THE EMBO JOURNAL

Manuscript EMBO-2016-95500

A postprandial FGF19-SHP-LSD1 regulatory axis mediates epigenetic repression of hepatic autophagy

Sangwon Byun, Young-Chae Kim, Yang Zhang, Bo Kong, Grace Guo, Junichi Sadoshima, Jian Ma, Byron Kemper andJongsook Kemper

Corresponding author: Jongsook Kemper, University of Illinois at Urbana-Champaign

Review timeline:

Submission date:	15 August 2016
Editorial Decision:	19 September 2016
Revision received:	21 December 2016
Editorial Decision:	18 January 2017
Revision received:	02 February 2017
Additional Correspondence:	14 February 2017
Additional Correspondence:	16 February 2017
Additional Correspondence:	20 February 2017
Additional Correspondence:	21 February 2017
Editorial Decision:	29 March 2017
Revision received:	31 March 2017
Accepted:	04 April 2017

Editor: Andrea Leibfried

Transaction Report:

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

1st Editorial Decision 19 September 2016

Thank you for submitting your manuscript to us. I have now received all reports on your work, which I enclose below.

As you will see, referee #1 appreciates your data but requests extension to include analysis of the modulation of SHP nuclear translocation or Creb/crtc2/shp interactions via mTORC1 or AMPK. Referee #2 points out that the current data set lacks appropriate controls, precluding proper judgment of whether your claims are sufficiently supported by data. It is thus very difficult at this stage to conclude whether a revised version of your work would be appropriate for publication in The EMBO Journal. However, given the constructive input provided by both referees, I can offer to consider a revised version. This clearly would demand a lot of time and effort, since many experiments would have to be repeated and / or reanalyzed. I can extend the revision time to six months, should that be helpful. 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.

REFEREE REPORTS

Referee #1:

In this work Byun et al. show that Small Heterodimer Partner (SHP) mediates postprandial epigenetic repression of hepatic autophagy in response to a late fed-state hormone, FGF19 (hFGF19, mFGF15). Mechanistically, they demonstrate that FGF19 induces the SHP-mediated recruitment of LSD1 to CREB-bound autophagy genes resulting in dissociation of the CREB activator CRTC2, LSD1-mediated demethylation of gene-activation histone marks, H3K4-me2/3, and subsequent repressive histone modifications.

This is a very nice study that further expands our knowledge of transcriptional (and epigenetic) mechanisms involved in the regulation of liver autophagy in response to nutrients. In addition it also suggests co-regulations between the lysosome-autophagy pathway and bile acid metabolism. The conclusions rely on experiments performed by using multiple and physiologically relevant approaches (e.g. mouse models).

My only comment is that currently this work does not investigate how this transcriptional/epigenetic mechanism is coregulated with others, post-translational ones controlling liver autophagy in response nutrient fluctuations. A crosstalk must exist, and it may occur at multiple levels. In my opinion it is very likely that SHP nuclear translocation or Creb/crtc2/shp interactions are also modulated by nutrient sensitive kinase such as mTORC1 or AMPK. The authors should try to address this issue.

The data shown in Supplementary Fig. 2B represent an attempt in that direction, however I found them not compelling. It is very strange that FGF19 administration blunted completely the effect of Rapamycin, since Rapamycin works within minutes while FGF19 should take hours. If my interpretation of the experiment is correct, this result suggests that FGF19 signaling acts by inhibiting some downstream component of the mTORC1 pathway involved in autophagy regulation. The phosphorylation levels of ULK1 and of S6K should be monitored rather than the one of s6 (indirect mTORC1 substrate). The authors should perform co-treatment of FGF19 and Rapamycin also in vivo, in mice and evaluate autophagy.

In addition, also the AMPK status and the phosphorylation of ULK1 by AMPK should be monitored upon FGF19 treatment.

Additional (minor) points:

Quantification in Figure 5A should include co-localization between lipids and GFP-LC3 vesicles.

Which criteria were used in the electron microscopy experiments to define autophagosomes? I believe that CLEM or GFP-LC3 immunogold approaches will certainly represent a better way to clearly label autophagosomes.

Does liver size and appearance (color) changes during fed/fasting transition in SHP and FGF15 KO mice compared to control mice?

Referee #2:

Byun et al. have performed extensive molecular analyses of the postprandial transcriptional control of autophagic genes in the mouse liver. They propose that SHP is induced by FGF19 and recruits LSD1 to decrease H3K4me3 and transcriptional levels of autophagic genes in a late phase after feeding. This study builds upon recent discoveries from this laboratory and others showing that autophagy is transcriptionally controlled by the feeding status.
While of potential interest, lack of appropriate controls including confounding effects in knock-out mice together with poor usage of statistical analyses do not allow to appropriately judge the scientific merit of the study.

Major concerns :
1) Confounding factors for the interpretation of data obtained using knock-out or shRNA expressing mice were not appropriately controlled, e.g. intact FGF19 signalling in liver of SHP-/- mice and shLSD1 mice ; CREB, CRTC2 and LSD1 expression levels in SHP-/- mice...

2) Most data are antibody-based (IHC, western blot, ChIP...) but control for specific signal is lacking in several instances.

3) Fig.2C : Requires a control panel showing SHP western blot on the IPed material. The Co-IP procedure is poorly described. Does it involve removing contaminating DNA ?

4) The ChIP and re-ChIP procedures should be described as the references provided do not provide details. In all these assays, no negative control is shown and therefore the non-specific rate of DNA immunoprecipitation can not be appreciated.

6) Results of H3 ChIP data should be shown in all instances they were used to normalize H3K4me and H3K9/14ac ChIP data.

7) All reporter assays throughout the manuscript fail to show results for all the potential combinations of plasmids and therefore lack essential controls for interpretation of the data.

8) Fig.5B : Quantification on several hepatocytes should be shown (as performed in Seok and Fu et al Nature 2014).

9) Binding peaks in ChIP-seq data can be of variable strength. Therefore, identifying peaks and target genes of several factors may be highly dependent upon the specific parameters used for peak calling. No specific details and rationale is provided here regarding the peak lists which were used. This issue may impact on several analyses performed in the manuscript and may alter the conclusion of Fig.2A, which was used to justify CREB as the main factor of interest in this study.
In the same line, the authors should clarify how the different FXR ChIP-seq data were used to identify target genes. The authors need to provide an extended view of the ChIP-seq for SHP and FXR which verify the classification of genes analyzed in Fig.7. Here, as in all panels showing ChIP-seq data, the background should be visible so that one can judge of the peak height. This is essential to know which % input recovery represents actual binding.

10) Fig7: No change in H3K4me3 levels was reported for atg4. However, H3K4me3 levels are much lower than at Uvrag or Tfeb. Are all regions analyzed promoters ? The authors need to show ChIP-seq profiles across those genes and surroundings and identify regions which were further analyzed by ChIP.

11) The authors state that both FXR and SHP cause dissociation of CRTC2 from CREB. Importance of SHP is unclear since Fig.7 indicates that CRTC2 is already largely dismissed when SHP binding occurs.

12) Inappropriate statistical analyses (Student t-test) were performed for all presented data.
The number of independent replicates is often not provided (e.g; for ChIP assays).
Authors should use SD rather than SEM in all bar graphs (What to use to express the variability of data: Standard deviation or standard error of mean? Mohini P. Barde and Prajakt J. Barde Perspect Clin Res. 2012 Jul-Sep; 3(3): 113-116).

Minor points :

1) What was the feeding status of mice used in Fig.6B ?

2) P20 : correct typo : $P > 0.001$

1st Revision - authors' response 21 December 2016

Reviewer #1:

In this work Byun et al. show that Small Heterodimer Partner (SHP) mediates postprandial epigenetic repression of hepatic autophagy in response to a late fed-state hormone, FGF19 (hFGF19, mFGF15). Mechanistically, they demonstrate that FGF19 induces the SHP-mediated recruitment of LSD1 to CREB-bound autophagy genes resulting in dissociation of the CREB activator CRTC2, LSD1-mediated demethylation of gene-activation histone marks, H3K4-me2/3, and subsequent repressive histone modifications.

This is a very nice study that further expands our knowledge of transcriptional (and epigenetic) mechanisms involved in the regulation of liver autophagy in response to nutrients. In addition it also suggests co-regulations between the lysosome-autophagy pathway and bile acid metabolism. The conclusions rely on experiments performed by using multiple and physiologically relevant approaches (e.g. mouse models).

My only comment is that currently this work does not investigate how this transcriptional/epigenetic mechanism is coregulated with others, post-translational ones controlling liver autophagy in response nutrient fluctuations. A crosstalk must exist, and it may occur at multiple levels. In my opinion it is very likely that SHP nuclear translocation or Creb/crtc2/shp interactions (rapa ChIP) are also modulated by nutrient sensitive kinase such as mTORC1 or AMPK. The authors should try to address this issue. The data shown in Supplementary Fig. 2B represent an attempt in that direction, however I found them not compelling.

It is very strange that FGF19 administration blunted completely the effect of Rapamycin, since Rapamycin works within minutes while FGF19 should take hours. If my interpretation of the experiment is correct, this result suggests that FGF19 signaling acts by inhibiting some downstream component of the mTORC1 pathway involved in autophagy regulation.
The phosphorylation levels of ULK1 and of S6K should be monitored rather than the one of s6 (indirect mTORC1 substrate). The authors should perform co-treatment of FGF19 and Rapamycin also in vivo, in mice and evaluate autophagy. In addition, also the AMPK status and the phosphorylation of ULK1 by AMPK should be monitored upon FGF19 treatment.

Response: We thank the reviewer for raising this insightful and important issue about a potential crosstalk between acute nutrient-sensing kinase pathway and longer-term transcriptional pathway in regulation of hepatic autophagy. As we noted in the discussion, regulation of autophagy by feeding is complex and multiple pathways are involved. In the revision work, we investigated potential cross-talk between mTORC1-induced inhibition of autophagy and FGF19-induced inhibition of autophagy. To this end, we carried out several major experiments, including in vivo FGF19 and rapamycin co-treatment experiments as suggested by the reviewer. The responses to the specific comments from the reviewer are below:

In my opinion it is very likely that SHP nuclear translocation or Creb/crtc2/shp interactions are also modulated by nutrient sensitive kinase such as mTORC1 or AMPK.

Response: We performed three independent liver ChIP (rapa ChIP) assays in mice to examine the effect of treatment with FGF19, rapamycin, or both to examine the functional CREB/CRTC2/SHP interaction at autophagy-related genes (Supplemental Fig. S5). To avoid confounding effects of fed state hormones, we treated fasted mice with FGF19 as in previous studies by us and other investigators. We examined the occupancy of SHP, CREB, and CRTC2 at *Tfeb* (a key gene activator of autophagy) gene. FGF19 treatment increased SHP occupancy and decreased CRTC2 occupancy and CREB occupancy was not changed. Treatment with rapamycin did not result in significant changes in the occupancies of these factors at *Tfeb* and cotreatment of rapamycin with FGF19 did not affect the FGF19-mediated changes. Occupancy of these proteins at *Gapdh* as a control was not detected, i.e. the same as the IgG control (Supplemental Fig. S5). These results suggest that FGF19 inhibition of autophagy gene expression is likely independent of mTORC1 and that mTOR signaling doesn't directly affect the functional interactions of SHP, CREB, and CRTC2 at least at the *Tfeb* gene promoter.

It is very strange that FGF19 administration blunted completely the effect of Rapamycin, since Rapamycin works within minutes while FGF19 should take hours. If my interpretation of the experiment is correct, this result suggests that FGF19 signaling acts by inhibiting some downstream component of the mTORC1 pathway involved in autophagy regulation.

Response: This may be a result of confusion between the response to direct treatment with FGF19 and to feeding. After feeding, FGF19 is a late fed-state hormone and its serum levels peak 3-4 h after feeding much later than insulin signaling, so its action takes hours. Directly administered FGF19, however, acting through its membrane coreceptor complex quickly triggers the activation of intracellular signaling, including activation of ERK. Direct administered FGF19, therefore, would be expected to rapidly inhibit autophagy including that induced by rapamycin.

Alternatively, the reviewer's concern may be related to our conclusion that FGF19 acts via transcriptional regulation which would be slower than the inhibition of autophagy by the mTOR signaling pathway. This is a valid point and FGF19 signaling may affect a downstream component in the mTORC1 pathway. As noted below, more studies will be required to examine potential cross-talk between mTOR and FGF19 signaling. In the physiological setting, since mTORC1 signaling occurs early after feeding and FGF19 effects are delayed, the significance of FGF19 impacting the mTOR pathway may not be significant. Further, while mTOR signaling would be expected to be very rapid, substantial activation of autophagy induced by rapamycin requires an hr or more after addition of rapamycin. For example, see Zhou et al, Cell Research (2013) 23:508–523.

The phosphorylation levels of ULK1 and of S6K should be monitored rather than the one of s6 (indirect mTORC1 substrate). The authors should perform co-treatment of FGF19 and Rapamycin also in vivo, in mice and evaluate autophagy. In addition, also the AMPK status and the phosphorylation of ULK1 by AMPK should be monitored upon FGF19 treatment.

Response: In response to the reviewer's suggestions, we performed FGF19 and rapamycin experiments in mice in vivo. Since mTORC1 inhibits autophagy by phosphorylation of ULK1 at S757 (Ki et al., Nature Cell Biol, 2011), we examined the effect of treatment with rapamycin, FGF19, or both on p-S757-ULK1 levels and autophagic flux as assessed by the ratio of LC3-II/LC3-I in mice (3 mice/group). Treatment with rapamycin decreased p-S757-ULK1 levels and increased autophagic flux as expected (Supplemental Fig. S2B). FGF19 treatment inhibited autophagic flux without changing the levels of mTORC1 targets, p-S6K or p-ULK1, and co-treatment with rapamycin and FGF19 largely blunted the rapamycin-induced autophagic flux without changing the p-S757-ULK1 levels (Supplemental Fig. S2B). FGF19 treatment also increased p-ERK levels as expected, whereas p-AMPK levels is not changed (Supplemental Fig. S2C). These results suggest that FGF19 inhibition of autophagic flux is likely largely independent of mTORC1.

Finally, as noted in the discussion, since the temporal dissociation of CRTC2 correlates closely with the early binding of FXR in FXR target genes and with the late binding of SHP in the SHP target genes in temporal ChIP assays (Fig. 7), phosphorylation of CRTC2 does not appear to be sufficient for dissociation of CRTC2 from autophagy genes after feeding. However, we also observed that inhibition of autophagy genes, at least two tested genes, *Tfeb and Atg3*, were attenuated with the phosphor-defective CRTC2 S171A mutant that remains constitutively in the nucleus (Supplemental Fig. S13). Thus, these results, taken together, suggest that both phosphorylation status of CRTC2 regulated by acute nutrient-sensing kinases and FXR- or SHP-binding at CREB-target autophagy genes contribute to the dissociation of CRTC2 from autophagy genes. We agree with the reviewer that nutrient regulation of autophagy is complex and cross-talk between these two pathways likely exists. A number of studies have shown the role of acute nutrient-sensing kinase in autophagy regulation, and the role of transcriptional regulation in autophagy has been emerging. Further studies will be required for clear understanding of functional crosstalk between these two (acute kinase and longer term transcriptional) pathways in nutrient regulation of autophagy.

Additional (minor) points:

Quantification in Figure 5A should include co-localization between lipids and GFP-LC3 vesicles.

Response: As suggested, we measured co-localization between lipids and GFP puncta and quantitation is now presented in Fig. 5A right bottom.

Which criteria were used in the electron microscopy experiments to define autophagosomes? I believe that CLEM or GFP-LC3 immunogold approaches will certainly represent a better way to clearly label autophagosomes.

Response: Lipophagy is detected by the presence of autophasic vesicles within (inside) lipid vesicles as reported in recent lipophagy papers (Sinha et al., JCI, 2012; Lee et al., Nature, 2014). This can be detected by EM and also by co-staining of autophagic vesicles with BODIPY lipid staining (as we did in Fig. 5A). In the revision, we have quantified the co-localization of lipids and GFP-LC3 autophagic puncta (Fig. 5A, right bottom panel). While other approaches like GFP-LC3 immunogold staining are better, we believe that the data from the EM studies are sufficient to estimate the relative levels of lipophagy in different experimental groups.

Does liver size and appearance (color) changes during fed/fasting transition in SHP and FGF15 KO mice compared to control mice?

Response: Although color and appearance of liver in SHP-KO and FGF15-KO mice were not notably changed, lipid droplets in liver section detected by Oil Red-O staining were reduced and serum and liver triglyceride levels were significantly decreased in SHP-KO mice (Supplemental Fig. S8B-C). Notably, neutral lipid levels were decreased in fed mice but these effects were not observed SHP-KO mice (Supplemental Fig. S8D) and only modestly affected in FGF15-KO mice (Supplemental Fig. S8E).

Referee #2:

Byun et al. have performed extensive molecular analyses of the postprandial transcriptional control of autophagic genes in the mouse liver. They propose that SHP is induced by FGF19 and recruits LSD1 to decrease H3K4me3 and transcriptional levels of autophagic genes in a late phase after feeding. This study builds upon recent discoveries from this laboratory and others showing that autophagy is transcriptionally controlled by the feeding status.
While of potential interest, lack of appropriate controls including confounding effects in knock-out mice together with poor usage of statistical analyses do not allow to appropriately judge the scientific merit of the study.

Major concerns:
1) Confounding factors for the interpretation of data obtained using knock-out or shRNA expressing mice were not appropriately controlled, e.g. intact FGF19 signaling in liver of SHP-/- mice and shLSD1 mice; CREB, CRTC2 and LSD1 expression levels in SHP-/- mice

Response: We measured p-ERK levels as an indicator of intact FGF19 signaling. P-ERK levels were similar in WT, SHP-KO, and LSD1-downregulated mice, indicating that FGF19 signaling is intact and results from these studies in SHP-KO and LSD1-downregulated mice are presented in Supplemental Fig. S2A and S7H, respectively. Protein levels of CREB, CRTC2 and LSD1 in WT and SHP-KO mice were also measured and presented in Supplemental Fig. S7B.

2) Most data are antibody-based (IHC, western blot, ChIP...) but control for specific signal is lacking in several instances.

Response: In this study, we have used SHP antibody purchased from Santa Cruz Biotech (SC-30169, H-160). The antibody quality is adequate, although not superb, and works reasonably well for IB, IHC, IP (including ChIP) and has been used in our previous studies, including a recent SHP ChIP-seq study (Genome Biology 16:268 2015), in which we identified SHP binding sites globally in hepatic chromatin in mice treated with vehicle or FGF19. Using ChIP and IB studies in SHP-KO

mice as a control, we have verified the specificity and quality of the SHP antibody in published studies in WT and SHP-KO mice (Kim et al. Genome Biology, 2015). In the revision, we also detected SHP using the SHP antibody by IB in WT, FXR-KO, and SHP-KO mice (Supplemental Fig. S10A), and by IHC in WT and SHP-KO mice and showed that protein was detected only in the WT mice (Supplemental Fig. S8A). In addition, the specificity of the antibody has been confirmed in published studies which are referenced in the method part of the paper (page 19). Validation of an antibody to detect LSD1 was also examined using IHC and the data is presented in Supplemental Fig. S7G.

3) Fig.2C : Requires a control panel showing SHP western blot on the IPed material. The Co-IP procedure is poorly described. Does it involve removing contaminating DNA ?

Response: A more detailed description of the CoIP procedure is now presented in the Methods section. Detection of SHP levels by IB is now presented in Fig. 2E.

4) The ChIP and re-ChIP procedures should be described as the references provided do not provide details. In all these assays, no negative control is shown and therefore the non-specific rate of DNA immunoprecipitation can not be appreciated.

Response: In response to the reviewer's comments, ChIP and re-ChIP procedures have been described in more detail in the Methods section. Also, an IgG control for ChIP assays has been included in Fig. 1A, 2C, 3B, 5F, 5H, 7A, and Supplemental Fig. S5 in the revised manuscript to provide representative information about the non-specific immunoprecipitation of DNA. Other experiments are expressed as a fraction of input, which may be considered a better control than relative to IgG. At least in ChIP-seq studies, the input samples have been shown to be a better negative control than IgG. Further, in the new ChIP assays that examine the effect of FGF19 and rapamycin treatment on occupancy of SHP, CREB, and CRTC2, occupancy at the *Gapdh* gene has been included as an additional control (Supplemental Fig. S5, right).

6) Results of H3 ChIP data should be shown in all instances they were used to normalize H3K4me and H3K9/14ac ChIP data.

Response: We thank the reviewer for raising this technically important issue. In response to the author's comment, we measured total histone H3 occupancy and the histone H3 modifications reported have been normalized to total histone H3 levels at all the data from ChIP assays determining levels of modified histone H3 (Fig. 3B, 3C, 7C, Supplemental Fig. S7A, S7D, S7E, S9A, S9C, S12A-E)

7) All reporter assays throughout the manuscript fail to show results for all the potential combinations of plasmids and therefore lack essential controls for interpretation of the data.

Response: We carried out new luciferase reporter assays that include additional combinations of expression plasmids for CREB, CRTC2, SHP, and LSD1 as the reviewer suggested and the data are now presented in Supplemental Fig. S7C.

8) Fig.5B : Quantification on several hepatocytes should be shown (as performed in Seok and Fu et al Nature 2014).

Response: Lipophagy is detected by the presence of autophagic vesicles within (inside) lipid vesicles. This can be detected by EM and also by co-staining of autophagic vesicles with BODIPY lipid staining (as we did in Fig. 5). In the revision, we have quantified GFP puncta and the co-localization of lipid staining (Fig. 5A, right bottom panel).

9) Binding peaks in ChIP-seq data can be of variable strength. Therefore, identifying peaks and target genes of several factors may be highly dependent upon the specific parameters used for peak calling. No specific details and rationale is provided here regarding the peak lists which were used. This issue may impact on several analyses performed in the manuscript and may alter the conclusion of Fig.2A, which was used to justify CREB as the main factor of interest in this study.
In the same line, the authors should clarify how the different FXR ChIP-seq data were used to identify target genes. The authors need to provide an extended view of the ChIP-seq for SHP and

FXR which verify the classification of genes analyzed in Fig.7. Here, as in all panels showing ChIP-seq data, the background should be visible so that one can judge of the peak height. This is essential to know which % input recovery represents actual binding.

Response: We have now provided additional details of the procedures for analyzing ChIP-seq peaks in methods in the revision. We downloaded the ChIP-seq peaks directly from GEO database and peaks were intersected with target genes except for CREB for which we first converted peak coordinates from the mouse sequence database mm8 to mm9 using liftOver. The detailed peak calling methods for each TF are listed in Supplemental Table. S6 as published for each factor. We did not re-analyze data using same software and parameters for several reasons. First, as previous study suggested by evaluating different ChIP-seq callers using different bench-mark data: "There was no clear winner among the methods that would have outperformed the other approaches systematically in each dataset. Instead, the choice of the best method was strongly dependent on the data under analysis" (BMC Genomics. 2009; 10: 618). For the peaks we used, different labs already did quality control to make sure the peaks were reliable. For example, authors compared HOMER, MACSs and GLITR for CREB ChIP-seq data and found that the results are comparable. Since we are only interested in whether there are peaks located within 10kb of the TSS, using the same software would not likely provide significantly different results. Furthermore, we manually checked that peaks of different TFs near autophagy genes to make sure the ChIP-seq peaks are obvious.

Supplemental Table S6

Method to call ChIP-seq peaks for Transcription factors (TF) used in manuscript as described for in the published papers for each factor

TF	Method
CREB	For each condition (fasted, re-fed), non-redundant reads from all five biological replicates were pooled into a single read set, and initial peak-calling was performed with HOMER v3.0 (5% FDR), using a large pool of previous experimental inputs as the background control. To identify peaks that are not dependent on any single replicate, additional read subsets were created by pooling every possible combination of four replicates from each condition (fasted or re-fed), and peak-calling was repeated with the same parameters. Initial peak-calls from the 5-replicate pool were discarded if they failed to be called in any of the 4-replicate pools (SSI test). The entire SSI peak-calling procedure (using both the complete 5-replicate sets and the 4-replicate subsets) was also repeated with GLITR (5% FDR) or MACS v1.4 (default parameters) with PeakSplitter v0.1 in place of HOMER, for the purpose of comparing algorithm performance only. The SSI strategy can be applied using any peak-calling algorithm; we tested the SSI filter using the previously published peak-calling tools HOMER, GLITR, and MACS. While the results were comparable using any of these peak-calling algorithms, the HOMER+SSI combination resulted in the smallest number of algorithm-specific peaks, and the set of sites with the highest occurrence of known CREB motifs.
PPARα	Peaks were called using FindPeaks 4 with the following parameters: 0.1 for subpeaks and 0.3 for trim-peaks. All peaks with less than 10 overlapping reads were discarded.
SHP	Peaks were called by MACS2 version 2.0.10.20130501 using options '-g 1.87e9 -B'. Peaks with False Discovery Rate (FDR) < 0.05 were kept.
SREBP2	To determine where the SREBP-2 bound to the genome, we looked for areas where there were significantly more enriched reads mapped in the ChIP sample than in the IgG. This was accomplished using GLITR.

10) Fig7: No change in H3K4me3 levels was reported for atg4. However, H3K4me3 levels are much lower than at Uvrag or Tfeb. Are all regions analyzed promoters ? The authors need to show ChIP-seq profiles across those genes and surroundings and identify regions which were further analyzed by ChIP.

Response: FXR binding peaks, but not SHP peaks are detected at *Atg4* gene (Supplemental Fig. S11). We have shown that the majority (over 70%) of global SHP binding peaks were localized at the proximal promoter regions (Kim et al., Genome Biol, 16:268, 2015). Also, FXR binding was detected mostly at the intronic and intergenic regions (Lee et al., Hepatology, 2012). Regions analyzed were at the promoter regions for genes containing only SHP binding peaks or SHP and FXR both binding peaks and intron regions for FXR only binding peaks.

11) The authors state that both FXR and SHP cause dissociation of CRTC2 from CREB. Importance of SHP is unclear since Fig.7 indicates that CRTC2 is already largely dismissed when SHP binding occurs.

Response: The reviewer is presumably referring to the analysis of *Tfeb gene* which binds both SHP and FXR, in which case, the reviewer is correct since the action of FXR precedes that of SHP. For genes containing only SHP binding sites (e.g., *Uvrag* gene), however, CRTC2 dissociation occurs only after SHP binding (Fig. 7). After feeding, the inhibition of autophagy in WT mice is not observed in SHP-KO mice demonstrating that SHP is required for the inhibition of autophagy after feeding. Similarly autophagy after feeding is inhibited in FXR-KO mice, demonstrating both that FXR is required as well and that the effects on autophagy by the two nutrient-sensing transcriptional factors, FXR and SHP, is mutually interdependent.

12) Inappropriate statistical analyses (Student t-test) were performed for all presented data.
The number of independent replicates is often not provided (e.g; for ChIP assays).
Authors should use SD rather than SEM in all bar graphs (What to use to express the variability of data: Standard deviation or standard error of mean? Mohini P. Barde and Prajakt J. Barde Perspect Clin Res. 2012 Jul-Sep; 3(3): 113-116).

Response: We thank the reviewer for pointing out this important issue. In all of the data with statistical significance presented in the revised manuscript, the number of independent replicates is now indicated. Furthermore, SD is used to indicate variability and the n values are presented in the figure legends.

Minor points:

1) What was the feeding status of mice used in Fig. 6B?

Response: GW4064 is a synthetic specific agonist for the feeding-sensing bile acid nuclear receptor FXR. To test the pharmacological effect of this compound, mice are fasted for 12 h to avoid confounding activation of FXR by feeding. This has been indicated in the figure legend (Fig. 6B).

2) P20 : correct typo : $P > 0.001$

Response: The typo has been corrected.

2nd Editorial Decision 18 January 2017

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by the original referees again whose comments are shown below.

As you will see, referee #1 appreciates the revision. Referee #2, however, notes several issues with the revision that still need to be addressed. The issues relate directly to the work performed during revision and they preclude publication of your manuscript in the EMBO Journal at this stage. Though we usually only allow a single round of revision, I can offer to consider a further revised manuscript, should you be able to address the remaining concerns. Please get in touch with me to discuss the revision further if needed.

REFEREE REPORTS

Referee #1:

The authors have sufficiently addressed my requests. I have no additional concerns.

Referee #2:

In my opinion, several points of concern remain or emerge from the control experiments shown:

1) Reporter assays :
Since SHP and LSD1 repress basal reporter activities (Fig.S7C), how can one be confident that the effects observed in the main figures (Fig.2G, 3D) are specifically due to repression of CREB ?

2) Statistical analysis:
While the authors indicate that SD is used instead of SEM, all error bars look identical to the previous version of the manuscript,
Student t tests are used throughout the manuscript while this test does not apply in most instances.
No correction for multiple testing.

3) Genes defined as FXR or SHP only in Fig.7 actually appear to be bound by both factors in Fig.S11 (at same sites (uvrag, atg9b) or different sites (mapk8, atg4a)). This challenges the conclusions regarding the role exerted by FXR and SHP including the proposed temporal changes in repression mechanisms, which is one of the important conclusions.

4) Related to this point, the authors indicate on p14 that ' In sharp contrast to the genes with SHP binding sites, occupancy of LSD1 was not detected, and H3K4-me3 levels did not change'.
However, the authors indicate that for these genes intronic regions bound by FXR were analyzed.
Since H3K4me3 is low at enhancers compared to promoters (Heintzman et al. Nat G 2007), which is in line with the low H3K4me3 levels at the atg4 intronic region in Fig.7, the relevance of this observation is questionable.

2nd Revision - authors' response 02 February 2017

Response to Reviewers

Referee #1:

The authors have sufficiently addressed my requests. I have no additional concerns.

Response: We thank reviewer 1 for his/her comment on our revision work.

Referee #2:

In my opinion, several points of concern remain or emerge from the control experiments shown:

1) Reporter assays:
Since SHP and LSD1 repress basal reporter activities (Fig.S7C), how can one be confident that the effects observed in the main figures (Fig.2G, 3D) are specifically due to repression of CREB?

Response: CREB is a transcription factor driving hepatic fasting metabolic responses that promotes glucose production in the liver. It is well known that CREB is abundantly expressed in hepatic cells and hepatocytes and we also observed CREB is expressed in Hepa1c1c7 cells in the current study (Fig. S6C) and in previous studies (Fig. 3, Seok et al, Nature, 2014), so that inhibition of "basal activity" mediated by these endogenous factors would be expected. Importantly, SHP and LSD1 inhibit the CREB/CRTC2-mediated increase in transcription when CREB and CRTC2 are overexpressed, providing strong evidence that SHP and LSD1 inhibit CREB/CRTC2-mediated transcriptional activity. Furthermore, additional evidence that CREB is a target of SHP and LSD1 is provided in the bioinformatics analysis (Fig. 2B, Table S2), molecular and biochemical analyses (Fig. 2D-F), and immunofluorescence analysis (Fig. 2H) presented in this study. We feel these

experiments utilizing multiple approaches provide substantial and strong evidence supporting the conclusion that SHP and LSD1 inhibit CREB/CRTC2-mediated transcription.

2) Statistical analysis:
While the authors indicate that SD is used instead of SEM, all error bars look identical to the previous version of the manuscript,
Student t tests are used throughout the manuscript while this test does not apply in most instances.
No correction for multiple testing.

Response: The reviewer is correct that in nearly all cases, the error bars did not change. In preparing the first revision, we found that for experiments in the original manuscript, SD had been calculated and we mistakenly indicated in the figure legends that the SEM had been calculated. Changes were needed only for experiments in Fig.1A. We are sorry that we did not clearly point this out in the first revision. We have used the Student t-test to examine statistical significance between two experimental groups as indicated in the figures. We believe this is an appropriate use of this statistical method.

3) Genes defined as FXR or SHP only in Fig.7 actually appear to be bound by both factors in Fig.S11 (at same sites (uvrag, atg9b) or different sites (mapk8, atg4a)). This challenges the conclusions regarding the role exerted by FXR and SHP including the proposed temporal changes in repression mechanisms, which is one of the important conclusions.

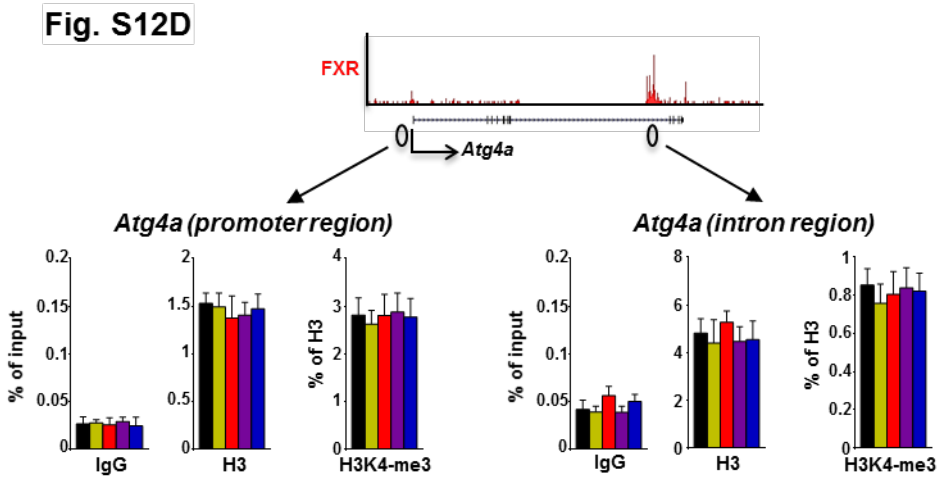
Response: The conclusions of which genes were selectively bound by FXR or SHP were based on the presence of statistically significant peaks i.e. “called peaks”. In the displays, there are often small peaks which are not statistically significant binding sites based on the criteria used for calling peaks.

4) Related to this point, the authors indicate on p14 that ' In sharp contrast to the genes with SHP binding sites, occupancy of LSD1 was not detected, and H3K4-me3 levels did not change'. However, the authors indicate that for these genes intronic regions bound by FXR were analyzed. Since H3K4me3 is low at enhancers compared to promoters (Heintzman et al. Nat G 2007), which is in line with the low H3K4me3 levels at the atg4 intronic region in Fig.7, the relevance of this observation is questionable.

Response:
This is an important issue and we thank the reviewer for pointing out this issue. To address the reviewer’s concern, we determined histone H3K4me3 levels at the proximal promoter region of the *Atg4a* gene using the same genomic DNA from the ChIP assays used in the intron region (Fig. 7A) and include the data for the information of the reviewers below and also has been presented in supplemental Fig. S12D in the revision manuscript. In spite of the inhibition of these genes by FXR and in contrast to the promoter regions of genes inhibited by SHP, the levels of histone H3K4me3 at the promoter did not change with time after feeding. This experiment provides further evidence that, compared to FXR repression of autophagy genes, a major and unique mechanism of SHP repression of autophagy genes is through recruitment of LSD1, which leads to epigenetic repression of these genes by decreasing histone H3K4me3 levels.

Effects of feeding times on histone H3K4-me3 levels at *Atg4a:* Mice were fasted for 12 h and then, fed for 1, 2, 4, or 8 h and livers were collected for ChIP assays. Histone H3K4-me3 levels were determined both at the intron region of *Atg4a* (chrX:137586310-137586816) having FXR binding peaks and the promoter region (chrX:137491147-137491430) as control. The intron data are also presented in Fig. 7A, middle.

Fig. S12D



Editor: Pre-Decision Consultation 14 February 2017

Thank you for submitting your revised manuscript to our journal.
As pointed out in my previous decision letter, we usually only allow a single round of revision. I nevertheless offered a further revision, pending satisfactory responses to the remaining concerns of referee #2. I also asked you to get in touch with me in case of trouble addressing the concerns.

As you will see below, referee #2 is not satisfied with the revision and thinks that her/his constructive comments, aimed at improving your manuscript, were not taken seriously. The following issues still remain:
- use of student's t-test as performed is inappropriate (used in all figures: 1-A-H; 2-C,D,G,H; 3-B-E; 4-A-F; 5-A-I; 6-A-E; 7C)
- the conclusion of figure S11 is not supported (meta-analysis of published ChIP-seq data grouped into peaks occurring for one transcription factor or another or both)

I consulted with an additional statistician and ChIP-seq expert, who thinks that these are valid concerns. Please see the comments of this expert below.

Since the very same issues were raised in the previous round of peer-review and have not been resolved, it is impossible for me to commit to another round of revision until I know clearly that the issues can be resolved. Therefore, before I can take a decision on your manuscript, I would need to see another version of it and of a detailed point-by-point response. This would allow you to show how you would incorporate the requested changes (potentially with the help of a statistician at your institute; and see also http://emboj.embopress.org/content/35/16/1726.long). A positive decision on your manuscript will thus depend on complete incorporation of the requested changes and obviously on the data still being supported when using appropriate statistical tests. Please also note that table S6 does not list FXR at the moment, and that potential issues regarding the conclusions drawn from the meta-analysis are not sufficiently mentioned in the manuscript.

Please send me the revised manuscript and point-by-point draft by reply email so that I can take an editorial decision on your manuscript. Thank you very much.

REFEREE REPORTS

Referee #2:

In their rebuttal, the authors have either not convincingly addressed my points (point3; definition of FXR and SHP only target genes remain an issue) or disregarded my comments (statistics are still wrong). Since these issues remain after 2 rounds of reviews, I do not feel confident the authors are willing to take these comments into consideration.

Statistician / ChIP-seq expert:

Stats issues:
The referee is correct: the authors use ratios everywhere. They are inherently skewed and not suitable for a t-test. One should instead work with log2 ratios. A t-test can then be used as this makes the distribution of the ratios more symmetric.
Also, a lot of tests are performed, but no multiple testing correction is ever applied (as the reviewer also notes). One needs to apply this to each set of tests, such as the ones presented in Figs. 1A, B, 2C, 3B, C etc.
The authors should report log2-transformed ratios, us a t-test and perform multiple testing correction (e.g. FDR, Holm, Bonferroni etc.)

ChIP Seq comparison figure issue:
The ChIP Seq figures are of really bad quality: they have a very low resolution, there are no genomic coordinates on the x-axis and there is no y-Axis, so one cannot compare the peak heights. They definitely need to be redone in my opinion.

Pre-Decision Consultation - authors' response 16 February 2017

Thank you for your patience with this paper. We have consulted an expert in statistics who agrees with reviewer 2 that the Student t-test is not appropriate for many of our experiments (excepting cases of direct comparison of two groups) and that a post-test is needed for the multiple samples.

This individual suggested using a 1-way or 2-way ANOVA with a Bonferroni post-test which is what we are proposing without doing the log transformation of the data. The log transformed data (see below) are not intuitive (see example, please see below) and in reviewing analysis in many papers with similar data (relative mRNA levels, ChIP-seq related to input) log transformation is not usually used for this type of analysis.

We are working with our bioinformatic co-authors to provide a better figure for the ChIp-seq browser display (Fig. S11) and will provide a better description of the rationale and criteria for selecting the FXR-only, SHP-only, and FXR and SHP genes for analysis.

If this approach is satisfactory, we will make the changes and resubmit the manuscript.

We look forward to hearing your answer and advice.

Pre-Decision Consultation - authors' response 20 February 2017

Response to reviewer concerns.

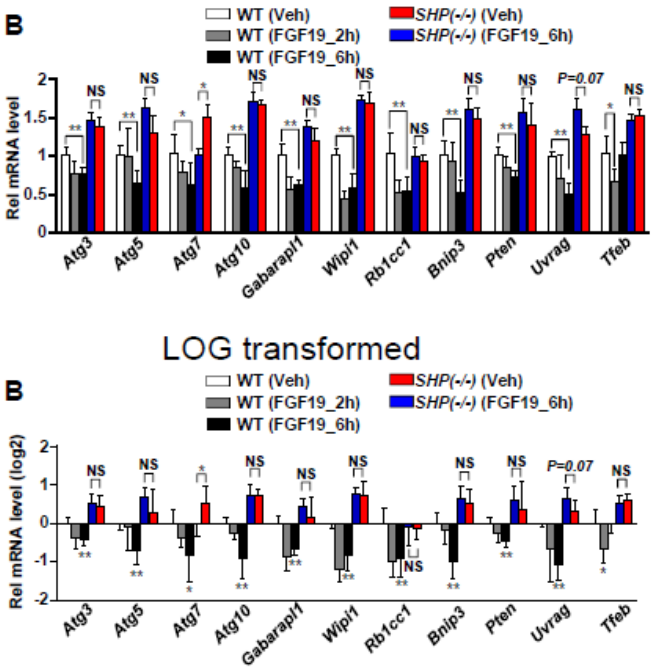
We thank the reviewers and the editor for their patience with this submission and their additional constructive comments. We are sorry for the slow response, but we have tried to address each point very carefully, particularly the statistical analysis which has taken some time.

Editor Letter: *use of student's t-test as performed is inappropriate (used in all figures: 1-A-H; 2-C,D,G,H; 3-B-E; 4-A-F; 5-A-I; 6-A-E; 7C)*
Reviewer 2: *disregarded my comments (statistics are still wrong)*
Statistician / ChIP-seq expert: *the authors use ratios everywhere. They are inherently skewed and not suitable for a t-test. One should instead work with log2 ratios. A t-test can then be used as this makes the distribution of the ratios more symmetric. Also, a lot of tests are performed, but no multiple testing correction is ever applied (as the reviewer also notes). One needs to apply this to each set of tests, such as the ones presented in Figs. 1A, B, 2C, 3B, C etc. The authors should report log2-transformed ratios, us a t-test and perform multiple testing correction (e.g. FDR, Holm, Bonferroni etc.)*

We have consulted an expert in statistics who agrees with reviewer 2 that the Student t-test is not appropriate for many of our experiments (excepting cases of direct comparison of two groups) and that a post-test is needed for the multiple samples. As suggested we have reanalyzed the statistical significance of all the data. GraphPad Prism 6 (GraphPad software version 6.01) was used for data analysis. Statistical significance was determined by the Student's two-tailed t test, Mann-Whitney test or one- or two-way ANOVA with the Bonferroni post-test for single or multiple comparisons as appropriate. Whenever relevant, the assumptions of normality were verified using the Shapiro–Wilk test, Kolmogorov-Smirnov test or the D'agostino-Pearson omnibus test. P-values < 0.05 were considered as statistically significant. We have modified the figures to indicate the results of this analysis and have indicated in the figure legends the specific methods used for each experiment in the figure.

We have not done the log transform of the data as suggested by the Statistician/ChIP-seq expert since the presentation of the data in this form is not intuitive and we believe would be confusing to the reader. In reviewing a number of papers presenting data similar to ours, this is not generally done.

An example of the transform that results in confusing presentation is below:



Editor Letter: *the conclusion of figure S11 is not supported (meta-analysis of published ChIP-seq data grouped into peaks occurring for one transcription factor or another or both)*
Editor Letter: *that potential issues regarding the conclusions drawn from the meta-analysis are not sufficiently mentioned in the manuscript.*
Referee 2: *point3; definition of FXR and SHP only target genes remain an issue*
Statistician / ChIP-seq expert: *The ChIP Seq figures are of really bad quality: they have a very low resolution, there are no genomic coordinates on the x-axis and there is no y-Axis, so one cannot compare the peak heights. They definitely need to be redone in my opinion.*

In response to the Statistician/ChIP-seq expert we have redrawn the browser images presented in Figures 2B, S1B, S11, and S12 with the X and Y axes defined properly. We have also better explained in the legend to Figure S11 the rationale for categorizing the genes for SHP or FXR binding. In particular, we have clarified that the categorization of the binding sites in genes into SHP only, FXR only, and both SHP and FXR was based on only SHP peaks at the promoter for SHP only, both SHP and FXR peaks at the promoter for both SHP and FXR, or no SHP peaks at the promoter with FXR peaks in an intron for the FXR only. We further clarified that the ChIP data in Figs. 7 and S12 analyzed these binding peak regions for FXR or SHP in the text and in the heading within the figures for each category. Thus, the ChIP assays analyzed protein binding and histone modification in the region of the specific binding sites.

Editor Letter: *table S6 does not list FXR at the moment*

We have added the description for FXR to table S6.

Editor: 2nd Pre-Decision Consultation 21 March 2017

Thank you very much again for sending another revised version of your manuscript for my assessment.
I went through the text and associated figures and I noted the following issues:

- Figure 1: In my view it would make sense to also analyze the significance of the difference between +/- FGF19/feeding treatment for SHP-/- and FGF15-/- groups in panel C, E, G, H to better match your ms text.
- Figure 2D and S6: You note 'Further, siRNA-mediated downregulation of CREB reduced the occupancy of SHP at Tfeb, Atg3, and Atg7'. In my view, this conclusion is not supported: you pull with CREB (which is downregulated), so obviously you shouldn't be able to re-ChIP with SHP, CRTC2 and CREB in the KD condition.
- Figure 4 E and F: You note 'Further, FGF19 or feeding dramatically decreased the number of LC3 puncta in mouse liver, and downregulation of LSD1 partially reversed the decrease'. In my view, this conclusion is not supported: there is a strong decrease in LC3 puncta upon FGF19/feeding treatment in the KD condition.
- Figure 5 G, I: legends are missing
- Figure 6 C: In my view it would be good to also analyze the significance of the difference between +/- GW4064 treatment in the SHP-/- condition to better match your ms text.
- Figure 6 D, F: You note: 'Conversely, the decreased LC3-II puncta and decreased ratio of LC3-II to LC3-I after FGF19 treatment in WT mice were attenuated in FXR-/- mice'. In my view, this conclusion is not supported: there is a strong decrease in LC3 puncta/LC3II/I ratio upon FGF19 treatment in the KD condition.
- Figure S3: In my view it would be good to also analyze the significance of the difference between +/- feeding treatment in the SHP-/- condition to better match your ms text.
- Figure S9B: In my view it would be good to also analyze the significance of the difference between +/- feeding treatment in the SHP-/- condition to better match your ms text.

Prior to taking an editorial decision, I would appreciate if you could further comment on these issues via response email. Thank you very much.

Pre-Decision Consultation- authors' 2nd response 28 March 2017

Thank you very much for your careful reading of our manuscript and constructive comments. We made the following changes in response to your suggestions (detailed below) which significantly improved the manuscript.

We have placed the revised manuscript and figures in a dropbox as before in a folder called EMBOJ-2016-95500-3-27-2017.

We hope the manuscript will now be acceptable for publication.

Response to concerns.

- Figure 1: In my view it would make sense to also analyze the significance of the difference between +/- FGF19/feeding treatment for SHP-/- and FGF15-/- groups in panel C, E, G, H to better match your ms text.

All of these significance differences had been analyzed, but we initially did not add a lot of the NS (statistically not significant) comparisons just to simplify the figures which were quite noisy. We have now put the suggested significance comparisons in the figure.

- Figure 2D and S6: You note 'Further, siRNA-mediated downregulation of CREB reduced the occupancy of SHP at Tfeb, Atg3, and Atg7'. In my view, this conclusion is not supported: you pull with CREB (which is downregulated), so obviously, you shouldn't be able to re-ChIP with SHP, CRTC2 and CREB in the KD condition.

You are right that this was badly worded. The important result of the re-ChIP is that SHP and CREB co-occupy the genes and the siCREB is basically a control showing that the SHP detected is not from non-specific binding, but is dependent on CREB. We have rewritten this as follows:

"Further, in re-ChIP assays, SHP was detected in CREB-bound chromatin indicating the co-occupancy of SHP and CREB at these genes, and as a control, downregulation of CREB showed that SHP occupancy in the CREB-bound chromatin was dependent on CREB (Fig. 2D and Supplemental Fig. S6A)."

- Figure 4 E and F: You note 'Further, FGF19 or feeding dramatically decreased the number of LC3 puncta in mouse liver, and downregulation of LSD1 partially reversed the decrease'. In my view, this conclusion is not supported: there is a strong decrease in LC3 puncta upon FGF19/feeding treatment in the KD condition.

We believe that in this case, these data show that there is a partial reversal after LSD downregulation so that the FGF19-mediated decrease is reduced from about 80% (82% in E; 74% in F) to 45% (40% in E; 49% in F) by LSD downregulation and also that basal levels are increased by LSD downregulation. We have rewritten this sentence as follows:

"Further, FGF19 or feeding dramatically decreased the number of LC3 puncta in mouse liver, and downregulation of LSD1 substantially increased basal puncta numbers and partially reversed the decrease in the numbers of puncta after FGF19 or feeding from about 80% to 45% (Fig. 4E, F)."

- Figure 5 G, I: legends are missing

Legends have been added.

- Figure 6 C: In my view it would be good to also analyze the significance of the difference between +/- GW4064 treatment in the SHP-/- condition to better match your ms text.

Suggested comparisons have been added-see first comment.

- Figure 6 D, F: You note: 'Conversely, the decreased LC3-II puncta and decreased ratio of LC3-II to LC3-I after FGF19 treatment in WT mice were attenuated in FXR-/- mice'. In my view, this conclusion is not supported: there is a strong decrease in LC3 puncta/LC3II/I ratio upon FGF19 treatment in the KD condition.

We agree that the way this is stated is incorrect. We have reworded this to be consistent with the results as follows:

"Conversely, basal numbers of LC3-II puncta and the basal ratio of LC3-II to LC3-I were increased in $FXR^{-/-}$ mice and were decreased after FGF19 treatment in both WT and $FXR^{-/-}$ mice, but were significantly higher after FGF19 treatment in $FXR^{-/-}$ mice compared to WT mice (Fig. 6D, E)."

- Figure S3: In my view it would be good to also analyze the significance of the difference between +/- feeding treatment in the SHP-/- condition to better match your ms text.

Suggested comparisons have been added-see first comment.

- Figure S9B: In my view it would be good to also analyze the significance of the difference between +/- feeding treatment in the SHP-/- condition to better match your ms text.

Suggested comparisons have been added-see first comment.

3rd Editorial Decision 29 March 2017

Many thanks for sending a further revised version of your manuscript to me. I have not involved the original referees or the advisor again, but have assessed myself your responses to the issues I noted. I appreciate the introduced changes, and I am happy to say that we can now move towards publication with your manuscript.

3rd Revision - authors' response 31 March 2017

(Authors uploaded the data from the pre-decision consultation.)

4th Editorial Decision 04 April 2017

Thank you for submitting the final version of your manuscript to us. This is a version containing the data you had made available earlier for pre-decision assessment. As mentioned before, I appreciate the introduced changes addressing the issues referee #2, a statistician, and myself noted, and I am happy to inform you that your manuscript has been accepted for publication in the EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Jongsook Kemper
Journal Submitted to: EMBO J
Manuscript Number: EMBOJ-2016-95500

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

USEFUL LINKS FOR COMPLETING THIS FORM

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

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

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

http://datadryad.org

http://figshare.com

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

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

http://biomodels.net/

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

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?	Power analysis was not done. In general, three or more independent determinations were done to permit statistical analysis
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For animal studies, at least 3 mice (3-6 mice/group) were used for each experimen as indoicated in the mthod of the paper (page 19). The n values are also given in the Figure legends.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Animals were not excluded from any of the reported experiments.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Animals were chosen at random for groups within the experiments, but no formal randomization methods were used.
For animal studies, include a statement about randomization even if no randomization was used.	Animals were chosen at random for groups within the experiments, but no formal randomization methods were used. Statement is included in methods P 19.
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.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding of the investigator was done in group allocation or when assessing results. This statement is included in method, p. 19.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Not tested
Is there an estimate of variation within each group of data?	No
Is the variance similar between the groups that are being statistically compared?	Not tested

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).	Source and catalog numbers of antibodies are listed in Methods, Page 20. The statement for the Antibody validation is in page 20.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Hepa1c1c7 cells were obtained from the ATCC. No STR profiling or tests for mycoplasma contamination were done.

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

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Species, strain, gender, age, and genetic modification staus and is reported in Methods, Page 20.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Statement is in Methods that the experiments are approved by the University of Illinois IACUC, Page 19-20.
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.	The compliance is as stated as in #9.

E- Human Subjects

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

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under '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	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	N/A
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

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