nature research

Peer Review Information

Journal: Nature Cell Biology
Manuscript Title: YAP/TAZ drives cell proliferation and tumor growth via a polyamine-eIF5A hypusination-LSD1 axis
Corresponding author name(s): Professor Duojia Pan

Reviewer Comments & Decisions:

Decision Letter, initial version:

Subject: Decision on Nature Cell Biology submission NCB-P46013
Message:

*Please delete the link to your author homepage if you wish to forward this email to co-authors.

Dear Professor Pan,

Your manuscript, "YAP/TAZ drives cell proliferation and tumor growth via a polyamine-eIF5A hypusination-LSD1 axis", has now been seen by 3 referees, who are experts in YAP/TAZ in cancer (referee 1 and 2); and polyamine metabolism with epigenetic expertise (referee 3). As you will see from their comments (attached below) they find this work of potential interest, but have raised substantial concerns, which in our view would need to be addressed with considerable revisions before we can consider publication in Nature Cell Biology.

Nature Cell Biology editors discuss the referee reports in detail within the editorial team, including the chief editor, to identify key referee points that should be addressed with priority, and requests that are overruled as being beyond the scope of the current study. To guide the scope of the revisions, I have listed these points below. We are committed to providing a fair and constructive peer-review process, so please feel free to contact me if you would like to discuss any of the referee comments further.

In particular, it would be essential to:

A) Strengthen the proposed mechanism as requested by all three referees:

Reviewer 1
"How specific is LSD1 activation for the YAP induced program? Is LSD1 activated in other, YAP independent liver overgrowth situations, e.g. by TCPOBOP administration?"

"How does Odc1 KD or LSD1 KO inhibit YAP induced overgrowth? Does it cause apoptosis or simply restrain cell proliferation?"

"Does Odc1 inhibition suppress the growth of cells that do not depend on YAP/TAZ?"

Reviewer 2
" Do authors have an explanation as to why arginine is highly enriched by YAP overexpression (Fig.1E)?..."

"...Thus, the observed decrease in hepatomegaly due to Odc1 knockdown before YAP overexpression seems to be somewhat obvious. To assess the genetic hierarchy of Odc1 as a downstream target of YAP, the authors should overexpress YAP first, then deplete Odc1. The same logic applies to LSD knockout (Fig.5)."

"The induction of Odc1 by YAP, as shown by the authors, is generally not dramatic, and thus doubts remain as to whether it is a bona fide YAP target gene. The reviewer suggests considering the following to add credibility to this claim..."

"The induction of Odc1 and polyamines appears much greater in actual tissues although only wild-type YAP was expressed, as opposed to YAP 5SA/TAZ 4SA (hyperactive mutants) in earlier cell line-based experiments wherein this induction was not very striking. Perhaps the authors can observe a much greater liver phenotype/polyamine production if AAV-YAP 5SA is used. Conversely, why does OE or wild-type YAP in cell lines do not have much effect on Odc1 expression (Extended Fig. 3A)? The authors should validate whether Odc1 expression is directly proportional to YAP activity, for example by expressing increasing amounts of YAP (e.g. using Tet-On system). Also, the authors should show the levels of Odc protein from liver tissues (Fig 3d)"

"The overall molecular mechanism portrayed here, although novel, appears too stretched out since it involves so many different cellular processes (e.g. transcription, metabolomic change, hypusination, translation) which sheds doubts to whether this 'axis' is truly linear and intact. Importantly, the authors do not provide sufficient explanation as to how candidate targets were chosen for further analysis, leaving doubts to whether the authors truly took an unbiased approach. For example, in the text pertaining to Fig.4 the authors simply 'choose' LSD1 as one of the enzymes regulating histone methylation or acetylation, since chromatin remodeling is important in YAP/TAZ-induced transcriptional

programs. What was the rationale for this decision, and why were other candidates (e.g. KDM6B, SETD2) excluded?"

"Extended Fig. 4 shows data in which overexpression of OAZ1, which binds to ODC1 and promotes its proteasomal degradation, rescues YAP overexpression-induced increase in liver size/polyamine production/cell proliferation. However, it is evident from Extended Fig. 4E that OAZ1 expression did not lead to even a modest decrease in ODC1 protein levels, leaving doubts to whether the reversal of phenotypes upon OAZ1 overexpression is indeed due to the suppression of ODC1."

"The authors claimed that ~50% of all YAP-downregulated genes depended on LSD1, while ~30% of YAP-upregulated genes depended on LSD1. Also, they showed that H3K4me1/2 peaks were decreased signal in YAP OE as compared to wildtype control livers and identified 727 putative LSD1 target genes. What are the 30% of YAP-upregulated genes depended on LSD1 ? Are they involved in proliferation or anti-cell death ? Without experimental evidences, I am not sure whether these too many LSD1 target genes are truly downregulated in YAP-OE and are associated with unfavorable liver cancer."

Reviewer 3
"Most importantly, the measurement of total polyamines is insufficient to make any specific claims about the role of the individual polyamines. The increase in total polyamines measured in response to overexpression of YAP in mouse livers is predominantly represented by acetylputrescine, a catabolic product of excess putrescine which, likely a response to increased ODC1 activity beyond that necessary to maintain functional levels of the higher polyamines, spermidine and spermine. Also, effects in mouse liver are not necessarily mimicked in vitro cell culture. Consequently, measurement of the individual polyamines is necessary in all experiments to determine any specific changes in polyamine pools in response to the various manipulations used here. This is particularly important in the experiments that use DFMO or siRNA knockdown of ODC1... Since the authors claim that hypusinated eIF-5A is a critical factor in the synthesis of LSD1 and thus is a through line in the YAP/TAZ-polyamine-eIF5A-LSD1 axis, it is important that actual changes in spermidine occur as a result of their manipulations rather than off-target effects..."

"...however, it provides no insight as to whether polyamines are involved in the histone methylation changes. It is not clear why equivalent data were not provided for the ODC1 knockdown, YAP overexpression livers or better yet, DFMO treated, YAP overexpressing animals."

"...What is the evidence that YAP/TAZ enhancer activity and not MYC activation is responsible for the increased ODC1 expression in the authors' systems?
"

B) Clarify the difference between in vitro and in vivo observations:

Reviewer 1
"The effects of SP-2577 are quite impressive in the in vitro assays indicating not only inhibition of proliferation but cell death. However, the effects are much less impressive in vivo. Do the authors have an explanation for this? Is the dose very different? Is the inhibitor toxic in vivo?
Etc?"

C) All other referee concerns pertaining to strengthening existing data, providing controls, methodological details, clarifications and textual changes as applicable should also be addressed.

D) Finally please pay close attention to our guidelines on statistical and methodological reporting (listed below) as failure to do so may delay the reconsideration of the revised manuscript. In particular please provide:

- a Supplementary Figure including unprocessed images of all gels/blots in the form of a multi-page pdf file. Please ensure that blots/gels are labeled and the sections presented in the figures are clearly indicated.

- a Supplementary Table including all numerical source data in Excel format, with data for different figures provided as different sheets within a single Excel file. The file should include source data giving rise to graphical representations and statistical descriptions in the paper and for all instances where the figures present representative experiments of multiple independent repeats, the source data of all repeats should be provided.

We would be happy to consider a revised manuscript that would satisfactorily address these points, unless a similar paper is published elsewhere, or is accepted for publication in Nature Cell Biology in the meantime.

When revising the manuscript please:

- ensure that it conforms to our format instructions and publication policies (see below and www.nature.com/nature/authors/).

- provide a point-by-point rebuttal to the full referee reports verbatim, as provided at the end of this letter.

- provide the completed Editorial Policy Checklist (found here https://www.nature.com/authors/policies/Policy.pdf), and Reporting Summary (found here https://www.nature.com/authors/policies/ReportingSummary.pdf). This is essential for reconsideration

of the manuscript and these documents will be available to editors and referees in the event of peer review. For more information see http://www.nature.com/authors/policies/availability.html or contact me.

Nature Cell Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

Please submit the revised manuscript files and the point-by-point rebuttal to the referee comments using this link:

[REDACTED]

*This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We would like to receive a revised submission within six months. We would be happy to consider a revision even after this timeframe, however if the resubmission deadline is missed and the paper is eventually published, the submission date will be the date when the revised manuscript was received.

We hope that you will find our referees' comments, and editorial guidance helpful. Please do not hesitate to contact me if there is anything you would like to discuss.

Best wishes,

Zhe Wang

Zhe Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: zhe.wang@nature.com

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
Li et al. uncovered a role for YAP/TAZ in the biosynthesis of the regulatory metabolite polyamine. Mechanistically, they found that YAP/TAZ directly activates the transcription of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis, and that the increased polyamine levels promote the hypusination of eIF5A to support translation of the transcriptional repressor LSD1. Notably, inhibition of Odc1, polyamine biosynthesis or LSD1 suppressed the ectopic proliferation induced by YAP/TAZ in the mouse liver and to some degree in cultured human cells. The manuscript uses a variety of sophisticated and technically sound in vivo, in vitro and biochemical methods. The data are of high quality and well presented and of interest to a general audience. In particular, these findings add a new dimension to the understanding of YAP/TAZ and open new strategies for anti-cancer approaches.

Comments:

1. Fig1: It would be informative to compare the absolute levels of expression for paralogous genes.

2. The authors mention that YAP/TAZ can induce Odc1 in various cell lines. Did they find cell lines where this was not the case even though YAP/TAZ could induce their canonical target genes? What about HEK293, MCF10A and other cell lines that are often used to look at YAP/TAZ gain of function phenotypes?

3. line 164: should read “ORFs encoding short polyproline tracks”

4. What are the levels of LSD1 in the LSD1 ko livers compared to normal livers?

5. What is the effect of Odc1 KD or LSD1 KO on normal (embryonic) liver growth? For example, the authors could knockout LSD1 using alb-Cre.

6. How specific is LSD1 activation for the YAP induced program? Is LSD1 activated in other, YAP independent liver overgrowth situations, e.g. by TCPOBOP administration?

7. How does Odc1 KD or LSD1 KO inhibit YAP induced overgrowth? Does it cause apoptosis or simply restrain cell proliferation?

8. The effects of SP-2577 are quite impressive in the in vitro assays indicating not only inhibition of proliferation but cell death. However, the effects are much less impressive in vivo. Do the authors have an explanation for this? Is the dose very different? Is the inhibitor toxic in vivo?
Etc?

9. Does Odc1 inhibition suppress the growth of cells that do not depend on YAP/TAZ?

Reviewer #2:
Remarks to the Author:
In this manuscript, Li and colleagues report a mechanism by which YAP/TAZ promotes the transcription of ornithine decarboxylase 1, the rate-limiting enzyme in polyamine biosynthesis. This was found by surveying the metabolic profile in YAP/TAZ-activated transgenic mouse models and human cancer cells. Increased polyamine levels then promote the hypusination of eIF5A, which enhances the efficient translation of histone demethylase LSD1. They further showed that LSD1 downregulates the expression of a significant portion of YAP/TAZ-downregulated genes in YAP/TAZ-activated cells.

The relationship between Hippo/YAP and polyamines is a novel finding, which warrants attention. However, even if the mechanisms of YAP/TAZ-polyamine-eIF5A hypusination-LSD1 is potentially interesting, the reviewer feels that the overall this mechanism is far too overreaching, and as a result, the study lacks depth. Also, some of the results presented are modest or marginal at best and seriously suggest that the authors should downplay their findings.

The followings are comments and suggestions.

Major points:

1. Do authors have an explanation as to why arginine is highly enriched by YAP overexpression (Fig.1E)? This is the most upstream precursor to polyamine production, even before the metabolic regulation by the polyamine-related enzymes, some of whose expression are regulated by YAP.
2. The authors deplete Odc1 before YAP overexpression (Fig.3A). Polyamines are claimed to be generally important for tumorigenesis. Thus, the observed decrease in hepatomegaly due to Odc1 knockdown before YAP overexpression seems to be somewhat obvious. To assess the genetic hierarchy of Odc1 as a downstream target of YAP, the authors should overexpress YAP first, then deplete Odc1. The same logic applies to LSD knockout (Fig.5).
3. The induction of Odc1 by YAP, as shown by the authors, is generally not dramatic, and thus doubts remain as to whether it is a bona fide YAP target gene. The reviewer suggests considering the following to add credibility to this claim:

a. Compare induction of Odc1 from overexpression of YAP 5SA TEAD-binding defective mutant (S94A or delta-C-terminal) with YAP 5SA. Although the authors identify TEAD binding sites within Odc1 distal elements, the authors never directly tested the enrichment of TEADs, or really assessed the importance of TEAD in YAP-mediated induction of Odc1.
b. Using CRISPR, generate a cell line with mutations in the endogenous YAP-binding site of Odc1 enhancer (#3, #4, or both) and assess whether this cell line is refractory to polyamine biosynthesis upon YAP overexpression.
c. MYC is also responsible for Odc1 transcription (Bello-Fernandez et al. PNAS, 1993), and co-regulation of YAP/MYC target genes have been reported (Croci et al. Genes Dev. 2017). It was also demonstratedathat YAP regulates MYC transcription/activation (Mori et al. Cell 2014). Based on these reports, it could be possible that YAP does not directly promote Odc1 transcription per se, but rather depends upon genetic interaction with MYC.

4. Phenotype-wise, the authors only depicted hepatomegaly and changes in cellular proliferation. The physiological implications from this story would be improved if they also looked at the hepatocyte properties (e.g. loss of hepatocyte properties or conversion into biliary-like cells) and whether Odc1 knockdown or inhibition of eIF5A hypusination reverses this YAP-driven process.
5. The induction of Odc1 and polyamines appears much greater in actual tissues although only wild-type YAP was expressed, as opposed to YAP 5SA/TAZ 4SA (hyperactive mutants) in earlier cell line-based experiments wherein this induction was not very striking. Perhaps the authors can observe a much greater liver phenotype/polyamine production if AAV-YAP 5SA is used. Conversely, why does OE or wild-type YAP in cell lines do not have much effect on Odc1 expression (Extended Fig. 3A)? The authors should validate whether Odc1 expression is directly proportional to YAP activity, for example by expressing increasing amounts of YAP (e.g. using Tet-On system). Also, the authors should show the levels of Odc protein from liver tissues (Fig 3d)
6. The induction of eIF5AHyp appears to be very marginal, compared to induction of Odc1 or polyamine production observed previously in the liver. Perhaps the authors would observe greater induction using AAV-YAP 5SA in the liver or YAP 5SA overexpression in cell lines. Also, it would facilitate visualization to add a third set of YAP overexpression + Odc1 knockdown or YAP overexpression + polyamine inhibitor to show that the effect promoted by YAP is indeed reversed by inhibition of this downstream polyamine pathway.
7. The overall molecular mechanism portrayed here, although novel, appears too stretched out since it involves so many different cellular processes (e.g. transcription, metabolomic change, hypusination, translation) which sheds doubts to whether this ‘axis’ is truly linear and intact. Importantly, the authors do not provide sufficient explanation as to how candidate targets were chosen for further analysis, leaving doubts to whether the authors truly took an unbiased approach. For example, in the text pertaining to Fig.4 the authors simply ‘choose’ LSD1 as one of the enzymes regulating histone methylation or acetylation, since chromatin remodeling is important in YAP/TAZ-induced transcriptional

programs. What was the rationale for this decision, and why were other candidates (e.g. KDM6B, SETD2) excluded?
8. Extended Fig. 4 shows data in which overexpression of OAZ1, which binds to ODC1 and promotes its proteasomal degradation, rescues YAP overexpression-induced increase in liver size/polyamine production/cell proliferation. However, it is evident from Extended Fig. 4E that OAZ1 expression did not lead to even a modest decrease in ODC1 protein levels, leaving doubts to whether the reversal of phenotypes upon OAZ1 overexpression is indeed due to the suppression of ODC1.
9. The authors claimed that ~50% of all YAP-downregulated genes depended on LSD1, while ~30% of YAP-upregulated genes depended on LSD1. Also, they showed that H3K4me1/2 peaks were decreased signal in YAP OE as compared to wildtype control livers and identified 727 putative LSD1 target genes. What are the 30% of YAP-upregulated genes depended on LSD1 ? Are they involved in proliferation or anti-cell death ? Without experimental evidences, I am not sure whether these too many LSD1 target genes are truly downregulated in YAP-OE and are associated with unfavorable liver cancer.
10. The authors stated, “How YAP/TAZ confers gene downregulation is currently unknown”, but this is not the case. In fact, previous reports showed that YAP/TAZ, as transcriptional co-repressors, repressed expression of antiproliferative, cell-death-inducing genes, and lineage-specific genes (Cell reports 11.2 (2015): 270-282. Nature communications 7.1 (2016): 1-14, Cell Reports 36.2 (2021): 109347). The NuRD complex mediates the repressor function of YAP/TAZ. Thus, it would be better for the authors to explain or discuss the difference or the relationship between the NuRD complex-mediated and LS1-mediated repressive role of YAP/TAZ.

Minor points:

1. The authors utilize primarily AML12 (normal hepatocyte cell line) for representative eIF5A hypusination experiments (Fig.4), whereas in other Figures mostly HCC cell lines are used. The reason for this sudden change in cell line usage is not explained.
2. The reviewer personally feels that Fig.6 should not be considered as a main figure, but rather a supplementary figure for Fig.5.
3. We noticed some typos (underlined):
a. Line 96: by OAZ1 binding, which indues ODC1 degradation by proteasome.
b. Line 128: YAP/TAZA
c. Line 133: short hairpin RAN (shRNA)
d. Extended Fig. 6J: MDA-MB-31 cell

Reviewer #3:
Remarks to the Author:

The current manuscript by Li et al. presents interesting data to tie together the transcriptional co-activators YAP/TAZ, polyamine metabolism, eIF5A hypusination, and LSD1 activity in human malignancies. The data presented indicating that YAP/TAZ maybe upstream regulators of polyamine biosynthesis that act as enhancers for ODC1 transcription is strong and well justified. Ruther, the indication that in some cells this increased expression of ODC1 leads to an increase in total polyamine content of the cells is clearly demonstrated. The authors demonstrate that inhibition of polyamine biosynthesis with DFMO or ODC1 knockdown unsurprisingly reduces tumor cell growth that can be prevented by co-addition of putrescine.

However, there are several issues that must be addressed to validate the hypothesis of a direct role of the YAP/TAZ-polyamine-eIF5A-LSD1 axis in malignancies.

1) Most importantly, the measurement of total polyamines is insufficient to make any specific claims about the role of the individual polyamines. The increase in total polyamines measured in response to overexpression of YAP in mouse livers is predominantly represented by acetylputrescine, a catabolic product of excess putrescine which, likely a response to increased ODC1 activity beyond that necessary to maintain functional levels of the higher polyamines, spermidine and spermine. Also, effects in mouse liver are not necessarily mimicked in vitro cell culture. Consequently, measurement of the individual polyamines is necessary in all experiments to determine any specific changes in polyamine pools in response to the various manipulations used here. This is particularly important in the experiments that use DFMO or siRNA knockdown of ODC1.

The reason that the measurement of the individual polyamines is so critical is that spermidine is the only polyamine that can be used in the synthesis of hypusinated eIF-5A. Since the authors claim that hypusinated eIF-5A is a critical factor in the synthesis of LSD1 and thus is a through line in the YAP/TAZ-polyamine-eIF5A-LSD1 axis, it is important that actual changes in spermidine occur as a result of their manipulations rather than off-target effects.

Additionally, although the authors indicate the method they used for the determination of total polyamine pools, no information was provided as to how individual polyamines were measured.

2) The authors provide H3K4me1/2 ChIP-seq data from their YAP overexpressing livers. These data are consistent with YAP modulating LSD1 expression/activity; however, it provides no insight as to whether polyamines are involved in the histone methylation changes. It is not clear why equivalent data were not provided for the ODC1 knockdown, YAP overexpression livers or better yet, DFMO treated, YAP overexpressing animals.

3) Authors do not indicate how the Western blots were quantified. It is stated ECL was used to visualize the blots, but ECL can only be used to quantify differences with in a very narrow linear range and it is not clear from the presentation that the measured values are within that narrow range.

4) It is not clear that the OAZ1 expression vector used contained the necessary frame shift mutation that would be necessary for the functional OAZ1 protein to be expressed.

5) It should be noted that increased polyamine biosynthesis is an absolutely required event for induced cell proliferation. Consequently, although YAP/TAZ have binding sites in the enhancer region of ODC1 they also regulate a multitude of genes that can ultimately result in increased growth rate, thus the increase in polyamine biosynthesis may be an indirect result of increased YAP/TAZ. This possibility should be discussed. See point 6 below.

6) YAP/TAZ activation upregulates the expression of MYC, a known transcriptional activator of ODC1. What is the evidence that YAP/TAZ enhancer activity and not MYC activation is responsible for the increased ODC1 expression in the authors' systems?

GUIDELINES FOR MANUSCRIPT SUBMISSION TO NATURE CELL BIOLOGY

READABILITY OF MANUSCRIPTS – Nature Cell Biology is read by cell biologists from diverse backgrounds, many of whom are not native English speakers. Authors should aim to communicate their findings clearly, explaining technical jargon that might be unfamiliar to non-specialists, and avoiding non-standard abbreviations. Titles and abstracts should concisely communicate the main findings of the study, and the background, rationale, results and conclusions should be clearly explained in the manuscript in a manner accessible to a broad cell biology audience. Nature Cell Biology uses British spelling.

MANUSCRIPT FORMAT – please follow the guidelines listed in our Guide to Authors regarding manuscript formats at Nature Cell Biology.

TITLE – should be no more than 100 characters including spaces, without punctuation and avoiding technical terms, abbreviations, and active verbs..

AUTHOR NAMES – should be given in full.

AUTHOR AFFILIATIONS – should be denoted with numerical superscripts (not symbols) preceding the names. Full addresses should be included, with US states in full and providing zip/post codes. The corresponding author is denoted by: "Correspondence should be addressed to [initials]."

ABSTRACT AND MAIN TEXT – please follow the guidelines that are specific to the format of your manuscript, as listed in our Guide to Authors (http://www.nature.com/ncb/pdf/ncb_gta.pdf) Briefly, Nature Cell Biology Articles, Resources and Technical Reports have 3500 words, including a 150 word abstract, and the main text is subdivided in Introduction, Results, and Discussion sections. Nature Cell Biology Letters have up to 2500 words, including a 180 word introductory paragraph (abstract), and the text is not subdivided in sections.

ACKNOWLEDGEMENTS – should be kept brief. Professional titles and affiliations are unnecessary. Grant numbers can be listed.

AUTHOR CONTRIBUTIONS – must be included after the Acknowledgements, detailing the contributions of each author to the paper (e.g. experimental work, project planning, data analysis etc.). Each author should be listed by his/her initials.

FINANCIAL AND NON-FINANCIAL COMPETING INTERESTS – the authors must include one of three declarations: (1) that they have no financial and non-financial competing interests; (2) that they have financial and non-financial competing interests; or (3) that they decline to respond, after the Author Contributions section. This statement will be published with the article, and in cases where financial and non-financial competing interests are declared, these will be itemized in a web supplement to the article. For further details please see https://www.nature.com/licenceforms/nrg/competing-interests.pdf.

REFERENCES – are limited to a total of 70 for Articles, Resources, Technical Reports; and 40 for Letters. This includes references in the main text and Methods combined. References must be numbered sequentially as they appear in the main text, tables and figure legends and Methods and must follow the precise style of Nature Cell Biology references. References only cited in the Methods should be numbered consecutively following the last reference cited in the main text. References only associated with Supplementary Information (e.g. in supplementary legends) do not count toward the total reference limit and do not need to be cited in numerical continuity with references in the main text. Only published papers can be cited, and each publication cited should be included in the numbered reference list, which should include the manuscript titles. Footnotes are not permitted.

METHODS – Nature Cell Biology publishes methods online. The methods section should be provided as a separate Word document, which will be copyedited and appended to the manuscript PDF, and incorporated within the HTML format of the paper.

Methods should be written concisely, but should contain all elements necessary to allow interpretation and replication of the results. As a guideline, Methods sections typically do not exceed 3,000 words. The Methods should be divided into subsections listing reagents and techniques. When citing previous methods, accurate references should be provided and any alterations should be noted. Information must be provided about: antibody dilutions, company names, catalogue numbers and clone numbers for monoclonal antibodies; sequences of RNAi and cDNA probes/primers or company names and catalogue numbers if reagents are commercial; cell line names, sources and information on cell line identity and authentication. Animal studies and experiments involving human subjects must be reported in detail, identifying the committees approving the protocols. For studies involving human subjects/samples, a statement must be included confirming that informed consent was obtained. Statistical analyses and information on the reproducibility of experimental results should be provided in a section titled "Statistics and Reproducibility".

All Nature Cell Biology manuscripts submitted on or after March 21 2016 must include a Data availability statement at the end of the Methods section. For Springer Nature policies on data availability see http://www.nature.com/authors/policies/availability.html; for more information on this particular policy see http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf. The Data availability statement should include:

• Accession codes for primary datasets (generated during the study under consideration and designated as "primary accessions") and secondary datasets (published datasets reanalysed during the study under consideration, designated as "referenced accessions"). For primary accessions data should be made public to coincide with publication of the manuscript. A list of data types for which submission to community-endorsed public repositories is mandated (including sequence, structure, microarray, deep sequencing data) can be found here http://www.nature.com/authors/policies/availability.html#data.

• Unique identifiers (accession codes, DOIs or other unique persistent identifier) and hyperlinks for datasets deposited in an approved repository, but for which data deposition is not mandated (see here for details http://www.nature.com/sdata/data-policies/repositories).

• At a minimum, please include a statement confirming that all relevant data are available from the authors, and/or are included with the manuscript (e.g. as source data or supplementary information), listing which data are included (e.g. by figure panels and data types) and mentioning any restrictions on availability.

• If a dataset has a Digital Object Identifier (DOI) as its unique identifier, we strongly encourage including this in the Reference list and citing the dataset in the Methods.

We recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange. More details can found at www.nature.com/protocolexchange/about.

DISPLAY ITEMS – main display items are limited to 6-8 main figures and/or main tables for Articles, Resources, Technical Reports; and 5 main figures and/or main tables for Letters. For Supplementary Information see below.

FIGURES – Colour figure publication costs $600 for the first, and $300 for each subsequent colour figure. All panels of a multi-panel figure must be logically connected and arranged as they would appear in the final version. Unnecessary figures and figure panels should be avoided (e.g. data presented in small tables could be stated briefly in the text instead).

All imaging data should be accompanied by scale bars, which should be defined in the legend. Cropped images of gels/blots are acceptable, but need to be accompanied by size markers, and to retain visible background signal within the linear range (i.e. should not be saturated). The boundaries of panels with low background have to be demarked with black lines. Splicing of panels should only be considered if unavoidable, and must be clearly marked on the figure, and noted in the legend with a statement on whether the samples were obtained and processed simultaneously. Quantitative comparisons between samples on different gels/blots are discouraged; if this is unavoidable, it should only be performed for samples derived from the same experiment with gels/blots were processed in parallel, which needs to be stated in the legend.

Figures should be provided at approximately the size that they are to be printed at (single column is 86 mm, double column is 170 mm) and should not exceed an A4 page (8.5 x 11"). Reduction to the scale that will be used on the page is not necessary, but multi-panel figures should be sized so that the whole figure can be reduced by the same amount at the smallest size at which essential details in each panel are visible. In the interest of our colour-blind readers we ask that you avoid using red and green for contrast in figures. Replacing red with magenta and green with turquoise are two possible colour-safe alternatives. Lines with widths of less than 1 point should be avoided. Sans serif typefaces, such as Helvetica (preferred) or Arial should be used. All text that forms part of a figure should be rewritable and removable.

We accept files from the following graphics packages in either PC or Macintosh format:

- For line art, graphs, charts and schematics we prefer Adobe Illustrator (.AI), Encapsulated PostScript (.EPS) or Portable Document Format (.PDF). Files should be saved or exported as such directly from the application in which they were made, to allow us to restyle them according to our journal house style.

- We accept PowerPoint (.PPT) files if they are fully editable. However, please refrain from adding PowerPoint graphical effects to objects, as this results in them outputting poor quality raster art. Text used for PowerPoint figures should be Helvetica (preferred) or Arial.

- We do not recommend using Adobe Photoshop for designing figures, but we can accept Photoshop generated (.PSD or .TIFF) files only if each element included in the figure (text, labels, pictures, graphs, arrows and scale bars) are on separate layers. All text should be editable in 'type layers' and line-art such as graphs and other simple schematics should be preserved and embedded within 'vector smart objects' - not flattened raster/bitmap graphics.

- Some programs can generate Postscript by 'printing to file' (found in the Print dialogue). If using an application not listed above, save the file in PostScript format or email our Art Editor, Allen Beattie for advice (a.beattie@nature.com).

Regardless of format, all figures must be vector graphic compatible files, not supplied in a flattened raster/bitmap graphics format, but should be fully editable, allowing us to highlight/copy/paste all text and move individual parts of the figures (i.e. arrows, lines, x and y axes, graphs, tick marks, scale bars etc.). The only parts of the figure that should be in pixel raster/bitmap format are photographic images or 3D rendered graphics/complex technical illustrations.

All placed images (i.e. a photo incorporated into a figure) should be on a separate layer and independent from any superimposed scale bars or text. Individual photographic images must be a minimum of 300+ DPI (at actual size) or kept constant from the original picture acquisition and not decreased in resolution post image acquisition. All colour artwork should be RGB format.

FIGURE LEGENDS – must not exceed 350 words for each figure to allow fit on a single printed NCB page together with the figure. They must include a brief title for the whole figure, and short descriptions of each panel with definitions of the symbols used, but without detailing methodology.

TABLES – main tables should be provided as individual Word files, together with a brief title and legend. For supplementary tables see below.

SUPPLEMENTARY INFORMATION – Supplementary information is material directly relevant to the conclusion of a paper, but which cannot be included in the printed version in order to keep the manuscript concise and accessible to the general reader. Supplementary information is an integral part of a Nature Cell Biology publication and should be prepared and presented with as much care as the main display item, but it must not include non-essential data or text, which may be removed at the

editor's discretion. All supplementary material is fully peer-reviewed and published online as part of the HTML version of the manuscript. Supplementary Figures and Supplementary Notes are appended at the end of the main PDF of the published manuscript.

Supplementary items should relate to a main text figure, wherever possible, and should be mentioned sequentially in the main manuscript, designated as Supplementary Figure, Table, Video, or Note, and numbered continuously (e.g. Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2 etc.).

Unprocessed scans of all key data generated through electrophoretic separation techniques need to be presented in a supplementary figure that should be labelled and numbered as the final supplementary figure, and should be mentioned in every relevant figure legend. This figure does not count towards the total number of figures and is the only figure that can be displayed over multiple pages, but should be provided as a single file, in PDF or TIFF format. Data in this figure can be displayed in a relatively informal style, but size markers and the figures panels corresponding to the presented data must be indicated.

The total number of Supplementary Figures (not including the “unprocessed scans” Supplementary Figure) should not exceed the number of main display items (figures and/or tables (see our Guide to Authors and March 2012 editorial http://www.nature.com/ncb/authors/submit/index.html#suppinfo; http://www.nature.com/ncb/journal/v14/n3/index.html#ed). No restrictions apply to Supplementary Tables or Videos, but we advise authors to be selective in including supplemental data.

Each Supplementary Figure should be provided as a single page and as an individual file in one of our accepted figure formats and should be presented according to our figure guidelines (see above). Supplementary Tables should be provided as individual Excel files. Supplementary Videos should be provided as .avi or .mov files up to 50 MB in size. Supplementary Figures, Tables and Videos much be accompanied by a separate Word document including titles and legends.

GUIDELINES FOR EXPERIMENTAL AND STATISTICAL REPORTING

REPORTING REQUIREMENTS – To improve the quality of methods and statistics reporting in our papers we have recently revised the reporting checklist we introduced in 2013. We are now asking all life sciences authors to complete two items: an Editorial Policy Checklist (found here https://www.nature.com/authors/policies/Policy.pdf) that verifies compliance with all required editorial policies and a reporting summary (found here https://www.nature.com/authors/policies/ReportingSummary.pdf) that collects information on experimental design and reagents. These documents are available to referees to aid the evaluation of the manuscript. Please note that these forms are dynamic ‘smart pdfs’ and must therefore be

downloaded and completed in Adobe Reader. We will then flatten them for ease of use by the reviewers. If you would like to reference the guidance text as you complete the template, please access these flattened versions at http://www.nature.com/authors/policies/availability.html.

STATISTICS – Wherever statistics have been derived the legend needs to provide the n number (i.e. the sample size used to derive statistics) as a precise value (not a range), and define what this value represents. Error bars need to be defined in the legends (e.g. SD, SEM) together with a measure of centre (e.g. mean, median). Box plots need to be defined in terms of minima, maxima, centre, and percentiles. Ranges are more appropriate than standard errors for small data sets. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test used needs to be stated in the legend. Statistics such as error bars must not be derived from n<3. For sample sizes of n<5 please plot the individual data points rather than providing bar graphs. Deriving statistics from technical replicate samples, rather than biological replicates is strongly discouraged. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test stated in the legend.

Information on how many times each experiment was repeated independently with similar results needs to be provided in the legends and/or Methods for all experiments, and in particular wherever representative experiments are shown.

We strongly recommend the presentation of source data for graphical and statistical analyses as a separate Supplementary Table, and request that source data for all independent repeats are provided when representative experiments of multiple independent repeats, or averages of two independent experiments are presented. This supplementary table should be in Excel format, with data for different figures provided as different sheets within a single Excel file. It should be labelled and numbered as one of the supplementary tables, titled “Statistics Source Data”, and mentioned in all relevant figure legends.

--------- Please don't hesitate to contact NCB@nature.com should you have queries about any of the above requirements ---------

Author Rebuttal to Initial comments

Response to reviewers' comments (NCB-P46013):

Dear reviewers and editor,

Thank you very much for your favorable review and your insightful comments to improve our manuscript. We have conducted additional experiments and extensively revised the manuscript accordingly. The following is a point-by-point response to reviewers' comments. For your convenience, we highlight NCB editor's "key referee points that should be addressed with priority" in blue font. We hope you will find the revised manuscript satisfactory.

We include most of the new results in the revised manuscript. However, due to space limit and , some results and explanations are included only in this rebuttal letter, which we wish to publish as **Peer Review Information** accompanying our paper.

Reviewer #1:

Remarks to the Author:
Li et al. uncovered a role for YAP/TAZ in the biosynthesis of the regulatory metabolite polyamine. Mechanistically, they found that YAP/TAZ directly activates the transcription of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis, and that the increased polyamine levels promote the hypusination of eIF5A to support translation of the transcriptional repressor LSD1. Notably, inhibition of Odc1, polyamine biosynthesis or LSD1 suppressed the ectopic proliferation induced by YAP/TAZ in the mouse liver and to some degree in cultured human cells. The manuscript uses a variety of sophisticated and technically sound in vivo, in vitro and biochemical methods. The data are of high quality and well presented and of interest to a general audience. In particular, these findings add a new dimension to the understanding of YAP/TAZ and open new strategies for anti-cancer approaches.

Response:
We thank the reviewer for support of our work. Below we address the reviewer's specific points.

Comments:

1. Fig1: It would be informative to compare the absolute levels of expression for paralogous genes.

Response:
In Fig. 1g, we followed the convention of presenting the fold-changes of various metabolic genes in YAP OE vs. control livers. The absolute expression levels of these genes vary by >10,000 fold, making it impossible to display their absolute levels in the same graph.

For the reviewer's reference, we present in the table below the absolute levels of expression for paralogous genes, normalized to *Gapdh*. Note that *Arg1* was decreased while *Arg2* increased by YAP overexpression. Of note, Arg1 is a cytosolic form that is primarily involved in ureagenesis, and Arg2 is the mitochondrial form involved in ornithine biosynthesis (Cederbaum, Stephen D.,

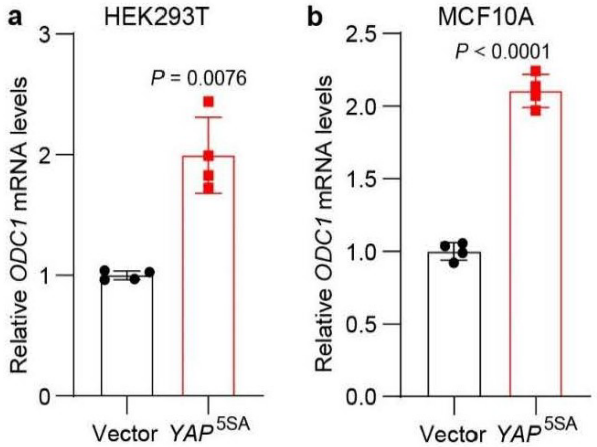
et al. Molecular genetics and metabolism 81 (2004): 38-44). Thus, the upregulation of *Arg2* is consistent with the increased polyamine biosynthesis. Both *Azin1* and *Azin2* expression were increased by *YAP* overexpression. *Oaz1* expression was decreased while *Oaz2* unchanged by *YAP* overexpression. Please note that the expression level of *Oaz1* is much higher than that of *Oaz2*. Thus, the total pool of Oaz is primarily determined by *Oaz1* expression level. Together, these results are consistent with increased polyamine biosynthesis in *YAP* OE livers.

	Control				YAP OE			
Arg1	1.427057	2.202118	2.558386	1.52121	0.446797	0.900095	0.582287	0.685304
Arg2	0.000142	0.000102	0.000223	0.000144	0.003064	0.004579	0.004594	0.004735
Azin1	0.001004	0.001373	0.001253	0.001210	0.003051	0.003358	0.002957	0.003138
Azin2	0.00014	0.000131	0.000153	0.000057	0.000941	0.001017	0.001041	0.001151
Oaz1	0.246964	0.252571	0.250279	0.250185	0.179782	0.180023	0.162667	0.175719
Oaz2	0.00756	0.006719	0.008115	0.007457	0.007652	0.008197	0.008328	0.008059

2. The authors mention that YAP/TAZ can induce Odc1 in various cell lines. Did they find cell lines where this was not the case even though YAP/TAZ could induce their canonical target genes? What about HEK293, MCF10A and other cell lines that are often used to look at YAP/TAZ gain of function phenotypes?

Response:

ODC1 was induced by YAP/TAZ in all cell lines we have examined, including HLE, SNU-886, HPNE, MDA-MB-231 and ES-2. To further address the reviewer's question, we tested HEK293T and MCF10A cells. As in the other cell lines we have examined, *ODC1* was induced by YAP^{5SA} in both HEK293T and MCF10A cells. These data are added as **Extended Data Fig. 3b, c** in the revision (also shown below as **Response Fig. 1** for the reviewer's convenience). In addition, *ODC1* was also induced by YAP^{5SA} in the human haploid cell line HAP1 (Please see our answer to question 3b of Reviewer #2 and **Extended Data Fig. 3j**). Together, these results implicate YAP/TAZ as a general regulator of *ODC1*.



Response Fig. 1| (**a**) HEK293T cells were transiently transfected with empty vector or YAP^{5SA} construct, and relative mRNA levels of *ODC1* were measured by RT-qPCR. Data are represented as mean ± SD, $n = 4$, unpaired two-tailed Student's *t*-test. (**b**) Relative mRNA levels of *ODC1* in MCF10A cells transfected with lentivirus expressing an empty vector or YAP^{5SA}

9

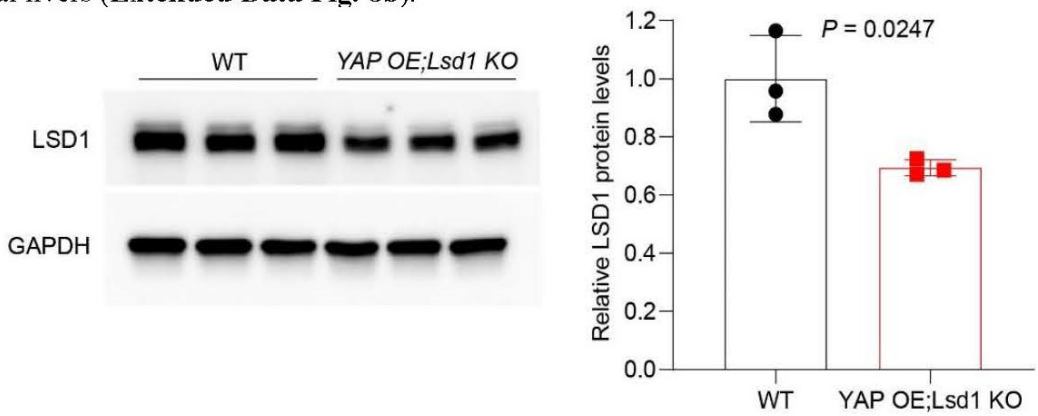
were examined by RT-qPCR. Data are represented as mean ± SD, n = 4, unpaired two-tailed Student's t-test.

3. line 164: should read "ORFs encoding short polyproline tracks"

Response:
Corrected.

4. What are the levels of LSD1 in the LSD1 ko livers compared to normal livers?

Response:
The levels of LSD1 in the *YAP OE;Lsd1 KO* livers are ~70% of that in wild type livers (**Response Fig. 2**). The levels of LSD1 in the *Lsd1 KO* liver are ~15% of that in wild type normal livers (**Extended Data Fig. 8b**).



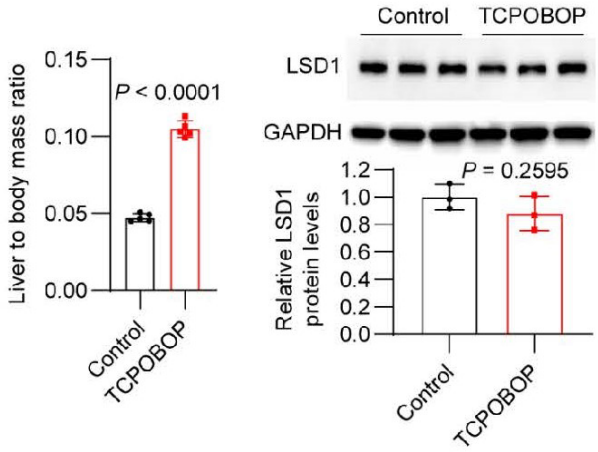
Response Fig. 2| Relative LSD1 protein levels in normal livers (WT) and *YAP OE;Lsd1* KO livers. Data are represented as mean ± SD, n = 3, unpaired two-tailed Student's t-test.

5. What is the effect of Odc1 KD or LSD1 KO on normal (embryonic) liver growth? For example, the authors could knockout LSD1 using alb-Cre.

Response:
Since the YAP OE liver model used in our study is induced specifically in adult mice, it is important to also apply *Odc1* KD or *Lsd1* KO in adult livers in order to assess the direct genetic requirement of *Odc1* or *Lsd1* in YAP OE livers. This should minimize complications caused by indirect, developmental defects resulting from KD or KO these genes from embryonic stage.

6. How specific is LSD1 activation for the YAP induced program? Is LSD1 activated in other, YAP independent liver overgrowth situations, e.g. by TCPOBOP administration?

Response:
Thank you for this good suggestion. As suggested, we measured LSD1 levels in livers of control and TCPOBOP treated animals. The results show that TCPOBOP did not increase LSD1 expression, despite that it induced hepatomegaly. This data is included as **Extended Data Fig. 6p** and **r** in the revision (shown below as **Response Fig. 3** for the reviewer's convenience).

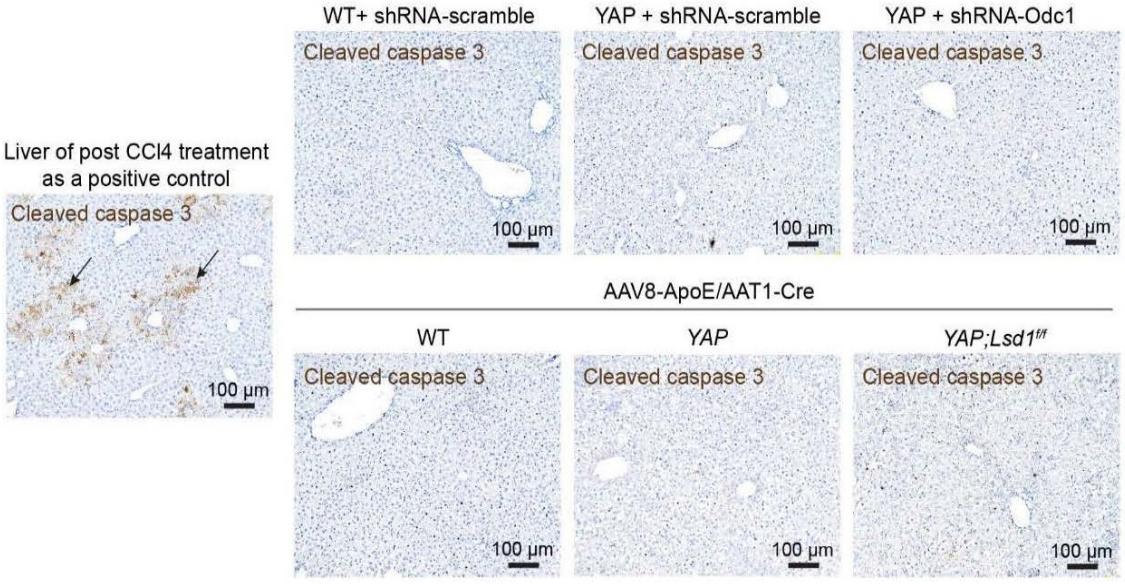


Response Fig. 3| Wild type mice were treated with vehicle (Control) and a single dose of 3 mg/kg TCPOBOP prepared in 10% DMSO/ 90% corn oil (TCPOBOP). Mice were sacrificed 7 days post TCPOBOP treatment and LSD1 protein levels in the liver were measured by western blot. Data are represented as mean ± SD, $n = 3$, unpaired two-tailed Student's *t*-test.

7. How does Odc1 KD or LSD1 KO inhibit YAP induced overgrowth? Does it cause apoptosis or simply restrain cell proliferation?

Response:

We did liver IHC staining to examine apoptosis marker cleaved caspase 3. We did not see significant apoptosis caused by *Odc1* KD or *Lsd1* KO when compared to the two corresponding controls. Therefore, *Odc1* KD or *Lsd1* KO most likely simply restrain cell proliferation. The results are shown in **Response Fig. 4**. The left panel is a positive control.



Response Fig. 4| Representative images of cleaved caspase 3 IHC staining of liver samples from the indicated mice.

8. The effects of SP-2577 are quite impressive in the in vitro assays indicating not only inhibition of proliferation but cell death. However, the effects are much less impressive in vivo. Do the authors have an explanation for this? Is the dose very different? Is the inhibitor toxic in vivo? Etc?

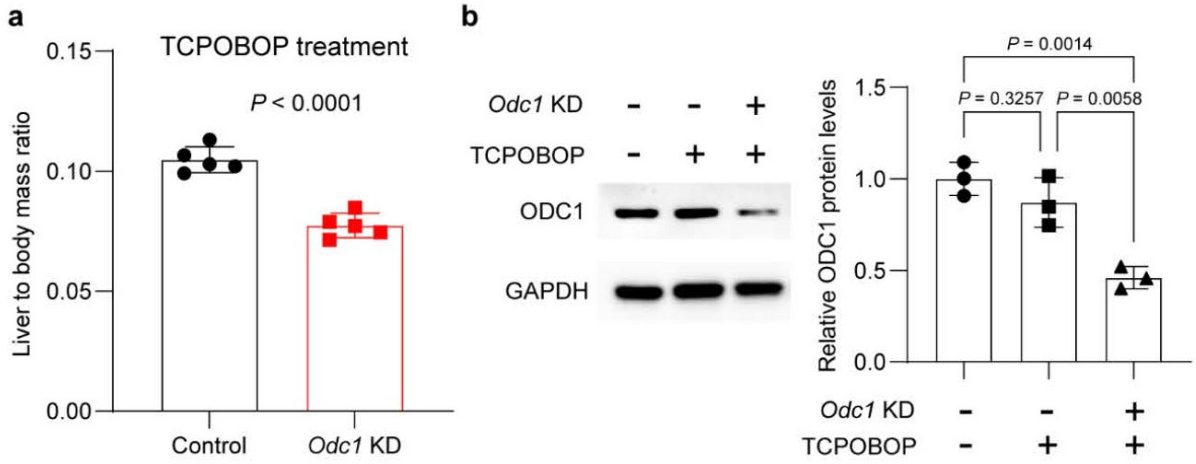
Response:

It is common to observe higher potency of drug treatment *in vitro* than *in vivo*. As described in our paper, we used 1-5 μM for *in vitro* assays and 50 mg/kg daily injection for *in vivo* testing. While the SP-2577 concentration was sufficient to inhibit cell proliferation *in vitro*, due to drug pharmacokinetics, such as *in vivo* half-life time and tissue distribution, the final concentration of SP-2577 *in vivo* may be much lower than *in vitro*. Nevertheless, we did observe benefits for SP-2577 treatment *in vivo*. We believe SP-2577 has minimal toxicity, since animal body weight did not change significantly during treatment (**Fig. 6i**).

9. Does Odc1 inhibition suppress the growth of cells that do not depend on YAP/TAZ?

Response:

As suggested by the reviewer in question #6, we induced liver overgrowth using TCPOBOP (a single dose of 3 mg/kg TCPOBOP prepared in 10% DMSO/ 90% corn oil) in the control and *Odc1* knockdown (KD) mice. The results show that Odc1 inhibition also suppresses the liver overgrowth induced by TCPOBOP administration (**Response Fig. 5a**), consistent with a general role of polyamines in cell proliferation. However, different from YAP overexpression, TCPOBOP treatment did not upregulate ODC1 levels (**Response Fig. 5b**), emphasizing the specificity of ODC1 induction by YAP.



Response Fig. 5| (**a**) Wild type mice were injected first with AAV-shRNA-scramble (Control) or AAV-shRNA-Odc1 (*Odc1* KD), then with TCPOBOP 5 days after AAV injection. Mice were sacrificed 7 days post TCPOBOP treatment, and liver size was quantified as liver to body mass ratio. (**b**) Relative ODC1 protein levels in the indicated mouse livers. (n = 3, One-way ANOVA)

Reviewer #2:

Remarks to the Author:

In this manuscript, Li and colleagues report a mechanism by which YAP/TAZ promotes the transcription of ornithine decarboxylase 1, the rate-limiting enzyme in polyamine biosynthesis. This was found by surveying the metabolic profile in YAP/TAZ-activated transgenic mouse models and human cancer cells. Increased polyamine levels then promote the hypusination of eIF5A, which enhances the efficient translation of histone demethylase LSD1. They further showed that LSD1 downregulates the expression of a significant portion of YAP/TAZ-downregulated genes in YAP/TAZ-activated cells.

The relationship between Hippo/YAP and polyamines is a novel finding, which warrants attention. However, even if the mechanisms of YAP/TAZ-polyamine-eIF5A hypusination-LSD1 is potentially interesting, the reviewer feels that the overall this mechanism is far too overreaching, and as a result, the study lacks depth. Also, some of the results presented are modest or marginal at best and seriously suggest that the authors should downplay their findings.

Response:

We thank the reviewer for recognizing the novelty of our findings and very much appreciate the suggestions for improvement. Below we address the reviewer's specific points.

The followings are comments and suggestions.

Major points:

1. Do authors have an explanation as to why arginine is highly enriched by YAP overexpression (Fig.1E)? This is the most upstream precursor to polyamine production, even before the metabolic regulation by the polyamine-related enzymes, some of whose expression are regulated by YAP.

Response:

Arginine is a semi-essential amino acid, which is especially important for proliferating cells. Arginine is mainly obtained from diet, and a small amount is synthesized in the kidney. Thus, most proliferating tissues *in vivo* rely heavily on arginine uptake from their environment. We speculate that arginine enrichment in *YAP* OE livers could be due to increased arginine uptake.

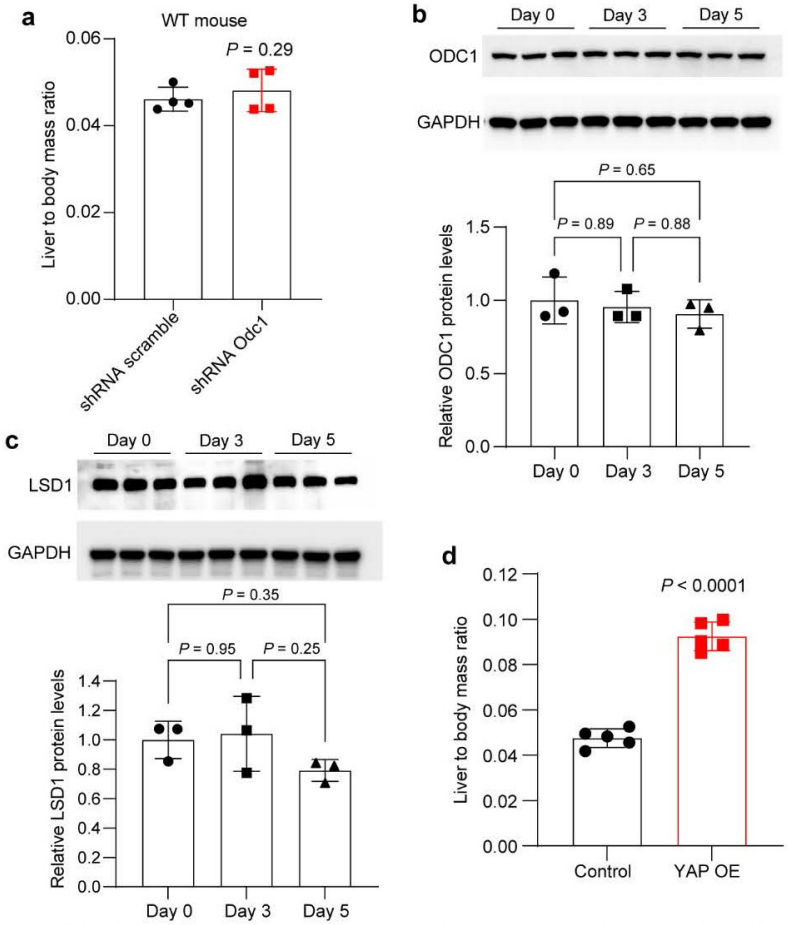
2. The authors deplete Odc1 before YAP overexpression (Fig.3A). Polyamines are claimed to be generally important for tumorigenesis. Thus, the observed decrease in hepatomegaly due to Odc1 knockdown before YAP overexpression seems to be somewhat obvious. To assess the genetic hierarchy of Odc1 as a downstream target of YAP, the authors should overexpress YAP first, then deplete Odc1. The same logic applies to LSD knockout (Fig.5).

Response:

First, AAV-based vectors are often delivered first for *in vivo* experiments because it takes time for AAV to enter and express efficiently in the liver. For example, a similar protocol was used by Yuan *et al* (Nat Commun. 2018 Nov 16;9(1):4834., Figure 2f).

Second, please note that neither *Odc1* knockdown (**Response Fig. 6a**) nor *Lsd1* knockout (**Extended Data Fig. 8**) alone by AAV had discernable effects on liver size in normal mice. However, *Odc1* knockdown or *Lsd1* knockout by AAV markedly suppressed liver overgrowth induced by *YAP* overexpression.

Third, we did examine *Odc1* expression level after knockdown using AAV-shRNA targeting *Odc1* in mouse liver and now the results are shown in **Response Fig. 6b** for reviewer's reference. *Odc1* expression levels only began to show signs of decrease 5 days after AAV injection, and similar results were found for *Lsd1* expression in AAV-Cre treated $Lsd1^{f/f}$ mice (**Response Fig. 6c**). However, *YAP* induction by doxycycline was much earlier since 5-day treatment already induced dramatic liver enlargement (**Response Fig. 6d**). Thus, these results show that *YAP* induction was much earlier than *Odc1* knockdown and *Lsd1* knockout, and *Odc1* and *Lsd1* were not depleted before YAP overexpression in our experiments, supporting the genetic hierarchy of *Odc1* and *Lsd1* as downstream target of YAP.

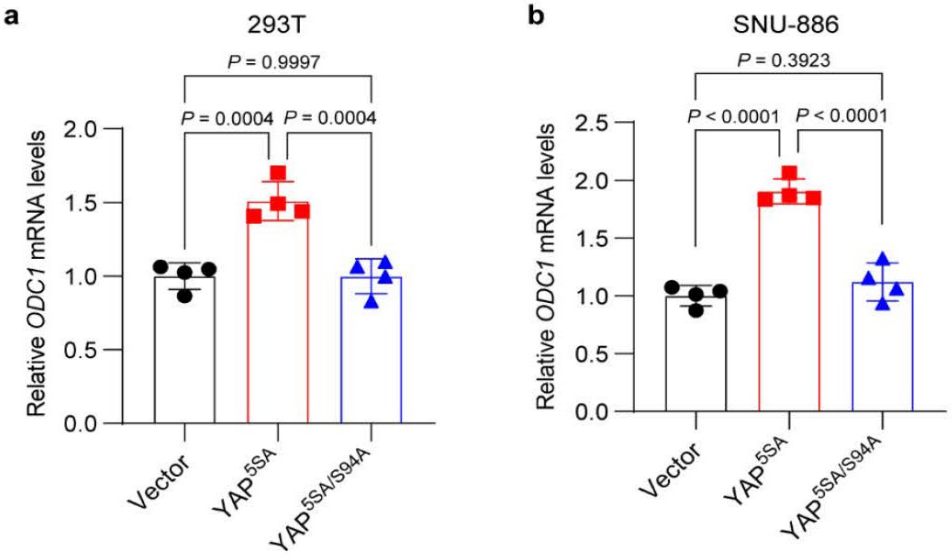


Response Fig. 6| (a) The liver to body mass ratio between control and *Odc1* KD in wild type mice. Data are represented as mean ± SD, $n = 4$, unpaired two-tailed Student's *t*-test. (**b**) Relative ODC1 protein levels at different time points after AAV-shRNA-Odc1 treatment. Data are represented as mean ± SD, $n = 3$, One-way ANOVA. (**c**) Relative LSD1 protein levels at different time points post AAV-Cre treatment. Data are represented as mean ± SD, $n = 3$, One-

way ANOVA. (**d**) Liver to body mass ratio after 5-day doxycycline treatment. Data are represented as mean ± SD, $n = 5$, unpaired two-tailed Student's t-test.

3. The induction of Odc1 by YAP, as shown by the authors, is generally not dramatic, and thus doubts remain as to whether it is a bona fide YAP target gene. The reviewer suggests considering the following to add credibility to this claim:
a. Compare induction of Odc1 from overexpression of YAP 5SA TEAD-binding defective mutant (S94A or delta-C-terminal) with YAP 5SA. Although the authors identify TEAD binding sites within Odc1 distal elements, the authors never directly tested the enrichment of TEADs, or really assessed the importance of TEAD in YAP-mediated induction of Odc1.

Response:
Good suggestion. As suggested, we compared the induction of *ODC1* by $YAP^{5SA/S94A}$ or YAP^{5SA} in 293T and SNU-886 cells. The results show that YAP^{5SA} induced *ODC1* expression significantly in both cell lines, but $YAP^{5SA/S94A}$ did not induce *ODC1* in either 293T or SNU-886 cells, suggesting that YAP binding to TEADs is required for the induction of *ODC1*. We include this data as **Extended Data Fig. 3h** and **i** in the revision (also shown here as **Response Fig. 7a,b** for reviewer's convenience).

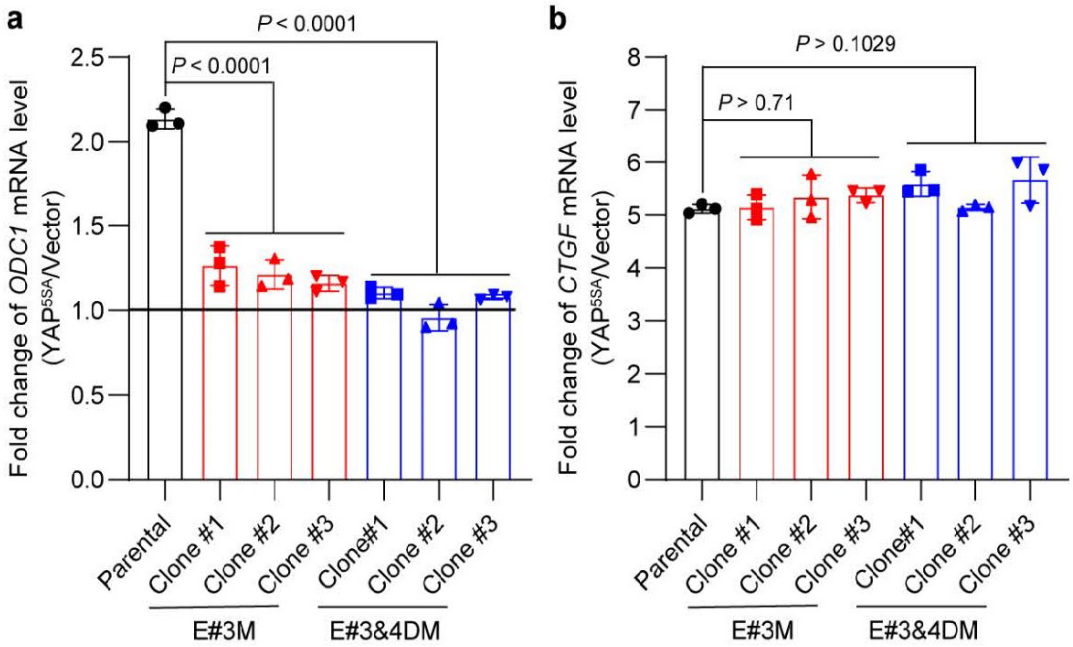


Response Fig. 7| (**a**) Relative mRNA levels of *ODC1* in 293T cells, which were transiently transfected with the indicated plasmids. (**b**) Relative mRNA levels of *ODC1* in SNU-886 cells, which were transiently transfected with the indicated plasmids. (Data are represented as mean ± SD, $n = 4$, One-way ANOVA)

b. Using CRISPR, generate a cell line with mutations in the endogenous YAP-binding site of Odc1 enhancer (#3, #4, or both) and assess whether this cell line is refractory to polyamine biosynthesis upon YAP overexpression.

Response:
We thank the reviewer for this good suggestion. As suggested, we disrupted the endogenous YAP/TEAD-binding sites of *ODC1* enhancer using CRISPR/CAS9 in the haploid cell line

HAP1. The results show that disrupting #3 YAP/TEAD-binding site largely suppressed, and disrupting both #3 and #4 YAP/TEAD-binding sites nearly abolished, YAP-induced *ODC1* transcription, while *CTGF* was not affected further supporting *ODC1* as a direct target of YAP. We include this data as **Extended Data Fig. 3j,k** in the revision (also shown here as **Response Fig. 8a,b** for reviewer's convenience).



Response Fig. 8| Human HAP1 parental cells, with mutations in the endogenous YAP/TEAD-binding site of *ODC1* enhancer #3 (E#3M) or both #3 and #4 (E#3&4DM) were transfected by lentivirus expressing an empty vector or YAP^{5SA}. Relative mRNA levels of *ODC1* and *CTGF* were examined by RT-qPCR. Data are represented as mean ± SD, $n = 3$, one-way ANOVA, Dunnett's multiple comparisons test.

c. MYC is also responsible for Odc1 transcription (Bello-Fernandez et al. PNAS, 1993), and co-regulation of YAP/MYC target genes have been reported (Croci et al. Genes Dev. 2017). It was also demonstrated that YAP regulates MYC transcription/activation (Mori et al. Cell 2014). Based on these reports, it could be possible that YAP does not directly promote Odc1 transcription per se, but rather depends upon genetic interaction with MYC.

Response:

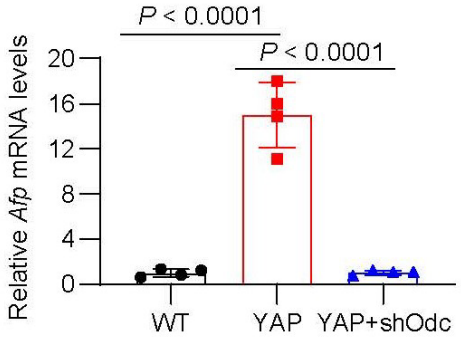
As described above, we demonstrate that YAP/TEAD directly regulates *ODC1* transcription, since mutating two TEAD-binding sites in endogenous *ODC1* locus abolished its induction by YAP. We do not exclude the possibility that besides this direct role, YAP may also cooperate with MYC to induce *ODC1* expression.

4. Phenotype-wise, the authors only depicted hepatomegaly and changes in cellular proliferation. The physiological implications from this story would be improved if they also looked at the hepatocyte properties (e.g. loss of hepatocyte properties or conversion into biliary-

26

like cells) and whether Odc1 knockdown or inhibition of eIF5A hypusination reverses this YAP-driven process.

Response:
Since our study mainly focused on cell proliferation and tumor growth, we did not characterize hepatocyte properties in great details. To address the reviewer's question, we measured the expression of alpha-fetoprotein (Afp), a fetal liver gene that is normally turned off in adult livers but is potently induced by YAP OE in adult livers. The results show that *Odc1* knockdown reversed the YAP OE-driven *Afp* expression, which is consistent with *Odc1* knockdown suppressing YAP-driven de-differentiation of hepatocytes. We include this data as **Fig. 3i** in the revision (also shown here as **Response Fig. 9** for reviewer's convenience).



Response Fig. 9 | Relative *Afp* mRNA levels of livers of WT+shRNA-scramble, YAP OE+shRNA-scramble and YAP OE+shRNA-Odc1 mice. (Data are represented as mean ± SD, *n* = 4, One-way ANOVA)

5. The induction of Odc1 and polyamines appears much greater in actual tissues although only wild-type YAP was expressed, as opposed to YAP 5SA/TAZ 4SA (hyperactive mutants) in earlier cell line-based experiments wherein this induction was not very striking. Perhaps the authors can observe a much greater liver phenotype/polyamine production if AAV-YAP 5SA is used. Conversely, why does OE or wild-type YAP in cell lines do not have much effect on Odc1 expression (Extended Fig. 3A)? The authors should validate whether Odc1 expression is directly proportional to YAP activity, for example by expressing increasing amounts of YAP (e.g. using Tet-On system).

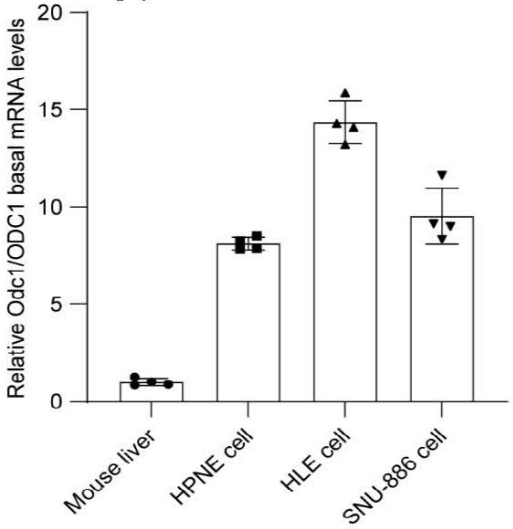
Response:
The YAP transgenic model originally generated by our laboratory (Dong *et al*, *Cell*. 2007 Sep 21;130(6):1120-33.) used an optimized liver-specific expression cassette that produces very high transgene expression levels, which essentially evades any negative regulation by the upstream kinases and results in YAP activation. Thus, even though wildtype YAP was overexpressed, it resulted in massive hepatomegaly in adult mice and liver tumors. This is analogous to the dramatic overgrowth induced by overexpressing wildtype Yki (the *Drosophila* homologue of YAP) in flies. Thus it is not surprising that great induction of *Odc1* and polyamines can be induced in our YAP OE mouse model.

27

The results shown in **Extended Data Fig. 3a** are actually the induced fold change of *ODC1* mRNA level relative to the basal level. It is conceivable that it is much easier to induce more

fold changes when the basal level is low than when the basal level is high. We thus compared the basal *ODC1* mRNA level of cell lines to that of mouse liver and found that these cell lines had much higher basal levels than mouse liver (**Response Fig. 10**).

The results in **Extended Data Fig. 3a** indeed support that *ODC1* expression is positively correlated with YAP activity. For YAP activity: YAP^{S127A} > YAP > empty vector. Similarly, for *ODC1* levels: YAP^{S127A} > YAP > empty vector.

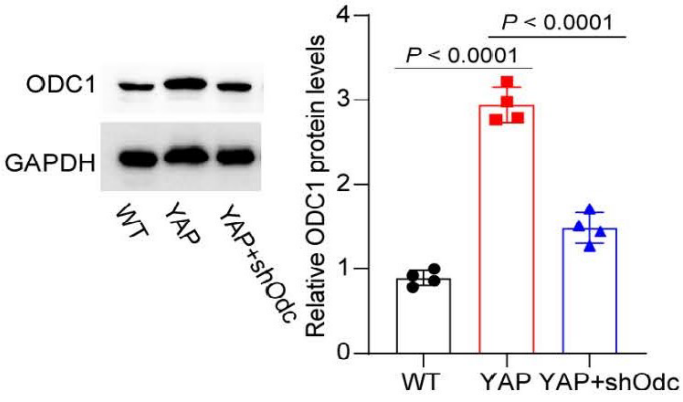


Response Fig. 10| The basal levels of *Odc1/ODC1* mRNA in mouse liver and different proliferating cell lines. All levels of mRNA were normalized to Gapdh/GAPDH.

Also, the authors should show the levels of Odc protein from liver tissues (Fig 3d)

Response:

We have added the data showing the levels of ODC1 protein from liver tissues as **Fig. 3d** in the revision (also shown here as **Response Fig. 11** for reviewer's convenience). Overall, these results are consistent with the mRNA levels.



28

Response Fig. 11| The relative levels of ODC1 protein in mouse livers with the indicated genotype and treatment. Data are represented as mean ± SD, $n = 4$, One-way ANOVA.

6. The induction of eIF5AHyp appears to be very marginal, compared to induction of Odc1 or polyamine production observed previously in the liver. Perhaps the authors would observe greater induction using AAV-YAP 5SA in the liver or YAP 5SA overexpression in cell lines. Also, it would facilitate visualization to add a third set of YAP overexpression + Odc1 knockdown or YAP overexpression + polyamine inhibitor to show that the effect promoted by YAP is indeed reversed by inhibition of this downstream polyamine pathway.

Response:
Please note that the induction of eIF5A^{Hyp} in mouse liver, although mild, is consistent and statistically significant, as evidenced by a small *P* value (**Fig. 4a**).

As mentioned in our answer to question #5 above, our YAP transgenic model results in very high YAP activity as indicated by dramatic liver overgrowth. The extent of liver overgrowth in our model is actually greater than that described for a YAP S127A model generated by another lab (using less optimized expression vector) (*Curr Biol*, 2007 Dec 4;17(23):2054-60). Therefore, the relatively mild induction of eIF5A^{Hyp} is unlikely due to insufficient YAP activity. Meanwhile, we did a literature search and found two studies that also examined the induction of eIF5A^{Hyp} in solid tissues. Consistent with our observations, these two studies also observed mild induction of eIF5A^{Hyp} in solid tissues *in vivo* (Fig. 3b of *Sci Signal. 2019 Dec 3;12(610):eaax0715*; Fig.2 j of *Cell Rep. 2021 Apr 13;35(2):108941.*) Together these results suggest that it is probably difficult to induce dramatic increase in eIF5A^{Hyp} level in solid tissues, especially given the already high basal level of eIF5A^{Hyp}.

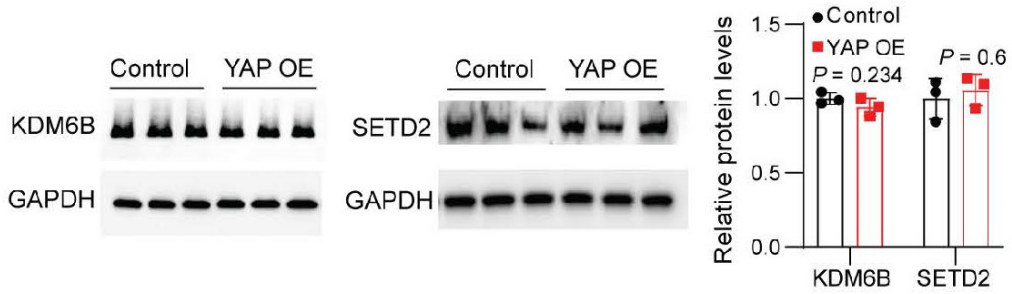
The reviewer might have missed this particular data in our paper, but we did express polyamine inhibitor Oaz1 in YAP transgenic mouse liver and examined the induction of LSD1 and eIF5A^{Hyp} (**Fig. 4h**). The results show that the induction of LSD1 and eIF5A^{Hyp} by YAP overexpression in mouse liver was indeed reversed by inhibition of the downstream polyamine biosynthesis pathway.

7. The overall molecular mechanism portrayed here, although novel, appears too stretched out since it involves so many different cellular processes (e.g. transcription, metabolomic change, hypusination, translation) which sheds doubts to whether this 'axis' is truly linear and intact. Importantly, the authors do not provide sufficient explanation as to how candidate targets were chosen for further analysis, leaving doubts to whether the authors truly took an unbiased approach. For example, in the text pertaining to Fig.4 the authors simply 'choose' LSD1 as one of the enzymes regulating histone methylation or acetylation, since chromatin remodeling is important in YAP/TAZ-induced transcriptional programs. What was the rationale for this decision, and why were other candidates (e.g. KDM6B, SETD2) excluded?

Response:
The mechanism proposed in this study is evidence-based as we show that: 1) YAP induces Odc1 expression and polyamine biosynthesis (Fig. 1-3 and Extended Data Fig. 3); 2) Polyamines increase eIF5A hypusination to increase LSD1 level (Fig. 4 and Extended Data Fig. 6); 3) Lsd1 is required for YAP-induced liver overgrowth and transcription change (Fig. 5 and 7). In the revision, we provide further evidence showing that *ODC1* is a direct target of YAP/TEAD

(Extended Data Fig. 3h-j), and that YAP-induced eIF5A hypusination and LSD1 upregulation are suppressed by inhibition of polyamine biosynthesis (Fig. 4h).

We excluded KDM6B and SETD2 because KDM6B and SETD2 protein levels did not show significant difference between control and YAP overexpression livers. We include this data as **Extended Data Fig. 6b** in the revision (also shown below as **Response Fig. 12** for reviewer's convenience).



Reviewer Fig. 12| The relative KDM6B and SETD2 protein levels in control and YAP OE livers. Data are represented as mean ± SD, $n = 3$, unpaired two-tailed Student's t-test.

8. Extended Fig. 4 shows data in which overexpression of OAZ1, which binds to ODC1 and promotes its proteasomal degradation, rescues YAP overexpression-induced increase in liver size/polyamine production/cell proliferation. However, it is evident from Extended Fig. 4E that OAZ1 expression did not lead to even a modest decrease in ODC1 protein levels, leaving doubts to whether the reversal of phenotypes upon OAZ1 overexpression is indeed due to the suppression of ODC1.

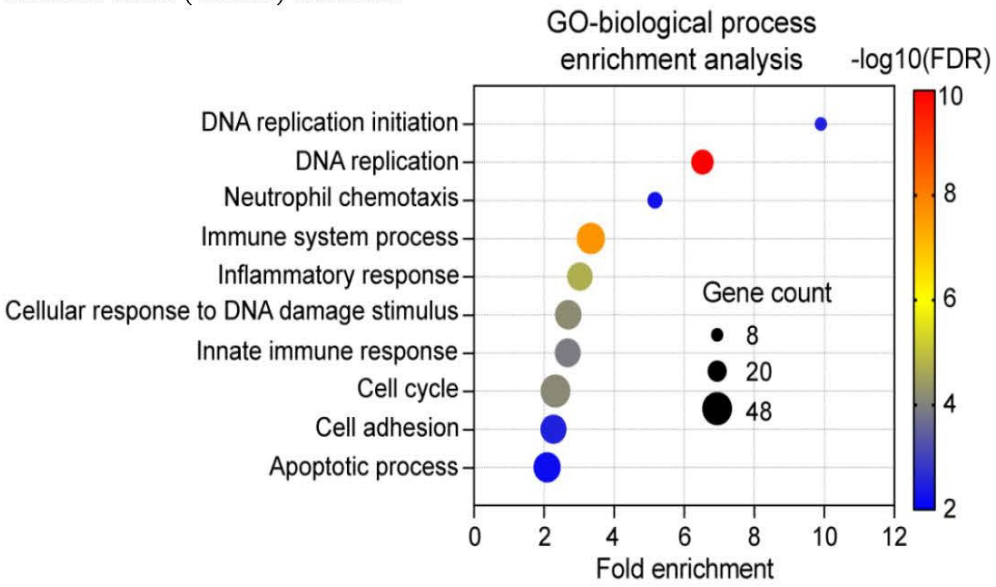
Response:
ODC1 functions as a homodimer. OAZ1 regulates ODC1 activity via two mechanisms. First, OAZ1 and ODC1 form heterodimer to disrupt ODC1 homodimer, thus inactivating the activity of ODC1. Second, OAZ1 binds to ODC1, leading to ODC1 degradation. Our results suggest that the first mechanism may play a major role in our model.

9. The authors claimed that ~50% of all YAP-downregulated genes depended on LSD1, while ~30% of YAP-upregulated genes depended on LSD1. Also, they showed that H3K4me1/2 peaks were decreased signal in YAP OE as compared to wildtype control livers and identified 727 putative LSD1 target genes. What are the 30% of YAP-upregulated genes depended on LSD1? Are they involved in proliferation or anti-cell death? Without experimental evidences, I am not sure whether these too many LSD1 target genes are truly downregulated in YAP-OE and are associated with unfavorable liver cancer.

Response:
The gene list of the 30% of YAP-upregulated genes are included as **Supplementary Table 1** in the revision. GO enrichment analysis shows that these genes are mainly involved in DNA replication, immune response, cell cycle and apoptosis (**Response Fig. 13**). Downregulation of the 727 putative LSD1 target genes in YAP OE was confirmed by the RNA-seq data provided in **Supplementary Table 1** (triplicate per group). Among them, 58 genes are prognostic favorable

30

genes in liver cancer, meaning that downregulation of these genes is associated with unfavorable outcomes. The prognostic favorable gene list in liver cancer was obtained from analysis of The Cancer Genome Atlas (TCGA) database.



Reviewer Fig. 13| GO-biological process enrichment analysis of YAP-upregulated genes depended on LSD1, GO terms with FDR < 0.01 were shown.

10. The authors stated, "How YAP/TAZ confers gene downregulation is currently unknown", but this is not the case. In fact, previous reports showed that YAP/TAZ, as transcriptional co-repressors, repressed expression of antiproliferative, cell-death-inducing genes, and lineage-specific genes (Cell reports 11.2 (2015): 270-282. Nature communications 7.1 (2016): 1-14, Cell Reports 36.2 (2021): 109347). The NuRD complex mediates the repressor function of YAP/TAZ. Thus, it would be better for the authors to explain or discuss the difference or the relationship between the NuRD complex-mediated and LS1-mediated repressive role of YAP/TAZ.

Response:
We thank the reviewer for reminding us these papers, which we cite and discuss in the revision (see line 276-283).

Minor points:

1. The authors utilize primarily AML12 (normal hepatocyte cell line) for representative eIF5A hypusination experiments (Fig.4), whereas in other Figures mostly HCC cell lines are used. The reason for this sudden change in cell line usage is not explained.

Response:
The rationale of our study is that we first characterize eIF5A hypusination in mouse livers and mouse hepatocyte cell line **(Fig. 4)**. We then validate these findings using human cancer cell lines (**Extended Data Fig. 6e-o**). Importantly, the results are consistent across all different models. Together, these data show a conserved mechanism between mouse and human.

31

2. The reviewer personally feels that Fig.6 should not be considered as a main figure, but rather a supplementary figure for Fig.5.

Response:
We'd like to keep the content in Fig. 6 as a main figure, because this figure is important evidence showing that LSD1 is a potential target for human cancers with active YAP/TAZ.

3. We noticed some typos (underlined):
a. Line 96: by OAZ1 binding, which indues ODC1 degradation by proteasome.

Response:
Corrected.

b. Line 128: YAP/TAZA

Response:
Corrected.

c. Line 133: short hairpin RAN (shRNA)

Response:
Corrected.

d. Extended Fig. 6J: MDA-MB-31 cell

Response:
Corrected.

Reviewer #3:

Remarks to the Author:

The current manuscript by Li et al. presents interesting data to tie together the transcriptional co-activators YAP/TAZ, polyamine metabolism, eIF5A hypusination, and LSD1 activity in human malignancies. The data presented indicating that YAP/TAZ maybe upstream regulators of polyamine biosynthesis that act as enhancers for ODC1 transcription is strong and well justified. Ruther, the indication that in some cells this increased expression of ODC1 leads to an increase in total polyamine content of the cells is clearly demonstrated. The authors demonstrate that inhibition of polyamine biosynthesis with DFMO or ODC1 knockdown unsurprisingly reduces tumor cell growth that can be prevented by co-addition of putrescine.

However, there are several issues that must be addressed to validate the hypothesis of a direct role of the YAP/TAZ-polyamine-eIF5A-LSD1 axis in malignancies.

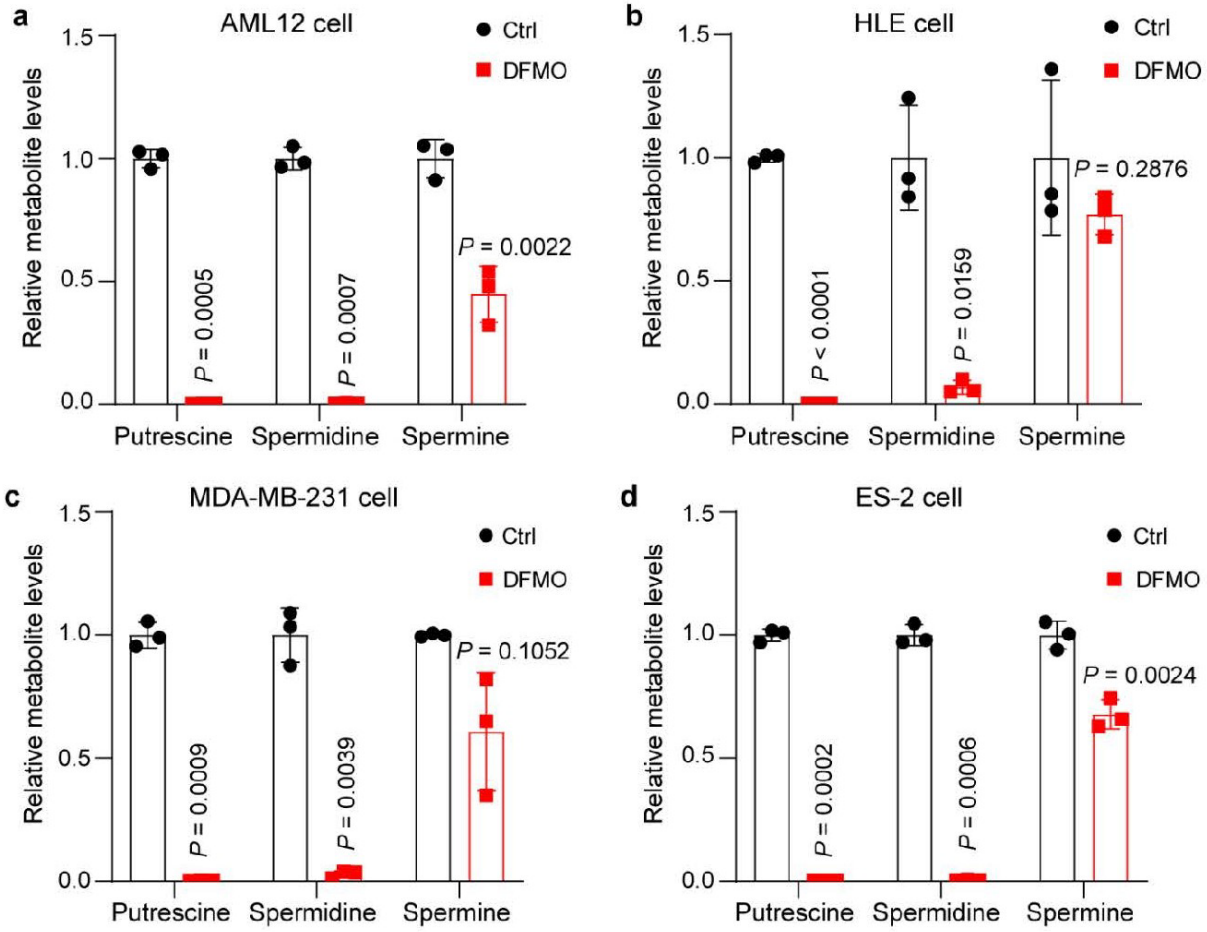
Response:

We thank the reviewer for support of our work and appreciate the insightful comments. Below we address the reviewer's specific points.

1) Most importantly, the measurement of total polyamines is insufficient to make any specific claims about the role of the individual polyamines. The increase in total polyamines measured in response to overexpression of YAP in mouse livers is predominantly represented by acetylputrescine, a catabolic product of excess putrescine which, likely a response to increased ODC1 activity beyond that necessary to maintain functional levels of the higher polyamines, spermidine and spermine. Also, effects in mouse liver are not necessarily mimicked in vitro cell culture. Consequently, measurement of the individual polyamines is necessary in all experiments to determine any specific changes in polyamine pools in response to the various manipulations used here. This is particularly important in the experiments that use DFMO or siRNA knockdown of ODC1. The reason that the measurement of the individual polyamines is so critical is that spermidine is the only polyamine that can be used in the synthesis of hypusinated eIF-5A. Since the authors claim that hypusinated eIF-5A is a critical factor in the synthesis of LSD1 and thus is a through line in the YAP/TAZ-polyamine-eIF5A-LSD1 axis, it is important that actual changes in spermidine occur as a result of their manipulations rather than off-target effects.

Response:

We did show individual polyamines in control and YAP OE mouse livers in our original submission (**Fig. 1e**). The results show that the levels of spermidine and putrescine were increased while spermine level was not changed significantly in YAP OE livers. Following the suggestion by the reviewer, we measured individual polyamine levels in AAV-shOdc treated YAP OE livers. This data is included as **Fig. 3e** in the revision. We also added data showing levels of individual polyamines in DFMO treated cell lines (**Extended Data Fig. 7**) (the same data are also provided here as **Response Fig. 14** for reviewer's convenience). Consistent with our model, both AAV-shOdc and DFMO treatments significantly reduced the level of spermidine.



Response Fig. 14| Relative levels of individual polyamine in DFMO-treated cell lines.

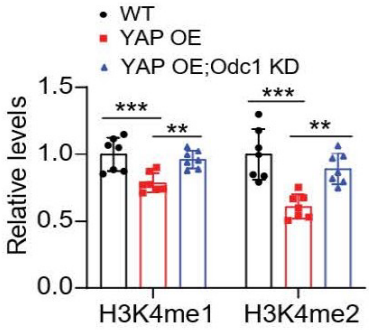
Additionally, although the authors indicate the method they used for the determination of total polyamine pools, no information was provided as to how individual polyamines were measured.

Response:
We include the method in the revision (see line 732-743).

2) The authors provide H3K4me1/2 ChIP-seq data from their YAP overexpressing livers. These data are consistent with YAP modulating LSD1 expression/activity; however, it provides no insight as to whether polyamines are involved in the histone methylation changes. It is not clear why equivalent data were not provided for the ODC1 knockdown, YAP overexpression livers or better yet, DFMO treated, YAP overexpressing animals.

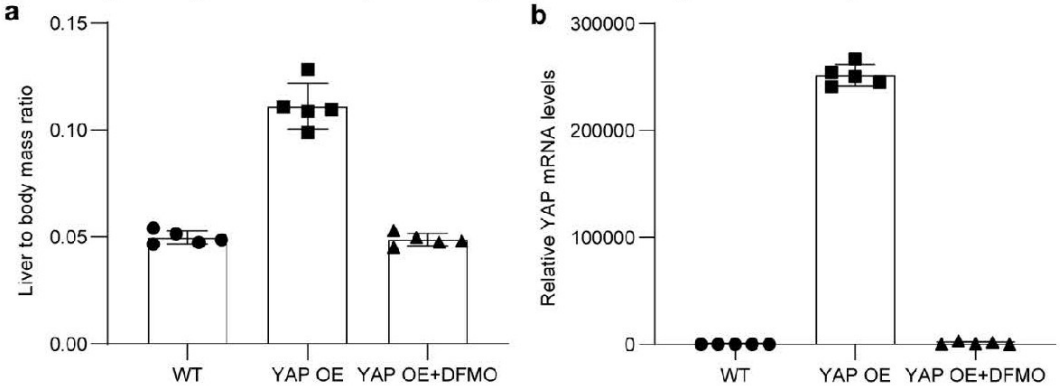
Response:
We have added the data of H3K4me1/2 levels for *Odc1* knockdown, *YAP* OE livers in revised **Extended Data Fig. 6d** (also provided below as **Response Fig. 15** for reviewer's convenience). The results show that *Odc1* knockdown greatly suppressed the reduction of H3K4me1/2 induced

by *YAP* overexpression. Moreover, ectopic expression of the Odc1 endogenous inhibitor Oaz1 rescued the increased levels of H3K4me1/2 demethylase LSD1 in *YAP* overexpression livers (**Fig. 4h**). Taken together, these results support that polyamines are involved in the histone methylation (H3K4me1/2) changes induced by *YAP* overexpression.



Response Fig. 15| Relative levels of H3K4me1/2 in control, YAP OE and YAP OE;Odc1 KD livers. Data are represented as mean ± SD, $n = 7$, $^{**}P < 0.0046$, $^{***}P < 0.0009$, One-way ANOVA.

We also treated YAP OE mice with DFMO and found that DFMO completely suppressed YAP-induced liver overgrowth (**Response Fig. 16a**). However, this experiment is inconclusive since DFMO also inhibited the induction of YAP transgene in our mouse models for unknown reasons (**Response Fig. 16b**). This observation is unique to DFMO, since we did not observe inhibition of YAP transgene expression in any other experiments throughout our study.



Response Fig. 16| (**a**) Liver to body mass ratio of WT, YAP OE and YAP OE mice treated with DFMO (YAP OE+DFMO). (**b**) Relative mRNA levels of YAP in the indicated mouse livers.

3) Authors do not indicate how the Western blots were quantified. It is stated ECL was used to visualize the blots, but ECL can only be used to quantify differences with in a very narrow linear range and it is not clear from the presentation that the measured values are within that narrow range.

5

Response:

We added more information on Western blots (see line 756-759). Specifically, we use SuperSignal West Dura as chemiluminescent substrates, which is recommended for quantitative

western blotting by the manufacturer. Unlike substrates with signals that decline to barely detectable levels in 30–60 minutes, the signal produced with SuperSignal West Dura chemiluminescent substrate is stable for 24 hours. Images were captured and analyzed by a ChemiDoc MP imaging system under non-saturation condition.

4) It is not clear that the OAZ1 expression vector used contained the necessary frame shift mutation that would be necessary for the functional OAZ1 protein to be expressed.

Response:
Thank you for reminding us this key information. The Oaz1 expression vector used contained the necessary frameshift mutation that would be necessary for the functional Oaz1 protein to be expressed. We include this information in the revision (please see line 853-854).

5) It should be noted that increased polyamine biosynthesis is an absolutely required event for induced cell proliferation. Consequently, although YAP/TAZ have binding sites in the enhancer region of ODC1 they also regulate a multitude of genes that can ultimately result in increased growth rate, thus the increase in polyamine biosynthesis may be an indirect result of increased YAP/TAZ. This possibility should be discussed. See point 6 below.

Response:
Thank you for this great question. We will answer this question together with question 6) below.

6) YAP/TAZ activation upregulates the expression of MYC, a known transcriptional activator of ODC1. What is the evidence that YAP/TAZ enhancer activity and not MYC activation is responsible for the increased ODC1 expression in the authors' systems?

Response:
This is a very good point. As shown in our response to Reviewer #2's point 3b-c, we have added data in the revision showing that YAP/TEAD directly regulates *ODC1* transcription, since mutating two TEAD-binding sites in endogenous *ODC1* locus abolished its induction by YAP (**Extended Data Fig. 3j**). We do not exclude the possibility that besides this direct role, YAP may also cooperate with MYC to induce *ODC1* expression.

Decision Letter, first revision:

Subject: NCB: Your manuscript, NCB-P46013A
Message: Our ref: NCB-P46013A

27th December 2021

Dear Dr. Pan,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Cell Biology manuscript, "YAP/TAZ drives cell proliferation and tumor growth via a polyamine-eIF5A hypusination-LSD1 axis" (NCB-P46013A). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within one week). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

In recognition of the time and expertise our reviewers provide to Nature Cell Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "YAP/TAZ drives cell proliferation and tumor growth via a polyamine-eIF5A hypusination-LSD1 axis". For those reviewers who give their assent, we will be publishing their names alongside the published article.

Nature Cell Biology offers a Transparent Peer Review option for new original research manuscripts submitted after December 1st, 2019. As part of this initiative, we encourage our authors to support increased transparency into the peer review process by agreeing to have the reviewer comments, author rebuttal letters, and editorial decision letters published as a Supplementary item. When you submit your final files please clearly state in your cover letter whether or not you would like to

participate in this initiative. Please note that failure to state your preference will result in delays in accepting your manuscript for publication.

Cover suggestions

As you prepare your final files we encourage you to consider whether you have any images or illustrations that may be appropriate for use on the cover of Nature Cell Biology.

Covers should be both aesthetically appealing and scientifically relevant, and should be supplied at the best quality available. Due to the prominence of these images, we do not generally select images featuring faces, children, text, graphs, schematic drawings, or collages on our covers.

We accept TIFF, JPEG, PNG or PSD file formats (a layered PSD file would be ideal), and the image should be at least 300ppi resolution (preferably 600-1200 ppi), in CMYK colour mode.

If your image is selected, we may also use it on the journal website as a banner image, and may need to make artistic alterations to fit our journal style.

Please submit your suggestions, clearly labeled, along with your final files. We'll be in touch if more information is needed.

Nature Cell Biology has now transitioned to a unified Rights Collection system which will allow our Author Services team to quickly and easily collect the rights and permissions required to publish your work. Approximately 10 days after your paper is formally accepted, you will receive an email in providing you with a link to complete the grant of rights. If your paper is eligible for Open Access, our Author Services team will also be in touch regarding any additional information that may be required to arrange payment for your article.

Please note that Nature Cell Biology is a Transformative Journal (TJ). Authors may publish their research with us through the traditional subscription access route or make their paper immediately open access through payment of an article-processing charge (APC). Authors will not be required to make a final decision about access to their article until it has been accepted. Find out more about Transformative Journals

Authors may need to take specific actions to achieve compliance with funder and institutional open access mandates. For submissions from January 2021, if your research is supported by a funder that requires immediate open access (e.g. according to Plan S principles) then you should select the gold OA route, and we will direct you to the compliant route where possible. For authors selecting the

subscription publication route our standard licensing terms will need to be accepted, including our self-archiving policies. Those standard licensing terms will supersede any other terms that the author or any third party may assert apply to any version of the manuscript.

Please note that you will not receive your proofs until the publishing agreement has been received through our system.

For information regarding our different publishing models please see our Transformative Journals page. If you have any questions about costs, Open Access requirements, or our legal forms, please contact ASJournals@springernature.com.

Please use the following link for uploading these materials:
[REDACTED]

If you have any further questions, please feel free to contact us. Many thanks!

Best regards,

Ziqian Li
Editorial Assistant
Nature Cell Biology

On behalf of

Zhe Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: zhe.wang@nature.com

Reviewer #1:
Remarks to the Author:
The authors have answered my questions.

Reviewer #2:
Remarks to the Author:
The revised manuscript by Li et al. addressed many of my primary concerns. But I have one concern that was not fully addressed, and I respectfully ask the authors for further clarification:

In response to our comment #8 regarding Ext. Figure 4, the authors simply presume that OAZ overexpression did not significantly downregulate ODC1 protein levels due to the predominance of the interference of ODC homodimerization mechanism. However, the authors do not provide any experimental evidence to support this claim. The absence of any difference in ODC1 levels in Ext. Figure 4d makes it difficult for readers to accept the integrity of the authors' proposed mechanism. Thus, to address whether the dimerization mechanism is favored in the authors' scenario, the authors could run a native (non-denaturing) gel to assess this (or if the degradation mechanism is favored, MG132 or poly-ubiquitination experiments). Or, as mentioned in response, the authors need to propose the possible mechanism of the OAZ-mediated ODC1 inhibition in the main text.

Reviewer #3:
Remarks to the Author:
The authors have sufficiently addressed my concerns.

Author Rebuttal, first revision:

Reviewer #1:
Remarks to the Author:
The authors have answered my questions.

Response:
We are glad that the reviewer is satisfied with our revision. Thanks again for helping to improve our manuscript.

Reviewer #2:
Remarks to the Author:
The revised manuscript by Li et al. addressed many of my primary concerns. But I have one concern that was not fully addressed, and I respectfully ask the authors for further clarification:

In response to our comment #8 regarding Ext. Figure 4, the authors simply presume that OAZ overexpression did not significantly downregulate ODC1 protein levels due to the predominance of the interference of ODC homodimerization mechanism. However, the authors do not provide any experimental evidence to support this claim. The absence of any difference in ODC1 levels in Ext. Figure 4d makes it difficult for readers to accept the integrity of the authors' proposed mechanism. Thus, to address whether the dimerization mechanism is favored in the authors' scenario, the authors could run a native (non-denaturing) gel to assess this (or if the degradation mechanism is favored, MG132 or poly-ubiquitination experiments). Or, as mentioned in response, the authors need to propose the possible mechanism of the OAZ-mediated ODC1 inhibition in the main text.

Response:
We would like to emphasize that OAZ1 is known to inhibit ODC1 by preventing ODC1 homodimer formation or by inducing ODC1 degradation. Both mechanisms are well known and accepted in the polyamine field (see reference 13 and 14). In our experiment, we simply employed OAZ1 overexpression as a way to suppress ODC1 activity. Our emphasis was not about the specific mechanism by which OAZ1 suppresses ODC1 activity in this particular context, but rather the fact that OAZ1 is a well-established inhibitor of ODC1, although our data is certainly more consistent with OAZ1 suppressing ODC1 homodimerization. Regardless of the specific mechanism involved, the polyamine levels shown in Extended Data Fig. 4e clearly shows that OAZ1-mediated inhibition of polyamine synthesis was successful.

We did try to address this particular point by Reviewer #2 in our revision submitted to you and the reviewers. As shown in page 5 line 94-96, we wrote the following:

“ODC1 functions as a homodimer and is normally inhibited by OAZ1, which competitively binds to ODC1 to prevent ODC1 homodimer formation and induces ODC1 degradation.”

To make this clearer and avoid any possible confusion, we propose to change this sentence to the following:

“ODC1 functions as a homodimer and its enzymatic activity is inhibited by OAZ1, which competitively binds to ODC1 to prevent ODC1 homodimer formation or to induce ODC1 degradation.”

To further drive home this message, we propose to change the following sentence on page 9 line 196:

“Lastly, we examined YAP OE livers that ectopically expressed the ODC1 antizyme OAZ1.”

To the following:
“Lastly, we examined YAP OE livers that ectopically expressed the ODC1 antizyme OAZ1, a well-known inhibitor of ODC1 enzyme.”

We hope these revisions could address Reviewer #2's concerns to his/her satisfaction.

Reviewer #3:
Remarks to the Author:
The authors have sufficiently addressed my concerns.

Response:
We are glad that the reviewer is satisfied with our revision. Thanks again for helping to improve our manuscript.

Final Decision Letter:

Subject: Decision on Nature Cell Biology submission NCB-P46013B
Message:

Dear Dr Pan,

I am pleased to inform you that your manuscript, "YAP/TAZ drives cell proliferation and tumor growth via a polyamine-eIF5A hypusination-LSD1 axis", has now been accepted for publication in Nature Cell Biology.

Thank you for sending us the final manuscript files to be processed for print and online production, and for returning the manuscript checklists and other forms. Your manuscript will now be passed to our production team who will be in contact with you if there are any questions with the production quality of supplied figures and text.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Cell Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

After the grant of rights is completed, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at rjsproduction@springernature.com immediately.

You will not receive your proofs until the publishing agreement has been received through our system.

Due to the importance of these deadlines, we ask that you please let us know now whether you will be difficult to contact over the next month. If this is the case, we ask you provide us with the contact

information (email, phone and fax) of someone who will be able to check the proofs on your behalf, and who will be available to address any last-minute problems.

If you have any questions about our publishing options, costs, Open Access requirements, or our legal forms, please contact ASJournals@springernature.com

Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details. An online order form for reprints of your paper is available at https://www.nature.com/reprints/author-reprints.html. All co-authors, authors' institutions and authors' funding agencies can order reprints using the form appropriate to their geographical region.

Publication is conditional on the manuscript not being published elsewhere and on there being no announcement of this work to any media outlet until the online publication date in Nature Cell Biology.

Please note that Nature Cell Biology is a Transformative Journal (TJ). Authors may publish their research with us through the traditional subscription access route or make their paper immediately open access through payment of an article-processing charge (APC). Authors will not be required to make a final decision about access to their article until it has been accepted. Find out more about Transformative Journals

Authors may need to take specific actions to achieve compliance with funder and institutional open access mandates. For submissions from January 2021, if your research is supported by a funder that requires immediate open access (e.g. according to Plan S principles) then you should select the gold OA route, and we will direct you to the compliant route where possible. For authors selecting the subscription publication route our standard licensing terms will need to be accepted, including our self-archiving policies. Those standard licensing terms will supersede any other terms that the author or any third party may assert apply to any version of the manuscript.

To assist our authors in disseminating their research to the broader community, our SharedIt initiative provides you with a unique shareable link that will allow anyone (with or without a subscription) to read the published article. Recipients of the link with a subscription will also be able to download and print the PDF.

If your paper includes color figures, please be aware that in order to help cover some of the additional cost of four-color reproduction, Nature Research charges our authors a fee for the printing of their color figures. Please contact our offices for exact pricing and details.

As soon as your article is published, you will receive an automated email with your shareable link.

If you have not already done so, we strongly recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange (www.nature.com/protocolexchange), an open online resource established by Nature Protocols that allows researchers to share their detailed experimental know-how. All uploaded protocols are made freely available, assigned DOIs for ease of citation and are fully searchable through nature.com. Protocols and the Nature and Nature research journal papers in which they are used can be linked to one another, and this link is clearly and prominently visible in the online versions of both papers. Authors who performed the specific experiments can act as primary authors for the Protocol as they will be best placed to share the methodology details, but the Corresponding Author of the present research paper should be included as one of the authors. By uploading your Protocols to Protocol Exchange, you are enabling researchers to more readily reproduce or adapt the methodology you use, as well as increasing the visibility of your protocols and papers. You can also establish a dedicated page to collect your lab Protocols. Further information can be found at www.nature.com/protocolexchange/about

You can use a single sign-on for all your accounts, view the status of all your manuscript submissions and reviews, access usage statistics for your published articles and download a record of your refereeing activity for the Nature journals.

Please feel free to contact us if you have any questions.

With kind regards,

Zhe Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: zhe.wang@nature.com

Click here if you would like to recommend Nature Cell Biology to your librarian
http://www.nature.com/subscriptions/recommend.html#forms

** Visit the Springer Nature Editorial and Publishing website at www.springernature.com/editorial-and-publishing-jobs for more information about our career opportunities. If you have any questions please click here.**