EMBO reports

FACT maintains nucleosomes during transcription and stem cell viability in adult mice

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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.)

Dear Katerina,

Thank you for the submission of your manuscript to EMBO reports. I have now read and discussed it with my colleagues here, and I am sorry to say that we all agree that it is not well suited for us.

We note that your study reports that loss of FACT in adult mice is lethal, that it causes damage in multiple tissues, that it reduces proliferation and increases cell death, that it leads to loss of stem cells in the bone marrow and intestine, that it reduces the expression of genes with a role in RNA and protein synthesis, and induces genes with a role in IFN signaling, that it prevents the proliferation of stem cells in vitro, and that it changes chromatin accessibility.

While we recognize the observation that FACT loss seems to affect stem cells more than differentiated cells, we also note that it remains unclear why this is. It was further reported before that FACT is mainly expressed in stem and progenitor cells and that it regulates the expression of core stem cell transcription factors. FACT is also known to regulate chromatin accessibility and transcription. It is further unclear to me from how you describe the results, which of the effects on the mouse tissues in response to FACT loss are due to tamoxifen treatment and which are due to FACT loss. A more thorough description would be helpful here. I understand that there is no tamoxifen-treated control mouse? If this is the case, it remains unclear whether tamoxifen or FACT loss cause the effects.

Taken together, given the current literature on FACT, we think that novel insight into why FACT affects stem cells more than other cells, or very clean and unambiguous data showing that FACT is required for the maintenance of different types of stem cells in vivo would be required for the publication of your manuscript here.

As it stands, I am sorry to say that we have decided not to proceed with in-depth review. I am sorry to disappoint you on this occasion, also given that we pre-discussed your work, but the context of the FACT literature and your exact set of data was not fully clear to me at that point.

Kind regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Dear Esther,

thank you for the considering of our manuscript, I just wanted to clarify several issues, which you mentioned below:

1. First we treated with tamoxifen control mice and cells in all experiments, in vitro and in vivo, which we run.
2. "It was further reported before that FACT is mainly expressed in stem and progenitor cells and that it regulates the expression of core stem cell transcription factors." You are right expression was shown, but the function of FACT in these cells was still unclear. Our manuscript is the first to show that viability of the earliest progenitors in two tissues bone marrow and intestine depends on FACT, while viability of later progenitors which also express FACT is not dependent on FACT.
3. "FACT is also known to regulate chromatin accessibility and transcription." This is also true, but how it regulates is also quite unclear and as you remember from the conference this topic caused a lot of debates. In our previous paper (https://pubmed.ncbi.nlm.nih.gov/32498018/), we showed that FACT loss did not affect chromatin accessibility in normal fibroblasts. Here for the first time we show that in mesenchymal stem cells loss of FACT increases accessibility in transcription dependent manner. FACT was first proposed to play an opposite role, "facilitating" chromatin accessibility. Based on this FACT removal would be expected to reduce chromatin accessibility. Actually, recent preprint from Studitsky/Formosa groups (https://www.biorxiv.org/content/10.1101/2021.07.13.452273v1) shows that in cell free system yeast FACT indeed opens nucleosomes and thus may facilitate transcription, while our data show that mammalian FACT protects nucleosomes during transcription in cells. Three existing publications approaching FACT role in stem cells (https://pubmed.ncbi.nlm.nih.gov/27146025/, https://pubmed.ncbi.nlm.nih.gov/30456357/, https://pubmed.ncbi.nlm.nih.gov/30078731/) - all used knockdown or partial reduction of FACT levels in mammalian cells and did not see toxicity of this manipulation for stem cells as we also do not see toxicity of removal of one allele of Ssrp1, suggesting that data from experiments with partial reduction of FACT needs to be treated with caution. So, I just wanted to explain these issues before you make your final decision.

Thank you,
Katerina

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Dear Dr. Gurova,

Thank you for the submission of your manuscript to EMBO reports. We have now received the full set of enclosed referee reports on it.

As you will see, the referees find the data potentially interesting. However, they also point out that the writing is clearly below standard, and that the data and the assays need to be described and performed much more thoroughly. This is an important point, as the unclear presentation of the data might preclude a clear assessment of the experimental evidence provided. I think that referee 1's comment that several of the analyses are superficial is particularly important, as well as that more quality controls for the experiments are required. I agree that the data showing an effect of FACT loss on stem cell pools should be strengthened. All referees note that significant revisions are required.

This manuscript is a borderline case and referees 1 and 3 indicate in the manuscript summary table that the manuscript "might be" published post-revision. We can therefore only offer to publish your manuscript, if the referees will be convinced by the revised study. It might also be helpful to have the revised manuscript proof-read by a colleague.

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

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss this further.

Regarding data quantification, please specify the number "n" for how many independent experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. This information must be provided in the figure legends. Please also include scale bars in all microscopy images.

IMPORTANT NOTE: we perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:
1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.
2) Your manuscript contains statistics and error bars based on n=2. Please use scatter blots in these cases. No statistics should be calculated if n=2.

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

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).
See https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf for more info on how to prepare your figures.

3) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

4) 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.

5) a complete author checklist, which you can download from our author guidelines <https://www.embopress.org/page/journal/14693178/authorguide>. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

6) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines <https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>

7) Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (see https://www.embopress.org/page/journal/14693178/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 & Method (see also https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study. * Note - All links should resolve to a page where the data can be accessed. *
If your study has not produced novel datasets, please mention this fact in the Data Availability Section.

8) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at <https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

9) Our journal also 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 https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

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

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File (RPF) to accompany accepted manuscripts. 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.

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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,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

In the current manuscript, Goswami and colleagues explore the intriguing question why the histone chaperone FACT is dispensable in some cell types, but essential in others. This question is also of importance as FACT has been suggested as a potential draggable target in cancer cells. For the first time, the authors use an inducible knock-out to address the dependency organismal-wide. The authors conclude that mainly the stem-cell pool is affected by FACT depletion, mainly based on sc-RNA-seq. There is a lot of data in the manuscript and the assumptions made based on experimental data are reasonable, but my

main concern at the moment is the fact that (potentially because of various experimental strategies) the analysis is superficial. If the authors would like to make statements about the stem cell pools, then scRNA-seq experiments will have to be validated. Also the RNA- and ATAC-seq parts are still superficial and not ideally planned. I have put more specific points below.

Major points:

scRNA-seq: The description for the analysis of single-cell RNA sequencing should be expanded, particularly which features and cut-offs were used for gene/UMI detection - it is also unclear how cell cycle stage was calculated, Either feature plots for the marker genes or heatmaps used should be shown for all scRNA-seq datasets to understand how well the clusters were called and how well marker genes differentiated cell types. Quantitative cell numbers from scRNA-seq are not really reliable and usually qualitative rather than quantitative and would not allow for precise quantification of changes as the authors imply (e.g. lines 209/210)- if the authors would like to make a point about the lineage dependency and see where cell numbers are decreasing/increasing, then this result should be verified by classical FACS-based approaches where standard marker sets are available for the various blood cell types; similar thoughts hold true for the intestinal dataset, where a immunohistochemistry with the specfic markers would back-up the scRNA-seq data. The resolution for Figure 4A/B is not good enough to evaluate the data. What stem cells are the authors referring to? HSC, multipotent progenitors? This is not clear. From the colour code in Fig. 4B it also appears that eosinophils are missing? How does this fit?

As FACT depletion dramatically changes the chromatin landscape, it cannot be ruled out that transcription is globally changed. This should have been controlled using spike-ins during the RNA-seq. I could not find the number of replicates used and whether these were technical or biological.

From the data presented I am not convinced that the ATAC-seq worked well. Figure S11B shows a read distribution in which the nucleosome free reads are uncharacteristically low. This might also explain the lack of correlation between RNA- and ATAC-seq. It would be essential to show some more quality control for this experiment, incl. number of mapped reads, number of duplicates and mitochondrial reads. I could not find the number of replicates used and whether these were technical or biological.

One major difference between stem and differentiated cells is the extent of cell cycle. While LT-HSCs do not cycle as much, intestinal stem cells cycle constantly. Thus, the explanation of a transcriptional effect of FACT on chromatin seems plausible. To be able to make this point, the authors should perform ATAC-seq under cell cycle arrested stages to exlude cell cycle as a confounding variable. This is particularly true as RNA-seq i not the best choice to directly look at transcription as it measures steady-state RNA levels rater than transcription itself. Therefore, traditionally the field has used GRO-seq like approaches to investigate the crosstalk between chromatin and transcription.

Minor points:

Figure 4C and D are overlapping

As there is a clear ageing dependency of FACT depletion on weight loss and viability, the authors should be more careful when claiming that FACT loss is lethal at all ages. As it is not reasonable to ask for a repetition of the experiment in geriatric mice, the authors should tone down this statement and subheading (e.g. line 99)

The authors state that no change in blood biochemistry was observed upon FACT depletion, but do no present data. This should be included as part of supplement (line 117).

Not clear why skin was excluded as a classical stem cell niche - the authors state that not all mice showed a phenotype, but no statistics shown - this point should be made clearer (line 124)

Referee #2:

Goswami et al. provide the first description of the global effects of conditional deletion of FACT in a developed mammal. FACT has been observed to be essential for viability in some single-celled models and for early steps mammalian development, but its accumulation and contribution to viability in developed cell lines and mature tissues have provided a confusing picture. The catalog of effects provided in this manuscript spanning a broad range of cell types in mature mice therefore provides significant new insights into FACT function in various circumstances. The results largely support recent developments in this field, indicating a role for FACT in restoring chromatin after transcription, but the global analysis provides the opportunity to begin to answer questions about why some cells require FACT function for their proliferation and viability and others do not. This work will have significant impact on answering these questions. The analysis of effects extends from gross anatomy to genomic measurements, providing a large dataset for workers in this field. The primary weakness of the manuscript is the writing itself, which will need extensive editing for standard usage, and would also benefit from clarification or better organization in many places.

1) Description of the statistical analyses needs to be improved. For example, Fig 1B shows "p<0.05" but it is not clear which populations this applies to or whether this is relative to untreated animals or other cohorts. Similarly, 3B shows a p value of "0.000" which should be corrected to a non-zero value. In all cases, the statistical test used should be stated and the number of samples analyzed should be given in the legend.

2) A coordinated effort to edit passages for clarity and standard usage is needed, as many grammatical errors also lead to ambiguity of intent. This is particularly true of the genomic analysis, where further attempts to lead the reader to understand the support for the conclusions stated is needed.

3) As a minor point, the authors state in the introduction in line 64 that "most of them are essential for viability" but the references cite work on non-essential chaperones. The intention here should be clarified. Also, SSRP1 is "Structure-Specific Recognition Protein 1."

4) As recombination did not appear to occur in testes, it seems that discussion of this tissue can be omitted after stating this observation.

Referee #3:

Goswami et al. describe in this manuscript their study of the histone chaperone FACT by using conditional mouse knock-out lines.
FACT is a highly conserved chromatin regulator consisting of two subunits. In the past, it has been considered primarily as a facilitator of transcription, but it plays also important roles for the maintenance of nucleosomes and thereby the chromatin signature. To investigate which particular tissues are affected, when the FACT subunit Sssrp1 is ubiquitously depleted the authors used mice carrying Ssrp1 alleles, (Ssrp1fl/fl; CreERT2) and compared the effects of Ssrp1 depletion with heterozygotes or wild type animals.
Depletion of Ssrp1 in homozygous animals led to a reduction of hematopoietic and intestinal cells. The authors conclude that the loss of these cells is mainly based on reduced proliferation. Interestingly, the opposite is the case for hepatocytes, which started proliferating upon Ssrp1 depletion.

By performing single-cell RNA-seq (scRNA-seq) they found that stem cells are sensitive to loss of the FACT subunit Ssrp1 but not differentiated cells, suggesting that intestinal stem cells and some bone marrow progenitors disappeared.

Together with additional testing for organoid formation and by immunostaining for Ssrp1, the authors conclude that stem cells are dependent on FACT for viability.

To test whether loss of the FACT subunit Ssrp1 in stem cells may lead to changes in chromatin accessibility thereby decreasing the viability of stem cells the authors performed ATAC-seq in parallel to RNA-seq. Their results suggest that a number of genes were upregulated upon Ssrp1 loss while a smaller fraction is downregulated.

Upregulated genes relate to interferon response, which may reflect the observed accumulation of immune cells in some of the organs that were affected upon Ssrp1 depletion.

Overall, the study supports current knowledge about the important chromatin regulator FACT. The data is mostly convincing and provides some important observations regarding the impact on tissues in mice upon loss of the Ssrp1 subunit. Yet, in some case it is not clear how many replicate experiments have been conducted and which type of statistics were applied (just noting 'we used GraphPad' is not sufficient).
Additionally, but the manuscript has immense shortcomings which need improvement and corrections. The writing quality is far below standard and requires in-depth editing - due to the high amount of grammar/language mistakes I stopped quite early with correcting and indicating all the mistakes.

Comments:

Page2-line38: "...although whether it disassemble or assemble...
> grammar (it disassembles...)

Page2-line40:... deleted FACT subunit... (?)
> grammar

Page2-line40: FACT loss was lethal due to the failure of hematopoietic and intestinal tissue...
> failure of what? function, or it seems like a term like 'development' or 'specification' before 'failure' is missing.

Page2-line44: dene bodies
> dense bodies

Page3-line69: "Histone chaperon FACT"
> chaperone

Page3-lin83 "survive died upon FACT knockdown"
> remove died

Page3-lin89-90; several grammar mistakes and words missing!!

Page14-Line308-311: "There were very few peaks which emerged de novo (17 out of 47620 peaks detected in cells without FACT) or completely disappeared (4 out of 65041 total peaks in cells with FACT) upon FACT depletion, suggestion that FACT loss results in quantitative rather than qualitative changes in chromatin accessibility".
> the other way around: qualitative, not quantitative!?
> suggestion to suggesting

....

Generally, the quality of the manuscript in terms of language and grammar is very poor - also on the following pages! Due to the vast amount of grammar mistakes, missing words, articles, and many more, I am stopping at this point to correct/indicate these mistakes! The manuscript needs massive improvements and careful editing!

Figure 2 legend. A. > what is indicated by the blue arrow?

Figure 2 legend. C. H&E stating of liver sections > staining

Figure 3: Scale bars missing in panels A, B

Figure 6-J: Loading of Western Blots for osteocytes needs improving. Based on the current data, it is not very clear whether SSRP1 is lost in these cells!

Indication of the type of statistical analyses that were applied is missing in most figures! Importantly, in the Methods section, the only description of how statistics have been conducted is: "All statistical analysis was performed on GraphPad Prism 8. Data was considered significant if p-value was < 0.05".
This is not acceptable! Mentioning the actual types of statistical analysis and number of replicates for each data set is crucial.

What is the number of biological or technical replicates of the ATAC/RNA-Seq! This is nowhere indicated but is required for proper statistics and conclusions!

The authors should indicate/discuss that additional functions of Ssrp1 apart from being a subunit of FACT cannot be fully excluded. Ssrp1 depletion alone may cause the observed phenotypes due

Dear reviewers, thank you very much for the critical reading of our manuscript and for providing insightful suggestions. We tried to answer all your questions and ran several of the experiments that you recommended. Please see our responses below, which were also incorporated in the revised manuscript.

Referee #1:
Major points:

"***scRNA-seq: The description for the analysis of single-cell RNA sequencing should be expanded, particularly which features and cut-offs were used for gene/UMI detection - it is also unclear how cell cycle stage was calculated"***

We added the details of the scRNA-seq analysis to the Materials and Methods, including the filtering procedure and the gene lists used to identify cells in the different phases of the cell cycle. The cell-cycle scores for the S and G2/M phases were calculated using the CellCycleScoring method based on the orthologous mouse genes for cc.genes in the Seurat package.

"Either feature plots for the marker genes or heatmaps used should be shown for all scRNA-seq datasets to understand how well the clusters were called and how well marker genes differentiated cell types."

We built feature plots for all the major known bone marrow and intestine cell markers and a heatmap of the genes/clusters and included them as Supplementary Figures (Appendix, Figures S1-S7). We did not include most of them into Level 1 and Level 2 data due to the following surprising discovery. Though the cell types within the clusters were identified with high confidence using methods based on whole transcriptome analysis, such as the singleR R package using the Immgen database as a reference and MSigDB-GSEA with 'immunologic signature' and 'cell type signature' gene sets, when we used individual

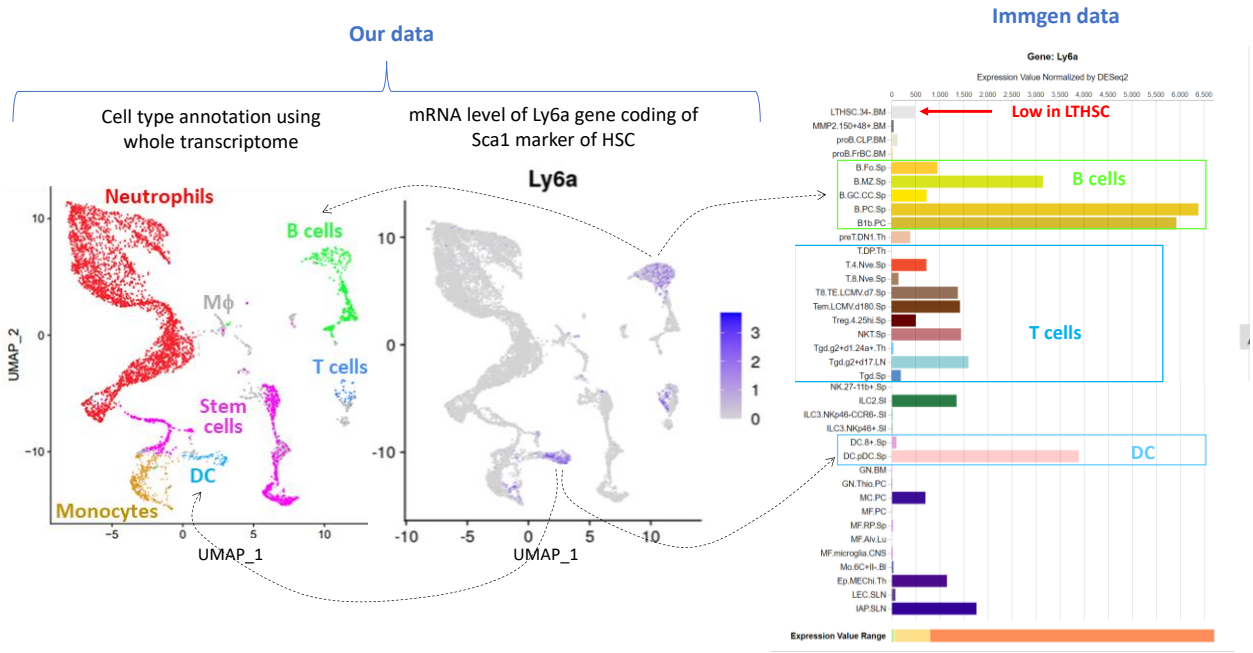

Figure R1. Correspondence of Ly6A coding Sca1 marker of HSC between our and Immgen data.

markers of the cell types for feature plots, it only worked well for few markers but far from all of them including major markers of the hematopoietic stem cells (HSC) (see Fig.R1 below). First, we thought that this was a problem related to our data; however, we then looked at the expression of the same genes in the Immgen bulk RNA-seq samples, sorted based on antibody staining of the protein expressed by this marker. We also did not see marker expression in the cell type where it was expected based on antibody staining. The genes for several well-established markers used to identify and sort specific cell types were not expressed at a high level in this cell type but were expressed in those cells where it was not detected by the antibody. See, for example, Sca1 marker (*Ly6a* gene). The most likely explanation for this discrepancy is that the antibody staining measures the presence of a protein/cell surface antigen, while scRNA-seq measures the presence of a rather abundant transcript, as only genes expressed at relatively high levels can be detected by the 10X scRNA-seq approach. Therefore, these data may not always correspond to each other due to different half-lives, the kinetics of expression/presentation, or the presence of posttranslational modifications. Because this discrepancy is beyond our investigation, we used cell identification only based on the transcriptional signature but confirmed the data using different methods and two different datasets. We included just a few examples of the gene-feature plots (Fig.5D, E).

"Quantitative cell numbers from scRNA-seq are not really reliable and usually qualitative rather than quantitative and would not allow for precise quantification of changes as the authors imply (e.g., lines 209/210)".

We agree with the reviewer and have excluded several quantitation plots. We have now included pie charts that show the distribution of the cell types (Fig.4C,E and Fig.5C). Pie charts have been widely used for the description of scRNA data in different publications. We left only one quantitation plot showing the fold-change in the proportion of bone marrow cell types (Fig.4D) because the bar diagram allows for easier appreciation of the moderate but important changes in cell abundance compared to the visual impression from the UMAP plot.

Remark from the editor related to this analysis: "Referee 1 further suggests that you "avoid showing fold-changes of abundances. The absences of the stem cell clusters are fairly obvious in the data. I would suggest though to make much better use of the data and expand the scRNA analysis using approaches such as pseudotime plots to understand better where the block is in differentiation."

As mentioned above, we left just one fold-change graphs to support the visible impressions of the mild redistribution of cluster abundance within the neutrophils and B cells. We also ran several iterations of pseudotime analysis (see example below, Fig.R2). Unfortunately, neither of these analyses made the data clearer. They failed to establish a correct hierarchy of cell differentiation in the control samples. Because the hierarchy for bone marrow and intestine is well known and FACT depletion affects the cell types at the top of the differentiation cascade in both cases, we think that using pseudotime analysis only complicates the data interpretation unnecessarily. Our data show that in FACT depleted tissues both LTHSC and Lgr5+ cells become practically undetectable, what suggest that *Ssrp1 KO* directly affects these cells, and the direct/indirect role of FACT in the viability/activity of the descendent cells needs to be established in future time-dependent experiments. To make these parts of the manuscript clearer, we completely rewrote the text and remade Figures 4 and 5.

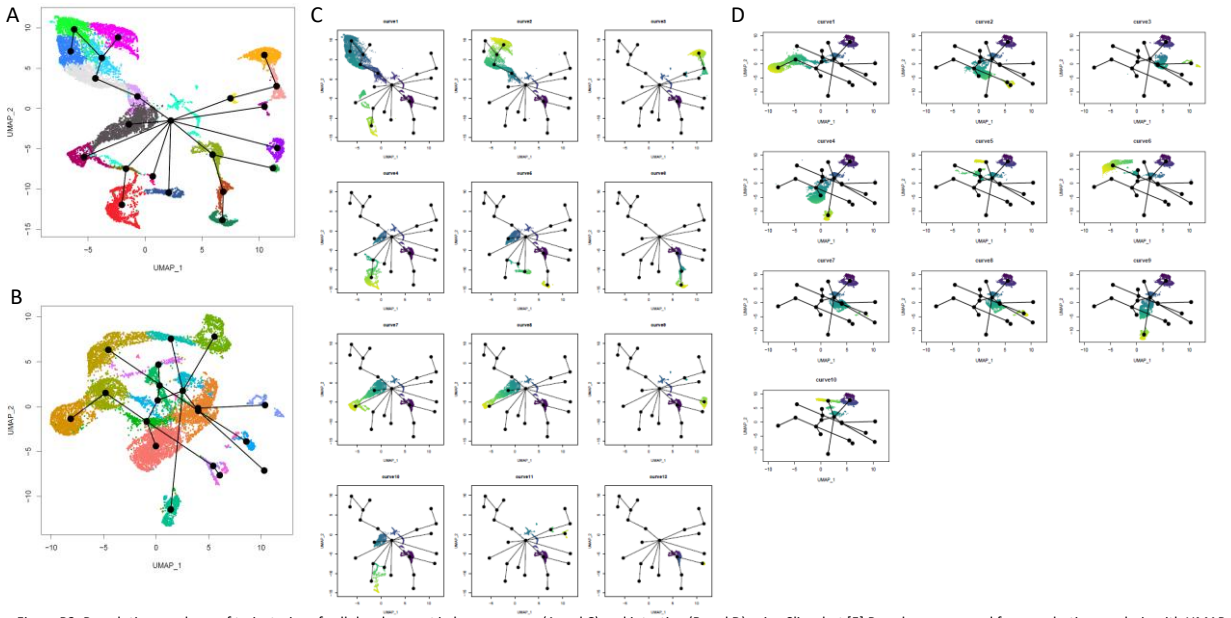

Figure R2. Pseudotime analyses of trajectories of cell development in bone marrow (A and C) and intestine (B and D) using Slingshot [5] R package was used for pseudo-time analysis with UMAP embedding. The stem cells cluster in intestine data and the LTHSC cluster in bone marrow data are selected as roots respectively. For intestine data, the immune cells (Ptprc/CD45 positive cells) were removed as they are not on the same development path with other intestine cells; then the remaining cells are re-normalized, and clustered for pseudotime analysis. A and B. All identified trajectories for bone marrow (A) and intestine (B) cells. C and D. Potential lineages of differentiation of bone marrow (C) and intestinal (D) cells.

"if the authors would like to make a point about the lineage dependency and see where cell numbers are decreasing/increasing, then this result should be verified by classical FACS-based approaches where standard marker sets are available for the various blood cell types; similar thoughts hold true for the intestinal dataset, where a immunohistochemistry with the specific markers would back-up the scRNA-seq data".

Unfortunately, we could not perform the suggested experiments due to the lack of mice with the necessary genotypes. All mouse work was put on hold in our facility because of the COVID19 pandemic and related restrictions. Sperm from the mice were frozen; however, we are still far from having enough mice for the requested experiments. Thus, we softened our conclusions from the experiments we had already performed and provided a discussion of their limitations.

New text in the Results section: *scRNA-seq data suggest* that the cell types most sensitive to FACT loss are the undifferentiated stem cells. More mature differentiated cells tolerate FACT depletion; however, *these findings need to be confirmed by additional methods*.

"The resolution for Figure 4A/B is not good enough to evaluate the data. What stem cells are the authors referring to? HSC, multipotent progenitors? This is not clear. From the colour code in Fig. 4B it also appears that eosinophils are missing? How does this fit?"

We have significantly modified this figure and its legend. Eleven types of bone marrow stem cells were identified, and they are all shown and properly labeled in the revised figure (Fig. 4E, F) unless they were present at very low numbers in both samples.
The reviewer also correctly noticed that the eosinophils were missing from the plot. There were very few eosinophils detected before and zero after FACT depletion. These cells are normally at very low level in bone marrow. Moreover, reanalysis of the data after additional filtering did not identify eosinophils. (Fig, 4B, C).

"FACT depletion dramatically changes the chromatin landscape, it cannot be ruled out that transcription is globally changed. This should have been controlled using spike-ins during the RNA-seq. I could not find the number of replicates used and whether these were technical or biological."

We added a quantitative measurement of global transcription using the EU incorporation assay (Fig.7A and EV4A, B).

"From the data presented I am not convinced that the ATAC-seq worked well. Figure S11B shows a read distribution in which the nucleosome free reads are uncharacteristically low. This might also explain the lack of correlation between RNA- and ATAC-seq. It would be essential to show some more quality control for this experiment, incl. number of mapped reads, number of duplicates and mitochondrial reads. I could not find the number of replicates used and whether these were technical or biological."

We ran the ATAC-seq again under growth-arrested conditions to eliminate the influence of the cell cycle phase on the data using two replicates (independently grown and treated plates of MSC) for each condition. We are grateful for this suggestion because the data are clearer with a more striking difference between the wild-type and *Ssrp1* null cells, with almost identical results from the two biological replicates. Moreover, previously existing issue of different direction of changes at TSS and gene body was not reproduced in growth-arrested cells. In growth arrested cells changes at both regions are in the same direction (Fig.8). As for the fragment length distribution issue, both homozygous and heterozygous growth-arrested cells demonstrated an even stronger skew towards mononucleosome-sized fragments, which might reflect a more compact chromatin state in the growth-arrested cells. We did not find any literature data that compared ATAC-seq-measured chromatin accessibility between proliferating and quiescent cells) However, the fragment distribution was the same between all conditions (Appendix, Fig.S8), suggesting that the tagmentation conditions were similar. We also discussed this issue with a couple of ATAC-seq experts, and they both confirmed that this should not be a problem if we see clear peaks that are reproducible between the replicates. To mitigate potential problems, we separately analyzed the reads of fragments of all lengths, shorter than nucleosomes, and mononucleosomes. All these approaches produced very similar results.
Alignment statistics is shown in Appendix Fig.S9.

"***One major difference between stem and differentiated cells is the extent of cell cycle. While LT-HSCs do not cycle as much, intestinal stem cells cycle constantly. Thus, the explanation of a transcriptional effect of FACT on chromatin seems plausible. To be able to make this point, the authors should perform ATAC-seq under cell cycle arrested stages to exlude cell cycle as a confounding variable. This***

is particularly true as RNA-seq is not the best choice to directly look at transcription as it measures steady-state RNA levels rather than transcription itself. Therefore, traditionally the field has used GRO-seq like approaches to investigate the crosstalk between chromatin and transcription."

As discussed above, we repeated the ATAC-seq experiments using growth-arrested cells. We also completely agree with the reviewer that although bulk RNA-seq is widely used to evaluate gene expression changes, it is not the most accurate method to measure transcription. Thus, we added the EU incorporation assay, which uses the amount of EU incorporated over 60 minutes to measure of RNA synthesis. We measured EU incorporation in individual cells using flow cytometry. In parallel, we stained the same cells for DNA content to address the effect of the cell cycle and SSRP1 to confirm *Ssrp1* deletion (Fig.7A and EV4A, B).

Minor points:

"Figure 4C and D are overlapping"

We have significantly modified Figure 4 and removed this overlap***.***

"As there is a clear ageing dependency of FACT depletion on weight loss and viability, the authors should be more careful when claiming that FACT loss is lethal at all ages. As it is not reasonable to ask for a repetition of the experiment in geriatric mice, the authors should tone down this statement and subheading (e.g., line 99)"

We made the suggested modifications and changed the subheading to the following: "**FACT loss is lethal in adult mice.**"

"The authors state that no change in blood biochemistry was observed upon FACT depletion, but do no present data. This should be included as part of supplement (line 117)."

We provided this information in Table EV1.

"Not clear why skin was excluded as a classical stem cell niche - the authors state that not all mice showed a phenotype, but no statistics shown - this point should be made clearer (line 124)"

For this study, we excluded skin from the analysis because skin lesions were observed in most but not all mice very close to the time of critical weight loss when mice had to be euthanized. Therefore, we could not make an accurate statistical assessment. Expression of $CreER^{T2}$ under a keratinocyte-specific promoter is needed for an accurate assessment of FACT's role in the skin.

Referee #2:

"Goswami et al. provide the first description of the global effects of conditional deletion of FACT in a developed mammal. FACT has been observed to be essential for viability in some single-celled models and for early steps mammalian development, but its accumulation and contribution to viability in

developed cell lines and mature tissues have provided a confusing picture. The catalog of effects provided in this manuscript spanning a broad range of cell types in mature mice therefore provides significant new insights into FACT function in various circumstances. The results largely support recent developments in this field, indicating a role for FACT in restoring chromatin after transcription, but the global analysis provides the opportunity to begin to answer questions about why some cells require FACT function for their proliferation and viability and others do not. This work will have significant impact on answering these questions. The analysis of effects extends from gross anatomy to genomic measurements, providing a large dataset for workers in this field."

Thank you very much for the positive assessment of our study.

"The primary weakness of the manuscript is the writing itself, which will need extensive editing for standard usage, and would also benefit from clarification or better organization in many places."

We apologize for this shortcoming. The revised manuscript has been edited by a professional scientific editor.

1) "Description of the statistical analyses needs to be improved. For example, Fig 1B shows "p<0.05" but it is not clear which populations this applies to or whether this is relative to untreated animals or other cohorts. Similarly, 3B shows a p value of "0.000" which should be corrected to a non-zero value. In all cases, the statistical test used should be stated, and the number of samples analyzed should be given in the legend".

We carefully edited the manuscript and added all the details for the statistical analyses.

2) "A coordinated effort to edit passages for clarity and standard usage is needed, as many grammatical errors also lead to ambiguity of intent. <u>This is particularly true of the genomic analysis, where further attempts to lead the reader to understand the support for the conclusions stated is needed."</u>

We have significantly modified the description of the data and analyses, modified some figures, and used a professional scientific editor to improve the clarity and grammar of the manuscript.

3) "As a minor point, the authors state in the introduction in line 64 that "most of them are essential for viability" but the references cite work on non-essential chaperones. The intention here should be clarified. Also, SSRP1 is "Structure-Specific Recognition Protein 1." "

The text has been corrected.

4) "As recombination did not appear to occur in testes, it seems that discussion of this tissue can be omitted after stating this observation."

We removed the text, as suggested.

Referee #3:

"Generally, the quality of the manuscript in terms of language and grammar is very poor - also on the following pages! Due to the vast amount of grammar mistakes, missing words, articles, and many more, I am stopping at this point to correct/indicate these mistakes! The manuscript needs massive improvements and careful editing!"

We have had the revised manuscript reviewed by a professional scientific editor.

"Indication of the type of statistical analyses that were applied is missing in most figures! Importantly, in the Methods section, the only description of how statistics have been conducted is: "All statistical analysis was performed on GraphPad Prism 8. Data was considered significant if p-value was < 0.05". This is not acceptable! Mentioning the actual types of statistical analysis and number of replicates for each data set is crucial. What is the number of biological or technical replicates of the ATAC/RNA-Seq! This is nowhere indicated but is required for proper statistics and conclusions!"

All the requested information has been added to the Material and Methods, Results, and Figure Legends.

"Additionally, but the manuscript has immense shortcomings which need improvement and corrections. The writing quality is far below standard and requires in-depth editing - due to the high amount of grammar/language mistakes I stopped quite early with correcting and indicating all the mistakes."

Comments:

"Generally, the quality of the manuscript in terms of language and grammar is very poor - also on the following pages! Due to the vast amount of grammar mistakes, missing words, articles, and many more, I am stopping at this point to correct/indicate these mistakes! The manuscript needs massive improvements and careful editing!"

In response to all grammar comments, we hired a professional scientific editor to edit the revised manuscript.

"Figure 2 legend. A. > what is indicated by the blue arrow?"

We apologize for the confusion. The arrow was put by in the image by our pathologist, who indicated "intestinal mucosa with crypts (blue arrow)" in his report. The arrow was left there by mistake when we created the figure.

"Figure 3: Scale bars missing in panels A, B"

We added scale bars to the figure panels.

"Figure 6-J: Loading of Western Blots for osteocytes needs improving. Based on the current data, it is not very clear whether SSRP1 is lost in these cells!?"

We agree that based on the actin signal, it looks like there is less protein in the 4-OHT-treated samples; however, this is observed for both genotypes. In all experiments we observed slightly higher actin levels in untreated osteocytes compared to adipocytes and 4-OHT-treated osteocytes. This observation may be a real biological phenomenon, which we have not investigated. Total protein was carefully normalized for the loading.
For the SSRP1 protein levels, there is a clear reduction in SSRP1 in the differentiated cells versus the undifferentiated cells, as described by us and others. Since the SSRP1 levels are almost undetectable in osteocytes even before 4-OHT treatment, it is hard to see a further reduction. However, these data show that SSRP1 is not essential for the survival of differentiated osteocytes.

"Indication of the type of statistical analyses that were applied is missing in most figures! Importantly, in the Methods section, the only description of how statistics have been conducted is: "All statistical analysis was performed on GraphPad Prism 8. Data was considered significant if p-value was < 0.05". This is not acceptable! Mentioning the actual types of statistical analysis and number of replicates for each data set is crucial."

We added the details for the statistical methods used to all figures presenting quantitative data.

"What is the number of biological or technical replicates of the ATAC/RNA-Seq! This is nowhere indicated but is required for proper statistics and conclusions! "

There were two biological replicates for each type of experiment. We added this information to the revised manuscript.

"The authors should indicate/discuss that additional functions of Ssrp1 apart from being a subunit of FACT cannot be fully excluded. Ssrp1 depletion alone may cause the observed phenotypes due"

We have added this to the discussion of the revised manuscript: "Although in this study we genetically excised only *Ssrp1* gene, we have never observed in mammalian cells existence of SPT16 in the absence of SSRP1 and unbound to SSRP1. Thus, we discuss below the consequences of the loss of the whole complex rather than SSRP1 subunit, though theoretically SSRP1 may have independent activity in some cells or conditions."

Dear Katerina,

Thank you for the submission of your revised manuscript. We have now received the enclosed reports from the referees that were asked to assess it, and I am happy to say that both support its publication now. Referee 1 still has one more suggestion that I would like you to incorporate before we can proceed with the official acceptance of your manuscript. Please let me know where the new text changes are, or if new data have been added to the final version of your manuscript.

A few other editorial requests also need to be addressed:

- Please use the subheading "Data Availability Section" for your newly deposited data, all other info needs to be deleted from this section (the mouse model and the dataset tables).

- Please use the subheading "Conflict of Interest" for this statement.

- Please correct the REFERENCE FORMAT to the new EMBO reports (Harvard) style. Not more than 10 authors may be listed.

- The funding info needs to be included in the Acknowledgement section.

- Fig EV1D callout is missing. Fig EV4D callout is missing. Fig EV5C callout is missing. Please add.

- The DATASET EV LEGENDS are OK, but the text callouts need to be corrected to "Dataset EV 1" etc.

- The author checklist is mainly filled with "yes"es. Please either provide more specific information in the checklist itself, or add page numbers to indicate where in the manuscript file the information can be found.

I would like to suggest a few minor changes to the title and abstract that needs to be written in present tense. Please let me know whether you agree with the following, and please check my comments in the text below:

FACT maintains nucleosomes during transcription and is essential for stem cell viability

Preservation of nucleosomes during replication has been extensively studied, while the maintenance of nucleosomes during transcription has gotten less attention. The histone chaperone FACT is involved in transcription elongation, although whether it disassembles or assembles nucleosomes during this process is unclear. In order to elucidate the function of FACT in mammals, we deleted a FACT subunit [Did you delete all of FACT or a single subunit? What is it called?] in adult mice. FACT loss is lethal, possibly due to the loss of the earliest progenitors in bone marrow and intestine, while more differentiated cells are not affected. Using cells isolated from several tissues, we show that FACT loss is lethal only for stem cells but not for differentiated cells in vitro. FACT depletion increases chromatin accessibility in a transcription-dependent manner [does this exclusively happen in stem cells?], suggesting that nucleosomes are lost during transcription in the absence of FACT. The most prominent response to the loss of nucleosomes is the activation of interferon signaling and the accumulation of immunocytes in organs sensitive to FACT loss [OK?]. FACT maintains chromatin integrity during transcription in mammalian adult stem cells, suggesting that chromatin transcription in stem cells and differentiated cells is different.

EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is exactly 550 pixels wide and 200-600 pixels high (the height is variable). You can either show a model or key data in the synopsis image. Please note that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

I look forward to seeing a final version of your manuscript as soon as possible. Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Best wishes,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The revised manuscript is significantly improved and I welcome the additional experimentation and changes to the text. I do not

fully understand the explanation why feature plots are not working in this case. Particularly in the case for the hematopoietic system, this is well established and rather speaks to issues of depth in the experiment. If it really is impossible to provide further data and evidence for LTHSCs to be depleted (lines 206-226) and Figure 4F, I would down-tone this section even further. The fact that stem cells are impacted the most, is made now very clear. Thus I do support publication of the current manuscript.

Referee #3:

The concerns I raised in my evaluation of the original manuscript version were addressed in the revised version. I support publication of the revised manuscript.

Dear Editor,

Thank you again for considering our manuscript. We incorporated all editorial and reviewer #1 suggestions. Please see below the list of our modifications.

Best regards,

Katerina

Editorial requests:

- Please use the subheading "Data Availability Section" for your newly deposited data, all other info needs to be deleted from this section (the mouse model and the dataset tables). DONE

- Please use the subheading "Conflict of Interest" for this statement. DONE

- Please correct the REFERENCE FORMAT to the new EMBO reports (Harvard) style. Not more than 10 authors may be listed. DONE

- The funding info needs to be included in the Acknowledgement section. DONE

- Fig EV1D callout is missing DONE.. Fig EV4D callout is missing THIS ONE WAS NOT MISSING. Fig EV5C callout is missing. Please add. DONE

- The DATASET EV LEGENDS are OK, but the text callouts need to be corrected to "Dataset EV 1" etc. DONE

- The author checklist is mainly filled with "yes"es. Please either provide more specific information in the checklist itself, or add page numbers to indicate where in the manuscript file the information can be found. DONE

I would like to suggest a few minor changes to the title and abstract that needs to be written in present tense. Please let me know whether you agree with the following, and please check my comments in the text below:

FACT maintains nucleosomes during transcription and is essential for stem cell viability ACCEPTED

Preservation of nucleosomes during replication has been extensively studied, while the maintenance of nucleosomes during transcription has gotten less attention. The histone chaperone FACT is involved in transcription elongation, although whether it disassembles or assembles nucleosomes during this process is unclear. In order to elucidate the function of FACT in mammals, we deleted a FACT subunit [Did you delete all of FACT or a single subunit? What is it called?] in adult mice. FACT loss is lethal, possibly due to the loss of the earliest progenitors in bone marrow and intestine, while more differentiated cells are not affected. Using cells isolated from several tissues, we show that FACT loss is lethal only for stem cells but not for differentiated cells in vitro. FACT depletion increases chromatin accessibility in a transcription-dependent manner [does this exclusively happen in stem cells?], suggesting that nucleosomes are lost during transcription in the absence of FACT. The most prominent

response to the loss of nucleosomes is the activation of interferon signaling and the accumulation of immunocytes in organs sensitive to FACT loss [OK?]. FACT maintains chromatin integrity during transcription in mammalian adult stem cells, suggesting that chromatin transcription in stem cells and differentiated cells is different.
MOSTLY ACCEPTED – SEE BELOW:

Preservation of nucleosomes during replication has been extensively studied, while the maintenance of nucleosomes during transcription has gotten less attention. The histone chaperone FACT is involved in transcription elongation, although whether it disassembles or assembles nucleosomes during this process is unclear. To elucidate the function of FACT in mammals, we deleted Ssrp1 subunit of FACT in adult mice. FACT loss is lethal, possibly due to the loss of the earliest progenitors in bone marrow and intestine, while more differentiated cells are not affected. Using cells isolated from several tissues, we show that FACT loss is lethal only for stem cells but not for differentiated cells *in vitro*. FACT depletion increases chromatin accessibility in a transcription-dependent manner in stem cells, suggesting that nucleosomes are lost in these cells during transcription in the absence of FACT. The most prominent response to the loss of nucleosomes was the activation of interferon signaling and the accumulation of immunocytes in in organs sensitive to FACT loss. FACT maintained chromatin integrity during transcription in mammalian adult stem cells, suggesting that chromatin transcription in stem cells and differentiated cells is different.

OK?

EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is exactly 550 pixels wide and 200-600 pixels high (the height is variable). You can either show a model or key data in the synopsis image. Please note that text needs to be readable at the final size. Please send us this information along with the revised manuscript. DONE

Referee #1:

The revised manuscript is significantly improved and I welcome the additional experimentation and changes to the text. I do not fully understand the explanation why feature plots are not working in this case. Particularly in the case for the hematopoietic system, this is well established and rather speaks to issues of depth in the experiment. If it really is impossible to provide further data and evidence for LTHSCs to be depleted (lines 206-226) and Figure 4F, I would down-tone this section even further. The fact that stem cells are impacted the most, is made now very clear. Thus I do support publication of the current manuscript.

KG: As reviewer requested, we included feature plots with markers of individual cell populations, as well as heatmaps in Supplementary Materials (Layer 3 data as they are now named in the author guidance). We tried to say more explicitly in the last version that LTHSC were identified using gene expression signature approach.

Katerina Gurova
Roswell Park Cancer Institute
Cell Stress Biology
Elm and Carlton Str
Buffalo, NY 14263
United States

Dear Dr. Gurova,

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

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

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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.

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

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Corresponding Author Name: Katerina Gurova
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2021-53684V3

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 $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For in vitro experiments (excluding next generation sequencing) the strength of the effect were tested in preliminary experiment. Then based on this sample size was calculated using alpha = 0.05.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Animla experiments were first run with 5 mice per group (survival) or 3 mice per group (tissue collection). Since difference between control and experimental groups were maximal possible (death of experimental animals and no change in control) there was no need to calculate sample size. Experiments were repeated with the same number of animals.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	There was no exclusion of animals in the experiments.
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.	Randomization was not applicable, since all animals had to be genotyped before taken into experiment.
For animal studies, include a statement about randomization even if no randomization was used.	Randomization was not applicable, since all animals had to be genotyped before taken into experiment.
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.	yes, pathologist doing tissue evaluation was blinded.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Examination of tissue sections was done by pathologist blindly (only ID of animals were provided, but not age or genotype).
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, where appropriate.
Is there an estimate of variation within each group of data?	Variation was estinated in the preliminary experiments and then used for calculation of a sample size.

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http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

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http://figshare.com

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https://osp.od.nih.gov/biosafety-biosecurity-and-emerging-biotechnology/
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	yes for cell-based experiments. No - for animal experiments. Control groups as expected had less variance in survival (100% survived) and weight (stable), while experimental animals died between 5 and 50 days after start of treatment and their weight loss was also uneven.

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).	All used antibodies were validated in the lab using positive and negative samples.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* 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.	yes. See Material and Method section: Animal experiments
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	yes. See Material and Method section: Animal experiments
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.	yes

E- Human Subjects

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

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	yes, see Data Availability Section: The datasets supporting the conclusions in this article are available in the GEO Datasets repository. scRNA-seq data are available as GSE189866, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE189866; ATAC-seq and RNA-seq data are available as GSE189663, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE189663.
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).	NA
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).	NA
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.	NA

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.	NA