

Resident macrophages restrain pathological adipose tissue remodeling and protect vascular integrity in obese mice

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Referee #1 Review

Received: 10th Feb 21

Report for Author:

Summary

The current manuscript by Chen et al. explores the interaction between adipose tissue macrophages (ATM) and their surrounding microenvironment within white adipose tissue (WAT) in mice. In particular, the authors demonstrate that MHCII^{high}-Cd11⁻ and MHCII^{low}-Cd11⁻ cells represent the main ATM sub-populations in lean animals while Cd11⁺ cells become prominent during the development of high fat diet (HFD)-induced obesity. These latter cells also showed higher lipid and energy mobilizing capacity as compared with the other sub-types. Depletion of all

Cd169 positive ATMs by a diphtheria toxin- based approach led to adipocyte hypertrophy, aberrant adipokine secretion and an overall increase in eWAT mass upon HFD feeding. These physiological changes correlated with alterations in genes involved in lipid homeostasis under both lean and obese conditions. Depletion of ATMs was found to perturb the Cd45- cell fraction under obese conditions, overall contributing to a fibrosis-like gene expression signature. Confirming previous findings, the MHCII^{high}/low cell populations were documented to reside in close proximity to blood vessels. Ablation of ATMs led to a significant loss of endothelial cells, vascular remodeling, and vascular leakiness under obese conditions. In addition, ATM-depleted WAT showed signs of enhanced extracellular matrix remodeling, fiber deposition and fibrosis. Overall, the authors conclude that ATMs are critical for the maintenance of WAT integrity and thus play a rather beneficial role in adipose tissue function.

General comments

Adipose tissue dysfunction is at the core of obesity and its related complications. Indeed, beyond its simple mass, proper WAT function is known to contribute to systemic energy homeostasis by acting as an endocrine organ, further underlining the notion that WAT is more than a simple energy storage organ. In this setting, the cellular composition of WAT is critical for its function and a number of studies have highlighted the importance of immune cells in this context, particularly focusing on ATMs. Here, Chen et al. provide a detailed and thorough description of WAT alterations in response to ATM depletion, thus providing support for the critical importance of ATMs for tissue integrity. The study employs state-of-the-art technology, is well-written and structured, and key conclusions are supported by experimental data. However, the following main concerns require additional attention by the authors: a) The study suffers from a principal lack of mechanistic understanding, i.e. how do ATMs in fact communicate with their microenvironment to cause all the described changes? Thus, the current manuscript remains at a rather descriptive level and can be seen more as a resource paper with rather limited conceptual/mechanistic advance. The study would significantly benefit from an example case describing the ATMs impact on lipid metabolism, absence of CD31 cells etc. b) The manuscript would further benefit from a verification of main phenotypes in independent mouse models. While crossing DTR mice into db or ob backgrounds would admittedly go beyond the scope of this study, the authors could at least try to correlate ATM gene signatures with functional WAT properties in these models. In this respect, demonstrating relevance for human adipose tissue, e.g. using human adipocytes with co-cultures of ATM-like cells, would further underline the relevance of the reported findings.

Minor comment

1. How does ATM depletion affect brown adipose tissue function? Please provide data on BAT histology and gene expression profiles.
2. The metabolic characterization of the employed HFD mouse model is insufficient. The authors should provide a minimum of metabolic data to define the state of obesity investigated, e.g. GTT, ITT, body weight curves, fasting glucose, serum insulin, serum TGs.

Referee #2 Review

Received: 18th Feb 21

Report for Author:

The manuscript by Chen et al. entitled "Resident macrophages restrain pathological adipose tissue remodelling and protect vascular integrity in obese mice" investigates the role of white adipose tissue macrophages during high fat diet-induced obesity. The authors first profiled the myeloid

landscape during obesity development similar to their previous publication (Chen & Ruedl, 2020). Next they analysed different myeloid subsets by various techniques including RNA-Seq, histology and OCR, thereby identifying CD11c⁺ ATM as cells with the highest lipid metabolism. Taking advantage of different myeloid depletion system (the CD169-DTR mouse line and anti-Csf1r treatment), the authors show that depletion of CD11c⁺ ATMs affects the lipid composition in the fat tissue and disrupts the vascular integrity. Also an increase in fibrosis and immune response was observed after CD11c⁺ ATM depletion, indicating that these cells are important for eWAT remodelling.

The paper is well written and the amount of work substantial. The techniques throughout the manuscript are of high standard and the experiments are well performed. However, there are a couple of issues that need attention.

Major issues:

1. The DTx depletion system is a very sensitive way to induce cell death. For instance, the CD11c-DTR mouse cannot be treated for a long period of time with DTx, before the mouse develops a lethal myocarditis, which is not the case in BM chimeric animals (Männ et al., 2016; 27184067). This observation is not explained in detail, maybe leakage of the CD11c-DTR construct or expression of CD11c by non-hematopoietic cells, but it shows, how careful transgenic DTx-based system should be used and controlled - recently summarised by the last author herself (Ruedl & Jung, 2018). In the here presented paper, the authors show a drastic depletion of capillary endothelial cells in DTx-treated CD169-DTR animals, resulting in vascular leakage (Fig. 7D). This effect was clearly visible in HFD CD169-DTR mice (Fig. 5D, 7B), but the scRNA-Seq data also emphasises a reduction of VEC1 cells in ND CD169-DTR mice (Fig. 5D). The CD169-DTR mouse in fact also shows higher leakage in other diseases and other organs as shown by the authors (Gupta et al., 2016; 27477286; Purnama et al., 2014; 26487623). In order to rule out that this is a direct depletion effect of DTx on non-hematopoietic cells (namely endothelial cells), the authors should convincingly show that CD31⁺ cells are not depleted by repeated DTx treatment in CD169-DTR mice. This could be done for various organs by flow cytometric quantification of CD31⁺ cells and Evan blue staining in DTx-treated ND CD169-DTR mice. However, a much more convincing approach would be the usage of BM chimeric mice: BM of CD169-DTR mice should be transferred into irradiated, fat tissue-protected WT (CD45.1/1) mice. After HFD, the mice should receive DTx and the depletion of capillary endothelial cells and vascular leakage needs to be analysed. Histology or flow analysis for CD169 to prove that stromal cells are CD169 negative is not enough to clarify this point. Since only one molecule is enough to kill a cell by DTx (Yamaizumi et al., 1978; 699044), FACS and histology do not provide sufficient depth to exclude off-target effects.

2. The Tim-4 flow cytometry data presented in Fig. 1B was already used in an earlier publication of the authors (Chen & Ruedl, 2020; Fig. 5A).

3. Please present in Fig. 5C also the expression of Siglec1. It is important to know, if some CD45^{neg} cells express this gene, which might influence the depletion results (see point 1).

4. It seems that the transcriptomic data of Fig. 2 will be saved for another publication. However, the bulk RNA data is presented here for the first time and therefore should be shown entirely (the specified GSE148386 number does not exist). It is important to see, how the cells cluster and relate to each other and what kind of genes - other than lipid-related genes - are deregulated between the ATMs. Also provide a more detailed analysis of the genes involved in lipid metabolism.

5. The authors write in line 378-381 that the inflammatory expression was increased in non-hematopoietic cells isolated from depleted HFD animals (Fig. 8G). However, this is not really visible

from the violin blots. Can the authors represent statistic to support their statement?

Minor things (typos and clarifications):

- page 5 line 114: "CD11c+ cells, which are almost undetectable in young lean mice (Fig 1A and C), only start to infiltrate eWAT with increasing age" This is not really visible through the figure; either delete or quantify or reference.
- page 8; line 171-173: "...restricted to the three ATM subpopulations and is not seen in any other cell types (Chen & Ruedl, 2020)" Please specify here. In the mentioned publication, only hematopoietic cells were investigated by CD169 flow cytometry.
- page 14; line 319: "CD169-DT"
- page 15; line 330 & 332: "mmp12"
- page 15; line 341-342 "CD11c+ ATM fraction is the fraction most closely associated with blood vessels, in particular capillaries, and supports blood vessel tone and integrity." Tone was actually not shown here.
- page 16; line 373 "Fig. 8G" Please add "and Fig EV7A"
- The "CX3CR1GFP" line (page 23, line 516) was not used in the study
- Please add a short protocol of cell isolation on page 23, line 535 and do not just refer to another publication
- Please check gene names vs. protein names throughout the manuscript. For instance, figure 8G+H indicates protein, but it should be transcript. Also "MMP12" in figure 7H or EV7 etc.
- Check the spelling of "heatmap" throughout the figure
- It would be more appropriate to show the SD and not SEM of the data. The SD quantifies the scatter/variability of the data. SEM does not actually give information about data variability but quantifies how accurately one knows the true mean of a population. And also please use appropriate statistical test for comparison of 3 or more groups and not t test (Fig. 2B/J; 3F; 7F/H (one-way ANOVA)).
- Fig 1b: indicate what's on y-axis
- Page 36; line 859 and page 40; line 935: Please add "mean +/-" before SEM
- Page 39; line 913: "Sixteen" it's actually seventeen
- Please indicate in Fig. 4A that the HFD WT and ND WT were treated with DTx
- Fig 4C: What lipids are shown in the heatmap? Was a p-value or FC used?
- Fig. 5A: It is unclear, why the authors decided on the presented gating strategy for the scRNA-Seq experiment. Why did the authors gate out all important F4/80+ ATM cell populations and included other leukocytes?
- Page 42; line 985: ...ASC1 (top, ...) and stromal cell clusters (bottom,...) - NOT left & right
- Fig. 8D+E. What are ASC1, what are stromal cells?
- The authors use the word "dramatic" occasionally, which should be avoided
- Why did the authors use for Fig EV5 a MHCII staining for the HFD instead of CD11c as done before? How can they discriminate between MHCII+ ATMs and CD11c+ ATMs?

Dear Christiane,

Thank you for transferring your manuscript to EMBO Reports, which was previously reviewed at The EMBO Journal.

Having read the manuscript and the referee reports, I would like to invite you to submit a revised manuscript to EMBO Reports.

Please address all referee concerns except for the ones regarding the mechanism (referee #1, general comment b) and the BAT related phenotypes (referee #1, minor comment 1). Regarding the point 2 of referee #2, please refrain from re-using previously published data and consider referencing the previous publication instead. As for the point 4 of referee #2, please make sure to make GSE148386 dataset is available. Or at least provide a referee password at this stage. The dataset will have to be made publicly available prior to publication. Please see point 9 below.

Please revise your manuscript with the understanding that the referee concerns (as in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

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.

*** Temporary update to EMBO Press scooping protection policy:

We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic and have therefore extended our 'scooping protection policy' to cover the period required for a full revision to address the experimental issues highlighted in the editorial decision letter. Please contact the scientific editor handling your manuscript to discuss a revision plan should you need additional time, and also if you see a paper with related content published elsewhere.***

IMPORTANT NOTE: we perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:

1. A data availability section providing access to data deposited in public databases is missing (where applicable).
2. Your manuscript contains statistics and error bars based on $n=2$ or on technical replicates. Please use scatter plots in these cases.

You can submit the revision either as a Scientific Report or as a Research Article. For Scientific Reports, the revised manuscript can contain up to 5 main figures and 5 Expanded View figures. If the revision leads to a manuscript with more than 5 main figures it will be published as a Research Article. In this case the Results and Discussion section should be separate. If a Scientific Report is submitted, these sections have to be combined. This will help to shorten the manuscript text by

eliminating some redundancy that is inevitable when discussing the same experiments twice. In either case, all materials and methods should be included in the main manuscript file.

Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page with page numbers, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

Please note that for all articles published beginning 1 July 2020, the EMBO Reports reference style will change to the Harvard style for all article types. Details and examples are provided at <https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

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).

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. For more details on our Transparent Editorial Process, please visit our website:

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You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

4) a complete author checklist, which you can download from our author guidelines (<http://embor.embopress.org/authorguide>). 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 (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<http://embor.embopress.org/authorguide>).

6) 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. See detailed instructions regarding expanded view here: <<http://embor.embopress.org/authorguide#expandedview>>.

- 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.

7) We would also encourage you to include the source data for figure panels that show essential data.

Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available <<http://embor.embopress.org/authorguide#sourcedata>>.

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

9) Please make sure to include a Data Availability Section before submitting your revision - if it is not applicable, make a statement that no data were deposited in a public database. Primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see <<http://embor.embopress.org/authorguide#dataavailability>>).

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

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

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

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

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

10) Regarding data quantification, please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

Please note that error bars and statistical comparisons may only be applied to data obtained from at least three independent biological replicates.

Please also include scale bars in all microscopy images.

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

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

Kind regards,

Deniz

Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

Point-By-Point Reply

We thank the reviewers for their constructive comments on our work. We have tried to address as well as we can on the experimental concerns and provide detailed answers to the raised remarks. Amendments in the manuscript have been highlighted in red.

We hope that with the described changes and responses, the paper is suitable for publication in EMBO Reports

Referee #1:

Summary

The current manuscript by Chen et al. explores the interaction between adipose tissue macrophages (ATM) and their surrounding microenvironment within white adipose tissue (WAT) in mice. In particular, the authors demonstrate that MHCII^{high}-Cd11⁻ and MHCII^{low}-Cd11⁻ cells represent the main ATM sub-populations in lean animals while Cd11⁺ cells become prominent during the development of high fat diet (HFD)-induced obesity. These latter cells also showed higher lipid and energy mobilizing capacity as compared with the other sub-types. Depletion of all Cd169 positive ATMs by a diphtheria toxin- based approach led to adipocyte hypertrophy, aberrant adipokine secretion and an overall increase in eWAT mass upon HFD feeding. These physiological changes correlated with alterations in genes involved in lipid homeostasis under both lean and obese conditions. Depletion of ATMs was found to perturb the Cd45⁻ cell fraction under obese conditions, overall contributing to a fibrosis-like gene expression signature. Confirming previous findings, the MHCII^{high}/low cell populations were documented to reside in close proximity to blood vessels. Ablation of ATMs led to a significant loss of endothelial cells, vascular remodeling, and vascular leakiness under obese conditions. In addition, ATM-depleted WAT showed signs of enhanced extracellular matrix remodeling, fiber deposition and fibrosis. Overall, the authors conclude that ATMs are critical for the maintenance of WAT integrity and thus play a rather beneficial role in adipose tissue function.

General comments

Adipose tissue dysfunction is at the core of obesity and its related complications. Indeed, beyond its simple mass, proper WAT function is known to contribute to systemic energy homeostasis by acting as an endocrine organ, further underlining the notion that WAT is more than a simple energy storage organ. In this setting, the cellular composition of WAT is critical for its function and a number of studies have highlighted the importance of immune cells in this context, particularly focusing on ATMs. Here, Chen et al. provide a detailed and thorough description of WAT alterations in response to ATM depletion, thus providing support for the critical importance of ATMs for tissue integrity. The study employs state-of-the-art technology, is well-written and structured, and key conclusions are supported by experimental data. However, the following main concerns require additional attention by the authors: a) The study suffers from a principal lack of mechanistic understanding, i.e. how do ATMs in fact communicate with their microenvironment to cause all the described changes? Thus, the current manuscript remains at a rather descriptive level and can be seen more as a resource paper with rather limited conceptual/mechanistic advance. The study would significantly benefit from an example case describing the ATMs impact on lipid metabolism, absence of CD31 cells etc. b) The manuscript would further benefit from a verification of main phenotypes in independent mouse models. While crossing DTR mice into db or ob backgrounds would admittedly go beyond the scope of this study, the authors could at least try to correlate ATM gene signatures with functional WAT properties in these models. In this respect, demonstrating

relevance for human adipose tissue, e.g. using human adipocytes with co-cultures of ATM-like cells, would further underline the relevance of the reported findings.

Minor comment

1. How does ATM depletion affect brown adipose tissue function? Please provide data on BAT histology and gene expression profiles.

2. The metabolic characterization of the employed HFD mouse model is insufficient. The authors should provide a minimum of metabolic data to define the state of obesity investigated, e.g. GTT, ITT, body weight curves, fasting glucose, serum insulin, serum TGs.

We have included some metabolic data obtained from the serum collected from obese WT and CD169-DTR mice with their resident macrophages depleted for 12 days. Adiponectin, leptin, insulin and FFAs serum levels are now included in a new supplementary figure (Fig. EV2). In addition, we have included the body weight curve as well as the GTT results obtained from mice which ATMs were depleted for 12 days.

Referee #2:

The manuscript by Chen et al. entitled "Resident macrophages restrain pathological adipose tissue remodelling and protect vascular integrity in obese mice" investigates the role of white adipose tissue macrophages during high fat diet-induced obesity. The authors first profiled the myeloid landscape during obesity development similar to their previous publication (Chen & Ruedl, 2020). Next they analysed different myeloid subsets by various techniques including RNA-Seq, histology and OCR, thereby identifying CD11c⁺ ATM as cells with the highest lipid metabolism. Taking advantage of different myeloid depletion system (the CD169-DTR mouse line and anti-Csf1r treatment), the authors show that depletion of CD11c⁺ ATMs affects the lipid composition in the fat tissue and disrupts the vascular integrity. Also an increase in fibrosis and immune response was observed after CD11c⁺ ATM depletion, indicating that these cells are important for eWAT remodelling.

The paper is well written and the amount of work substantial. The techniques throughout the manuscript are of high standard and the experiments are well performed. However, there are a couple of issues that need attention.

Major issues:

1. The DTx depletion system is a very sensitive way to induce cell death. For instance, the CD11c-DTR mouse cannot be treated for a long period of time with DTx, before the mouse develops a lethal myocarditis, which is not the case in BM chimeric animals (Männ et al., 2016; 27184067). This observation is not explained in detail, maybe leakage of the CD11c-DTR construct or expression of CD11c by non-hematopoietic cells, but it shows, how careful transgenic DTx-based system should be used and controlled - recently summarised by the last author herself (Ruedl & Jung, 2018).

In the here presented paper, the authors show a drastic depletion of capillary endothelial cells in DTx-treated CD169-DTR animals, resulting in vascular leakage (Fig. 7D). This effect was clearly visible in HFD CD169-DTR mice (Fig. 5D, 7B), but the scRNA-Seq data also emphasises a reduction of VEC1 cells in ND CD169-DTR mice (Fig. 5D). The CD169-DTR mouse in fact also shows higher leakage in other diseases and other organs as shown by the authors (Gupta et al., 2016; 27477286; Purnama et al., 2014; 26487623). In order to rule out that this is a direct depletion effect of DTx on non-hematopoietic cells (namely endothelial cells), the authors

should convincingly show that CD31⁺ cells are not depleted by repeated DTx treatment in CD169-DTR mice. This could be done for various organs by flow cytometric quantification of CD31⁺ cells and even blue staining in DTx-treated ND CD169-DTR mice. However, a much more convincing approach would be the usage of BM chimeric mice: BM of CD169-DTR mice should be transferred into irradiated, fat tissue-protected WT (CD45.1/1) mice. After HFD, the mice should receive DTx and the depletion of capillary endothelial cells and vascular leakage needs to be analysed. Histology or flow analysis for CD169 to prove that stromal cells are CD169 negative is not enough to clarify this point. Since only one molecule is enough to kill a cell by DTx (Yamaizumi et al., 1978; 699044), FACS and histology do not provide sufficient depth to exclude off-target effects.

To address the concern regarding a possible expression of CD169 (Siglec1) by non-haematopoietic cells we re-analysed our scRNAseq data and clearly show that this molecule is not expressed by any CD45^{neg} cell type in the adipose tissue, inclusive VEC1 and VEC2. Therefore we are convinced that the disappearance of CD31⁺ cells is due to a protective effect of macrophages on vasculature, in particular during metabolic dysfunction (shown in this manuscript) as well as during infection (shown in Gupta et al, 2016 and Purnama et al 2014). A new Fig. EV7 was added.

2. The Tim-4 flow cytometry data presented in Fig. 1B was already used in an earlier publication of the authors (Chen & Ruedl, 2020; Fig. 5A).

We have taken away the Tim-4 expression profile shown in Fig. 1B.

3. Please present in Fig. 5C also the expression of Siglec1. It is important to know, if some CD45^{neg} cells express this gene, which might influence the depletion results (see point 1).

We have added a new supplementary figure (Fig. EV7), showing the CD169 (Siglec1) expression profile in all CD45^{neg} adipose tissue cells, inclusive VEC1 and VEC2.

4. It seems that the transcriptomic data of Fig. 2 will be saved for another publication. However, the bulk RNA data is presented here for the first time and therefore should be shown entirely (the specified GSE148386 number does not exist). It is important to see, how the cells cluster and relate to each other and what kind of genes - other than lipid-related genes - are deregulated between the ATMs. Also provide a more detailed analysis of the genes involved in lipid metabolism.

The data have been correctly deposited under GSE148386 and we asked GEO to released them to the public, which was done Thursday 11 of March 2021. Data should be now available.

5. The authors write in line 378-381 that the inflammatory expression was increased in non-hematopoietic cells isolated from depleted HFD animals (Fig. 8G). However, this is not really visible from the violin blots. Can the authors represent statistic to support their statement?

We have included the statistic (Wilcoxon) done between the clusters 0 (ASC1), 1 (ASC2) and 6 (stromal cells) and rephrased the sentence.

Minor things (typos and clarifications):

- page 5 line 114: "CD11c⁺ cells, which are almost undetectable in young lean mice (Fig 1A and C), only start to infiltrate eWAT with increasing age" This is not really visible through the figure; either delete or quantify or reference.

We disagree with the reviewer, it is clear from the Fig. 1C that in young mice the absolute numbers (y-axis) are quite low and they are increasing with age and development of obesity.

- page 8; line 171-173: "...restricted to the three ATM subpopulations and is not seen in any other cell types (Chen & Ruedl, 2020)" Please specify here. In the mentioned publication, only hematopoietic cells were investigated by CD169 flow cytometry.

We have taken away the sentence "and is not seen in any other cell types" and included a new Fig. EV7 which shows the lack of expression in CD45^{neg} eWAT cells.

- page 14; line 319: "CD169-DT" **CD169-DT was corrected in CD169-DTR**

- page 15; line 330 & 332: "mmp12" **corrected**

- page 15; line 341-342 "CD11c+ ATM fraction is the fraction most closely associated with blood vessels, in particular capillaries, and supports blood vessel tone and integrity." Tone was actually not shown here. **"Tone" was taken away**

- page 16; line 373 "Fig. 8G" Please add "and Fig EV7A" . **A new Fig EV9A, instead of "old" Fig. EV7, was accordingly added**

- The "CX3CR1GFP" line (page 23, line 516) was not used in the study : **The staining profile of Cx3CR1 shown in Fig. 1B was obtained using the CX₃CR-1^{GFP} transgenic mouse.**

- Please add a short protocol of cell isolation on page 23, line 535 and do not just refer to another publication. **A short isolation description was added to the M&M section.**

- Please check gene names vs. protein names throughout the manuscript. For instance, figure 8G+H indicates protein, but it should be transcript. Also "MMP12" in figure 7H or EV7 etc.

- Check the spelling of "heatmap" throughout the figure. **Corrected**

- It would be more appropriate to show the SD and not SEM of the data. The SD quantifies the scatter/variability of the data. SEM does not actually give information about data variability but quantifies how accurately one knows the true mean of a population. And also please use appropriate statistical test for comparison of 3 or more groups and not t test (Fig. 2B/J; 3F; 7F/H (one-way ANOVA)). **To visualize the scatter/variability of the data, we have now converted the original bar charts to combined scatter and bar charts. Therefore, SEM was not changed into SD. The statistics was amended accordingly.**

- Fig 1b: indicate what's on y-axis : **Added (relative cell counts) and legend accordingly corrected.**

- Page 36; line 859 and page 40; line 935: Please add "mean +/-" before SEM . **Added**

- Page 39; line 913: "Sixteen" it's actually seventeen . **Sixteen was corrected in seventeen.**

- Please indicate in Fig. 4A that the HFD WT and ND WT were treated with DTx. **This was already indicated in M&M section: "CD169-DTR⁺ and CD169-DTR⁻ mice were intraperitoneally (i.p.) injected with 20 ng/g DT (Sigma) every 3 to 4 days to maintain the depletion of CD169⁺ ATMs". We have now added (WT) after CD169-DTR⁻**

- Fig 4C: What lipids are shown in the heatmap? Was a p-value or FC used?

We have now included the names of the lipids (modified figure 4). The values used were abundance values.

- Fig. 5A: It is unclear, why the authors decided on the presented gating strategy for the scRNA-

Seq experiment. Why did the authors gate out all important F4/80⁺ ATM cell populations and included other leukocytes?

The have intentionally excluded the F4/80^{hi} ATM fraction since this population represents more than 80% of the total CD45⁺ cells in the WAT and the remaining cells would have been not “fairly” represented in our scRNA seq analysis. The main purpose of the scRNAseq was to see the consequences of the depletion of ATMs on other CD45⁻ and CD45⁺ cells. In this manuscript, we have focused mainly on the CD45⁻ fraction, we are currently analysing in more detail the CD45⁺ fraction and the results will be presented in a separate manuscript.

- Page 42; line 985: ...ASC1 (top, ...) and stromal cell clusters (bottom,...) - NOT left & right .
corrected.

- Fig. 8D+E. What are ASC1, what are stromal cells?

ASC1 represents adipocyte stem cell 1 (mentioned at line 246). In the eWAT there are two major populations of ASCs, ASC1 and ASC2. Each ASC cluster shows specific expressed genes as well as common ones. They can be distinguished from each other by differential expression of genes associated with ECM production and turnover (Rayanne B. Burl et al., *Cell Metabolism*, 2018).

Stromal cells are adipose-derived multipotent mesenchymal stromal cells, including fibroblasts. This information was now added at line 246.

- The authors use the word "dramatic" occasionally, which should be avoided . Dramatic was exchanged in “major”.

- Why did the authors use for Fig EV5 a MHCII staining for the HFD instead of CD11c as done before? How can they discriminate between MHCII⁺ ATMs and CD11c⁺ ATMs?

In our evaluation of the proximity of ATMs to the nerves, we were forced to use the anti-MHC II antibody, since anti CD11c and anti Tuj1 Abs are from the same species hence these two antibodies can't be paired together in the same staining. We opted to visualize the whole MHC class II⁺ population which includes both CD11c negative as well as positive fractions.

Dear Christiane,

Thank you for submitting your revised manuscript. It has now been seen by both of the original referees.

As you can see, the referees find that the study is significantly improved during revision and recommend publication. However, referee #1 (former referee #2) has outstanding concerns, which were also brought up in the first round of revision. I have discussed these concerns further with the referee #2 (former referee 1#).

Referee #2 agrees with referee #1 that analyzing the CD31+ cells in DTx-treated ND CD169-DTR mice by FACS and quantify the Evans blue influx into various organs are required for ruling out the leakage of the CD11c-DTR and for publication here. We agree that this concern needs to be addressed.

Also, please address the remaining minor points of referee #1.

In addition, I need you to address the editorial points below before I can accept the manuscript:

- We can accommodate maximum 5 keywords. Thus, please select 5 of the keywords provided and delete the rest.
- Please rename the 'Declaration of Interests' section as 'Conflict of Interests'.
- As per our format requirements, in the reference list, citations should be listed in alphabetical order and then chronologically, with the authors' surnames and initials inverted; where there are more than 10 authors on a paper, 10 will be listed, followed by 'et al.'. Please see <https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>
- We noted that the panels of Figures EV1 - EV7 are not called out in the text.
- The movies should be ZIPed with their legends. The legends need to be removed from the text file. The nomenclature needs to be corrected to 'Movie EV#'.
- For technical reasons, our limit for expanded view (EV) figures is 5 (please see our author guidelines <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>). You currently have 9 EV figures. You can either combine figures into 5 expanded view figures, or this could be turned into an appendix file, with the correct nomenclature "Appendix Figure S1" etc. and a table of contents added to the first page. Either way, please update the callouts in the text.
- Papers published in EMBO Reports include a 'synopsis' and 'bullet points' to further enhance discoverability. Both are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short standfirst summarizing the study in 1 or 2 sentences that summarize the paper and are provided by the authors and streamlined by the handling editor. I would therefore ask you to include your synopsis blurb and 3-5 bullet points listing the key experimental findings.
- In addition, please provide an image for the synopsis. This image should provide a rapid overview of the question addressed in the study but still needs to be kept fairly modest since the image size cannot exceed 550x400 pixels.
- Our production/data editors have asked you to clarify several points in the figure legends (see attached document). Please incorporate these changes in the attached word document and return it with track changes activated.

Thank you again for giving us to consider your manuscript for EMBO Reports, I look forward to your

minor revision.

Kind regards,

Deniz

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Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

Referee #1:

The authors answered most of my concerns adequately. However, some important issues were insufficiently corrected.

-One of my major concerns was connected to side effects of the CD169-DTR mouse. I mentioned various points including:

A) quantification of VEC cells in ND CD169-DTR mice (original statement: "This effect was clearly visible in HFD CD169-DTR mice (Fig. 5D, 7B), but the scRNA-Seq data also emphasises a reduction of VEC1 cells in ND CD169-DTR mice (Fig. 5D)"),

B) analysis of CD31+ cells in various organs of DTx treated CD169-DTR mice (original statement: "This could be done for various organs by flow cytometric quantification of CD31+ cells and even blue staining in DTx-treated ND CD169-DTR mice.") and

C) the generation of bone marrow chimera.

I stated that RNA analysis is not enough to exclude the possible leakiness of the CD169-DTR system, which might lead to the unexpected loss of CD31+ cells in HFD CD169-DTR mice. The authors now show in their revised version only the RNA single cell data of Siglec1 expression and claim that Siglec1 is not expressed by VEC cells. Based on this data, I agree that the 10X Chromium system did not pick up any endogenous Siglec1 molecule in VECs, but again, this does not rule out any leakiness of the transgenic BAC-based CD169-DTR system. Furthermore, due to its shallow sequencing, low abundant expressed genes are likely to be missed in 10X experiments (see for instance PMID: 33662621). Since it is uncertain, where the BAC integrated into the genome, the endogenous Siglec1 expression is nothing more than a rough estimate of the BAC-based Siglec1 promoter activity.

I agree that the BM chimera approach is a long (even though the ultimate) experiment and due to the pandemic situation maybe hard to perform, however, analysing CD31+ cells in DTx-treated ND CD169-DTR mice by FACS (!) and quantify the evans blue influx into various organs is an appropriate demand. Also the reanalysis of Fig. 5D+E in order to show the abundance of VEC1 and VEC2 cells in ND CD169-DTR mice is possible (this point is hard to get from the Fig. 5E heatmap). Last but not least, the authors could annotate Dtr expression in their scRNA-Seq data and additionally to Siglec1 show Dtr expression.

-Please mention in Fig. 1b that the CX3CR1 signal is actually expression and not protein (accordingly mention in Figure 1b: Cx3cr1-Gfp).

-In order to judge the variability of their data, the authors changed their graph style. However, this does not justify the continuous use of SEM. SEM quantifies uncertainty in estimate of the mean whereas SD indicates dispersion of the data from mean (see for instance PMID: 21206631,

15311405, 25354300, 33402813...). Here, the authors show dispersion from mean and should use throughout the study SD.

-In my previous review I asked "Fig. 8D+E. What are ASC1, what are stromal cells?" This question was connected to the Figure and the Figure legends. It's not stated, which graph in the Figure represents ASC1 and which one stromal cells. Please indicate.

Referee #2:

The authors responded appropriately to the initial concerns and have improved the manuscript significantly.

We acknowledge both reviewers final comments/requests. We have tried to address as well as we can the experimental concerns on the DTR mouse model used in our work and provided detailed answers to the raised remarks. Amendments in the manuscript have been highlighted in red. The editorial points have been also addressed and corrected.

We hope that with the described changes and responses, the paper is now suitable for publication in EMBO Reports.

Referee #1:

The authors answered most of my concerns adequately. However, some important issues were insufficiently corrected.

-One of my major concerns was connected to side effects of the CD169-DTR mouse. I mentioned various points including:

A) quantification of VEC cells in ND CD169-DTR mice (original statement: "This effect was clearly visible in HFD CD169-DTR mice (Fig. 5D, 7B), but the scRNA-Seq data also emphasises a reduction of VEC1 cells in ND CD169-DTR mice (Fig. 5D)"),

B) analysis of CD31+ cells in various organs of DTx treated CD169-DTR mice (original statement: "This could be done for various organs by flow cytometric quantification of CD31+ cells and evan blue staining in DTx-treated ND CD169-DTR mice.") and

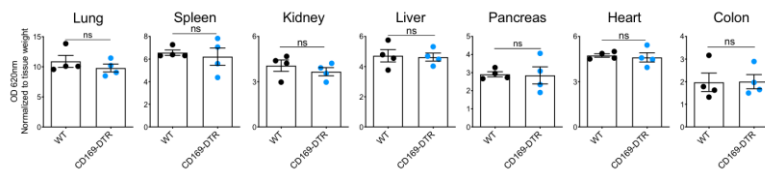
C) the generation of bone marrow chimera.

I stated that RNA analysis is not enough to exclude the possible leakiness of the CD169-DTR system, which might lead to the unexpected loss of CD31+ cells in HFD CD169-DTR mice. The authors now show in their revised version only the RNA single cell data of Siglec1 expression and claim that Siglec1 is not expressed by VEC cells. Based on this data, I agree that the 10X Chromium system did not pick up any endogenous Siglec1 molecule in VECs, but again, this does not rule out any leakiness of the transgenic BAC-based CD169-DTR system. Furthermore, due to its shallow sequencing, low abundant expressed genes are likely to be missed in 10X experiments (see for instance PMID: 33662621). Since it is uncertain, where the BAC integrated into the genome, the endogenous Siglec1 expression is nothing more than a rough estimate of the BAC-based Siglec1 promoter activity. I agree that the BM chimera approach is a long (even though the ultimate) experiment and due to the pandemic situation maybe hard to perform, however, analysing CD31+ cells in DTx-treated ND CD169-DTR mice by FACS (!!) and quantify the evans blue influx into various organs is an appropriate demand. Also the reanalysis of Fig. 5D+E in order to show the abundance of VEC1 and VEC2 cells in ND CD169-DTR mice is possible (this point is hard to get from the Fig. 5E heatmap). Last but not least, the authors could annotate Dtr expression in their scRNA-Seq data and additionally to Siglec1 show Dtr expression.

We understand the concern of the reviewers regarding a possible expression of human HBEGF on CD31 endothelial cells, but we are still confident that the disappearance of these cells is caused by the absence of CD169⁺ macrophages, for following reasons:

- 1) The role of vasculature-associated macrophages (also called perivascular macrophages) on vascular permeability as well as integrity has been already described in many other tissues/organs (here a recent review of DePalma et al, 2018). For example ablation of macrophages in the intestine leads to loss VE-Cadherin⁺ blood vessels and enhanced vascular leakage (De Schepper, Verheijden et al., 2018).
In our work, we extend this observation to the white adipose tissue. As described in our manuscript, we observed that absence of adipose tissue macrophages leads to vascular leakage, phenomenon which is more pronounced under HFD than under ND feeding regiment.
- 2) To further solidify this point, we have added an Evans blue analysis of white adipose tissue obtained from 7 day DT-treated CD169 DTR male mice and WT controls kept under ND (6 months) (Fig EV4), data not shown in our previous manuscript version. A slight, however non-significant increase of vasculature leakage was observed in macrophage-depleted lean mice.

- 3) Additionally, we have added a flow cytometry analysis of CD31⁺ cells obtained from adipose tissue collected from ND treated CD169 DTR lean male mice and WT mice (6 months old) which were maintained 7 days without macrophages (Fig. EV4).
- 4) To visualize a possible expression of human HBEGF in the adipose tissue, our bioinformaticians extensively reanalysed the scRNAseq data. Cellranger-3.1.0 was used to align reads against human and mouse hybrid reference genome (refdata-cellranger-hg19-and-mm10-3.0.0 downloaded from 10x website). No expression of human HBEGF was detected as shown in a violin plot included now in Appendix Fig S3. Please note that F4/80^{hi} macrophages were sorted out and were not present in our samples.
- 5) We have analysed also the Evans blue “leakage” in other organs too, see figure below. We have tested 4 mice with the same age and sex used for the scRNA seq analysis (6 months old, and males). CD169⁺ macrophages were maintained depleted for 7 days and organs were collected and processed at day 7. As shown in the figure below, under this condition, no significant difference was detected between DT-injected WT and CD169-DTR mice.



Since we are already exceeding the figure numbers as well as for a better flow of the manuscript, we would prefer not to include this analysis to the our current work, only in case the reviewer still requests this.

- 6) We have included in the discussion section a paragraph mentioning the contribution of macrophages on vasculature permeability/integrity in other organs.

We hope that we could finally convince the reviewers that the reduction of endothelial cells is not an artefact of the used DTR mouse model.

- Please mention in Fig. 1b that the CX3CR1 signal is actually expression and not protein (accordingly mention in Figure 1b: Cx3cr1-Gfp).

Done, as requested.

-In order to judge the variability of their data, the authors changed their graph style. However, this does not justify the continuous use of SEM. SEM quantifies uncertainty in estimate of the mean whereas SD indicates dispersion of the data from mean (see for instance PMID: 21206631, 15311405, 25354300, 33402813...). Here, the authors show dispersion from mean and should use throughout the study SD.

Done, as requested.

-In my previous review I asked "Fig. 8D+E. What are ASC1, what are stromal cells?" This question was connected to the Figure and the Figure legends. It's not stated, which graph in the Figure represents ASC1 and which one stromal cells. Please indicate.

Done, as requested.

Dear Christiane,

Thank you for submitting your revised manuscript. We have now received the input from referee #1 (please see below). Also, I have now looked at everything else and all is fine. Therefore, I am very pleased to accept your manuscript for publication in EMBO Reports.

Congratulations on a nice work!

Kind regards,

Deniz

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Deniz Senyilmaz Tiebe, PhD

Editor

EMBO Reports

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Referee #1:

The authors sufficiently answered all my question and added the important control experiment. I congratulate the authors to this nice manuscript.

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Journal Submitted to: EMBO reports

Manuscript Number: EMBOR-2021-52835

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

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
 - common tests, such as t-test (please specify whether paired vs. unpaired), simple 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.

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?	The sample size was chosen accordingly to the test performed and after the total samples are also pooled cells/tissue obtained from more mice.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Animals used were aged gender matched depending on the availability of the mice. Minimum of 4-5 mice were used and often for some analysis such as scRNAseq pooled adipose tissue cells obtained from more mice were analysed.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	High fat diet treated mice were considered obese when their body weight was above 40- 42 g. Only males were used throughout the study.
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.	no randomization was used.
For animal studies, include a statement about randomization even if no randomization was used.	no randomization was used.
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	no blinding was done
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.	N/A
Is there an estimate of variation within each group of data?	N/A

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<http://ClinicalTrials.gov>

<http://www.consort-statement.org>

<http://www.consort-statement.org/checklists/view/32-consort/66-title>

<http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumour-research>

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<http://www.selectagents.gov/>

Is the variance similar between the groups that are being statistically compared?	N/A
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C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	antibody clones as well as company names were added.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

* 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.	Done
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Included
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.	confirm

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
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.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
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.	N/A
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.	N/A

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'.	Done
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	
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).	done
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).	N/A
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 Biocompare (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.	N/A

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.	N/A
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