucan may facilitate their enhanced affinity to and phagocytosis by myeloid cells, therefore inducing more profound effects.

We have expanded the *Discussion* regarding this point (lines 370-375 and 469-488) in the revised manuscript.

Major Point 2- In the same figure 8 A-B-C-D the authors conclude that HR-TPT might not be stable due to lack of glycan. If this is the case, should not the TPT become freely available to the microglia cells and thus have similar effect then TPT alone?

Reply to Major Point 2:

We thank the reviewer for raising this concern. The stability of the HR DNA origami is context-dependent, and it also depends on the reference object. As presented in Figure 7E in the manuscript, the TPT release profile of both HR-TPT and TopoGami at 37℃ is similar, with around 50% of TPT being released from the DNA origami after 84 hours. However, when mass DNase (0.5U/μL Benzonase) and $MgCl_2$ (2mM) were added, the effect of glucan-coating in preventing the degradation of DNA origami and the uncontrolled release of TPT was clearly evident. We assume there could be a certain level of nucleases or nucleic acid-degrading molecules in the cell culture medium, but this is not comparable to the experimental conditions we used in Figure 7E. We therefore speculate that there could be a very low amount of freely available TPT when using HR-TPT *in vitro* within the timeframe we analyzed, and this agrees with our results in Figure 8 in which HR-TPT is slightly more effective than HR in inhibiting the expression of inflammatory cytokines. However, the stability and the targeted delivery of uncoated DNA origami *in vivo* could be much more compromised (Cassinelli *et al*, 2015), and while outside the scope of the current study, is a pressing issue for researchers within the nano research field.

Major Point 3-In figure 8G and F, the difference between MyloGami and TopoGami seems higher than in the previous figure. Could that be due to the instability of their delivery system in

vivo and consequently a better release of TPT compare to the short time in vitro experiment where the glycan would have a preferred response? It would add to the discussion if the authors could speculate on that or even design an experiment to address that.

Reply to Major Point 3:

This is a very important point raised by the reviewer. We have elaborated the discussion in the revised manuscript (see the highlighted texts in Lines 469-488). It has been previously reported that although DNA nanostructures could prime macrophages and attenuate their inflammatory responses following LPS/IFNɣ stimulation, it could also induce inflammatory responses in naïve macrophages (Zhang *et al*, 2018; Fukui *et al*, 2018; Pohar *et al*, 2017). For myeloid cells cultured *in vitro*, LPS/IFNγ stimulation dominantly drives all cells towards the same activation phenotype, whereas in an *in vivo* setting the cells can be much more heterogeneously activated. MyloGami could therefore modulate the inflammatory activation of some myeloid cells, but may also potentiate the inflammatory response in *other* subsets (e.g. the resting macrophages), and therefore the net effect is not as significant as with TopoGami.

We speculate that the release of TPT from TopoGami *in vivo* is relatively low. On the one hand, even under very extreme pro-degradation conditions (e.g. addition of high concentration of DNase), β-glucan coating still partially preserves the integrity of the DNA origami (Figure 7E). In comparison, the physiological brain milieu should be less harsh and therefore less efficient at disrupting the β-glucan-coated structure. Conversely, as we delivered TopoGami into the CNS via intracisternal injection, and as we know that the turnover of mouse cerebrospinal fluid (around 40μL) is about 2h (Oshio *et al*, 2005; Johanson *et al*, 2008), TopoGami that is not delivered to myeloid cells could be quickly expelled from the CNS. We therefore do not consider it likely that within this short time window there will be a substantial release of TPT from the β-glucan-coated DNA origami. In addition, the data presented in Rebuttal Figure. 6A depicts the IVIS *in vivo* imaging of drug distribution when MyloGami is administered i.v; the conjugated Cy-5 fluorescent signal is maintained strongly in the liver and abdominal adipose tissue after 4h, as previously reported using other β-glucan particles(Sharma *et al*, 2019), whereas the Cy5 fluorescence conjugated in bare DNA origami (HR-Cy5) faded quickly, which was similar to that of free Cy5. This indicates a slower clearance of DNA origami *in vivo* when coated with β-glucan. We therefore believe that the stability of TopoGami/MyloGami is acceptable when delivered *in vivo*.

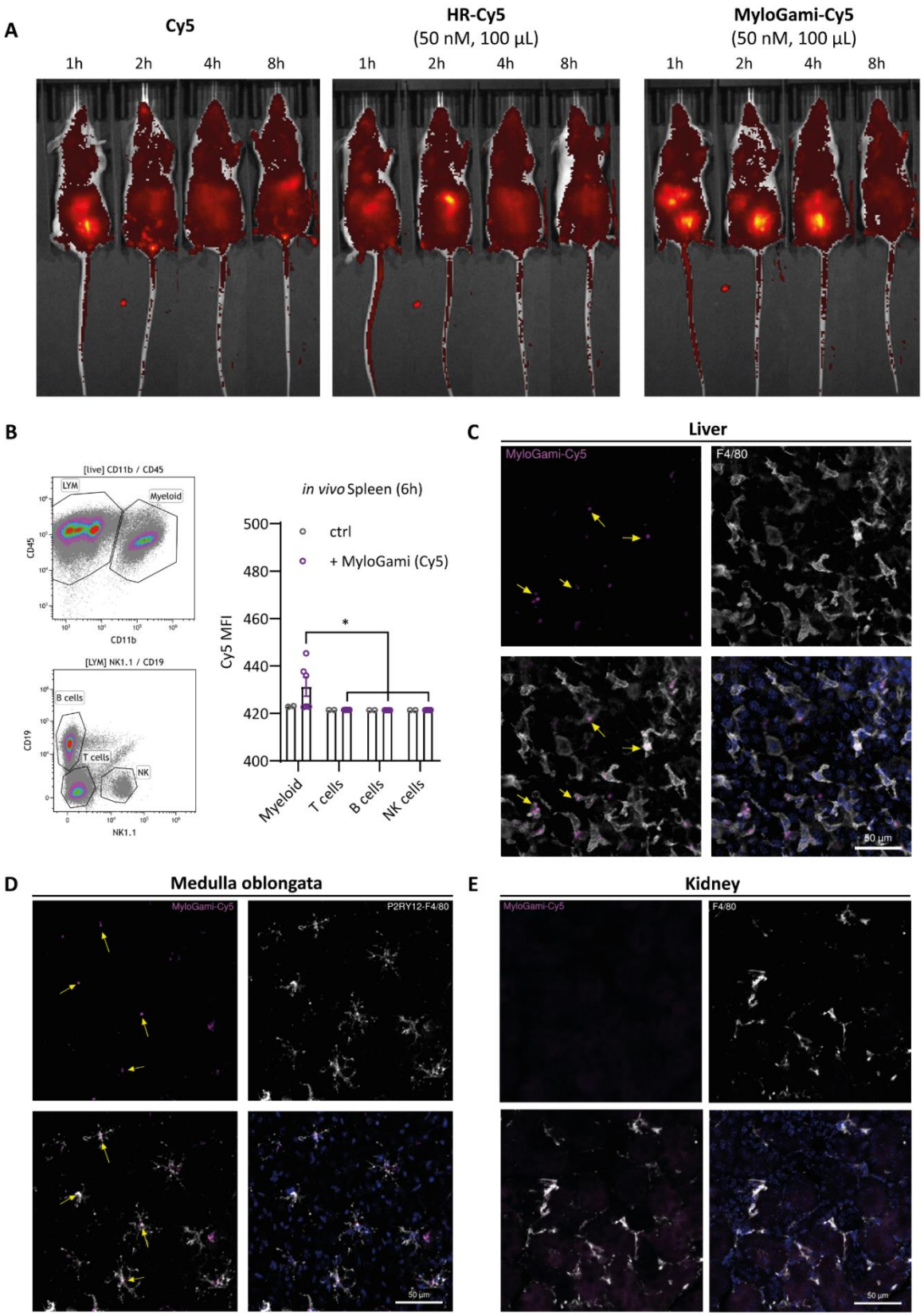
Major Point 4- An analysis of the uptake of TopoGami after in vivo delivery rather than using the cells isolated in vitro would greatly strengthen their conclusion on the preferential uptake of TopoGami by myeloid cells.

Reply to Major Point 4:

We agree with this suggestion from the reviewer. We have thus performed additional experiments during the revision to characterize the *in vivo* distribution and uptake of MyloGami. We performed kinetic IVIS *in vivo* imaging of mice receiving **i.v** injection of Cy5, HR-Cy5 or MyloGami-Cy5, respectively, and there was a clear difference in the distribution patterns of Cy5 fluorescence signals in the MyloGami-injected mice compared to in the HR-injected mice. Free Cy5 and HR-Cy5 were quickly distributed *in vivo* over time, while MyloGami-Cy5 rapidly

accumulated in the liver after 1h (Rebuttal Figure. 6A). This is expected and consistent with what Myriam Aouadi's lab has previously reported. They found the glucan-encapsulated siRNA particles target macrophages with the highest efficiency in the liver Kupffer cells (Tencerova, 2020; Aouadi *et al*, 2009). Glucan particles accumulate not only in the liver but also in abdominal adipose tissues (Sharma *et al*, 2019; Aouadi *et al*, 2013). We also noted a stronger accumulation of Cy5 signals in the liver and abdominal region even after 4h, which may also indicate that the glucan-coated MyloGami is less degraded in the circulation. To better confirm the experimental protocols we used in our project, we injected 100µL 50nM MyloGami-Cy5 (**i.p**) and analyzed the splenocytes using flow cytometry after 6h. Although the association of Cy5 signal within myeloid cells compared to in other cell compartments is not as obvious as we observed *ex vivo* (Figure 7 J-Q in the manuscript), there is still an increased Cy5 fluorescent intensity in myeloid cells compared to in T cells, B cells and NK cells (Rebuttal Figure. 6B). We also harvested the livers and kidneys from these mice and identified Cy5+ staining mostly in the F4/80-expressing myeloid cells in the liver, but not in the macrophages in the kidney (Rebuttal Figure. 6C, E). We used F4/80 and P2RY12 to label microglia/macrophages in the CNS, and when intracisternally injected (**i.c**) into the CNS, MyloGami-Cy5 signals were also observed to colocalize with microglia/macrophages in the CNS (Rebuttal Figure. 6D). With these additional *in vivo* results, the *ex vivo* results presented in the manuscript, and published evidence, we conclude that the glucan-coated MyloGami/TopoGami preferentially targets myeloid cells.

Changes to the revised manuscript: Rebuttal Figure.6 is included in the revised manuscript as *Figure EV4* with corresponding figure legends; added texts in Lines 349-363.



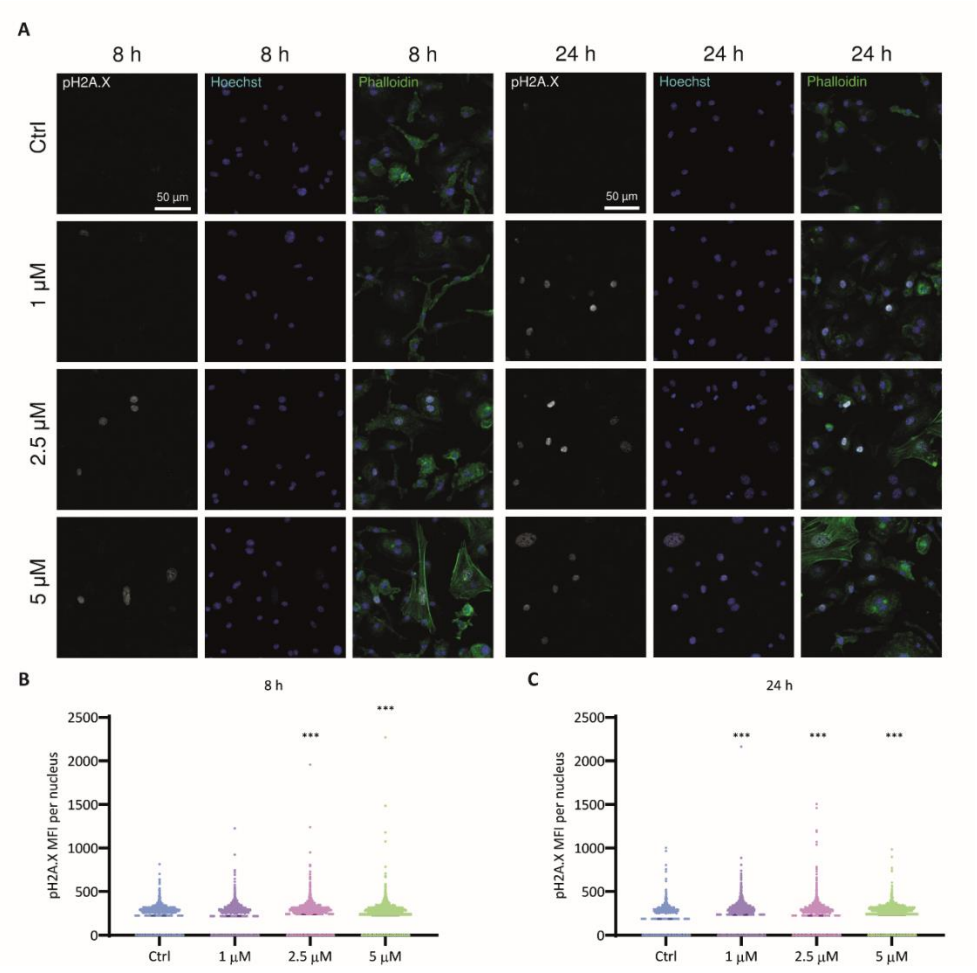
Rebuttal Figure. 6 *In vivo* distribution and uptake of MyloGami.

Minor points:

Minor Point 1-CPT/TPT induce DNA damage and as such could potentially cause mutations during long term experiments. DNA damage could easily be analysed by microscopy to determine if at the concentration they used, cells display increase markers of DNA damage (like p-yH2AX). This marker could also be used to determine if TPT/CPT is indeed release in their in vitro experiments using TopoGami.

Reply to Minor Point 1:

We thank the reviewer for this advice, and we have assessed the expression of the DNA damage marker phospho-Histone H2A.X (pH2A.X) during the revision. As expected, TPT at 2.5 and 5µM concentrations significantly induced DNA damage in primary microglia after 8h incubation and the effect was maintained after 24h. However, short-term exposure (8h) to 1µM TPT did not induce DNA damage in microglia, although long-term exposure (24h) also significantly induced the expression of pH2A.X (Rebuttal Figure. 7). We have now referenced the long-term side-effect of topoisomerase 1 inhibitors in the revised manuscript (Lines 294-296), and the Rebuttal Figure.7 is also included in the manuscript as *Appendix Figure S4*.

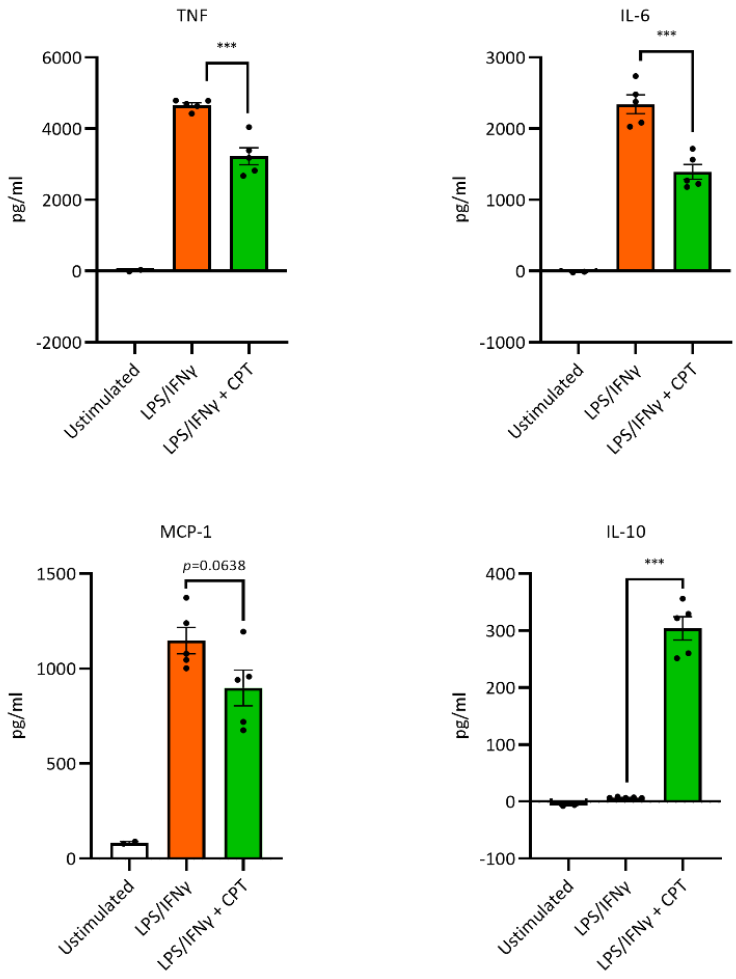


Rebuttal Figure. 7: DNA damage marker expression.

Minor Point 2-In figure 2, fold change is used for the Y axis of the different ELISA graph. To better appreciate the variation, concentration (pg/ml) is usually preferred.

Reply to Minor Point 2:

We appreciate this suggestion by the reviewer. The expression of these cytokines was analyzed using a cytometric bead assay (CBA). We ordered new kits and standard samples and repeated the experiment another time. As presented in Rebuttal Figure. 8, the Y-axes now depict concentrations (pg/ml). We have included the results in the revised manuscript (Figure 3F).



Rebuttal Figure. 8: Another repetition of CBA assay presented with concentrations.

Minor Point 3: The conclusion on the role of Izkf1 and Hdac2 would benefit from experimental data. As such, the data do not support the conclusion even though the authors mitigated their claim on this subject.

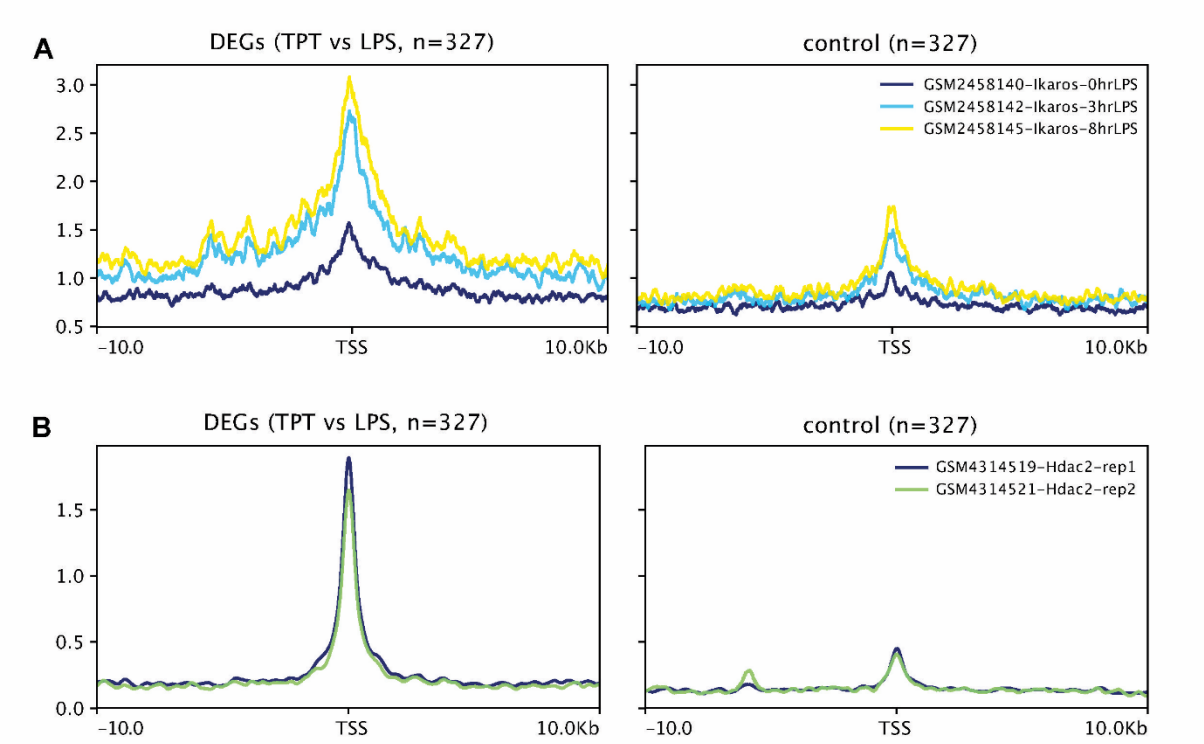
Reply to Minor Point 3:

We thank the reviewer for this comment, which is also a concern raised by another reviewer. We have therefore limited the emphasis and discussion of this part in the manuscript, and have provided more data in the revised manuscript.

To address whether the anti-inflammatory response of TPT is mediated through transcriptional regulation of inflammatory genes by Ikzf1, we firstly took advantage of publicly available ChIP-seq data (Oh *et al*, 2018). In that study the authors profiled the genome-wide binding of Ikaros, encoded by IKAROS Family Zinc Finger 1 (Ikzf1), in mouse macrophages before and after LPS treatment. We plotted the occupancy of Ikaros (Ikzf1) at the transcriptional starting sites (TSS) of the differentially expressed genes (DEGs) when comparing the TPT group with the LPS group, as well as of a random set of annotated genes with the same sample size as the control by using our RNA-seq data (Fig. 5B, Rebuttal Figure. 9A). First, Ikaros (Ikzf1) occupancy at the TSS of DEGs was much higher when compared to the controls. Additionally, we found that the binding of Ikaros (Ikzf1) at DEGs TSS sites increased after LPS treatment, indicating the close association between transcriptomic alteration caused by TPT treatment in the LPS model and Ikaros (Ikzf1) binding (Rebuttal Figure. 9A). Importantly, following TPT treatment the expression of Ikaros (Ikzf1) was reduced (Fig. 5J). We therefore reasoned that by lowering the expression of Ikaros (Ikzf1), TPT could partially reverse the transcriptomic changes induced by LPS.

Similar to Ikaros (Ikzf1), we inspected the occupancy of Histone deacetylase 2 (Hdac2) using a published dataset GSE137666 (Tanaka *et al*, 2020). We profiled the binding of Hdac2 at the TSS of genes that were deregulated when comparing the TPT and LPS groups according to our RNA-seq data, as well as the same number of randomly selected annotated genes (Rebuttal Figure. 9B). We determined a stronger enrichment of Hdac2 binding to the DEGs when compared to controls, suggesting involvement of Hdac2 in the anti-inflammatory functions of TPT.

We became interested in the involvement of Ikzf1 (Ikaros) as it is less studied in myeloid cells, and

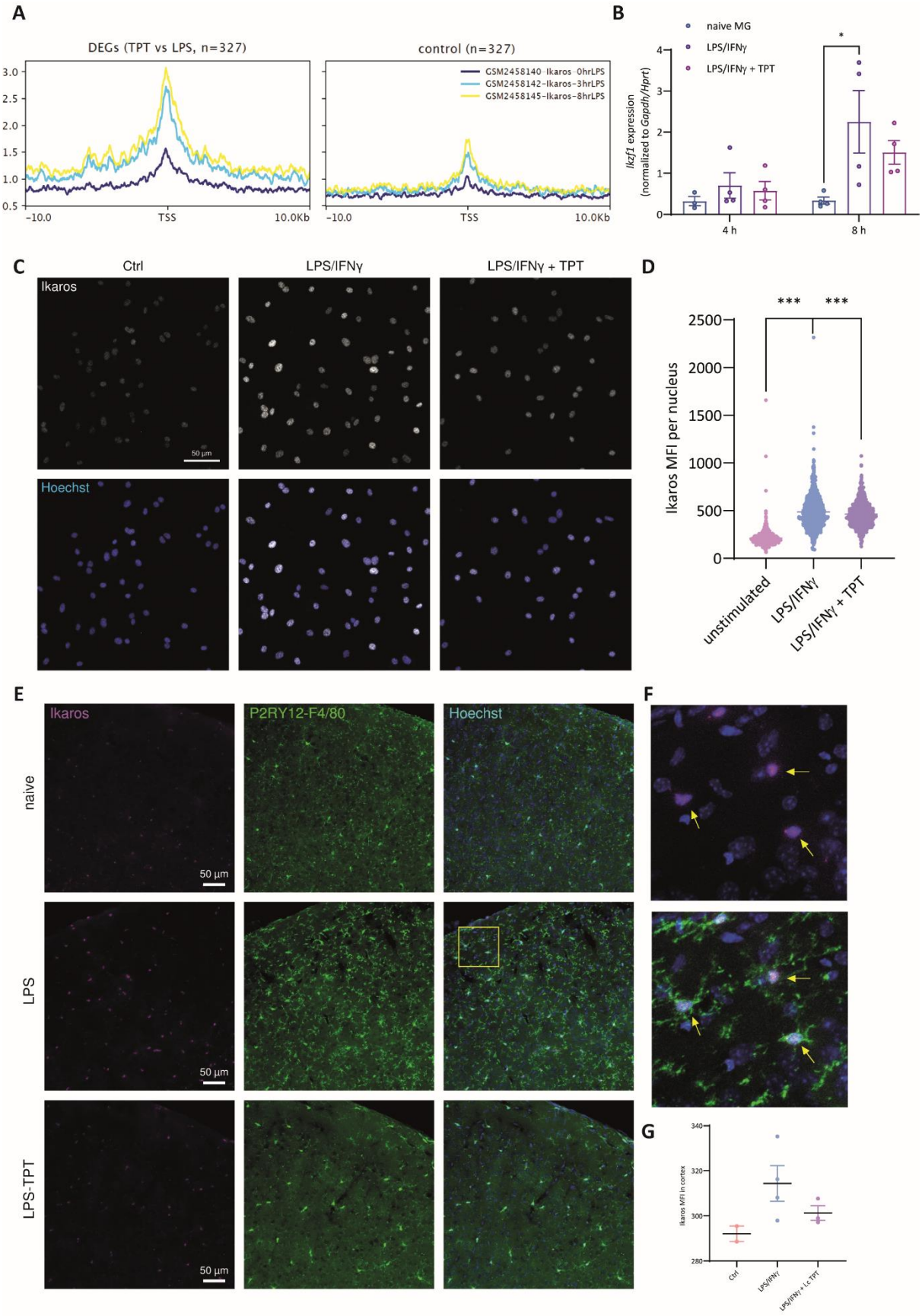


Rebuttal Figure. 9: Transcriptional start site (TSS) analysis of Ikaros and Hdac2 on DEGs (TPT vs LPS) or random genes.

we therefore performed additional experiments investigating its change following inflammatory responses and TPT treatment. We observed a significant increase in the mRNA expression of *Ikzf1* in primary microglia culture following LPS/IFNɣ stimulation, indicating involvement of *Ikzf1* during microglial activation; there is a decreased tendency of Ikzf1 expression following TPT treatment, although this was not significant due to small sample size and sample variation (Rebuttal Figure. 10 B). We then analyzed Ikaros protein expression in nuclei in cultured primary microglia after 12 h treatment (984, 1186, and 1145 cells were analyzed in the unstimulated group, LPS/IFNɣ group, and LPS/IFNɣ+TPT group, respectively.). Consistent with the RT-PCR data, Ikaros expression was upregulated following LPS/ IFNɣ-induced microglial activation but was decreased when cells were treated with TPT (Rebuttal Figure. 10 C, D). We also histologically examined Ikaros expression in brain sections, and also noted an increased Ikaros expression in the nuclei of microglia in the cortex of the LPS-challenged mice, compared to in the naïve mice. The i.c delivery of TPT also inhibited the expression of Ikaros to some extent (Rebuttal Figure. 10 E-G).

In summary, we agree with the reviewer and despite the experimental data provided during the revision, we still reduced the discussion about *Ikzf1* in the revised manuscript, reflecting that it is not the main focus of the current project. However, as we previously proposed in the original version of the manuscript, the role of *Ikzf1* in determining the function of microglia is of significant interest in the field of neuroinflammation, and we noted the previous generation of an *IkE5*$^{fl/fl}$ mouse line by inserting loxP sites flanking the *Ikzf1* exon 5 (Joshi *et al*, 2014). We are currently contacting this group and intend to breed their mice with *Cx3cr1*^{CreERT} or *Tmem119*^{CreERT} mice in order to provide a more in-depth understanding of the role of Ikaros in regulating microglial function.

Changes made in the revised manuscript: removed the findings regarding Ikaros in the *Abstract*, downsized the texts regarding Ikaros in the *Discussion* (only retained Lines 425-431), added a *Figure EV2* (aka Rebuttal Figure.10) and its corresponding figure legend.



Rebuttal Figure. 10: Ikaros expression following inflammatory response and TPT treatment.

Referee #2:

Comment 1:

The manuscript by the Harris lab and collaborators tackles an important biological question related to myeloid biology and neuroinflammation, and, at large, how to ameliorate disease associated with hyperinflammation by suppressing immune response/activation.

Their study is timely, novel and extensive, and point toward a potential use of inhibitors of Topoisomerase 1 to suppress microglia activation/ hyperinflammatory response. The experimental plan and execution have been conducted successfully and two most notable results (the suppression of microglia inflammation by TPT and the use of novel technology to ameliorate targeting and toxicity of TPT) are important for both the immunology and neurobiology community).

Response to Comment 1:

We are grateful that our current project is appreciated by the reviewer.

Comment 2:

The part that is less impressive or better, less developed, is the work on the level of Top1 in cells and patients (MS and ALS), which could be placed in Supplementary Figures. Same goes for some of the control of the Topogomi (example: electropheresis).

Response to Comment 2:

We highly appreciate this comment from the reviewer. In the revised manuscript we have reorganized the results in the main figures and removed the results of TOP1 expression mined from public datasets from the main figures, and placed them into *Appendix Figure S1*. However, we consider the data presented in *Figure 7* regarding the biophysical validation of the DNA origami to be an important proof of the physical properties, as well as the successful production of the DNA origami nanostructure. We therefore choose to keep them in the main figures. Changes made: graphs from the main figure (*Figure 6* in the original manuscript) were moved to *Appendix Figure S1* with texts in Lines 156-165 being modified.

Comment 3:
The Discussion is well constructed, and fair with respect to the challenges ahead like make Topogami traverse the BBB. The part about TF and especially the discussion about Ikaros is speculative and do not add much, in my opinion.

Response to Comment 3:

We appreciate the suggestion by the reviewer, which is also commented by another reviewer. We have performed additional experiments during the revision to briefly understand the involvement

of Ikaros following microglial activation and TPT treatment (Rebuttal Figure.10). Despite this, we agree with the reviewer and have downsized the discussions about Ikaros in the revised manuscript.
Changes made in the revised manuscript: removed the findings regarding Ikaros in the *Abstract*, downsized the text regarding Ikaros in the *Discussion* (only in Lines 425-431), and added a *Figure EV2* (aka Rebuttal Figure.10) and its corresponding figure legend.

Comment 4:

As the authors mentioned, there are challenges ahead but their study is very extensive and important. This reviewers thinks that this manuscript might have been a manuscript that underwent review in another journal as the amount of experiments and quality is very high. Regardless of whether that is true, I do not think the authors should do more for this manuscript aside from potential edits suggested and I recommend publication.

Response to Comment 4:
We sincerely hope the revised version will now be acceptable for publication.

Referee #3:

The authors used Connectivity Map to screen for drugs that can regulate microglial inflammation. They found that TOP1 inhibitor CPT could modulate microglial activation. TOP1 expression increased in M1 microglia. TOP1 inhibitor CPT reduced proinflammatory cytokine expression and increased IL-10 expression in LPS/INFg stimulated microglia, and therefore dampened inflammation in microglia. TPT, a FDA-approved analog of CPT, was protective in a LPS induced neuroinflammation model and the authors provided evidence that microglia from TPT treated mice were less inflammatory. In addition, the authors showed that TPT protected mice from the EAE model and TOP1 expression was elevated in the nerve system in mice or human with neuroinflammatory conditions. Last, the authors designed TopoGami that could target myeloid cells and dampen their inflammation. The study is based on a screening of immunoregulatory drugs of microglia and translates into a bioengineered TOP1 inhibitor. It is of interest to people in the neuroinflammation field.

There are a few questions that need to be clarified.

Comment 1:

1. In the two neuroinflammatory models the authors used, monocytes are recruited. Recruited monocytes are usually more inflammatory than resident microglia. Did TPT mainly reduce monocyte recruitment or reduce recruited monocyte/microglia inflammation? Can M1/M2 macrophages or their markers be measured?

Response to Comment 1:

We thank the reviewer for the question. Indeed, both the EAE model and the LPS challenge model lead to severe neuroinflammatory responses in the CNS, although the respective underlying mechanisms are different. The LPS challenge model efficiently stimulates an innate immune inflammatory response mainly characterized by the activation of myeloid cells; the CNS infiltration of peripheral immune cells (e.g. monocytes) is moderate, especially when the mice were euthanized for analysis as early as 4 h after the LPS challenge. The blood-brain barrier is relatively resistant to LPS-induced disruption, which hinders the influx of monocyte recruitment into the brain parenchymal (Banks *et al*, 2015). Unlike the LPS challenge model in which myeloid cells are the main effector cells, the MOG-induced EAE model is more complicated starting with the presentation of myelin antigen by peripheral myeloid cells (mostly dendritic cells) to naive T lymphocytes via MHC class II binding to TCR, followed by autoreactive T lymphocyte priming, activation, expansion, and migration to the CNS accompanied by significant disruption of blood-brain barrier integrity; the infiltrated Th1/Th17 cells will re-encounter CNS myelin antigen presented by CNS antigen presenting cells, and thus be restimulated and produce mass inflammatory cytokines. This inflammatory cascade activates resident microglia and leads to the production of inflammatory cytokines and chemokines such as CCL-2, MIP-1 and IFN-ɣ, which further recruits the infiltration of inflammatory peripheral immune cells (e.g. monocytes) to the CNS, causing axonal and myelin damage, meanwhile further activating resident microglia and infiltrated Th1/Th17 cells (Constantinescu *et al*, 2011). This vicious inflammatory loop comprises the functionality of different immune cell compartments (e.g. peripheral myeloid cells,

T lymphocytes, resident microglia), and targeting either compartment may help cut off the inflammatory loop and mitigate EAE.

The effect of TPT could be ascribed to both the reduction of monocyte recruitment (in EAE) and the mitigation of myeloid cell inflammation (in the EAE/LPS model).

Monocyte recruitment is mostly CCL2/CCR2-driven and CCL2 (aka MCP-1) is primarily produced by myeloid cells. Both our *in vitro* (Figure 3F) and *in vivo* (Figure 4J, K) data show that the TOP1 inhibitor could inhibit CCL2 production, and we also recorded reduced infiltration of monocytes into the spinal cords in EAE mice treated with TPT (Figure 6I-K). Meanwhile, our *in vitro* data and results from the myeloid cell-driven LPS challenge model also confirmed the effect of TPT in alleviating myeloid cell inflammatory responses, which in turn might also reduce the infiltration of inflammatory monocytes.

As we mentioned in the manuscript (Lines 101-106), the M1/M2 dichotomy of microglia and macrophages is less favored as the emergency of single-cell RNA sequencing and CyTOF mass spectrometry has revealed the profound heterogeneity of the cells. The functionality of the cells, instead of M1/M2 marker expression, is more biologically relevant (Schwabenland *et al*, 2021). Nevertheless, for *in vitro* experiments, we have included M1 markers such as iNOS/Nos2, TNF-α, IL-6, and IL-1b, and the anti-inflammatory M2 marker IL-10. As presented in the results (Figure 3), CPT/TPT downregulated the M1 marker expression and potentiated IL-10 expression. We also noted an increased IL-10 production in myeloid cells from EAE mice treated with TPT, compared to in the EAE control mice (Figure EV3), which was consistent with our *in vitro* findings.

Comment 2:

Can the author comment on how topoisomerase up-regulation enhances inflammation?

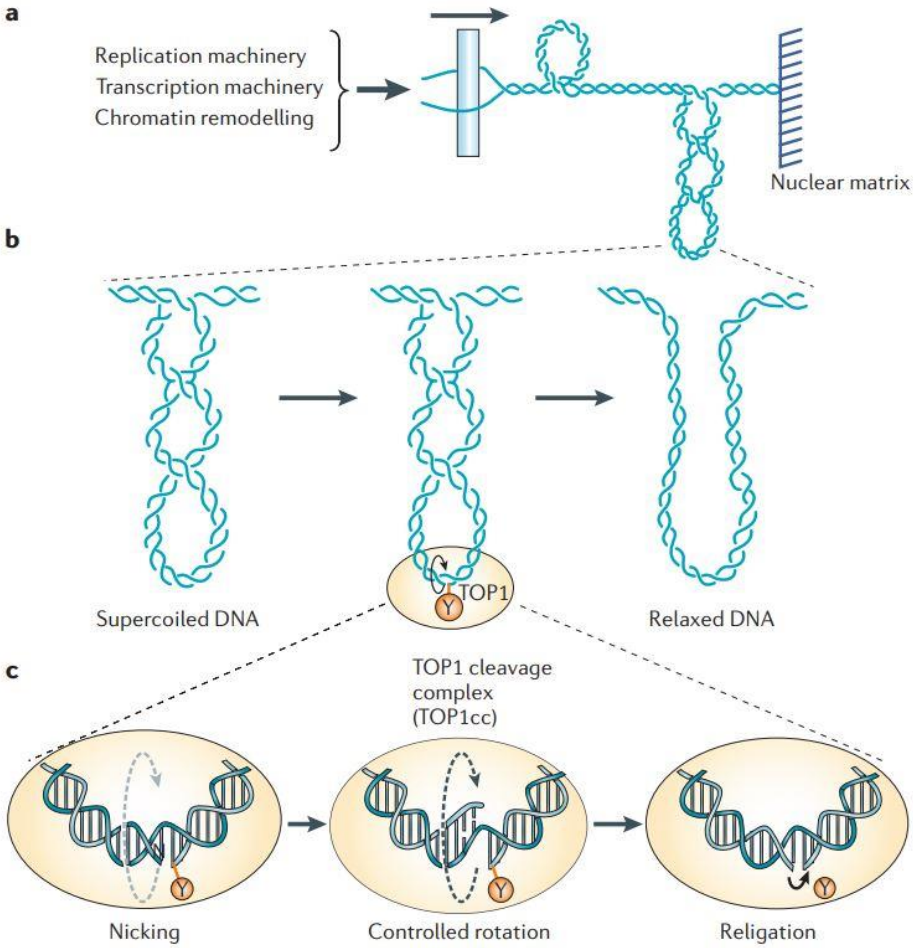
Response to Comment 2:

This is a very relevant question. Topoisomerases disentangle supercoiled DNA during transcription by nicking one single strand, allowing it to rotate around the intact DNA single strand, followed by religation. Their activities are required for all genes to resolve topological torsion during transcription, DNA replication, and chromatin remodelling (Pommier, 2006) (Rebuttal Figure.11). However, the dependence on TOP1 activity for different genes during transcription is heterogeneous, and there is a gene-specific activator-like role for TOP1. TOP1 activities at the promoters of housekeeping genes could be redundant, whereas for genes whose transcription requires more chromatin remodelling and topological adjustment, an active and sufficient TOP1 involvement is indispensable (Rialdi *et al*, 2016).

The innate immune system can be triggered by pathogen-associated molecular patterns (PAMPs) such as LPS to induce inflammatory responses. This leads to the activation of PAMP-inducible inflammatory genes whose expression usually requires RNAPII-initiated transcriptional elongation, histone modifications, and nucleosome remodelling (Smale & Natoli, 2014; Pope & Medzhitov, 2016). Upon stimulation, the selective binding of transcription factors to the promoters of PAMP-inducible genes of the inflammatory signalling pathways (e.g. the NF-κB signaling pathway) triggers their activation, with a high demand for the transcriptional machinery, topoisomerase-dependent topological adjustment as well as the transcriptional elongation (Baranello *et al*, 2016).

Simply put, compared to housekeeping genes most inflammatory genes are PAMP-inducible genes that are highly dependent on topoisomerase activities to facilitate their efficient transcription. Adequate topoisomerase activities therefore ensure the expression of inflammatory genes. Under proinflammatory stimulations, increased topoisomerase activities will be required and are possibly induced through the kinase activity of BRD4 (Baranello *et al*, 2016) to allow for inflammatory gene expression to defend the host cell from the invasion of pathogens or danger signals. However, since TOP1 activities at the promoter of housekeeping genes are redundant but are highly required for inducible-genes, TOP1 inhibitors are therefore more efficient in dampening the transcription of the inducible-genes such as genes encoding inflammatory cytokines, and thus mitigate inflammation.

We have expanded the description of how TOP1 is involved in inflammation in Lines 68-77 in the revised manuscript.



Rebuttal Figure. 11 TOP1 relaxes supercoiled DNA and facilitates ongoing transcription, replication and chromatin remodelling. (Figure is modified from Pommier *Nat Rev Cancer* 2006.)

Comment 3:

In recent years, the Netea group has found that B-glucan can prime macrophages. I wonder why MyloGami is anti-inflammatory in this study.

Response to Comment 3:

This is a very important and relevant question that is also raised by another reviewer, and we have discussed it thoroughly in the section 'Response to Major Point 1' as well as providing new data. β-glucan can indeed prime naïve macrophages and induce inflammatory response as we also showed in Rebuttal Figure. 4A that at a concentration of 50μg/mL β-glucan can induce iNOS expression in naïve macrophages. However, when macrophages were primed with β-glucan, subsequent LPS/IFNɣ challenge failed to induce a strong inflammatory response as evidenced by lower iNOS expression (Rebuttal Figure. 4B). We speculate that β-glucan may prime and reprogram macrophages and induce innate immune tolerance, desensitizing their response to secondary pro-inflammatory stimuli.

However, the dose of β-glucan used to coat the DNA origami is relatively low (with a final concentration of only around 2μg/mL β-glucan), and as we showed and discussed above (Rebuttal Figure. 4), this concentration may not induce any significant immunological responses. The unexpected anti-inflammatory effect of MyloGami may be ascribed to enhanced delivery of DNA nanostructure into the cells, as previous studies have shown that DNA nanostructure *per se* could inhibit the inflammatory response in macrophages treated with LPS/IFNɣ, though the exact underlying mechanism is not fully understood (Zhao *et al*, 2020; Zhang *et al*, 2018; Ma *et al*, 2021).

As pointed out by the Netea group, the immunological imprinting of innate immune cells (trained immunity or immune tolerance) is becoming a focal point (Divangahi *et al*, 2021). The phenomenon we observed during the revision (as shown in Rebuttal Figure. 4) has also drawn our interest to further investigate innate immune memory in the future.

We have included more discussions regarding this reviewer's point in lines 469-488 and 370-375 in the revised manuscript.

References:

Aouadi M, Tencerova M, Vangala P, Yawe JC, Nicoloro SM, Amano SU, Cohen JL & Czech MP (2013) Gene silencing in adipose tissue macrophages regulates whole-body metabolism in obese mice. *Proc Natl Acad Sci U S A* 110: 8278–8283

Aouadi M, Tesz GJ, Nicoloro SM, Wang M, Chouinard M, Soto E, Ostroff GR & Czech MP (2009) Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation. *Nature* 458: 1180–1184

Banks WA, Gray AM, Erickson MA, Salameh TS, Damodarasamy M, Sheibani N, Meabon JS, Wing EE, Morofuji Y, Cook DG, *et al* (2015) Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. *J Neuroinflammation* 12: 223

Baranello L, Wojtowicz D, Cui K, Devaiah BN, Chung H-J, Chan-Salis KY, Guha R, Wilson K, Zhang X, Zhang H, *et al* (2016) RNA polymerase II regulates topoisomerase 1 activity to favor efficient transcription. *Cell* 165: 357–371

Camilli G, Bohm M, Piffer AC, Lavenir R, Williams DL, Neven B, Grateau G, Georgin-Lavialle S & Quintin J (2020) β-Glucan-induced reprogramming of human macrophages inhibits NLRP3 inflammasome activation in cryopyrinopathies. *J Clin Invest* 130: 4561–4573

Cassinelli V, Oberleitner B, Sobotta J, Nickels P, Grossi G, Kempter S, Frischmuth T, Liedl T & Manetto A (2015) One-step formation of “chain-armor”-stabilized DNA nanostructures. *Angew Chem Int Ed Engl* 54: 7795–7798

Constantinescu CS, Farooqi N, O’Brien K & Gran B (2011) Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol* 164: 1079–1106

Divangahi M, Aaby P, Khader SA, Barreiro LB, Bekkering S, Chavakis T, van Crevel R, Curtis N, DiNardo AR, Dominguez-Andres J, *et al* (2021) Trained immunity, tolerance, priming and differentiation: distinct immunological processes. *Nat Immunol* 22: 2–6

Fukui R, Yamamoto C, Matsumoto F, Onji M, Shibata T, Murakami Y, Kanno A, Hayashi T, Tanimura N, Yoshida N, *et al* (2018) Cleavage of toll-like receptor 9 ectodomain is required for in vivo responses to single strand DNA. *Front Immunol* 9: 1491

Heng Y, Zhang X, Borggrewe M, van Weering HRJ, Brummer ML, Nijboer TW, Joosten LAB, Netea MG, Boddeke EWGM, Laman JD, *et al* (2021) Systemic administration of β-glucan induces immune training in microglia. *J Neuroinflammation* 18: 57

Horvath RJ, Nutile-McMenemy N, Alkaitis MS & Deleo JA (2008) Differential migration, LPS-induced cytokine, chemokine, and NO expression in immortalized BV-2 and HAPI cell lines and primary microglial cultures. *J Neurochem* 107: 557–569

Johanson CE, Duncan JA 3rd, Klinge PM, Brinker T, Stopa EG & Silverberg GD (2008) Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. *Cerebrospinal Fluid Res* 5: 10

Joshi I, Yoshida T, Jena N, Qi X, Zhang J, Van Etten RA & Georgopoulos K (2014) Loss of Ikaros DNA-binding function confers integrin-dependent survival on pre-B cells and progression to acute lymphoblastic leukemia. *Nat Immunol* 15: 294–304

Ma W, Zhan Y, Zhang Y, Mao C, Xie X & Lin Y (2021) The biological applications of DNA nanomaterials: current challenges and future directions. *Signal Transduct Target Ther* 6: 351

Maezawa T, Ohtsuki S, Hidaka K, Sugiyama H, Endo M, Takahashi Y, Takakura Y & Nishikawa M (2020) DNA density-dependent uptake of DNA origami-based two-or three-dimensional nanostructures by immune cells. *Nanoscale* 12: 14818–14824

Moorlag SJCFM, Khan N, Novakovic B, Kaufmann E, Jansen T, van Crevel R, Divangahi M & Netea MG (2020) B-glucan induces protective trained immunity against Mycobacterium tuberculosis infection: A key role for IL-1. *Cell Rep* 31: 107634

Moussaud S & Draheim HJ (2010) A new method to isolate microglia from adult mice and culture them for an extended period of time. *J Neurosci Methods* 187: 243–253

Oh K-S, Gottschalk RA, Lounsbury NW, Sun J, Dorrington MG, Baek S, Sun G, Wang Z, Krauss KS, Milner JD, *et al* (2018) Dual roles for Ikaros in regulation of macrophage chromatin state and inflammatory gene expression. *J Immunol* 201: 757–771

Oshio K, Watanabe H, Song Y, Verkman AS & Manley GT (2005) Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-1. *FASEB J* 19: 76–78

Pohar J, Lainšček D, Ivičak-Kocjan K, Cajnko M-M, Jerala R & Benčina M (2017) Short single-stranded DNA degradation products augment the activation of Toll-like receptor 9. *Nat Commun* 8: 15363

Pommier Y (2006) Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer* 6: 789–802

Pope SD & Medzhitov R (2016) GENE EXPRESSION. Unwinding inducible gene expression. *Science* 352: 1058–1059

Rialdi A, Campisi L, Zhao N, Lagda AC, Pietzsch C, Ho JSY, Martinez-Gil L, Fenouil R, Chen X, Edwards M, *et al* (2016) Topoisomerase 1 inhibition suppresses inflammatory genes and protects from death by inflammation. *Science* 352: aad7993–aad7993

Schwabenland M, Brück W, Priller J, Stadelmann C, Lassmann H & Prinz M (2021) Analyzing microglial phenotypes across neuropathologies: a practical guide. *Acta Neuropathol* 142: 923–936

Sharma Y, Ahmad A, Yavvari PS, Kumar Muwal S, Bajaj A & Khan F (2019) Targeted SHP-1 silencing modulates the macrophage phenotype, leading to metabolic improvement in dietary obese mice. *Mol Ther Nucleic Acids* 16: 626–636

Sierra A, Gottfried-Blackmore AC, McEwen BS & Bulloch K (2007) Microglia derived from aging mice exhibit an altered inflammatory profile. *Glia* 55: 412–424

Smale ST & Natoli G (2014) Transcriptional control of inflammatory responses. *Cold Spring Harb Perspect Biol* 6: a016261

Stothers CL, Burelbach KR, Owen AM, Patil NK, McBride MA, Bohannon JK, Luan L, Hernandez A, Patil TK, Williams DL, *et al* (2021) B-glucan induces distinct and protective innate immune memory in differentiated macrophages. *J Immunol* 207: 2785–

Tanaka S, Ise W, Inoue T, Ito A, Ono C, Shima Y, Sakakibara S, Nakayama M, Fujii K, Miura I, *et al* (2020) Tet2 and Tet3 in B cells are required to repress CD86 and prevent autoimmunity. *Nat Immunol* 21: 950–961

Tencerova M (2020) Glucan-encapsulated siRNA particles (GeRPs) for specific gene silencing in Kupffer cells in mouse liver. *Methods Mol Biol* 2164: 65–73

Xu J, Liu D, Yin Q & Guo L (2016) Tetrandrine suppresses β-glucan-induced macrophage activation via inhibiting NF-κB, ERK and STAT3 signaling pathways. *Mol Med Rep* 13: 5177–5184

Xu X, Yasuda M, Nakamura-Tsuruta S, Mizuno M & Ashida H (2012) β-Glucan from Lentinus edodes inhibits nitric oxide and tumor necrosis factor-α production and phosphorylation of mitogen-activated protein kinases in lipopolysaccharide-stimulated murine RAW 264.7 macrophages. *J Biol Chem* 287: 871–878

Zhang Q, Lin S, Shi S, Zhang T, Ma Q, Tian T, Zhou T, Cai X & Lin Y (2018) Anti-inflammatory and antioxidative effects of tetrahedral DNA nanostructures via the modulation of macrophage responses. *ACS Appl Mater Interfaces* 10: 3421–3430

Zhao D, Cui W, Liu M, Li J, Sun Y, Shi S, Lin S & Lin Y (2020) Tetrahedral framework nucleic acid promotes the treatment of bisphosphonate-related osteonecrosis of the jaws by promoting angiogenesis and M2 polarization. *ACS Appl Mater Interfaces* 12: 44508–44522

Dear Dr. Zhu,

Thank you for the submission of your revised manuscript to our editorial offices. I have now received the reports from the referees that were asked to re-evaluate your study, you will find below. As you will see, the referees now fully support the publication of your study in EMBO reports. Referee #2 has some further suggestions to improve the manuscript, I ask you to address in a final revised manuscript.

Moreover, I have these editorial requests I also ask you to address:

- Please provide a more comprehensive and shorter title with not more than 100 characters (including spaces).

- Please provide the abstract written in present tense. Moreover, please shorten the abstract to not more than 175 words.

- Please add up to 5 keywords to the title page.

- 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 policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement' and put it after the Acknowledgements section.

- Please make sure that all figure panels are called out sequentially (as they show up in the figure) and separately. Please check or change the order of the panels in the figures. Presently, the callouts for the panels of Figs. 5,6 and EV3 panel callouts are mix-up and not alphabetical. Moreover, there are no separate callouts for the panels of Figs. EV5 and S2-S5. Finally, please add a callout for Appendix Table S1.

- Please remove the referee token from the Data Availability section and make sure that the dataset is public latest upon publication of the study.

- Author Andre Gueirrero Cacais seems missing from the author contributions. Please check.

- Please add scale bars of similar style and thickness to the microscopic images (main, EV and Appendix figures), using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend. Presently, the scale bars for many images are too thin.

- Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (main, EV and Appendix figures), and that statistical testing has been done where applicable. Please avoid phrases like 'independent experiment', but clearly state if these were biological or technical replicates. Please add complete statistical testing to all diagrams. Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant.

- Please make sure that all the funding information is entered into the online submission system and is complete and similar to the one in the manuscript text file.

- Please also note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please do that for co-corresponding author Harris. Please find instructions on how to link the ORCID ID to the account in our manuscript tracking system in our Author guidelines:
http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see any modifications done.

In addition, I would need from you:
- a short, two-sentence summary of the manuscript (not more than 35 words).
- two to four short bullet points highlighting the key findings of your study.
- a schematic summary figure (in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels) that can be used as a visual synopsis on our website.

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

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

Best,

Achim Breiling
Senior Editor
EMBO Reports

Referee #1:

The authors have done a tremendous work to answer this reviewer comments. In addition, they extensively explained concepts, details mechanisms and added references. This reviewer believes that this latest version should be published and that the work in this current form is excellent.

Referee #2:

The authors did a good job in clarifying/performing the requested experiments. Few minor edits:
- The paper below by Ho et al. in Cell that uses TPT in suppressing SARS2 inflammation is included but not properly referenced. It should be referenced when talking about TPT being better and less toxic than CPT.

- Together with (or instead of) the review from Natoli and Smale, another review from Guccione et al. (PMID 29184195) should be cited that explains the idea of housekeepers vs inducible gene activation and the effect of epigenetic and DNA topology inhibitors.

Ho JSY, Mok BW-Y, Campisi L, Jordan T, Yildiz S, Parameswaran S, Wayman JA, Gaudreault NN, Meekins DA, Indran SV, et al (2021) TOP1 inhibition therapy protects against SARS-CoV-2-induced lethal inflammation. Cell

---
Referee #3:

The authors have addressed my concerns.

EMBOR-2021-54499V3 Point-by-point Response

Thank you for the submission of your revised manuscript to our editorial offices. I have now received the reports from the referees that were asked to re-evaluate your study, you will find below. As you will see, the referees now fully support the publication of your study in EMBO reports. Referee #2 has some further suggestions to improve the manuscript, I ask you to address in a final revised manuscript.

Moreover, I have these editorial requests I also ask you to address:

- Please provide a more comprehensive and shorter title with not more than 100 characters (including spaces).

Response: We have changed the title to *Myeloid cell-specific topoisomerase 1 inhibition using DNA origami mitigates neuroinflammation.* Change has been made to the latest version of the manuscript.

- Please provide the abstract written in present tense. Moreover, please shorten the abstract to not more than 175 words.

Response: This has been changed in the revised manuscript.

- Please add up to 5 keywords to the title page.

Response: 5 keywords were added to the title page.

- 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 policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement' and put it after the Acknowledgements section.

Response: This has been addressed accordingly.

- Please make sure that all figure panels are called out sequentially (as they show up in the figure) and separately. Please check or change the order of the panels in the figures. Presently, the callouts for the panels of Figs. 5,6 and EV3 panel callouts are mix-up and not alphabetical. Moreover, there are no separate callouts for the panels of Figs. EV5 and S2-S5. Finally, please add a callout for Appendix Table S1.

Response: Thank you for the suggestion. Changes have been made in the manuscript. Appendix Figure S5 shows the gating strategy and there are no separate panels, and therefore we have added the callout as *Appendix Figure S5*.

- Please remove the referee token from the Data Availability section and make sure that the dataset is public latest upon publication of the study.

Response: This has been changed and the dataset E-MTAB-10679 is now publicly available.

- Author Andre Gueirrero Cacais seems missing from the author contributions. Please check.

Response: 'AO' was used to indicate the author, and was changed to 'AG-C'.

- Please add scale bars of similar style and thickness to the microscopic images (main, EV and Appendix figures), using clearly visible black or white bars (depending on the background). Please

place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend. Presently, the scale bars for many images are too thin.

Response: The scale bars throughout the manuscript have been modified and aligned to the lower right corner of the images with enhanced thickness.

- Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (main, EV and Appendix figures), and that statistical testing has been done where applicable. Please avoid phrases like 'independent experiment', but clearly state if these were biological or technical replicates. Please add complete statistical testing to all diagrams. Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant.

Response: Changes have been made throughout the manuscript.

- Please make sure that all the funding information is entered into the online submission system and is complete and similar to the one in the manuscript text file.

Response: Most of the funding information has been entered into the online submission system except for the internal grant ‘the StratNeuro funding for Collaborative Neuroscience Projects at Karolinska Institutet’ which is not available in the system, but otherwise stated in the acknowledgment.

- Please also note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please do that for co-corresponding author Harris. Please find instructions on how to link the ORCID ID to the account in our manuscript tracking system in our Author guidelines:
http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

Response: The ORCID ID for Prof. Harris has been added.

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see any modifications done.

Response: All the comments were addressed in the revised manuscript. We appreciate the great help from the publisher.

In addition, I would need from you:

- a short, two-sentence summary of the manuscript (not more than 35 words).

- two to four short bullet points highlighting the key findings of your study.

- a schematic summary figure (in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels) that can be used as a visual synopsis on our website.

Response: These have been added to the revised manuscript.

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

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

Best,

Achim Breiling

Senior Editor

EMBO Reports

Referee #1:

The authors have done a tremendous work to answer this reviewer comments. In addition, they extensively explained concepts, details mechanisms and added references. This reviewer believes that this latest version should be published and that the work in this current form is excellent.

Referee #2:

The authors did a good job in clarifying/performing the requested experiments. Few minor edits:

- The paper below by Ho et al. in Cell that uses TPT in suppressing SARS2 inflammation is included but not properly referenced. It should be referenced when talking about TPT being better and less toxic than CPT.

Response: We appreciate the suggestion and have removed one sentence with an unnecessary citation of this paper in Lines 152-154. After reading this reference thoroughly again, we don't think the paper compares TPT with CPT in terms of their efficacy and toxicity; CPT was not mentioned throughout the reference.

- Together with (or instead of) the review from Natoli and Smale, another review from Guccione et al. (PMID 29184195) should be cited that explains the idea of housekeepers vs inducible gene activation and the effect of epigenetic and DNA topology inhibitors.

Response: We thank the reviewer for the advice and this reference has been cited in the latest version.

Ho JSY, Mok BW-Y, Campisi L, Jordan T, Yildiz S, Parameswaran S, Wayman JA, Gaudreault NN, Meekins DA, Indran SV, et al (2021) TOP1 inhibition therapy protects against SARS-CoV-2-induced lethal inflammation. Cell

---

Referee #3:

The authors have addressed my concerns.

Dr. Keying Zhu
Karolinska Institutet
Department of Clinical Neuroscience
Center for Molecular Medicine, L8:04
Stockholm, Please Select 17176
Sweden

Dear Dr. Zhu,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, 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.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: 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." Please note that the author checklist will still be published even if you opt out of the transparent process.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Achim Breiling
Senior Editor
EMBO Reports

****

THINGS TO DO NOW:

Once your article has been received by Wiley for production, the corresponding author will receive an email from Wiley's Author Services system which will ask them to log in and will present them with the appropriate license for completion.

You will receive proofs by e-mail approximately 2-3 weeks after all relevant files have been sent to our Production Office; you should return your corrections within 2 days of receiving the proofs.

Please inform us if there is likely to be any difficulty in reaching you at the above address at that time. Failure to meet our deadlines may result in a delay of publication, or publication without your corrections.

All further communications concerning your paper should quote reference number EMBOR-2021-54499V3 and be addressed to emboreports@wiley.com.

Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Keying Zhu & Robert A. Harris
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2021-54499V1

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- 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:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	A general overall Study Design is provided in the manuscript. We did not use explicit power analysis; the sample size for different experiments was based on previous publications and the availability of samples.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	In accordance with the 3R principle for animal research, we usually have around 10 mice in each group for each replicate of an EAE batch (typically we repeat 3 EAE batches for key conclusions). For validations using Western blot, flow cytometry, immunostaining, RT-PCR, and so forth, we usually include 3 biological replicates with at least 2 technical replicates (e.g. 2 technical repeats for each sample of each gene in RT-PCR experiments, 3-5 randomly selected fields under the microscope from each sample for immunofluorescent stainings.), which usually give sufficient power to show significant differences. The sample size for each individual experiment was indicated in the figure legends, and the number of repetitions was also indicated.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	This has been stated in the Study Design section in the manuscript.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	We randomly allocate mice into control & experimental groups.
For animal studies, include a statement about randomization even if no randomization was used.	This has been stated in the Study Design section in the manuscript. Mice were randomly assigned to different treatment or control groups.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	For EAE scoring, we ask another group member who does not know the grouping information to score the mice. Histological quantification and behavioral analysis were performed blinded. For other experiments with objective quantitative readouts, raw data will be provided. This information can be found in the Study Design section in the Materials & Methods part.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA.
5. For every figure, are statistical tests justified as appropriate?	The detailed statistics and sample size were stated in each figure legend.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	We included a Statistical analysis section in the manuscript with descriptions.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-report

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
https://osp.od.nih.gov/biosafety-biosecurity-and-emerging-biotechnology/
http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	Yes
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Detailed antibody catalogs and epitopes (for FACS antibodies) were provided in the Materials and Methods section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA. We did not use cell line; we used primary cells instead.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Stated in the Experimental animals section in the manuscript.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Stated in the Ethics statement secion in the manuscript.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We comply with the ARRIVE reporting guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. 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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	RNA-seq results are available in ArrayExpress repository (E-MTAB-10679), with the following login details (for reviewers): Username: Reviewer_E-MTAB-10679<; a password reviewers is provided in the manuscript at the Data and materials availability section.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	All data associated with this study are present in the paper or the Supplementary Materials; raw data will be uploaded once the manuscript is accpeted.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA