EMBO reports

Tissue-like Environments Shape Functional Interactions of HIV-1 with Immature Dendritic Cells

Lara Gallucci, Tobias Abele, Raffaele Fronza, Bettina Stolp, Vibor Laketa, Samy Ahmed, Annica Flemming, Barbara Müller, Kerstin Göpfrich, and Oliver Fackler
DOI: 10.15252/embr.202356818

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Review Timeline:

Submission Date:	12th Jan 23
Editorial Decision:	16th Jan 23
Revision Received:	9th Feb 23
Editorial Decision:	16th Mar 23
Revision Received:	21st Mar 23
Accepted:	29th Mar 23

Editor: Achim Breiling

Transaction Report: This manuscript was transferred to EMBO reports following peer review at The EMBO Journal.

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

Dear Prof. Fackler,

Thank you for transferring your study to EMBO reports. I now went through your manuscript and the referee reports on your revised manuscript at The EMBO Journal (attached below again). The referees have remaining concerns and suggestions to improve the manuscript, or to strengthen the data and the conclusions drawn. Looking also through your appeal letter sent to the The EMBO Journal and the referee cross-commenting, I now ask you to address these concerns in a final revised manuscript by clarifying the remaining points and by a major re-writing of the text (as indicated in your appeal letter). As referee #1 indicated during cross-commenting, s/he agrees with referee #2 that the "authors could either provide the controls asked for or proceed to a major re-writing of the manuscript so that their conclusions are supported by the data with no ambiguity". Thus, please do that and also provide a final p-b-p-response addressing the remaining points.

Revised manuscripts should be submitted within three months of a request for revision. Please contact me to discuss the revision (also by video chat) if you have questions or comments.

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

- We allow up to 8 main figures and 5 EV figures for an article published in EMBO reports. Thus, please try to combine the data you show in the present EV figures to have 5 or supply additional supplementary material as a single pdf file labeled Appendix. The Appendix should have page numbers and needs to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.

- Please provide a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.

- Please add a 'Data Availability' section (DAS) to the manuscript. Please list there the accession numbers and database that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study. Please make sure the data is public latest upon publication of the study.

Data availability section

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

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])
- We now request the publication of original source data with the aim of making primary data more accessible and transparent to the reader. It seems you have been contacted already by our source data coordinator, who indicated which figure panels we would need source data for. I attach again the source data checklist. Please make sure that all the requested source data is provided. Please upload all source data for one figure as one pdf per figure (main or EV figures) or (if there is more than one file) ZIPed together as one folder. Please provide the source data for the Appendix as one file or folder. Finally, please upload the filled in source data checklist with your final revised files.

- Regarding data quantification and statistics, please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also for potential EV figures and all those in the final Appendix). Please also check that all the p-values are explained in the legend, and that these fit to those shown in the figure. Please provide statistical testing where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates. See also:
http://www.embopress.org/page/journal/14693178/authorguide#statisticalanalysis

Please add complete statistical testing to all diagrams (main, EV and Appendix figures). Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant. In case n=2, please show the data as separate datapoints without error bars and statistics.

Most importantly: If n<5, please show single datapoints for diagrams!

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

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

- Please name the movie files 'Movie EVx' and add callouts for these using these names to the manuscript text. Please upload the movie files ZIPed together with a text file containing a title and a legend. Finally, please remove the movie legends from the manuscript text file.

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

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

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

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

Kind regards,

Achim

Achim Breiling
Senior Editor
EMBO Reports

Referee #1:

The authors have addressed all my questions and performed additional controls that sustain their findings. I thank the authors for clarifying some aspects of their work that were less clear to me in the first manuscript, and for improving their paper accordingly.

Referee #2:

While the study is interesting, the links between the different observations are still not clear. Unfortunately, the authors were not able to answer the referee's concerns, and some of the controls they now provide such as morphology on 2D collagen or T cell / DC encounter in 3D vs 2D strongly suggests their observations cannot be explain by 3D vs 2D environment as it is now stated.

The authors should provide the controls asked for, or proceed to a major re-writing of the manuscript so that their conclusions

are supported by the data with no ambiguity. The observations are still interesting, but as such, the reviewer thinks there is a lot of interpretation and even though the model proposed is "nice", it is not supported by the data at this stage.

Referee #3:

In the original manuscript, they authors made a series of interesting observations regarding the biology of human immature dendritic cells and their response to HIV infection, my main concern was the lack of functional link between these different observations.

Unfortunately, the authors did not or could not perform the suggested experiments to provide some these links (in particular with respect to mechanosensing/morphological changes). As we suggested, the authors tested the impact of collagen and showed that culturing DCs in 2D in the presence of collagen is sufficient to induce the observed changes in 3D. This raises the question of whether the 3D environment itself is in fact critical (as stated in the title and in the manuscript). Thus, despite some other efforts made by the authors, the revised manuscript has minimally addressed my concerns.

Rebuttal Letter

Referee #1:

The authors have addressed all my questions and performed additional controls that sustain their findings. I thank the authors for clarifying some aspects of their work that were less clear to me in the first manuscript, and for improving their paper accordingly.
Reply: Thank you very much – we are glad that we were able to address all previous comments.

Referee #2:

While the study is interesting, the links between the different observations are still not clear. Unfortunately, the authors were not able to answer the referee's concerns, and some of the controls they now provide such as morphology on 2D collagen or T cell / DC encounter in 3D vs 2D strongly suggests their observations cannot be explain by 3D vs 2D environment as it is now stated.
Reply: The reviewer requested experiments to assess the functional role of DC-SIGN upregulation for increased permissivity for infection to link our different observations. To adress this point we had included new results with the anti-DC-SIGN blocking antibody that support that upregulation of DC-SIGN cell surface levels in 3D collagen is mechanistically linked to these effects. Similarly, the newly provided iDC / T cell encounter analysis by two-photon microscopy shows that iDCs in 2D suspension move at higher displacement speed and contact CD4 T cells with higher frequency as compared to 3D collagen. In our opinion, this explains why we observe higher virus transfer rates in 2D suspension. To avoid any misunderstanding, the text in which these results are described and discussed was carefully revised and limitations to the interpretation of these findings emphasized. Regarding the impact of 3D and 2D collagen matrices, we believe that there were a couple of misunderstandings, which we attempted to resolve in the revised manuscript. First with respect to the microscopy analysis of iDC morphology, we think that the referee might have been inadvertently misled by the fact that, in the merged pdf in figure 8B of the former revision, the panel of iDC morphology in 2D collagen did not appear. This panel was included in the submitted source file of the former revision, but was somehow lost during pdf conversion and we apologize for not having spotted this mistake. As can be seen in the full figure now included and in line with the description in the text, the morphology of iDCs in 2D collagen is visibly different from that in 3D collagen. This suggests that to acquire a fully elongated morphology reminiscent of that of iDCs in vivo, a 3D environment is required.
Second, we fully agree that adhesion to 2D collagen is sufficient to recapitulate some of the functional adaptations of iDC observed in 3D collagen including DC-SIGN upregulation and enhanced permissivity to productive HIV infection. We therefore describe that relevant functional properties of iDCs are altered by two different modes of interaction with a tissue environment encountered by a migrating iDC. Importantly, these properties are distinct from that of iDCs kept in 2D suspension cultures traditionally used in HIV research. We significantly revised the text and manuscript title to emphasize this and made sure that labels in all figures unambiguously designate 2D suspension, 2D collagen or 3D collagen cultures.

The authors should provide the controls asked for, or proceed to a major re-writing of the manuscript so that their conclusions are supported by the data with no ambiguity. The observations are still interesting, but as such, the reviewer thinks there is a lot of interpretation and even though the model proposed is "nice", it is not supported by the data at this stage.
Reply: as suggested by the reviewer, we proceeded to a major re-writing of the manuscript and adjusted its title for more accurate description and interpretation of our observations (also see reply to previous point). We also amended the model figure by including the 2D collagen condition.

Referee #3:

In the original manuscript, they authors made a series of interesting observations regarding the biology of human immature dendritic cells and their response to HIV infection, my main concern was the lack of functional link between these different observations.

Unfortunately, the authors did not or could not perform the suggested experiments to provide some these links (in particular with respect to mechanosensing/morphological changes).
Reply: As mentioned in our previous reply, we were unfortunately unable to address this aspect due to cytotoxicity and lack of feasibility of the micropatterning approach in the BSL3 laboratory within the time frame allocated for the revision. In the discussion with the editor offered with the invitation to submit a revised version of the manuscript, four main concerns raised by the three reviewers were identified to be addressed with high priority by new experimentation: effect on HIV-2 naturally expressing Vpx, mechanistic insight in the role of DC-SIGN upregulation, comparison of 2D and 3D collagen conditions and assessment of cell motility/interaction frequency. The two last points were major concerns also raised by referee#3. Considering this discussion and the technical difficulties described above, we have addressed the mechanosensing aspects in the text and proceeded to a major re-writing as also suggested by referee#2.

As we suggested, the authors tested the impact of collagen and showed that culturing DCs in 2D in the presence of collagen is sufficient to induce the observed changes in 3D. This raises the question of whether the 3D environment itself is in fact critical (as stated in the title and in the manuscript). Thus, despite some other efforts made by the authors, the revised manuscript has minimally addressed my concerns.
Reply: We fully agree that polarized contact to 2D collagen is sufficient to recapitulate some of the functional adaptations of iDC observed in 3D collagen including DC-SIGN upregulation and enhanced permissivity to productive HIV infection. We therefore describe that relevant functional properties of iDCs are altered by two different modes of interaction with a tissue environment encountered by a migrating iDC. Importantly, these properties are distinct from that of iDCs kept in 2D suspension cultures traditionally used in HIV research. To emphasize these differences between 2D/3D collagen and 2D suspension, we significantly revised the text and manuscript title to emphasize this and made sure that labels in all figures unambiguously designate 2D suspension, 2D collagen or 3D collagen cultures.

Editorial Requests

- We allow up to 8 main figures and 5 EV figures for an article published in EMBO reports. Thus, please try to combine the data you show in the present EV figures to have 5 or supply additional supplementary material as a single pdf file labeled Appendix. The Appendix should have page numbers and needs to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.
Reply: We moved four EV figures to the Appendix in a single pdf: Former FigureEV2 is now Appendix Figure 1; Former Figure EV3 is now Appendix Figure 2; Former FigureEV7 is now Appendix Figure 3; Former Figure EV8 is now Appendix Figure 4.

- Please provide a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.
Reply: Author checklist is provided

- Please add a 'Data Availability' section (DAS) to the manuscript. Please list there the accession numbers and database that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study. Please make sure the data is public latest upon publication of the study.

Data availability section

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

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])
Reply: A data availability section is included with reference to the accession number and link to the database used to deposit the microarray data generated in our study. As of now, data are not publicly available but reviewers have access using the token: spwbisqipncjbmv. The data will be made available upon publication of the study.

- We now request the publication of original source data with the aim of making primary data more accessible and transparent to the reader. It seems you have been contacted already by our source data coordinator, who indicated which figure panels we would need source data for. I attach again the source data checklist. Please make sure that all the requested source data is provided. Please upload all source data for one figure as one pdf per figure (main or EV figures) or (if there is more than one file) ZIPed together as one folder. Please provide the source data for the Appendix as one file or folder. Finally, please upload the filled in source data checklist with your final revised files.
Reply: Original source data is provided as requested.

- Regarding data quantification and statistics, please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also for potential EV figures and all those in the final Appendix). Please also check that all the p-values are explained in the legend, and that these fit to those shown in the figure. Please provide statistical testing where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates. See also:
http://www.embopress.org/page/journal/14693178/authorguide#statisticalanalysis

Please add complete statistical testing to all diagrams (main, EV and Appendix figures). Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant. In case n=2, please show the data as separate datapoints without error bars and statistics.

Most importantly: If n<5, please show single datapoints for diagrams!

Reply: Figures and text have been revised accordingly. SD and statistical analyses have been removed from data sets with only two replicates. Statistical tests have been explained and corrected according to the indications provided as comment in the manuscript file. n.s. has been added in the Figure 3C.

- Please add scale bars of similar style and thickness to the microscopic images using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend.
Reply: Scale bars of the same thickness have been added to the lower right corner of all microscopy images and their sizes are defined in the figure legends.

- Please note our reference format:
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat
Reply: References were formatted accordingly.

- 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 add a statement declaring your competing interests. Please name that section 'Disclosure and Competing Interests Statement' and put it after the acknowledgements section.
Reply: A respective statement was included.

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https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines
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- Please order the manuscript sections like this using these names:
Title page - Abstract - Keywords - Introduction - Results - Discussion - Materials and Methods - Data availability section (DAS) - Acknowledgements - Disclosure and Competing Interests Statement - References - Figure legends - Expanded View Figure legends
Reply: Paragraphs are ordered accordingly.

- Please also make sure that all the funding information is also entered into the online submission system and is complete and similar to the one in the manuscript text file (acknowledgements).
Reply: Done as requested.

- Please name the movie files 'Movie EVx' and add callouts for these using these names to the manuscript text. Please upload the movie files ZIPed together with a text file containing a title and a legend. Finally, please remove the movie legends from the manuscript text file.
Reply: Movies are named, called out and provided accordingly.

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text and comments. Please use the attached file as basis for further revisions and provide your final manuscript file with track changes, in order that we can see any modifications done.
Reply: We used this file as basis for our revision and tracked all changes made.

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

Dear Prof. Fackler,

Thank you for the submission of your revised manuscript to our editorial offices. I have now received the reports from the two referees that were asked to re-evaluate your study, you will find below. As you will see, both referees now fully support the publication of your study in EMBO reports.

Before we can proceed with formal acceptance, I have these further editorial requests I ask you to address in a final revised version of the manuscript:

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

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

- Please also provide full scientific titles for the EV figures describing the data shown (not just 'related to Figure x').

- Please remove the heading 'Expanded View' from the manuscript text file. Please order the manuscript sections like this, using (only) these names:
Title page - Abstract - Keywords - Introduction - Results - Discussion - Materials and Methods - Data availability section - Acknowledgements - Disclosure and Competing Interests Statement - References - Figure legends - Expanded View Figure legends

- The Data Availability Section should be restricted to information on datasets submitted to external repositories. Thus, please remove any information regarding requests and lead contacts from this section. Moreover, please make sure that the GEO dataset is public latest on the online publication date of the manuscript.

- Please remove the author information from the Appendix file. It is enough to state 'Appendix for ...' followed by the table of contents. Please provide this file as pdf and please use the nomenclature Appendix Figure Sx for names and titles of the figures (the S is missing at some places) and for the callouts in the manuscript text.

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

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

Best,

Achim Breiling
Senior Editor
EMBO Reports

Referee #1 (Referee #3 from The EMBO Journal submission):

The authors proceeded to a major rewriting of the manuscript, which makes it both clearer and more rigorous scientifically in the description of the results.

Referee #2 (Referee #2 from The EMBO Journal submission):

By rewriting and clarifications the authors have improved the manuscript and the link between the data and the conclusions of the paper.

I believe that this interesting manuscript is suitable for publication in EMBO reports.

- Please provide individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission. Please consult our guide for figure preparation:
http://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf
Reply: Figures have been scaled/resized to comply with the figure guide lines, and are provided as individual tiff files.

- Please also provide full scientific titles for the EV figures describing the data shown (not just 'related to Figure x').
Reply: Full titles were included for all figures.

- Please remove the heading 'Expanded View' from the manuscript text file. Please order the manuscript sections like this, using (only) these names:
Title page - Abstract - Keywords - Introduction - Results - Discussion - Materials and Methods - Data availability section - Acknowledgements - Disclosure and Competing Interests Statement - References - Figure legends - Expanded View Figure legends
Reply: The expanded view heading as well as the title of the subparagraph “Limitation of the study” were removed. Data availability is now called data availability section and the order of the paragraphs is as outlined above.

- The Data Availability Section should be restricted to information on datasets submitted to external repositories. Thus, please remove any information regarding requests and lead contacts from this section. Moreover, please make sure that the GEO dataset is public latest on the online publication date of the manuscript.
Reply: Information regarding leading contact and requests were removed and the process to render the GEO data set available launched.

- Please remove the author information from the Appendix file. It is enough to state 'Appendix for ...' followed by the table of contents. Please provide this file as pdf and please use the nomenclature Appendix Figure Sx for names and titles of the figures (the S is missing at some places) and for the callouts in the manuscript text.
Reply: Author information were removed, Figure are now referred to as Appendix Figure Sx and scientific titles are included.

- Please make sure that all figure panels (main, EV and Appendix figures) are called out separately and sequentially. Presently, a callout for Appendix Fig. S3E seems missing. Please check.
Reply: Appendix Figure S3E is now also called out in the text.

- Please upload the movies separately. Each movie should be ZIPed with its legend and uploaded as one ZIP per movie.
Reply: Done as requested.

- Thanks for providing the source data. Please upload all source data for one figure as one pdf per figure or (if there is more than one file) ZIPed together as one folder. There should then be one ZIPed folder for each main figure (named Source data fro Fig x). Moreover, please upload the EV and Appendix SD as separate folders, one with the source data for all the EV Figures ZIPed together (with separate folders for each figure), and one for the Appendix (with separate folders for each figure).
Reply: Done as requested.

- Please upload the synopsis image as a separate file in jpeg or tiff format and please note the size (exact width of 550 pixels and a height of not more than 400 pixels). Please also make sure that fonts are not too small in that format/size.

Reply: Figure is provided accordingly

Prof. Oliver Fackler
University Hospital Heidelberg
Department of Infectious Diseases, Integrative Virology
INF 344
Heidelberg 69120
Germany

Dear Prof. Fackler,

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

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

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Senior Editor
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Abridged guidelines for figures

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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 Presentation.

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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?	Yes	Dara Availability Section

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	Materials and Methods

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	Materials and methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Materials and methods (peripheral blood from anonymous donors)
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	cells are routinely tested for mycoplasma contamination

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.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

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.	Yes	Materials and Methods

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?	Yes	Material and Methods and Acknowledgments

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	
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? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	Figure Legends
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	Material and Methods and Figure Legends

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

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.	Yes	Materials and methods (receipt of peripheral blood from anynymous donors, ethocs vote S-024/2022)
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.	Not Applicable	
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	