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p53 protects against alcoholic fatty liver disease via ALDH2 inhibition

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Transaction Report:

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

Thank you for submitting your manuscript for consideration by the EMBO Journal. I apologise for the protracted assessment of your manuscript due to delays in review submission. We have now received comments from two reviewers, which are included below for your information.

As you will see from the reports, both reviewers find the study of interest, while also indicating a number of concerns regarding the presented analysis that would have to be addressed before they can support publication here. In particular, reviewer #1 raises concerns regarding the use of HepG2 cells in the analysis and the role of p53 in regulation of pyruvate levels and histone acetylation. Reviewer #3 was not able to submit their report, but also raised concerns regarding the HepG2 cell data, since these cells lack ethanol metabolising enzymes (ADH and CYP2E1). Therefore, addressing this important issue will be required for further consideration here.

If you are able to address the issues raised by the reviewers, I would be happy to consider a revised version of the manuscript. I think it would be helpful to discuss the revision in more detail via email or phone/videoconferencing.
I should also add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve the main concerns at this stage.

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

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

Please feel free to contact me if you have any further questions regarding the revision. Thank you for the opportunity to consider your work for publication. I look forward to receiving the revised manuscript.

Referee #1:

Comments to Authors

In this paper, the authors examine whether p53 has a role in the development of alcoholic fatty liver disease. The authors conclude that p53 represses ethanol oxidation by directly inhibiting ALDH2, which reduces acetate and acetyl CoA levels that

results in epigenetic inhibition of SCD1 and fatty liver. Further conclusion is that p53 also indirectly inhibits ALDH2 by p53-mediated regulation of glucose metabolism, resulting in decreased pyruvate levels and consequently, decreased pyruvate-mediated activation of ALDH2. The studies in HepG2 cells represent a comprehensive analysis of the effects of p53 on ethanol metabolism. Studies in p53-null and ALDH2-null mice provide in vivo confirmation of the cell studies and models for effects on development of alcoholic fatty liver. Overall, this is an interesting study that is generally well done and supports the conclusions. The pyruvate studies are relatively weaker and not developed completely and there are some other concerns.

1. Most studies were done in HepG2 cells and while reasonable, HepG2 cells as transformed cells that have been extensively cultured, may not reflect normal metabolic processes in the liver. Some of the key finding should be confirmed in primary mouse hepatocyte, mice, or preferably, human hepatocytes to provide some human relevance. In addition, it is not clear that physiological/pathological levels of pyruvate are used in the HepG2 cell studies.
2. The evidence that pyruvate activates ALDH2 is clear. However, the mechanism by which p53 reduces pyruvate is not entirely clear resting on the correlation between p53-mediated inhibition of glucose metabolism and reduced pyruvate with glucose expression. The relative importance of the pyruvate vs direct ALDH2 p53 effects is not clear.
3. The idea that p53 reduces histone acetylation is interesting, but general effects on histone acetylation should have global effects on multiple genes. It is not clear how gene-specific effects on metabolism genes, and Scd1 in particular, can result from general histone acetylation effects. Effects on other direct or non-direct p53 target genes, such as Aldh2, would be helpful.
4. The development of fatty liver is assessed by H&E and Oil Red O staining and ALT and AST levels. Analysis of hepatic inflammation and/or apoptosis in alcoholic fatty liver disease should be included in this study.
5. The discussion is relatively short and should be expanded to include, for example, discussion of human relevance, why low ALDH2 correlates to a poor prognosis for HCC, and the known role of p53 in hepatic lipid/BA metabolism and how that relates to effects on alcohol metabolism.

Minor
1. A model figure would be helpful to the reader and should be included.
2. The histology images should be shown at higher magnification (insets possibly) since it is hard to evaluate the changes as shown in the manuscript.
3. Western blots in general are not quantified. To establish changes in levels, some key gels should be quantified, and statistical analysis applied.
4. The text relevant to Fig. 3C and S4A-S4B in Results should say that "pyruvate can directly increase (not inhibit) purified ALDH activity."
5. The statements that p53 had no effect on protein levels of ALDH2 is not entirely accurate. In fact, the levels seem to increase (see Fig. S1A) so a better description is that P53 did not decrease ALDH2 levels.
6. The bioinformatic analysis of the ChIP-seq studies is not described in methods.
7. Details of the methods for p53 KO in HepG2 cells are not described in methods.

Referee #2:

Yao et al present a comprehensive set of data to establish an important role of p53 in the development of alcohol-induced liver disease. With various detailed binding assays, they prove that p53 directly binds to Aldh2 to prevent oligomer formation and to reduce Aldh2 activity, with accordant increases in cellular and tissue acetate and acetyl-CoA upon p53 knock-down. In addition, starvation experiments in HepG2 cells indicate that p53 is also contributing to Aldh2 activity regulation through its known impact on glycolysis because pyruvate binds to Aldh2, which is another novel finding. Systemically, the authors use elegant experiments in mouse models to show that the effect of p53 in ethanol-induced steatosis is mediated through liver Aldh2. Downstream, acetyl-CoA levels, modulated by the p53/Aldh2-axis, are shown to influence global histone acetylation levels and the authors focus on the desaturase SCD1, connecting ethanol-induced steatosis with accumulation of MUFAs in case of p53 knock-out.
Overall, using a wide array of models and assays, spanning from cell-free to mice, the authors draw a convincing picture of p53 as central factor in alcohol-related hepatic steatosis in mice which was previously undescribed. Throughout the manuscript, the authors use proper controls and employ complimentary systems or methods to corroborate individual findings. The writing is clear and easy to follow and the results are interpreted with adequate caution.
The only concern apparent to this reviewer is the low number of mice used in some experiments (n=3) like the ones in Figure 6. Moreover, in Figure 4, liver weight is reported from five mice per group, while all other data is shown only for 3 mice. It would we more transparent to show the data from all 5 mice, plotting the single data points in the bar graphs (i.e. as scatter dot plot).
Other than that, this reviewer only has minor comments regarding the discussion paragraph:
The discussion could be extended to include a comparison of the in vivo findings in this study to the clinical situation. E.g.: How can mice with complete Aldh2 knock-out be compared to mutated ALDH2 in humans in terms of ALDH2 activity and disease severity? And is the population with mutated ALDH2 also presenting with less alcohol-induced fatty liver disease as in Figure 4 (if this is known)?
Can the authors speculate why different p53 mutants do not affect Aldh2 activity despite the fact that they are still binding Aldh2?
Furthermore, other candidates involved in TG synthesis from their ChIP-seq data could be discussed and/or made available via

public databases like NCBI GEO.
Writing and wording:
In the paragraph pertaining to Figure 4: The authors write that "...that p53 inhibits ethanol-induced fatty liver through ALDH2..." at two positions in this paragraph. Correct would be to use "alleviate" instead of "inhibit" as liver TGs are increased already in the wildtype control with intact p53 upon ethanol feeding.
Finally, the text contains some typos and syntactic mistakes (e.g. singular/plural, a few incorrect sentences).

Response to Referee #1:

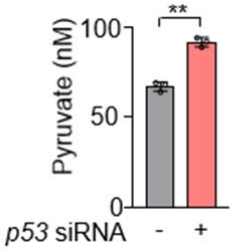
In this paper, the authors examine whether p53 has a role in the development of alcoholic fatty liver disease. The authors conclude that p53 represses ethanol oxidation by directly inhibiting ALDH2, which reduces acetate and acetyl CoA levels that results in epigenetic inhibition of SCD1 and fatty liver. Further conclusion is that p53 also indirectly inhibits ALDH2 by p53-mediated regulation of glucose metabolism, resulting in decreased pyruvate levels and consequently, decreased pyruvate-mediated activation of ALDH2. The studies in HepG2 cells represent a comprehensive analysis of the effects of p53 on ethanol metabolism. Studies in p53-null and ALDH2-null mice provide in vivo confirmation of the cell studies and models for effects on development of alcoholic fatty liver. Overall, this is an interesting study that is generally well done and supports the conclusions. The pyruvate studies are relatively weaker and not developed completely and there are some other concerns.

We thank the referee for the positive comments on our work and have work extensively to address the concerns. As detailed below, we have performed numerous experiments to address these comments, which we believe improve the manuscript greatly.

1. Most studies were done in HepG2 cells and while reasonable, HepG2 cells as transformed cells that have been extensively cultured, may not reflect normal metabolic processes in the liver. Some of the key finding should be confirmed in primary mouse hepatocyte, mice, or preferably, human hepatocytes to provide some human relevance. In addition, it is not clear that physiological/pathological levels of pyruvate are used in the HepG2 cell studies.

We thank the referee for raising this important issue. As suggested by the referee, we have examined the activity of ALDH2 in $p53^{+/+}$ and $p53^{-/-}$ mouse primary hepatocytes and human normal liver cells (CCC-HEL-1). Consistent with the data in mice and HepG2 cells, depletion of p53 led to an increase in ALDH enzymatic activity in mouse primary hepatocytes and CCC-HEL-1 cells (revised Figures 1L and 1M). Consistent with the observations in HepG2 cells, p53 knockout led to an increase in the levels of acetyl-CoA in mouse primary hepatocytes and CCC-HEL-1 cells, and this effect was largely blocked by ALDH2 inhibition (revised Figures 1S and 1T). We also confirmed the binding of p53 to ALDH2 in mouse primary hepatocytes and CCC-HEL-1 cells (revised Figures EV2C and EV2D). Furthermore, we examined the expression of SCD1 and histone acetylation in mice, and found that loss of p53 led to elevated SCD1 expression and histone acetylation in mouse liver (revised Figure 5G). Notably, when ALDH2 was absent, p53 failed to affect acetyl-CoA production and SCD1 expression, as well as the histone acetylation (revised Figures 1S, 1T, 4O, 5B, and 5G). Thus, these data, together with our previous observations in mouse livers and HepG2 cells, support our findings regarding the inhibitory effect of p53 on ethanol metabolism through ALDH2.

In addition, we have determined the absolute levels of pyruvate in HepG2 cells and CCC-HEL-1 cells. The absolute amount of pyruvate in these cells was 40-80 nM (revised Figures 3K, and Response Figure 1 shown below). As suggested, we treated HepG2 cells and mouse primary hepatocytes and CCC-HEL-1 cells with physiological levels (40 nM) of pyruvate and examined ALDH2 activity in the presence or absence of p53. In all cells lines, depletion of p53 increased ALDH2 activity even in the presence of exogenous pyruvate, and similarly, pyruvate addition still resulted in increased ALDH2 activity in p53-deficient cells (revised Figures EV3K-M).



Response Figure 1. Related to revised Figure EV3M. CCC-HEL-1 cells were treated with p53 siRNA or control siRNA (-) for 48 hours, and cellular levels of pyruvate were determined by LC-MS analysis. n=3 independent wells. p53 expression was measured by Western blot analysis as shown in revised Figure EV3M. Data are the mean ± SD. P values were determined by unpaired two-tailed Student's t tests. ** $p < 0.01$.

2. The evidence that pyruvate activates ALDH2 is clear. However, the mechanism by which p53 reduces pyruvate is not entirely clear resting on the correlation between p53-mediated inhibition of glucose metabolism and reduced pyruvate with glucose expression. The relative importance of the pyruvate vs direct ALDH2 p53 effects is not clear.

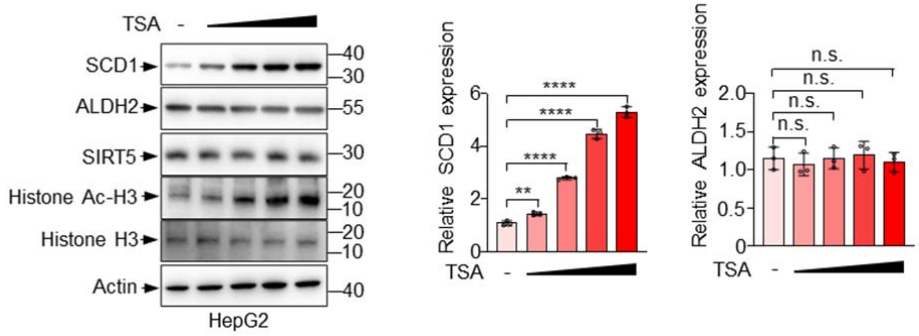
We thank the referee for these insightful comments. Numerous studies have reported that p53 can inhibit glycolytic fluxes both directly and indirectly(Bensaad, Tsuruta et al., 2006, Kawauchi, Araki et al., 2008, Madan, Gogna et al., 2011, Mai, Gosa et al., 2017). Consistent with this, we found that p53 expression or activation reduced cellular pyruvate production in both HepG2 cells and CCC-HEL-1 cells (revised Figures 3K, and Response Figure 1 shown above). To determine the relative importance of the pyruvate vs direct ALDH2-p53 effects, we first treated mouse primary hepatocytes with the glucose analogue 2DG (2-Deoxy-D-glucose), a competitive glycolysis inhibitor that can reduce pyruvate production. We found that ALDH2 enzymatic activity was reduced by approximately 30% in $p53^{+/+}$ cells compared to $p53^{-/-}$ cells (revised Figure EV3N column 1 vs column 2), whereas 2DG treatment reduced ALDH2 enzymatic activity by approximately 10% in $p53^{-/-}$ cells (revised Figure EV3N column 4 vs column 2). Interestingly, p53 inhibited ALDH2 enzymatic activity in 2DG-treated cells (revised Figure EV3N column 3 vs column 4) even more so than in control cells (revised Figure EV3N column 1 vs column 2).

Similarly, more inhibition of ALDH2 by p53 was observed in HepG2 cells treated with PKM2 siRNA (revised Figure EV3O). Thus, it appears that compared to pyruvate, p53 plays a dominant role in the inhibition of ALDH2.

3. The idea that p53 reduces histone acetylation is interesting, but general effects on histone acetylation should have global effects on multiple genes. It is not clear how gene-specific effects on metabolism genes, and Scd1 in particular, can result from general histone acetylation effects. Effects on other direct or non-direct p53 target genes, such as Aldh2, would be helpful.

We agree with the referee that general effects on histone acetylation should have global effects on multiple genes. KEGG analysis of ChIP-seq data revealed that the related genes of increased peaks in liver from ethanol-treated *p53*$^{-/-}$ mice were mainly enriched in metabolic pathways including fatty acid metabolism. Given the strong influence of the p53-ALDH2 axis on hepatic lipid accumulation, we focused on genes directly involved in fatty acid metabolism. By analyzing the promoter regions of fatty acid synthesis-related genes enriched by histone H3K9 and H3K27 acetylation, we found that the enrichment level of the stearoyl-CoA desaturase-1 (SCD1) gene promoter region was most significantly affected by p53 depletion (revised Figure 5D). Moreover, inspection of SCD gene showed that alcohol-induced histone acetylation at SCD1 gene was induced by p53 depletion (revised Figure 5E). Additionally, results of ChIP assays using anti-H3K9ac and anti-H3K27ac antibodies showed significantly increased levels of acetylated histones bound to the SCD1 gene in the liver of *p53*$^{-/-}$ mice compared to wildtype mice, particularly in ethanol-treated *p53*$^{-/-}$ mice (revised Figure 5F). Thus, these findings provide additional evidence that regulation of SCD1 expression may be an important mechanism for the regulation of alcohol-induced hepatic lipid accumulation by p53 loss.

As also suggested by the referee, we examined whether the expression of p21, which is a p53 direct target gene, was affected by the ethanol-derived histone acetylation. As shown in revised Figure 5G, ethanol treatment had no effect on p21 expression, and ALDH2 expression was also unaffected. Furthermore, we treated HepG2 cells with TSA, which is an inhibitor of HDACs, to increase histone acetylation. TSA treatment did increase SCD1 expression, but had no effect on ALDH2 or SIRT5, which are not the direct transcriptional target genes of p53, suggesting that SCD1 expression is specifically regulated by p53-mediated histone acetylation (please see the Response Figure 2 shown below). Together, these data demonstrate that SCD1, a non-direct transcriptional target gene of p53, is regulated by p53 through histone acetylation.



Response Figure 2. HepG2 cells were treated with increasing amounts (0, 5, 10, 20, 50μM) of TSA for 24 hours, and protein expression was determined by western blot analysis. The mRNA levels of SCD1 and ALDH2 was measured by qRT-PCR analysis. n=3 independent wells. Data are the mean ± SD. P values were determined by unpaired two-tailed Student's t tests. ** $p < 0.01$, **** $p<0.0001$, n.s. not significant.

4. The development of fatty liver is assessed by H&E and Oil Red O staining and ALT and AST levels. Analysis of hepatic inflammation and/or apoptosis in alcoholic fatty liver disease should be included in this study.

We thank the referee for this excellent comment. To address this comment, we first have assessed inflammation score based on H&E staining of mice livers. Increased inflammation scores were obtained in ethanol-fed *p53*$^{-/-}$ mice, but not in mice deficient in ALDH2 (revised Figure 4D). To further confirm this, we have also analyzed the expression of hepatic inflammatory cytokines, such as IL-6, IL-1β, TNF-α, Cxcl1, Cxcl2. Consistently, the expressions of these inflammatory cytokines were increased in ethanol-fed wildtype mice and especially in *p53*$^{-/-}$ mice, whereas knockout of ALDH2 completely abolished this effect (revised Figure 4E-I). In addition, we determined apoptosis by examining the expression of cleaved caspase 3 in alcoholic fatty liver. As shown in revised Figure 5G, p53 deficiency abrogated ethanol-induced hepatic apoptosis.

5. The discussion is relatively short and should be expanded to include, for example, discussion of human relevance, why low ALDH2 correlates to a poor prognosis for HCC, and the known role of p53 in hepatic lipid/BA metabolism and how that relates to effects on alcohol metabolism.

The role of ALDH2 in cancer appears to be different from that in fatty liver. Although greatly dependent on tissue type, ALDH2 dysfunction contributes to the development and progression of cancer.

ALDH2 polymorphism or mutations is correlated to an increased risk of alcohol-related cancers including HCC(Brooks, Enoch et al., 2009, Seo, Gao et al., 2019). However, ALDH2 rs671 mutation (Glu to Lys mutation at 487) was also found to be significantly associated with low risk of HCC related to alcohol drinking (Liu,

Yang et al., 2016), and also plays a protective role in ovarian cancer unrelated to alcohol consumption(Ugai, Kelemen et al., 2018). Thus, the regulatory effects of ALDH2 on tumors are complex, may be tissue type-specific, and are also likely to be context-dependent, such as whether alcohol is present and under what types of signaling stimulus. Given the lack of a direct mechanistic link between fatty liver and HCC, we currently do not know whether the regulation of ALDH2 by p53 directly leads to HCC and further mechanistic studies are needed.

Recently, a role of p53 in mediating lipid metabolism has emerged, with intriguing metabolic roles in regulating cholesterol homeostasis and lipid droplet formation. Genetically or pharmacologically activated p53 is capable of regulating genes involved in multiple aspects of lipid metabolism, including intracellular regulators of ceramide and fatty acids, systemic lipid uptake, and lipoprotein metabolism (Goldstein, Ezra et al., 2012). However, whether hepatic p53 plays a role in alcohol metabolism remains unclear. In this work, we identify a key role for the p53-ALDH2-SCD1 axis in regulating alcohol metabolism and its associated fatty liver disease, adding a new chapter to the understanding of the function of hepatic p53 in regulating lipid metabolism.

As suggested, we have now incorporated these discussions into the revised version.

Minor

1. A model figure would be helpful to the reader and should be included.

As suggested, a working model has been provided (revised Figure 6K).

2. The histology images should be shown at higher magnification (insets possibly) since it is hard to evaluate the changes as shown in the manuscript.

As suggested, images with higher dpi have been provided.

3. Western blots in general are not quantified. To establish changes in levels, some key gels should be quantified, and statistical analysis applied.

As suggested, we have quantified some of the important western blot data, and conducted statistical analysis accordingly.

4. The text relevant to Fig. 3C and S4A-S4B in Results should say that "pyruvate can directly increase (not inhibit) purified ALDH activity."

We thank the referee for carefully reading the manuscript. We have now thoroughly checked the manuscript. We apologize for these mistakes.

5. The statements that p53 had no effect on protein levels of ALDH2 is not entirely accurate. In fact, the levels seem to increase (see Fig. S1A) so a better description is

that P53 did not decrease ALDH2 levels.

We thank the referee for this excellent suggestion. As suggested, we have now changed the statement to “p53 depletion did not decrease ALDH2 levels”.

6. The bioinformatic analysis of the ChIP-seq studies is not described in methods.

We apologize for not including this method in the previous version of manuscript. We have now described it in methods. Please see page 23 of the revised manuscript.

7. Details of the methods for p53 KO in HepG2 cells are not described in methods.

We are sorry for not including this method in the previous version of manuscript. We have now described it in methods. Please see page 17 of the revised manuscript.

Response to Referee #2:

Yao et al present a comprehensive set of data to establish an important role of p53 in the development of alcohol-induced liver disease. With various detailed binding assays, they prove that p53 directly binds to Aldh2 to prevent oligomer formation and to reduce Aldh2 activity, with accordant increases in cellular and tissue acetate and acetyl-CoA upon p53 knock-down. In addition, starvation experiments in HepG2 cells indicate that p53 is also contributing to Aldh2 activity regulation through its known impact on glycolysis because pyruvate binds to Aldh2, which is another novel finding. Systemically, the authors use elegant experiments in mouse models to show that the effect of p53 in ethanol-induced steatosis is mediated through liver Aldh2. Downstream, acetyl-CoA levels, modulated by the p53/Aldh2-axis, are shown to influence global histone acetylation levels and the authors focus on the desaturase SCD1, connecting ethanol-induced steatosis with accumulation of MUFAs in case of p53 knock-out.
Overall, using a wide array of models and assays, spanning from cell-free to mice, the authors draw a convincing picture of p53 as central factor in alcohol-related hepatic steatosis in mice which was previously undescribed. Throughout the manuscript, the authors use proper controls and employ complimentary systems or methods to corroborate individual findings. The writing is clear and easy to follow and the results are interpreted with adequate caution.

We are very grateful to the referees for the positive and constructive comments, which encouraged us and improved the manuscript considerably.

The only concern apparent to this reviewer is the low number of mice used in some experiments (n=3) like the ones in Figure 6. Moreover, in Figure 4, liver weight is reported from five mice per group, while all other data is shown only for 3 mice. It would we more transparent to show the data from all 5 mice, plotting the single data points in the bar graphs (i.e. as scatter dot plot).

We thank the referee for this excellent comment. As suggested, we have now included the data analyzed from all the 5 mice used, for example, in revised Figures 4 and 6, and the single data points are also shown in the bar graphs. Please see revised Figures 4 and 6 for details.

In addition, we also repeated some of these experiments again and similar results were obtained. We are sorry that these data were not included as the animal facility here was closed for a while due to the large-scale Omicron virus outbreak and some of the mice died.

Other than that, this reviewer only has minor comments regarding the discussion paragraph:
The discussion could be extended to include a comparison of the in vivo findings in

this study to the clinical situation. E.g.: How can mice with complete Aldh2 knock-out be compared to mutated ALDH2 in humans in terms of ALDH2 activity and disease severity? And is the population with mutated ALDH2 also presenting with less alcohol-induced fatty liver disease as in Figure 4 (if this is known)?

The role of ALDH2 deficiency in the pathogenesis of alcoholic liver injury remains still obscure and even controversial. Although pharmacological activation of Aldh2 or global overexpression of Aldh2 appears to ameliorate chronic alcohol-induced hepatic steatosis(Guo, Xu et al., 2015, Zhong, Zhang et al., 2015), improved alcoholic fatty liver in ALDH2 deficient mice has also been reported(Chaudhry, Samak et al., 2015) (Kwon, Won et al., 2014). Deficiency of ALDH2 in humans leads to the accumulation of acetaldehyde in the blood, but there are few reports on its association with steatosis. Nevertheless, different degrees of variation in ALDH2 expression levels might have different effects on fatty liver, which may result from a combination of complex mechanisms.

We have discussed these in the revised version.

Can the authors speculate why different p53 mutants do not affect Aldh2 activity despite the fact that they are still binding Aldh2?

We thank the referee for the insightful question on why different p53 mutants do not affect ALDH2 activity. We are sorry that currently we do not have a clear explanation, yet we envision a possibility for this observation is that tumor-associated mutations impair the native conformation of p53, the inhibition on ALDH2 is probably attributed to the native conformation of p53. We have also included this possible explanation in the revised version.

Furthermore, other candidates involved in TG synthesis from their ChIP-seq data could be discussed and/or made available via public databases like NCBI GEO.

As recommended by the referee, we have analyzed the data of ChIP-seq and found that the acetylation modification of histones H3K9 and H3K27 in other candidates involved in TG synthesis including Lpin1, Gpam, Scarb1, Apoc2, Ldlr, and Sorl1 was also significantly regulated by p53. However, we noted that the enrichment level of the stearoyl-CoA desaturase-1 (SCD1) gene promoter region was most significantly affected by p53 deletion (revised Figure 5D), and subsequent mechanistic studies revealed that the regulation of SCD1 expression may be an important mechanism for p53 deletion-mediated alcohol-induced hepatic lipid accumulation.

Certainly, once the article is accepted, we will upload these ChIP-seq data to a public database. We thank the referee for this great suggestion.

Writing and wording:
In the paragraph pertaining to Figure 4: The authors write that "...that p53 inhibits

ethanol-induced fatty liver through ALDH2..." at two positions in this paragraph. Correct would be to use "alleviate" instead of "inhibit" as liver TGs are increased already in the wildtype control with intact p53 upon ethanol feeding.
Finally, the text contains some typos and syntactic mistakes (e.g. singular/plural, a few incorrect sentences).

We thank the referee for carefully reading the manuscript. We have now thoroughly checked the manuscript. We apologize for the mistakes.

Reference

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Thank you for submitting a revised version of your manuscript. Your study has now been seen by both original referees, who find that their previous concerns have been addressed and now recommend publication of the manuscript. There remain only a couple of minor editorial points that have to be addressed before I can extend formal acceptance of the manuscrip.:

Please let me know if you have any further questions regarding any of these points. You can use the link below to upload the revised files.

Thank you again for giving us the chance to consider your manuscript at The EMBO Journal. I look forward to receiving the final version.

Referee #1:

With the additional experiments presented and discussion of the issues, the authors have adequately addressed all my concerns and comments.

Referee #2:

The authors sufficiently addressed the concerns of this reviewer. A temporarily non-public GEO upload link for the ChIP-seq data could be demanded before acceptance. But this depends on journal policy.

The authors addressed the minor editorial issues.

Thank you for addressing the final editorial issues. I am now pleased to inform you that your manuscript has been accepted for publication.

EMBO Press Author Checklist

Corresponding Author Name: Peng Jiang and Wenjing Du
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2022-112304

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Reporting Checklist for Life Science Articles (updated January 2022)
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	

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

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Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Materials and Methods

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Materials and Methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Materials and Methods
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Materials and Methods

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
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Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

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If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

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Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Materials and Methods
Include a statement about **blinding** even if no blinding was done.	Not Applicable	
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Not Applicable	
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Figures

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In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Figures
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figures

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Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
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Reporting

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

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