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Viral nucleoprotein antibodies activate TRIM21 and induce T cell immunity

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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.)

1st Editorial Decision 8th Sep 2020

Dear Leo,

Thank you for for submitting your manuscript to The EMBO Journal and also apologises for the delay in getting back to you with a decision. I have now received the three reports on your study that I have enclosed below.

As you can see from the comments, the referees find the analysis very interesting, valuable and very well done. They all support publication here. It makes me very happy to see such positive comments from three good experts in the field. They raise relative minor concerns with the study and most can be addressed with a better discussion and text changes. I like the suggestions provided by referee #2 - point 2 and 3. Point # 3 will also go towards addressing the concern raised by referee #1. Do you have any data on hand to address those points? Would be good to discuss this further and we can do so via email or a video call.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

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 discussing the revisions further with you

best Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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

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Referee #1:

Manuscript from Caddy et al. describes a mechanism by which anti-Nucleoprotein Abs against LCMV may inhibit virus replication. This mechanism involves TRIM21 FcR intracellular binding of virus Ab immune complexes that triggers efficient TCTL response. Authors use either in vitro transfection of Abs in cells or in vivo treatment of mouse KO for TRIM21. The results described are properly conducted and highly convincing. According to their results, authors proposed an additional model for virus inhibition linking Ab and T cell response.
However, they are some limitation of the experiments proposed. For example, in vitro transfection of the Abs bypass all the first steps of Ab virus entry into the cytosol. If macrophages are doing the jobs as proposed by in vivo experiments, co-culture with macrophages may be envisaged.
Also, it was proposed that Ab/ TRIM21 interaction is dependent on both Ab Fc domain and TRIM21 polymorphism. The role of this interaction was not analyzed by using different Abs, modifying Fc domain or TRIM21 polymorphism. The role of this interaction should at least be discussed.
Authors may design a schematically representation to depict the mechanistic mode proposed of Ab/TRIM21 inhibitory activity. A black box on how Ab enter into cytosol should be symbolized.

Referee #2:

Caddy et al present an interesting study that demonstrates, at least in the LCMV mouse model, that non neutralizing antibodies against nucleocapsid provide some levels of protection in vivo by promoting rapid activation of specific CTLs in a TRIM21 recognition dependent manner, contributing to viral clearance. The authors conduct appropriate mechanistic studies that lead to this conclusion. These observations put together pieces of data that we consistent with what we previously know and offer a well-supported mechanism to explain the "mysterious" ability of nucleocapsid antibodies to provide protection in vivo.
Specific questions

1. One still wonders how important N antibodies are in the presence of existing CD8 memory, as all the studies are conducted during primary infections and therefore, the contribition of N antibodies to protection in the presence of T cell memory is not very clear.

2. The authors might consider conducting loss of function experiments to strengthen the conclusions. For example, by using a virus that has a point mutation in N that prevents binding to the N antibody used in the antibody passive immunization experiments, they could clearly demonstrate that binding of the antibody to N is required for the protection. Alternatively, they might show that TRIM21 mediated protection is compromised in the context of mice lacking B cells unable to generate N antibodies.

3. The use of Fab control antibody in the antibody transfer experiments will confirm that the TRIM21-mediated effect is through recognition of the Fc portion of the antibody-antigen complexes.

Referee #3:

In a beautifully written manuscript the authors show convincingly that antibodies directed against N protein of CMV which are non-neutralizing promote viral clearance by another mechanism. Anti-N antibodies protect the host because they engage cytosolic TRIM21 which facilitates degradation of the attached N protein and priming of LCMV-specific T cells.
1. As a minor suggestion the authors may consider explaining better how they think the immune-complexes might be internalized and whether or not MHC class II also presents N-derived peptides. This comment does not suggest new experiments but would provide a more integrated view of the development of the protective cellular immune response to LCMV.
2. In figure 2A the WT weight is almost 10% higher than KO weight while in figure 1 the two points perfectly overlap. A brief explanation would be helpful.

Referee #1:

Manuscript from Caddy et al. describes a mechanism by which anti-Nucleoprotein Abs against LCMV may inhibit virus replication. This mechanism involves TRIM21 FcR intracellular binding of virus Ab immune complexes that triggers efficient TCTL response. Authors use either in vitro transfection of Abs in cells or in vivo treatment of mouse KO for TRIM21. The results described are properly conducted and highly convincing. According to their results, authors proposed an additional model for virus inhibition linking Ab and T cell response. However, they are some limitation of the experiments proposed. For example, in vitro transfection of the Abs bypass all the first steps of Ab virus entry into the cytosol. If macrophages are doing the jobs as proposed by in vivo experiments, co-culture with macrophages may be envisaged. Also, it was proposed that Ab/ TRIM21 interaction is dependent on both Ab Fc domain and TRIM21 polymorphism. The role of this interaction was not analyzed by using different Abs, modifying Fc domain or TRIM21 polymorphism. The role of this interaction should at least be discussed. Authors may design a schematically representation to depict the mechanistic mode proposed of Ab/TRIM21 inhibitory activity. A black box on how Ab enter into cytosol should be symbolized.

We thank the reviewer for their comments. The mechanism of cytosolic import in antigen presenting cells (APCs) is poorly understood. Recent work suggests that import is an inefficient process and APCs require as yet uncharacterised stimulus (Kozik et al., 2020). We are currently working towards an *in vitro* model that we can use to investigate this mechanism further. While the details of IgG:TRIM21 interaction are not investigated in detail in the current study, we have dissected this in previous work. Previously we have shown that TRIM21 has broad antibody specificity, interacting with all IgG subtypes and IgM (Mallery et al., 2010). TRIM21 is highly conserved and human polymorphisms are restricted to rare variants; a recent study based on empirical testing of all variants identified in the 1000 genomes collection concluded that complete loss-of-function would only be predicted in ~1 in a billion individuals (Zeng, Slodkowicz, & James, 2019). TRIM21 is also highly conserved between mammals, with mouse TRIM21 binding human IgG and vice-versa (Keeble, Khan, Forster, & James, 2008). TRIM21:IgG interaction has been characterised in detail by x-ray crystallography (James, Keeble, Khan, Rhodes, & Trowsdale, 2007) and a single IgG point mutation, H433A, is sufficient to prevent interaction (Foss et al., 2016) and specifically abolish TRIM21 function: Antibodies with mutation H433A lose TRIM21 antiviral activity *in vitro,* in both cell lines (McEwan et al., 2012) and primary human macrophages (Labzin et al., 2019), and *in vivo* in a mouse model of infection (Bottermann et al., 2018). Importantly, in the present study, we show that an H433A mutation prevents anti-N antibody KL53 from inducing TRIM21-mediated intracellular degradation of LCMV nucleoprotein. This is consistent with a direct interaction between TRIM21 and IgG being required to generate nucleoprotein peptides for MHC Class I presentation.

We have added new text into the discussion (Lines 222-231) and, as suggested, a schematic giving an overview of our model for TRIM21s involvement in antigen presentation.

Referee #2:

Caddy et al present an interesting study that demonstrates, at least in the LCMV mouse model, that non neutralizing antibodies against nucleocapsid provide some levels of protection in vivo by promoting rapid activation of specific CTLs in a TRIM21 recognition dependent manner, contributing to viral clearance. The authors conduct appropriate mechanistic studies that lead to this conclusion. These observations put together pieces of data that we consistent with what we previously know and offer a well-supported mechanism to explain the "mysterious" ability of nucleocapsid antibodies to provide protection in vivo.

Specific questions

Q1. One still wonders how important N antibodies are in the presence of existing CD8 memory, as all the studies are conducted during primary infections and therefore, the contribition of N antibodies to protection in the presence of T cell memory is not very clear.

A1. This is an important point and, while the present study does not address this directly, on the basis of our findings we speculate that the same mechanism of TRIM21-mediated antigen presentation may help in the restimulation of memory T cells. We have added a sentence to highlight this in the discussion (Lines 193-196).

Q2.1. The authors might consider conducting loss of function experiments to strengthen the conclusions. For example, by using a virus that has a point mutation in N that prevents binding to the N antibody used in the antibody passive immunization experiments, they could clearly demonstrate that binding of the antibody to N is required for the protection.

A2.1 This is a great suggestion and in previous work we have introduced mutations into adenovirus hexon to reduce antibody binding and prevent TRIM21 function (Bottermann et al., 2016). In that case, we had a crystal structure of the antibody:antigen complex. Unfortunately we don't have this information for the binding of KL53 to N and are unable to make a loss-of-binding point mutation.

Q2.2 Alternatively, they might show that TRIM21 mediated protection is compromised in the context of mice lacking B cells unable to generate N antibodies.

A2.2 This is also an excellent suggestion. Previous studies have used a variety of antibody-deficient mouse backgrounds to study LCMV infection. Experiments have been performed in B cell restricted MD4 and T11 µMT mice, in the B cell deficient JHT strain and in IgMi, which produce little soluble IgG. In each case there is a divergence from wild-type only after the 1st week of infection, with viraemia remaining high. This phenomenon closely matches what we observe in TRIM21 knockouts. We don't currently have double knockout mice in these strains but are establishing them for future work. Our prediction would be that removing TRIM21 from antibody-deficient mice will have no additive effect on LCMV infection, as TRIM21 forms a subset of antibody protection. This prediction is based on previous *in vivo* work where we made a point mutation in a potent antiviral IgG to prevent TRIM21 binding (Bottermann et al., 2018). The mutant antibody no longer blocked adenovirus infection in wild-type mice. In contrast, TRIM21 KO animals were similarly infected whether given unmutated or mutant antibody. These experiments demonstrate that TRIM21 immune protection is dependent upon antibodies.

3. The use of Fab control antibody in the antibody transfer experiments will confirm that the TRIM21-mediated effect is through recognition of the Fc portion of the antibody-antigen complexes.

As described above, we have previously shown in an adenovirus infection model that TRIM21 must interact with the Fc region of IgG in order to mediate its effects *in vivo*. We tested an antibody with mutation H433A, which is located in the Fc, and found that it no longer provided TRIM21-mediated protection. As discussed in detail in the response to reviewer 1, we have extensively characterised this mutant and shown that it specifically abolishes TRIM21 binding and activity. The Fab experiment is a good suggestion, although a Fab may have reduced affinity for LCMV N protein compared to the IgG because it cannot bind bivalently, and this could contribute to reduced protection. It may be possible to clone the KL53 hybridoma, mutate it and produce sufficient recombinant antibody for *in vivo* study and this is something we are actively pursuing.

Referee #3:

In a beautifully written manuscript the authors show convincingly that antibodies directed against N protein of CMV which are non-neutralizing promote viral clearance by another mechanism. Anti-N antibodies protect the host because they engage cytosolic TRIM21 which facilitates degradation of the attached N protein and priming of LCMV-specific T cells.

Q1. As a minor suggestion the authors may consider explaining better how they think the immune-complexes might be internalized and whether or not MHC class II also presents N-derived peptides. This comment does not suggest new experiments but would provide a more integrated view of the development of the protective cellular immune response to LCMV.

A1. We thank the reviewer for their positive comments. We have added a new paragraph into the discussion to explain how immune complexes may be internalized (Lines 198-224), including a sentence on MHC Class II (Lines 213-215)

Q2. In figure 2A the WT weight is almost 10% higher than KO weight while in figure 1 the two points perfectly overlap. A brief explanation would be helpful.

A2. In the experiment in Figure 1, the WT and KO weights diverge at day 9 whereas in the experiment in Figure 2 the weights diverge at day 8. This may be due to slightly different doses being used in these experiments ($10^{\wedge 5}$ FFU for Figure 1 and $0.5 \times 10^{\wedge 5}$ FFU for Figure 2). At the higher dose, the weight loss is slightly steeper and the recovery in WT body weight slightly delayed.

References

Bottermann, M., Foss, S., van Tienen, L. M., Vaysburd, M., Cruickshank, J., O'Connell, K., … James, L. C. (2018). TRIM21 mediates antibody inhibition of adenovirus-based gene delivery and vaccination. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(41), 10440–10445. https://doi.org/10.1073/pnas.1806314115

Bottermann, M., Lode, H. E., Watkinson, R. E., Foss, S., Sandlie, I., Andersen, J. T., & James, L. C. (2016). Antibody-antigen kinetics constrain intracellular humoral immunity. *Scientific Reports*, *6*, 37457. https://doi.org/10.1038/srep37457

Foss, S., Watkinson, R. E., Grevys, A., McAdam, M. B., Bern, M., Høydahl, L. S., … Andersen, J. T. (2016). TRIM21 Immune Signaling Is More Sensitive to Antibody Affinity Than Its Neutralization Activity. *Journal of Immunology (Baltimore, Md. : 1950)*, *196*(8), 3452–3459. https://doi.org/10.4049/jimmunol.1502601

James, L. C., Keeble, A. H., Khan, Z., Rhodes, D. A., & Trowsdale, J. (2007). Structural basis for PRYSPRY-mediated tripartite motif (TRIM) protein function. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(15), 6200–6205. https://doi.org/10.1073/pnas.0609174104

Keeble, A. H., Khan, Z., Forster, A., & James, L. C. (2008). TRIM21 is an IgG receptor that is structurally, thermodynamically, and kinetically conserved. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(16). https://doi.org/10.1073/pnas.0800159105

Kozik, P., Gros, M., Itzhak, D. N., Joannas, L., Heurtebise-Chrétien, S., Krawczyk, P. A., … Amigorena, S. (2020). Small Molecule Enhancers of Endosome-to-Cytosol Import Augment Anti-tumor

Immunity. *Cell Reports*, *32*(2), 107905. https://doi.org/10.1016/j.celrep.2020.107905

Labzin, L. I., Bottermann, M., Rodriguez-Silvestre, P., Foss, S., Andersen, J. T., Vaysburd, M., … James, L. C. (2019). Antibody and DNA sensing pathways converge to activate the inflammasome during primary human macrophage infection. *The EMBO Journal*, *38*(21), e101365. https://doi.org/10.15252/embj.2018101365

Mallery, D. L., McEwan, W. A., Bidgood, S. R., Towers, G. J., Johnson, C. M., & James, L. C. (2010). Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21). *Proceedings of the National Academy of Sciences of the United States of America*, *107*(46). https://doi.org/10.1073/pnas.1014074107

McEwan, W. A. ., Hauler, F., Williams, C. R., Bidgood, S. R., Mallery, D. L., Crowther, R. A., & James, L. C. (2012). Regulation of virus neutralization and the persistent fraction by TRIM21. *Journal of Virology*, *86*(16). https://doi.org/10.1128/JVI.00728-12

Zeng, J., Slodkowicz, G., & James, L. C. (2019). Rare missense variants in the human cytosolic antibody receptor preserve antiviral function. *ELife*, *8*. https://doi.org/10.7554/eLife.48339

1st Revision - Editorial Decision 6th Oct 2020

Dear Leo,

Thanks for submitting your revised manuscript to the EMBO Journal. I have now had a chance to take at the revised version and your response. I appreciate the introduced changes and I am happy to let you know that we will accept the manuscript for publication here.

Before sending you the final accept letter there are just a few last things that we need to resolve.

- Figure 5 is missing

- When you resubmit will you remove the figures from the MS text. As long as we have the figures uploaded as separate files then we are good. The figure legends should be at the very end

- We require a Data Availability section. As far as I can see no data is generated that needs to be deposited in a database. If this is correct please state: This study includes no data deposited in external repositories

- Can you double check that the reference format is OK

- Please also make sure you add the funding info to submission system

- We encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. It would be great if you could provide me with a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files.

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That should be all - let me know if we need to discuss anything further

Best Karin

Karin Dumstrei, PhD
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Please 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:
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- 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
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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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The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 4th Jan 2021.

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2nd Revision - Editorial Decision 26th Oct 2020

Dear Leo,

Thank you for submitting your revised manuscript. I have now had a chance to take a look at it and all looks good. I am therefore very pleased to accept the manuscript for publication here.

Congratulations on a nice study!

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
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Corresponding Author Name: Leo James
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2020-106228

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- ➔ a specification of the experimental system investigated (eg cell line, species name).
- ➔ the assay(s) and method(s) used to carry out the reported observations and measurements
- ➔ an explicit mention of the biological and chemical entity(ies) that are being measured.
- ➔ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- ➔ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- ➔ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- ➔ a statement of how many times the experiment shown was independently replicated in the laboratory.
- ➔ definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ 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.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Power analysis
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We performed a power analysis in order to determine sample sizes for each group.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	We did not exclude any animals from our data sets.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Different investigators dosed and monitored the animals than those who performed the tissue analysis.
For animal studies, include a statement about randomization even if no randomization was used.	We did not use randomization.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	We did not use blinding
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes
Is there an estimate of variation within each group of data?	There is a measured determination of error
Is the variance similar between the groups that are being statistically compared?	Yes

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6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	We used the anti-nucleoprotein antibody KL53, as described in: Zeller, W., Bruns, M., & Lehmann-Grube, F. (1988). Lymphocytic choriomeningitis virus X. Demonstration of nucleoprotein on the surface of infected cells. Virology, 162(1), 90–97. https://doi.org/10.1016/0042-6822(88)90397-2
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Sources of all cells lines are ATCC

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

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	We used C57BL/6 mice of both genders. Animals were between 8 to 12 weeks of age. Animals were housed at the MRC ARES facility and husbandry was performed according to the Home Office Animals (Scientific Procedures) Act (1986) and approved by the Medical Research Council Animal Welfare and Ethical Review Body. Animals were bred within the facility.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Experiments were conducted in accordance with the 19.b.7 moderate severity limit protocol and Home Office Animals (Scientific Procedures) Act (1986) and approved by the Medical Research Council Animal Welfare and Ethical Review Body.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We did not use ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Not applicable
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not applicable
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Not applicable
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Not applicable
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not applicable
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not applicable
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not applicable

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Provided
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	All data is contained within the work.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	Not applicable
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	Checked

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No