EMBO reports

MiR-342 controls Mycobacterium tuberculosis susceptibility by modulating inflammation and cell death

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

1st Editorial Decision 20th Jan 2021

Dear Dr. Wu,

Thank you for the submission of your research manuscript to EMBO reports. We have now received the reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, the referees think that these findings are of interest. However, they have several comments, concerns and suggestions, indicating that a major revision of the manuscript is necessary to allow publication of the study in EMBO reports. As the reports are below, and I think all their points need to be addressed, I will not detail them here.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript or in the detailed point-by-point response. Acceptance of your 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 of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision. We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic and we have therefore extended our 'scooping protection policy' to cover the period required for full revision. Please contact me to discuss the revision should you need additional time, and also if you see a paper with related content published elsewhere.

When submitting your revised manuscript, please also carefully review the instructions that follow below.

PLEASE NOTE THAT upon resubmission revised manuscripts are subjected to an initial quality control prior to exposition to re-review. Upon failure in the initial quality control, the manuscripts are sent back to the authors, which may lead to delays. Frequent reasons for such a failure are the lack of the data availability section (please see below) and the presence of statistics based on n=2 (the authors are then asked to present scatter plots or provide more data points).

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the final manuscript text (including legends for main figures, EV figures and tables), but without the figures included. Please make sure that changes are highlighted to be clearly visible. Figure legends should be compiled at the end of the manuscript text.

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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.

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Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines: http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq and array data) are deposited in an appropriate public database. If no primary datasets have been deposited, please also state this in the respective section (e.g. 'No primary datasets have been generated and deposited'), see below.

See also: http://embor.embopress.org/authorguide#datadeposition

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 & Methods) that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

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

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

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

Moreover, I have these editorial requests:

6) We strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. If you want to provide source data, please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

7) 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:
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8) Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many independent experiments (biological replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable, and also add a paragraph detailing this to the methods section. See:
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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,

Achim Breiling
Editor
EMBO Reports

Referee #1:

In the present report, Fu et al., provide a comparative study aiming to decipher the mechanism of resistance/susceptibility to M. tuberculosis (Mtb). They compared the C3HeB/FeJ (C3H) mouse

model, known to be highly sensitive to Mtb infection with the C57BL/6 mouse model, which is normally resistant to Mtb infection. They identified that the resistance phenotype depends on the expression of the microRNA miRNA 342-3p Furthermore, they show that miRNA 342-3p, down regulate SOCS6 leading to the STAT1 dependent induction of pro-inflammatory cytokine as well as inducing macrophage death through apoptosis.

Overall the data are clear, well presented and supports most of the claims. The authors have performed a substantial number of experiments with appropriate controls presented in 8 exhaustive figures. The amount of work as well as the tools and techniques used in the study is very impressive. However, most of the relevant studies in the field are somehow not considered, which affects the interpretation of some the data. It is thus critical that the authors include more key references about macrophage death, or the SOCS-STAT signaling in the context of TB disease.

The authors claim in the manuscript that SOCS6 is the major regulator of cytokine signaling in their infection model and this is a bit an overstatement. It is crucial for this study to clearly show how the miRNA-342-3p and SOCS6 silencing impact other SOCS and STAT family members. This should help to clarify the message of the paper and bring more scientific perspectives to this work. Please find below my comments, which must be addressed before considering the manuscript for publication.

Major comments:

It is critical for the main message of this work to show if both mRNAi-342-3p and the silencing of Socs6 impact the expression of other Socs family members and the activation of the other STATs family members.

Line 210: specific STAT1 inhibitor called Fludarabine. How specific is this inhibitor? Could the authors provide a western blot showing that Fludarabine inhibit only STAT1. I may have missed it but at which concentration the inhibitor was used? Can you add this information in the legend Figure 5 page 36.
Overall manuscript is well written but some parts require editing. Importantly, the authors showed the role of SOCS 6 in the control of TB but both in the introduction and the discussion, the literature regarding the role of JAK-STAT-SOCS signaling in tuberculosis is very poor. Authors must introduce and discuss briefly the previous studies. Some papers are listed below:

Masood KI, Rottenberg ME, Salahuddin N, Irfan M, Rao N, Carow B, Islam M, Hussain R, Hasan Z.BMC Infect Dis. 2013 Jan 15;13:13. doi: 10.1186/1471-2334-13-13.PMID: 23320781

Masood KI, Hussain R, Rao N, Rottenberg ME, Salahuddin N, Irfan M, Hasan Z.J Infect Dev Ctries. 2014 Jan 15;8(1):59-66. doi: 10.3855/jidc.3412.PMID: 24423713

Queval CJ, Song OR, Carralot JP, Saliou JM, Bongiovanni A, Deloison G, Deboosère N, Jouny S, Iantomasi R, Delorme V, Debrie AS, Park SJ, Gouveia JC, Tomavo S, Brosch R, Yoshimura A, Yeramian E, Brodin P.Cell Rep. 2017 Sep 26;20(13):3188-3198. doi: 10.1016/j.celrep.2017.08.101.PMID: 28954234

Masood KI, Rottenberg ME, Carow B, Rao N, Ashraf M, Hussain R, Hasan Z.Scand J Immunol. 2012 Oct;76(4):398-404. doi: 10.1111/j.1365-3083.2012.02731.x.PMID: 22670716

Carow B, Gao Y, Terán G, Yang XO, Dong C, Yoshimura A, Rottenberg ME.Tuberculosis (Edinb). 2017 Dec;107:175-180. doi: 10.1016/j.tube.2017.09.007. Epub 2017 Sep 22.PMID: 29050767

Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, Murray PJ, Barry C, Kaplan G.J Interferon Cytokine Res. 2005 Nov;25(11):694-701. doi: 10.1089/jir.2005.25.694.PMID: 16318583

Kumar R, Sahu SK, Kumar M, Jana K, Gupta P, Gupta UD, Kundu M, Basu J.Cell Microbiol. 2016 May;18(5):679-91. doi: 10.1111/cmi.12540. Epub 2015 Nov 27.PMID: 26513648

Queval CJ, Song OR, Deboosère N, Delorme V, Debrie AS, Iantomasi R, Veyron-Churlet R, Jouny S, Redhage K, Deloison G, Baulard A, Chamaillard M, Locht C, Brodin P.Sci Rep. 2016 Jul 7;6:29297. doi: 10.1038/srep29297.PMID: 27384401

Lastrucci C, Bénard A, Balboa L, Pingris K, Souriant S, Poincloux R, Al Saati T, Rasolofo V, González-Montaner P, Inwentarz S, Moraña EJ, Kondova I, Verreck FA, Sasiain Mdel C, Neyrolles O, Maridonneau-Parini I, Lugo-Villarino G, Cougoule C.Cell Res. 2015 Dec;25(12):1333-51. doi: 10.1038/cr.2015.123. Epub 2015 Oct 20.PMID: 26482950

The authors are also not citing the key publications regarding the modulation of macrophage death by Mtb. I recommend the authors to introduce the current knowledge about JAK-STAT-SOCS signaling in Tuberculosis and then discuss the potential role of other SOCS in the context of TB. As an example, discussion line 392-393, the author claim that SOCS6 is "the main inhibitor of cytokines" without discussing the role of the other SOCS, known to regulate the immune response during Mtb infection. Other publications regarding the role of apoptosis in the cell spread are missing in the discussion (line 344.) For example, Aguilo et al., Cell Microbiol. 2013 and Lerner et al., 2017 JCB should be cited.

Overall, the figures are very clear and the results are convincing. However, the data interpretation Page 11-Line 214 to 219 and Figure 5 is confusing. The fact that, in Socs6-/- BMDM there is more nuclear translocation of STAT1, does not mean that STAT1 is a "downstream transcription factor of Socs6". SOCS are known to inhibit STAT signaling. In that context, by repressing Stat1, Socs6 prevents inflammation and promotes bacterial growth and not the other way around. Please clarify this statement in the manuscript.

In the results sections page 10 lines 204-206, the authors state they examined the phosphorylation levels of the STAT family in Mtb-infected Socs6+/+ and Socs6-/- BMDMs but did not provide any data about it. Furthermore, in the discussion, page 19, lines 390 to 393, the authors stated that SOCS6 is the inhibitor of cytokines in their infection model. This statement is not convincing considering the multiple previous studies showing the role of other SOCS or STAT proteins in the Mtb replication, susceptibility and immune response to TB (please see my first comment). The SOCS-STAT cascades are complex and could be inter-regulated. It is thus possible that the loss of Socs6 expression indirectly impact the signaling cascades of other SOCS and STAT members.

Minor comments:
Some sentence from the abstract and the end of the discussion look very similar (e.g. lines 43-46). Please rephrase them and provide a bit more details regarding the mechanism highlighted in your study.
The authors used the term "multiplication" to refer to the bacterial growth. I would replace "multiplication" by "replication".
line 37-38: replace the sentence by: apoptosis/necrosis balance is known to be critical for the host

susceptibility
Line 39: replace "unknow" by "unclear"
Line 41: remove the word substrain
line 59: replace the sentence by [...] "Key genes, contributing to the regulatory network."
Line 61: replace the sentence by: "Although there are evidences that host miRNAs my impact on microbial life cycle and pathogenesis, it has been..."
Line 62, remove the word "firmly"
Line 64-65: modify the sentence as follow: "Thus, miRNAs likely influence the relation between the hosts and pathogens".
Line 77: after "targeted Socs6" precise if it is expressed or repressed.
Line 109: should it be "which were" instead of "which was"
Line 173: "The data indicated that Socs6 is a target of miRNA-342-3p" for degradation? Could the authors be more specific?
Line 176: would it be more accurate to replace "abrogated in the presence of SOCS6" by "abrogated when we restore SOCS6 expression"?

Referee #2:

Fu et al. have disclosed an important role of miR-342 in the regulation of cell death in M.tb infected macrophages. In addition, the authors describe that forced expression of miR-342-3p in mice confers resistance to M.tb, which is associated with increased cytokine and chemokine. Moreover, the paper links SOCS6, and processing of A20 and RIPK3 with the modulation of macrophage cell death pathways. Finally, some evidence for similar regulatory mechanisms in human PBMC is presented.

Given the medical importance of M.tb. infections and the still largely enigmatic immunology of M.tb. control, unraveling the role of immunological regulators in tuberculosis is of great importance.

miR-342 is not a new kid on the block of immune regulation in infection. It has largely been explored in viral infections. Similarly, miR-342 has already been investigated in macrophages and in the context of cell death, the latter in view of carcinogenesis. Moreover, although the description of a role of miR-342 in M.tb. infections and the detailed signaling effects are novel, the literature on other miRs (and LncRNA regulating them) in macrophage apoptosis is substantial. Accordingly, the concept that miR regulate macrophage apoptosis in M.tb infection has been repeatedly explored before. Still, the comparison of M.tb resistant and susceptible mouse strains is valid, and the strain specific switch between apoptosis and necrosis is interesting. The sheer quantity of provided data is impressive. Moreover, generating Mir342+/+C3H mice and analyzing them in an M.tb infection model is commendable.

Yet, several important issues concerning the execution of the study remain.

Major points:
• Although the comparison of C3H/FeJ and B6 mice is interesting, it is similarly challenging. While miR-342-3p is expressed at substantially higher level in macrophages from B6 mice than from C3H/FeJ mice, it is expressed in C3H/FeJ macrophages as well. However, the genetically modified mouse strains go in different directions. In other words, miR-342-3p is overexpressed in C3H/FeJ mice and knocked-out in B6 mice. This is valid, as long as all other controls are performed in each strain. This is - however -not the case. In particular in the case of SOCS6, where it is not - side-by-

side- overexpressed and suppressed in both strains.

- Fig. 1G: Adding miR-342-3p inhibitor to BL6 BMDMs induces the switch from apoptosis to necrosis (fig. 1F). Why is a significantly lower survival rate only seen if a caspase inhibitor is added (fig. 1G) and not for Mtb alone? How can this be explained? And why is this difference presented in 1G significant but not for ctrl and NC mimic in fig. 1E?
- Fig . 2: It seems unclear how related the Mir342+/+C3H mice are with the controls. Littermates should be used.
- Fig. 5: The authors declare that the increase of Caspase 8 in Socs-/- is STAT1 mediated. This direct link is not experimentally shown. Why did the authors exclusively study expression of Caspases 3, 7 and 8, instead of e.g. caspase 2 (Sironi and Ouchi, 2004)? Is IFNγ expression/production unchanged in Socs6-/- and Socs+/+ treated and untreated BMDM?
- Fig. 8: Authors suggest that the A20-mediated death cell response is completely independent of cytokine production. Why can overexpression of A20 then partly compensate for the cytokine lack in Socs6+/+ fig. 8A?
- The authors write that throughout the manuscript "data are representative of three independent experiments" or "three independent experiments with n = 3 technical replicates are shown". Does this mean that 3 independent experiments with 3 technical replicates were performed? As an example the legend to fig. 2 states "Individual data points represent individual technical replicates": I am not sure what is meant here. It seems obvious that statistics must be performed from biological replicates and not from technical replicates. Technical replicates should not be depicted as individual data points.
- The analysis of cell death mechanisms is depicted in a complicated way. Moreover, it is stated in the methods that Annexin V/PI staining was performed, however, I cannot find the data. Please provide verification of apoptosis in M.tb infected macrophages with methods other than the ApoTox-Glo Triplex Assay.

Minor:

- Fig. S1A: If cell death mechanisms are analyzed, the control condition without M.tb infection is in general missing. Please correct the figure legend S1A: stimulation instead of stimulated.
- Fig. 4E+F Is the scale bar the same for all images?
- Fig. 5 How is cytokine production affected in Mir342-/- B6 + si-Socs6 or Mir342+/+ C3H + si-Socs6?
- Fig. S5 Figure legend indicates that data were analyzed by student´s t-test, but no statistic analysis is shown in the figure.
- Student´s t-test should be used for comparison of two groups only. E.g. 1C, 1E, 1G, 2C, 2D, 2G, 2H should rather be analyzed by multiple t-tests or ANOVA (2C, 2D, 2G, 2H) as more than two groups are shown in one graph. Overall, in some graphs statistical analysis is missing, e.g. fig. 8D.
- Authors analyze PBMC in humans and find similar regulatory mechanisms. PBMC, when extracted from the blood have perhaps not been in contact with M.tb. In vitro BMDMs were directly infected with M.tb. The authors should discuss how the upregulation of miR-342 is initiated and if it is dependent on the direct contact or even phagocytosis of M.tb.
- Line 109 fig. 1A does not show any statistics, so changes cannot be declared as significant.
- Line 353 A limited set of inflammatory cytokines is analyzed in the study, thus the conclusion drawn here is too radical. Please rephrase.
- Line 37 This statement is too categorical. Apoptosis/necrosis are definitely essential factors in the understanding of host susceptibility. But mycobacteria influence the host immune response of macrophages at different levels, including cell division, cell fate, cell metabolism, etc., and not only with respect to cell death mechanisms. Please rephrase this sentence.
- Reference Prosser HM seems to be wrong (James Dooley et al 2017 No Functional Role for microRNA-342 in a Mouse Model of Pancreatic Acinar Carcinoma Front Oncol. 2017 May 18;7:101).

Referee #3:

General comments:
In their paper entitled "MicroRNA 1 controls susceptibility to Mycobacterium tuberculosis by modulating inflammation and cell death mechanism", Fu et al. identify a specific microRNA, miR-342-3p, which appears to regulate cytokine production and switching from Mtb-induced necrosis and apoptosis of macrophages. They nicely complement in vitro work with mouse model work and analyses of clinical samples from TB patients and healthy controls. Overall, the study makes a potentially important contribution to our understanding of TB pathogenesis, and has implications for diagnostics and host-directed therapies. However, there are some issues outlined below, which, if adequately addressed, could further strengthen the conclusions.

Specific comments:
1. Abstract: It is stated that "apoptosis/necrosis is known to be the ultimate factor affecting host susceptibility to Mtb" (lines 37-38). This is an overstatement, and should be moderated by something like the following: "apoptosis/necrosis is an important factor affecting host susceptibility to Mtb." It is stated that these findings may lead to "...the development of novel therapeutic approaches for drug-resistant TB" (line 48); however, M. tuberculosis antibiotic resistance has nothing to do with the study's findings (i.e., any emerging host-directed therapies would be expected to be equally as effective against drug-susceptible TB). This is also mentioned in the last sentence of the Introduction (lines 85-87). Finally, "Mycobacterium tuberculosis" should be italicized in line 35.
2. Introduction: The literature review for the role of host/bacterial miRNAs and SOCS6 and SOCS7 is incomplete. More specific references/information should be included to contextualize how each of these factors are known to regulate innate immune responses. Also, the authors should include a justification for why they focused specifically on miR-342 and SOCS6. The introduction is the weakest section of the paper.
3. Introduction: The authors state it is "firmly established that microbe-derived miRNAs have an unfavorable effect on host anti-microbial immunity" (lines 62-63). However, this is certainly an overstatement, as this is still a relatively new area of research. Also, it is not clear in the next sentence (lines 64-65) if they are referring to bacterial miRNAs or host miRNAs or both.
4. Results: The section describing the mouse models (lines 91-102) would better fit in the Introduction.
5. Results: The rationale for using different mouse strains is clearly stated, given that baseline expression of the miRNA of interest is different in each strain. However, whatever interventions are performed in one strain, should also be performed in the other to enable direct comparisons. This is because miR-342 expression is not the only difference between these mouse strains. Recognizing that this would potentially represent a substantial amount of work, this limitation should at least be very clearly stated in the Discussion.
6. Results (Figure 1): There are some important controls missing. Specifically, the authors should show the effects of both mimic and inhibitor in each cell type, not simply one treatment in each cell type. Even though the expression is opposite in each cell type, they should show results for both to allow for direct comparisons. Since PBMCs (rather than pure macrophages) were used from clinical samples, it should be mentioned that the source of miR-342-3p may also be other cell types (Fig. 1J; lines 127-131).
7. Results (Figure 2): The histology results are not particularly convincing. The authors should consider performing a quantitative stain-based assessment of apoptosis vs necrosis in these

samples.
8. Results (Figure 3): The authors state that Socs6 was selected for further investigation due to putative regions that match the miR-342-3p seed sequence (lines 155-157). However, this justification is not adequate, since all miR-342-3p targets should have seed sequence complementarity.
9. have some important missing controls that will be critical for the accurate interpretation of their results.
10. Results: The section describing the various factors involved in cell death pathways (lines 236-244) belongs in the Introduction.
11. Discussion: It is stated that "... miR-342-3p has the potential to develop into a TB susceptibility marker" (line 339). However, if a biomarker for TB susceptibility was being sought, the study would have benefited from more of an unbiased approach, using various clinical groups and validation sets. The study's findings have more implications for understanding TB pathogenesis, i.e., a new regulatory pathway involved in tissue necrosis, and for developing novel host-based therapies targeting the apoptosis-necrosis switch.
12. General: All of the figures are relatively difficult to follow. The authors should consider simplifying the data, or only presenting that data that are essential to the story. For examples, some of the immunoblots could be moved to the supplemental figures.
13. General: The text requires some attention to syntax/grammar. Also, some of the transitions are abrupt. For example, in the Introduction, SOCS6 is introduced abruptly (line 66) after describing miRNAs.

Responses to comments from Reviewer #1:

Comments: In the present report, Fu et al., provide a comparative study aiming to decipher the mechanism of resistance/susceptibility to M. tuberculosis (Mtb). They compared the C3HeB/FeJ (C3H) mouse model, known to be highly sensitive to Mtb infection with the C57BL/6 mouse model, which is normally resistant to Mtb infection. They identified that the resistance phenotype depends on the expression of the microRNA miRNA 342-3p Furthermore, they show that miRNA 342-3p, down regulate SOCS6 leading to the STAT1 dependent induction of pro-inflammatory cytokine as well as inducing macrophage death through apoptosis.

Overall the data are clear, well presented and supports most of the claims. The authors have performed a substantial number of experiments with appropriate controls presented in 8 exhaustive figures. The amount of work as well as the tools and techniques used in the study is very impressive. However, most of the relevant studies in the field are somehow not considered, which affects the interpretation of some the data. It is thus critical that the authors include more key references about macrophage death, or the SOCS-STAT signaling in the context of TB disease.

The authors claim in the manuscript that SOCS6 is the major regulator of cytokine signaling in their infection model and this is a bit an overstatement. It is crucial for this study to clearly show how the miRNA-342-3p and SOCS6 silencing impact other SOCS and STAT family members. This should help to clarify the message of the paper and bring more scientific perspectives to this work. Please find below my comments, which must be addressed before considering the manuscript for publication.

RESPONSE: We thank Reviewer 1 for the encouraging words and thoughtful comments on our paper. In accordance with your suggestions, we have included discussions and references regarding macrophage death and the SOCS-STAT signaling in the context of TB disease. In addition, we have added relevant experiments to validate the specificity of miRNA-342-3p on SOCS6, and the potential effect of SOCS6 on other SOCS and STAT family members in the revised manuscript. In the following lines, we provide detailed responses to your concerns and comments. Thank you for taking time and effort to help us improve the paper, and we hope that our revisions can meet your requirements.

Major comments:

1. It is critical for the main message of this work to show if both mRNAi-342-3p and the silencing of Socs6 impact the expression of other Socs family members and the activation of the other STATs family members.

RESPONSE: We thank the reviewer for pointing this out, and we agree with the reviewer that the statement was not convincing enough without testing other SOCS and STAT family members during Mtb infection. Considering that other SOCS family members have structures similar to SOCS6, we examined whether they could be

regulated by miR-342-3p. The results showed that miR-342-3p had no significant effect on the 3'UTR activity of other SOCS family members (Appendix Fig. S4A), indicating that miR-342-3p has a relatively specific regulation on SOCS6. Further, the effects of SOCS6 on other SOCS and STAT family members were examined (Appendix Fig. S4B-D, Fig. EV3A). The results showed that SOCS6 could negatively regulate the expression of SOCS7 (Appendix Fig. S4B); however, siRNA knockdown experiments indicated that SOCS7 was dispensable for the Mtb-induced cell death mechanism in CH3 and B6 mice (Appendix Fig. S4C, D). In addition, among the STAT family, STAT3 activation was observed in *Socs6*$^{+/+}$ macrophages infected with Mtb (Fig. EV3A). Nonetheless, the similar tendency of STAT3 activation was also detected in *Socs6*$^{-/-}$ cells (Fig. EV3A). Thus, we speculate that the activation of STAT3 has nothing to do with the existence of SOCS6, and the pathway that SOCS6 mediates the downstream STAT1 is relatively independent of other STAT family members. However, this needs to be validated with further experiments. The corresponding revisions are on Page 13, Line 257-263 and Page 19, Line 388-404.

2. Line 210: specific STAT1 inhibitor called Fludarabine. How specific is this inhibitor? Could the authors provide a western blot showing that Fludarabine inhibit only STAT1. I may have missed it but at which concentration the inhibitor was used? Can you add this information in the legend Figure 5 page 36.

RESPONSE: Fludarabine is a specific inhibitor of STAT1. It has no similar effect on the other STATs according to the manufacturer's instructions (Selleck, USA). Fludarabine has been widely used as a STAT1 specific inhibitor in previous studies (Frank, Mahajan et al., 1999, Wang, Marinis et al., 2018). In this study, we showed that the phosphorylation of STAT1 and STAT3 was upregulated in Mtb-infected *Socs6*$^{-/-}$ BMDMs (Fig. EV3A). Therefore, we have performed additional Western Blotting experiments to check the specificity of Fludarabine on STAT1, and the result showed that Fludarabine only inhibits STAT1, rather than STAT3 (Fig. EV3B). The working concentration of Fludarabine was indicated in Figure 5C (Figure 5B in the revised manuscript) (10 μM), and we have added this information in the corresponding Figure legend (Page 42, Line 986-987).

References:

Frank DA, Mahajan S, Ritz J (1999) Fludarabine-induced immunosuppression is associated with inhibition of STAT1 signaling. Nat Med 5: 444-7

Wang W, Marinis JM, Beal AM, Savadkar S, Wu Y, Khan M, Taunk PS, Wu N, Su W, Wu J, Ahsan A, Kurz E, Chen T, Yaboh I, Li F, Gutierrez J, Diskin B, Hundeyin M, Reilly M, Lich JD et al. (2018) RIP1 Kinase Drives Macrophage-Mediated Adaptive Immune Tolerance in Pancreatic Cancer. Cancer cell 34: 757-774

3. Overall manuscript is well written but some parts require editing. Importantly, the authors showed the role of SOCS 6 in the control of TB but both in the introduction

and the discussion, the literature regarding the role of JAK-STAT-SOCS signaling in tuberculosis is very poor. Authors must introduce and discuss briefly the previous studies. Some papers are listed below:

Masood KI, Rottenberg ME, Salahuddin N, Irfan M, Rao N, Carow B, Islam M, Hussain R, Hasan Z.BMC Infect Dis. 2013 Jan 15;13:13. doi: 10.1186/1471-2334-13-13.PMID: 23320781

Masood KI, Hussain R, Rao N, Rottenberg ME, Salahuddin N, Irfan M, Hasan Z.J Infect Dev Ctries. 2014 Jan 15;8(1):59-66. doi: 10.3855/jidc.3412.PMID: 24423713

Queval CJ, Song OR, Carralot JP, Saliou JM, Bongiovanni A, Deloison G, Deboosère N, Jouny S, Iantomasi R, Delorme V, Debrie AS, Park SJ, Gouveia JC, Tomavo S, Brosch R, Yoshimura A, Yeramian E, Brodin P.Cell Rep. 2017 Sep 26;20(13):3188-3198. doi: 10.1016/j.celrep.2017.08.101.PMID: 28954234

Masood KI, Rottenberg ME, Carow B, Rao N, Ashraf M, Hussain R, Hasan Z.Scand J Immunol. 2012 Oct;76(4):398-404. doi: 10.1111/j.1365-3083.2012.02731.x.PMID: 22670716

Carow B, Gao Y, Terán G, Yang XO, Dong C, Yoshimura A, Rottenberg ME.Tuberculosis (Edinb). 2017 Dec;107:175-180. doi: 10.1016/j.tube.2017.09.007. Epub 2017 Sep 22.PMID: 29050767

Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, Murray PJ, Barry C, Kaplan G.J Interferon Cytokine Res. 2005 Nov;25(11):694-701. doi: 10.1089/jir.2005.25.694.PMID: 16318583

Kumar R, Sahu SK, Kumar M, Jana K, Gupta P, Gupta UD, Kundu M, Basu J.Cell Microbiol. 2016 May;18(5):679-91. doi: 10.1111/cmi.12540. Epub 2015 Nov 27.PMID: 26513648

Queval CJ, Song OR, Deboosère N, Delorme V, Debrie AS, Iantomasi R, Veyron-Churlet R, Jouny S, Redhage K, Deloison G, Baulard A, Chamaillard M, Locht C, Brodin P.Sci Rep. 2016 Jul 7;6:29297. doi: 10.1038/srep29297.PMID: 27384401

Lastrucci C, Bénard A, Balboa L, Pingris K, Souriant S, Poincloux R, Al Saati T, Rasolofo V, González-Montaner P, Inwentarz S, Moraña EJ, Kondova I, Verreck FA, Sasiain Mdel C, Neyrolles O, Maridonneau-Parini I, Lugo-Villarino G, Cougoule C.Cell Res. 2015 Dec;25(12):1333-51. doi: 10.1038/cr.2015.123. Epub 2015 Oct 20.PMID: 26482950

RESPONSE: We greatly appreciate the reviewer for this suggestion. We have carefully read the above references, and introduced and discussed them accordingly in the revised manuscript. In addition, we have added and clarified some other relevant literatures to improve the paper. The corresponding revisions are in the introduction and discussion sections of the revised manuscript.

4. The authors are also not citing the key publications regarding the modulation of macrophage death by Mtb. I recommend the authors to introduce the current knowledge about JAK-STAT-SOCS signaling in Tuberculosis and then discuss the potential role of other SOCS in the context of TB. As an example, discussion line

392-393, the author claim that SOCS6 is "the main inhibitor of cytokines" without discussing the role of the other SOCS, known to regulate the immune response during Mtb infection. Other publications regarding the role of apoptosis in the cell spread are missing in the discussion (line 344.) For example, Aguilo et al., Cell Microbiol. 2013 and Lerner et al., 2017 JCB should be cited.
RESPONSE: We thank the reviewer for this comment. We have added relevant publications about JAK-STAT-SOCS signaling in the context of tuberculosis, and discussed the potential role of other SOCS in regulating the immune response during Mtb infection (Page 5-6, Line 88-101). In addition, we have added and discussed the above mentioned papers regarding the role of apoptosis in the cell spread. The corresponding revision is on Page 20, Line 408-414.

5. Overall, the figures are very clear and the results are convincing. However, the data interpretation Page 11-Line 214 to 219 and Figure 5 is confusing. The fact that, in Socs6-/- BMDM there is more nuclear translocation of STAT1, does not mean that STAT1 is a "downstream transcription factor of Socs6". SOCS are known to inhibit STAT signaling. In that context, by repressing Stat1, Socs6 prevents inflammation and promotes bacterial growth and not the other way around. Please clarify this statement in the manuscript.
RESPONSE: We thank the reviewer for pointing this out, and we have modified this sentence into "The above results suggested that SOCS6 might prevent inflammation and promote bacterial growth by repressing STAT1". The corresponding revision is on Page 13, Line 270-272.

6. In the results sections page 10 lines 204-206, the authors state they examined the phosphorylation levels of the STAT family in Mtb-infected Socs6$^{+/+}$ and Socs6-/- BMDMs but did not provide any data about it. Furthermore, in the discussion, page 19, lines 390 to 393, the authors stated that SOCS6 is the inhibitor of cytokines in their infection model. This statement is not convincing considering the multiple previous studies showing the role of other SOCS or STAT proteins in the Mtb replication, susceptibility and immune response to TB (please see my first comment). The SOCS-STAT cascades are complex and could be inter-regulated. It is thus possible that the loss of Socs6 expression indirectly impact the signaling cascades of other SOCS and STAT members.
RESPONSE: We thank the reviewer for raising this insightful comment, and have included the results of phosphorylation levels of the STAT family members in the revised manuscript (Fig. EV3A). Combined with the previous comments and suggestions raised by the reviewer, we have added experiments and discussions regarding the possibility of inter-regulation of SOCS and STAT members in Mtb infection. Also, the relevant sentence has been rephrased on Page 22, Line 464-465.

Minor comments:
7. Some sentence from the abstract and the end of the discussion look very similar (e.g. lines 43-46). Please rephrase them and provide a bit more details regarding the mechanism highlighted in your study.
RESPONSE: Thanks for this considerate advice. The Abstract and Discussion parts have been modified accordingly in the revised manuscript.

8. The authors used the term "multiplication" to refer to the bacterial growth. I would replace "multiplication" by "replication".
RESPONSE: This issue has been corrected as suggested.

9. line 37-38: replace the sentence by: apoptosis/necrosis balance is known to be critical for the host susceptibility
RESPONSE: This issue has been corrected as suggested.

10. Line 39: replace "unknow" by "unclear"
RESPONSE: This issue has been corrected as suggested.

11. Line 41: remove the word substrain
RESPONSE: This issue has been corrected as suggested.

12. line 59: replace the sentence by [...] "Key genes, contributing to the regulatory network."
RESPONSE: This issue has been corrected as suggested.

13. Line 61: replace the sentence by: "Although there are evidences that host miRNAs my impact on microbial life cycle and pathogenesis, it has been..."
RESPONSE: This issue has been corrected as suggested.

14. Line 62, remove the word "firmly"
RESPONSE: This issue has been corrected as suggested.

15. Line 64-65: modify the sentence as follow: "Thus, miRNAs likely influence the relation between the hosts and pathogens".
RESPONSE: This issue has been corrected as suggested.

16. Line 77: after "targeted Socs6" precise if it is expressed or repressed.
RESPONSE: This issue has been corrected as suggested.

17. Line 109: should it be "which were" instead of "which was"
RESPONSE: This issue has been corrected as suggested.

18. Line 173: "The data indicated that Socs6 is a target of miRNA-342-3p" for

degradation? Could the authors be more specific?
RESPONSE: This sentence has been modified to “These data indicated that *Socs6* is a specific target of miR-342-3p for degradation”.

19. Line 176: would it be more accurate to replace "abrogated in the presence of SOCS6" by "abrogated when we restore SOCS6 expression"?
RESPONSE: This issue has been corrected as suggested. We appreciate the reviewer for all these insightful comments, as well as the detailed modifications of words and sentences.

Responses to comments from Reviewer #2:

Fu et al. have disclosed an important role of miR-342 in the regulation of cell death in M.tb infected macrophages. In addition, the authors describe that forced expression of miR-342-3p in mice confers resistance to M.tb, which is associated with increased cytokine and chemokine. Moreover, the paper links SOCS6, and processing of A20 and RIPK3 with the modulation of macrophage cell death pathways. Finally, some evidence for similar regulatory mechanisms in human PBMC is presented.

Given the medical importance of M.tb. infections and the still largely enigmatic immunology of M.tb. control, unraveling the role of immunological regulators in tuberculosis is of great importance.

miR-342 is not a new kid on the block of immune regulation in infection. It has largely been explored in viral infections. Similarly, miR-342 has already been investigated in macrophages and in the context of cell death, the latter in view of carcinogenesis. Moreover, although the description of a role of miR-342 in M.tb. infections and the detailed signaling effects are novel, the literature on other miRs (and LncRNA regulating them) in macrophage apoptosis is substantial. Accordingly, the concept that miR regulate macrophage apoptosis in M.tb infection has been repeatedly explored before. Still, the comparison of M.tb resistant and susceptible mouse strains is valid, and the strain specific switch between apoptosis and necrosis is interesting. The sheer quantity of provided data is impressive. Moreover, generating Mir342+/+C3H mice and analyzing them in an M.tb infection model is commendable.

Yet, several important issues concerning the execution of the study remain.

RESPONSE: We thank Reviewer 2 for your kind words pertaining to our paper. We appreciate the time and effort spent by you to provide such insightful comments on this study. In the following lines, we provide detailed responses to your concerns and comments. We hope that the revised paper will meet your requirements.

Major points:

1. Although the comparison of C3H/FeJ and B6 mice is interesting, it is similarly challenging. While miR-342-3p is expressed at substantially higher level in macrophages from B6 mice than from C3H/FeJ mice, it is expressed in C3H/FeJ macrophages as well. However, the genetically modified mouse strains go in different directions. In other words, miR-342-3p is overexpressed in C3H/FeJ mice and knocked-out in B6 mice. This is valid, as long as all other controls are performed in each strain. This is - however -not the case. In particular in the case of SOCS6, where it is not - side-by-side- overexpressed and suppressed in both strains.

RESPONSE: We thank the reviewer for raising this concern. We used macrophages derived from SOCS6-knockout C3H mice (*Socs6*$^{-/-}$) and their wild-type littermates (*Socs6*$^{+/+}$) as cell models to study the regulation of SOCS6 on downstream signaling pathways and cell death mode. Although the amount of SOCS6 protein was exquisitely manipulated by the interventions as expected, and these models served to

achieve the purpose of experimental design very well in this study, we agree with the reviewer that it is not a perfect side-by-side case compared with miR-342-3p. To support our argument, we have included Western Blotting experiments to test the protein levels of SOCS6 in macrophages obtained from different mouse strains (Appendix Fig. S4E).
We are aware that this is a limitation of this study, thus we have included a summary of limitations and unanswered questions left hanging in the Discussion section of revised manuscript and hope that our justification is suitable.

2. Fig. 1G: Adding miR-342-3p inhibitor to BL6 BMDMs induces the switch from apoptosis to necrosis (fig. 1F). Why is a significantly lower survival rate only seen if a caspase inhibitor is added (fig. 1G) and not for Mtb alone? How can this be explained? And why is this difference presented in 1G significant but not for ctrl and NC mimic in fig. 1E?
RESPONSE: We thank the reviewer for raising this interesting question. In this study, z-VAD, a pan-caspase inhibitor, was used to distinguish cell death mechanisms. MiR-342-3p inhibitor induced the switch from apoptosis to necrosis in Mtb-infected B6 BMDMs. Interestingly, the cell survival rate was further decreased when z-VAD was added. This observation is consistent with previous reports (He et al., 2009, Zhang et al., 2009). We speculate that in addition to caspase inhibition, z-VAD might be involved in the regulation of cell death patterns via veiled mechanisms. The potential roles of z-VAD in cell death should be determined. We have included this part in the Discussion of revised manuscript. The corresponding revision is on Page 24, Line 497-504.
The Ctrl and NC mimic groups in Fig. 1E also showed the similar phenomenon significantly, and we have added significance symbols in the figure.

References:

He SD, Wang L, Miao L, Wang T, Du FH, Zhao LP, Wang XD (2009) Receptor Interacting Protein Kinase-3 Determines Cellular Necrotic Response to TNF-alpha. Cell 137: 1100-1111

Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han JH (2009) RIP3, an Energy Metabolism Regulator That Switches TNF-Induced Cell Death from Apoptosis to Necrosis. Science 325: 332-336

3. Fig. 2: It seems unclear how related the Mir342+/+C3H mice are with the controls. Littermates should be used.
RESPONSE: In Fig. 2 and Fig. 4, the control groups of *Mir342*$^{+/+}$C3H mice and *Mir342*$^{-/-}$B6 mice (C3H and B6 in the Figure) were indeed the corresponding littermates during the construction of transgene mice. We have clarified that C3H and B6 were wild-type littermates in the Figure Legends as well as in the main text (Page 9, Line 186-187, and Page 38-39, Line 916-927) to avoid potential confusion.

4. Fig. 5: The authors declare that the increase of Caspase 8 in Socs-/- is STAT1 mediated. This direct link is not experimentally shown. Why did the authors exclusively study expression of Caspases 3, 7 and 8, instead of e.g. caspase 2 (Sironi and Ouchi, 2004)? Is IFNγ expression/production unchanged in Socs6-/- and Socs+/+ treated and untreated BMDM?

RESPONSE: According to the reviewer's suggestion, we have added experiments to prove that the increase of Caspase 8 in *Socs6*$^{-/-}$ cells is STAT1-dependent (Fig. EV3J, K). We have included experiments to examine the expressions of Caspase 2 and Caspase 9, which are also known to be related with apoptosis (Hong, Chung et al., 2019, Sironi & Ouchi, 2004). The results showed that SOCS6 had no significant effect on the expression of Caspase 2 and Caspase 9 (Fig. EV3H, I).

Since the release of IFN-γ is closely related to Mtb infection, we have included experiments to test whether SOCS6 regulated IFN-γ expression. The results showed that, IFN-γ expression was increased in both *Socs6*$^{+/+}$ and *Socs6*$^{-/-}$ cells after Mtb infection, however, there was no significant difference between the two cell types (Fig. EV3G). Therefore, SOCS6 was not related to the expression of IFN-γ in Mtb infection. The corresponding revision is on Page 14, Line 280-284.

References:

Hong JY, Chung KS, Shin JS, Lee JH, Gil HS, Lee HH, Choi E, Choi JH, Hassan AHE, Lee YS, Lee KT (2019) The Anti-Proliferative Activity of the Hybrid TMS-TMF-4f Compound Against Human Cervical Cancer Involves Apoptosis Mediated by STAT3 Inactivation. Cancers (Basel) 11: 1927

Sironi JJ, Ouchi T (2004) STAT1-induced apoptosis is mediated by caspases 2, 3, and 7. The Journal of biological chemistry 279: 4066-74

5. Fig. 8: Authors suggest that the A20-mediated death cell response is completely independent of cytokine production. Why can overexpression of A20 then partly compensate for the cytokine lack in Socs6+/+ fig. 8A?

RESPONSE: We thank the reviewer for raising this interesting question. In fact, we have already discussed this phenomenon in the original manuscript. The corresponding content is on Page 22, Line 459-470.

Interestingly, the presence or absence of A20 in *Socs6*$^{+/+}$ macrophages exerted different effects on cytokine release, which however, was not observed in *Socs6*$^{-/-}$ macrophages. We speculate that this may be because SOCS6 has a stronger inhibitory effect on cytokines than A20. Although A20 can be used as a negative feedback regulator of NF-κB to inhibit the production of cytokines, its effect is far less than SOCS6. Therefore, the absence of SOCS6 will results in a large amount of cytokines expression, in this case, the regulatory effect of A20 appears to be minimal. However, this hypothesis needs to be verified by further experiments.

6. The authors write that throughout the manuscript "data are representative of three independent experiments" or "three independent experiments with n = 3 technical replicates are shown". Does this mean that 3 independent experiments with 3 technical replicates were performed? As an example the legend to fig. 2 states "Individual data points represent individual technical replicates": I am not sure what is meant here. It seems obvious that statistics must be performed from biological replicates and not from technical replicates. Technical replicates should not be depicted as individual data points.

RESPONSE: We thank the reviewer for pointing this issue out. In this study, only qRT-PCR data, such as Fig. 1C, 3C, were the REPRESENTATIVE of three biological replicates (mean ± s.e.m. of n = 3 technical duplicates). The remaining data were obtained from three biological replicates (mean ± s.e.m. of n = 3 biological duplicates). In accordance with the reviewer's suggestions, we have checked the statistical methods throughout the manuscript, and have included the details of statistics in the Figure Legends to avoid potential confusion and misunderstanding.

7. The analysis of cell death mechanisms is depicted in a complicated way. Moreover, it is stated in the methods that Annexin V/PI staining was performed, however, I cannot find the data. Please provide verification of apoptosis in M.tb infected macrophages with methods other than the ApoTox-Glo Triplex Assay.

RESPONSE: We thank the reviewer for this comment. Annexin V/PI staining was performed to verify the data obtained from ApoTox-Glo Triplex Assay. Therefore, Annexin V/PI staining data were presented in the Expanded View Figures and Appendix Figures (Fig. EV1C, D, Fig. EV2E, F, Fig. EV4C, G, Appendix Fig. S3).

Minor:

8. Fig. S1A: If cell death mechanisms are analyzed, the control condition without M.tb infection is in general missing. Please correct the figure legend S1A: stimulation instead of stimulated.

RESPONSE: We thank the reviewer for raising this concern. The purpose of this study was to investigate the cell death mechanism underlying the phenotypic differences between C3H and B6 mice- derived BMDMs during Mtb infection. However, cell death rarely happened (survival rates >95%) under control condition (without Mtb infection), regardless of whether SOCS6 was involved or not. Therefore, we did not show the control condition in the figures for simplicity.

The grammar error has been corrected accordingly.

9. Fig. 4E+F Is the scale bar the same for all images?

RESPONSE: Yes, the scale bar was the same for all images. To avoid misunderstanding, we have added a scale bar to each of the panels.

10. Fig. 5 How is cytokine production affected in Mir342-/- B6 + si-Socs6 or Mir342+/+ C3H + si-Socs6?

RESPONSE: We have followed the reviewer's request and supplemented experiments to detect cytokine production in *Mir342*$^{-/-}$ B6 + si-*Socs6* and *Mir342*$^{+/+}$ C3H + SOCS6 macrophages. The results showed that the secretion of TNF-α, IL-1, IL-6 and CXCL15 was enhanced by SOCS6 knockdown in *Mir342*$^{-/-}$B6 mice, and was hampered by SOCS6 overexpression in *Mir342*$^{+/+}$C3H mice, as expected. The corresponding revision is in Fig. EV2J, K, and Page 12, Line 243-246.

11. Fig. S5 Figure legend indicates that data were analyzed by student´s t-test, but no statistic analysis is shown in the figure.

RESPONSE: We have supplemented the statistical analysis in Fig. S5 (Appendix Fig. S3 in the revised manuscript), and also in Fig. EV1C, D, Fig. EV2E, F, Fig. EV4C, G.

12. Student´s t-test should be used for comparison of two groups only. E.g. 1C, 1E, 1G, 2C, 2D, 2G, 2H should rather be analyzed by multiple t-tests or ANOVA (2C, 2D, 2G, 2H) as more than two groups are shown in one graph. Overall, in some graphs statistical analysis is missing, e.g. fig. 8D.

RESPONSE: In accordance with the reviewer's suggestion, we have gone through the full text and re-analyzed the statistics by ANOVA for comparison of more than two groups. The missing statistical analysis in some graphs have been added in the revised manuscript.

13. Authors analyze PBMC in humans and find similar regulatory mechanisms. PBMC, when extracted from the blood have perhaps not been in contact with M.tb. In vitro BMDMs were directly infected with M.tb. The authors should discuss how the upregulation of miR-342 is initiated and if it is dependent on the direct contact or even phagocytosis of M.tb.

RESPONSE: We thank the reviewer for this insightful comment. Indeed, PBMC might not have been in contact with Mtb when it is extracted from blood. It is worth noting how the upregulation of miR-342-3p is initiated, and whether it depends on the direct contact with Mtb. We speculate that the infection signals could be transmitted through cellular communication such as endocrine, which means that the signals could be transmitted throughout the organism via the circulatory system. However, more clinical data and experiments are required for this hypothesis. We have included this discussion into the revised manuscript on Page 23-24, Line 491-496.

14. Line 109 fig. 1A does not show any statistics, so changes cannot be declared as significant.

RESPONSE: We have included the quantification of gels in Fig. 1A, and the word "significantly" has been deleted from the sentence as the reviewer suggested.

15. Line 353 A limited set of inflammatory cytokines is analyzed in the study, thus the conclusion drawn here is too radical. Please rephrase.
RESPONSE: We thank the reviewer for this advice. We have rephrased this sentence into "In our current work, we found that RIPK3 had nothing to do with the production of several inflammatory cytokines, including TNF-α and IL-1, and this is consistent with Stutz's findings".

16. Line 37 This statement is too categorical. Apoptosis/necrosis are definitely essential factors in the understanding of host susceptibility. But mycobacteria influence the host immune response of macrophages at different levels, including cell division, cell fate, cell metabolism, etc., and not only with respect to cell death mechanisms. Please rephrase this sentence.
RESPONSE: In accordance with the reviewer's suggestion, we have rephrased this sentence into "As a result of the encounter between macrophages and Mtb, apoptosis/necrosis balance is known to be critical for the host susceptibility to Mtb".

17. Reference Prosser HM seems to be wrong (James Dooley et al 2017 No Functional Role for microRNA-342 in a Mouse Model of Pancreatic Acinar Carcinoma Front Oncol. 2017 May 18;7:101).
RESPONSE: We thank the reviewer for pointing this out. We have replaced it with the correct reference.

Responses to comments from Reviewer #3:

General comments:

In their paper entitled "MicroRNA 1 controls susceptibility to Mycobacterium tuberculosis by modulating inflammation and cell death mechanism", Fu et al. identify a specific microRNA, miR-342-3p, which appears to regulate cytokine production and switching from Mtb-induced necrosis and apoptosis of macrophages. They nicely complement in vitro work with mouse model work and analyses of clinical samples from TB patients and healthy controls. Overall, the study makes a potentially important contribution to our understanding of TB pathogenesis, and has implications for diagnostics and host-directed therapies. However, there are some issues outlined below, which, if adequately addressed, could further strengthen the conclusions.

RESPONSE: We thank Reviewer 3 for sparing the time and energy to offer us these constructive comments related to our paper. The manuscript has been greatly improved as a result of your valuable feedback. In the following lines, we detail our responses to each of your comments. We hope that the revised paper will meet your requirements.

Specific comments:

1. Abstract: It is stated that "apoptosis/necrosis is known to be the ultimate factor affecting host susceptibility to Mtb" (lines 37-38). This is an overstatement, and should be moderated by something like the following: "apoptosis/necrosis is an important factor affecting host susceptibility to Mtb." It is stated that these findings may lead to "...the development of novel therapeutic approaches for drug-resistant TB" (line 48); however, M. tuberculosis antibiotic resistance has nothing to do with the study's findings (i.e., any emerging host-directed therapies would be expected to be equally as effective against drug-susceptible TB). This is also mentioned in the last sentence of the Introduction (lines 85-87). Finally, "Mycobacterium tuberculosis" should be italicized in line 35.

RESPONSE: We thank the reviewer for these great suggestions. The sentence in Abstract has been rephrased into “apoptosis/necrosis balance is known to be critical for the host susceptibility to Mtb”.

The statement of the study's findings has also been rephrased as follows, “Our findings reveal several strategies for host innate immunity to diminish intracellular Mtb via miRNA-mRNA network, and indicate that emerging host-directed therapies would be expected to be equally as effective against drug-susceptible TB.” The corresponding revisions are on Page 3, Line 47-49 and Page 7, Line 133-137.

The italics of “*Mycobacterium tuberculosis*” has been modified accordingly.

2. Introduction: The literature review for the role of host/bacterial miRNAs and SOCS6 and SOCS7 is incomplete. More specific references/information should be included to contextualize how each of these factors are known to regulate innate

immune response. Also, the authors should include a justification for why they focused specifically on miR-342 and SOCS6. The introduction is the weakest section of the paper.
RESPONSE: We really appreciate this constructive comment, which was also raised by Reviewer 1. To make the introduction section more complete, we have added a lot of literatures to introduce the role of miRNAs and SOCS family members in the regulation of innate immune responses. The corresponding revision is on Page 4-6, Line 60-105 of the revised manuscript.
The reasons why we focused specifically on miR-342 and SOCS6 have already been stated in the original manuscript. The corresponding contents are on Page 7-8, Line 142-146, and Page 10, Line 200-204.

3. Introduction: The authors state it is "firmly established that microbe-derived miRNAs have an unfavorable effect on host anti-microbial immunity" (lines 62-63). However, this is certainly an overstatement, as this is still a relatively new area of research. Also, it is not clear in the next sentence (lines 64-65) if they are referring to bacterial miRNAs or host miRNAs or both.
RESPONSE: We thank the reviewer for pointing this issue out, and we share the same opinion with the reviewer regarding the overstated description. To ensure that we are being as clear as possible, we have revised this part as follows:
MicroRNAs (miRNAs) act by negatively regulating the expression of key genes, contributing to the regulatory network(Bartel, 2018). There are evidences that host miRNAs may impact on microbial life cycle and pathogenesis (Huang, Wang et al., 2007, Jopling, Yi et al., 2005, Liu, da Cunha et al., 2016). More commonly, bacteria can regulate the expression of host specific miRNAs, weaken the host's immunity, so as to achieve the purpose of promoting survival and immune evasion (Fu, Xue et al., 2020, Kumar, Sahu et al., 2015, Liu, Chen et al., 2018). Recently, it has been also reported that microbial-derived miRNAs have an unfavorable effect on host anti-microbial immunity(Choy, Siu et al., 2008, Sullivan, Grundhoff et al., 2005). Thus, miRNAs, both from bacteria and host, are likely to influence the relation between hosts and pathogens.

4. Results: The section describing the mouse models (lines 91-102) would better fit in the Introduction.
RESPONSE: We thank the reviewer for this suggestion, and this section has now been moved to the Introduction part of revised paper on Page 6-7, Line 119-129.

5. Results: The rationale for using different mouse strains is clearly stated, given that baseline expression of the miRNA of interest is different in each strain. However, whatever interventions are performed in one strain, should also be performed in the other to enable direct comparisons. This is because miR-342 expression is not the

only difference between these mouse strains. Recognizing that this would potentially represent a substantial amount of work, this limitation should at least be very clearly stated in the Discussion.
RESPONSE: We thank the reviewer for this valuable suggestion. We have included this limitation in the Discussion part of the revised manuscript. The corresponding revision is on Page 23, Line 479-483.

6. Results (Figure 1): There are some important controls missing. Specifically, the authors should show the effects of both mimic and inhibitor in each cell type, not simply one treatment in each cell type. Even though the expression is opposite in each cell type, they should show results for both to allow for direct comparisons. Since PBMCs (rather than pure macrophages) were used from clinical samples, it should be mentioned that the source of miR-342-3p may also be other cell types (Fig. 1J; lines 127-131).
RESPONSE: We have followed the reviewer's suggestion and included experiments to show the effects of mimic and inhibitor in each cell type for direct comparisons. As a result, treatment with miR-342-3p inhibitor in C3H BMDMs resulted in a more serious necrosis, and treatment with miR-342-3p mimic in B6 BMDMs resulted in a more serious apoptosis (Fig. EV1E-H).
In addition, we agree with the reviewer about the limitation of using PBMCs from clinical samples, and we have discussed this issue in the revised manuscript on Page 23, Line 488-491.

7. Results (Figure 2): The histology results are not particularly convincing. The authors should consider performing a quantitative stain-based assessment of apoptosis vs necrosis in these samples.
RESPONSE: We thank the reviewer for this comment. The purpose of histology results was to show the susceptible degree of mice to Mtb infection. In general, Mtb-susceptible mice are often accompanied by severe necrotic TB nodules, which are caused by necrotic death of cells and tissues. In contrast, the Mtb-resistant phenotype shows inflammations (sometimes mild TB nodules), which represent an increased cell apoptosis and a decreased necrosis. Although we believe it is not that perfect, this criterion is generally accepted to determine susceptibility to Mtb infection. In addition, we had performed a pathology scoring system of the histology results. The scoring system was described in Materials and Methods (Page 29-30, Line 618-629), and the scoring results were shown in Appendix Table S1. In this study, we mainly used BMDMs derived from mice to quantitatively assess the cell death mode (apoptosis/necrosis). We hope this explanation could be understood by the reviewer.

8. Results (Figure 3): The authors state that Socs6 was selected for further investigation due to putative regions that match the miR-342-3p seed sequence (lines

155-157). However, this justification is not adequate, since all miR-342-3p targets should have seed sequence complementarity.
RESPONSE: We thank the reviewer for this good point. In fact, we chose *Socs6* for further investigation through a designed siRNA screening strategy. The results showed that among the potential candidates, *Socs6* was involved in the switching between Mtb-induced apoptosis and necrosis. We have now included the siRNA screening data in the revised paper (Fig. EV2A, B).

9. have some important missing controls that will be critical for the accurate interpretation of their results.
RESPONSE: Consider it in conjunction with Comment 6 raised by the reviewer, we have included experiments to show the effects of mimic and inhibitor in each cell type for direct comparisons. The corresponding revision is in Fig. EV1E-H.

10. Results: The section describing the various factors involved in cell death pathways (lines 236-244) belongs in the Introduction.
RESPONSE: We thank the reviewer for this suggestion, and we have moved this part into introduction on Page 6, Line 107-117.

11. Discussion: It is stated that "... miR-342-3p has the potential to develop into a TB susceptibility marker" (line 339). However, if a biomarker for TB susceptibility was being sought, the study would have benefited from more of an unbiased approach, using various clinical groups and validation sets. The study's findings have more implications for understanding TB pathogenesis, i.e., a new regulatory pathway involved in tissue necrosis, and for developing novel host-based therapies targeting the apoptosis-necrosis switch.
RESPONSE: We thank the reviewer for pointing this issue out. The description of study's findings has been modified into “These data indicated that miR-342-3p participates in a new regulatory pathway involved in tissue necrosis”. The corresponding revision is on Page 23, Line 476-477.

12. General: All of the figures are relatively difficult to follow. The authors should consider simplifying the data, or only presenting that data that are essential to the story. For examples, some of the immunoblots could be moved to the supplemental figures.
RESPONSE: We thank the reviewer for this suggestion. In the revised manuscript, we have rearranged the figures, Fig. 5B, D, E, J, K, Fig. 6B, D, I-L, Fig. 7H-L in the original version have been moved to Expanded View Figures and Appendix Figures. We hope that this revision is easy to read and understand.

13. General: The text requires some attention to syntax/grammar. Also, some of the transitions are abrupt. For example, in the Introduction, SOCS6 is introduced abruptly (line 66) after describing miRNAs.
RESPONSE: We thank the reviewer for pointing this out. Consider it in conjunction with Comment 2 raised by the reviewer, we have included a lot of literatures to introduce the role of miRNAs and SOCS family members in the regulation of innate immune responses (Page 4-6, Line 60-105). Moreover, we have carefully examined and modified the entire manuscript, and we hope the revised paper is smooth and fluent to the readers.
The grammatical mistakes have also been corrected in the revised manuscript.

1st Revision - Editorial Decision 18th May 2021

Dear Dr. Wu,

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the three referees that were asked to re-evaluate your study, you will find below. As you will see, the referees now support the publication of your study in EMBO reports. Nevertheless, referees #2 and #3 have remaining concerns and/or suggestions to improve the manuscript, we ask you to address in a final revised manuscript.

Most importantly, please address the points regarding statistics by referee #2. Indeed, you repeatedly use the term 'biological/technical duplicates' in the legends, which makes no sense if n is larger 2 (as you indicate). I guess this should be 'biological/technical replicates'. Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also of the EV and Appendix figures), and that statistical testing has been done and is explained in detail where applicable. If n is indeed 2, statistical testing would make no sense!

Please also provide point-by-point response to the remaining referee points and to the editorial requests below.

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

- Please have your final manuscript text carefully proofread by a native speaker. In its present form this cannot be published. See also the comments of referee #3.

- The title is presently too long. Moreover, I think it would make sense to mention the name of the microRNA here. I would suggest this shortened title:
miR-342 controls Mycobacterium tuberculosis susceptibility by modulating inflammation and cell death

- Please order the manuscript sections like this:
Title page - Abstract - Keywords - Introduction - Results - Discussion - Materials and Methods - Data availability section - Acknowledgements - Author contributions - Conflict of interest statement - References - Figure legends - Expanded View Figure legends.

- As the Western blots shown are significantly cropped, could you provide the source data for all the blots (main, EV figures and Appendix figures). The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. Please submit the source data (scans of entire blots) together with the revised manuscript. Please include size markers for scans of entire blots, label the scans with figure and panel number and send one PDF file per figure. The images for the Appendix should be combined in one file.

- The panel for STAT5 in EV3A looks very similar to the lower panel for Caspase 9 in EV3I (maybe at a different exposure). Moreover Caspase 9 in the upper panel in EV3I looks similar p-STAT5 in EV3A. Please check and confirm that these are different experiments.

- In the Appendix file, please move the legends below the respective figures and the title above the figures. I think this is easier for readers to comprehend.

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

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

Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please do that for corresponding authors Li and Wang. Please find instructions on how to link the ORCID ID to the account in our manuscript tracking system in our Author guidelines:
http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

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

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

Referee #1:

I am very grateful the authors took the time to address my concerns. This is a very important work with some novel concepts that will open some new research avenues. I am happy to recommend publication in EMBOR Reports.

Referee #2:

The manuscript is improved, but - in my view - needs a thorough revision by a statistical expert. Problems may be in part related to language.

1) I am still not sure how many experiments were done and analyzed.
What are "three duplicates"?
E.g., were three independent experiments performed in 1C?
In 1D: What does each point represent? One independent measurement? Or means of duplicates?
And wat does independent mean exactly?
Why are parametric and not parametric tests mixed?

I have a hard time believing that the variability in cell death between independent (i.e. experiments on different days with bone-marrow from independent mice) is so small. This is a decisive experiment. The set up needs to be explained in detail. Statistics must only be performed where at

least three independent biological replicates have been the case.
If not: Please remove asterixis and adjust wording, where you show representative data, i.e. technical replicates.

2) Please provide representative FACS blots for 1D as well as for Annexin V stainings in the supplement.

3) The introduction is very - too - long.

Referee #3:

General comments:
In their revised manuscript "MicroRNA 1 controls susceptibility to Mycobacterium tuberculosis by modulating inflammation and cell death mechanism", Fu et al. have satisfactorily addressed the major concerns raised during the original review.
There are several minor comments, primarily related to the language of the revised text:

Minor comments:
1. Abstract, Line 38: Delete "the" before "host susceptibility".
2. Abstract, Lines 42 and 44: Add "the" before "miR-342-3p/SOCS6 axis".
3. Abstract, Line 43: Change "cytokines and chemokines" to "cytokine and chemokine" or "...by increasing the production of inflammatory cytokines and chemokines."
4. Abstract, Line 46: Change "diminish intracellular Mtb" to "control intracellular Mtb growth".
5. Abstract, Line 49: It is not clear why "drug-susceptible" is mentioned since such HDTs would presumably work equally well irrespective of antibiotic susceptibility pattern of the infecting isolate. Also, consider replacing "and indicate that emerging host-directed therapies would be expected to be equally as effective against drug-susceptible TB" with "and pave the way for host-directed therapies targeting these pathways."
6. Introduction, lines 79-80: Change "makes contribution" to "contributes".
7. Introduction, lines 107-108: Change "...cell death mode is known to be critical for the host susceptibility" to "the mode of cell death is known to be critical for host susceptibility."
8. Introduction, line 116: Change "complex" to "complexes".
9. Introduction, line 134: Insert "the" before "miR-342-3p/SOCS6 axis".
10. Introduction, lines 136-137: This is a strong statement. I would change to: "These findings suggest that new host-based therapies targeting these pathways might help combat drug-susceptible and drug-resistant TB".
11. Results, Lines 169-170: Change "more serious necrosis" to "more severe necrosis".
12. Results, Lines 170-171: Change "more serious apoptosis" to "a greater degree of apoptosis".
13. Results, Line 260: Delete "the" before "SOCS6 signaling".
14. Results, Line 283: Change "hypnotize" to "hypothesize". Change "is not related to the expression of IFN-γ" to "does not play a role in the expression of IFN-γ".
15. Results, Line 286: Change "/" to commas between each caspase.
16. Discussion, line 402: Change "has nothing to do with the existence..." to "has no effect on..."
17. Discussion, line 423: Change "had nothing to do with" to "had no role in".
18. Discussion, lines 478-479: Change "unanswered questions left hanging" to "remaining questions".
19. Discussion, line 488: Change "case compared" to "comparison".
20. Discussion, line: 503: Change "veiled" to "additional".

21. Discussion, line 504: Change "should be determined" to "require further investigation".

Responses to comments from Editor:

Comments: Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the three referees that were asked to re-evaluate your study, you will find below. As you will see, the referees now support the publication of your study in EMBO reports. Nevertheless, referees #2 and #3 have remaining concerns and/or suggestions to improve the manuscript, we ask you to address in a final revised manuscript.

Response: Thank you very much for offering us these important comments and suggestions related to our paper. We would also like to extend our appreciation to the reviewers for their excellent work during the revisions. In the following lines, we provide detailed responses to your comments and requests, and we hope that our revisions meet your requirements.

Comments: Most importantly, please address the points regarding statistics by referee #2. Indeed, you repeatedly use the term 'biological/technical duplicates' in the legends, which makes no sense if n is larger 2 (as you indicate). I guess this should be 'biological/technical replicates'. Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also of the EV and Appendix figures), and that statistical testing has been done and is explained in detail where applicable. If n is indeed 2, statistical testing would make no sense!

Response: Thank you for this comment. The biological/technical "duplicates" in the text should indeed be biological/technical "replicates", and we apologize for this confusion caused by typos. In accordance with your as well as the Reviewer's suggestion, we have included information to explain in detail about how the experiments were performed and their nature, as well as how the statistical test was carried out for each panel of the figures (including EV and Appendix figures). We hope that this revision is easy to read and understand.

Comments: Please also provide also a point-by-point response to the remaining referee points and to the editorial requests below.

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

- Please have your final manuscript text carefully proofread by a native speaker. In its present form this cannot be published. See also the comments of referee #3.

Response: Thank you for this suggestion. The final manuscript text has been carefully proofread by native speakers from the professional language editing company.

[Image of the editing certificate removed]

- The title is presently too long. Moreover, I think it would make sense to mention the name of the microRNA here. I would suggest this shortened title:
miR-342 controls Mycobacterium tuberculosis susceptibility by modulating inflammation and cell death
Response: We thank the Editor for this suggestion and have accordingly revised the title to “MiR-342 controls Mycobacterium tuberculosis susceptibility by modulating inflammation and cell death”.

- Please order the manuscript sections like this:
Title page - Abstract - Keywords - Introduction - Results - Discussion - Materials and Methods - Data availability section - Acknowledgements - Author contributions - Conflict of interest statement - References - Figure legends - Expanded View Figure legends.
Response: The order of the manuscript sections has been modified as suggested.

- As the Western blots shown are significantly cropped, could you provide the source data for all the blots (main, EV figures and Appendix figures). The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. Please submit the source data (scans of entire

blots) together with the revised manuscript. Please include size markers for scans of entire blots, label the scans with figure and panel number and send one PDF file per figure. The images for the Appendix should be combined in one file.
Response: We have provided all the source data of Western blots as required.

- The panel for STAT5 in EV3A looks very similar to the lower panel for Caspase 9 in EV3I (maybe at a different exposure). Moreover Caspase 9 in the upper panel in EV3I looks similar p-STAT5 in EV3A. Please check and confirm that these are different experiments.
Response: Thank you for this reminder. We have carefully checked the above-mentioned blots. Although the bands look very similar, by checking the experimental records and comparing the image features under different exposure conditions (such as the background features of PVDF membrane), we are sure that they come from different experiments. In order to avoid any potential misunderstanding, we have included the comparisons of the above-mentioned bands under different exposure conditions and re-uploaded the source data of Fig. EV3A (Source Data Fig. EV3) in the revised manuscript.

- In the Appendix file, please move the legends below the respective figures and the title above the figures. I think this is easier for readers to comprehend.
Response: This issue has been modified as suggested.

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see any modifications done.
Response: We have provided the final manuscript file with track changes and have addressed the comments from the publisher as required. The files have been uploaded as “Manuscript” and “Responses to queries from the publisher”, respectively.

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

Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please do that for corresponding authors Li and Wang.

Response: The ORCID ID of corresponding authors Zhifeng Li and Xingsheng Wang have been supplied as required.

Responses to comments from referee #2:

General comments: The manuscript is improved, but - in my view - needs a thorough revision by a statistical expert. Problems may be in part related to language.

Response: We would like to thank the Reviewer for the excellent work during the revisions. As for the statistics, the problems are partly caused by the language just as you and the editor mentioned, and we apologized for the confusion. The final manuscript has been reviewed by statistical experts and proofread by native speakers as suggested. In the following lines, we provide detailed responses to your concerns and comments, and we hope that our revisions can meet your requirements.

Comment: 1) I am still not sure how many experiments were done and analyzed. What are "three duplicates"?

E.g., were three independent experiments performed in 1C?

In 1D: What does each point represent? One independent measurement? Or means of duplicates? And wat does independent mean exactly?

Response: Thank you for this comment, which was also raised by the Editor. The biological/technical "duplicates" in the text should indeed be biological/technical "replicates". This typo has been corrected in the revised manuscript.

We performed three independent experiments in Figure 1C and 1D. The data came from three biological replicates. We had one biological replicate in one independent experiment. Each point represents mean value of technical replicates in one biological replicate. Independent means independent experiment.

In order to avoid potential confusion caused by general description, we have included information to explain how the experiments were carried out in detail, and how the statistical analysis was done for each panel of the figures. We hope we are being as clear as possible.

Comment: 2) Why are parametric and not parametric tests mixed?

Response: We believe that the use of parametric testing or non-parametric testing depends on the characteristics of the data. Most of the experimental data in the paper are suitable for parametric testing. However, we found that the *in vitro* CFU assays are not suitable for parametric testing by analyzing the distribution characteristics of the original data. Therefore, we used medians ± interquartile ranges followed by Mann-Whitney U test for statistical analysis in CFU assays as previously described (Zheng et al, 2018, Wang et al, 2020).

Zheng R, Liu H, Zhou Y, Yan D, Chen J, Ma D, Feng Y, Qin L, Liu F, Huang X et al (2018) Notch4 Negatively Regulates the Inflammatory Response to Mycobacterium tuberculosis Infection by Inhibiting TAK1 Activation. J Infect Dis 218: 312-323

Wang L, Wu J, Li J, Yang H, Tang T, Liang H, Zuo M, Wang J, Liu H, Liu F et al (2020)

Host-mediated ubiquitination of a mycobacterial protein suppresses immunity. Nature 577: 682-688

Comment: 3) I have a hard time believing that the variability in cell death between independent (i.e. experiments on different days with bone-marrow from independent mice) is so small. This is a decisive experiment. The set up needs to be explained in detail. Statistics must only be performed where at least three independent biological replicates have been the case.
If not: Please remove asterixis and adjust wording, where you show representative data, i.e. technicaI replicates.
Response: We agree with the Reviewer that cell death detection is decisive and we used two methods to detect cell death in this paper for the purpose of mutual confirmation. The first one is ApoTox-Glo Triplex Assay (shown in the main figures). The cell death mode was determined by the measurement of fluorescence and luminescence using a VICTOR X5 Multilabel Plate Reader (such as Fig. 1D). The data came from three biological replicates, which were collected from three independent experiments. As for the small variability in cell death between independent experiments, we have carefully checked our data and found that the variability is relatively small only in Fig. 1D, data in other figures (such as Fig. 1F, 3F, 6E, et.al) are fluctuated within the normal range. We assume that the small variability in Fig. 1D may be partly related to the small sample size (n = 3). We wish the reviewer could take the accidental event into consideration and hope our justification is acceptable.

The second method is flow cytometry analysis by using the Annexin V/PI staining (such as Fig. EV1C). In order to better represent the comparison of apoptosis/necrosis, flow data were extracted to make a stacked histogram. Fig. EV1C shows one representative data of three biological replicates. We do agree with the reviewer that statistics must be performed from biological replicates and not from technical replicates. The asterixis have been removed accordingly.

Comment: 4) Please provide representative FACS blots for 1D as well as for Annexin V stainings in the supplement.
Response: The experiment performed in Fig. 1D was ApoTox-Glo Triplex Assay instead of flow cytometry, as we explained in Comment 3). In the revised manuscript, we have provided the source data collected by VICTOR X5 Multilabel Plate Reader (Source Data Fig. 1D), and the representative flow cytometry data for Annexin V/PI stainings (Source Data Fig. EV1C).

Comment: 5) The introduction is very - too - long.
Response: We have deleted part of the content in the introduction, and we hope this modification is easy to read and understand.

Responses to comments from referee #3:

General comments:

In their revised manuscript "MicroRNA 1 controls susceptibility to Mycobacterium tuberculosis by modulating inflammation and cell death mechanism", Fu et al. have satisfactorily addressed the major concerns raised during the original review.

Response: We really appreciate the Reviewer for the excellent work during the revisions. The detailed modifications of words and sentences have been modified as suggested.

Minor comments:

1. Abstract, Line 38: Delete "the" before "host susceptibility".

Response: This issue has been modified as suggested.

2. Abstract, Lines 42 and 44: Add "the" before "miR-342-3p/SOCS6 axis".

Response: This issue has been modified as suggested.

3. Abstract, Line 43: Change "cytokines and chemokines" to "cytokine and chemokine" or "...by increasing the production of inflammatory cytokines and chemokines."

Response: This issue has been modified as suggested.

4. Abstract, Line 46: Change "diminish intracellular Mtb" to "control intracellular Mtb growth".

Response: This issue has been modified as suggested.

5. Abstract, Line 49: It is not clear why "drug-susceptible" is mentioned since such HDTs would presumably work equally well irrespective of antibiotic susceptibility pattern of the infecting isolate. Also, consider replacing "and indicate that emerging host-directed therapies would be expected to be equally as effective against drug-susceptible TB" with "and pave the way for host-directed therapies targeting these pathways."

Response: The phrase “drug-susceptible” has been deleted and this sentence has been modified as suggested.

6. Introduction, lines 79-80: Change "makes contribution" to "contributes".

Response: This issue has been modified as suggested.

7. Introduction, lines 107-108: Change "...cell death mode is known to be critical for the host susceptibility" to "the mode of cell death is known to be critical for host susceptibility."

Response: We do agree that this change would be better. However, this sentence has been deleted during this revision according to the comments. We wish the reviewer would understand this modification and hope our justification is suitable.

8. Introduction, line 116: Change "complex" to "complexes".
Response: We do agree that this change would be better. However, this sentence has been deleted during this revision according to the comments. We wish the reviewer would understand this modification and hope our justification is suitable.

9. Introduction, line 134: Insert "the" before "miR-342-3p/SOCS6 axis".
Response: This issue has been modified as suggested.

10. Introduction, lines 136-137: This is a strong statement. I would change to: "These findings suggest that new host-based therapies targeting these pathways might help combat drug-susceptible and drug-resistant TB".
Response: This issue has been modified as suggested.

11. Results, Lines 169-170: Change "more serious necrosis" to "more severe necrosis".
Response: This issue has been modified as suggested.

12. Results, Lines 170-171: Change "more serious apoptosis" to "a greater degree of apoptosis".
Response: This issue has been modified as suggested.

13. Results, Line 260: Delete "the" before "SOCS6 signaling".
Response: This issue has been modified as suggested.

14. Results, Line 283: Change "hypnotize" to "hypothesize". Change "is not related to the expression of IFN-γ" to "does not play a role in the expression of IFN-γ".
Response: This issue has been modified as suggested.

15. Results, Line 286: Change "/" to commas between each caspase.
Response: This issue has been modified as suggested.

16. Discussion, line 402: Change "has nothing to do with the existence..." to "has no effect on..."
Response: This issue has been modified as suggested.

17. Discussion, line 423: Change "had nothing to do with" to "had no role in".
Response: This issue has been modified as suggested.

18. Discussion, lines 478-479: Change "unanswered questions left hanging" to "remaining questions".
Response: This issue has been modified as suggested.

19. Discussion, line 488: Change "case compared" to "comparison".
Response: We appreciate this suggestion. However, this sentence has been revised by the native speakers as the editor suggested. We hope this modification would meet your approval.

20. Discussion, line: 503: Change "veiled" to "additional".
Response: This issue has been modified as suggested.

21. Discussion, line 504: Change "should be determined" to "require further investigation".
Response: This issue has been modified as suggested.

2nd Revision - Editorial Decision 18th Jun 2021

Haibo Wu
Chongqing University
School of Life Sciences, Chongqing University, Chongqing 401331, China
Chongqing 401331
China

Dear Dr. Wu,

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

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

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: 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."

Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

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Corresponding Author Name: Haibo Wu

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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 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.

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)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was based on empirical data from pilot experiments.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Ten of age- and sex-matched mice were used in each group.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Exclusion criteria were pre-established, e.g. any experiment in which technical problems occurred that might affect the data itself were excluded. Mice showing clear signs of abnormal sickness were excluded from 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.	Mice were randomly grouped and treated accordingly in each experimental condition.
For animal studies, include a statement about randomization even if no randomization was used.	Six-week-old mice were randomly allocated into groups (n=10) and aerosol infected with approximately 400 CFU Mtb.
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.	The investigators were blinded during data collection and analysis.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Data collection and analysis for animal experiments were blinded.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, we used Kolmogorov-Smirnov (KS) in SPSS to test normal distribution.
Is there an estimate of variation within each group of data?	We included SEM for groups in experiments unless otherwise indicated.

Is the variance similar between the groups that are being statistically compared?	Yes

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).	The source and validation of antibodies were reported in the materials and methods.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	We used cell lines acquired from ATCC. All cell lines were recently authenticated and tested for mycoplasma contamination.

* 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.	C3HeB/FeJ and C57BL/6J mice were purchased from Jackson Laboratory. Mir342+/+C3H mice (C3HeB/FeJ-Tg(mROSA-Mir342)1cyagen) and Mir342-/-B6 mice (C57BL/6J-Mir342tm1cyagen) were constructed and identified by Cyagen Biosciences (Guangzhou, China).The Socs6-/- mice (C3HeB/FeJ-Socs6em1cyagen) were purchased from Cyagen Biosciences (Guangzhou, China). Littermate wild-type mice were used as controls. The pBROAD3-A20 vector was microinjected into the zygotes from Socs6+/+ (wild-type) and Socs6-/- mice to generate A20-overexpressing mice (A20+/+Socs6+/+, A20+/+Socs6-/-). A20-knockout mice (A20-/-Socs6+/+, A20-/-Socs6-/-) were generated from Socs6+/+ and Socs6-/- mice using CRISPR/Cas9. Littermate wild-type mice were used as controls. Six-week-old mice were randomly allocated into groups (n=10) for experiments.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	This study was carried out in strict accordance with the Guidelines for the Care and Use of Animals of Chongqing University. All animal experimental procedures were approved by the Animal Ethics Committees of the School of Life Sciences, Chongqing University.
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.	Other relevant aspects of animal studies were adequately reported according to ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Blood samples were obtained from Chongqing Public Health Medical Center in accordance with the guidelines of the local ethics committee. Patients providing blood samples were given informed consent. The ethics committee approved this consent procedure.
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.	Informed consent was obtained from all subjects and the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	None
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	None
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	None
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.	None
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.	None

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	"Data Availability" section has been previded in the manuscript.
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).	No primary datasets were generated and deposited.
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).	No human clinical and genomic datasets were generated and deposited.
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	No computational models were created.

G- Dual use research of concern

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