THE
EMBO
JOURNAL

Hepatic Sel1L-Hrd1 ER-Associated Degradation (ERAD) manages FGF21 levels and systemic metabolism via CREBH

Asmita Bhattacharya, Shengyi Sun, Heting Wang, Ming Liu, Qiaoming Long, Lei Yin, Sander Kersten, Kezhong Zhang, Ling Qi

Review timeline:

Submission date:	19th Feb 2018
Editorial Decision:	19th Mar 2018
Revision received:	15th Jul 2018
Editorial Decision:	7th Aug 2018
Revision received:	8th Aug 2018
Editorial Decision:	15th Aug 2018
Revision received:	15th Aug 2018
Accepted:	5th Sep 2018

Editor: Elisabetta Argenzio

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 19th Mar 2018

Thank you for submitting your manuscript on the regulation of FGF21 levels through SEL1L-HRD1 axis in mouse liver. We have now received three referee reports on your study, which are included below for your information.

As you can see, while all referees consider the findings novel and interesting, referee #2 and #3 also raise some critical points that need to be addressed before they can support publication here.
In particular, referees #2 is concerned that the study did not investigate the role of SEL1L in CREBH degradation, nor adequately test the kinetic of HRD1-dependent CREBH degradation. Referee #3 points out that the clarity of the manuscript would be greatly improved if you would edit and streamline the text incorporating his/her suggestions.

Addressing these issues through additional data and altered presentation as suggested by the referees would be essential to warrant publication in The EMBO Journal. Given the overall interest of your study, I would thus like to invite you to revise the manuscript in response to the referee reports.

REFEREE REPORTS:

Referee #1:

In their manuscript "Hepatic ER-Associated Degradation manages FGF21 levels and metabolism via CREBH during fasting-feeding and growth" Asmita Bhattacharya et. al. address the regulation of FGF21 via Sel1L-Hrd1 ERAD.

© European Molecular Biology Organization

The phenotypic investigation of the liver specific Sel1L-knockout mouse exhibits a defect in growth and female fertility. To examine where this growth retardation of the Sel1LAlb mice originates the authors performed a transcriptomic analysis of the liver. They identified Fgf21 to be elevated in the Sel1LAlb mice in contrast to WT mice. They could confirm these elevated levels on mRNA as well as protein level. Through a comparison of genome-wide expression analysis in genes that are altered in Fgf21-overexpressing transgenic mice with the Sel1LAlb mice they were able to show that the Sel1LAlb mice phenocopy Fgf21-gain-of-function mice.
The authors identified Crebh to be responsible for the high levels of Fgf21. They were able to show that Crebh is interacting with Hrd-1 and that it is stabilized upon inhibition of ERAD or the proteasome but not through inhibition of the lysosome. Therefore, they conclude that Crebh is an ERAD substrate. Through conditional liver knockdown of Crebh the authors were able to rescue the phenotypes of the Sel1LAlb mice such as growth retardation, Fgf21 protein levels and glucose uptake. Finally the authors were able to show that Fgf21 levels are tightly regulated via Sel1L dependent control of Crebh during fasting-feeding and growth.

The data shown in this manuscript presents a new pathway to regulate the liver-derived, fasting-induced hormone Fgf21 via the conserved ERAD branch Sel1L-Hrd1. This represents a new function of the ERAD pathway in metabolism as well as new way to control the hormone Fgf21. Therefore, this topic will attract the broad readership of EMBO J.

Referee #2:

This manuscripts reports the phenotype of mice with liver specific deletion of Sel1L. These mice are smaller and the females are infertile. Fgf21 mRNA and protein levels are increased. Indeed these mice share similar phenotype as Fgf21 transgenic mice. Knockdown of Fgf21 partially rescues some of the the defects. The authors then show that Crebh levels are increased in the liver of these mice. Crebh is an ER-anchored transcription factor, which is cleaved in the Golgi to release the active transcription factor for many genes including Fgf21. Crebh degradation is reduced in 293 cells lacking Hrd1. Finally knockdown of Crebh partially rescues some of the defects observed in these animals.

Overall the study is interesting and provides useful information about the role of Sel1L in liver. However, some issues need to be addressed to clarify the results of the study.

Major Concerns:

Sel1L is not associated only with Hrd1. Surprisingly, the requirement for Sel1L in Crebh degradation is not addressed inthis study. Instead the authors try to show that Hrd1 is required for Crebh degradation. Since other transcription factors of this family have been reported to be Hrd1 substrates, Crebh may well be a Hrd1 substrate. However, this does not address the role of Sel1L in this process.

In addition, this reviewer is not able to find information for the HEK293 cells lines with Hrd1 knockout using CRISPR cited in ref 25. Instead ref 25 describes a Sel1L CRISPR cell line. It is not clear why this Sel1L knockout cell line is not used. Specificity should be address by re-expressing Sel1L in these cells.

The exposure for Fig 5a looking at the degradation of Crebh in Hrd1 knockout cells makes it difficult to compare the kinetics of degradation between WT and KO. The quantitation also does not seem to reflect the density shown in the blot. Pulse chase experiments will be better for direct comparision. The cycloheximide experiment is further complicated by formation of the cleaved fragment of Crebh. Since these experiments are performed with transfected Crebh, it will be more useful to compare a Crebh mutant that cannot be cleaved.

In Fig 5b, the conditions for IP is too mild. This can be seen in the co-IP of Hrd1 with Crebh. This design is flawed as the Hrd1 associated with Crebh could account for the difference din ubiquitination. Stringent conditions should be used to strip off co-associated proteins for

uiquitination assays. Co-IP experiments may well require a less stringent buffer and should be part of a separate experiment.

In Fig 6, knockdown of Crebh is partial. This results in a reduction in the levels of Fgf21, but the remaining levels are at least 10 fold over control. It is surprising that growth (as measured by weight gain) is fully restored in these animals and their blood glucose levels are almost indistinguishable from those of control animals.

Referee #3:

In the study entitled "Hepatic ER-Associated Degradation manages FGF21 levels and metabolism via CREBH during fasting-feeding and growth" Bhattacharya and colleagues discover that, in mice, the Sel1L-Hrd1 ERAD complex plays a crucial role in regulating Fgf21 transcription and growth in a Crebh-dependent manner. They first show that the Hrd1 and Sel1L protein levels in the liver fluctuate in response to growth and fasting-feeding. To further investigate the role of the hepatic Hrd1-Sel1L degradation axis in vivo, the authors use hepatocyte-specific Sel1L-deficient mice and find that these mice are growth retarded. Moreover, liver-specific Sel1L KO showed highly increased Fgf21 expression in the liver with concomitant increase of circulating Fgf21. Interestingly, it is shown that in many aspects this mice phenocopies Fgf21-transgenic mice. By further exploring the Sel1 Fgf21 connection, it is shown that the previously described short half-life of the transcription factor Crebh is due to the activity of Sel1L-Hrd1 complex and that intracellular Crebh accumulation in Sel1L KO leads to increased Fgf21. This was further confirmed by Crebh depletion experiments. Lastly, it is shown that the hepatic Sel1L-Hrd1 protein complex is dynamically regulated during growth and fasting-feeding and as such regulates the activity of the Crebh-Fgf21 axis. Thus, this study identifies the Sel1L-Hrd1 ERAD complex as a key repressor of Fgf21 transcription in the liver.

This reviewer finds that throughout the study, experiments are well controlled and that most of the data is convincing. The findings are interesting and make valuable contributions to the field. However, there are some minor issues this reviewer would like to see resolved. Thus, I consider this work suitable for the audience of the EMBO Journal once the authors address the concerns raised below.

Minor issues:
Throughout the manuscript, the authors refer to Hrd1-Sel1L degradation axis using the term 'ERAD'. Examples are the titles and text of first and last paragraph in the Results section. In this way, the authors give the impression that ERAD only consists of one axis, namely the Hrd1-Sel1L degradation complex. As the Hrd1-Sel1L axis is not the only axis in ERAD, the authors should be more specific in their phrasing.

The authors state that Os9 is a substrate for the Hrd1-Sel1L degradation complex. While there is circumstantial evidence hinting that OS-9 may be degraded in a SEL1L/Hrd1-dependent manner, no published data exist (including the referenced paper) that convincingly shows that this is indeed the case.

The immunofluorescence images in the manuscript should also show the single channels for clarity. Nuclear accumulation of Crebh is not obvious.

Supplementary Figure 5 is critical to solidify the role of Fgf21 as main culprit in the Sel1L liver specific phenotypes. I would recommend the inclusion of (at least part of) the data in a main figure.

Could the authors comment on why in some cases they observe a doublet for Crebh on Western blot, and in other cases is a singlet. Is this because different cell lines/types were used?

In the final figure, the authors indicate that metabolic signals during fasting-feeding and growth influence the Hrd1-Sel1L degradation axis. While the data shown in figure 1 supports this, the authors do not discuss how these metabolic signals during fasting-feeding and growth might influence the Hrd1-Sel1L degradation axis, and what the identify of these metabolic signals might be.

© European Molecular Biology Organization

In Figure 5A the authors forgot to add 'light' and 'dark' annotations for the two exposures shown.

Typo in the second paragraph of the Discussion section. "Indeed, Crebh of Fgf32" should probably read "Indeed, Crebh or Fgf21".

1st Revision - authors' response 15th Jul 2018

Referee #1:

In their manuscript "Hepatic ER-Associated Degradation manages FGF21 levels and metabolism via CREBH during fasting-feeding and growth" Asmita Bhattacharya et. al. address the regulation of FGF21 via Sel1L-Hrd1 ERAD.

The phenotypic investigation of the liver specific Sel1L-knockout mouse exhibits a defect in growth and female fertility. To examine where this growth retardation of the Sel1LAlb mice originates the authors performed a transcriptomic analysis of the liver. They identified Fgf21 to be elevated in the Sel1LAlb mice in contrast to WT mice. They could confirm these elevated levels on mRNA as well as protein level. Through a comparison of genome-wide expression analysis in genes that are altered in Fgf21-overexpressing transgenic mice with the Sel1LAlb mice they were able to show that the Sel1LAlb mice phenocopy Fgf21-gain-of-function mice.
The authors identified Crebh to be responsible for the high levels of Fgf21. They were able to show that Crebh is interacting with Hrd-1 and that it is stabilized upon inhibition of ERAD or the proteasome but not through inhibition of the lysosome. Therefore, they conclude that Crebh is an ERAD substrate. Through conditional liver knockdown of Crebh the authors were able to rescue the phenotypes of the Sel1LAlb mice such as growth retardation, Fgf21 protein levels and glucose uptake. Finally the authors were able to show that Fgf21 levels are tightly regulated via Sel1L dependent control of Crebh during fasting-feeding and growth.

The data shown in this manuscript presents a new pathway to regulate the liver-derived, fasting-induced hormone Fgf21 via the conserved ERAD branch Sel1L-Hrd1. This represents a new function of the ERAD pathway in metabolism as well as new way to control the hormone Fgf21. Therefore, this topic will attract the broad readership of EMBO J.

We thank Referee #1 for their positive comments on our manuscript and deeply appreciate his/her support.

Referee #2:

This manuscripts reports the phenotype of mice with liver specific deletion of Sel1L. These mice are smaller and the females are infertile. Fgf21 mRNA and protein levels are increased. Indeed these mice share similar phenotype as Fgf21 transgenic mice. Knockdown of Fgf21 partially rescues some of the the defects. The authors then show that Crebh levels are increased in the liver of these mice. Crebh is an ER-anchored transcription factor, which is cleaved in the Golgi to release the active transcription factor for many genes including Fgf21. Crebh degradation is reduced in 293 cells lacking Hrd1. Finally knockdown of Crebh partially rescues some of the defects observed in these animals.

Overall the study is interesting and provides useful information about the role of Sel1L in liver. However, some issues need to be addressed to clarify the results of the study.

We thank Referee #2 for his/her insightful critique. Below we have detailed how we have addressed each of these concerns specifically in the revised manuscript.

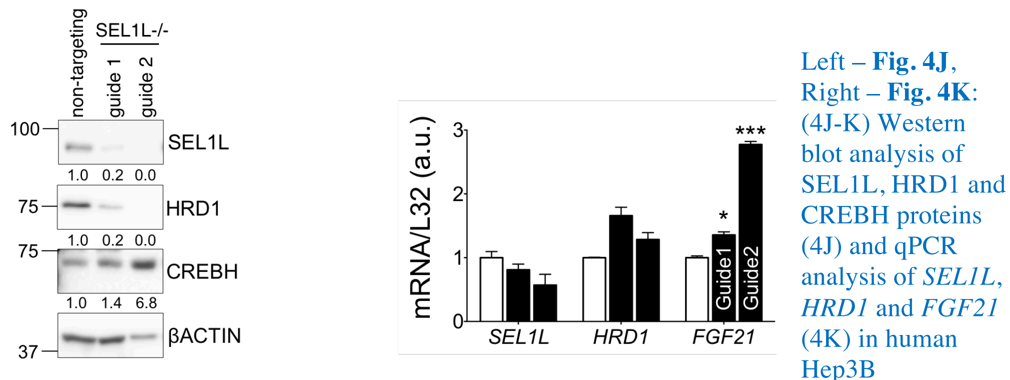
Major Concerns:

Sel1L is not associated only with Hrd1. Surprisingly, the requirement for Sel1L in Crebh

© European Molecular Biology Organization

degradation is not addressed in this study. Instead the authors try to show that Hrd1 is required for Crebh degradation. Since other transcription factors of this family have been reported to be Hrd1 substrates, Crebh may well be a Hrd1 substrate. However, this does not address the role of Sel1L in this process.

1. We now show that Sel1L CRISPR in human hepatocyte line Hep3B leads to Crebh accumulation and FGF21 induction (**Figure 4J-K**). Furthermore, we show here and in previous publications that Sel1L depletion leads to significant reduction of Hrd1 protein levels. Sel1L being a co-factor integral for Hrd1 stability and function, the specific function of Sel1L here is to facilitate the Hrd1-centered ERAD complex to target the ER-resident protein CREBH for proteasomal degradation. Whether Sel1L is involved in substrate recruitment remains an open question for the field.



Left – **Fig. 4J**, Right – **Fig. 4K**: (4J-K) Western blot analysis of SEL1L, HRD1 and CREBH proteins (4J) and qPCR analysis of *SEL1L*, *HRD1* and *FGF21* (4K) in human Hep3B hepatocytes upon CRISPR deletion of SEL1L with two different guides. β-Actin, loading control for Western blot analysis. Ribosomal *L32*, loading control for qPCR analysis. Values, mean ± SEM; *, p<0.05; **, p<0.01; ***, p<0.001; n.s., non-significant by Student's t test.

2. Additionally, new data from a co-IP experiment (**Figure EV4F**) now shows Sel1L to be pulled down with Crebh, further underscoring the interaction and importance of this protein in Crebh ERAD.

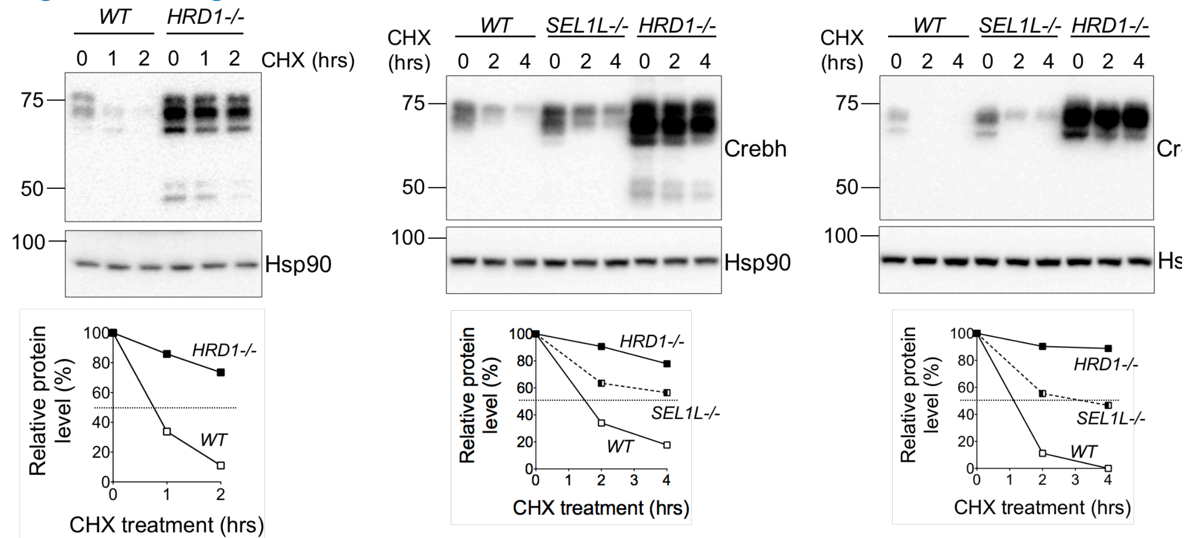
In addition, this reviewer is not able to find information for the HEK293 cells lines with Hrd1 knockout using CRISPR cited in ref 25. Instead ref 25 describes a Sel1L CRISPR cell line. It is not clear why this Sel1L knockout cell line is not used. Specificity should be address by re-expressing Sel1L in these cells.

1. We have deleted the erroneous reference and included the correct reference for generation of the HRD1 knockout HEK293T cells in the methods section. *HRD1-/-* HEK293T cells have been previously described in PMID: 28920920, and *Sel1L-/-* and *Hrd1-/-* N2a cells have been previously described in PMID: 29457782.
2. We have now included data showing Crebh stabilization in both Sel1L-/- and Hrd1-/- cells (**Figure 5A and Figure EV4A-B**), with the effect being much more pronounced in the Hrd1-/- cells, as Sel1L-/- cells presumably still retain residual Hrd1 activity.
3. Upon over-expression of Sel1L, cells appeared very unhealthy and did not grow well. We suspect it could be due to altering the ratio between Hrd1 and Sel1L in these cells. Hence, we were unable to include this particular rescue experiment in this manuscript. However, as a whole, our data including several new pieces of data shown in **Figure 4-5** is sufficient to support the notion that Sel1L-Hrd1 ERAD targets ER-resident CREBH for proteasomal degradation.

The exposure for Fig 5a looking at the degradation of Crebh in Hrd1 knockout cells makes it difficult to compare the kinetics of degradation between WT and KO. The quantitation also does not seem to reflect the density shown in the blot. Pulse chase experiments will be better for direct comparison. The cycloheximide experiment is further complicated by formation of the cleaved fragment of Crebh. Since these experiments are performed with transfected Crebh, it will be more useful to compare a Crebh mutant that cannot be cleaved.

© European Molecular Biology Organization

1. A new data is now shown in Figure 5A.
2. We have tried the pulse-chase experiment for the last three months for CREBH. Unfortunately, our lab not being well set up for pulse chase analysis, we are facing technical challenges with carrying out a successful pulse chase experiment.
3. To circumvent this issue, we now have generated a Crebh construct where the RNNNRNL of the S1P cleavage site and the LP of the S2P cleavage site have been mutated to Alanines. Using this and the Sel1L/Hrd1 CRISPR knockout cells, we have re-done our cycloheximide analysis to study and better delineate the half-life/stabilization of Crebh (**Figure 5A and Figure EV4A-B**).

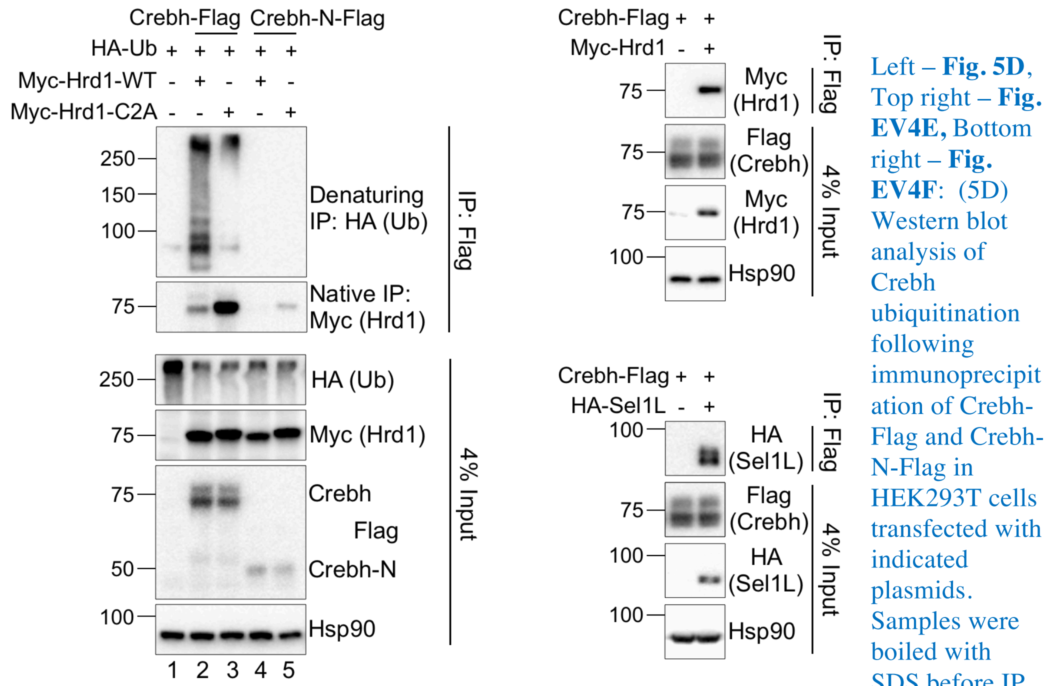


Left – **Fig. 5A**, Centre – **Fig. EV4A,** Right – **Fig. EV4B**: (5A) Western blot analysis of Crebh protein half-life in transfected WT and HRD1-/- HEK293T cells treated with cycloheximide (CHX) for indicated times. (EV4A-B) Western blot analysis of Crebh (A) and cleavage-defective-Crebh (B, Crebh*) half-life in transfected *WT, Sel1L-/-* and *Hrd1-/-* N2a cells treated with cycloheximide (CHX) for indicated times. The decay of Crebh proteins are shown below each panel. All cell culture experiments were done in 2-3 independent repeats with cells passaged less than 3 times. Hsp90, loading control for Western blot analysis.

In Fig 5b, the conditions for IP is too mild. This can be seen in the co-IP of Hrd1 with Crebh. This design is flawed as the Hrd1 associated with Crebh could account for the difference in ubiquitination. Stringent conditions should be used to strip off co-associated proteins for ubiquitination assays. Co-IP experiments may well require a less stringent buffer and should be part of a separate experiment.

1. We have now repeated the experiment with more stringent IP conditions ("Denaturing IP") to strip off associated proteins. New data is now shown in **Figure 5D** and below, which demonstrates that CREBH is ubiquitinated in an Hrd1-dependent manner. We have also added co-IP data separately (**Figure 5D** under "Native IP" **and EV4E-F**) to show interaction between CREBH and Sel1L/Hrd1.

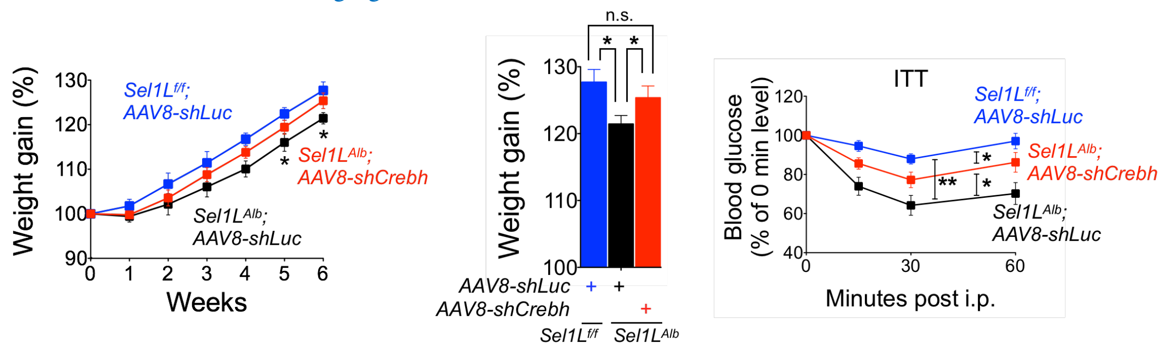
© European Molecular Biology Organization



Left – **Fig. 5D**, Top right – **Fig. EV4E,** Bottom right – **Fig. EV4F**: (5D) Western blot analysis of Crebh ubiquitination following immunoprecipitation of Crebh-Flag and Crebh-N-Flag in HEK293T cells transfected with indicated plasmids. Samples were boiled with SDS before IP for denaturing IP and not so for native IP. These cells were treated with proteasomal inhibitor for the last 6 hrs prior to immunoprecipitation. (EV4E-F) Co-immunoprecipitation analysis of Crebh with Hrd1 (E) and Sel1L (F) when co-expressed in HEK293T cells. All cell culture experiments were done in 2-3 independent repeats with cells passaged less than 3 times. Hsp90, loading control for Western blot analysis.

In Fig 6, knockdown of Crebh is partial. This results in a reduction in the levels of Fgf21, but the remaining levels are at least 10 fold over control. It is surprising that growth (as measured by weight gain) is fully restored in these animals and their blood glucose levels are almost indistinguishable from those of control animals.

1. We have now added more n number to the AAV-shCrebh (now n=10) experiment to make these experiments more reliable. With all the data taken together from all these mice (**Figure EV5E and Figure 6E-F**), the weight gain and ITT curves for these experiments show an intermediate pattern, suggestive of a partial rescue in response to the partial reduction in circulating Fgf21 levels in these mice.



Left – **Fig. EV5E**, Centre – **Fig. 6E,** Right – **Fig. 6F**: Data from the rescue experiments where 5-week-old *Sel1L$^{f/f}$* and *Sel1L^{Alb}* mice were injected i.v. with AAV8-*shCrebh* or control AAV8-*shLuc*. (EV5E) Weekly weight gain post injection (n=10 per group). (6E) Weight gain 6 weeks post injection (n=10 per group). (6F) Insulin tolerance test (ITT) 5-weeks after injection (n=10 per group). Values, mean ± SEM; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s., non-significant by 2-way ANOVA analysis.

Referee #3:

In the study entitled "Hepatic ER-Associated Degradation manages FGF21 levels and metabolism via CREBH during fasting-feeding and growth" Bhattacharya and colleagues discover that, in mice, the Sel1L-Hrd1 ERAD complex plays a crucial role in regulating Fgf21 transcription and growth in a Crebh-dependent manner. They first show that the Hrd1 and Sel1L protein levels in the liver fluctuate in response to growth and fasting-feeding. To further investigate the role of the hepatic Hrd1-Sel1L degradation axis in vivo, the authors use hepatocyte-specific Sel1L-deficient mice and find that these mice are growth retarded. Moreover, liver-specific Sel1L KO showed highly increased Fgf21 expression in the liver with concomitant increase of circulating Fgf21. Interestingly, it is shown that in many aspects this mice phenocopies Fgf21-transgenic mice. By further exploring the Sel1 Fgf21 connection, it is shown that the previously described short half-life of the transcription factor Crebh is due to the activity of Sel1L-Hrd1 complex and that intracellular Crebh accumulation in Sel1L KO leads to increased Fgf21. This was further confirmed by Crebh depletion experiments. Lastly, it is shown that the hepatic Sel1L-Hrd1 protein complex is dynamically regulated during growth and fasting-feeding and as such regulates the activity of the Crebh-Fgf21 axis. Thus, this study identifies the Sel1L-Hrd1 ERAD complex as a key repressor of Fgf21 transcription in the liver.

This reviewer finds that throughout the study, experiments are well controlled and that most of the data is convincing. The findings are interesting and make valuable contributions to the field. However, there are some minor issues this reviewer would like to see resolved. Thus, I consider this work suitable for the audience of the EMBO Journal once the authors address the concerns raised below.

We thank Referee #3 for his/her helpful comments on our manuscript. Below we have detailed how we have attempted to improve the discussion of specific parts of the manuscript as per these suggestions.

Minor issues:
Throughout the manuscript, the authors refer to Hrd1-Sel1L degradation axis using the term 'ERAD'. Examples are the titles and text of first and last paragraph in the Results section. In this way, the authors give the impression that ERAD only consists of one axis, namely the Hrd1-Sel1L degradation complex. As the Hrd1-Sel1L axis is not the only axis in ERAD, the authors should be more specific in their phrasing.

We agree with the reviewer’s comment here. We have now changed the title to reflect Sel1L-Hrd1 ERAD, and edited our text to ensure more accurate and judicious use of the terms “Sel1L-Hrd1” and “ERAD” by referring specifically to Sel1L-Hrd1 ERAD except when discussing the ERAD machinery of the cell in general.

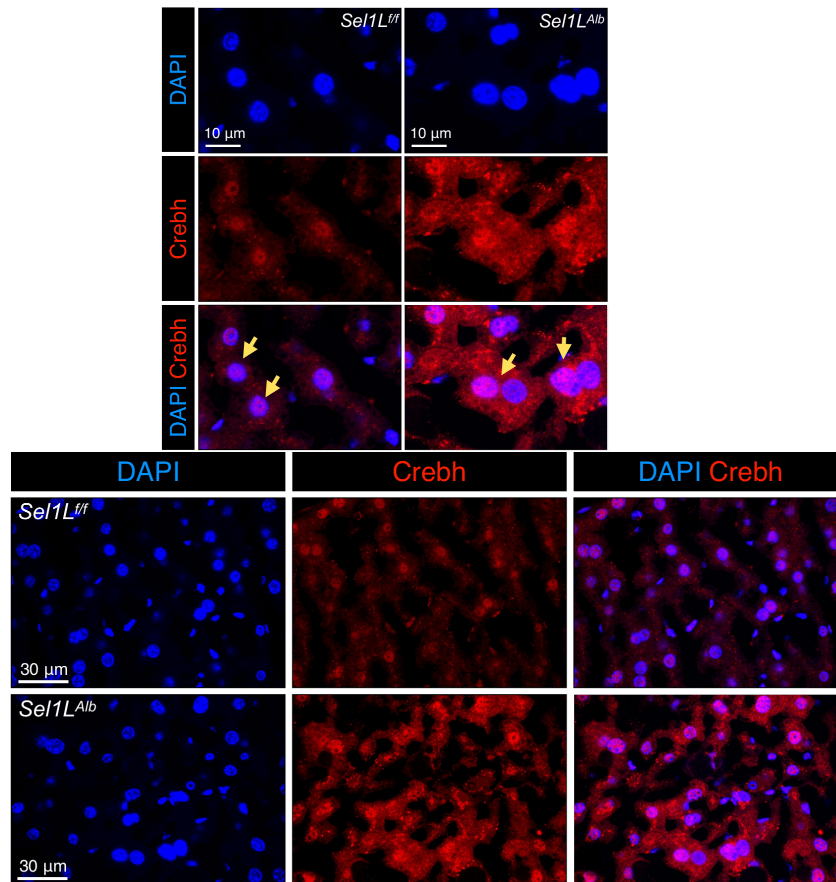
The authors state that Os9 is a substrate for the Hrd1-Sel1L degradation complex. While there is circumstantial evidence hinting that OS-9 may be degraded in a SEL1L/Hrd1-dependent manner, no published data exist (including the referenced paper) that convincingly shows that this is indeed the case.

We agree with the reviewer’s statement completely and have edited the manuscript to make our text more scientifically precise and correct.

The immunofluorescence images in the manuscript should also show the single channels for clarity. Nuclear accumulation of Crebh is not obvious.

We have now edited the figures to include single channel images for Crebh in the current data panels (**Figure 4H and Figure EV3F**).

© European Molecular Biology Organization



Left – **Fig. 4H**, Rottom right – **Fig. EV3F**: Representative confocal images (4H) and zoomed out versions (EV3F) of Crebh in the liver cryosections of 8-week-old mice. Note that a fraction of hepatocytes is binucleated.

Supplementary Figure 5 is critical to solidify the role of Fgf21 as main culprit in the Sel1L liver specific phenotypes. I would recommend the inclusion of (at least part of) the data in a main figure.

We have now moved some of the Fgf21-KD data to main **Figure 2J-L** and revised the text accordingly.

Could the authors comment on why in some cases they observe a doublet for Crebh on Western blot, and in other cases is a singlet. Is this because different cell lines/types were used?

This is likely due to the different gel running conditions (% of the gel and running time) as well as different cell types. Crebh is a protein with 4 glycosylation sites. We have noted different amounts of glycosylation in different cell types, leading to bands with different mobility on SDS-PAGE.

In the final figure, the authors indicate that metabolic signals during fasting-feeding and growth influence the Hrd1-Sel1L degradation axis. While the data shown in figure 1 supports this, the authors do not discuss how these metabolic signals during fasting-feeding and growth might influence the Hrd1-Sel1L degradation axis, and what the identify of these metabolic signals might be.

The reviewer made a great point. We have now discussed the point in the discussion on page 13, to highlight the openness and importance of this topic.

In Figure 5A the authors forgot to add 'light' and 'dark' annotations for the two exposures shown.

We have now edited the figure to correct this oversight.

© European Molecular Biology Organization

Typo in the second paragraph of the Discussion section. "Indeed, Crebh of Fgf32" should probably read "Indeed, Crebh or Fgf21".

We have now edited the text to correct this error.

2nd Editorial Decision 7th Aug 2018

Thank you for submitting a revised version of your manuscript. It has now been seen by the original referees whose comments are shown below.

As you will see, while they find that the criticisms have been sufficiently addressed, referee #2 and referee#3 ask you to further discuss the effect of: i) Sel1L overexpression on cell death; and ii) Hrd1 and Sel1L depletion on CREBH degradation. In addition, before we can officially accept the manuscript there are a few editorial issues concerning text and figures that I need you to address:

REFEREE REPORTS:

Referee #1:

The authors thoroughly addressed the remaining critical questions/points raised by all reviewers; the manuscript provides a very strong contribution for EMBO J.

Referee #2:

The authors have greatly improved their manuscript and addressed my concerns. The authors noted that overexpression of Sel1L induced cell death, which complicated their experiment to rescue Sel1L knockout cells. This information is important and should be discussed in the text.

Referee #3:

The manuscript has been improved in this revised version and I would highly recommend its publication in the embo journal.

I would just request the authors to add a line to comment on the fact that depletion of Hrd1 has a much stronger effect on CRBH degradation than depletion of Sel1l. The authors always mention Hrd1/Sel1L axis as if they had similar phenotypes but the magnitude of the effect appears susbstantially different

2nd Revision - authors' response 8th Aug 2018

Thank you for your kind decision. Here we wish to submit our revised manuscript titled "Hepatic Sel1L-Hrd1 ER-Associated Degradation manages FGF21 levels and systemic metabolism via CREBH".

In this revised version, we have added comments on two points raised by the reviewers 2 and 3:
(i) cell survival issues regarding our current Sel1L overexpression system (on page 20 we added: *An attempt to overexpress Sel1L in cells resulted in complications in cell survival, an issue currently under further investigation*.)
(ii) more pronounced substrate stabilization in Hrd1-KO cells as compared to Sel1L-KO systems [on page 10 we added: *Notably, accumulation and stabilization of Crebh protein were more pronounced in HRD1-/- cells than in SEL1L-/- cells, potentially owing to the residual Hrd1 protein in SEL1L-/- cells (Figure 1C) (33)*.]

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: Ling Qi
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2018-99277

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

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

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

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)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Based on sample size formula of power analysis, N=8(CV)2[1+(1-PC)2]/(PC)2, to reach the error 0.05, Power 0.80, percentage change in means (PC) is 20%, co-efficient of variation (CV) is 10 ~ 15% (varies between the experiments).
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	4-6 mice per group is the minimal number of animals to obtain statistical significance. We routinely used a total of 4-10 mice in each study to ensure adequate power.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples or animals were excluded from analyses.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Mice in each group were randomly chosen matching in the age, genotype and gender.
For animal studies, include a statement about randomization even if no randomization was used.	See above.
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.	The investigators were not blinded during experiments and result assessment.
4.b. For animal studies, include a statement about blinding even if no blinding was done	See above.
5. For every figure, are statistical tests justified as appropriate?	Yes, as stated in each figure legend.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Student's t-test and 2-way ANOVA, as appropriate.
Is there an estimate of variation within each group of data?	Yes.

Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	All antibodies used have been described with company and catalog number in the Materials and Methods section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	All cell lines sources are described in the Materials and Methods section with references. All cell lines used were free of mycoplasma.

* 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.	Mice were all of C57BL/6J background with genotypes as described in labels. Both males and females were used. Mouse age information is indicated in legends.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal procedures were as per regulations by the Institutional Animal Care and Use Committee of both Cornell University and University of Michigan Medical School. Further information regarding the same is provided in Materials and Methods section.
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 studies were in compliance with guidelines.

E- Human Subjects

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

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The accession codes for microarray data will be made available for public via GEO database upon acceptance of this manuscript.
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).	Details of microarray are described in Materials and Methods section.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

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