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Ageing induces tissue-specific transcriptomic changes in *Caenorhabditis elegans*

Yidong Shen, Xueqing Wang, Quanlong Jiang, Yuanyuan Song, Zhidong He, Hongdao Zhang, Mengjiao Song, Xiaona Zhang, Yumin Dai, Oezlem Karalay, Christoph Dieterich, Adam Antebi, Ligang Wu, and Jing-Dong Han
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Transaction Report:

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

1st Editorial Decision **6th Oct 2021**

Dear Dr Shen,

Thank you again for the submission of your manuscript (EMBOJ-2021-109633) to The EMBO Journal. Please accept my apologies for the delay with the peer-review of your work due to protracted referee input and detailed discussions in the team. Your study has been sent to three referees, and we have received reports from all of them, which I enclose below.

As you will see, the referees acknowledge the potential interest and value of your resource analysis and results, although they also express major concerns, which need to be addressed before they can be supportive of publication at the EMBO Journal. In more detail, referee #3 states that the tissue-specific data is not sufficiently integrated with whole worm analyses, and requests consolidation of this aspect (ref#3, pt.1). Further, this expert points to incomplete characterisation of the roles of irx-1 and myrf-2, which in his/her view decreases the impact of the current results (ref#3, pt.2). Reviewer #1 agrees in that the functional exploration of newly identified tissue-specific regulators and their contribution control of ageing processes remains too premature. Finally, all three referees list a number of issues related to missing controls, as incomplete data presentation and methods annotation, as well as a need for a revised discussion or the findings that would have to be addressed to achieve the level of robustness needed for The EMBO Journal.

Given the referees' overall positive recommendations and detailed comments, I would like to invite you to submit a revised version of the manuscript, addressing the issues raised. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

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

Thank you for the opportunity to consider your work for publication.
I look forward to your revision.

Kind regards,

Daniel Klimmeck

Daniel Klimmeck, PhD
Senior Editor
The EMBO Journal

Instruction for the preparation of your revised manuscript:

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 response 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 (https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/Author Checklist%20-%20EMBO%20J-1561436015657.xlsx). 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.

6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary

datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/14602075/authorguide#datadeposition).
In case you have no data that requires deposition in a public database, please state so in this section. 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. ***

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

8) We would also encourage you to include the source data for figure panels that show essential data. Numerical data can 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 or a single pdf per main figure 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 .

9) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online (see examples in https://www.embopress.org/doi/10.15252/embj.201695874). A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc. in the text and their respective legends should be included in the main text after the legends of regular figures.

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

- Additional Tables/Datasets should be labelled 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.

10) When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
http://bit.ly/EMBOPressFigurePreparationGuideline

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

11) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

Further information is available in our Guide to Authors: https://www.embopress.org/page/journal/14602075/authorguide

Revision to The EMBO Journal should be submitted online within 90 days, unless an extension has been requested and approved by the editor; please click on the link below to submit the revision online before 4th Jan 2022:

Link Not Available

Referee #1:

The study of Wang et al, which describes tissue-specific transcriptome changes during aging in C. elegans is very cleanly carried out, with a very well written text and lovely, clear illustrations.

The study, which appears technically flawless, makes forcefully and convincingly one single point: that the transcriptome changes during aging are different in each tissue studied. To a degree, this was to be expected. Each tissue has a unique transcriptome thus the changes with age are expected to be different from other tissues, as the starting point is a different transcriptome. However, in theory, it could have been possible that what changes during aging is a common core group of genes that could have been the same in each tissue. This seems unlikely but it still needed to be shown that this was not the case.

In some sense the paper is purely descriptive, but this description provides and establishes an interesting point.
I am less impressed by the results of attempting to analyze the functional significance of the changes in figures 3-5. Nor by the little new we have learned about irx-1 and myrf-2. None of this tells us anything about what drives the aging process per se or even what makes it different from tissue to tissue. Nonetheless, the techniques and data of Wang et al. could definitely contribute to functional studies in the future by allowing focus on the aging process one tissue at a time.
In summary this is a very good descriptive study that strongly makes an important point.

Referee #2:

In "Ageing induces tissue specific transcriptomic changes in Caenorhabditis elegans", Wang and colleagues, unsurprisingly find, as stated in the title, that transcriptomic changes with age are tissue specific. I say these results are unsurprising as this is reasonably well documented in human studies which clearly demonstrate that loss of homeostasis (e.g. disease) is underlied by tissue specific changes in these tissues (which is also why there are disease specific treatments). That said, as correctly stated by Wang and colleagues, this is not an issue that has received much attention in animal model. Moreover, the ageing community as a whole seems to have moved away from the revelation 40 and, again, 20 years ago that healthspan is a more important metric than lifespan having, once again, decided to push toward funding lifespan extending research and often ignoring the fact that long life <> long health. In this context, this paper is extremely important in refocusing the ageing, and particularly the C. elegans ageing, research community on the fact that "health" is tissue specific and that ageing research should focus on not just "health" but also tissue specific health with age.

The authors use fairly new/novel techniques to effectively perform tissue and age specific transcriptomic analysis of C. elegans tissues much as one might do with rodents, or in some instances people (importantly nervous system biopsies similar to those performed here are difficult in people barring disease). Thus, the technical limitations of the approach are similar to those in mammalian species and the authors have done a good job of addressing the concerns about cross cell/tissue contamination via their analysis. The authors have also used fairly standard bioinformatic approaches to examine differential gene expression and also more advanced biological process/network analysis as well as controlling transcription factor analysis. Unsurprisingly, using these more advanced techniques the authors find much more biological insight into transcriptomic changes with age and tissue including: i) similar biological processes change with age despite non identical genes underlying these changes; ii) tissue specific changes with age are more pronounced than common changes with age between tissues; iii) genes and biological processes previously identified as changing with age (in C. elegans and other species) are changing in this study (confirming the results against past studies, and therefore the underlying approach); iv) specific transcription factors may be associated with changes with age in specific tissues (not surprising given the same is true for cell development); v) Solute carriers appear to be an important class of genes that have not previously be highlighted as important for loss of cellular homeostasis with age (despite them potentially underlying the metabolic and mitochondrial gene expression changes previously identified and their clear relevance to human diseases). Lastly, the authors validate the importance of two transcription factors to tissue specific health with age, focusing on two tissues of importance in the clinic, intestine and muscle. In the case of the intestine the authors demonstrate the importance of a transcription factor for intestinal integrity/barrier function, a key issue with ageing people. In the case of the muscles, the authors demonstrate the importance of a transcription factor for sarcomere integrity, a potentially key issue with ageing people (NB loss of strength with age in people is not completely explainable via structural changes to muscle).

In sum this is an important paper for the fields of ageing and C. elegans ageing, the studies are well conducted, and the results are both validated by uncovering changes as expected from the past literature and by some further experimentation with two transcription factors/tissues.

Minor thoughts for improving the manuscript:

Justification for D1 and D8 as time points might be helpful.

The paper is very bioinformatic heavy any areas or increased highlighting of biology/physiology might be helpful for the non specialist (the only area where I saw an opportunity to do this, however, was maybe more discussion of Figure 3 and even that is limited to a few sentences).

A bit more on the relevance to humans either in the introduction, or particularly in the discussion, might be helpful for the non specialist. For example, possibly citing some tissue specific/common ageing signatures from human studies and possibly noting loss of the intestinal barrier is an important problem in human ageing.

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In this manuscript entitled "Ageing induces tissue specific transcriptomic changes in Caenorhabditis elegans", Wang et al. profiled tissue-specific transcriptomic changes using youn and old C. elegans after isolating cells from five somatic tissues in C. elegans. The authors found that each tissue displayed distinct ageing-regulated differentially expressed genes (Age-DEGs) and biological processes (Age-BPs). The authors also showed that different sets of transcription factors affected the Age-DEGs and the Age-BPs in a tissue-specific manner. By using tissue-specific RNAi knockdown, they determined the functional roles of two genes, irx-1 and myrf-2, in the aging of two tissues, the intestine and muscles, respectively. This paper provides comprehensive information to understand tissue-specific aging in C. elegans. Following are my concerns the authors should address.

Major comments:
1) Although their analysis is informative, I recommend that the authors should further analyze their tissue-specific age-dependent gene expression changes with respect to the age-dependent gene expression changes in whole worm body, by using their own data and also previously published data.
2) The functional characterization of irx-1 and myrf-2 is nice, but looks incomplete. Are these genes required for maintaining lifespan? Are they required for longevity caused by interventions, such as daf-2 mutations and dietary restriction? Are they differentially regulated in long lived animals? Addressing these issues will make this paper much more complete.

Minor comments:
1) In the "Results" section, the authors wrote "Nevertheless, tissue ageing is more similar from the perspective of Age-BPs than of Age-DEGs (Fig 2 and 3).", but these figures tend to qualitatively, not quantitatively, support the sentence. Please add quantitative results that support the sentence.
2) Are there more quantitative and unbiased analyses for DEGs, similar to the analysis shown in Fig. 1C and D?
3) The authors used worms at days 1 and 7 of adulthood for whole worm RNA-Seq, but those at days 1 and 8 for tissues Smart-Seq2. The authors should describe why they used different ages of old worms.
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11) Page 6, Change "Principle" to "Principal".

We would like to thank the referees for their precious time reviewing our manuscript and their valuable comments to improve this study. Our detailed responses to their concerns are listed below on a point-by-point basis.

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-- Many thanks for your appreciation of our study! Our tissue-specific transcriptomic datasets in ageing worm tissues found that the five analysed tissues shared no common upregulated genes and only three common downregulated genes. In contrast, thousands of genes were changed in these tissues during ageing (Figure 2). The three are all collagen genes, namely *col-93*, *col-103*, and *col-159*. Therefore, it is unlikely that a common core group of genes changes during ageing in each tissue.

Instead, we did find a common core group of biological pathways changes during ageing throughout the five analysed tissues (Figure 3 and Dataset EV2). By WormCat category 3, which is the most detailed categorisation by this algorithm, 1.3% and 1.9% Age-BPs are respectively up- and down-regulated in all five tissues. The three

commonly downregulated collagen genes are in one of these Age-BPs, “extracellular material – collagen” (Dataset EV2).

Taken together, our findings indicate that each tissue undergoes specific transcriptomic changes during ageing, as you pointed out, and in turn causes similar and distinctive biological changes.

As you suggested, we have further clarified this issue in the revised manuscript.

In some sense the paper is purely descriptive, but this description provides and establishes an interesting point. I am less impressed by the results of attempting to analyse the functional significance of the changes in figures 3-5. Nor by the little new we have learned about irx-1 and myrf-2. None of this tells us anything about what drives the aging process per se or even what makes it different from tissue to tissue. Nonetheless, the techniques and data of Wang et al. could definitely contribute to functional studies in the future by allowing focus on the aging process one tissue at a time.

In summary this is a very good descriptive study that strongly makes an important point.

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stated by Wang and colleagues, this is not an issue that has received much attention in animal model. Moreover, the ageing community as a whole seems to have moved away from the revelation 40 and, again, 20 years ago that healthspan is a more important metric than lifespan having, once again, decided to push toward funding lifespan extending research and often ignoring the fact that long life <> long health. In this context, this paper is extremely important in refocusing the ageing, and particularly the C. elegans ageing, research community on the fact that "health" is tissue specific and that ageing research should focus on not just "health" but also tissue specific health with age.

-- Many thanks for your appreciation! We do hope that the findings and datasets in this manuscript could promote and facilitate future studies on tissue-specific health with age, especially in *C. elegans*.

The authors use fairly new/novel techniques to effectively perform tissue and age specific transcriptomic analysis of C. elegans tissues much as one might do with rodents, or in some instances people (importantly nervous system biopsies similar to those performed here are difficult in people barring disease). Thus, the technical limitations of the approach are similar to those in mammalian species and the authors have done a good job of addressing the concerns about cross cell/tissue contamination via their analysis. The authors have also used fairly standard bioinformatic approaches to examine differential gene expression and also more advanced biological process/network analysis as well as controlling transcription factor analysis. Unsurprisingly, using these more advanced techniques the authors find much more biological insight into transcriptomic changes with age and tissue including: i) similar biological processes change with age despite non identical genes underlying these changes; ii) tissue specific changes with age are more pronounced than common changes with age between tissues; iii) genes and biological processes previously identified as changing with age (in C. elegans and other species) are

changing in this study (confirming the results against past studies, and therefore the underlying approach); iv) specific transcription factors may be associated with changes with age in specific tissues (not surprising given the same is true for cell development); v) Solute carriers appear to be an important class of genes that have not previously be highlighted as important for loss of cellular homeostasis with age (despite them potentially underlying the metabolic and mitochondrial gene expression changes previously identified and their clear relevance to human diseases).

-- Thanks for reminding us of the importance of SLCs. Corresponding information and discussion have been included in the revised manuscript.

Lastly, the authors validate the importance of two transcription factors to tissue specific health with age, focusing on two tissues of importance in the clinic, intestine and muscle. In the case of the intestine the authors demonstrate the importance of a transcription factor for intestinal integrity/barrier function, a key issue with ageing people. In the case of the muscles, the authors demonstrate the importance of a transcription factor for sarcomere integrity, a potentially key issue with ageing people (NB loss of strength with age in people is not completely explainable via structural changes to muscle).

In sum this is an important paper for the fields of ageing and C. elegans ageing, the studies are well conducted, and the results are both validated by uncovering changes as expected from the past literature and by some further experimentation with two transcription factors/tissues.

Minor thoughts for improving the manuscript:

Justification for D1 and D8 as time points might be helpful.

-- Sorry for the potential confusion. In the lab-cultured *C. elegans* hermaphrodites,

progeny production starts at day 1 of adulthood (D1) and ends by around day 8 of adulthood (D8). So D1 and D8 are two commonly recognised time points for young and aged worms, respectively. Corresponding clarification has been included in the revised manuscript as suggested. Many thanks for your suggestion!

The paper is very bioinformatic heavy any areas or increased highlighting of biology/physiology might be helpful for the non specialist (the only area where I saw an opportunity to do this, however, was maybe more discussion of Figure 3 and even that is limited to a few sentences).

-- Thank you for your suggestion! We have added more corresponding information in the revised manuscript, especially in the section describing Fig 3.

A bit more on the relevance to humans either in the introduction, or particularly in the discussion, might be helpful for the non specialist. For example, possibly citing some tissue specific/common ageing signatures from human studies and possibly noting loss of the intestinal barrier is an important problem in human ageing.

-- Many thanks for your suggestion! In the revised “Introduction” section, we have further introduced the relevance of insulin/IGF-1 signalling, which was first identified in *C. elegans* ageing, in human longevity. We also included more comparison with human ageing in the revised “Discussion”, including the loss of the intestinal barrier and sarcopenia.

Referee #3:

In this manuscript entitled "Ageing induces tissue specific transcriptomic changes in Caenorhabditis elegans", Wang et al. profiled tissue-specific transcriptomic changes

using young and old C. elegans after isolating cells from five somatic tissues in C. elegans. The authors found that each tissue displayed distinct ageing-regulated differentially expressed genes (Age-DEGs) and biological processes (Age-BPs). The authors also showed that different sets of transcription factors affected the Age-DEGs and the Age-BPs in a tissue-specific manner. By using tissue-specific RNAi knockdown, they determined the functional roles of two genes, irx-1 and myrf-2, in the aging of two tissues, the intestine and muscles, respectively. This paper provides comprehensive information to understand tissue-specific aging in C. elegans. Following are my concerns the authors should address.

Major comments:

1) Although their analysis is informative, I recommend that the authors should further analyse their tissue-specific age-dependent gene expression changes with respect to the age-dependent gene expression changes in whole worm body, by using their own data and also previously published data.

-- Many thanks for your advice! Following your suggestion, we have further analysed our data and the data from a previous report (Hou *et al*, 2016). Please see the revised manuscript (Fig EV3) for the detailed description. In short, the analysis using either dataset consistently showed that the Age-DEG sets in individual tissues are distinct from each other and from that in the whole worm.

2) The functional characterisation of irx-1 and myrf-2 is nice, but looks incomplete. Are these genes required for maintaining lifespan?

-- This is indeed an intriguing issue to pursue. Both TFs are upregulated with ageing in the intestine and BWM, respectively (Dataset EV1). Following your advice, we suppressed *irx-1* in the intestine and *myrf-2* in BWM using tissue-specific RNAi and examined their effects on lifespan. Whereas the intestinal suppression of *irx-1* did

not change worms lifespan, inhibiting *myrf-2* in BWM made worms live longer (Fig. 6 and 7). These data indicate that the impact of Age-TFs could either be restricted to a certain tissue or spread throughout the body. Please see the revised manuscript for detailed results and discussion.

Are they required for longevity caused by interventions, such as daf-2 mutations and dietary restriction? Are they differentially regulated in long lived animals? Addressing these issues will make this paper much more complete.

-- Following your advice, we examined *irx-1* and *myrf-2* expression in two well-established genetic longevity models, IIS (*daf-2*) and DR treatment (*eat-2*) (Kenyon, 2010) (Fig EV9).

In the case of *irx-1*, it is upregulated in *daf-2(-)* mutants but not regulated in *eat-2(-)* mutant at the level of whole worm. In contrast, both *daf-2(-)* and *eat-2(-)* mutation decreased *irx-1* transcription in the intestine (Fig EV9A-C). As *irx-1* is upregulated during WT ageing in the intestine (Dataset EV1), the downregulation of intestinal *irx-1* by *daf-2(-)* and *eat-2(-)* could be a counteraction of WT ageing by IIS and DR, respectively. *irx-1* therefore could be involved in the improved intestinal integrity by IIS and DR (Gelino *et al*, 2016).

In the case of *myrf-2*, DR did not affect its expression in either the whole worm or BWM (Fig EV9D-F). *daf-2* indeed reduced its expression in BWM (Fig EV9E and F). This suggests that *myrf-2* may not be involved in the longevity by DR whereas it could be a target of IIS-controlled longevity.

Please see the revised manuscript for a more detailed description and discussion.

Minor comments:

1) In the "Results" section, the authors wrote "Nevertheless, tissue ageing is more similar from the perspective of Age-BPs than of Age-DEGs (Fig 2 and 3).", but these figures tend to qualitatively, not quantitatively, support the sentence. Please add quantitative results that support the sentence.

-- Thanks for your suggestion! To quantitatively show that ageing is more similar in the aspect of Age-BPs than Age-DEGs, we calculated the ratio of Age-BPs and Age-DEGs shared by at least two tissues. 15.2% Age-DEGs whereas 31.8% Age-BPs at Category 3, which is the most detailed categorisation by WormCat, were shared in at least two tissues (Fig 2 and 3, Dataset EV1 and 2). We think these quantitative analyses further support our speculation that “Tissue ageing is more similar from the perspective of Age-BPs than of Age-DEGs”.

2) Are there more quantitative and unbiased analyses for DEGs, similar to the analysis shown in Fig. 1C and D?

-- As suggested, we analysed Age-DEGs using heatmap, an unbiased and widely used bioinformatics method (Fig EV2A). Similar to the results in Fig 1B and C (previously Fig 1C and D), the heatmap clearly shows the remarkable and various ageing-induced transcriptomic changes in the tissues.

3) The authors used worms at days 1 and 7 of adulthood for whole worm RNA-Seq, but those at days 1 and 8 for tissues Smart-Seq2. The authors should describe why they used different ages of old worms.

-- The two datasets used worms at two slightly different ages, as you pointed out. This is because two labs performed the whole worm RNA-Seq and tissue Smart-Seq2

in different years, the former by Antebi lab and the latter by Shen lab. But the adult worms at day 7 and day 8 of adulthood are very similar, both at the end of the reproductive period.

To further consolidate our comparison of the age-dependent transcriptomic changes between tissues and the whole worm, we introduced another dataset from a published study, as you insightfully suggested. The aged worm in this dataset is at day 8 of adulthood (Hou *et al.*, 2016) and gives similar results as our own whole worm RNA-Seq data (Fig EV3). Corresponding explanation and discussion have been included in the revised manuscript.

4) In Figure EV1, the authors need to match the lists of tissue-specific genes in EV1B and those examined by qRT-PCR in EV1C-G.

-- Many thanks for your suggestion to make the illustration clearer and more accurate! We have labelled the corresponding tissues to those tissue-specific genes in Fig EV1C-G.

5) In Figure 1, I recommend that the authors change the order of panel C, D and B to lay out from unsupervised analyses to supervised analyses.

-- Fig 1 has been reorganised as suggested. Thank you for your comment!

6) In Figure 1C, the authors need to add the proportion of variance explained by each axis and explain the different direction of age-dependent transcriptomic changes in the intestine from those in other tissues.

-- Fig 1B (previously Fig 1C) has been modified as suggested.

The different direction of age-dependent changes in the intestine is due to a label mistake. Many thanks for your careful reading! The mistake has been corrected in the revised Fig 1B.

In addition, it will be better to simultaneously compare tissue-specific upregulated genes and downregulated genes in Figure 2.

-- Thank you for your suggestion! As suggested, we have simultaneously compared the up- and down-regulated genes across the five examined tissues. Please see Fig EV2C in the revised manuscript for more details.

7) In the "Results" section, the authors wrote "50.1% of Age-DEGs have orthologs in mammals (Dataset EV1).". It will be more informative to provide statistics and supportive figures to show the number of mammalian orthologs of Age-DEGs in dataset EV1.

-- Thanks for the suggestion! A supportive figure is included in the revised manuscript as Figure EV2B to show the percentage of Age-DEGs with mammalian orthologs. Dataset EV1 was modified accordingly to provide detailed statistics in its first sheet.

8) It will be better to show statistics (e.g. p values or RF) for overlaps in Venn diagrams.

-- Thanks for your advice! We have calculated *P*-value by hypergeometric test for the Venn diagrams. Please see the revised figures for details.

9) The authors wrote the label "body length per 30 sec" on the y-axis in Figure 7E, while "Worm tracks in 30 sec" in 7D. Please use "Worm tracks in 30 sec" in Figure 7E.

-- Sorry for the confusing label! The y-axis in Fig 7E shows the length of worm tracks which is normalised against worm body length. The label has been corrected following your comment in the revised manuscript.

10) In Figure 6 and 7, the authors jumped to intestinal permeability and sarcomere structures without relevant rationales, respectively. The authors also need to explain whether the role of irx-1 is beneficial to longevity or not.

-- Corresponding explanations of intestinal permeability and sarcomere structure have been included in the revised manuscript as advised.

irx-1 is predicted to be a proAge-TF in the intestine (Fig 4). It is upregulated in the intestine during WT ageing, whereas downregulated in the intestine of long-lived *daf-2(-)* and *eat-2(-)* mutants (Fig EV9 and Dataset EV1). Suppressing *irx-1* improves the intestinal barrier in aged worms (Fig 6). Therefore, we conclude that *irx-1* is detrimental to longevity, especially in the intestine. The corresponding discussion has been included in the revised manuscript.

11) Page 6, Change "Principle" to "Principal".

-- The spelling mistake has been corrected. Thanks!

References

Gelino S, Chang JT, Kumsta C, She X, Davis A, Nguyen C, Panowski S, Hansen M (2016) Intestinal Autophagy Improves Healthspan and Longevity in C. elegans during Dietary Restriction. *PLoS genetics* 12: e1006135

Hou L, Wang D, Chen D, Liu Y, Zhang Y, Cheng H, Xu C, Sun N, McDermott J, Mair WB *et al* (2016) A Systems Approach to Reverse Engineer Lifespan Extension by Dietary Restriction. *Cell metabolism* 23: 529-540

Kenyon CJ (2010) The genetics of ageing. *Nature* 464: 504-512

Dear Dr Shen,

Thank you for submitting your revised manuscript (EMBOJ-2021-109633R) to The EMBO Journal. Your amended study was sent back to the three referees for re-evaluation, and we have received comments from all of them, which I enclose below. As you will see, the referees stated that the issues raised have been adequately addressed and they are now broadly in favour of publication.

Thus, we are pleased to inform you that your manuscript has been accepted in principle for publication in The EMBO Journal.

We need you to still take care of a number of minor issues related to formatting and data representation as detailed below, which should be addressed at re-submission.

Please contact me at any time if you have additional questions related to below points.

Thank you for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to your final revision.

Again, please contact me at any time if you need any help or have further questions.

Kind regards,

Daniel Klimmeck

Daniel Klimmeck PhD
Senior Editor
The EMBO Journal

Formatting changes required for the revised version of the manuscript:

>> Introduce ORCID IDs for all corresponding authors (JD.J.H.) via our online manuscript system. Please see below for additional information.

>>Adjust the title of the 'Competing Interests' section to 'Disclosure and Competing Interests Statement'.

>> Recheck the bioRxiv reference for Zhang et al. 2021 for updates on journal publication.

>> Remove the referee links from the data accessibility section and add dataset identifiers. Make sure the data is made publicly accessible.

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Please note that as of January 2016, our new EMBO Press policy asks for corresponding authors to link to their ORCID iDs. You can read about the change under "Authorship Guidelines" in the Guide to Authors here: http://emboj.embopress.org/authorguide

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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (9th May 2022). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

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Referee #1:

I am satisfied with the manuscript as it is. I have no special request for revisions.

Referee #2:

The authors have done an excellent job in considering my review of their excellent work and have improved the paper both for the non-specialist and for those wanting greater insight from the authors. I feel the revisions have not altered my initial assessment of the quality and importance of the work and therefore have no further comments beyond commending the authors.

Referee #3:

The authors addressed all my previous concerns successfully.

The authors performed the requested editorial changes.

Dear Dr Shen,

Thank you for submitting the revised version of your manuscript. I have now evaluated your amended manuscript and concluded that the remaining minor concerns have been sufficiently addressed.

Thus, I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper.

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Please let me know, should you be interested to engage in commissioning a similar video synopsis for your work. According operation instructions are available and intuitive.

If you have any questions, please do not hesitate to call or email the Editorial Office.

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

Kind regards,

Daniel Klimmeck

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Yidong Shen
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2021-109633R

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The sample size in the current study was determined by referring to well-accepted previous studies.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The sample size in the current study was determined by referring to well-accepted previous studies.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	For lifespan assays, the worms undergoing internal hatching, bursting vulva, crawling off the plates, or contamination were censored by referrig to well-accepted previous studies. The criteria were pre-established before performing assays.
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.	Yes. All samples were allocated into experimental groups randomly.
For animal studies, include a statement about randomization even if no randomization was used.	All worms were allocated into experimental groups randomly.
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.	Blinding of the investigator was performed when assessing results.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Blinding of the investigator was performed when assessing results.
5. For every figure, are statistical tests justified as appropriate?	Yes. The statistical tests in each figure were justified as appropriate following well-accepted studies.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The data meet the normal distribution and assessed by Kolmogorov-Smirnov test.
Is there an estimate of variation within each group of data?	Usually the within-group variation is used in ANOVA test. In the t-test, standard deviation of each group is calculated. It's similar with within-group variation.

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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).	NA
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

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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.	C. elegans hermaphrodites at various ages (up to day 36 of adulthood) were used in this study, following standard worm culture protocol. The detailed information of all used strains were included in Table EV1.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
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.	We confirm that all animal assays are compliant with the ARRIVE guidelines.

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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 RNA-Seq data from this publication have been deposited to the SRA database (https://www.ncbi.nlm.nih.gov/sra). Please see the "Data Availability" section for details.
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