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Plant YTHDF proteins are direct effectors of antiviral immunity against an N6-methyladenosine-containing RNA virus

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Review #1

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

The authors have shown previously that local and systemic infection of alfalfa mosaic virus (AMV) is inhibited and the relative abundance of m6A in the viral RNAs is increased in mutant Arabidopsis plants defective in the m6A demethylase gene ALKBH9B. Here the authors show that genetic inactivation of 2 or 3 m6A-binding proteins (ECTs or m6A readers) enhances the systemic, but not the local, infection of AMV. Notably, the systemic virus resistance of the demethylase mutant plants is largely eliminated by inactivating the same set of ECTs. Moreover, the authors detected in vivo association of ECT2 with AMV RNAs and identified a N-terminal motif of ECT2 that is necessary for AMV resistance, but dispensable for its role in endogenous developmental functions. These findings together provide further evidence to support their earlier conclusion for an antiviral role of m6A methylation of viral RNAs. Unfortunately, several key questions remain unknown and should be addressed to justify publication in EMBO J.

Major comments

1. The authors reported in 2017 that both the local and systemic infection of AMV is inhibited in the m6A demethylase mutant plants (PNAS 114:10755-60). In this work, they show that inactivation of ECTs enhances only the systemic AMV infection, but has no effect on the local infection (Fig. 2). Studies presented in neither Fig. 5 nor Fig. 6 examined possible effects on the local virus infection. Moreover, line 1 of page 13 mentioned a model that ECT binding "causes inhibition of viral replication". To resolve these contradictory descriptions on the step of the virus infection cycle targeted by m6A RNA methylation, it is essential to perform protoplast replication assays to determine whether the mutation of either the demethylase gene or ECTs affects viral RNA replication at single-cell level and to examine local infection for the studies presented in Figs 5 & 6.

2. The title and conclusion of this manuscript defined YTHDF proteins (ECTs) as "direct effectors of antiviral immunity", which is misleading. It remains completely unknown why m6A methylation of viral RNAs is inhibitory to virus infection. The available data suggest that it may act by blocking virus replication and/or movement or by enhancing any of the several known antiviral responses. Effector molecules of an antiviral immunity cannot be identified when the effector mechanism is unknown.

3. At the end of page 13 and elsewhere in this manuscript, the authors conclude that "the m6A-ECT axis constitutes a first, basal layer of antiviral defense", a conclusion that is not supported by the evidence presented. This conclusion will be incorrect if m6A methylation of viral RNAs does not inhibit virus accumulation levels in the protoplast virus replication assays as requested above.

4. In the first paragraph of page 17 and elsewhere in this manuscript, the authors question the relative importance of antiviral RNA silencing against AMV and related viruses as compared to m6A RNA methylation. It is important to determine if AMV becomes more virulent in RNAi-defective mutant plants such as dcl2/4 double mutant and if m6A RNA methylation also confers AMV resistance in RNAi-defective mutant plants.

Minor comments:

1. State the time post-inoculation when the samples were taken for the RNA-seq analysis in Fig.1.

2. State whether all of the northern blotting experiments used RNA extracted from single plant or pooled plants and had been repeated.

3. Verify that the statistical analysis method used in virus titer quantitative analysis is student t-test or one-way ANOVA.

4. The legend to Fig. 4F should be Fig. 4F and 4G.

5. Explain what Δ2 means in Fig. 6B.

6. If both the left and right bars correspond to 1 cm, 9-DAG-old plants would be much bigger than 16-DAG-old plants, which cannot be true.

2. Significance:

Significance (Required)

Advance:

The authors have shown previously that local and systemic infection of alfalfa mosaic virus (AMV) is inhibited and the relative abundance of m6A in the viral RNAs is increased in mutant Arabidopsis plants defective in the m6A demethylase gene

ALKBH9B. Here the authors show that genetic inactivation of 2 or 3 m6A-binding proteins (ECTs or m6A readers) enhances the systemic, but not the local, infection of AMV. Notably, the systemic virus resistance of the demethylase mutant plants is largely eliminated by inactivating the same set of ECTs. Moreover, the authors detected in vivo association of ECT2 with AMV RNAs and identified a N-terminal motif of ECT2 that is necessary for AMV resistance, but dispensable for its role in endogenous developmental functions. These findings together provide further evidence to support their earlier conclusion for an antiviral role of m6A methylation of viral RNAs.

Audience:

Broad, including those interested in RNA modifications, antiviral immunity, and plant biology.

Your expertise:

Antiviral immunity, plant biology

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 3 and 6 months

4. *Review Commons* values the work of reviewers and encourages them to get credit for their work. Select 'Yes' below to register your reviewing activity at Web of Science Reviewer Recognition Service (formerly Publons); note that the content of your review will not be visible on Web of Science.

Web of Science Reviewer Recognition

No

Review #2

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

Summary

In this manuscript, the authors asked the important question of how RNA modification is associated with viral defense in plants. Based on the previous findings that the infectivity of AMV relies on demethylation of viral RNA by recruitment of the cellular m6A demethylase ALKBH9B, in this study, they showed that inactivation of the m6A reader proteins, ECT2, ECT3, and ECT5, is sufficient to restore AMV infectivity in partially resistant alkbh9b mutants. Considering the potential roles of m6A modification in viral defense but the limited knowledge on this topic, the current study opens a new direction of research regarding the role of RNA modification in viral defense.

Major comments

- It is interesting to see that the IDR of ECT2 harbors two separable activities employed to achieve different goals: one that stimulates cellular proliferation by binding to endogenous m6A-containing mRNA, and one that effects basal antiviral resistance when ECT2 binds to hypermethylated viral RNA. Considering that the IDR of a protein contributes the chaperone activity of the protein and then can increase the binding capacity of the protein to different substrates, it would be more informative if the authors discuss whether the RNA chaperone activity of IDR of ECT2 is possibly involved in the different processes.

- It is interesting to propose a working model that m6A site multiplicity in AMV RNA may be a key factor distinguishing it from endogenous mRNA. In that sense, it would be clearer if the authors describe how many m6A sites are present in AMV RNA. Are these m6A sites clustered in certain regions of the viral RNA important for viral replication?

- It is not clear what the correlation between the phase separation capability of ECT proteins and viral infection is.

Minor comments

- In page 6, what is the alfalfa mosaic virus (AMV) RNA 3? Are there AMV RNA 1 and 2? What are their differences? Is the m6A-YTH module specific to AMV RNA 3?

- Name of the mutants; it would be better if same name was used for the mutant throughout the manuscript and in figures; for instance, ect2-1/ect5-2 and de25, as well as ect2-1/ect3-1/ect5-4 and te235 were used, which is not easy to follow.

- Figure 4G legend is missing.

2. Significance:

Significance (Required)

Considering the potential roles of m6A modification in viral defense but the limited knowledge on this topic, the current study opens a new direction of research regarding the role of RNA modification in viral defense.

The audience will be broad, including any persons who are working on epitranscriptomics, plant sciences, viral infection, and clinical application. I am working on epitranscriptomic RNA modification in plant development and abiotic stress responses.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Less than 1 month

4. *Review Commons* values the work of reviewers and encourages them to get credit for their work. Select 'Yes' below to register your reviewing activity at Web of Science Reviewer Recognition Service (formerly Publons); note that the content of your review will not be visible on Web of Science.

Web of Science Reviewer Recognition

Yes

Review #3

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

Previously, the authors showed evidence that m6A modifications of AMV RNAs erased by the host ALKBH9b enhances AMV spread in Arabidopsis. In this paper, the authors show by transcriptome analysis and RT-qPCR that the accumulation of m6A "reader" proteins, ECT2, 3 and 5 are increased during AMV infection in Arabidopsis. Combined mutations of ect2,3 and 5 led to increased AMV accumulation, suggesting that ECT2,3,5 are critical in inhibition of AMV accumulation in systemic leaves of Arabidopsis. Mutagenesis of ECT2 putative m6A-binding pocket did not restore AMV resistance in double-mutant de23 plant, arguing that m6A reader function of this protein is needed to provide resistance against AMV. Then, proximity-labeling was used to show that ECT2 binds to AMV RNA2 and likely RNA1 in planta. Finally, debilitating both the m6A eraser (ALKBH9b) and the reader (ect2,3,5) restored susceptibility to AMV infection in Arabidopsis, thus providing evidence that the m6A reader proteins are critical for resistance against AMV and that AMV exploits ALKBH9b to fight against ECT2,3,5 in plants. Altogether, these are novel and important findings. The paper is well-written.

I do not have main concerns.

Minor points:

- Abstract: "AMV replication" should be replaced by "AMV infection" or "AMV spread", because the locally infected leaves show similar AMV replication/accumulation in de23 and wt Arabidopsis.

- Alkbh9b mutation causes inhibition of local and systemic movement of AMV, whereas de23 mutant increases AMV accumulation only in systemic leaves (Fig. 1). What is the explanation that de23 does not affect local movement of AMV?

- The last chapter (p19-20) is too speculative and too long, it does not make the paper more interesting: I recommend shortening it and to minimize speculation.

2. Significance:

Significance (Required)

This paper shows evidence for a new antiviral strategy present in plants. Overall, this is a significant new finding.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Less than 1 month

4. *Review Commons* values the work of reviewers and encourages them to get credit for their work. Select 'Yes' below to register your reviewing activity at Web of Science Reviewer Recognition Service (formerly Publons); note that the content of your review will not be visible on Web of Science.

Web of Science Reviewer Recognition

Yes

Revision Plan

Manuscript number: #RC-2022-01728
Corresponding author(s): Vicente Pallas

1. General Statements [optional]

We greatly appreciate the valuable comments from the referees, which have generally been very positive and constructive. The three referees have emphasized the significance of our study that opens a new direction of research regarding the role of RNA modification in viral defense. In addition, the reviewers confirm our view that the audience of our work would be broad.

The major concerns of the reviewers are limited to four main points:
i) to be clearer in our description on the effect of the m6A-YTHDF axis on the viral infectivity and avoid making assumptions on effects on replication (ref. #1 and #3);
ii) reviewer 1 finds that the title and conclusion of this manuscript defining YTHDF proteins (ECTs) as "direct effectors of antiviral immunity" is misleading. Nonetheless, as detailed below, Reviewer 1 confuses mere knowledge of effects of m6A with those conferred by YTHDF proteins binding to m6A, and indeed overlooks nearly all evidence presented in the paper for how m6A in AMV confers antiviral resistance (i.e. mechanistic insight);
iii) the discussion on the relative importance of antiviral RNA silencing and m6A-YTHDF against AMV;
iv) to establish more clearly whether the phase separating capability of IDRs in the reading proteins correlates with the antiviral activity (reviewer 2).

We have already completed substantial experimental work to address several of these points. Nonetheless, we find it prudent to ask for an extension of the revision time beyond four weeks to allow for repeats of a few of the infection experiments in question. In the following section, we specify a plan of action for the revisions.

2. Description of the planned revisions

Regarding the four major concerns raised by the reviewers, we will experimentally address the last two, whereas we think the first two do not need any further experimental work, as explained in section 4. Thus, the working plan for points #3 and #4 will be as follows:

iii) the discussion on the relative importance of antiviral RNA silencing and m6A-YTHDF against AMV and related viruses

As we mention in the manuscript (discussion, first chapter), AMV *"is one of only very few studied plant RNA viruses for which no anti-RNAi effector has been identified. In addition, prunus necrotic ringspot virus (PNRSV), a virus genetically and functionally closely related to AMV (Pallas et al, 2013), does not induce easily detectable siRNAs, unlike nearly all other studied plant RNA viruses (Herranz et al, 2015)".*

Thus, we do not come up with a strong judgment on whether RNAi is more or less important than m6A-YTHDFs for AMV resistance.

In any case, although these indirect observations seem to be quite solid, we agree with the reviewer that conclusive evidence to discard RNAi as a defense layer against AMV, at least at the time where ECTs are acting, is lacking. Thus, we plan to evaluate how the absence of the main components of the RNAi machinery affects AMV infection and if this 'universal' defense layer interferes/overlaps with the ECTs antiviral defense observed here. Realistically, this will take us 8-10 weeks. The experiments within this topic are based on established and published methods and thus, on solid experience. We do not expect any fallback solution and the results will be conclusive in this sense. We also note that the very time-consuming part of constructing mutants defective in both RNAi and m^6A-ECT components (in this case, *ect2/ect3/rdr6*), as well as a first round of infection assays has already been completed at this point

iv) To establish more clearly whether the phase separating capability of IDRs in the reading proteins correlates with the antiviral activity (Reviewer 2).

We agree with Reviewer 2 that this is an interesting and important question. Hence, we have teamed up with the group of Prof. Kresten Lindorff-Larsen, expert in molecular simulations of protein folding and interaction. The Lindorff-Larsen group has recently published a powerful computational approach to simulate phase separation behavior of intrinsically disordered proteins (IDPs) or regions of proteins (IDRs) (Tesei et al., 2021, Accurate model of liquid-liquid phase behavior of intrinsically disordered proteins from optimization of single-chain properties, *PNAS* 118, (44) e2111696118). Applying this simulation method to the Arabidopsis ECT proteins establishes two facts that we will incorporate into a revised version:

(1) The IDR of ECT2 shows marked phase separation propensity, in agreement with the experimental evidence published in Arribas-Hernández et al., 2018, Plant Cell.
(2) The deletion mutant of ECT2 (ΔN5) with defective antiviral activity, yet unaffected ability to accelerate growth of leaf primordia shows markedly reduced phase separation propensity driven, in the main, by the many tyrosine residues in the region deleted in the mutant.

These results suggest that phase separation capability indeed correlates with antiviral activity.
Since not only ECT2, but also ECT3, ECT5 and, to some extent, ECT4, participate in AMV resistance, we plan further simulation work on these proteins during the first two weeks of January 2023 before submission of a revised version of the manuscript.

3. Description of the revisions that have already been incorporated in the transferred manuscript

All the minor concerns raised by the three reviewers have been addressed and we have incorporated all of their suggestions in this intermediate version.

4. Description of analyses that authors prefer not to carry out

As previously mentioned, we believe that points 1 and 2 do not require an experimental approach to be addressed for the following reasons:

i) to be clearer in our description on the effect of the m6A-YTHDF axis on the viral infectivity and avoid making assumptions on effects on replication (ref. #1 and #3)

We agree with the reviewer that the term 'inhibition of viral replication' was not very appropriate because the idea that was intended to be conveyed was that of viral accumulation. Hence, we will change this use of language, and we thank the reviewer for pointing out this inaccurate description.
When it comes to differences between effects on infection in inoculated and non-inoculated leaves, there may be a slight misunderstanding, perhaps because we were not clear enough in our originally submitted version. In reality, there are some differences even in inoculated leaves between wild type and *ect* mutants, especially in the triple mutant, but the slightly higher accumulation in *ect* mutants is not clearly observed in every experiment and hence, does not always rise to the level of significance. Although it is possible that, at local level, ALKBH9B-mediated m6A would have other ECTs-independent effects, similar to what has been described for some animal viruses (Baquero-Pérez et al., 2021. Viruses), we think that the most likely explanation for this phenomenon is a combination of infection titers and ECT redundancy.

The suggestion to use protoplasts is very accurate, but it would not resolve any doubt in this scenario, because ECTs are mainly expressed in mitotically active cells (Arribas-Hernández et al, 2020, 2018) and, since mature tissues make up the better part of the leaves used to isolate protoplasts, only few of the isolated cells would be useful. In

addition, we previously showed that AMV accumulation is reduced in *alkbh9b* protoplasts compared to WT (Martínez-Pérez et al., 2021. Front. Microbiol.), which suggests that m6A levels of vRNAs are critical for the first stages of the infection, but in that case no problems with the expression pattern of the demethylase were expected.

ii) The title and conclusion of this manuscript defined YTHDF proteins (ECTs) as "direct effectors of antiviral immunity", which is misleading. Effector molecules of an antiviral immunity cannot be identified when the effector mechanism is unknown;

In this regard, we have a very different vision from the one the reviewer proposes. We believe that it is not correct to say that the effector molecules of an antiviral immunity cannot be identified until its mechanism is demonstrated. In fact, RNA silencing effectors were discovered long before their mechanism was elucidated in detail. One molecular interpretation of the Flor's seminal gene-for-gene model, in terms of receptor/effector recognition, is that specific interaction between the receptor and its recognized (cognate) effector protein triggers resistance.
Furthermore, we strongly believe that we provide enough arguments to propose a model, although, as we comment in the end of the discussion, "we view this model as a conceptual framework of value in the design of future experiments to test its validity". The reasoning that we show here is the following:

1. The m6A binding proteins are necessary for the antiviral response.
2. At least ECT2 recognizes AMV RNAs *in vivo* and that its m6A-binding capacity is necessary to play a role in AMV infection.
3. Simply losing methylase activity – with the same developmental defects as ect2/3/ – does not lead to the same degree of loss of resistance, and you can affect AMV resistance without affecting developmental functions of ECT2.

Altogether, these observations justify the proposal that m6A exerts antiviral effects by acting as binding sites of ECT proteins in viral RNA, which we consider a clear mechanistic advance.

Bearing in mind that m6A-modified vRNAs might concentrate in replication complexes and that MeRIP-seq methodology to map m6A revealed site multiplicity in the genome of some RNA viruses (Gokhale et al., 2016. Cell Host&Microb; Martínez-Pérez et al., 2017; Lichinchi et al., 2016. Nat Microbiol; Lichinchi et al., 2016. Cell Host&Microb; Marquez-Molins et al, 2022), our results recalled the previously proposed model in which m6A sites multiplicity causes the phase separation of these RNAs through the interaction of the IDRs of the YTH proteins (Ries et al, 2019; Fu & Zhuang, 2020; Gao et al, 2019). Now, with the new simulations of phase separation behavior, although still a model that requires further experimental tests, we have better evidence to support the model that it is related to LLPS of ECT-bound viral RNA. Therefore, we firmly believe that our title conceptually reflects the basic concepts of resistance induction in virus-plant interactions.

1st Editorial Decision **16th Jan 2023**

Dear Dr. Pallas,

Thank you for transferring your manuscript with Review Commons referee reports and responses to The EMBO Journal.

After reading the referees' recommendations and your responses, I appreciate the importance of your findings and would like to invite you to submit a revised version of the manuscript. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

The editorial team here at EMBO Journal is very interested in your study and I am, in principle, happy with your revision plan. However, maybe we could Zoom to discuss how best to address the concerns of Referee 1? I will be available all week.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

William Teale

William Teale, PhD
Editor
The EMBO Journal
w.teale@embojournal.org

Instructions for preparing your revised manuscript:

Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
https://bit.ly/EMBOPressFigurePreparationGuideline
See also figure legend guidelines: https://www.embopress.org/page/journal/14602075/authorguide#figureformat

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IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
https://www.embopress.org/page/journal/14602075/authorguide#expandedview

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (16th Apr 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

Link Not Available

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Rev_Com_number: RC-2022-01728
New_manu_number: EMBOJ-2022-113378
Corr_author: Pallas
Title: Plant YTHDF proteins are direct effectors of antiviral immunity against an m6A-containing RNA virus

Manuscript EMBOJ-2022-113378 | [RC-2022-01728] [REV]

Dear Dr. Teale,

I hope this email finds you well and that everything is going well for you.

First of all, I would like to thank the great editorial work of the reviewers who have made this new version of the manuscript significantly improve.

We have dealt with most, if not all, of the concerns raised by the referees, which has meant doing new experiments and introducing a new section titled "The region deleted in ECT2 ΔN5 contributes to the propensity of ECT2 to phase separate". We have added two new figures, one dealing on how the absence of the main components of the RNAi machinery affects AMV infection and the other to show how the region deleted in ECT2ΔN5 contributes substantially to phase separation of the IDR of ECT2. Consequently, we have incorporated two new authors to the manuscript, as you probably have noticed. In this point-by-point response, the referees' statements appear in bold and our answers in normal style. In the word document, the main paragraphs that have significantly been modified appear on a yellow background while single words are highlighted in red.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

The authors have shown previously that local and systemic infection of alfalfa mosaic virus (AMV) is inhibited and the relative abundance of m6A in the viral RNAs is increased in mutant Arabidopsis plants defective in the m6A demethylase gene ALKBH9B. Here the authors show that genetic inactivation of 2 or 3 m6A-binding proteins (ECTs or m6A readers) enhances the systemic, but not the local, infection of AMV. Notably, the systemic virus resistance of the demethylase mutant plants is largely eliminated by inactivating the same set of ECTs. Moreover, the authors detected in vivo association of ECT2 with AMV RNAs and identified a N-terminal motif of ECT2 that is necessary for AMV resistance, but dispensable for its role in endogenous developmental functions. These findings together provide further evidence to support their earlier conclusion for an antiviral role of m6A methylation of viral RNAs. Unfortunately, several key questions remain unknown and should be addressed to justify publication in EMBO J.

Major comments

1. The authors reported in 2017 that both the local and systemic infection of AMV is inhibited in the m6A demethylase mutant plants (PNAS 114:10755-60). In this work, they show that inactivation of ECTs enhances only the systemic AMV infection, but has no effect on the local infection (Fig. 2). Studies presented in neither Fig. 5 nor Fig. 6 examined possible effects on the local virus infection. Moreover, line 1 of page 13 mentioned a model that ECT binding "causes inhibition of viral replication". To resolve these contradictory descriptions on the step of the virus infection cycle targeted by m6A RNA methylation, it is essential to perform protoplast replication assays to determine whether the mutation of either the demethylase gene or ECTs affects viral RNA replication at single-cell level and to examine local infection for the studies presented in Figs 5 & 6.

We greatly appreciate the reflections of the referee regarding the step of the infection cycle of the virus that may be affected by m^6A methylation.

Regarding the local infection, we were not clear enough in our originally submitted manuscript and we thank the reviewer for pointing this out. In reality, one can see the same tendency when comparing viral accumulation in inoculated leaves as in the systemic infection, but the slightly higher accumulation in *ect* mutants is not clearly observed in every experiment and hence, does not always rise to the level of significance. These results are quite expectable, since, as we explained in this new version of the manuscript, ECT2/3/4 are mostly expressed in rapidly dividing, young tissue (Arribas-Hernández et al, 2018, 2020) and their absence, therefore, has a greater effect in these tissues. This expression pattern is the reason why we do not think a protoplast experiment would be very informative, since mature tissues make up the better part of the leaves used to isolate protoplasts. In addition, as we have added in the introduction section, we previously showed a clear reduction in AMV levels detected upon infection of protoplasts isolated from wild type and *alkbh9b* plants (Martínez-Pérez et al, 2021), which suggests that m^6A levels of vRNAs are critical for the first stages of the infection. However, the strongest and more clear effect in *alkbh9b* plants was the reduction of systemic viral invasion and, especially, the lack of infection in the floral stems (See Martínez-Pérez et al., 2021 Front Microbiol. 2021 12:745576). Thus, since it would give us a more revealing result, we decided to check systemic infection to evaluate whether the partial resistance of *alkbh9b* plants was reverted by mutating ects (Figure 5). Likewise, in Figure 6 we performed a complementing experiment using ECT2 and $ECT2^{\Delta N5}$ and, thus, we needed to analyze the most evident phenotype observed in *te234* mutant, which was the higher susceptibility to AMV systemic infection.

2. The title and conclusion of this manuscript defined YTHDF proteins (ECTs) as "direct effectors of antiviral immunity", which is misleading. It remains completely unknown why m6A methylation of viral RNAs is inhibitory to virus infection. The available data suggest that it may act by blocking virus replication and/or movement or by enhancing any of the several known antiviral responses. Effector molecules of an antiviral immunity cannot be identified when the effector mechanism is unknown.

We understand “direct effectors” as the proteins that recognize m^6A vRNA and activate an antiviral response. In this sense, we described in a previous study that, while ALKBH9B does not play a relevant role on Arabidopsis mRNAs (Duan et al., 2017), it regulates AMV accumulation through their m^6A demethylation (Martínez-Pérez et al., 2017. PNAS). Thus, the fact that the mutation of the ECTs is enough to revert the partial resistance observed in *alkbh9b* (Figure 5) suggests that the ECTs act directly recognizing m^6A-modified vRNAs.

In this regard, we have a very different vision from the one the reviewer proposes. We believe that it is not correct to say that the effector molecules of an antiviral immunity cannot be identified until its mechanism is demonstrated. In fact, **RNA silencing effectors were discovered long before their mechanism was elucidated in detai**l. One molecular interpretation of the Flor’s seminal gene-for-gene model, in terms of receptor/effector recognition, is that specific interaction between the receptor and its recognized (cognate) effector protein triggers resistance.
Furthermore, we strongly believe that we provide enough arguments to propose a model, although, as we comment in the end of the discussion, “we view this model as a conceptual

framework of value in the design of future experiments to test its validity". The reasoning that we show here is the following:

i) The m^6A binding proteins are necessary for the antiviral response.
ii) At least ECT2 recognizes AMV RNAs *in vivo* and its m^6A-binding capacity is necessary to play a role in AMV infection.
iii) Simply losing methylase activity – with the same developmental defects as *ect2/3/4* – does not lead to the same degree of loss of resistance, and you can affect AMV resistance without affecting developmental functions of ECT2 (Figure 6).
iv) Altogether, these observations justify the proposal that m^6A exerts antiviral effects by acting as binding sites of ECT proteins in viral RNA, which we consider a clear mechanistic advance.
v) Moreover, we have teamed up with the group of Prof. Kresten Lindorff-Larsen and, in this version, we have added molecular simulations of the phase separation propensity of the IDR of ECT2. The new results (Figure 7) show that the IDR of ECT2 presents marked phase separation propensity, which is reduced in the deletion mutant with defective antiviral activity ($ECT2^{\Delta N5}$), yet unaffected ability to accelerate growth of leaf primordia. Thus, these results and the complementation experiments of Figure 6, together with what we know from previous studies (**m^6A levels of vRNAs are critical for the first stages of the infection**), give as several hints about a possible mechanism. Therefore, we consider that altogether our results point that ECTs would be direct effectors of the observed response.

3. At the end of page 13 and elsewhere in this manuscript, the authors conclude that "the m6A-ECT axis constitutes a first, basal layer of antiviral defense", a conclusion that is not supported by the evidence presented. This conclusion will be incorrect if m6A methylation of viral RNAs does not inhibit virus accumulation levels in the protoplast virus replication assays as requested above.

We have eliminated the word 'first' and we thank the referee for pointing out this inaccuracy. However, we really believe we have shown that **"the m^6A-ECT axis constitutes a basal layer of antiviral defense"**:

We have already mentioned above the inconvenience for a protoplast experiment in the case of ect mutants. But, as we have explained in the first point, experiments on protoplasts in hypermethylated plants (*alkbh9b* mutants) were already carried out, and it was verified that m^6A levels of vRNAs are critical for the first stages of the infection (see Fig. 6 in (Martínez-Pérez *et al*, 2021)). In turn, we show here that at least **ECT2 recognizes AMV RNAs *in vivo* and that its m^6A-binding capacity is necessary to play a role in AMV infection**, and, most importantly, the mutation of ects is enough to revert the partial resistant observed in *alkbh9b* plants (Figure 5).

4. In the first paragraph of page 17 and elsewhere in this manuscript, the authors question the relative importance of antiviral RNA silencing against AMV and related viruses as compared to m6A RNA methylation. It is important to determine if AMV becomes more virulent in RNAi-defective mutant plants such as dcl2/4 double mutant and if m6A RNA methylation also confers AMV resistance in RNAi-defective mutant plants.

We agree with the referee that this is a very interesting and hot topic. Following his/her suggestions, we have evaluated the levels of virus accumulation in different mutant backgrounds affected in different essential parts of the RNAi pathway. We chose to use the single mutants *dcl2*, *dcl4* and *rdr6*, and the double sgs3/rdr6 to cover siRNA production from dsRNAs and secondary amplification. Additionally, we developed triple mutants

rdr6/ect2/ect3 to check out the relation among RNAi and m^6A-ECT systems. Overall, this analysis reveals that m^6A-ECT system is independent of RNAi.:

These results are showed in a new Figure (Supplemental Figure 5) and discussed in the revised manuscript:

"Mutants with lesions in RDR6/SGS3, DCL4 or DCL2 did not show significant changes in AMV levels in non-inoculated leaves at 7 days post-inoculation (dpi) (Supplemental Figure 5A), a point in time in which the effect of ECTs on infection is evident. We next analyzed the role of the ECTs when RNAi is attenuated. We chose inactivation of RDR6 in the *de23* background, since cucumber mosaic virus, another m^6A-containing positive-strand RNA virus phylogenetically related to AMV, is silenced predominantly by the RDR6-dependent viral siRNAs (Wang et al, 2011; Martínez-Pérez et al, 2017; Dalmay et al, 2000; Mourrain et al, 2000). Even in the *rdr6* mutant background, inactivation of ECT2/ECT3 led to increased AMV titers (Supplemental Figure 5B), and, thus, we conclude that the action of m^6A-ECT system is independent of RNAi. These results do not, however, rule out that RNAi may affect the outcome of AMV infection in some setting of the virus-plant interaction, for instance with different strengths of inoculum or at different times post-inoculation."

Minor comments:

1. State the time post-inoculation when the samples were taken for the RNA-seq analysis in Fig.1.

- Already stated in methods, but it has been added to the legend.

2. State whether all of the northern blotting experiments used RNA extracted from single plant or pooled plants and had been repeated.

- This information has been added to materials and methods section:

"Inoculated leaves and non-inoculated aerial tissue were harvested at the corresponding dpi. Systemic infection was evaluated in single plants, while local viral accumulation was analyzed by pooling two plants per sample". "Every infection experiment was repeated at least twice"

3. Verify that the statistical analysis method used in virus titer quantitative analysis is student t-test or one-way ANOVA.

-It is indicated in each Figure. t-test comparing each genotype with WT.

4. The legend to Fig. 4F should be Fig. 4F and 4G.

- Modified in the text file.

5. Explain what â 2 means in Fig. 6B.

ΔN5. Modified

6. If both the left and right bars correspond to 1 cm, 9-DAG-old plants would be much bigger than 16-DAG-old plants, which cannot be true.

This has been modified; thanks!

Reviewer #1 (Significance (Required)):

Advance:

The authors have shown previously that local and systemic infection of alfalfa mosaic virus (AMV) is inhibited and the relative abundance of m6A in the viral RNAs is increased in mutant Arabidopsis plants defective in the m6A demethylase gene ALKBH9B. Here the

authors show that genetic inactivation of 2 or 3 m6A-binding proteins (ECTs or m6A readers) enhances the systemic, but not the local, infection of AMV. Notably, the systemic virus resistance of the demethylase mutant plants is largely eliminated by inactivating the same set of ECTs. Moreover, the authors detected in vivo association of ECT2 with AMV RNAs and identified a N-terminal motif of ECT2 that is necessary for AMV resistance, but dispensable for its role in endogenous developmental functions. These findings together provide further evidence to support their earlier conclusion for an antiviral role of m6A methylation of viral RNAs.
Audience:
Broad, including those interested in RNA modifications, antiviral immunity, and plant biology.
Your expertise: Antiviral immunity, plant biology

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

Summary

In this manuscript, the authors asked the important question of how RNA modification is associated with viral defense in plants. Based on the previous findings that the infectivity of AMV relies on demethylation of viral RNA by recruitment of the cellular m6A demethylase ALKBH9B, in this study, they showed that inactivation of the m6A reader proteins, ECT2, ECT3, and ECT5, is sufficient to restore AMV infectivity in partially resistant alkbh9b mutants. Considering the potential roles of m6A modification in viral defense but the limited knowledge on this topic, the current study opens a new direction of research regarding the role of RNA modification in viral defense.

Major comments

It is interesting to see that the IDR of ECT2 harbors two separable activities employed to achieve different goals: one that stimulates cellular proliferation by binding to endogenous m6A-containing mRNA, and one that effects basal antiviral resistance when ECT2 binds to hypermethylated viral RNA. Considering that the IDR of a protein contributes the chaperone activity of the protein and then can increase the binding capacity of the protein to different substrates, it would be more informative if the authors discuss whether the RNA chaperone activity of IDR of ECT2 is possibly involved in the different processes.

It is interesting to propose a working model that m6A site multiplicity in AMV RNA may be a key factor distinguishing it from endogenous mRNA. In that sense, it would be clearer if the authors describe how many m6A sites are present in AMV RNA. Are these m6A sites clustered in certain regions of the viral RNA important for viral replication? It is not clear what the correlation between the phase separation capability of ECT proteins and viral infection is.

We totally agree with the reviewer respect to the importance of identifying how many m^6A sites are present in AMV genome. In fact, we identified several discrete peaks distributed along the AMV genome containing putative DRACH consensus motifs in a previous work by using a MeRIP-seq approach of RNAs isolated from viral particles (Martínez-Pérez *et al*, 2017). Furthermore, we found that the one located at the most end of the 3'UTR of AMV RNA 3 is a key structural requirement for *in vivo* viral replication (Alvarado-Marchena *et al*, 2022). However, we cannot correlate that effect with binding to ECTs, since this 3'UTR DRACH

motif is part of a binding domain for the AMV coat protein, and the 3'UTR/CP interaction is critical for AMV infection. We are currently planning future experiments to directly identify m^6A residues in all viral RNAs (not only the encapsidated ones), to try to determine how many of them co-exist on the very same viral RNA molecule, which is, still nowadays, something difficult to address. Nonetheless, so far, MeRIP-seq methodology to map m^6A revealed site multiplicity in the genome of several RNA viruses (Gokhale et al., 2016. Cell Host&Microb; Martínez-Pérez et al., 2017; Lichinchi et al., 2016. Nat Microbiol; Lichinchi et al., 2016. Cell Host&Microb), and, recently, a computational approach to deduce RNA modifications in plant viruses made by some of us revealed a higher proportion of RNA chemical modifications in comparison with mRNAs of Arabidopsis (Marquez-Molins et al, 2022).

Respect to the correlation between the phase separation capability of ECT proteins and viral infection, we appreciate very much this suggestion. In fact, as we explained in point 2 of Reviewer #1, we have teamed up with specialists in simulation of molecular properties of IDRs to analyze computationally how the ECT IDRs, and the deletion that affects the antiviral activity, behave with respect to phase separation. As we mentioned above and it can be seen in the new Figure 7, applying this simulation method, we show the following:

1) The IDR of ECT2 shows marked phase separation propensity.

2). The deletion mutant of ECT2 with defective antiviral activity, yet unaffected ability to accelerate growth of leaf primordia, shows markedly reduced phase separation propensity driven, in the main, by the many tyrosine residues in the region deleted in the mutant.

Taken together, our genetic dissection of the IDR of ECT2 and our simulation of its biophysical properties suggest that the two separable activities of the IDR of ECT2 seem to be related with its ability to phase separate, which may be of particular importance for the antiviral function of ECT2. Thus, we have incorporated a new section entitled "The region deleted in ECT2 ΔN5 contributes to the phase separation propensity of ECT2" and consequently added two new authors in this paper.

Minor comments

In page 6, what is the alfalfa mosaic virus (AMV) RNA 3? Are there AMV RNA 1 and 2? What are their differences? Is the m6A-YTH module specific to AMV RNA 3?

Thanks for this comment. We have clarified now how the composition of the AMV genome is at the beginning of the paragraph: "among them alfalfa mosaic virus (AMV), which consists of three genomic RNA molecules (RNA1, RNA2, and RNA3) and a subgenomic RNA (RNA4) (Bujarski et al, 2019)"

Name of the mutants; it would be better if same name was used for the mutant throughout the manuscript and in figures; for instance, ect2-1/ect5-2 and de25, as well as ect2-1/ect3-1/ect5-4 and te235 were used, which is not easy to follow.

Thanks for the suggestion. We have clarified this issue through the text and in the Supplemental Table 1. The abbreviated form (double,de23, de24, de34; triple,te234, te235) is used for the main and recurrently used mutants to facilitate the reading. For the "extra" mutants used to complement our results (Supplemental Figures), we use the extended form specifying each allele (for example, ect2-3/ect3-2).

Figure 4G legend is missing.

Thanks a lot! Included now.

Reviewer #2 (Significance (Required)):

Considering the potential roles of m6A modification in viral defense but the limited knowledge on this topic, the current study opens a new direction of research regarding the role of RNA modification in viral defense.

The audience will be broad, including any persons who are working on epitranscriptomics, plant sciences, viral infection, and clinical application. I am working on epitranscriptomic RNA modification in plant development and abiotic stress responses.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

Previously, the authors showed evidence that m6A modifications of AMV RNAs erased by the host ALKBH9b enhances AMV spread in Arabidopsis. In this paper, the authors show by transcriptome analysis and RT-qPCR that the accumulation of m6A "reader" proteins, ECT2, 3 and 5 are increased during AMV infection in Arabidopsis. Combined mutations of ect2,3 and 5 led to increased AMV accumulation, suggesting that ECT2,3,5 are critical in inhibition of AMV accumulation in systemic leaves of Arabidopsis. Mutagenesis of ECT2 putative m6A-binding pocket did not restore AMV resistance in double-mutant de23 plant, arguing that m6A reader function of this protein is needed to provide resistance against AMV. Then, proximity-labeling was used to show that ECT2 binds to AMV RNA2 and likely RNA1 in planta. Finally, debilitating both the m6A eraser (ALKBH9b) and the reader (ect2,3,5) restored susceptibility to AMV infection in Arabidopsis, thus providing evidence that the m6A reader proteins are critical for resistance against AMV and that AMV exploits ALKBH9b to fight against ECT2,3,5 in plants. Altogether, these are novel and important findings. The paper is well-written.

I do not have main concerns

Minor points:

Abstract
"AMV replication" should be replaced by "AMV infection" or "AMV spread", because the locally infected leaves show similar AMV replication/accumulation in de23 and wt Arabidopsis.

Done. Thanks for the suggestion.

Alkbh9b mutation causes inhibition of local and systemic movement of AMV, whereas de23 mutant increases AMV accumulation only in systemic leaves (Fig. 1). What is the explanation that de23 does not affect local movement of AMV?

We have explained it in point 1 of referee #1 and, moreover, this issue has been clarified in the text: “The viral RNA levels in inoculated leaves (local infection) were slightly higher in mutant combinations involving *ect2*, but reached statistical significance only in the *ect2-1/ect3-1/ect4-2* (henceforth, *te234*) triple mutant (**Figure 2A, C**). However, these differences were much more evident in non-inoculated leaves (systemic infection), in which AMV titers were clearly elevated in *ect2-1/ect3-1* (henceforth, *de23*) and *te234* mutant plants compared

to wild type (Figure 2B) […] We note that several factors may underlie the fact that much clearer differences were observed in uninoculated young tissue compared to inoculated mature leaves, including the fact that ECT2/3/4 are mostly expressed in rapidly dividing, young tissue (Arribas-Hernández et al, 2018, 2020) and their absence, therefore, has a greater effect in these tissues".

The last chapter (p19-20) is too speculative and too long, it does not make the paper more interesting: I recommend shortening it and to minimize speculation

As we have explained above (point 2 of reviewer #1 and major comments of reviewer #2), we have added new data (Results, section "The region deleted in ECT2 ΔN5 contributes to the phase separation propensity of ECT2" and Figure 7) about the relation between the ability of the IDR of ECT2 to phase separate and the antiviral function of this protein. Thus, our discussion of the molecular basis of m^6A-ECT-mediated antiviral defense is considerably less speculative in the revised version. Furthermore, we have shortened a bit this last chapter.

Reviewer #3 (Significance (Required)):

This paper shows evidence for a new antiviral strategy present in plants. Overall, this is a significant new finding.

Dear Vicente,

We have now received re-review reports for the revised version of your manuscript from all three referees, which I have attached below. As you will see, you have addressed all concerns satisfactorily.

However, before we can proceed to publication, (and in addition to the minor corrections spotted by referee #2) there are some remaining editorial points which need to be addressed.

In this regard would you please:

• remove track changes, and highlighted text; there is a stray "€" in the legend to figure 2,
• enter funding information on our submission system for the following grants -PRISM (Protein Interactions and Stability in Medicine and Genomics), Novo Nordisk Foundation (to K.L.-L.; NNF18OC0033950), EMBO postdoctoral fellowship (ALTF 810-2022), Predoctoral Contract FPI-2015-072406 from the Subprograma FPI-MINECO (Formación de Personal Investigador-Ministerio de Economía y Competitividad), Spanish MCIN/AEI/10.13039/501100011033 granting agency, Fondo Europeo de Desarrollo Regional (FEDER),
• include up to five keywords,
• rename the conflict of interest statement as the "DISCLOSURE AND COMPETING INTERESTS STATEMENT",
• remove the author credit section form the manuscript,
• reorganize callouts from the text so that Figure 2B is referred to after 2A; there is a callout for Fig. EV1D, but no such panel; callouts for Fig EV2A-B are missing,
• ensure the author checklist is completed,
• include a table of contents in Appendix 1 with page numbers; the nomenclature for tables should be Appendix Table S1-S2 (also in their text callouts),
• correct the section order according to the Instructions to Authors section of our website; the main figure legends should only be in ms file, please remove them from the individually uploaded figures,
• rename "Summary" as "Abstract", and
• remove the "APPENDIX INFORMATION" section from the manuscript file.

In addition, could you please supply me with Source Data for gels and their quantification in Figure EV3.

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Best wishes,

William

William Teale, PhD
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- a word file of the manuscript text.
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- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (17th Aug 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

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Referee #1:

The revised manuscript is acceptable for publication.

Referee #2:

Dear Editor,

The authors addressed all of my comments, which improved and clarified the manuscript. I have no further major points but have a few minor points.

Minor points;

Fig. 1; at the end of the figure title, "at 8 days dpi" should be "at 8 dpi" and DPI should be defined (explained) in the legends of Fig. 1, 2, 3, and 5.

Fig. 3; the figure legend describes *: $p < 0.05$ and **: $p < 0.01$, but only two stars are labeled on the figure.

Fig. 7; for a statistical analysis, the error bars on Fig. 7C should be explained.

Fig. EV1; the figure legends describes "asterisks indicate $p < 0.05$", however, no star marks on the figure.

Referee #3:

In this revised manuscript, the authors have addressed major points, by adding new data on the RNAi-independent antiviral role of m6A "reader" proteins, ECT2, 3 and 5 in AMV infection in Arabidopsis.
They also added molecular simulation data on the IDR region of ECT2 and the delta5 mutant of ECT2, which showed reduced antiviral activity and lesser potential to self-associate. These data strengthened their argument on the possible role of ECT2 phase separation in antiviral function.
Altogether, the discovery of the new m6A readers-driven antiviral activity in Arabidopsis is novel and important and interest to broad readership. The paper is well-written.

I do not have new concerns.

The minor corrections spotted by referees have been addressed as follows:

Referee #1:

The revised manuscript is acceptable for publication.

Referee #2:

Dear Editor,

The authors addressed all of my comments, which improved and clarified the manuscript. I have no further major points but have a few minor points.

Minor points;

Fig. 1; at the end of the figure title, "at 8 days dpi" should be "at 8 dpi" and DPI should be defined (explained) in the legends of Fig. 1, 2, 3, and 5.
Done,

Fig. 3; the figure legend describes *: $p < 0.05$ and **: $p < 0.01$, but only two stars are labeled on the figure.
Corrected.

Fig. 7; for a statistical analysis, the error bars on Fig. 7C should be explained.

This has been clarified by adding: "Error bars indicate standard errors from block averaging across each simulation using the BLOCKING tool (github.com/fpesceKU/BLOCKING)".

Fig. EV1; the figure legends describes "asterisks indicate $p < 0.05$", however, no star marks on the figure.

Corrected.

Referee #3:

In this revised manuscript, the authors have addressed major points, by adding new data on the RNAi-independent antiviral role of m6A "reader" proteins, ECT2, 3 and 5 in AMV infection in Arabidopsis.
They also added molecular simulation data on the IDR region of ECT2 and the delta5 mutant of ECT2, which showed reduced antiviral activity and lesser potential to self-associate. These data strengthened their argument on the possible role of ECT2 phase separation in antiviral function.
Altogether, the discovery of the new m6A readers-driven antiviral activity in Arabidopsis is novel and important and interest to broad readership. The paper is well-written.

I do not have new concerns.

Dear Vicente,

I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

Congratulations on this manuscript - a really impressive achievement!

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Best wishes,

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- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
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- 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;
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Select "Not Applicable" only when the requested information is not relevant for your study.

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Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Appendix 1 Table S2. List of oligonucleotides used in this work

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Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Yes	Appendix 1 Table S1. List of Arabidopsis thaliana mutant lines employed in this work
Microbes: provide species and strain, unique accession number if available, and source.	Yes	Materials and Methods

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Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

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Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Not Applicable	
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For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Materials and Methods and figure legends

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In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figures

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Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	
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Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
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Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

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Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Data availability section
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Yes	Data availability section
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	