

Intramuscular self-amplifying RNA and adenoviral RSV vaccines program distinct systemic and respiratory mucosal immune architectures

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

2nd Jun 2026

Dear Dr. Cheng,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received feedback from the two reviewers who agreed to evaluate your manuscript. All referees recognize interest of the study but also raise important concerns that should be addressed in a major revision. If you would like to discuss further the points raised by the referees, I am available to do so via email or video. Let me know if you are interested in this option.

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

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

I look forward to receiving your revised manuscript.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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

We require:

- 1) A .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.
- 2) Individual production quality figure files as .eps, .tif, .jpg (one file per figure). For guidance, download the 'Figure Guide PDF': <https://media.springernature.com/original/springer-cms/rest/v1/content/27825798/data/v1>.
- 3) A .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.
- 4) A complete author checklist, which you can download from our author guidelines. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.
- 5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript.
- 6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public.

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

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

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

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

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

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

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

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

- the medical issue you are addressing,
- the results obtained and
- their clinical impact.

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

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

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

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

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

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

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

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

Referee #1 (Remarks for Author):

The goal of this study is to determine the effects of two different vaccine platforms on immune responses to a single antigen. The manuscript reports a detailed, direct comparison of the properties of immune responses to the pre-fusion F protein of RSV (respiratory syncytial virus) delivered by an adenovirus vector or delivered by a lipid nanoparticle (LNP) assembled with an mRNA encoding the pre-fusion RSV F protein. In contrast to most mRNA LNP vaccines, this mRNA encoded self-amplifying sequences as well as the pre-F sequence.

Delivery of both vectors was via intramuscular (IM) immunization of mice. Neutralizing antibody titers in the serum of the immunized animals and cell populations in their lungs, spleens, lymph nodes, and bone marrows were characterized in detail using antibodies specific for different subsets of B and T cells, presumably using flow cytometry for quantification. The expression levels of host proteins were measured in single cells from different cell types. The large amounts of data generated have led the authors to make conclusions that are somewhat different from previous studies of effects of different platforms for antigen delivery.

Similar comparisons of immune responses after IM immunization of the same antigen using with different platforms have been reported by a number of investigators. Most have concluded that the LNP platform results in an increase in the magnitude of responses compared to other platforms but not necessarily differences in the types of the responses. In contrast, this manuscript concludes that the adenovirus vector results primarily in superior induction of systemic immunity while the LNP vector promotes preferentially respiratory mucosal immunity (in spite of the delivery via the IM route). They conclude that the LNP platform does not necessarily result in increased responses by rather responses in different compartments of the immune system despite the same route of immunization. That is, the different platforms program immune responses in different ways.

These conclusions appear supported by the detailed and comprehensive data presented, which is an exhaustive characterization of the different populations of T and B cells induced by the different platforms and characterization of differential expression of host proteins. The conclusions are novel and the study is important for consideration of immunization protocols for protection from respiratory pathogens.

The manuscript could be considerably improved and more readable by presenting the figures and figure legends with increased clarity. For example:

1. Figure 1: Some panels are mislabeled. Panel G is not an immunoblot as it is described in the figure legend. Panel H is not a transmission EM of the LNPs as described by the legend.
2. Figure 2: The authors need to define titers of antibodies in a more quantitative manner. Are the titers ng/ml of Ab (preferred) or dilution of sera resulting in loss of signal?
3. Figures 3, 4: quantification of specific cell populations needs more quantitative measures. As presented, the axis is a percent. This is percent of what?
4. Figure 4: panel H description is missing from the legend.
5. Figure 5: what is meant by "proportions"
6. Statistics are missing from Figure 6, panels B and D and G.
7. Figure 7: Panels C and D have statistical analyses between a column with data and another with no data shown.
8. All figures are very difficult to read due to the very small font size used for labeling the axis of the graphs.

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

RSV vaccines have not yet been licensed for use in infants, who thus remain vulnerable to RSV-associated severe disease.

Referee #2 (Remarks for Author):

The authors compared the humoral and cellular immune responses induced following an intramuscular prime-boost immunization of mice with RSV-preF encoded by a saRNA or Ad5 vector. Whereas the study has its merits, there are several points that need to be clarified before the manuscript could be considered for re-review.

Major remarks:

1. The Ad5-RSV-PreF should be described in the Materials and Methods section. In addition, the comparison of the in vitro preF expression by the saRNA and Ad5 vaccines should be extended with an analysis of the cell culture supernatant because DS-Cav1 is a secreted, soluble protein. The immunoblot (which primary antibody was used?) in Figure 1H is most likely from a denaturing SDS-PAGE. If so, the authors should acknowledge that the immunoblot reveals little information on the prefusion

state of the expressed protein.

2. The source of preF that was used in the ELISA should be specified.

3. The description of the virus neutralization assay is confusing and incomplete. RSV A2 virus strain was used (which infectious dose?), mixed with 293T cells, which somehow led to the expression of luciferase. Please clarify. The RSV B neutralization assay should be described as well.

4. Figure 8 is confusing. Both vaccines were administered intramuscularly whereas this is only depicted in the left hand panel. The right hand panel suggests that the Ad5-RSV-preF viral particles migrate to the spleen and lymph nodes, for which no experimental evidence is shown.

Other remarks:

- The sentence "In animal models, F protein-based vaccines have been shown to overcome Th2 bias and promote the induction of neutralizing antibodies and T cells", in the introduction, requires a reference to support the statement.
- The sentence "Researchers have identified the prefusion conformation of the fusion glycoprotein (preF) as a highly immunogenic component of RSV", in the introduction, requires revision. preF is preferred as an RSV vaccine candidate because the majority of RSV neutralizing activity in human sera is directed against preF, not because preF is highly immunogenic.
- Clesrovimab has also been approved for the prevention of RSV lower respiratory tract disease in neonates and infants.
- Figure 1 legend: panels G and H seem to be swapped.
- "The results showed that the GMTs of LNP-RSV-preF saRNA against RSV A2 and RSV B were 4898 and 1558, respectively, while the GMTs of Ad5-RSV-preF were 1840 and 565 (Fig 2E)." Figure 2E shows the opposite, please check.
- Please specify the source and nature (length, tiled?) of the RSV F peptide pool that was used in the ELISPOT and flow cytometry experiments.
- Please check the position of the vertical dashed lines in Figure 6B.
- Figure 7D, right panel: IgA+ instead of IgG+?
- "In contrast, the LNP-RSV-preF saRNA vaccine induced more long-lived IgA+ ASCs specifically in the lung tissue, suggesting a preferential advantage in maintaining respiratory mucosal antibody memory (Fig. 7D and E)." Fig. 7E shows comparable IgA+ ASC numbers in the lung and higher IgG+ ASC numbers in the lung in the Ad5-RSV-preF immunized mice. Please clarify.
- Please specify the unit (endpoint titers?) of anti-preF IgG/IgA antibody levels in the Y-axis of the ELISA graphs.
- References 21 and 22 are the same.

Manuscript Number: EMM-2026-23938

Title: Intramuscular self-amplifying RNA and adenoviral RSV vaccines program distinct systemic and respiratory mucosal immune architectures

Dear editors and reviewers

We thank all the editors and reviewers for their valuable comments and suggestions. We have carefully revised the manuscript to enhance its clarity and facilitate the understanding of the readers. Our point-to-point responses are presented in the following. We hope that the revision would satisfactorily address the comments and concerns of the editors and reviewers.

Response Letter to Reviewers' Comments

Reviewer's comments

Referee #1 (Remarks for Author): **Concern # 1.** Figure 1: Some panels are mislabeled. Panel G is not an immunoblot as it is described in the figure legend. Panel H is not a transmission EM of the LNPs as described by the legend.

Author response: We sincerely thank the reviewer for identifying this error. We have corrected the labeling in the revised manuscript.

Author Action: We have revised the labels for Panels G and H in Figure 1 accordingly.

Concern # 2. Figure 2: The authors need to define titers of antibodies in a more quantitative manner. Are the titers ng/ml of Ab (preferred) or dilution of sera resulting in loss of signal?

Author response: We sincerely thank the reviewer for this valuable suggestion. Quantification in ng/mL would indeed be ideal. However, since our ELISA detects RSV preF-specific antibodies in mouse serum, a mouse-derived standard reference antibody is required for accurate quantitation. To our knowledge, no commercially available standard reference for mouse RSV preF-specific antibody currently exists. Therefore, we defined the antibody titer as the highest serum dilution at which the assay signal remained detectable. Accordingly, the endpoint titers are reported as the reciprocal of the highest dilution giving a positive signal.

Author Action: We have revised the presentation of all IgG and IgA binding antibody data throughout the manuscript. All corresponding figures now display antibody titers as endpoint titers (the highest serum dilution with a detectable signal). In addition, we have revised the Materials and Methods section by providing a more detailed description of the ELISA procedure, including the definition of endpoint antibody titers. (Page 18, Line 1-2)

Concern # 3. Figures 3, 4: quantification of specific cell populations needs more quantitative measures. As presented, the axis is a percent. This is percent of what?

Author response: We sincerely thank the reviewer for this valuable suggestion. To facilitate a more accurate quantification of specific cell populations, we have now indicated the corresponding parent cell population for each highlighted cell subset in all relevant figures.

Author Action: We have revised the flow cytometry quantification plots in Figures 3, 4, and 5 by updating the y-axis labels to specify the percentage of each cell population relative to its parent cell population, thereby providing a clearer representation of the data.

Concern # 4 Figure 4: panel H description is missing from the legend.

Author response: We sincerely thank the reviewer for identifying this omission.

Author Action: We have added the description for Panel H to the Figure 4 legend in the revised manuscript.

Concern # 5. Figure 5: what is meant by "proportions"

Author response: In the original figure, "proportions" referred to the percentage of each analyzed cell population relative to its corresponding parent cell population.

Author Action: To improve clarity, we have revised the description in Figure 5 by replacing the term "proportions" with "percentages".

Concern # 6. Statistics are missing from Figure 6, panels B and D and G.

Author response: We sincerely thank the reviewer for identifying this omission. We would like to clarify that the statistical analysis shown in Panel G is based on the data presented on the y-axis of the corresponding graph.

Author Action: We have added the corresponding statistical analyses to Panels B and D of Figure 6 in the revised manuscript.

Concern # 7. Figure 7: Panels C and D have statistical analyses between a column with data and another with no data shown.

Author response: We sincerely apologize for this error, which occurred during the figure preparation process. The column without data was mistakenly included; it actually corresponds to the data from the control group.

Author Action: We have modified Panels C and D of Figure 7 by adding the corresponding data from the control group.

Concern # 8. All figures are very difficult to read due to the very small font size used for labeling the

axis of the graphs.

Author response: We sincerely thank the reviewer for this valuable suggestion.

Author Action: To improve the readability and facilitate the review process, we have increased the font size of the axis labels in all figures.

Referee #2 (Remarks for Author):

Major remarks:

Concern # 1. The Ad5-RSV-PreF should be described in the Materials and Methods section. In addition, the comparison of the in vitro preF expression by the saRNA and Ad5 vaccines should be extended with an analysis of the cell culture supernatant because DS-Cav1 is a secreted, soluble protein. The immunoblot (which primary antibody was used?) in Figure 1H is most likely from a denaturing SDS-PAGE. If so, the authors should acknowledge that the immunoblot reveals little information on the prefusion state of the expressed protein.

Author response: We sincerely thank the reviewer for these detailed comments and for raising several important points regarding the characterization of our vaccines. Both the saRNA and adenoviral vector vaccines express a full-length, membrane-anchored preF protein that retains the transmembrane and cytoplasmic domains, rather than the soluble secreted DS-Cav1 form. The antigen sequences were designed by introducing the four DS-Cav1-stabilizing mutations (S155C, S290C, S190F, V207L) into the full-length wild-type RSV F backbone. Because the expressed preF is membrane-bound, it is not released into the cell culture supernatant, which precludes detection by supernatant analysis. For in vitro expression analysis, we therefore extracted total cellular protein using RIPA lysis buffer. We acknowledge that the denaturing detergents in RIPA buffer disrupt the prefusion conformation, and consequently the subsequent western blot—performed with an anti-RSV F protein antibody—detects total F protein regardless of its conformational state. As the reviewer correctly noted, this immunoblot cannot provide information on the prefusion status of the expressed protein. We fully agree with this limitation and have explicitly addressed it in the revised manuscript.

Author Action: We have revised the Materials and Methods section to include a detailed description of the construction of Ad5-RSV-preF construct design, including the specific DS-Cav1 mutations introduced (Page 16, Line 11-15 and Page 17, Line 2-10). We have also specified the primary antibody used for western blotting (Page 18, Line 13-22). In addition, we have added a statement in the relevant Results subsection (and/or figure legend) explicitly acknowledging that the denaturing and reducing

conditions of the SDS-PAGE immunoblot do not permit assessment of the prefusion conformation of the expressed antigen.(Page 26, Line 15-21)

Concern # 2. The source of preF that was used in the ELISA should be specified.

Author response: We sincerely thank the reviewer for this valuable suggestion.

Author Action: We have specified the source of the preF protein in the ELISA method description. (Page 17, Line 22-23)

Concern # 3. The description of the virus neutralization assay is confusing and incomplete. RSV A2 virus strain was used (which infectious dose?), mixed with 293T cells, which somehow led to the expression of luciferase. Please clarify. The RSV B neutralization assay should be described as well.

Author response: We sincerely thank the reviewer for this valuable suggestion. The infectious dose of the RSV A2 strain used in the neutralization assay was 2×10^4 TCID₅₀/mL. We have revised the description of the neutralization assay procedure, including the previous statement: “Subsequently, 293T cells (1.5×10^4 cells per well) were added to the plates and cultured for 2 days at 37° C under 5% CO₂ to allow luciferase expression. On the final day, the supernatant was removed, and 100 µL of luciferase substrate was added to each well. Luminescence was read using a multimode microplate reader (PerkinElmer, USA) with Kaleido 3.0 software.” In addition, we have rewritten this section to provide a clearer description of the RSV neutralization assay and supplemented the methodology for the RSV B strain neutralization assay in the revised Materials and Methods section.

Author Action: We have rewritten the description of the neutralizing antibody assay in the Materials and Methods section (Page 18, Line 4-10). The revised description now includes the infectious doses of both RSV A2 and RSV B strains, as well as the detailed procedure for the RSV B strain neutralization assay.

Concern # 4. Figure 8 is confusing. Both vaccines were administered intramuscularly whereas this is only depicted in the left hand panel. The right hand panel suggests that the Ad5-RSV-preF viral particles migrate to the spleen and lymph nodes, for which no experimental evidence is shown.

Author response: We sincerely thank the reviewer for this valuable suggestion. We have revised and improved the presentation of the data shown in Figure 8 to avoid any potential confusion and enhance the clarity of the figure.

Author Action: We have added an illustration of the intramuscular injection route in the right panel and revised the description regarding the migration of Ad5-RSV-preF viral particles to the spleen and lymph nodes.

Other remarks:

Concern # 5. The sentence "In animal models, F protein-based vaccines have been shown to overcome Th2 bias and promote the induction of neutralizing antibodies and T cells", in the introduction, requires a reference to support the statement.

Author response: We sincerely thank the reviewer for identifying this issue. We agree that the description in this section was inaccurate and have revised it accordingly in the revised manuscript.

Author Action: We have revised the corresponding section in the Introduction and replaced the original statement with the following text: "In animal models, modern prefusion F protein-based RSV vaccines have been shown to induce potent neutralizing antibody responses together with cellular immunity, while reducing the risk of Th2-skewed immune responses compared with formalin-inactivated RSV vaccines." An appropriate reference has been cited. (Page 3, Line 7-10)

Concern # 6. The sentence "Researchers have identified the prefusion conformation of the fusion glycoprotein (preF) as a highly immunogenic component of RSV", in the introduction, requires revision. preF is preferred as an RSV vaccine candidate because the majority of RSV neutralizing activity in human sera is directed against preF, not because preF is highly immunogenic.

Author response: We sincerely thank the reviewer for identifying this issue. We agree that the description in this section was inaccurate and have revised it accordingly in the revised manuscript.

Author Action: We have revised the original statement to: "Researchers have identified that the majority of RSV neutralizing activity in human serum is directed against the prefusion F protein (preF)." (Page 3, Line 11-12)

Concern # 7. Clesrovimab has also been approved for the prevention of RSV lower respiratory tract disease in neonates and infants.

Author response: We sincerely thank the reviewer for this valuable suggestion. We have incorporated this information into the Introduction section of the revised manuscript.

Author Action: We have added the following statement to the corresponding section of the revised manuscript: "To date, three humanized monoclonal antibodies (palivizumab, nirsevimab, and clesrovimab) and three vaccines (GSK's Arexvy, Pfizer's Abrysvo, and Moderna's mRESVIA) have been approved for use against RSV infection." (Page 3, Line 12-13)

Concern # 8. Figure 1 legend: panels G and H seem to be swapped.

Author response: We sincerely thank the reviewer for identifying this error.

Author Action: We have corrected the labels for Panels G and H in Figure 1 and updated the legend

accordingly.(Page 19, Line 19-24)

Concern # 9. "The results showed that the GMTs of LNP-RSV-preF saRNA against RSV A2 and RSV B were 4898 and 1558, respectively, while the GMTs of Ad5-RSV-preF were 1840 and 565 (Fig 2E)." Figure 2E shows the opposite, please check.

Author response: We sincerely thank the reviewer for identifying this error.

Author Action: We have corrected the GMT values for LNP-RSV-preF saRNA and Ad5-RSV-preF in the Results section, swapping the values for the LNP-RSV-preF saRNA and Ad5-RSV-preF groups to match Figure 2E.(Page 6, Line 17-18)

Concern # 10. Please specify the source and nature (length, tiled?) of the RSV F peptide pool that was used in the ELISPOT and flow cytometry experiments.

Author response: The peptide pool used for both the ELISpot and flow cytometry assays was the Human respiratory syncytial virus (RSV) (A2) Fusion glycoprotein F0 Peptide Pool (Sino Biological, Cat#PP004), which consists primarily of 15-mer peptides with an 11-amino-acid (aa) overlap.

Author Action: We have added the information regarding the RSV F peptide pool used in the Materials and Methods section of the revised manuscript.(Page 18, Line 29-30 and Page 19, Line 22-23)

Concern # 11. Please check the position of the vertical dashed lines in Figure 6B.

Author response: We sincerely thank the reviewer for identifying the issue with the vertical dashed line.

Author Action: We have corrected the position of the vertical dashed line in Figure 6B in the revised manuscript.

Concern # 12. Figure 7D, right panel: IgA⁺ instead of IgG⁺?

Author response: We sincerely thank the reviewer for identifying this figure preparation error. The right panel of Figure 7D corresponds to IgA⁺ cells.

Author Action: We have revised the y-axis label description in Figure 7D in the revised manuscript.

Concern # 13. "In contrast, the LNP-RSV-preF saRNA vaccine induced more long-lived IgA⁺ ASCs specifically in the lung tissue, suggesting a preferential advantage in maintaining respiratory mucosal antibody memory (Fig. 7D and E)." Fig. 7E shows comparable IgA⁺ ASC numbers in the lung and higher IgG⁺ ASC numbers in the lung in the Ad5-RSV-preF immunized mice. Please clarify.

Author response: We sincerely apologize for this error, which occurred during the description of the results. As the reviewer correctly pointed out, in Ad5-RSV-preF-immunized mice, the numbers of pulmonary IgA⁺ ASC were comparable, whereas the numbers of IgG⁺ ASC were higher.

Author Action: We have rewritten this section of the manuscript. The revised text is as follows:
“Notably, Ad5-RSV-preF elicited a higher frequency of long-lived IgG⁺ antibody-secreting cells (ASCs) in both the spleen and lungs than LNP-RSV-preF saRNA. In contrast, LNP-RSV-preF saRNA induced a greater frequency of long-lived IgA⁺ ASCs in the spleen. However, the two vaccine platforms generated comparable numbers of long-lived IgA⁺ ASCs in the lungs, suggesting that LNP-RSV-preF saRNA does not provide a measurable advantage over Ad5-RSV-preF in establishing long-term pulmonary IgA humoral memory.”(Page 11, Line 6-11)

Concern # 14. Please specify the unit (endpoint titers?) of anti-preF IgG/IgA antibody levels in the Y-axis of the ELISA graphs.

Author response: We sincerely thank the reviewer for this valuable suggestion. We have revised the corresponding section accordingly.

Author Action: We have revised the presentation of all IgG and IgA binding antibody measurements. All figures now uniformly display antibody titers as serum dilution endpoint titers. In addition, we have added detailed descriptions of the ELISA assay in the Materials and Methods section.(Page 18, Line 1-2)

Concern # 15. References 21 and 22 are the same.

Author response: We sincerely thank the reviewer for identifying the issue of the duplicated reference citation. We have corrected this error in the revised manuscript.

Author Action: We have removed the duplicated reference from the revised manuscript.(Page 22, Line 14-15)

9th Sep 2026

Dear Dr. Cheng,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now heard back from the two referee who we asked to evaluate your revised manuscript. I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

- 1) Please address referee #2 minor comments.
- 2) Author checklist: Please submit a complete checklist. <https://media.springernature.com/original/springer-cms/rest/v1/content/27825796/data/v1>
- 3) In the main manuscript file, please do the following:
 - Please address all comments suggested by our data editors listed below:
 - o Figure legends:
 1. Please note that the legend for figure 4A is missing in the manuscript. This needs to be rectified.
 2. Please note that the exact p values are not provided in the legends of figures 2B-E; 3A-D, E, F; 4B-H; 5B-I; 6B, D; 7B-F.
 3. Please note that the box plots need to be defined in terms of minima, maxima, centre, bounds of box and whiskers, and percentile in the legends of figures 3A-D, 7C, D, E.
 4. Please note that information related to n is missing in the legends of figures 1D-F; 4B, G, H; 5B-I; 6F, 7B-F.
 5. Please note that the error bars are not defined in the legends of figures 1D-F; 2B-E; 3E, F; 4B-H; 5B-I; 7B, F.
 - Please update the order and the headings of the manuscript sections to: Abstract / The Paper Explained / Introduction / Results / Discussion / Methods / Data Availability / Acknowledgements / Disclosure and Competing Interests Statement / References / Figure Legends /
 - Add callouts for Fig 1G, Fig 2A, Fig 4H, Fig 7A.
 - In Methods, a statistical paragraph should reflect all information that you have filled in the Authors Checklist, especially regarding randomization, blinding, replication.
 - Indicate in legends exact n and exact p values, not a range, along with the statistical test used. To keep the figures "clear" some authors found providing an Appendix table Sx with all exact p-values preferable. You are welcome to do this if you want to.
 - Please remove Reagents and Tools Table and upload it as a separate .docx file. More information on how to adhere to this format as well as downloadable templates (.docx) for the Reagents and Tools Table can be found in our author guidelines: <https://link.springer.com/journal/44321/submission-guidelines#structuredmethods>
 - Rename "Competing interest" to to "Disclosure and competing interests statement". 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.
 - Author contributions: Please remove it from the manuscript and specify author contributions in our submission system. CRediT has replaced the traditional author contributions section because it offers a systematic machine-readable author contributions format that allows for more effective research assessment. You are encouraged to use the free text boxes beneath each contributing author's name to add specific details on the author's contribution. More information is available in our guide to authors: <https://link.springer.com/journal/44321/submission-guidelines#authorshipguidelines>
 - Move "Ethics approval" statement to the "Animal studies" paragraph in the Methods section.
 - Remove the subheading "Declarations" and "Consent for publication" statement.
- 4) Funding: Please merge with Acknowledgements.
- 5) The Paper Explained: Please provide "The Paper Explained" and add it to the main manuscript text. Please check any of recently published EMBO Mol Med research articles for reference.
- 6) Synopsis:
 - Synopsis image: Please submit Figure 8 as a visual abstract. The image should be high-resolution jpeg file exactly 550 px-wide x 300 pixels high.
 - Please check your synopsis text and image before submission with your revised manuscript. Please be aware that in the proof stage minor corrections only are allowed (e.g., typos).
- 7) As part of the EMBO Publications transparent editorial process (see our Editorial at <http://embomolmed.embopress.org/content/2/9/329>), EMBO Molecular Medicine will publish online a Review Process File (RPF) to accompany accepted manuscripts. This file will be published in conjunction with your paper and will include the anonymous referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript. Let us know if you want to remove or not any figures from it prior to publication. Please note that the Authors checklist will be published at the end of the RPF.
- 8) Please provide a point-by-point letter INCLUDING my comments as well as the reviewer's reports and your detailed responses (as Word file).

I look forward to reading a new revised version of your manuscript as soon as possible.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

*** Instructions to submit your revised manuscript ***

*** PLEASE NOTE *** As part of the EMBO Publications transparent editorial process initiative (see our Editorial at <https://www.embopress.org/doi/pdf/10.1002/emmm.201000094>), EMBO Molecular Medicine will publish online a Review Process File to accompany accepted manuscripts.

In the event of acceptance, this file will be published in conjunction with your paper and will include the anonymous 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 at contact@embomolmed.org.

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

We require:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

- the medical issue you are addressing,
- the results obtained and
- their clinical impact.

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

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

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

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

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

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

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

*Additional important information regarding Figures

Each figure should be given in a separate file and should have the following resolution:

Graphs 800-1,200 DPI

Photos 400-800 DPI

Colour (only CMYK) 300-400 DPI"

Figures are not edited by the production team. All lettering should be the same size and style; figure panels should be indicated by capital letters (A, B, C etc). Gridlines are not allowed except for log plots. Figures should be numbered in the order of their appearance in the text with Arabic numerals. Each Figure must have a separate legend and a caption is needed for each panel.

*Additional important information regarding figures and illustrations can be found at
<https://media.springernature.com/original/springer-cms/rest/v1/content/27825798/data/v1>

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

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

The authors have responded satisfactorily to comments of the reviewers.

Referee #1 (Remarks for Author):

The authors have responded appropriately to all comments/issues of the reviewers.

Manuscript is not appropriate for a short report. The data are too complex to be adequately presented in a short report.

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

The Ad5 - saRNA comparison was thoroughly performed in a mouse model. As the authors acknowledge in the discussion, an RSV challenge experiment was not performed, which may have revealed a different outcome. For this, however, another animal model such as the cotton rat would be preferred over the mouse.

Referee #2 (Remarks for Author):

- Abstract, line 15-16: The statement "Vaccine platforms intrinsically program distinct compartmentalized immune responses against RSV independent of antigen" is unclear: which vaccine platforms? In case the authors refer to the Ad5 - saRNA comparison "independent of antigen" was not shown: only one antigen (pre-F) was studied. Please remove or adapt this statement from the abstract.
- Please specify the origin of the RSV A2 and B virus in the Materials and Methods section.

Manuscript Number: EMM-2026-23938

Title: Intramuscular self-amplifying RNA and adenoviral RSV vaccines program distinct systemic and respiratory mucosal immune architectures

Dear editors and reviewers,

We thank all the editors and reviewers for their valuable comments and suggestions. We have carefully revised the manuscript to enhance its clarity and facilitate the understanding of the readers. Our point-to-point responses are presented in the following. We hope that the revision would satisfactorily address the comments and concerns of the editors and reviewers.

Response Letter to the Editor and Reviewers' Comments

1) Referee #2 minor comments.

- Abstract, line 15-16: The statement "Vaccine platforms intrinsically program distinct compartmentalized immune responses against RSV independent of antigen" is unclear: which vaccine platforms? In case the authors refer to the Ad5 - saRNA comparison "independent of antigen" was not shown: only one antigen (pre-F) was studied. Please remove or adapt this statement from the abstract.

Author response: Thank you for the suggestions provided by the reviewers. We have revised the manuscript accordingly. The revised content is as follows: Ad5-vectored and LNP-saRNA vaccine platforms induce distinct compartmentalized immune responses to the same RSV preF antigen. (Page 2, Line 15-16)

- Please specify the origin of the RSV A2 and B virus in the Materials and Methods section.

Author response: In the materials and methods section, we have added the sources of RSV type A2 and type B viruses. (Page 17, Line 10-11)

2) Author checklist: Please submit a complete checklist.

<https://media.springernature.com/original/springer-cms/rest/v1/content/27825796/data/v1>

Author response: We have uploaded the list of authors as required.

All comments suggested by our data editors listed below:

Figure legends:

1. Please note that the legend for figure 4A is missing in the manuscript. This needs to be rectified.

Author response: We have added the annotation of figure 4A to the figure legends. (Page 26, Line 2)

2. Please note that the exact p values are not provided in the legends of figures 2B-E; 3A-D, E, F; 4B-H; 5B-I; 6B, D; 7B-F.

Author response: We have uploaded the Exact p-values of all the graphs in Table EV1 for readers to review late.

3. Please note that the box plots need to be defined in terms of minima, maxima, centre, bounds of box and whiskers, and percentile in the legends of figures 3A-D, 7C, D, E.

Author response: We have modified Figures 3A-D and 7C-E according to your suggestions.

4. Please note that information related to n is missing in the legends of figures 1D-F; 4B, G, H; 5B-I; 6F, 7B-F.

Author response: We have supplemented the relevant content of "n" as indicated in figure legends.

5. Please note that the error bars are not defined in the legends of figures 1D-F; 2B-E; 3E, F; 4B-H; 5B-I; 7B, F.

Author response: We have supplemented the definition of error bar in figure legends.

6. Please update the order and the headings of the manuscript sections to: Abstract / The Paper Explained / Introduction / Results / Discussion / Methods / Data Availability / Acknowledgements / Disclosure and Competing Interests Statement / References / Figure Legends /

Author response: According to the format requirements for uploading the manuscript, we have made the necessary revisions.

7. Add callouts for Fig 1G, Fih 2A, Fig 4H, Fig 7A.

Author response: We have added annotations to Fig. 1G (Page 6, line 14), Fig. 2A (Page 6, line 21), Fig. 4H (Page 8, line 23), and Fig. 7A (Page 11, line 13) to the manuscript.

8. In Methods, a statistical paragraph should reflect all information that you have filled in the Authors Checklist, especially regarding randomization, blinding, replication.

Author response: We have supplemented the significance Analysis methods used in the paper in the "Statistical Analysis" section.

9. Indicate in legends exact n and exact p values, not a range, along with the statistical test used. To keep the figures "clear" some authors found providing an Appendix table Sx with all exact p-values preferable. You are welcome to do this if you want to.

Author response: We have uploaded the P-values of all the graphs in a new supplementary file for readers to review late.

10. Please remove Reagents and Tools Table and upload it as a separate .docx file. More information on how to adhere to this format as well as downloadable templates (.docx) for the Reagents and Tools Table can be found in our author guidelines:

<https://link.springer.com/journal/44321/submission-guidelines#structuredmethods>

Author response: We have removed the "Reagents and Tools List" section from the manuscript and uploaded it as a separate.docx file.

11. Rename "Competing interest" to to "Disclosure and competing interests statement". 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.

Author response: We have changed "Competitive Interests" to "Disclosure and Competitive Interests Statement".

12. Author contributions: Please remove it from the manuscript and specify author contributions in our submission system. CRediT has replaced the traditional author contributions section because it offers a systematic machine-readable author contributions format that allows for more effective research assessment. You are encouraged to use the free text boxes beneath each contributing author's name to add specific details on the author's contribution. More information is available in our guide to authors:

<https://link.springer.com/journal/44321/submission-guidelines#authorshipguidelines>

Author response: We have deleted this part of the manuscript.

13. Move "Ethics approval" statement to the "Animal studies" paragraph in the Methods section.

Author response: We have moved "Ethics approval" statement to the "Animal studies" paragraph in the Methods section. (Page 15, line 17-20)

14. Remove the subheading "Declarations" and "Consent for publication" statement.

Author response: We have deleted this part of the manuscript.

15. Funding: Please merge with Acknowledgements.

Author response: We have combined the funding section with the acknowledgments.

16. The Paper Explained: Please provide "The Paper Explained" and add it to the main manuscript text. Please check any of recently published EMBO Mol Med research articles for reference.

Author response: We have added the "The Paper Explained" section to the manuscript. (Page 2, line 19-30; Page 3, line 1-14)

17. Synopsis:

- Synopsis image: Please submit Figure 8 as a visual abstract. The image should be high-resolution jpeg file exactly 550 px-wide x 300 pixels high.

- Please check your synopsis text and image before submission with your revised manuscript. Please be aware that in the proof stage minor corrections only are allowed (e.g., typos).

Author response: We uploaded Figure 8 in our manuscript as a visual abstract.

23rd Sep 2026

Dear Dr. Cheng,

We are pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

Your manuscript will be processed for publication by EMBO Press. It will be copy edited and you will receive page proofs prior to publication. Please note that you will be contacted by Springer Nature Author Services to complete licensing and payment information.

You may qualify for financial assistance for your publication charges - either via a Springer Nature fully open access agreement or an EMBO initiative. Check your eligibility: <https://link.springer.com/journal/44321/how-to-publish-with-us>

Should you be planning a Press Release on your article, please notify the production team via this form in order to coordinate publication and release dates: <https://forms.cloud.microsoft/e/eH4A8JVP6g>

If you have any questions, please do not hesitate to contact the Editorial Office. Thank you for your contribution to EMBO Molecular Medicine.

Yours sincerely,
Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

>>> Please note that it is EMBO Molecular Medicine policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here: <https://link.springer.com/partners/embo-press/editorial-policies#Peer%20review>

EMBO Press Author Checklist

Corresponding Author Name: Ping Cheng
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2026-23938-V2

USEFUL LINKS FOR COMPLETING THIS FORM

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[EMBO Reports - Author Guidelines](#)
[Molecular Systems Biology - Author Guidelines](#)
[EMBO Molecular Medicine - Author Guidelines](#)

Reporting Checklist for Life Science Articles (updated January

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: [10.31222/osf.io/9sm4x](https://doi.org/10.31222/osf.io/9sm4x)). Please follow the journal's guidelines in preparing your article. **Please note that a copy of this checklist will be published alongside your article.**

Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots 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. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

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 χ^2 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.

Please complete ALL of the questions below.

Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	
Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For antibodies provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Reagents and Tools Table
DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	
Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, and OR RRID.	Yes	Reagents and Tools Table
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Reagents and Tools Table
Report if the cell lines were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	
Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID.	Yes	Materials and Methods
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail housing and husbandry conditions .	Yes	Materials and Methods
Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	
Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	
Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Not Applicable	

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been pre-registered , provide DOI in the manuscript . For clinical trials, provide the trial registration number OR cite DOI.	Not Applicable	
Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if external detailed step-by-step protocols are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about sample size estimate even if no statistical methods were used.	Yes	Figures
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	
Include a statement about blinding even if no blinding was done.	Not Applicable	
Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Not Applicable	
If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.		
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	Figures

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was replicated in laboratory.	Yes	Figures
In the figure legends: define whether data describe technical or biological replicates .	Yes	Figures

Ethics

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	
Studies involving human participants : For publication of patient photos , include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental animals : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Materials and Methods
Studies involving specimen and field samples : State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

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	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the authority granting approval and reference number for the regulatory approval provided in the manuscript?	Not Applicable	

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.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., ICMJE, MIBBI, ARRIVE, PRISMA) have been followed or provided.	Not Applicable	
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.	Not Applicable	
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.	Not Applicable	

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?	Not Applicable	
If publicly available data were reused, provide the respective data citations in the reference list .	Not Applicable	