

## Network analyses identify liver-specific targets for treating liver diseases

Sunjae Lee, Cheng Zhang, Zhengtao Liu, Martina Klevstig, Bani Mukhopadhyay, Mattias Bergentall, Resat Cinar, Marcus Ståhlman, Natasha Sikanic, Joshua K. Park, Sumit Deshmukh, Azadeh M. Harzandi, Tim Kuijpers, Morten Grøtli, Simon J. Elsässer, Brian D. Piening, Michael Snyder, Ulf Smith, Jens Nielsen, Fredrik Bäckhed, George Kunos, Mathias Uhlen, Jan Borén & Adil Mardinoglu

*Corresponding authors: Jan Borén & Adil Mardinoglu, Chalmers University of Technology*

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### Review timeline:

|                     |              |
|---------------------|--------------|
| Submission date:    | 21 May 2017  |
| Editorial Decision: | 20 June 2017 |
| Revision received:  | 04 July 2017 |
| Editorial Decision: | 17 July 2017 |
| Revision received:  | 19 July 2017 |
| Accepted:           | 24 July 2017 |

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Editor: Maria Polychronidou

### 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

20 June 2017

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The reviewers' recommendations are rather clear so I think that there is no need to repeat the points listed below. Of course, feel free to contact me in case you would like to discuss any particular point in further detail.

On a more editorial level, we would like to ask you to address the following issues:

- We have replaced Supplementary Information by the Expanded View (EV) format, in which a limited number of Supplementary Figures (typically ~5) are included in the article as EV figures. In this case all additional figures can be displayed as EV Figures. EV Figures should be provided as individual files and their legends should be included in the main text. For detailed instructions regarding expanded view please refer to our Author Guidelines.
- Datasets should be provided as individual .xls files labeled and cited as Dataset EV1, Dataset EV2 etc. The description of each dataset should be included in a separate tab in the corresponding .xls file.
- The link <http://sysmedicine.com/cns> providing access to the networks generated in the study is not working. For long term archival, we would also ask you to provide these files as EV Datasets or to upload them in a public repository. For more information please refer to our author guidelines for Data Deposition.

- The related unpublished study by Mardinoglu et al, should be cited in the text as "Mardinoglu et al, in preparation" (unless it has in the meantime been accepted, and is currently in press). For more information regarding citation of unpublished work you can refer to our Author Guidelines.
- Please provide a "standfirst text" summarizing the study in one or two sentences (approximately 250 characters), three to four "bullet points" highlighting the main findings and a "synopsis image" (211x157 pixels, jpeg format) to highlight the paper on our homepage.
- When you resubmit your manuscript, please download our CHECKLIST and include the completed form in your submission. \*Please note\* that the Author Checklist will be published alongside the paper as part of the transparent process.

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REFeree REPORTS

Reviewer #1:

The authors present an elegant study where co-expression networks (CNs) were constructed for 46 human tissues and liver cancer, followed up by detailed analysis of liver CNs and subsequently by validation studies in vitro and in vivo.

The manuscript is well written and the presentation is clear.

There are two issues the authors may consider elaborating further.

There is little information what samples were used to construct CNs. Specifically for liver which has been studied in more detail than other tissues in the study, one would expect that the type of samples included in construction of CNs would affect the co-expression patterns. How were the covariates such as gender, age, amount of liver fat been taken into account?

The method for construction of CNs is rather straightforward and based on Pearson correlation. No particular statistic was used to evaluate significance of associations and instead a cutoff value was used based on the ranking of associations. The motivation for this choice needs to be explained. Furthermore, Pearson correlation would lead to large number of spurious associations. Ideally, one would rather apply alternative methods for construction of CNs such as those based on partial correlation. The authors should explain better the reasoning for their choice and perhaps discuss the limitation in the Discussion.

Reviewer #2:

In this study, Lee et al have generated tissue-specific co-expression networks for 46 major human tissues and human hepatocellular carcinoma (HCC), and deciphered their tissue-specific functions. The results indicate that hepatic fatty acid synthase (FASN) is co-expressed with a few liver-specific genes associated with de novo lipogenesis that usually occurs during non-alcoholic fatty liver disease (NAFLD) and HCC. In fact, their reduced expression was found to be associated with reduced FASN expression. In addition, the authors potentially identify the cannabinoid type-1 receptor (CB1R) as the upstream modulator of these genes. This work complements their previously documented findings that describe other genomic analysis methodologies (such as transcriptional regulatory and protein-to-protein interaction networks) to identify tissue-specific genes that are correlated with functionality during health and disease.

Overall, these are interesting findings that provide compelling evidence for using such strategy to identify novel genes that are dysregulated during diseases. The authors provide a wealth of novel data supporting the fact that FASN expression is associated with PKLR, PNPLA3, and PCSK9, inhibition of which might be used as a potential treatment for NAFLD and HCC. Having said that, there are numerous issues that need to be addressed to improve the quality of the manuscript:

1. To identify the functional relationship between FASN and the hepatic-specific genes identified during NAFLD progression, the authors oddly decided to use a two-week high sucrose diet (HSD) mouse model. Since NAFLD is a well-known chronic condition that affects the liver, it would be important to show that a similar pattern of expression exists following a long-term HSD feeding. Moreover, beside evaluating hepatic triglyceride content (Fig. 5A), the authors should fully characterize their model by providing histological evaluation of the liver, assessing NASH score, determining serum ALT and AST levels, etc.
2. In accordance with the reduced FASN and its associated targets in PCSK9-null mice, are they also resistant to the development of hepatic steatosis on regular chow and HSD feeding?
3. The authors mentioned that the attenuation of FASN and its related targets in CB1R-null mice treated with DEN is associated with decreased tumor growth. However, no data representing tumor size are provided. I understand that they used samples that have been already described (Mukhopadhyay et al., 2015), but at least correlations between tumor size and gene expression should be added.
4. Since peripheral CB1R blockade has been shown to ameliorate HCC (Mukhopadhyay et al., 2015), it would be important to determine whether this effect is associated with reduced FASN expression and its related genes.
5. Identifying PKLR, PCSK9, and PNPLA3 genes to be associated with FASN expression as well as NAFLD and HCC progression is a major novelty of the current paper. Indeed, the authors conclude that targeting these genes/proteins by using a chemical compounds or monoclonal antibodies could be considered as an effective treatment for such diseases. Therefore, to further validate their conclusion, they should consider testing PCSK9 inhibitor for reversing NAFLD or attenuating HCC in their mouse models.

Minor comments:

1. It isn't clear whether the data in all figures are presented as mean {plus minus} sem.
2. In figure 5D & E the gene expression is normalized to % of HCC, why? Isn't it better to describe it against the expression in the noncancerous tissues?

1st Revision - authors' response

04 July 2017

## RESPONSE TO REVIEWER COMMENTS:

### Reviewer #1:

The authors present an elegant study where co-expression networks (CNs) were constructed for 46 human tissues and liver cancer, followed up by detailed analysis of liver CNs and subsequently by validation studies in vitro and in vivo. The manuscript is well written and the presentation is clear. There are two issues the authors may consider elaborating further.

*We would like to thank the reviewer for careful reading of our manuscript and providing constructive comments.*

There is little information what samples were used to construct CNs. Specifically for liver which has been studied in more detail than other tissues in the study, one would expect that the type of samples included in construction of CNs would affect the co-expression patterns. How were the covariates such as gender, age, amount of liver fat been taken into account?

*From GTEx database, we obtained RNA-seq data of tissues of all deceased donors and we used data as it is, considering that they are from normal population without selection bias. Therefore, co-expression networks based on these cohorts are expected to identify more general functional relationships rather than associations with a specific clinical indication.*

*Considering the comment of the reviewer, we discussed the limitation of cohorts used in this study in the Discussion section. We have not considered the effect of covariates while constructing CNs because their effects would be distinct by tissues, especially reproductive tissues, and may increase biases while comparing different tissue CNs.*

The method for construction of CNs is rather straightforward and based on Person correlation. No particular statistic was used to evaluate significance of associations and instead a cutoff value was used based on the ranking of associations. The motivation for this choice needs to be explained.

Furthermore, Person correlation would lead to large number of spurious associations. Ideally, one would rather apply alternative methods for construction of CNs such as those based on partial correlation. The authors should explain better the reasoning for their choice and perhaps discuss the limitation in the Discussion.

*Considering unbiased investigations across diverse tissues, we preferred to choose straightforward Pearson's correlations. Partial correlation would be alternative method, but it is still limited given that controlling random variables for co-expressions are not known and would increase biases by different tissues. We have used cut-off value, but it is strict that average cut-off correlation value was 0.576 and p-values of cut off correlations among all 46 tissues were less than  $1E-5$ .*

*We agree that spurious associations would be found from Pearson's correlations and it is discussed in the Discussion section. In order to avoid possible bias based on Pearson's correlation method, we have checked FASN-coexpressed target genes in HCC tumor, such as PKLR, PNPLA3, and PCSK9, by not only raw expression values but also log-transformed values, checked their expressions between patient groups stratified by FASN expressions (Figure 3B) and found significant correlation of genes with the expression of FASN.*

*Based on the suggestion of the reviewer, we included the following section to the paper.*

*"Correlation analysis is used to identify co-expressions between different genes based on mRNA expression data. Although co-expression does not necessarily indicate a relationship among transcript levels, functional relationships between encoded protein coding genes have been shown in our study. Co-expression analysis is applied to identify important alterations related to lipid synthesis, and promotion of cell survival and cell growth. In order to avoid possible false positives from co-expressed genes, we applied strict cut-off of Pearson's correlation coefficients, but it would be necessary to develop advanced method to identify co-expressed genes for smaller number of false positives. Here, we calculated correlations of gene expressions using normal tissue samples obtained from general population (i.e. GTEx) without selection bias, thus expected to identify general functional relationships, not only associated with a specific clinical indication. However, it is noteworthy that RNA-seq data generated for large and extensively-phenotyped cohorts would improve our understanding of functional relationships between genes in a specific clinical indication."*

Reviewer #2:

In this study, Lee et al have generated tissue-specific co-expression networks for 46 major human tissues and human hepatocellular carcinoma (HCC), and deciphered their tissue-specific functions. The results indicate that hepatic fatty acid synthase (FASN) is co-expressed with a few liver-specific genes associated with de novo lipogenesis that usually occurs during non-alcoholic fatty liver disease (NAFLD) and HCC. In fact, their reduced expression was found to be associated with reduced FASN expression. In addition, the authors potentially identify the cannabinoid type-1 receptor (CB1R) as the upstream modulator of these genes. This work complements their previously documented findings that describe other genomic analysis methodologies (such as transcriptional regulatory and protein-to-protein interaction networks) to identify tissue-specific genes that are correlate with functionality during health and disease.

Overall, these are interesting findings that provide compelling evidence for using such strategy to identify novel genes that are dysregulated during diseases. The authors provide a wealth of novel data supporting the fact that FASN expression is associated with PKLR, PNPLA3, and PCSK9, inhibition of which might be used as a potential treatment for NAFLD and HCC. Having said that, there are numerous issues that need to be addressed to improve the quality of the manuscript:

*We would like to thank the reviewer for careful reading of our manuscript and providing constructive comments.*

1. To identify the functional relationship between FASN and the hepatic-specific genes identified during NAFLD progression, the authors oddly decided to use a two-week high sucrose diet (HSD) mouse model. Since NAFLD is a well-known chronic condition that affects the liver, it would be important to show that a similar pattern of expression exists following a long-term HSD feeding. Moreover, beside evaluating hepatic triglyceride content (Fig. 5A), the authors should fully characterize their model by providing histological evaluation of the liver, assessing NASH score, determining serum ALT and AST levels, etc.

*In our study, we investigated the relationship between hepatic steatosis and the expression of FASN and our target genes. In order to show this association, we fed the mice with HSD for two weeks and measured the Hepatic TG levels as well as the expression of the genes. We also found a reference reporting that it is reasonable for generation of a NAFLD mouse model after 2-3 weeks of HSD ("Koteish A, Diehl A M. Animal models of steatosis, Seminars in liver disease (2001).")*

*In another independent mouse experiment, we fed the mouse with HSD for longer period and measured the hepatic steatosis and the expression of the genes using RT-PCR. We found very similar results. These liver tissue samples will be sent out for RNA sequencing and the global metabolic alterations will be studied in a follow up study.*

2. In accordance with the reduced FASN and its associated targets in PCSK9-null mice, are they also resistant to the development of hepatic steatosis on regular chow and HSD feeding?

*It has been reported that lipid accumulation in hepatocytes of PCSK9 KO mice was markedly reduced under both chow and high-cholesterol diets, revealing that PCSK9 deficiency confers resistance to liver steatosis (PMID: 18666258).*

*Moreover, Ruscica et al. ([Ruscica et al, 2016](#)) have recently associated circulating PCSK9 levels with accumulated liver fat. In 201 consecutive patients biopsied for suspected NASH, liver damage has been quantified by NAFLD activity score, circulating PCSK9 by ELISA, and hepatic mRNA by qRT-PCR in 76 of the patients. Circulating PCSK9 has been found to be significantly associated with steatosis grade, necroinflammation, ballooning, and fibrosis stage. Circulating PCSK9 has also been found to be significantly associated with hepatic expression of SREBP-1c and FASN, whereas PCSK9 mRNA levels have been found to be significantly correlated with steatosis severity and hepatic APOB, SREBP-1c and FASN expression.*

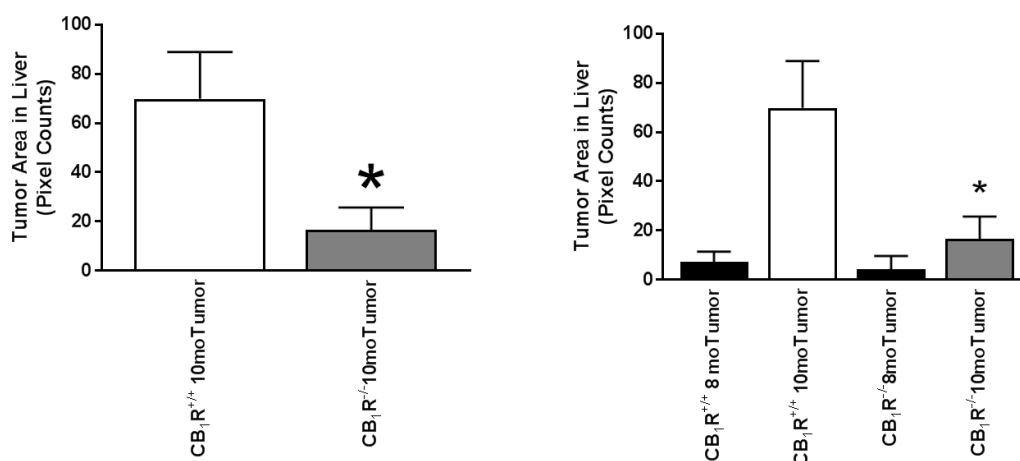
*Data in the literature supporting our findings presented in our manuscript.*

3. The authors mentioned that the attenuation of FASN and its related targets in CB1R-null mice treated with DEN is associated with decreased tumor growth. However, no data representing tumor

size are provided. I understand that they used samples that have been already described (Mukhopadhyay et al., 2015), but at least correlations between tumor size and gene expression should be added.

*The relationship between FASN and tumor size in mice may not have any significant correlation as FASN is a switching function (on or off) with tumor. In consistent with our hypothesis we have found that FASN is upregulated in mice which had detectable tumor in liver of mice.*

*Moreover, we have tumor size values from individual mice, but the FASN gene expression was measured by RNAseq using pooled tissue.*



4. Since peripheral CB1R blockade has been shown to ameliorate HCC (Mukhopadhyay et al., 2015), it would be important to determine whether this effect is associated with reduced FASN expression and its related genes.

*We observed that in the absence of CB1R, there is very low level FASN mRNA in the control and tumor of CB1R<sup>-/-</sup> mice. It further strengthens our hypothesis and FASN is detectable in higher level in HCC mice when tumor is present.*

5. Identifying PKLR, PCSK9, and PNPLA3 genes to be associated with FASN expression as well as NAFLD and HCC progression is a major novelty of the current paper. Indeed, the authors conclude that targeting these genes/proteins by using a chemical compounds or monoclonal antibodies could be considered as an effective treatment for such diseases. Therefore, to further validate their conclusion, they should consider testing PCSK9 inhibitor for reversing NAFLD or attenuating HCC in their mouse models.

*This is a very good point. However, there is no inhibitor available for any of the target genes including PKLR, PCSK9, and PNPLA3. We have started to work on the development of the small molecule and we will test it as soon as we have the first compounds available.*

Minor comments:

1. It isn't clear whether the data in all figures are presented as mean {plus minus} sem.

*Data are presented as the means  $\pm$  standard errors*

2. In figure 5D & E the gene expression is normalized to % of HCC, why? Isn't it better to describe it against the expression in the noncancerous tissues?

*We wanted to show the link between expressions of the genes not only between wild type HCC tumor and noncancerous samples but also in CB<sub>1</sub>R knockout HCC tumor and wild type HCC tumors.*

2nd Editorial Decision

17 July 2017

Dear Adil,

Thank you for sending us your revised manuscript. We have now heard back from reviewer #2 who was asked to evaluate your study. As you will see below, the reviewer is satisfied with some of the modifications made but has some remaining concerns, which we would ask you to address in a revision of the manuscript.

While we think that the experiments in mice fed a long-term HSD (point #1 of the reviewer) are not mandatory for acceptance of the paper, we think that points #4 and #5 need to be addressed, since they would indeed enhance the conclusiveness of the study.

On a more editorial level, I would like to draw your attention to the following points:

- I have made some changes in the standfirst text, bullet points and abstract (see attached file). Could you please let me know whether you agree with these changes or if you would prefer to further edit the text?

- I would also suggest the slightly modified title: "Network analyses identify liver-specific targets for treating liver diseases".

Please resubmit your revised manuscript online, with a covering letter listing amendments and responses to each point raised by the referees. Please resubmit the paper **\*\*within one month\*\*** and ideally as soon as possible. If we do not receive the revised manuscript within this time period, the file might be closed and any subsequent resubmission would be treated as a new manuscript. Please use the Manuscript Number (above) in all correspondence.

As a matter of course, please make sure that you have correctly followed the instructions for authors as given on the submission website.

Thank you for submitting this paper to Molecular Systems Biology.

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**REFeree REPORT**

Reviewer #2:

1. The authors have decided not to provide data, which evaluate their HSD-induced hepatic steatosis mouse model. Instead, they have chosen to cite a paper describing the model. As I mentioned before, NAFLD is a chronic condition, and I still believe that validating their data in a new set of animals would greatly add to the impact of the current paper.
2. Thank you for clarifying this point.
3. I am satisfied with the explanation provided.
4. The authors did not determine the expression levels of FASN in mice treated with a peripheral CB1 receptor blocker, which has been shown to reduce HCC occurrence.
5. There are currently two PCSK9 inhibitors, Evolocumab (Repatha) and Alirocumab (Praluent) that have been developed and tested, so stating that there is no inhibitor available is basically not too accurate.

2nd Revision - authors' response

19 July 2017

**RESPONSE TO REVIEWER COMMENT:**

*We would like to thank the reviewer for careful reading of our manuscript and providing constructive comments.*

1. The authors have decided not to provide data, which evaluate their HSD-induced hepatic steatosis mouse model. Instead, they have chosen to cite a paper describing the model. As I mentioned before, NAFLD is a chronic condition, and I still believe that validating their data in a new set of animals would greatly add to the impact of the current paper.

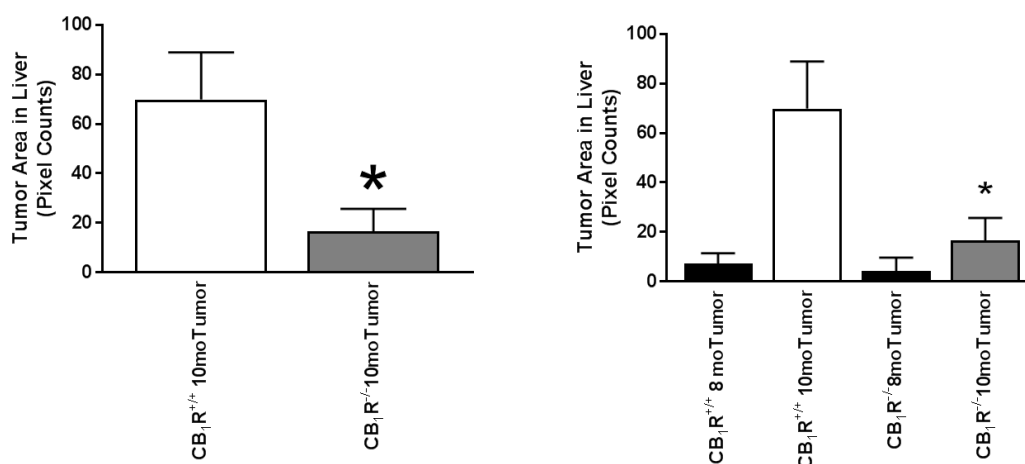
*We performed additional independent mouse experiment, generated RNAseq data and confirmed our results. Data has been presented in the thesis of Mattias Bergentall and will be published in a follow up study. Please let me know if you want me to send the thesis or the draft of the paper. Considering that we had similar results, we decided not to include in this paper.*

2. Thank you for clarifying this point.

3. I am satisfied with the explanation provided.

4. The authors did not determine the expression levels of FASN in mice treated with a peripheral CB1 receptor blocker, which has been shown to reduce HCC occurrence.

*We have already included the expression of FASN in the noncancerous and tumor samples obtained from wild type and CBR1 KO mice in Figure 5. Moreover, we had tumor size values from individual mice and the FASN gene expression was measured in these set of mice.*



5. There are currently two PCSK9 inhibitors, Evolocumab (Repatha) and Alirocumab (Praluent) that have been developed and tested, so stating that there is no inhibitor available is basically not too accurate.

*Thank you so much for pointing this. The drugs mentioned by the reviewer are not inhibitors. They are very well known monoclonal antibodies and target the circulation rather than liver tissue itself. That is the reason; there is a need for the development of inhibitors not only for PCSK9 but also for other tissues. We are developing PKL inhibitors and Pfizer is developing PCSK9 inhibitors. We are also filing a patent siRNA for PKL. Should be filed before August 15.*

3rd Editorial Decision

24 July 2017

Thank you again for sending us your revised manuscript. We are now satisfied with the modifications made and I am pleased to inform you that your paper has been accepted for publication.

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Adil Mardinoglu

Journal Submitted to: Molecular Systems Biology

Manuscript Number: MSB-17-7703

**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  $\chi^2$  tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

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).

**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?                                                                                     | In each mouse and cell line experiment we included more than 3 samples and adjusted p-values for multiple testing.                                                                                                                                                                                                        |
| 1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.                                                                       | We included 10 animals in each group of mice fed with CD and HSD and have also verified the results in an independent experiment.                                                                                                                                                                                         |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?                                                      | No samples were excluded from the analysis.                                                                                                                                                                                                                                                                               |
| 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.                | We have separated animals in two two group and supplemented one group with the HSD to see its effect on liver fat. The lipid and gene expression measurements were performed by a staff scientist with blinded samples.                                                                                                   |
| For animal studies, include a statement about randomization even if no randomization was used.                                                                                          | We have separated animals in two two group and supplemented one group with the HSD to see its effect on liver fat. The person who handled the mice did not perform the lipidomics analyses of the samples.                                                                                                                |
| 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 lipidomics analyses were performed with blinded samples. The clinical chemistry measurements were performed by a professional accredited clinical chemistry laboratory with blinded samples. The measurement of liver fat were performed by specialized clinical staff without knowing anything about the experiment. |
| 4.b. For animal studies, include a statement about blinding even if no blinding was done                                                                                                | We have not consider blinding of the investigator. The reason is that we wanted the same person fed the mice, in order to minimize variation. In addition, this person did not perform any subsequent analysis of the samples.                                                                                            |
| 5. For every figure, are statistical tests justified as appropriate?                                                                                                                    | Yes. We used appropriate statistical method.                                                                                                                                                                                                                                                                              |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.                                                                      | Yes. The data meet the assumptions of the tests.                                                                                                                                                                                                                                                                          |
| 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. We used appropriate statistical method.                                                                                                                                                                                                                                                                              |

**C- Reagents****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://jij.biochem.sun.ac.za>
[http://oba.od.nih.gov/biosecurity/biosecurity\\_documents.html](http://oba.od.nih.gov/biosecurity/biosecurity_documents.html)
<http://www.selectagents.gov/>

|                                                                                                                                                                                                                                                                                                                                                                  |                                                              |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| 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 ( <a href="#">see link list at top right</a> ), 1DegreeBio ( <a href="#">see link list at top right</a> ). | Mouse diets are defined.                                     |
| 7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.                                                                                                                                                                                                            | Cell lines have kindly provided by Human Protein Atlas team. |

\* 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.                                                                                                                                                                                                                                                            | Twenty male C57BL/6N mice were fed a standard mouse chow diet (Purina 7012, Harlan Teklad) and housed under a 12-h light-dark cycle. From 8 weeks of age, the mice were fed either a HSD diet (TD.88137, Harlan Laboratories, WI, USA) or CD for two weeks. The mice were housed at the University of Gothenburg animal facility (Lab of Exp Biomed) and supervised by university veterinarians and professional staff. The health status of our mice is constantly monitored according rules established by the Federation of European Laboratory Animal Science Associations. The experiments were approved by the Gothenburg Ethical Committee on Animal Experiments. |
| 9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.                                                                                                                                                                                                                                                                       | N/A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 10. We recommend consulting the ARRIVE guidelines ( <a href="#">see link list at top right</a> ) (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 ( <a href="#">see link list at top right</a> ) and MRC ( <a href="#">see link list at top right</a> ) recommendations. Please confirm compliance. | The ARRIVE Guidelines (Animal Research: Reporting In Vivo Experiments) are followed. This detailed information is included in the application for the animal ethics. Thus, all experiments are well documented.                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

#### E- Human Subjects

|                                                                                                                                                                                                                                                                                                                                                        |                                                                                |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| 11. Identify the committee(s) approving the study protocol.                                                                                                                                                                                                                                                                                            | No human study is performed.                                                   |
| 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.                                                                                                                                                                                                                                                          | Human data used in this study is publicly available in GTEx and TCGA databases |
| 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 ( <a href="#">see link list at top right</a> ) and submit the CONSORT checklist ( <a href="#">see link list at top right</a> ) 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 ( <a href="#">see link list at top right</a> ). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.                                                                                          | N/A                                                                            |

#### F- Data Accessibility

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| 18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'.<br><br>Data deposition in a public repository is mandatory for:<br>a. Protein, DNA and RNA sequences<br>b. Macromolecular structures<br>c. Crystallographic data for small molecules<br>d. Functional genomics data<br>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 ( <a href="#">see link list at top right</a> ) or Figshare ( <a href="#">see link list at top right</a> ).                                                                                                                                                                                                                                              | 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 ( <a href="#">see link list at top right</a> ) or EGA ( <a href="#">see link list at top right</a> ).                                                                                                                                                                                                                                       | 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.<br><br>Examples:<br><b>Primary Data</b><br>Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in <i>Shewanella oneidensis</i> MR-1. Gene Expression Omnibus GSE39462<br><b>Referenced Data</b><br>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<br>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 ( <a href="#">see link list at top right</a> ) and deposit their model in a public database such as Biocompare ( <a href="#">see link list at top right</a> ) or JWS Online ( <a href="#">see link list at top right</a> ). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information. | The networks used in this study are provided on an interactive web page at <a href="http://network.sysmedicine.com/">http://network.sysmedicine.com/</a> . Co-expression networks have also been uploaded to the NDEx ( <a href="http://indexbio.org">indexbio.org</a> ) and network IDs are provided in Dataset EV1. |

#### G- Dual use research of concern

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