EMBO reports

The glucocorticoid receptor in brown adipocytes is dispensable for the systemic control of energy homeostasis through brown adipose tissue

Christina Glantschnig, Frits Mattijssen, Elena Sophie Vogl, Asrar Ali Khan, Marcos Rios Garcia, Katrin Fischer, Timo Müller, Henriette Uhlenhaut, Peter Nawroth, Marcel Scheideler, Adam J. Rose, Natalia Pellegata, and Stephan Herzig

Review timeline:

Submission date:	24 May 2019
Editorial Decision:	12 June 2019
Revision received:	30 July 2019
Editorial Decision:	21 August 2019
Revision received:	2 September 2019
Accepted:	9 September 2019

Editor: Deniz Senyilmaz-Tiebe

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 12 June 2019

Thank you for the submission of your research manuscript to our journal. We have now received two referee reports that are copied below.

As you will see, both referees express interest in the findings demonstrating the effects of (or lack thereof) BAT specific GR depletion on metabolism. However, they also raise some concerns that need to be addressed for publication here.

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

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 the revisions further. As a matter of policy, competing manuscripts published during revision will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed.

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

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures

and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (<http://embor.embopress.org/authorguide>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

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

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

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

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

7) We would also encourage you to include the source data for figure panels that show essential data. <optional: We would also encourage you to include the source data for the following figure panels:
- Figure xA
- Figure zB

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

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

9) Regarding data quantification, please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure

legend. Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.
Please note that error bars and statistical comparisons may only be applied to data obtained from at least three independent biological replicates.
Please also include scale bars in all microscopy images.

10) As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File 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.

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

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

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

REFEREE REPORTS

Referee #1:

Glantschnig and colleagues have examined the effect of BAT-specific deficiency of the glucocorticoid receptor in mice on the metabolic response to cold, feeding/fasting, and diet-induced obesity. Overall, GR deficiency had minimal effect on the metabolic phenotype, leading the authors to conclude that GR in BAT is dispensable for the systemic control of energy homeostasis. Overall, the data are convincing and well presented. Although most of the results are "negative" data, this should not be used as an argument against publication of the paper. The results are certainly relevant given the rather inconsistent literature on the role of GR in white and brown adipose tissue.

Nevertheless, the paper has plenty of room for improvement, as outlined below.

Major comments:
1)The paper does not differentiate between the possibilities that GR in BAT has no role in metabolic regulation during cold, feeding/fasting, or diet-induced obesity, OR whether GR has no role in BAT, period. The transcriptomics analysis should enable the authors to determine whether deficiency of GR in BAT has any effects on gene expression beyond the genes mentioned and in particular whether it has any effect on expression of adipose tissue GR target genes (such as Pik3r1, Foxa3, Pnpla2, Angptl4). Ideally, it would be very interesting to study to the effect of corticosterone treatment on gene expression in flox and GRBATKO primary brown adipocytes or in BAT in vivo.

2) Since the authors did not study the metabolic response to glucocorticoid treatment in flox and GRBATKO mice, the title of the paper seems premature. The authors should consider studying the effect of pharmacological GR activation on metabolic regulation in flox and GRBATKO mice.

Minor comments:
1) The description of the experimental procedures is a bit sloppy, especially for the mouse studies. These studies need to be described in much more detail. For example, it is not indicated whether the experiments were conducted in male or female mice. Also, the age of the mice is not indicated. No information in given on ethical approval. The overall description of the mouse studies is brief and incomplete.

2) On page 3 it is stated that "In contrast to WAT, brown adipose tissue (BAT) contains fewer lipid

droplets, but..." This is incorrect. Brown adipocytes contain more lipid droplets than white adipocytes.

3) For GTT and ITT, it is not indicated in the methods when they were carried out. The legend of figure 5 says that the GTT was carried out after 19 weeks of HFD and the ITT after 20 weeks of HFD. However, in the discussion it is implied that these tests were carried out after 22 weeks of HFD. Please be clear and consistent.

4) It is not completely clear how the experiments shown in figure 1a and 1b were conducted. It seems that in figure 1a a higher dose of DEX was injected. But if that is the case, why is the increase in corticosterone lower in figure 1a than in figure 1b for the same groups of mice (flox and GRBATKO)? Frankly, I don't understand the purpose of figure 1b. Please make it more clear.

5) The paper uses Nr3c1 when describing gene expression and GR for the genotype. It would be preferable to be consistent and only use Nr3c1 or GR.

6) The second paragraph of the results section is redundant and can be removed.

7) My recommendation would be to delete any description of non-significant p values above the figures (for instance, figure 2d).

8) It is unclear what is shown in figure 2i. Is the heatmap based on mean expression values? Was RNA pooled from several animals? It would be useful to do a more extensive analysis of the cold-induced changes in gene expression in flox and GRbatKO mice (or were mice raised at room temperature not included in the analysis?)

9) It would be useful to have information on the expression of well-established GR target genes in BAT of the flox and GRbatKO mice. It would help determine whether GR deficiency in BAT has no effect on gene expression at all or whether there are changes in gene expression yet these changes do not translate into any changes in metabolic parameters.

10) Did the authors collect inguinal fat after cold exposure to enable studying the effect of GR deficiency on browning?

11) If the intraperitoneal glucose tolerance was done after 19 weeks of HFD, why are the bodyweight data only go until 10 weeks (Figure 4a).

12) Page 8. This sentence is confusing: So far, we had observed that loss of GR in BAT affected energy expenditure during a 10-week HFD intervention, but this did not lead to changes in glucose handling 10 weeks later. It suggest that there are two sequential HFD interventions, which is incorrect. Please rephrase to avoid confusion.

13) It seems that a number of separate cohorts of flox and GRbatKO mice were put on HFD. Please be more clear in describing these cohorts in the methods section.

14) Page 7: "To gain additional insight into the metabolic consequences of BAT GR deficiency under HFD conditions, we allowed the mice to gain body weight for an additional 6 weeks and..." Additional 6 weeks to what? Please clarify

15) The paragraph in the discussion starting with "GCs have been... GC excess" is difficult to follow. For instance, there is the need to elaborate on the BAT phenotype of 11β-HSD1 deficient mice.

16) The authors may consider changing the title to: The glucocorticoid receptor in brown adipocytes is dispensable for the systemic control of energy homeostasis. This title would better reflect the actual data.

17) Page 10. Please rephrase: "Overall, however, the effects observed are too small to be statistically detected, with a high standard error." Statistics does not detect anything.

18) The methods section contains at least two PMID that the authors forgot to include in the list of references.

19) In the discussion, the authors compare the GTT and ITT results from studies in GRadipoKO and GRbatko mice. However, results in GRbatko mice should not be directly compared with results in GRadipoKO mice, as the genotype of these mice is clearly distinct. Accordingly, it is premature to raise the suggestion that differences may be due to differences in age of the mice.

Referee #2:

The paper by Glantschnig et al. studies the role of the GR in systemic control of energy metabolism especially with regards to brown adipose tissue function. This is highly relevant since multiple studies (especially in vitro) have implicated the glucocorticoid axis in the regulation of BAT mediated thermogenesis, however the in vivo work is often hampered by the fact that glucocorticoids have other systemic effects which makes it difficult to dissect the contribution and function of BAT. IN summary the authors show through the use of many well-designed models that GR is dispensable for BAT function and might only have a slight influence under long term obesogenic conditions. Given the extremely careful way the work is conducted I think this paper would be very important for the scientific community. I have listed a few points below which would need to be addressed:

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Page 7: The changes in food intake are very interesting. Could the newly described secretin pathway be involved? The authors should check this maybe by measuring the expression in BAT of their animals.
Page 13: Which Ucp1ERCre mouse line was used, there are several out there.

1st Revision - authors' response 30 July 2019

Referee #1:

Glantschnig and colleagues have examined the effect of BAT-specific deficiency of the glucocorticoid receptor in mice on the metabolic response to cold, feeding/fasting, and diet-induced obesity. Overall, GR deficiency had minimal effect on the metabolic phenotype, leading the authors to conclude that GR in BAT is dispensable for the systemic control of energy homeostasis. Overall, the data are convincing and well presented. Although most of the results are "negative" data, this should not be used as an argument against publication of the paper. The results are certainly relevant given the rather inconsistent literature on the role of GR in white and brown adipose tissue.

Nevertheless, the paper has plenty of room for improvement, as outlined below.

We thank the referee for their interest and constructive criticism of the manuscript. We hope that the additional experiments performed as well as the revisions of the text in response to your comments strengthen the manuscript sufficiently and address all your concerns.

Major comments:

1)The paper does not differentiate between the possibilities that GR in BAT has no role in metabolic regulation during cold, feeding/fasting, or diet-induced obesity, OR whether GR has no role in BAT, period. The transcriptomics analysis should enable the authors to determine whether deficiency of GR in BAT has any effects on gene expression beyond the genes mentioned and in particular whether it has any effect on expression of adipose tissue GR target genes (such as Pik3r1, Foxa3, Pnpla2, Angptl4). Ideally, it would be very interesting to study to the effect of corticosterone treatment on gene expression in flox and GR^{BATKO} primary brown adipocytes or in BAT in vivo.

We agree with the referee that it is hard to differentiate between no role of GR in BAT in the conditions assayed, or no role of GR in BAT in general. However, we have provided substantial evidence for a lack of a physiological phenotype under a variety of conditions, in addition to gene expression assayed for thermogenic genes in the cold (Fig. 2I, Fig. EV 1H) and on HFD (Fig. 6F).

In order to address the referee's comment, we have now added measurements of both well-established, classic GR target genes (Gilz, Per1, Zbtb16, Fkbp5) as well as adipose tissue GR target genes (Pik3r1, Pnpla2, Angptl4) in Appendix Fig. S, exploring target gene responses under low-dose Dex exposure during the Dex suppression test. Under these conditions, the classical GR target gene Fkbp5 was significantly induced upon Dex treatment the effect of which was blunted in GR^{BATKO} mice, while other targets investigated did not respond to the low dose Dex. However, basal gene expression was found to be significantly lower in GR^{BATKO} animals as compared with controls, indicating that the GR in BAT indeed regulates gene transcription.

We acknowledge the limitation that these results do not preclude that effects would potentially have been visible given a higher Dex concentration. However, in this manuscript we aimed at elucidating the physiological effect of GR in BAT at baseline and in BAT-relevant (cold) and metabolic (HFD) challenges, and we believe that while it will be a fruitful area of investigation for the future, for this publication the pharmacological GR activation is out of scope.

2) Since the authors did not study the metabolic response to glucocorticoid treatment in flox and GR^{BATKO} mice, the title of the paper seems premature. The authors should consider studying the effect of pharmacological GR activation on metabolic regulation in flox and GR^{BATKO} mice.

While we agree with the referee on the importance of analyzing the effect of pharmacological GR activation in GR^{BATKO} mice, for our investigative purposes, pharmacological GR activation in the context of GR knockdown in the BAT was out of scope. To still address the referee's comment, we thus have measured 7 metabolites potentially relevant to GR function in the serum of mice harvested in the stressed condition of the Dexamethasone suppression test (Appendix Fig. S2). We were unable to detect any differences at the metabolite level, yet the above mentioned caveat applies.

Minor comments:

1) The description of the experimental procedures is a bit sloppy, especially for the mouse studies. These studies need to be described in much more detail. For example, it is not indicated whether the experiments were conducted in male or female mice. Also, the age of the mice is not indicated. No information in given on ethical approval. The overall description of the mouse studies is brief and incomplete.

We appreciate the constructive criticism and have amended the mouse methods accordingly. Male mice were used, as now stated in Materials & Methods in the "Animals" section, where the ethical approval information has been added now as well.

2) On page 3 it is stated that "In contrast to WAT, brown adipose tissue (BAT) contains fewer lipid droplets, but..." This is incorrect. Brown adipocytes contain more lipid droplets than white adipocytes.

We thank the referee for alerting us to this mistake, it has been corrected.

3) For GTT and ITT, it is not indicated in the methods when they were carried out. The legend of figure 5 says that the GTT was carried out after 19 weeks of HFD and the ITT after 20 weeks of HFD. However, in the discussion it is implied that these tests were carried out after 22 weeks of HFD. Please be clear and consistent.

We apologize for the oversight, this mistake has been corrected.

4) It is not completely clear how the experiments shown in figure 1a and 1b were conducted. It seems that in figure 1a a higher dose of DEX was injected. But if that is the case, why is the increase in corticosterone lower in figure 1a than in figure 1b for the same groups of mice (flox and

GR^{BATKO})? Frankly, I don't understand the purpose of figure 1b. Please make it more clear.

The referee is correct, the difference between Fig. 1A and 1B consists in the dosage used and in the fact that in Fig. 1B, only the Flox DEX vs GR^{BATKO} DEX groups are compared. For Fig. 1A, 0.1 mg/kg Dex were used, which is a higher dose compared to 0.02 mg/kg used in Fig. 1B. Given that a higher Dex dose would lead to more suppression and thus a lower increase in corticosterone, it is consistent that in Fig. 1A with the higher Dex dose, the increase of corticosterone is lower in the Flox DEX and GR^{BATKO} DEX groups, compared to Fig. 1B. Thus, the purpose of Fig. 1B was to make sure we do not overlook any subtle differences between the genotypes in the Dex suppression test due to higher dose of Dex. We apologize if the description of the experiment has been unclear and have amended the rationale in the results section.

5) The paper uses Nr3c1 when describing gene expression and GR for the genotype. It would be preferable to be consistent and only use Nr3c1 or GR.

We thank the referee for this constructive criticism and have changed the labelling to GR.

6) The second paragraph of the results section is redundant and can be removed.

We thank the referee for pointing this out, this paragraph has been removed.

7) My recommendation would be to delete any description of non-significant p values above the figures (for instance, figure 2d).

This has been implemented.

8) It is unclear what is shown in figure 2i. Is the heatmap based on mean expression values? Was RNA pooled from several animals? It would be useful to do a more extensive analysis of the cold-induced changes in gene expression in flox and GR^{BATKO} mice (or were mice raised at room temperature not included in the analysis?)

The heatmap is based on mean expression values of n=4, the RNA was not pooled. The most relevant thermogenic genes were picked from the data set to illustrate the lack of an effect of GR knockdown in GR^{BATKO} mice upon browning-related gene expression. Unfortunately, mice raised at room temperature were not included in the analysis. To satisfy the request for a more extensive analysis of the gene expression data, we have included a volcano plot of all detected gene expression changes in Fig. 2J. The cut-off values used were <0.1 adjusted p-value and >1.5 fold change.

9) It would be useful to have information on the expression of well-established GR target genes in BAT of the flox and GR^{BATKO} mice. It would help determine whether GR deficiency in BAT has no effect on gene expression at all or whether there are changes in gene expression yet these changes do not translate into any changes in metabolic parameters.

We agree with the referee and have added these measurements – they can be evaluated from the vehicle-treated groups now shown in Appendix Fig. S1.

10) Did the authors collect inguinal fat after cold exposure to enable studying the effect of GR deficiency on browning?

We have collected inguinal fat (iWAT) and measured thermogenic genes in these samples, as shown in Fig. EV1H.

11) If the intraperitoneal glucose tolerance was done after 19 weeks of HFD, why are the bodyweight data only go until 10 weeks (Figure 4a).

We have added the entire body weight curve up to week 24 including study design to a new Figure 4A

12) Page 8. This sentence is confusing: So far, we had observed that loss of GR in BAT affected

energy expenditure during a 10-week HFD intervention, but this did not lead to changes in glucose handling 10 weeks later. It suggest that there are two sequential HFD interventions, which is incorrect. Please rephrase to avoid confusion.

We thank the referee for this helpful comment, this has been rephrased.

13) It seems that a number of separate cohorts of flox and GRBATKO mice were put on HFD. Please be more clear in describing these cohorts in the methods section.

We appreciate that the description of the mouse experiments has been a bit confusing and we have made an effort to improve the method section accordingly.

14) Page 7: "To gain additional insight into the metabolic consequences of BAT GR deficiency under HFD conditions, we allowed the mice to gain body weight for an additional 6 weeks and..." Additional 6 weeks to what? Please clarify

This has been clarified in the text and in the legend of Figure EV4.

15) The paragraph in the discussion starting with "GCs have been... GC excess" is difficult to follow. For instance, there is the need to elaborate on the BAT phenotype of 11β-HSD1 deficient mice.

We thank the referee for this constructive criticism and have revised the paragraph accordingly in order to aid understanding.

16) The authors may consider changing the title to: The glucocorticoid receptor in brown adipocytes is dispensable for the systemic control of energy homeostasis. This title would better reflect the actual data.

We agree with the referee and have changed the title accordingly.

17) Page 10. Please rephrase: "Overall, however, the effects observed are too small to be statistically detected, with a high standard error." Statistics does not detect anything.

Again, we thank the referee for detecting this error and have rephrased the statement.

18) The methods section contains at least two PMID that the authors forgot to include in the list of references.

This oversight has been corrected.

19) In the discussion, the authors compare the GTT and ITT results from studies in GRadipoKO and GR^{BATKO} mice. However, results in GR^{BATKO} mice should not be directly compared with results in GRadipoKO mice, as the genotype of these mice is clearly distinct. Accordingly, it is premature to raise the suggestion that differences may be due to differences in age of the mice.

We agree with the referee regarding the lack of comparability between GR^{BATKO} and $GR^{adipoKO}$ mice. However, this was an attempt to clarify different findings in $GR^{adipoKO}$ mice, some of which correspond to our findings, while others do not. While the former (no effect in $GR^{adipoKO}$ and no effect in GR^{BATKO}) insinuates that GR in the adipose tissue has a negligible effect on systemic metabolism, the latter would imply that potentially, there is no effect of GR knockdown in BAT only, while a general adipose knockdown does affect metabolism e.g. on a HFD. It is hard to discuss these findings without analyzing why the different authors using $GR^{adipoKO}$ mice have come up with different data – for this, the age at the start of HFD might well be a factor. Nevertheless, we have tried to clarify our discussion of this issue for enhanced accuracy, and included the caveat suggested by the referee.

Referee #2:

The paper by Glantschnig et al. studies the role of the GR in systemic control of energy metabolism especially with regards to brown adipose tissue function. This is highly relevant since multiple studies (especially in vitro) have implicated the glucocorticoid axis in the regulation of BAT mediated thermogenesis, however the in vivo work is often hampered by the fact that glucocorticoids have other systemic effects which makes it difficult to dissect the contribution and function of BAT. IN summary the authors show through the use of many well-designed models that GR is dispensable for BAT function and might only have a slight influence under long term obesogenic conditions. Given the extremely careful way the work is conducted I think this paper would be very important for the scientific community. I have listed a few points below which would need to be addressed:

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Page 3: the intro on GR in adipogenesis and adipocyte function is a bit convoluted. I suggest to separate these two points and not mix them.

We thank the referee for this constructive criticism and have revised the paragraph accordingly.

Page 7: The changes in food intake are very interesting. Could the newly described secretin pathway be involved? The authors should check this maybe by measuring the expression in BAT of their animals.

We would like to preface this answer with the caveat that all food intake changes found in GR^{BATKO} mice were only trends and not found to be statistically significant.
In order to assess a potential involvement of the secretin pathway in GR^{BATKO} animals, we have consulted with the first author of the secretin study, Yongguo Li, who had recommended we measure if secretin receptor expression is increased in BAT, and assess Trpv-1 levels in the hypothalamus (Fig. EV2D-E), which were shown to rise upon secretin treatment. Both measurements do not support an involvement of the secretin pathway in the effects observed in GR^{BATKO} mice.

Page 13: Which Ucp1ERCre mouse line was used, there are several out there.
The UCP1 CreERT2 Line used originates from the Wolfrum lab (Lasar et al., 2018; Rosenwald et al., 2013). We thank the referee for mentioning this and have now added these references to the methods section.

2nd Editorial Decision 21 August 2019

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

As you can see, all referees find that the study is significantly improved during revision and recommend publication. Before I can accept the manuscript, I need you to address some editorial points below:

• We realized that the following panels are missing scale bars: Fig 2G, Fig 6D+G, Fig EV1G, Fig EV5B-E
• We noted the phrase 'data not shown' on page 7, which is not allowed as per journal policy. Please either show the data or remove the statement.
• Please make the GEO microarray gene expression data publicly available by removing the password protection.
• Our production/data editors have asked you to clarify several points in the figure legends (see attached document). Please incorporate these changes in the attached word document and return it with track changes activated.
• Papers published in EMBO Reports include a 'Synopsis' to further enhance discoverability. Synopses are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short standfirst - written by the handling editor - as well as 2-5 one sentence

bullet points that summarise the paper and are provided by the authors. I would therefore ask you to include your suggestions for bullet points
• In addition, please provide an image for the synopsis. This image should provide a rapid overview of the question addressed in the study but still needs to be kept fairly modest since the image size cannot exceed 550x400 pixels.
• EMBO Press is pleased to support the "minimum reporting standards in the life sciences" initiative (https://osf.io/preprints/metaarxiv/9sm4x/). This effort brings together a number of leading journals and reproducibility experts to develop minimum expectations for reporting information about Materials (including data and code), Design, Analysis and Reporting (MDAR) in published papers. We believe broad alignment on these standards will be to the benefit of authors, reviewers, journals and the wider research community and will help drive better practise in publishing reproducible research. We are therefore participating in a community pilot involving a small number of life science journals to test the MDAR checklist. The checklist is intended to help authors, reviewers and editors adopt and implement the minimum reporting framework. Since your manuscript fits the scope of the study, we very much hope that you will be willing to participate in this trial; the MDAR reporting checklist and an MDAR elaboration document providing context for the standards is attached. If you agree to participate, please complete the MDAR reporting checklist and return it to us within 7 days. We would also be very grateful if you could complete this author survey https://forms.gle/FRx7hpKS8g1QMNPR9.

Please note that your completed checklist and survey will be shared with the minimum reporting standards working group. However, the working group will not be provided with access to the manuscript or any other confidential information including author identities, manuscript titles or abstracts. Feedback from this process will be used to consider next steps, which might include revisions to the content of the checklist. Data and materials from this initial trial will be publicly shared in September 2019. Data will only be provided in aggregate form and will not be parsed by individual article or by journal, so as to respect the confidentiality of responses.

Please treat the checklist and elaboration as confidential as public release is planned for September 2019.

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

REFEREE REPORTS

Referee #1:

I have no further comments. The authors have appropriately addresses my comments.

Referee #2:

The authors addressed all my concerns I would suggest publication

2nd Revision - authors' response 2 September 2019

The authors performed all minor editorial changes.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Stephan Herzig
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-48552V1

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?	Sample size was based on experience from previous experiments performed in the lab and confirmed by biostatistical power calculation.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Typically, a sample size of 7-10 is sufficient to pick up meaningful differences.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Animals were excluded in case of sickness only.
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 stratified based on litter, then randomized over the different diets/treatments.
For animal studies, include a statement about randomization even if no randomization was used.	Mice were stratified based on litter, then randomized over the different diets/treatments.
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.	Mice were stratified based on litter, then randomized over the different diets/treatments. No blinding of investigator
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Not tested

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

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

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	Yes, as reported by standard error of the mean
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).	Catalog numbers are reported in the manuscript
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	n.a.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Brown adipose tissue (BAT) specific GR-knockout mice (GRBATKO) were generated by crossing Ucp1−CreERT2 mice with floxed GR mice. Throughout the experiments we used male mice aged 13 20 weeks old. All mice were maintained on a 12h light-dark cycle at 22°C with unlimited access to chow and water, unless stated otherwise.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Animal experiments were performed in accordance with the Directive 2010/63/EU from the European Union and the German Welfare Act, after approval by local authorities
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.	Ok

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	n.a.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	n.a.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	n.a.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	n.a.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	n.a.
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	n.a.
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	n.a.

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. 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	The dataset produced in this study is available in the following database: • Microarray gene expression data: Gene Expression Omnibus (GSE13554) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135544); reviewer access token: yfybeiewbjynloz
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).	n.a.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	n.a.
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as 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.	n.a.

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	n.a.