THE EMBO JOURNAL

Feeding activates FGF15-SHP-TFEB-mediated lipophagy in the gut

Sunmi Seok, Young-Chae Kim, Yang Zhang, Bo Kong, Grace Guo, Jian Ma, Byron Kemper, and Jongsook Kemper
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Editor: William Teale

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 **16th Nov 2021**

Dear Dr Kemper,

Thank you again for the submission of your manuscript (EMBOJ-2021-109997) entitled "Paradoxical feeding activation of gut lipophagy via an FGF15-SHP-TFEB axis limits postprandial lipids". As you know, we have asked three experts to assess your manuscript for the EMBO Journal. We have now received reports from all of them, which I have copied to the bottom of this message.

As you can see from their comments, the referees appreciate the significance of your central finding and are generally supportive of your work. However, as you will see there are a number of important issues that are consistently raised across all reports which will need to be addressed before we can progress towards publication in The EMBO Journal.

Firstly, the reviewers consistently request a more detailed characterization of the autophagy response to FGF15 in intestinal cells. Here, we agree that it will be important for you to test, through a more detailed molecular characterization, whether the feeding response is genuine autophagy or may be better characterized as "autophagy-like". Related to this, major concerns were raised regarding data inconsistencies with respect to the timing and kinetics of the feeding response (ref#1, pt.1). Please note that, for these points, characterization in cell culture appears will be satisfactory in our view.

Secondly, the referees state that the molecular details of why liver and intestinal cells respond differently to feeding remain unclear (ref#1, pt.2; ref#2) and ask you to engage in further in silico analyses of the data sets to gain additional insights into liver- and intestine-specific response pathways involved.

Thirdly, the exact cell type activated for intestinal autophagy remains unresolved (ref#1, pt.4). Please revise your discussion where appropriate to better account for this remaining ambiguity, also regarding your choice of cell lines used for the work.

Based on the overall interest expressed, I would like to invite you to address the comments of the referees in a revised version of the manuscript.

Considering the substantial amount of additional experiments required for the study, I would appreciate a direct exchange via a video or telephone call during the next few weeks in order to discuss how to best address the key concerns of the experts in your revision.

I should add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve these concerns at this stage. I believe the concerns of the referees are reasonable and addressable, but we are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic, so please contact me if you have any questions, need further input on the referee comments or if you anticipate any problems in addressing any of their points. Please, follow the instructions below when preparing your manuscript for resubmission.

I would also like to point out that as a matter of policy, competing manuscripts published during this period will not be taken into consideration in our assessment of the novelty presented by your study ("scooping" protection). We have extended this 'scooping protection policy' beyond the usual 3 month revision timeline to cover the period required for a full revision to address the essential experimental issues. Please contact me if you see a paper with related content published elsewhere to discuss the appropriate course of action.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

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

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

Kind regards,

William Teale

William Teale, Ph.D.
Editor
The EMBO Journal

When submitting your revised manuscript, please carefully review the instructions below and include the following items:

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) We require 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). If no data deposition in external databases is needed for this paper, please then state in this section: This study includes no data deposited in external repositories. 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) 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.

8) 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.).

9) 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 .

10) 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.

11) 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 .

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 14th Feb 2022:

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

The work entitled 'Paradoxical feeding activation of gut lipophagy via an FGF15-SHP-TFEB axis limits postprandial lipids' by Kemper group describes that feeding activates intestinal autophagy/lipophagy instead of inhibiting it. This is a very interesting observation. The authors propose that the activation of lipophagy in intestine is triggered by FGF15/19-mediated activation of PKC that in turn phosphorylates SHP and TFEB leading to their activation. Lipophagy induction is needed to degrade triglycerides and APOB48 during feeding.

The work is very well written and the data are of good quality. I have some questions that should be addressed/clarified experimentally to strength the main findings of the paper.

1) Timing of autophagy activation: the authors detected autophagy activation in intestine as early as 1h after feeding. To me this observation suggests that post-translational rather than transcriptional-based mechanism accounts for feeding mediated autophagy in intestine. The authors should study the timing of SHP and TFEB activation by FGF during feeding.

2) The role of SHP in intestine and in liver should be compared. It is important to further understand how does the very same protein can activate and repress autophagy in intestine and in liver, respectively. Which are SHP targets in liver? Does FGF15/19 also activates PKC in liver or selectively in intestine?

3) the degradation of APOB48 by lipophagy should be experimentally demonstrated.

4) It is unclear to me which is/are the intestinal cell population(s) that activates autophagy during feeding

Referee #2:

Seok et al. report that the signaling axis of FGF15/19-SHP-TFEB is responsible for unexpected activation of intestinal lipophagy upon feeding. They found that the fibroblast growth factor 15/19 (FGF 15/19) activates SHP/NR0B2 an orphan nuclear receptor that in turn interacts with TFEB. TFEB is also activated by FGF 15/19 through PCKβ/ζ-mediated phosphorylation, which leads to its nuclear translocation. SHP-TFEB interaction in the nucleus upregulates the expression of the autophagy factor Ulk1 as well as of the lipolysis factor Atgl, thereby limiting the postprandial intestinal triglycerides (TGs). By utilizing a mouse model system, the authors have found that feeding leads an opposite response between the liver and intestine in the protein levels of LC3 and p62 serving as markers for autophagy. While in the liver, feeding leads to reduced LC3 and increased p62 levels, in the intestine this is completely reversed. Evidence is provided to show that this phenotype is regulated by the FGF15-SHP-TFEB pathway. Furthermore, by using global ChIP-seq analysis after feeding, the SHP cistrome was implicated in the intestine-response to feeding. In addition, the mechanism of postprandial TFEB activation by PCKβ/ζ was fully characterized.
This is potentially interesting study describing a rather surprising phenomenon of differential organ response to feeding by the FGF15-SHP-TFEB pathway in the intestine. While the data concerning the signaling pathways are rather convincing the authors' model regarding regulation of autophagy/lipophagy is not well based. Using LC3 and p62 levels as the sole indication for activation or inhibition of autophagy is not sufficient. A more rigorous analysis as for a direct involvement of autophagy in this process is needed, using genetic and biochemical tools. Only in one case, shown in the supplementary data, the authors bothered to look for autophagy flux. This for example, should be performed for all the conditions described in this study. Another critical point is the lack of direct assessment of lipophagy in this study. Here too, the authors should employ available markers and genetic tools to challenge their model.
Additional comments
Figure 1 - The induction of intestinal autophagy was mainly based on cellular levels of autophagy markers LC3 and p62, lysosomal marker LAMP1 and accumulation of endogenous LC3 by IHC. Although the results obtained in SHP-KO or FGF15-KO mice were in line with these observations, the accumulation of LC3 and fluctuations in p62 do not always reflect on induction

of autophagy. The lysosomal protein LAMP1 is a TFEB transcriptional target and its protein levels is consistent with TFEB activation.
Figure 2B and Figure 3B - the authors should provide a clear explanation why they chose to ignored the pathways with lowest p-values.
Figure 5 -BODIPY dye serves well to stain neutral lipids but it is not an indicator of lipophagy as indicated by the authors. Moreover, its colocalization with GFP-LC3 is not convincing as it is staining the entire cytoplasm. The data leading to the conclusion that FGF19 promotes lipophagy are not convincing. The drop in BODIPY staining in response FGF19 + siATG7 treatment is somewhat puzzling and seem inconsistent with the suggested model. Finally, the overall quality of immunofluorescence should be improved.
To support the suggested model, the authors are encouraged to test for a differential autophagy response in vitro utilizing liver- and intestine-derived cells.

Referee #3:

The manuscript by Seok and colleagues demonstrates a novel tissue-specific regulation of autophagy, which is remarkably opposite in liver and intestine. This study uses robust animal models and the conclusions are obtained from in vivo systems (with exception of a couple of experiments carried out in a cell line).

The authors show that while autophagy is repressed in the liver after refeeding, it is activated in the intestine. This result is unexpected, and novel, demonstrating that the regulation of the autophagy pathwyay is tissue-specific. Furthermore, the authors detailed the mechanism at play, and showed conclusively, using knockout mouse models, that the hormone FGF15 regulates the repressor SHP. This brings another level of novelty, as SHP is usually described as a repressor but the authors show, using CHIPseq data cleverly crossed with transcriptome data from SHP-KO intestine, that SHP can also stimulate the expression of genes, and does this when associated with the transcription factor TFEB. The data is remarkably coherent along the manuscript, and the technical quality of the results is excellent. The results are often tested with multiple methods (as evidenced by the large amount of supplementary data!), thus further strengthening the conclusions.

One concern that I have is regarding the cell line chosen. This is relatively minor, because only few experiments were done with this line, and the key conclusions were all shown in vivo. But if the authors want to show a role for intestinal autophagy in the regulation of triacylglycerol homeostasis via chylomicrons, which is a process that takes place in the small intestine, it would have made sense to use an epithelial cell line that is more related to the small intestine rather than the HT29 which is colorectal. The authors should add a couple of sentences explaining the choice of cell line and its implications.

One (minor) weakness of the study is that while it finds the mechanism by which autophagy is differentially regulated between liver and intestine upon refeeding, the reason why those protein players are behaving in opposite manner in the two tissues remains unclear. In fairness, the authors recognize this in the discussion and speculated on possible reasons why this happens, and, anyway, this would be a follow-up project.

In summary, I recommend the publication of this excellent manuscript.

Response to the Reviewers:

Referee #1:

The work entitled 'Paradoxical feeding activation of gut lipophagy via an FGF15-SHP-TFEB axis limits postprandial lipids' by Kemper group describes that feeding activates intestinal autophagy/lipophagy instead of inhibiting it. This is a very interesting observation. The authors propose that the activation of lipophagy in intestine is triggered by FGF15/19-mediated activation of PKC that in turn phosphorylates SHP and TFEB leading to their activation. Lipophagy induction is needed to degrade triglycerides and APOB48 during feeding.

The work is very well written and the data are of good quality. I have some questions that should be addressed/clarified experimentally to strength the main findings of the paper.

1) Timing of autophagy activation: the authors detected autophagy activation in intestine as early as 1h after feeding. To me this observation suggests that post-translational rather than transcriptional-based mechanism accounts for feeding mediated autophagy in intestine. The authors should study the timing of SHP and TFEB activation by FGF during feeding.

Response: We thank the reviewer for this insightful comment on the kinetics of autophagic activation after feeding. We believe feeding-induced autophagic activation in the intestine occurs at both levels, acute post-translationally early after feeding as the reviewer commented, and then, sustained transcriptional activation relatively later after feeding. In this study, we focused on transcriptional control of intestinal autophagy by the late fed-state gut hormone, FGF15/19, via activation of SHP and TFEB. To further understand the timing of transcriptional activation of autophagy, WT littermates and FGF15-KO mice were fasted for 12 h and then, refed for 0, 2, 3, or 6 h. We found that autophagic activation determined by the LC3 II/I ratio and p62 levels was readily detected relatively later after feeding (2, 3, 6 h) in WT mice, but not in FGF15-KO mice. Notably, the increased nuclear levels of both TFEB and SHP, an indication of gene-regulatory functions of these proteins, were detected relatively later after feeding (3 or 6 h). These results, together with detailed studies presented in Fig. 7 and 8, indicate that feeding-induced endogenous FGF15 signaling is involved in transcriptional activation of intestinal autophagy via activation of TFEB and SHP in the late fed-state. These new data are now presented in Fig. 8C and 8D.

2) The role of SHP in intestine and in liver should be compared. It is important to further understand how does the very same protein can activate and repress autophagy in intestine and in liver, respectively. Which are SHP targets in liver? Does FGF15/19 also activates PKC in liver or selectively in intestine?

Response: The role of the orphan nuclear receptor SHP in repressing hepatic autophagy has been reported (EMBO J, 2017). In that published study, from genome-wide comparative ChIP-seq analyses of SHP and CREB binding, we showed that SHP interacts with and inhibits CREB in the liver, repressing hepatic autophagy after feeding. However, in ChIP-seq analysis

for SHP binding in mouse intestine and subsequent global motif analyses, the CREB motif was not detected within SHP binding peak regions, and instead, the TFEB motif was readily detected within the SHP binding regions globally (Fig. 6A). As we noted in the discussion (page 17), FGF15/19-activated SHP interacts with different sets of transcription factors in the gut and liver, activating TFEB to promote autophagy in the gut, whereas inhibiting CREB to repress it in the liver. Overall, this is a critical question and further mechanistic studies will be needed for fully understanding why the very same protein, SHP, can activate and repress autophagy in intestine and in liver, respectively.

With regard to the reviewer's second question, in published studies, we have shown that PKCζ is activated by FGF15/19 signaling in hepatocytes (Gastroenterology, 2018; Nat. Comm 2018; Nat. Commun, 2020). In a new study in the revised manuscript, we show that PKCβ is also activated in liver hepatocytes in response to FGF15/19 signaling (Supplemental Fig. S6A).

3) The degradation of APOB48 by lipophagy should be experimentally demonstrated.

Response: In response to the reviewer's suggestion, we examined the effect of FGF19 treatment on protein levels of ApoB48, the intestinal marker of TG-rich chylomicron, in HT29 intestinal cells after downregulation of the lysosomal membrane protein LAMP1. We found that FGF19 treatment resulted in decreased ApoB48 levels in a time-dependent manner, but these effects were not observed in LAMP1-downregulated cells (new data in Fig. 4I), suggesting that FGF19-induced autophagy is important for reduced ApoB48 levels. In the revision, we also performed extensive immunofluorescent studies to detect co-localization of LC3 puncta with PLIN2, a key protein of lipid droplets, and show that FGF19, together with SHP and TFEB, indeed activates lipophagy in HT29 cells (Fig. 4G, 5A, 7A).

4) It is unclear to me which is/are the intestinal cell population(s) that activates autophagy during feeding

Response: There are numerous cell types in intestine but increased LC3 puncta and decreased ApoB48 levels were detected mostly in enterocytes, although decreases were also detected in some epithelial cells. We noted this in the manuscript (page 8).

Referee #2:

Seok et al. report that the signaling axis of FGF15/19-SHP-TFEB is responsible for unexpected activation of intestinal lipophagy upon feeding. They found that the fibroblast growth factor 15/19 (FGF 15/19) activates SHP/NR0B2 an orphan nuclear receptor that in turn interacts with TFEB. TFEB is also activated by FGF 15/19 through PCKβ/ζ-mediated phosphorylation, which leads to its nuclear translocation. SHP-TFEB interaction in the nucleus upregulates the expression of the autophagy factor Ulk1 as well as of the lipolysis factor Atgl, thereby limiting the postprandial intestinal triglycerides (TGs). By utilizing a mouse model system, the authors have found that feeding leads an opposite response between the liver and intestine in the protein levels of LC3 and p62 serving as markers for autophagy. While in the liver, feeding leads to reduced LC3 and increased p62 levels, in the intestine this is completely reversed. Evidence is provided to show that this phenotype is regulated by the FGF15-SHP-TFEB pathway. Furthermore,

by using global ChIP-seq analysis after feeding, the SHP cistrome was implicated in the intestine-response to feeding. In addition, the mechanism of postprandial TFEB activation by PCKβ/ζ was fully characterized.

This is potentially interesting study describing a rather surprising phenomenon of differential organ response to feeding by the FGF15-SHP-TFEB pathway in the intestine. While the data concerning the signaling pathways are rather convincing the authors' model regarding regulation of autophagy/lipophagy is not well based. Using LC3 and p62 levels as the sole indication for activation or inhibition of autophagy is not sufficient. A more rigorous analysis as for a direct involvement of autophagy in this process is needed, using genetic and biochemical tools. Only in one case, shown in the supplementary data, the authors bothered to look for autophagy flux. This for example, should be performed for all the conditions described in this study. Another critical point is the lack of direct assessment of lipophagy in this study. Here too, the authors should employ available markers and genetic tools to challenge their model.

Response:

We thank the reviewer for these constructive comments. In response to the reviewer's comments, we carried out lipophagy studies in HT29 human intestinal cells. In the revision, we performed immunofluorescent studies in HT29 cells to examine the effects of FGF19 (new Fig. 4G,5A,7A), SHP (new Fig. 4G), TFEB (new Fig. 7A), and/or ATG7 (new Fig. 5A) on co-localization of LC3 puncta with PLIN2, a key protein for lipid droplets. We found that FGF19 treatment increases co-localization of LC3 puncta with PLIN2 and that downregulation of SHP, TFEB, or ATG7, decreased the colocalization, whereas overexpression of TFEB or SHP, substantially increased the colocalization. These results suggest that FGF19 promotes lipophgay and that TFEB, SHP, and ATG7 are important for activation of intestinal lipophagy triggered by FGF19 signaling. In response to the reviewer's second comment, we performed bafilomycin (BAF) experiments to test whether FGF15/19 or TFEB, a transcriptional activator of autophagy, increases autophagic flux in HT29 cells. These results (new Fig. 4H, 7B), together with the data presented in the initial manuscript (Fig. 1), suggest that FGF19-SHP-TFEB increases autophagic flux.

Additional comments

Figure 1 - The induction of intestinal autophagy was mainly based on cellular levels of autophagy markers LC3 and p62, lysosomal marker LAMP1 and accumulation of endogenous LC3 by IHC. Although the results obtained in SHP-KO or FGF15-KO mice were in line with these observations, the accumulation of LC3 and fluctuations in p62 do not always reflect on induction of autophagy. The lysosomal protein LAMP1 is a TFEB transcriptional target and its protein levels is consistent with TFEB activation.

Response: We agree with the reviewer. In the revision, we thus determined effect of FGF19 treatment on protein levels of ApoB48, the intestinal marker of TG-rich chylomicron, in HT29 intestinal cells after downregulation of the lysosomal membrane protein LAMP1. We found that FGF19 treatment resulted in decreased ApoB48 levels in a time-dependent manner, but these effects were not observed in LAMP1-dpownregulated cells (new data in Fig. 4I), suggesting

that FGF15/19 activates gut autophagy/lipophagy, which is associated with decreased ApoB48 levels. In the original manuscript, the protein levels of LAMP1 were measured in multiple IB studies (in the revised manuscript, presented in Fig. 1A-C)

Figure 2B and Figure 3B - the authors should provide a clear explanation why they chose to ignored the pathways with lowest p-values.

Response: We chose the pathways that were most relevant to the subject of our studies, but the lower p-value pathways are generally supportive of our choices. We believe they are parts of downstream or upstream of autophagy/lipophagy pathways. For example, in Fig. 2B, positive regulation of catabolic process is critical for autophagy/lipophagy process and protein dephosphorylation events and circadian regulation are likely involved in the overall lipophagic event in the intestine.

Figure 5 -BODIPY dye serves well to stain neutral lipids but it is not an indicator of lipophagy as indicated by the authors. Moreover, its colocalization with GFP-LC3 is not convincing as it is staining the entire cytoplasm. The data leading to the conclusion that FGF19 promotes lipophagy are not convincing. The drop in BODIPY staining in response FGF19 + siATG7 treatment is somewhat puzzling and seem inconsistent with the suggested model. Finally, the overall quality of immunofluorescence should be improved. To support the suggested model, the authors are encouraged to test for a differential autophagy response in vitro utilizing liver- and intestine-derived cells.

Response: As we responded to the reviewer's first comment, we carried out extensive additional immunofluorescent studies in HT29 intestinal cells. We examined the effects of FGF19 (Fig. 4G,5A,7A , SHP (Fig. 4G), TFEB (Fig. 7A), and/or ATG7 (Fig. 5A) on co-localization of LC3 puncta with PLIN2, a key protein for lipid droplets, instead of lipid droplets stained by BODIPY. The BODIPY experiments are now shown in Fig. S5. We found that FGF19 treatment increases co-localization of LC3 puncta with PLIN2 and that downregulation of SHP, TFEB, or ATG7, decreased the colocalization, whereas overexpression of TFEB or SHP, substantially increased the colocalization. We observed consistent results from multiple independent immunofluorescent studies.

With regard to autophagic studies in liver-derived mouse hepa1c1c7 cells, in published studies, we show that the late fed-state hormone FGF15/19 decreases colocalization of LC3 with lipids, inhibiting lipophagy (EMBO J, 2017), whereas the fasting hepatokine FGF21 increases it (Nat,. Commun 2020). We also showed that overexpression of CREB, a transcription activator of autophagy (Nature, 2014), increased the colocalization, whereas overexpression of SHP decreased it by inhibiting the CREB activity (EMBO J, 2017). We thank the reviewer for these helpful comments, and we believe that inclusion of these new studies strengthened our findings.

Referee #3:

The manuscript by Seok and colleagues demonstrates a novel tissue-specific regulation of autophagy, which is remarkably opposite in liver and intestine. This study uses robust

animal models and the conclusions are obtained from in vivo systems (with exception of a couple of experiments carried out in a cell line).
The authors show that while autophagy is repressed in the liver after refeeding, it is activated in the intestine. This result is unexpected, and novel, demonstrating that the regulation of the autophagy pathway is tissue-specific. Furthermore, the authors detailed the mechanism at play, and showed conclusively, using knockout mouse models, that the hormone FGF15 regulates the repressor SHP. This brings another level of novelty, as SHP is usually described as a repressor but the authors show, using CHIPseq data cleverly crossed with transcriptome data from SHP-KO intestine, that SHP can also stimulate the expression of genes, and does this when associated with the transcription factor TFEB. The data is remarkably coherent along the manuscript, and the technical quality of the results is excellent. The results are often tested with multiple methods (as evidenced by the large amount of supplementary data!), thus further strengthening the conclusions.
One concern that I have is regarding the cell line chosen. This is relatively minor, because only few experiments were done with this line, and the key conclusions were all shown in vivo. But if the authors want to show a role for intestinal autophagy in the regulation of triacylglycerol homeostasis via chylomicrons, which is a process that takes place in the small intestine, it would have made sense to use an epithelial cell line that is more related to the small intestine rather than the HT29 which is colorectal. The authors should add a couple of sentences explaining the choice of cell line and its implications.
Response: This is a very important issue, and we thank the reviewer for giving us for this opportunity to explain why we chose HT29 intestinal cells for in vitro autophagy/lipophagy studies. HT29 cells have been successfully utilized to elucidate in vitro mechanisms of intestinal chylomicron biogenesis and regulation of bile acid and cholesterol absorption and recycling (Kim et al., 2018a; Zhang et al, 2019). We have included this explanation in the revised manuscript (page 9).

One (minor) weakness of the study is that while it finds the mechanism by which autophagy is differentially regulated between liver and intestine upon refeeding, the reason why those protein players are behaving in opposite manner in the two tissues remains unclear. In fairness, the authors recognize this in the discussion and speculated on possible reasons why this happens, and, anyway, this would be a follow-up project.

In summary, I recommend the publication of this excellent manuscript.

Response: We greatly appreciate the reviewer's positive comments on our manuscript. As shown the responses to other reviewers' above, in this revision, we carried out extensive lipophagy studies using immunofluorescent studies to colocalize LC3 puncta with PLIN2, a key protein of lipid droplet. We also carried out in vivo experiments for better understanding of the timing and kinetics of feeding in control and FGF15-KO mice, and also further studied kinetics of ApoB48 reduction after FGF19 treatment in HT29 cells.

We thank all three reviewers for their insightful constructive comments. We believe our revisions in response to the comments have strengthened the manuscript and hope that our revised manuscript will now be acceptable for publication.

Dear Kim,

We have now received re-review reports from the two referees to whom I sent the manuscript back for a second time. You have addressed the concerns of referee 2 satisfactorily. However, I would like you to respond to referee 1, who has concerns regarding your immunofluorescence analysis and measurements of p62 responses.

In addition, when you submit your revised manuscript please also take care of the following points:

For the data availability section, please remove the referee token and make the datasets publicly available

Please rename the Conflict of Interest declaration as instructed in our new policy
Please change the reference format to include only the first ten authors for each reference (10 authors et al.)

We encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. It would be great if you could provide me with a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figures. The PDF files should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files. Source Data can also include Excel tables to accompany your graphs. We anticipate that their inclusion will make your work more discoverable and useable to scientists in the future.
There is a callout for Fig 7F but there is no such panel in the figure
We include a synopsis of the paper (see http://emboj.embopress.org/). Please provide me with a general summary statement and 3-5 bullet points that capture the key findings of the paper.
We also need a summary figure for the synopsis. The size should be 550 wide by [200-400] high (pixels). You can also use something from the figures if that is easier.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Yours sincerely,

William

William Teale, PhD
Editor
The EMBO Journal
w.teale@embojournal.org

Instructions for preparing your revised manuscript:

Please check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

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:
https://bit.ly/EMBOPressFigurePreparationGuideline
See also figure legend guidelines: https://www.embopress.org/page/journal/14602075/authorguide#figureformat

IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines
(https://www.embopress.org/page/journal/14602075/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
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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.

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

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 (22nd Jun 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:

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

The authors answered most of my questions. There are remaning concerns on the quality of the immunofluorescence analysis in 4G, 5A, 7A, that show no GFP signal in untreated or silenced samples, and this is very strange.
In addition figure 7B showed no effects of BAF on p62. Also P62 is a TFEB target that should be increase upon TFEB induction. These results are not convincing.

Referee #2:

The authors successfully addressed the comments raised by me and the other reviewers and in its present form the manuscript meets EMBO J. merit.

Response to reviewers

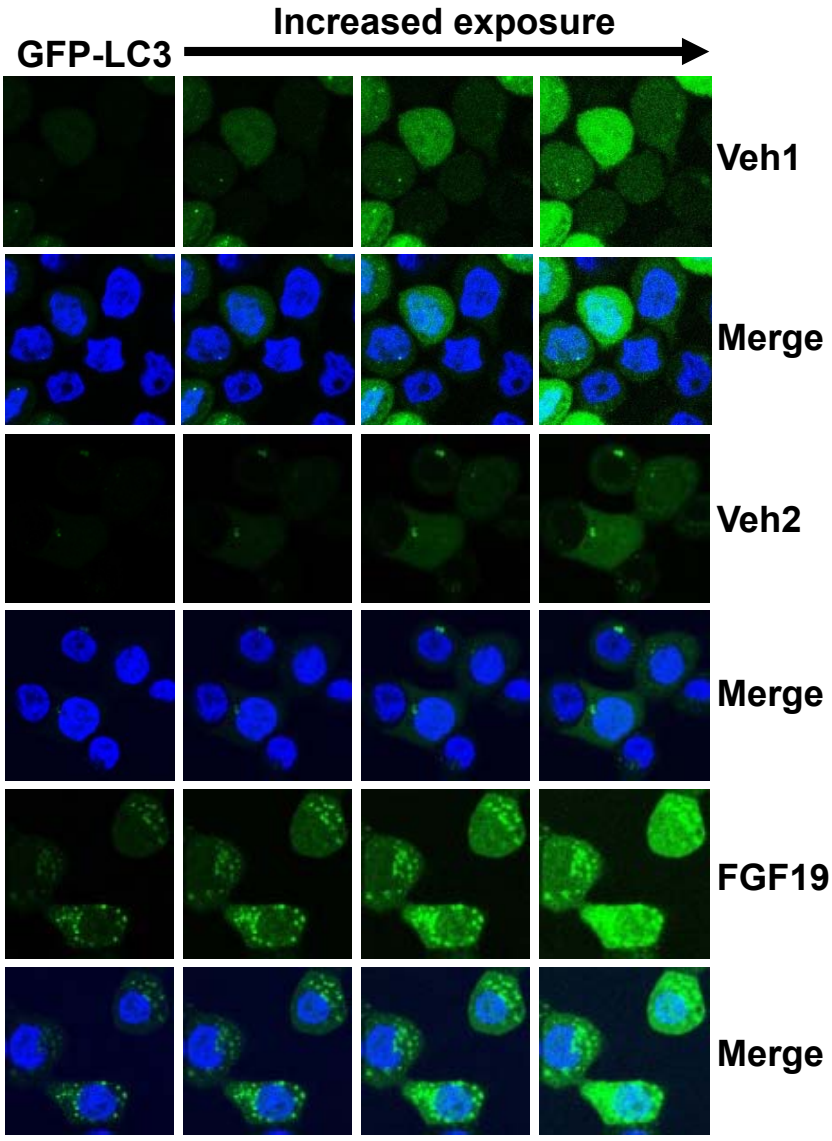
Seok et al., EMBOJ-2021-109997R

Referee #1:

The authors answered most of my questions.

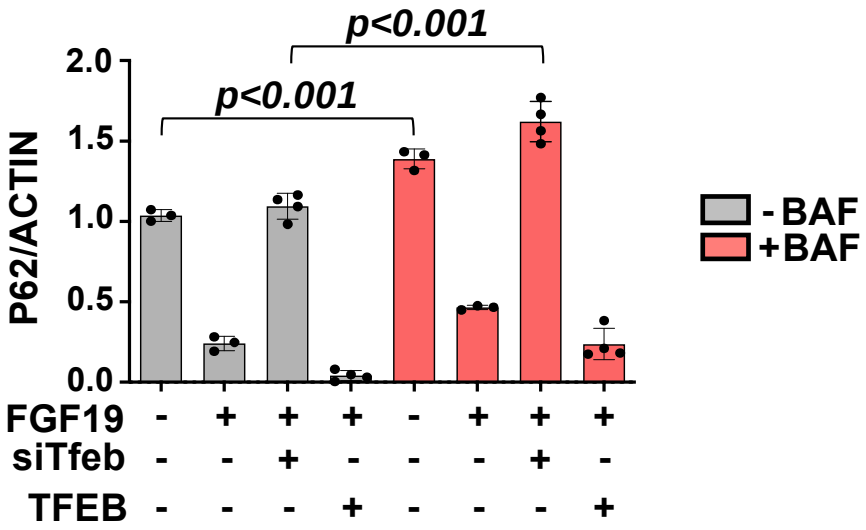
There are remaining concerns on the quality of the immunofluorescence analysis in 4G, 5A, 7A, that show no GFP signal in untreated or silenced samples, and this is very strange.

Response: The absence of signal in the control samples is due to the exposure. Because the signal is high in the FGF19-treated samples, exposures to prevent saturation of the signal in these samples result in little or no signal in the control and silenced samples. We have provided images for the reviewer's consideration with increased exposures that show the low level of GFP signal in these samples.



In addition, figure 7B showed no effects of BAF on p62.

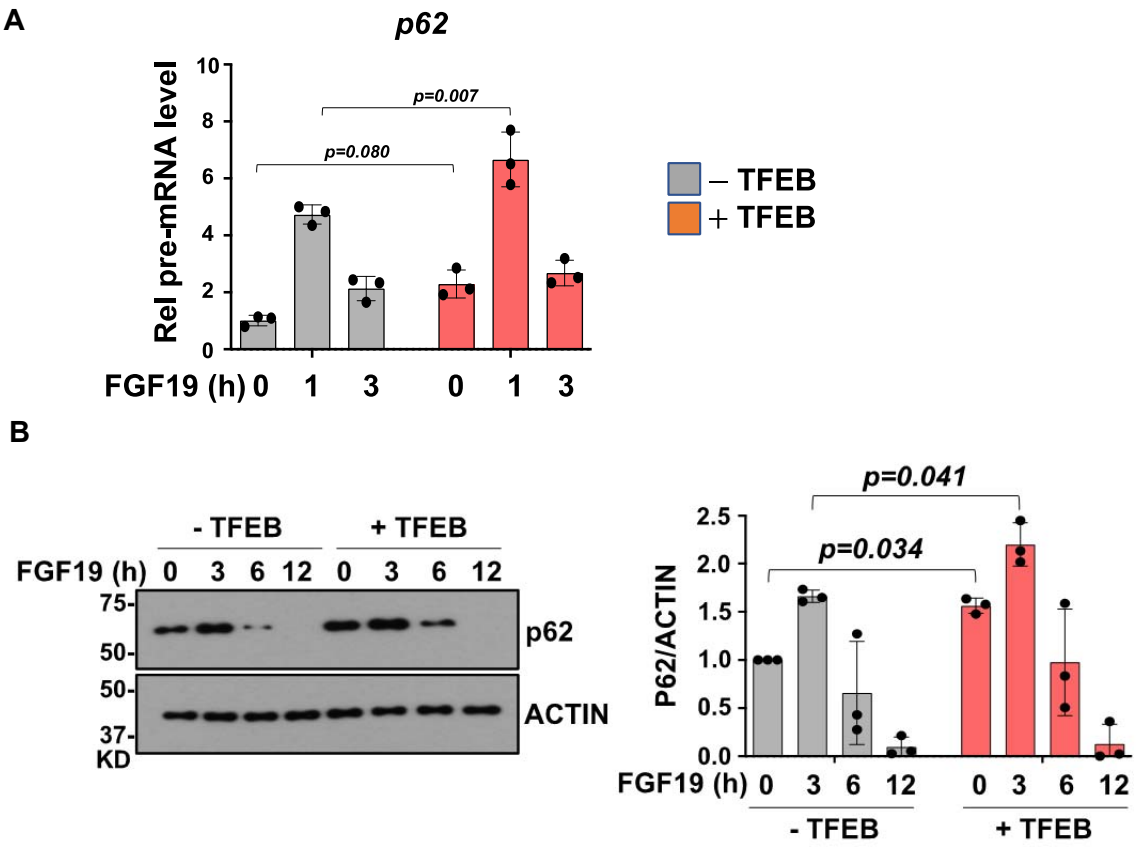
Response: Since the effects of BAF are relatively modest and perhaps not easily discerned from the blot as the reviewer noted, we have provided quantitation of the blots in quantitation (please see the below) in Fig. 7B to better illustrate the statistically significant Increase.



Also, P62 is a TFEB target that should be increase upon TFEB induction. These results are not convincing.

Response: In response to the reviewer's comment, we carried out additional experiments in HT29 intestinal cells. The effects of TFEB on p62 is dependent on the time after induction with FGF19 with or without expression of exogenous TFEB. In our manuscript, we proved data at 12 h after FGF19 treatment, which showed a decrease in p62. However, at earlier times, 1 to 3 h for pre-mRNA (an indicator of transcription), or 3 to 6 h for protein, p62 levels are increased after FGF19 treatment with or without expression of exogenous TFEB (see below, also presented in Appendix Fig. S6A, B). Thus, these results indicate that as the reviewer noted, p62 is a target of TFEB, and that p62 is induced by TFEB at early times after FGF19 treatment but is decreased by 12 h compared to zero time because of degradation of p62 protein due to FGF19-mediated autophagic activation by the time.

We thank the reviewer for his/her constructive and helpful comments.



Referee #2:

The authors successfully addressed the comments raised by me and the other reviewers and in its present form the manuscript meets EMBO J. merit.

Response: Thank you.

2nd Revision - Editorial Decision **21st Apr 2022**

Dear Kim,

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

Congratulations on a really nice paper!

Best wishes,

William

William Teale, PhD
Editor
The EMBO Journal
w.teale@embojournal.org

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EMBO Press Author Checklist

Jongsook Kim Kemper
EMBO J
EMBOJ-2021-109997

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Reporting Checklist for Life Science Articles (updated January 2022)

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

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

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:

  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Select response	No statement in the manscript. New materials and reagents will be provided on request for research purposes with minimal restriction as per the University Intellectual Transfer policy.

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Appendix, Table S5

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Appendix, table S7

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Material and Methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Select response	No authentication was done.

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Yes	Materials and Methods
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Select response	Mice are housed in accredited Univesity facilities administered by the Division of Animal Resources. No statement in manscript.

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Yes	Materials and Methods, "Mice" section
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Not in the ackowledgement section, but in the Materials and Methods section

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Materials and Methods, Experimental Design and Statistics section.
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Materials and Methods, Experimental Design and Statistics section.
Include a statement about **blinding** even if no blinding was done.	Yes	Materials and Methods, Experimental Design and Statistics section.
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	Materials and Methods, Experimental Design and Statistics section.
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Figure legends

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Figure legends
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figure legends

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approva**l (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Materials and Methods, "Mice" section
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE**, **MIBBI**, **ARRIVE**, **PRISMA**) have been followed or provided.	Select response	Not followed
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.	Not Applicable	
For **phase II and III randomized controlled trials**, please refer to the **CONSORT** flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Data avilability section
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Yes	Materials and Methods
If publicly available data were reused, provide the respective **data citations in the reference list**.	Yes	Databased accession number for publically available data is in the Data Availability section and the reference to the relevant published manuscript was provided in the reference list.