EMBO reports

FOXM1 network in association with TREM1 suppression regulates NET formation in diabetic foot ulcers

Andrew Sawaya, Rivka Stone, Spencer Mehdizadeh, Irena Pastar, Stephen Worrell, Nathan Balukoff, Mariana Kaplan, Marjana Tomic-Canic, and Maria Morasso
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Transaction Report:

(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 Dr. Morasso,

Thank you for submitting your preliminary point-by-point response. I have now looked at your points carefully. I appreciate that you can address many of the concerns raised and see that the proposed experiments will strengthen the manuscript.

As per your response to the 2nd concern of referee #1, the in vitro experiments you propose are sufficient for us and further in vivo analysis is not required.

Having looked at everything, I would like to invite you to submit a revised manuscript. However, I would like to point out that we need strong support from the referees to consider publication here. It is this aspect that is more difficult to assess at this stage.

Please see the guidelines for the revision below my signature.

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 Senyilmaz Tiebe

Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

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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.

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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. 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.

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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:
https://www.embopress.org/page/journal/14693178/authorguide#transparentprocess
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 (). 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 (). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines ().

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: .

- 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 .

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 .

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).

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.

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Referee #1:

Sawaya et al investigate the role of TREM1/FOXM1 on the formation of neutrophil extracellular traps and their role in impaired wound healing in diabetes. They bring some new information compared with the previous work of this group that might be of interest but needs more orthogonal evidence to secure the pathway proposed.
Major comments
The authors claim that FDI-6 has the same effect as PMA in induction of ROS and NET but in the figure 2B and 2C FDI-6 is the only treatment different from all the others.
Since NAC lacks any effect on the ROS levels and NET formation induced by PMA it is difficult to interpret it's effect in FDI-6 exposed cells (Fig 2b and 2c)?
To bring more evidence on the pathway proposed it is needed to explore the effect of TREM1 on wound healing and NET formation in vivo in the presence of FOXM1 inhibition.
The effect of TREM1 inhibition on wound healing in non diabetic condition should be explored.
Inhibition by FDI-6 should be confirmed by a more specific approach.
Unclear what type of DFU were used that makes difficult to interpret the results.

Referee #2:

Diabetic foot ulcer (DFU) is one of the major complications of the diabetes where wound closure /healing rate of ulcers is severely impaired and can result in lower limb amputation. Various cellular components including neutrophils play significant role in normal wound healing process. In DFU, neutrophil number drops significantly due to loss of the transcription factor FOXM1. They also form neutrophil extracellular traps (NETs) that contribute to tissue damage and impaired healing. Whether FOXM1 promote neutrophils to undergo NET formation is not known. Sawaya et al , has demonstrated here for the first time that TREM1/FOXM1 signalling regulate NET formation through ROS production during diabetic wound healing. This finding is very beneficial as targeting TREM1/FOXM1 signalling could recruit normal neutrophil numbers at the wound site and reduce NET formation to enhance the wound healing outcomes.
Comments:
1. Sawaya et al has compared the expression of various neutrophil signature genes of acute skin wounds with DFU in Figure F1C. However, it is not described anywhere if these were the human or mice samples, how the samples were processed, or how the RNA was isolated for PCR. Please provide the details in method section. Also there is only n=2 in the day3 skin wounds. Please increase the group size to improve the quality of the data.

2. Sawaya et in figure EV1B has provided images of FD1-6 treated neutrophils in glucose environment and diabetic wounds. These are just the representative images and actual quantification of the citH3 and elastase is required to confidently predict that inhibiting FOXM1 increases NET in FD1-6 treated neutrophils and diabetic wounds. Moreover, the magnification and quality of the images is very poor to predict if the staining of elastase and citH3 is real or not. Please provide higher magnification images to determine if the staining is nuclear/intracellular/cell surface. Isotype control should also be provided.
3. To quantify the NET formation by the neutrophils (figure2) , Sawaya et al isolated neutrophils of the peripheral blood of the healthy control subjects. However, its is not clear if the different blood donors were used to perform this study where n represents blood of individual donor, or their blood was pooled and then neutrophils was pooled into different well. How the study was performed will significantly impact the results therefore it is important to have this information. Please also provide n numbers as some of the data points in Fig 2 a, b and C donot have error bars, so does that mean it you only measure one sample for that data set?
4. It is important to provide the representative images of the quantification performed in Fig b and c of ROS production and NET formation to confidently validate the data. Please add them either in Fig 2 or in EV section.
5. Figure 4a, provide representative images showing the wound closure at different timepoint in different groups. The wound area in α-TREM1 treated mice is still large as compared to the vehicle diabetic mice and the wound is still not fully healed by day8. It is possible the neutrophil levels has still not reached the neutrophil levels of the non-diabletic wounds after the α-TREM1 treatment. It will be a good quality control to provide and quantify the FOXM1+ and citH3+ staining in non-diabetic wound and add the data set in figure b and c. The other factors not enabling the wound closure similar to non-diabetic wounds should also be discussed in the discussion. Also need higher magnification for all the images to determine the staining quality as per comment 2. Please provide images with DAPI because it is hard to determine the true staining.
6. Fig 5, need higher magnification images. To improve the quality of the manuscript it will be good to perform FoxM1, citH3 and neutrophil staining in the healing DFU and non healing DFU samples to show that there are more Foxm1+neutrophils and reduced NET formation in the healing samples as compared to the non-healing samples.
7. All the EV figures in the results are labelled as S1, S2 and so on. Change it to appropriate EMBOR format, which is EV1, EV2 etc.

Referee#1

Sawaya et al investigate the role of TREM1/FOXM1 on the formation of neutrophil extracellular traps and their role in impaired wound healing in diabetes. They bring some new information compared with the previous work of this group that might be of interest but needs more orthogonal evidence to secure the pathway proposed.

Major comments

1. The authors claim that FDI-6 has the same effect as PMA in induction of ROS and NET but in the figure 2B and 2C FDI-6 is the only treatment different from all the others. Since NAC lacks any effect on the ROS levels and NET formation induced by PMA it is difficult to interpret it's effect in FDI-6 exposed cells (Fig 2b and 2c)?

Response:

Thank you for pointing this out. We have re-formatted the original figures to separate the FDI-6 treatments from the PMA controls. Our experiments demonstrated that both FDI-6 and PMA induced ROS and NET formation, whereas combination treatment with NAC reversed their effects. The revised figure improves presentation of the data, such that the effects of FDI6 and PMA are clearly visible.

2. To bring more evidence on the pathway proposed it is needed to explore the effect of TREM1 on wound healing and NET formation in vivo in the presence of FOXM1 inhibition.

Response:

We have performed experiments assessing the effects of TREM1 in presence of FOXM1 inhibition in human neutrophils in vitro. We found that TREM1 treatment alone had no significant effect on NET formation compared to vehicle (See Figure EV4). However, combination treatment of TREM1 activator with FOXM1 inhibitor, FDI-6, significantly increased NET formation, further demonstrating FOXM1 to be downstream of TREM1 (See Figure EV4). We believe this directly addresses the reviewer's concerns regarding the effects of TREM1 in the presence of FOXM1 inhibition. However, performing additional experiments in vivo is not feasible in a timely fashion. Due to COVID-19 associated delays in obtaining additional mice and reagents, and restrictions imposed until recently for accessing animal facilities, repeating these experiments in our

diabetic murine model would require a minimum of 6 months. The experiment itself would require 40 mice with 8 treatment conditions and would likely show similar findings but would not provide major additional support for the pathways beyond what we have already demonstrated.

3. The effect of TREM1 inhibition on wound healing in non diabetic condition should be explored.

Response:
We have performed experiments in non-diabetic mice and consistently found no effect of TREM1 modulation on healing rate, as already shown in Figure 4A. We further performed stainings on NETosis readouts (citH3 and FOXM1 stainings) in the non-diabetic conditions and, similarly, found no effects (See Figure EV5).

4. Inhibition by FDI-6 should be confirmed by a more specific approach.
Response:
Other groups have performed a comprehensive biochemical and mechanistic characterization of FDI-6 as a specific inhibitor of FOXM1 via binding to DNA-binding domain (Gormally, MV et al, Nature Communications 2014). Our group further functionally tested FDI-6 as a FOXM1 inhibitor in diabetic murine wounding model (Sawaya et al, Nature Communications 2020). To address this comment specifically, we performed additional experiments to further demonstrate FDI-6 inhibition of FOXM1 in neutrophils, via qPCR of FOXM1 and its target genes. We found FDI-6 treatment inhibited expression FOXM1 and its target gene SOD2, confirming specificity of FDI-6 (See figure EV1).

5. Unclear what type of DFU were used that makes difficult to interpret the results.

Response:
We have expanded the Methods section to include details of how DFU specimens were obtained, inclusion/exclusion criteria, clinical characteristics of patients, and other details that will aid in interpreting results.

Referee #2

Diabetic foot ulcer (DFU) is one of the major complications of the diabetes where wound closure /healing rate of ulcers is severely impaired and can result in lower limb amputation. Various cellular components including neutrophils play significant role in normal wound healing process. In DFU, neutrophil number drops significantly due to loss of the transcription factor FOXM1. They also form neutrophil extracellular traps (NETs) that contribute to tissue damage and impaired healing. Whether FOXM1 promote neutrophils to undergo NET formation is not known. Sawaya et al, has demonstrated here for the first time that TREM1/FOXM1 signalling regulate NET formation through ROS production during diabetic wound healing. This finding is very beneficial as targeting TREM1/FOXM1 signalling could recruit normal neutrophil numbers at the wound site and reduce NET formation to enhance the wound healing outcomes.

Response:
We are grateful to the reviewer for recognizing the importance and potential clinical impact of our findings.

Comments:
1. Sawaya et al has compared the expression of various neutrophil signature genes of acute skin wounds with DFU in Figure F1C. However, it is not described anywhere if these were the human or mice samples, how the samples were processed, or how the RNA was isolated for PCR. Please provide the details in method section. Also there is only n=2 in the day3 skin wounds. Please increase the group size to improve the quality of the data.

Response:
The data in Figure 1 correspond to human datasets of acute skin wounds and diabetic foot ulcers (DFUs). We have updated the figure legend to state the samples are comparison of human acute and DFU wounds in vivo. In addition, we updated the method section to provide details of the sample processing and RNA isolation for qPCRs. Human in vivo sample collection is very limited. We have increased the sample size for the day 3 skin wounds to n=3.

2. Sawaya et in figure EV1B has provided images of FD1-6 treated neutrophils in glucose environment and diabetic wounds. These are just the representative images and actual quantification of the citH3 and elastase is required to confidently predict that inhibiting FOXM1 increases NET in FD1-6 treated neutrophils and diabetic wounds. Moreover, the magnification and quality of the images is very poor to predict if the staining of elastase and citH3 is real or not. Please provide higher magnification images to determine if the staining is nuclear/intracellular/cell surface. Isotype control should also be provided.

Response:
We have included quantification of NET formation and provided higher magnification images. This figure is now figure EV2. We have clarified that the appropriate DMSO vehicle control for experiments involving FDI-6 treatments was used for these experiments, as detailed by Gormally et al., Nature Communications 2014. Isotype IgG controls were used in experiments involving α-TREM1 treatments and have been clarified in methods section and figure legends.

3. To quantify the NET formation by the neutrophils (figure2), Sawaya et al isolated neutrophils of the peripheral blood of the healthy control subjects. However, its is not clear if the different blood donors were used to perform this study where n represents blood of individual donor, or their blood was pooled and then neutrophils was pooled into different well. How the study was performed will significantly impact the results therefore it is important to have this information. Please also provide n numbers as some of the data points in Fig 2 a, b and C do not have error bars, so does that mean it you only measure one sample for that data set?

Response:
Studies with neutrophils were isolated from 3 different blood donors and pooled together for experiments and performed in triplicate per condition. We have updated the figure legend to include sample size. The figure has been updated to more clearly display error bars, which were present in the original figure but difficult to visualize due to small standard deviation between samples at the earlier timepoints.

4. It is important to provide the representative images of the quantification performed in Fig b and c of ROS production and NET formation to confidently validate the data. Please add them either in Fig 2 or in EV section.

Response:
Representative images were added to EV3 to provide further validation of the data.

5. Figure 4a, provide representative images showing the wound closure at different timepoint in different groups. The wound area in α-TREM1 treated mice is still large as compared to the vehicle diabetic mice and the wound is still not fully healed by day8. It is possible the neutrophil levels has still not reached the neutrophil levels of the non-diabletic wounds after the α-TREM1 treatment. It will be a good quality control to provide and quantify the FOXM1+ and citH3+ staining in non-diabetic wound and add the data set in figure b and c. The other factors not enabling the wound closure similar to non-diabetic wounds should also be discussed in the discussion. Also need higher magnification for all the images to determine the staining quality as per comment 2. Please provide images with DAPI because it is hard to determine the true staining.

Response:
We have included images of wound closure at different timepoints as well as quantification of FOXM1 and citH3 in non-diabetic wounds (See EV5). Images with DAPI were added and provided at higher magnification. We have expanded the discussion section to highlight other factors not enabling wound closure.

6. Fig 5, need higher magnification images. To improve the quality of the manuscript it will be good to perform FoxM1, citH3 and neutrophil staining in the healing DFU and non healing DFU samples to show that there are more Foxm1+neutrophils and reduced NET formation in the healing samples as compared to the non-healing samples.

Response:
We have made numerous attempts to perform immunofluorescence stainings of FOXM1 and citH3 in DFU tissue sections. Specifically, we have performed staining with antibodies from both Cell Signaling and Abcam and have tried multiple dilution ranges, blocking reagents, and antigen retrieval approaches. Unfortunately, results in these human pathologic specimens (which we have in limited amount) have been unsuccessful, likely due to harsh protease and degradation environment of the actual wounds. Understanding the additional benefit of these stainings to support the manuscript, we have performed an immunohistochemical staining approach in an attempt to overcome the technical challenges. We were successful in citH3 stainings, however stainings with FOXM1 still remain unsuccessful. However, stainings with citH3 inversely correlated with the healing outcome in which healing DFUs showed decreased citH3 compared to nonhealing DFUs (See added stainings in revised figure 5C).

7. All the EV figures in the results are labelled as S1, S2 and so on. Change it to appropriate EMBOR format, which is EV1, EV2 etc.

Response:
All the labels to the EV figures have been made to the appropriate EMBO Reports format.

We hope that the revised manuscript that we strongly consider being of general interest and significant novelty will be considered for publication.

Dear Dr. Morasso,

Thank you for submitting your revised manuscript. It has now been seen by one of the original referees, whose comments have been copied below.

I apologize for this unusual delay in getting back to you, which was due to the unexpected delay in getting the referee report and protracted internal discussions.

As you can see, the referee appreciates that that more insight has been provided during revision. However, he/she still does not find that the proposed pathway is supported by the data and does not recommend publication. However, following further internal evaluations and discussions, we would like to offer publication pending satisfactory revisions as outlined below.

Given the referee's comments, I have looked into your point-by-point response in detail. I appreciate that you addressed many of the points raised by the referees. However, I have also evaluated the newly provided Figure EV4 in depth, as this is the key panel to support the proposed epistasis between TREM1 and FOXM1 in regulation of NET formation, and I agree with referee #1 that it does not satisfactorily address his/her major comment 2. This was a point that was particularly highlighted in the previous decision. As you also mention in your point-by-point response and the Results section, in Figure EV4, α-TREM1 treatment alone does not affect NET formation. This is expected because there is almost no NET formation detected under basal conditions (vehicle treated sample). As such, in my view, this panel does not support the proposed effect of α-TREM1 on NET formation. I appreciate that the combined treatment of α-TREM1 and FDI-6 is able to induce NET formation. However, in the absence of a condition where there is baseline NET formation, which can be blocked by α-TREM1, and the demonstration that α-TREM1 cannot inhibit NET formation in the presence of FDI-6, I do not find that this panel supports an epistatic relationship between TREM1 and FOXM1 in regulation of NET formation, in my view. I also note that a condition with only FDI-6 treatment is not provided. I have discussed my evaluation with the other members of our editorial team including our chief editor Dr. Bernd Pulverer and they are in agreement. As such, we arrived at similar conclusions to those of the referee's and we are not convinced that there is sufficient evidence supporting that TREM1 regulates NET formation by activating FOXM1.

However, given the demonstration that TREM1 restricts NET formation, promotes recruitment of FOXM1+ neutrophils to the wound site and accelerates diabetic wound healing in vivo, we would like to offer publication pending satisfactory revisions as follows. Please either replace Figure EV4 with more compelling evidence supporting this link, or remove Figure EV4 given the reasons explained above and revise the text to tone down the direct claims on TREM1 being upstream of FOXM1 throughout the manuscript including the title and the abstract. If you choose to do the latter, please perform the textual changes with 'track changes on'.

Moreover, I need you to address the editorial points below.

• We note that the format of the study is better suited for our 'Reports' format. Therefore, please combine the Results and Discussion sections.
• Please provide 3-5 keywords for your study. These will be visible in the html version of the paper and on PubMed and will help increase the discoverability of your work.
• We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Also, please rename the 'Conflict of Interests' section as 'Disclosure statement and competing interests'.
• Regarding the Author Contributions, we now use CRediT to specify the contributions of each author in the journal submission system. CRediT replaces the author contribution section. Please use the free text box to provide more detailed descriptions. See also guide to authors https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines.
• Please make sure that the funding information is complete in the manuscript submission system.
• We note that Fig EV3A&B are currently not called out 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 (max 35 words) 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 Senyilmaz Tiebe

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Deniz Senyilmaz Tiebe, PhD
Scientific Editor
EMBO Reports

Referee #1:

The authors brought some new information but more evidence is needed for ensure the biological relevance of the pathway proposed.

Deniz Senyilmaz Tiebe, PhD
Scientific Editor
EMBO Reports

Dear Dr. Deniz Senyilmaz Tiebe,

We thank you for your thorough review and instructions and for the opportunity to submit a revised manuscript EMBOR-2021-54558V1 previously entitled "**TREM1/FOXM1 signaling regulates formation of neutrophil extracellular traps contributing to diabetic wound healing clinical outcome,"** and we propose a revised title of **"FOXM1 network in association with TREM1 suppression regulates NET formation in diabetic foot ulcers"** that follows your indications and more accurately reflects the data presented in the manuscript.

All the points have been addressed, with the incorporation of the recommended comments and are tracked in the text. Specifically, we have:

1) removed Figure EV4;
2) revised the text throughout the manuscript to tone down the direct claims on role of TREM1 as an upstream of FOXM1 in the DFU healing
3) revised the text to integrate and combine the Results and Discussion sections. All our revisions are highlighted/tracked in the revised file;
4) provided keywords for the study;
5) edited author contributions based on the CRediT;
6) referred to the Fig. EV3A&B in the manuscript text;
7) reviewed and finalized competing interests (no revision was needed);
8) provided a 'synopsis' and 'bullet points';
9) provided an image for synopsis;
10) reviewed and responded to editors' points in figure legends.

We hope that these revisions improved the manuscript's clarity, that is now suitable and acceptable for publication in EMBO reports.
Best regards,

Maria I. Morasso PhD
Senior Investigator
Chief, Laboratory of Skin Biology
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health

Marjana Tomic-Canic, PhD Professor,
Vice Chair of Research
William H. Eaglstein Chair in Wound Healing
Director, Wound Healing and Regenerative
Medicine Research Program
Dr Phillip Frost Department of Dermatology
& Cutaneous Surgery

2nd Revision - Editorial Decision **8th Jun 2022**

Dear Dr. Morasso,

Thank you for submitting your revised manuscript. I have now looked at everything 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 Senyilmaz Tiebe
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Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

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Reporting Checklist for Life Science Articles (updated January 2022)

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data 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?	Not Applicable	

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Materials and Methods

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Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Materials and Methods

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Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Not Applicable	
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Materials and Methods
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

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Please detail **housing and husbandry conditions**.	Yes	Materials and Methods

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Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	
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?	Yes	Materials and Methods
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	Materials and Methods
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Materials and Methods and figure legends

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

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Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Materials and Methods
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If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
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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)
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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	