nature portfolio

Peer Review File

Interplay between acetylation and ubiquitination controls PSAT1 protein stability in lung adenocarcinoma

Corresponding Author: Dr Tianyu Han

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Major issues
As stated in the introduction, the claimed effects of PSAT1 on different cellular pathways in different cancers are very broad and are more correlation than causation. The introduction needs to be simplified and become more interpretive than just reporting from other sources.
The author need to provide a rationale on why a Histone deacetylase was suspected to deacetylate PSAT1. And they must show this effect is unique to PSAT1 and not other protein (a few negative controls are necessary).
Is the effect of cisplatin on acetylation and ubiquitination of PSAT1 unique? Other chemotherapy classes should be tested (positive and negative controls are needed).
"…pre-tested using the biomarkers proven", what biomarkers? NAM is used in high millimolar concentration that is not pharmacologically relevant.
Figure 1A: is the difference between TSA and TSA+NAM statistically significant?
Figures 1, 1D: please provide cell viability data with increasing time and concentration of TSA. The decrease in PSAT1 may be due to dead cells.
Figure 1G: Needs densitometry and statistical analysis. The difference between A549 and H1299 with BEAS does not appear to be significant. Moreover, was it a difference between two lung cancer cells?
Figure 1H (particularly H1299) does not support this conclusion: "We next examined the degradation rates of PSAT1 in these cells and found that the degradation of PSAT1 was attenuated in LUAD cells (Figure 1H), suggesting that acetylation affected PSAT1 protein stability."
Figure 1I: please provide the rationale for the doses of MG132 and chloroquine being 20 and 10 micromolar? Does chloroquine at 10 micromolar effectively inhibit autophagy?
Figures 1K-1M: the quality of Western blots are poor.
Is the effect of HDAC7 on PSAT1 exclusive? What is its effect on some other proteins (e.g. PHGDH, ASNS, ATF4, etc) involved in amino acid metabolism?
Please explain the process of choosing USP14 from your mass spec data "we performed mass spectrometry analysis and identified USP14 as a putative PSAT1-interacting protein (Supplemental Table 1)."
Is the HDAC7-USP14-PSAT1 axis exclusive for lung adenocarcinoma or is it a universal pathway in several cancer cells?

Minor issues
The source of Serine is more complicated than "Serine can be obtained from various sources: it can be taken up directly from the extracellular environment, derived from intracellular protein via autophagy, or synthesized de novo via the serine synthesis pathway (SSP)." Please see Hameed KM et al. Front Oncol. 2024 Apr 16;14:1326754.
All abbreviations should be defined when used for the first time: PSAT1 (line 68), EGFR (line 81), ROS (line 82), GSK3 (line 84), ATF4 (line 89), NRF2 (line 90), LUAD (line 104), HDAC7 (line 106), USP14 (line 106), UBE4B (line 108), HDAC (line 123)
This is sentence does not appear to be scientific: "PSAT1 is closely associated with the occurrence, development, treatment, and prognosis of various tumors." How PSAT1 is associated with treatment?!
What does this sentence mean "Acetylation plays an important role in cancer metabolism." One can argue that methylation, sulfation, phosphorylation, etc. plays an important role in cancer metabolism and other hallmarks of cancer. Please refrain

from general statements in the article that do not provide any specific information.
Figures 6J, 6K are not readable.
The authors should provide comments in Discussion on how this discovery can be exploited for treating patients with NSCLC and potentially other cancers?

Reviewer #2

(Remarks to the Author)
The manuscript by Dr Han and colleagues explores the post-translational regulation by acetylation and its interplay with ubiquitination of the metabolic enzyme PSAT1 in the context of lung adenocarcinoma. The authors show that PSAT1 acetylation is linked to its protein stability. They identified that PSAT1 acetylation promotes its ubiquitination by the E3 ligase UBE4B, leading to its proteasomal degradation. On the other side of this post-translational regulation mechanism, HDAC7 deacetylates PSAT1 promoting the recruitment of USP14, that then deubiquitinates PSAT1 resulting to the increase of its protein stability. This stabilization of PSAT1 seems to have an effect in the serine metabolism and cell proliferation of lung cancer cells. Overall, this is a convincing work, the manuscript is clear and well-written, the experiments are sound, the authors provide the necessary controls and their conclusions are not overstated.

I have the following comments:

1. Line 157: MG132 treatment also reversed the decreased expression of PSAT1 caused by TSA treatment (Figure 1J). This is not reflected clearly in the provided blot, should provide a quantification graph.

2. To explore the role of HDAC7 in PSAT1 protein expression, we overexpressed HDAC7 in H1299 cells and PSAT1 expression was examined. The result showed that the protein expression of PSAT1 increased significantly (Figure 2M). To further validate the above results, we transfected the HDAC7 specific siRNAs into H1299 cells followed by dectecting PSAT1 expression. Knocking down HDAC7 reduced PSAT1 expression (Figure 2N). What is the effect of overexpression/knock down of HDAC7 on the mRNA levels of PSAT1?

3. Figure 6A showed that the acetylation level of PSAT1-K51R was reduced compared with PSAT1-WT. The blot provided here is not very convincing. An additional in vitro acetylation assay using a recombinant wt and mutant PSAT1 might be a good option for a stronger clarification. Also, an immunofluorescence experiment showing the localization of the PSAT1 wt and mutants should be added as a supplementary figure.

4. Neither TSA treatment or HDAC7 overexpression affected the acetylation of PSAT1-K51R, indicating that PSAT1-K51R might be the main acetylation site (Figure 6B and C). In Figure 6B seems like there is a slight increase in the acetylation levels of PSAT1-K51R?

5. In text corrections:
- Line 188:… dectecting PSAT1 expression. Knocking down HDAC7 reduced PSAT1…. (detecting)
- Line 250: … detected the ubiquCitin chain types using antibodies specific for…. (ubiquitin)
- Line 408:… and EV4A-C showed that overexpressing UBE4B reduced the protein… (Figure S4A-C)
- Line 426:… and EV4F revealed that acetylation affected the ubiquitination of… (Figure S4F)
- Figures 6J and 6K are unreadable: Should get bigger sized letters

Reviewer #3

(Remarks to the Author)
Overall, the manuscript by Liu, Han, et al is a very interesting, comprehensive (dense) and well-written piece of work. The authors are very methodical in their experiments and present multiple lines of evidence for most conclusions. I have very few minor issues and no real major issues with this work.

Minor concerns:
-The only grammatical error i found is on line 250 "ubiquCitin".
-I feel that the cisplatin portion of the work takes away from the overall mechanistic and biochemical theme of the work. all the cisplatin work is buried in supplemental and I feel that if it was removed it wouldn't diminish the work.

Major-ish concern:
-My only slight major concern is that, with the exception of some very early experiments, all work is done in a single cell line. the authors do present 2 other cell lines in figure 1, however the rigor and reproducibility of the work would be strengthened by showing a few other very key findings in another cell line.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors answered the majority of my queries sufficiently. They have performed several new experiments and improved the text. I have no further comments.

Reviewer #2

(Remarks to the Author)
I have carefully reviewed the revisions made by the authors, as well as their responses to all of the referees comments. The authors have addressed all of the concerns I raised in my initial review. The revisions have significantly improved the quality and clarity of the manuscript, particularly the text clarity and figure / data presentation.

Given these improvements, I believe that the manuscript is now suitable for publication. I recommend that it be accepted for publication.

Reviewer #3

(Remarks to the Author)
The authors have done a very nice job addressing my comments, as well as the other reviewers comments. The manuscript has been much improved.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

Major issues

ν As stated in the introduction, the claimed effects of PSAT1 on different cellular pathways in different cancers are very broad and are more correlation than causation. The introduction needs to be simplified and become more interpretive than just reporting from other sources.

Response:

Thank you very much for your comments on our manuscript. We have simplified and modified the relevant sentences in the revised manuscript.

(pages 3-4, lines 59-74)

As a pivotal metabolic enzyme, upregulation of PSAT1 has been observed in cancer cell lines, and elevated PSAT1 expression enables cancer cells to survive under serine starvation condition and promote tumorigenesis. The protein abundance is tightly controlled by transcriptional, post-transcriptional, translational, and post-translational regulatory mechanisms. PSAT1 can be regulated by multiple upstream proteins and signaling molecules. A key regulator of SSP gene expression is Activating transcription factor 4 (ATF4), which directly binds to and activates the promoters. Also, nuclear factor erythroid 2-related factor 2 (NRF2) controls the expression of PSAT1 via ATF4 to support glutathione and nucleotide production. Moreover, a recent study demonstrated that c-Myc stimulated SSP activation by transcriptionally upregulating the expression of multiple SSP enzymes. PSAT1 is also subject to epigenetic control. The histone H3 lysine 9 methyltransferases (G9A) can transcriptionally activate PSAT1. However, little is known about the roles of post-translational modification in regulating PSAT1.

ν The author need to provide a rationale on why a Histone deacetylase was suspected to deacetylate PSAT1. And they must show this effect is unique to PSAT1 and not other

protein (a few negative controls are necessary).

Response:

TSA is the inhibitor of histone deacetylase (HDAC) classI/II family deacetylases. Recent studies have demonstrated that histone deacetylase could also deacetylate non-histone protein to regulate their function [1]. Our previous study also showed that HDAC4 deacetylated glutaminase to regulate tumorigenesis of lung cancer [2]. In this study, the results in Fig.1 demonstrated that TSA treatment decreased the expression of PSAT1. This indicated that histone deacetylase could also regulate serine metabolism through PSAT1. We verified that HDAC7 was the specific deacetylase for PSAT1, but not for the other enzymes in serine metabolism like PHGDH, PSPH and SHMT1/2 (Supplementary Fig. 2a-f). Thus, the deacetylation induced by HDAC7 was unique for PSAT1 in serine metabolism.

Ref. 1> Narita, Takeo et al. "Functions and mechanisms of non-histone protein acetylation." Nat Rev Mol Cell Biol. 2019;20(3):156-174.

Ref. 2> Wang, Tao et al. "Deacetylation of Glutaminase by HDAC4 contributes to Lung Cancer Tumorigenesis." Int J Biol Sci. 2022;18(11):4452-4465.

v Is the effect of cisplatin on acetylation and ubiquitination of PSAT1 unique? Other chemotherapy classes should be tested (positive and negative controls are needed).

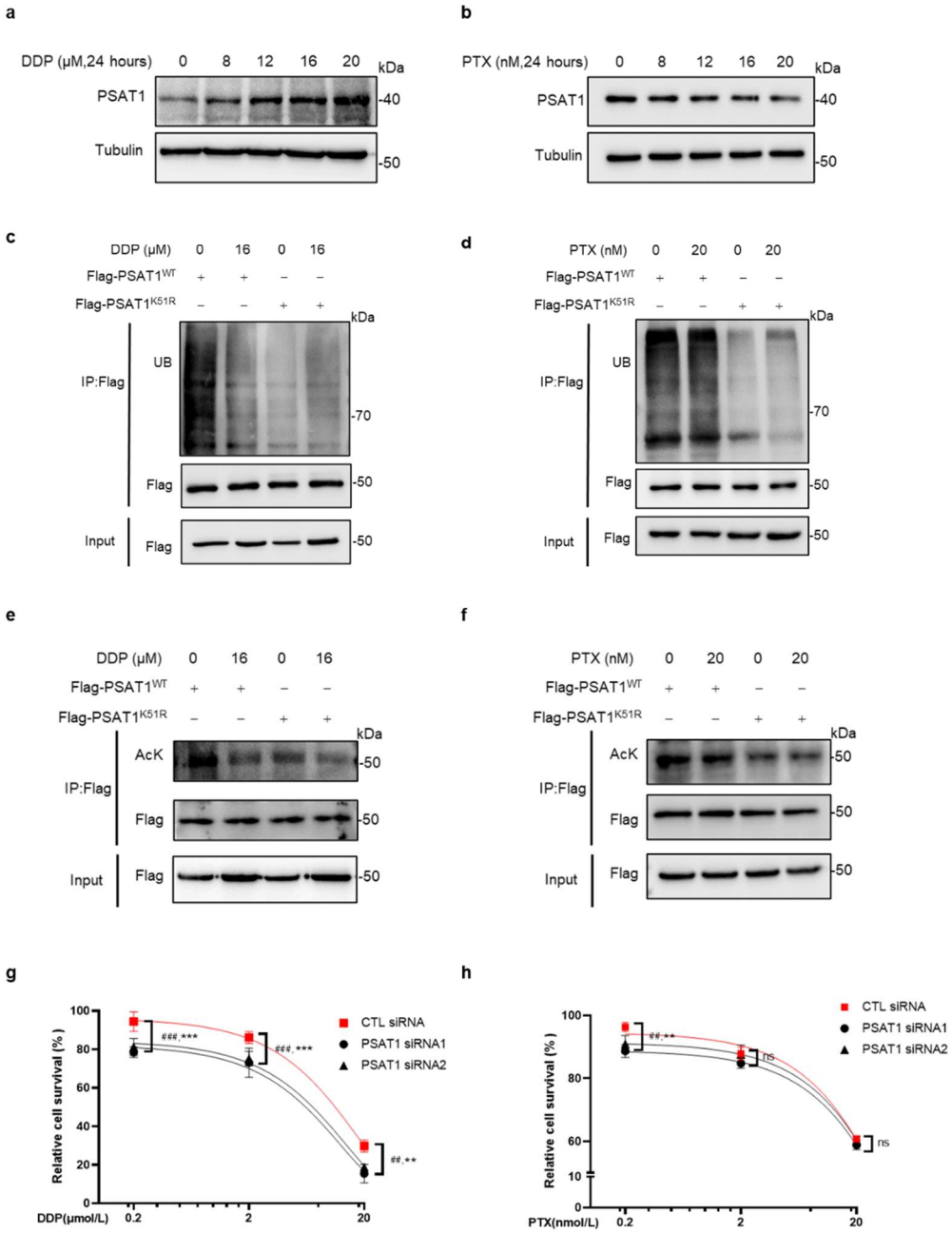
Response:

In order to clarify if the effect of cisplatin on acetylation and ubiquitination of PSAT1 was unique, we used another type of chemotherapy drug—paclitaxel (PTX), which targets the microtubule cytoskeleton. We found that PTX treatment did not have significant effects on the acetylation and ubiquitination of PSAT1. Knocking down PSAT1 also did not increased the sensitivity of cancer cells to PTX treatment. This indicated that the effects on acetylation and ubiquitination of PSAT1 might be unique to platinum-based drugs, which led to DNA damage.

(pages 19-20, lines 380-404)

Cisplatin (DDP) and paclitaxel (PTX) are two first-line chemotherapy drugs for the clinical treatment of non-small cell lung cancer (NSCLC). Mechanically, as a platinum chemotherapeutic agent, DDP can enter cells to cause DNA cross-linking, leading DNA damage. Unlike DDP, PTX targets the microtubule cytoskeleton, inducing cell cycle arrest and apoptosis. To investigate the effects of PSAT1 on chemotherapy treatment, we first measured the protein expression of PSAT1 when treating cells with different concentrations of DDP or PTX. Supplementary Fig. 7a, b showed that PSAT1 protein levels significantly increased in a DDP concentration dependent manner, while the protein levels slightly decreased with PTX treatment. We subsequently tested whether these chemotherapy drugs regulated the acetylation and ubiquitination levels of PSAT1. Supplementary Fig. 7c-f showed that DDP treatment had no significant effect on the acetylation and ubiquitination levels of PSAT1-K51R cells, whereas it led to a remarkable decrease in PSAT1-WT cells. We also found that PTX treatment did not alter the acetylation and ubiquitination levels. These results indicated that DDP affected PSAT1 expression through regulating the interplay between acetylation and ubiquitination, while PTX did not have these effects. Then, we tested if PSAT1 expression affected the therapeutic effect of DDP or PTX on lung cancer cells. Knocking down PSAT1 increased the sensitivity of cancer cells to DDP treatment rather than PTX (Supplementary Fig. 7g, h). Thus, we speculated that the different anticancer mechanism between DDP and PTX led to the unique effects for PSAT1. These results exhibit the potential application of PSAT1 as a therapeutic target for cancer treatment.

Supplementary Fig. 7



v "...pre-tested using the biomarkers proven", what biomarkers? NAM is used in high millimolar concentration that is not pharmacologically relevant.

Response:

The biomarkers indicated the acetylated proteins affected by NAM or TSA that have been proven by other studies. We detected the acetylation level of ACLY in cells

treated with NAM and the acetylation level of STAT3 in cells treated with TSA [1, 2]. The NAM treatment significantly increased the acetylation of ACLY, and TSA treatment elevated the acetylation of STAT3 (Supplementary Fig. 1a, b), indicating that both treatments were effective.

The millimolar doses of NAM were commonly used in studies focusing on acetylation [3-5].

Ref. 1> Zhou, Feifei et al. "ARHGEF3 regulates the stability of ACLY to promote the proliferation of lung cancer." Cell death & disease vol. 13,10 870. 14 Oct. 2022

Ref. 2> Zhong, Yu et al. "The HDAC10 instructs macrophage M2 program via deacetylation of STAT3 and promotes allergic airway inflammation." Theranostics vol. 13,11 3568-3581. 19 Jun. 2023

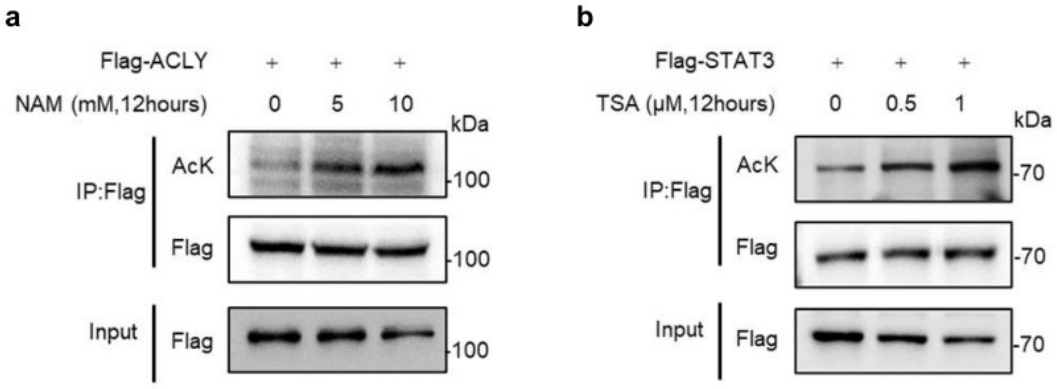
Ref. 3> Lin, Li et al. "Role of SIRT1 in Streptococcus pneumoniae-induced human β-defensin-2 and interleukin-8 expression in A549 cell." Molecular and cellular biochemistry vol. 394,1-2 (2014): 199-208

Ref. 4> Francesca Scatozza, et al. "Nicotinamide inhibits melanoma in vitro and in vivo." J Exp Clin Cancer Res. 2020 Oct 7;39(1):211.

Ref. 5> Zhang Y, et al. "Nicotinamide promotes pancreatic differentiation through the dual inhibition of CK1 and ROCK kinases in human embryonic stem cells." Stem Cell Res Ther. 2021 Jun 25;12(1):362.

(page 6, lines 97-99)

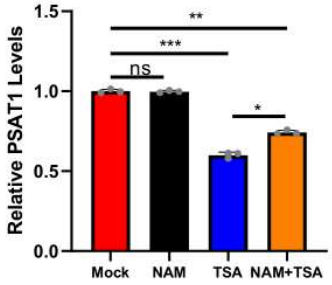
The treatment efficiencies of the inhibitors were pre-tested using the biomarkers proven by other studies (Supplementary Fig. 1a, b).



v Figure 1A: is the difference between TSA and TSA+NAM statistically significant?

Response:

According to your advice, we analyzed the difference between TSA and TSA+NAM. Fig. 1a showed that there were statistically significant between TSA and TSA+NAM.



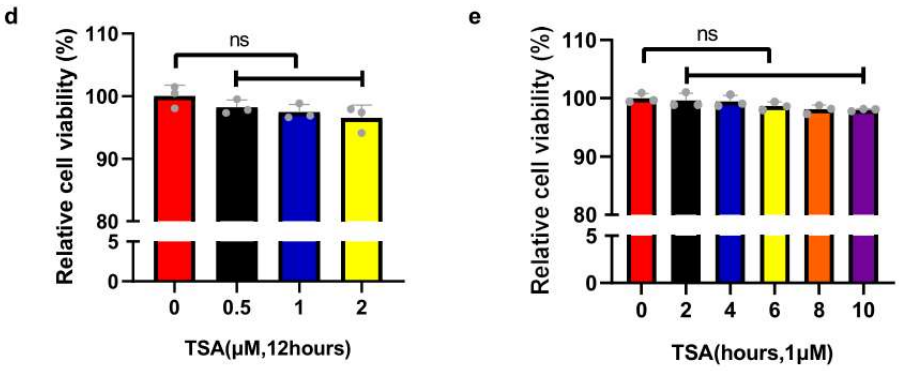
v Figures 1D: please provide cell viability data with increasing time and concentration of TSA. The decrease in PSAT1 may be due to dead cells.

Response:

According to your advice, we detected the cell viability with increasing time and concentration of TSA. Supplementary Fig. 1d and 1e showed that the cells could tolerate TSA treatment well under these conditions.

(page 7, lines 112-114)

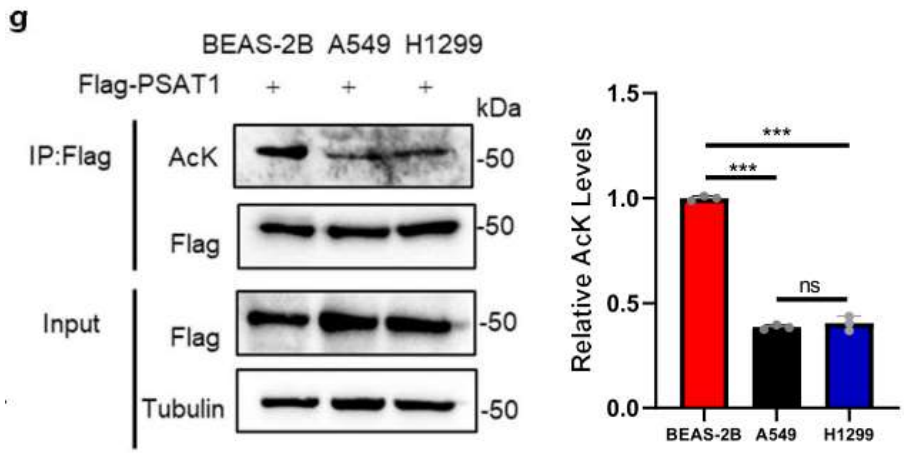
The cell viability was also determined under these conditions and Supplementary Fig. 1d, e showed that these treatments did not affect the cell viability significantly.



v Figure 1G: Needs densitometry and statistical analysis. The difference between A549 and H1299 with BEAS does not appear to be significant. Moreover, was it a difference between two lung cancer cells?

Response:

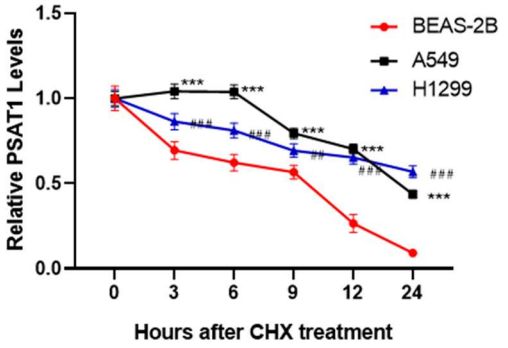
According to your advice, we made statistical analysis on PSAT1 acetylation between A549 and H1299 with BEAS-2B based on the results of three independent experiments. Fig. 1g showed that the acetylation levels of PSAT1 in A549 and H1299 cells were significantly lower than that in BEAS-2B cells, but there were no differences between A549 and H1299 cells.



v Figure 1H (particularly H1299) does not support this conclusion: "We next examined the degradation rates of PSAT1 in these cells and found that the degradation of PSAT1 was attenuated in LUAD cells (Figure 1H), suggesting that acetylation affected PSAT1 protein stability."

Response:

In Fig. 1i, we made statistical analysis and the result showed that the degradation rates of PSAT1 in BEAS-2B cells were faster than that in LUAD cells.



v Figure 1I: please provide the rationale for the doses of MG132 and chloroquine being 20 and 10 micromolar? Does chloroquine at 10 micromolar effectively inhibit autophagy?

Response:

The doses of MG132 and chloroquine used in our study were based on previous studies [1-3]. In Supplementary Fig. 1g, we measured the autophagy inhibition effect of CQ (10 μM) by testing the autophagy-related proteins expression levels.

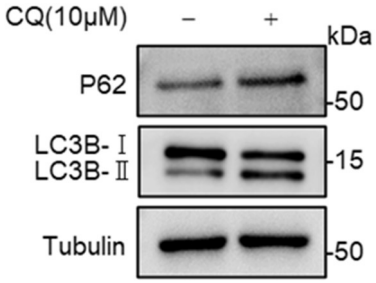
Ref. 1> Liu, Xiangguo et al. "Cellular FLICE-inhibitory protein down-regulation contributes to celecoxib-induced apoptosis in human lung cancer cells." Cancer research vol. 66,23 (2006): 11115-9.

Ref. 2> Datta, Satabdi et al. "Autophagy inhibition with chloroquine reverts paclitaxel resistance and attenuates metastatic potential in human nonsmall lung adenocarcinoma A549 cells via ROS mediated modulation of β-catenin pathway." Apoptosis vol. 24,5-6 (2019): 414-433.

Ref. 3> Lu, Yu-Ting et al. "Sulfuretin protects hepatic cells through regulation of ROS levels and autophagic flux." Acta pharmacologica Sinica vol. 40,7 (2019): 908-918.

(page 8, lines 135-137)

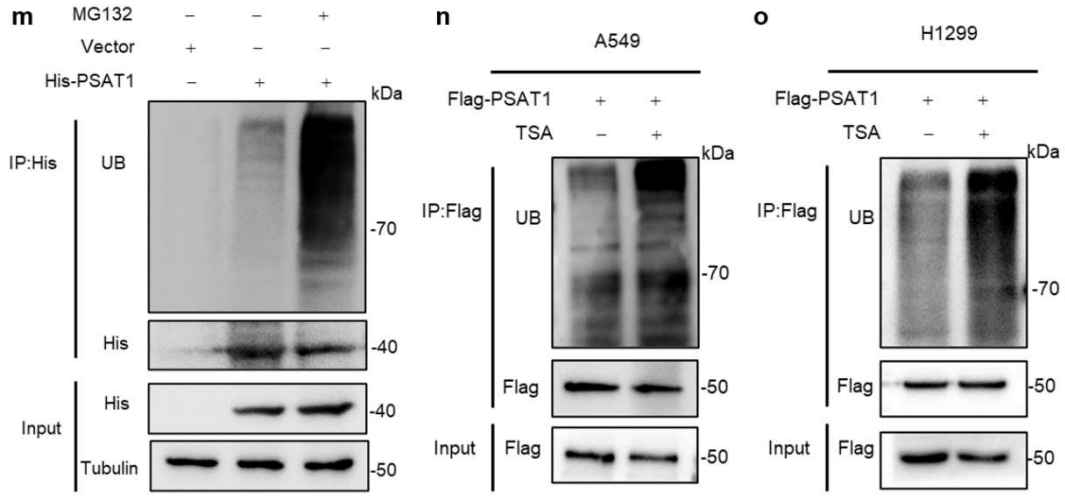
The treatment efficiency of CQ was pre-tested using the autophagy-related proteins by western blot (Supplementary Fig. 1g).



v Figures 1K-1M: the quality of Western blots are poor.

Response:

According to your advice, we performed these experiments again. The clearer results could be seen in Fig .1m-o.



v Is the effect of HDAC7 on PSAT1 exclusive? What is its effect on some other proteins (e.g. PHGDH, ASNS, ATF4, etc) involved in amino acid metabolism?

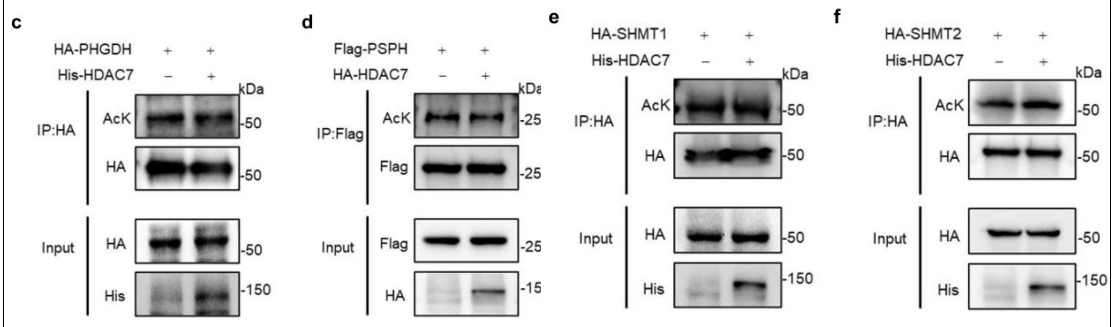
Response:

In the revised manuscript, we also detected the effects of HDAC7 on the acetylation of other enzymes in serine metabolism. The results showed that HDAC7 did not

affected the acetylation of other enzymes in serine metabolism like PHGDH, PSPH and SHMT1/2 (Supplementary Fig. 2c-f). Thus, the deacetylation induced by HDAC7 was unique for PSAT1 in serine metabolism.

(page 9, lines 160-164)

Then we detected the deacetylation efficiencies of some other proteins involved in serine synthesis by HDAC7 overexpression. Supplementary Fig. 2c-f showed that HDAC7 did not affect the acetylation levels of PHGDH, PSPH or serine hydroxymethyltransferase1/2 (SHMT1/2).



v Please explain the process of choosing USP14 from your mass spec data "we performed mass spectrometry analysis and identified USP14 as a putative PSAT1-interacting protein (Supplemental Table 1)."

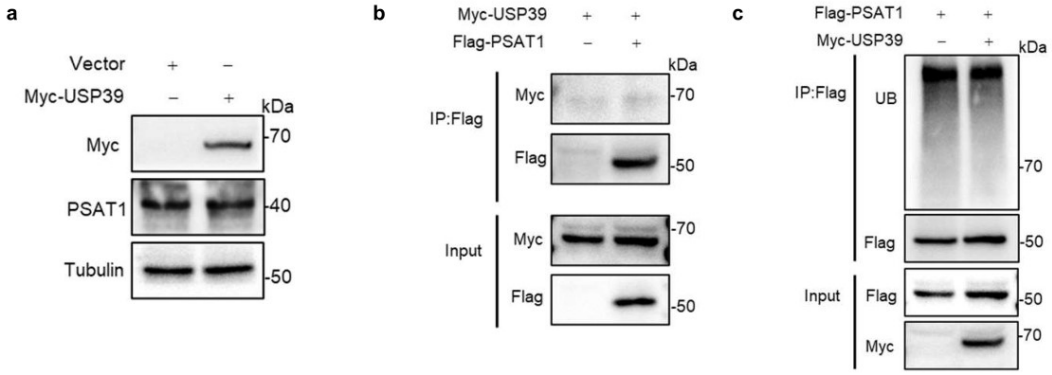
Response:

In our mass spectrometry data, we found two deubiquitinases USP14 and USP39. We demonstrated that USP39 did not interact with PSAT1 and regulate the protein or ubiquitination levels of PSAT1. However, USP14 interacted with PSAT1 and regulated its protein stability. Thus, we selected USP14 for further study. We have added these results in our revised manuscript.

(page 10, lines 190-197)

To explore the potential E3-ligases or deubiquitinases for PSAT1, we performed mass spectrometry analysis. Ultimately, two proteins of DUBs, named USP39 and USP14, were identified as a putative PSAT1-interacting protein (Supplementary data 1). Supplementary Fig. 3a-c showed USP39 could not interact with PSAT1 and regulate the protein or ubiquitination levels of PSAT1.

Supplementary Fig. 3



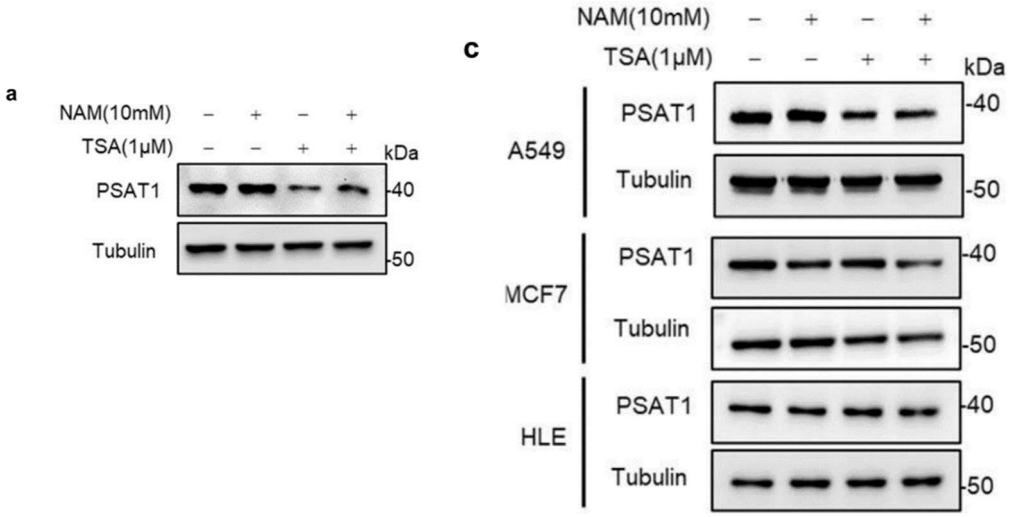
v Is the HDAC7-USP14-PSAT1 axis exclusive for lung adenocarcinoma or is it a universal pathway in several cancer cells?

Response:

According to your advice, we detected the effects of NAM and TSA in LUAD cells (H1299 and A549), breast cancer cells (MCF7) and hepatocellular carcinoma cells (HLE). Fig. 1a and Supplementary Fig. 1c showed that TSA treatment only decreased PSAT1 expression in LUAD cells, but not in breast cancer and liver cancer cells. These results indicated that HDAC7 did not regulate the expression of PSAT1 in other cancer cells, at least in breast cancer and liver cancer cells. Thus, we think that the HDAC7-USP14-PSAT1 axis is unique for LUAD cells.

(page 6, lines 99-104)

The protein expression of PSAT1 was significantly decreased when treating cells with TSA but not NAM in lung cancer cells, however these effects were not observed in breast cancer cell (MCF7) or hepatocellular carcinoma cell (HLE) (Fig. 1a and Supplementary Fig. 1c), suggesting that the phenomenon was specific to LUAD cells.



Minor issues

v The source of Serine is more complicated than "Serine can be obtained from various sources: it can be taken up directly from the extracellular environment, derived from intracellular protein via autophagy, or synthesized de novo via the serine synthesis pathway (SSP)." Please see Hameed KM et al. Front Oncol. 2024 Apr 16;14:1326754.

Response:

According to your advice, we have improved the description of this section.

Ref. 1> Hameed, Kanwal M et al. "Dual targeting of glutamine and serine metabolism in acute myeloid leukemia. " Frontiers in oncology vol. 14 1326754. 16 Apr. 2024, doi:10.3389/fonc.2024.1326754

(page 3, lines 46-49)

Serine can be obtained from various sources: it can be taken up directly from the extracellular environment, derived from intracellular protein via autophagy,

produced by glycine conversion or synthesized de novo via the serine synthesis pathway (SSP).

ν All abbreviations should be defined when used for the first time: PSAT1 (line 68), EGFR (line 81), ROS (line 82), GSK3 (line 84), ATF4 (line 89), NRF2 (line 90), LUAD (line 104), HDAC7 (line 106), USP14 (line 106), UBE4B (line 108), HDAC (line 123)

Response:

According to your advice, all abbreviations was defined when used for the first time.

ν This is sentence does not appear to be scientific: "PSAT1 is closely associated with the occurrence, development, treatment, and prognosis of various tumors." How PSAT1 is associated with treatment?!

Response:

There are currently no reports proving that PSAT1 is associated with treatment. We have deleted "treatment" in this sentence.

ν What does this sentence mean "Acetylation plays an important role in cancer metabolism." One can argue that methylation, sulfation, phosphorylation, etc. plays an important role in cancer metabolism and other hallmarks of cancer. Please refrain from general statements in the article that do not provide any specific information.

Response:

According to your advice, we have corrected similar statements in the revised manuscript.

ν Figures 6J, 6K are not readable.

Response:

Thank you for your advice, we made appropriate changes to make the figures clearer. (Fig. 6j, 6k and Supplementary Fig. 5).

v The authors should provide comments in Discussion on how this discovery can be exploited for treating patients with NSCLC and potentially other cancers?

Response:

According to your advice, we have provided the related comments in Discussion part of the revised manuscript.

(page 26-27, lines 520-534)

Given that PSAT1 is a key enzyme in de novo serine synthesis, which provides important cellular metabolic precursors for biosynthetic metabolism, we examined the effects of PSAT1 acetylation on the serine synthesis pathway. In our study, we found that acetylation of PSAT1 on Lysine 51 regulates serine metabolism and proliferation of LUAD cells. Importantly, the chemotherapy drug DDP could increase PSAT1 expression in LUAD cells by decreasing its acetylation on K51 and enhancing its protein stability. DDP is a DNA-damaging agent. We found PSAT1 could regulate nucleotides synthesis which was required for DNA biosynthesis. Knocking down PSAT1 increased the sensitivity of LUAD cells to DDP. However, treating LUAD cells with PTX, another chemotherapy drugs targeting the microtubule cytoskeleton, did not show this effect. These results indicated that inhibiting the function of PSAT1 is an effective strategy to enhance the therapeutic effect of chemotherapy drugs for DNA damage.

Reviewer #2 (Remarks to the Author):

The manuscript by Dr Han and colleagues explores the post-translational regulation by acetylation and its interplay with ubiquitination of the metabolic enzyme PSAT1 in the context of lung adenocarcinoma. The authors show that PSAT1 acetylation is linked to its protein stability. They identified that PSAT1 acetylation promotes its ubiquitination by the E3 ligase UBE4B, leading to its proteasomal degradation. On the other side of this post-translational regulation mechanism, HDAC7 deacetylates PSAT1 promoting the recruitment of USP14, that then deubiquitinates PSAT1 resulting to the

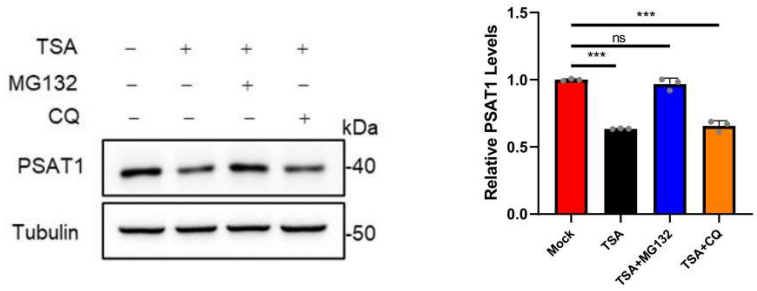
increase of its protein stability. This stabilization of PSAT1 seems to have an effect in the serine metabolism and cell proliferation of lung cancer cells. Overall, this is a convincing work, the manuscript is clear and well-written, the experiments are sound, the authors provide the necessary controls and their conclusions are not overstated.

I have the following comments:

1. Line 157: MG132 treatment also reversed the decreased expression of PSAT1 caused by TSA treatment (Figure 1J). This is not reflected clearly in the provided blot, should provide a quantification graph.

Response:

Thank you very much for your comments on our manuscript. According to your advice, we made statistical analysis based on the results of three independent experiments. The result showed that MG132 treatment reversed the decreased expression of PSAT1 caused by TSA treatment (Fig.1l).



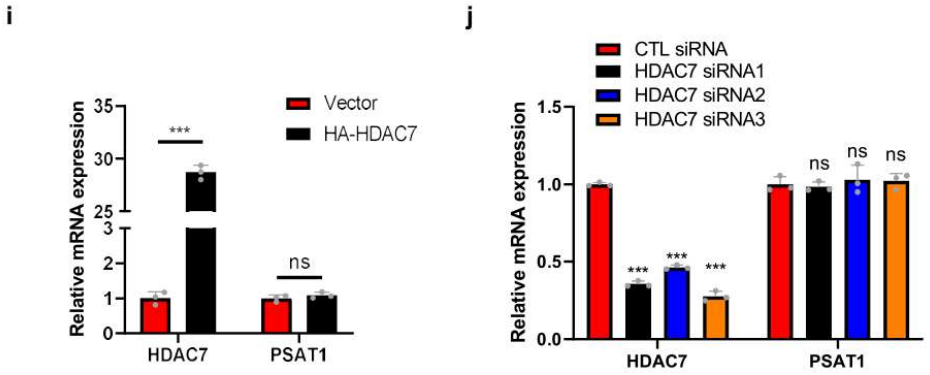
2. To explore the role of HDAC7 in PSAT1 protein expression, we overexpressed HDAC7 in H1299 cells and PSAT1 expression was examined. The result showed that the protein expression of PSAT1 increased significantly (Figure 2M). To further validate the above results, we transfected the HDAC7 specific siRNAs into H1299 cells followed by dectecting PSAT1 expression. Knocking down HDAC7 reduced PSAT1 expression (Figure 2N). What is the effect of overexpression/knock down of HDAC7 on the mRNA levels of PSAT1?

Response:

According to your advice, we detected the mRNA level of PSAT1 in cells with HDAC7 overexpression/knockdown. Supplementary Fig. 2i and 2j showed that HDAC7 overexpression/knockdown did not affected the mRNA level of PSAT1.

(page 9, lines 175-176)

Supplementary Fig. 2i, j showed HDAC7 did not affect the transcription of PSAT1.



3. Figure 6A showed that the acetylation level of PSAT1-K51R was reduced compared with PSAT1-WT. The blot provided here is not very convincing. An additional in vitro acetylation assay using a recombinant wt and mutant PSAT1 might be a good option for a stronger clarification. Also, an immunofluorescence experiment showing the localization of the PSAT1 wt and mutants should be added as a supplementary figure.

Response:

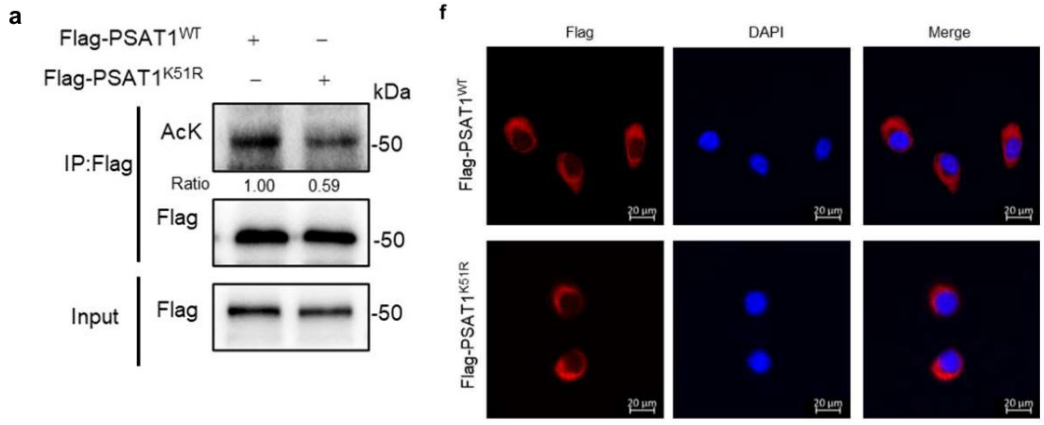
According to your advice, we performed this experiment again and the intensity of protein acetylation was analyzed. The clearer results could be seen in Fig. 6a.

In our study, we did not investigate the acetyltransferase of PSAT1. Thus, it is difficult for us to perform in vitro acetylation assay using the recombinant PSAT1-WT and PSAT1-K51R.

We also detected the localization of PSAT1-WT and PSAT1-K51R using immunofluorescence. Supplementary Fig. 4f showed that both PSAT1-WT and PSAT1-K51R located in cytoplasm and no significant changes were observed.

(page 16, lines 324-327)

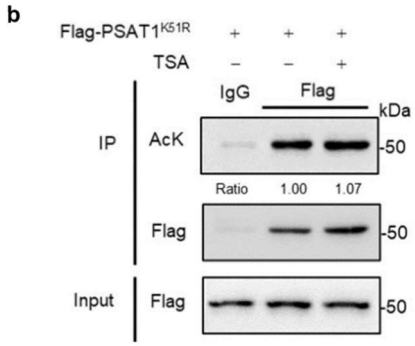
The immunofluorescence assay was performed and Supplementary Fig. 4f demonstrated that the localization of PSAT1-WT and PSAT1-K51R was consistent, both located in the cytoplasm.



4. Neither TSA treatment or HDAC7 overexpression affected the acetylation of PSAT1-K51R, indicating that PSAT1-K51R might be the main acetylation site (Figure 6B and C). In Figure 6B seems like there is a slight increase in the acetylation levels of PSAT1-K51R?

Response:

According to your advice, we performed the experiment in Fig.6b again and analyzed the intensity of protein acetylation. The results showed that the acetylation levels of PSAT1 remained almost unchanged in Fig. 6b.



5. In text corrections:

- Line 188:… dectecting PSAT1 expression. Knocking down HDAC7 reduced PSAT1…. (detecting)

- Line 250: … detected the ubiquCitin chain types using antibodies specific for…. (ubiquitin)

- Line 408:… and EV4A-C showed that overexpressing UBE4B reduced the protein… (Figure S4A-C)

- Line 426:… and EV4F revealed that acetylation affected the ubiquitination of… (Figure S4F)

- Figures 6J and 6K are unreadable: Should get bigger sized letters

Response:

Thank you for your advice, we made appropriate changes to make the figures clearer. (Fig. 6j, 6k and Supplementary Fig. 5). Meanwhile, according to your advice, we have made these corrections in the revised manuscript.

Reviewer #3 (Remarks to the Author):

Overall, the manuscript by Liu, Han, et al is a very interesting, comprehensive (dense) and well-written piece of work. The authors are very methodical in their experiments and present multiple lines of evidence for most conclusions. I have very few minor issues and no real major issues with this work.

Minor concerns:

-The only grammatical error i found is on line 250 "ubiquCitin".

Response: Thank you very much for your comments on our manuscript. We have corrected this error in our revised manuscript.

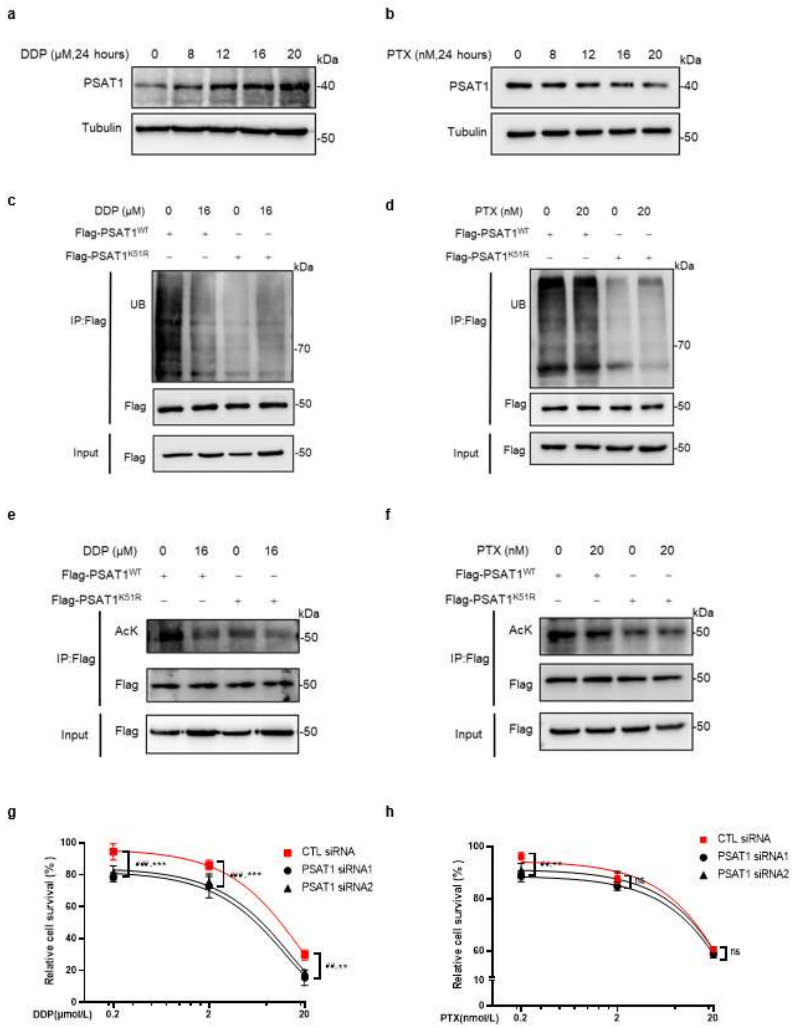
-I feel that the cisplatin portion of the work takes away from the overall mechanistic and biochemical theme of the work. all the cisplatin work is buried in supplemental and

I feel that if it was removed it wouldn't diminish the work.

Response:

Thank you for your advice. Clarifying the relationship between PSAT1 and lung cancer treatment is of great significance. Chemotherapy is one of the main treatment methods for lung adenocarcinoma, and platinum-based drugs combined with paclitaxel are commonly used first-line chemotherapy drugs. To preliminarily clarify the clinical significance of PSAT1 acetylation, we chose cisplatin and paclitaxel to test their effects on PSAT1 acetylation, and detected the effects of PSAT1 on drugs sensitivity. We believe that this may provide potential assistance for the clinical treatment of lung adenocarcinoma, so we retained these results in our manuscript.

Supplementary Fig. 7



Major-ish concern:

-My only slight major concern is that, with the exception of some very early experiments, all work is done in a single cell line. the authors do present 2 other cell lines in figure 1, however the rigor and reproducibility of the work would be strengthened by showing a few other very key findings in another cell line.

Response:

According to your advice, we repeated the key experiments in A549 cell lines (Supplementary Figures). Similar results were observed in both H1299 and A549 cell lines.

Manuscript # COMMSBIO-24-2810B

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors answered the majority of my queries sufficiently. They have performed several new experiments and improved the text. I have no further comments.

Response:

We thank the reviewer for the kind support.

Reviewer #2 (Remarks to the Author):

I have carefully reviewed the revisions made by the authors, as well as their responses to all of the referees comments. The authors have addressed all of the concerns I raised in my initial review. The revisions have significantly improved the quality and clarity of the manuscript, particularly the text clarity and figure / data presentation.

Given these improvements, I believe that the manuscript is now suitable for publication. I recommend that it be accepted for publication.

Response:

We thank the reviewer for the kind support.

Reviewer #3 (Remarks to the Author):

The authors have done a very nice job addressing my comments, as well as the other reviewers comments. The manuscript has been much improved.

Response:

We thank the reviewer for the kind support.

Manuscript # COMMSBIO-24-2810A

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

Major issues

ν As stated in the introduction, the claimed effects of PSAT1 on different cellular pathways in different cancers are very broad and are more correlation than causation. The introduction needs to be simplified and become more interpretive than just reporting from other sources.

Response:

Thank you very much for your comments on our manuscript. We have simplified and modified the relevant sentences in the revised manuscript.

(pages 3-4, lines 59-74)

As a pivotal metabolic enzyme, upregulation of PSAT1 has been observed in cancer cell lines, and elevated PSAT1 expression enables cancer cells to survive under serine starvation condition and promote tumorigenesis. The protein abundance is tightly controlled by transcriptional, post-transcriptional, translational, and post-translational regulatory mechanisms. PSAT1 can be regulated by multiple upstream proteins and signaling molecules. A key regulator of SSP gene expression is Activating transcription factor 4 (ATF4), which directly binds to and activates the promoters. Also, nuclear factor erythroid 2-related factor 2 (NRF2) controls the expression of PSAT1 via ATF4 to support glutathione and nucleotide production. Moreover, a recent study demonstrated that c-Myc stimulated SSP activation by transcriptionally upregulating the expression of multiple SSP enzymes. PSAT1 is also subject to epigenetic control. The histone H3 lysine 9 methyltransferases (G9A) can transcriptionally activate PSAT1. However, little is known about the roles of post-translational modification in regulating PSAT1.

ν The author need to provide a rationale on why a Histone deacetylase was suspected

to deacetylate PSAT1. And they must show this effect is unique to PSAT1 and not other protein (a few negative controls are necessary).

Response:

TSA is the inhibitor of histone deacetylase (HDAC) classI/II family deacetylases. Recent studies have demonstrated that histone deacetylase could also deacetylate non-histone protein to regulate their function [1]. Our previous study also showed that HDAC4 deacetylated glutaminase to regulate tumorigenesis of lung cancer [2]. In this study, the results in Fig.1 demonstrated that TSA treatment decreased the expression of PSAT1. This indicated that histone deacetylase could also regulate serine metabolism through PSAT1. We verified that HDAC7 was the specific deacetylase for PSAT1, but not for the other enzymes in serine metabolism like PHGDH, PSPH and SHMT1/2 (Supplementary Fig. 2a-f). Thus, the deacetylation induced by HDAC7 was unique for PSAT1 in serine metabolism.

Ref. 1> Narita, Takeo et al. "Functions and mechanisms of non-histone protein acetylation." Nat Rev Mol Cell Biol. 2019;20(3):156-174.

Ref. 2> Wang, Tao et al. "Deacetylation of Glutaminase by HDAC4 contributes to Lung Cancer Tumorigenesis." Int J Biol Sci. 2022;18(11):4452-4465.

v Is the effect of cisplatin on acetylation and ubiquitination of PSAT1 unique? Other chemotherapy classes should be tested (positive and negative controls are needed).

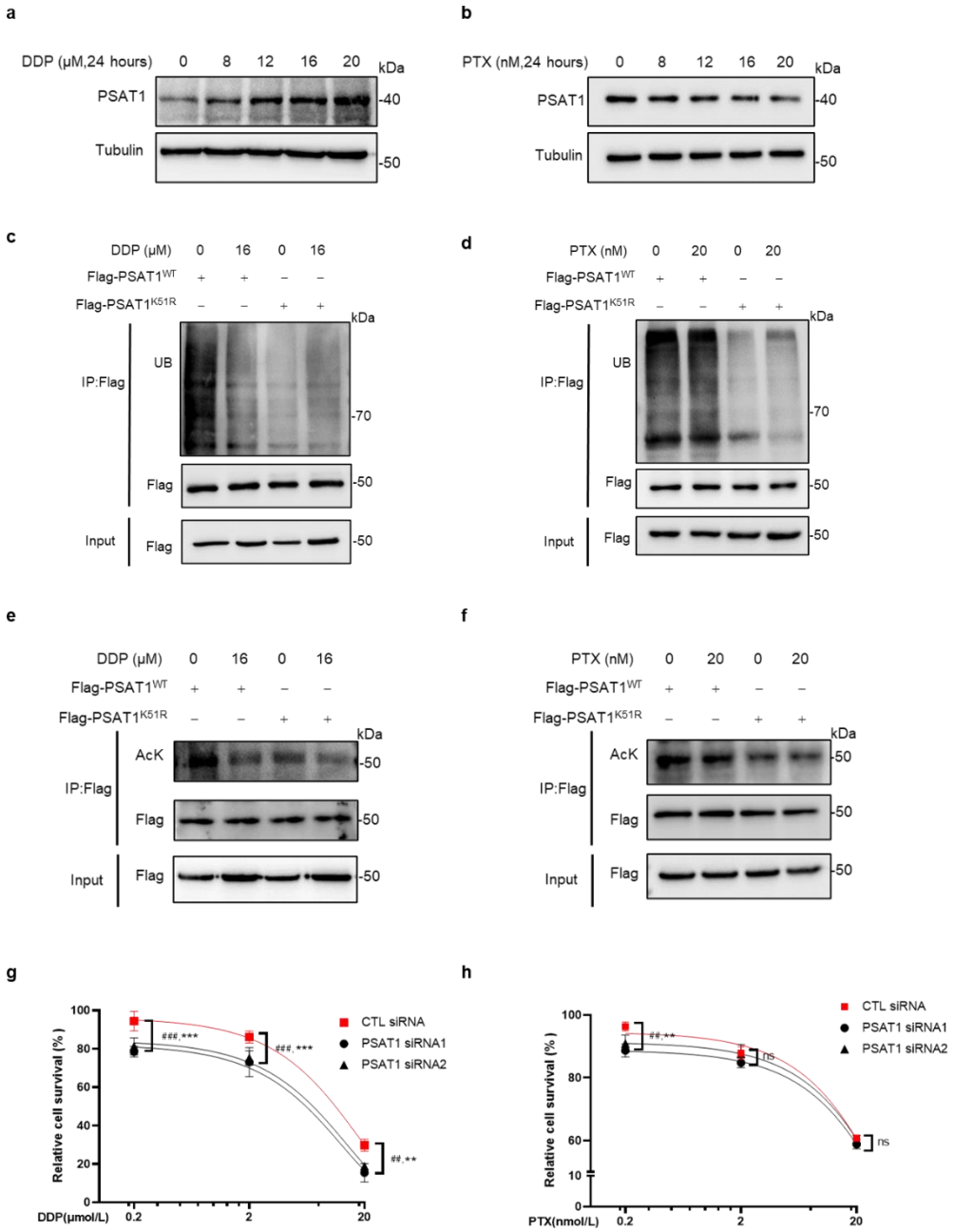
Response:

In order to clarify if the effect of cisplatin on acetylation and ubiquitination of PSAT1 was unique, we used another type of chemotherapy drug—paclitaxel (PTX), which targets the microtubule cytoskeleton. We found that PTX treatment did not have significant effects on the acetylation and ubiquitination of PSAT1. Knocking down PSAT1 also did not increased the sensitivity of cancer cells to PTX treatment. This indicated that the effects on acetylation and ubiquitination of PSAT1 might be unique to platinum-based drugs, which led to DNA damage.

(pages 19-20, lines 380-404)

Cisplatin (DDP) and paclitaxel (PTX) are two first-line chemotherapy drugs for the clinical treatment of non-small cell lung cancer (NSCLC). Mechanically, as a platinum chemotherapeutic agent, DDP can enter cells to cause DNA cross-linking, leading DNA damage. Unlike DDP, PTX targets the microtubule cytoskeleton, inducing cell cycle arrest and apoptosis. To investigate the effects of PSAT1 on chemotherapy treatment, we first measured the protein expression of PSAT1 when treating cells with different concentrations of DDP or PTX. Supplementary Fig. 7a, b showed that PSAT1 protein levels significantly increased in a DDP concentration dependent manner, while the protein levels slightly decreased with PTX treatment. We subsequently tested whether these chemotherapy drugs regulated the acetylation and ubiquitination levels of PSAT1. Supplementary Fig. 7c-f showed that DDP treatment had no significant effect on the acetylation and ubiquitination levels of PSAT1-K51R cells, whereas it led to a remarkable decrease in PSAT1-WT cells. We also found that PTX treatment did not alter the acetylation and ubiquitination levels. These results indicated that DDP affected PSAT1 expression through regulating the interplay between acetylation and ubiquitination, while PTX did not have these effects. Then, we tested if PSAT1 expression affected the therapeutic effect of DDP or PTX on lung cancer cells. Knocking down PSAT1 increased the sensitivity of cancer cells to DDP treatment rather than PTX (Supplementary Fig. 7g, h). Thus, we speculated that the different anticancer mechanism between DDP and PTX led to the unique effects for PSAT1. These results exhibit the potential application of PSAT1 as a therapeutic target for cancer treatment.

Supplementary Fig. 7



v "…pre-tested using the biomarkers proven", what biomarkers? NAM is used in high millimolar concentration that is not pharmacologically relevant.

Response:

The biomarkers indicated the acetylated proteins affected by NAM or TSA that have been proven by other studies. We detected the acetylation level of ACLY in cells

treated with NAM and the acetylation level of STAT3 in cells treated with TSA [1, 2]. The NAM treatment significantly increased the acetylation of ACLY, and TSA treatment elevated the acetylation of STAT3 (Supplementary Fig. 1a, b), indicating that both treatments were effective.

The millimolar doses of NAM were commonly used in studies focusing on acetylation [3-5].

Ref. 1> Zhou, Feifei et al. "ARHGEF3 regulates the stability of ACLY to promote the proliferation of lung cancer." Cell death & disease vol. 13,10 870. 14 Oct. 2022

Ref. 2> Zhong, Yu et al. "The HDAC10 instructs macrophage M2 program via deacetylation of STAT3 and promotes allergic airway inflammation." Theranostics vol. 13,11 3568-3581. 19 Jun. 2023

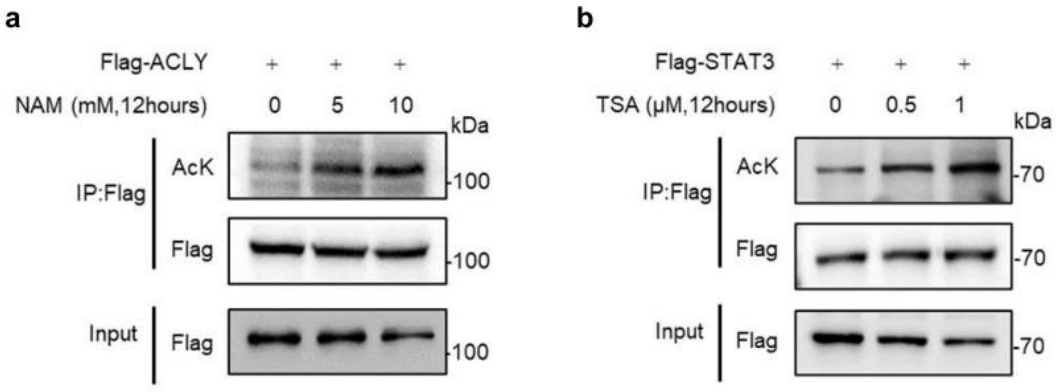
Ref. 3> Lin, Li et al. "Role of SIRT1 in Streptococcus pneumoniae-induced human β-defensin-2 and interleukin-8 expression in A549 cell." Molecular and cellular biochemistry vol. 394,1-2 (2014): 199-208

Ref. 4> Francesca Scatozza, et al. "Nicotinamide inhibits melanoma in vitro and in vivo." J Exp Clin Cancer Res. 2020 Oct 7;39(1):211.

Ref. 5> Zhang Y, et al. "Nicotinamide promotes pancreatic differentiation through the dual inhibition of CK1 and ROCK kinases in human embryonic stem cells." Stem Cell Res Ther. 2021 Jun 25;12(1):362.

(page 6, lines 97-99)

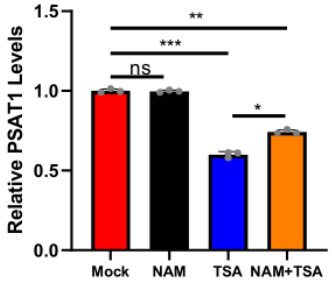
The treatment efficiencies of the inhibitors were pre-tested using the biomarkers proven by other studies (Supplementary Fig. 1a, b).



v Figure 1A: is the difference between TSA and TSA+NAM statistically significant?

Response:

According to your advice, we analyzed the difference between TSA and TSA+NAM. Fig. 1a showed that there were statistically significant between TSA and TSA+NAM.



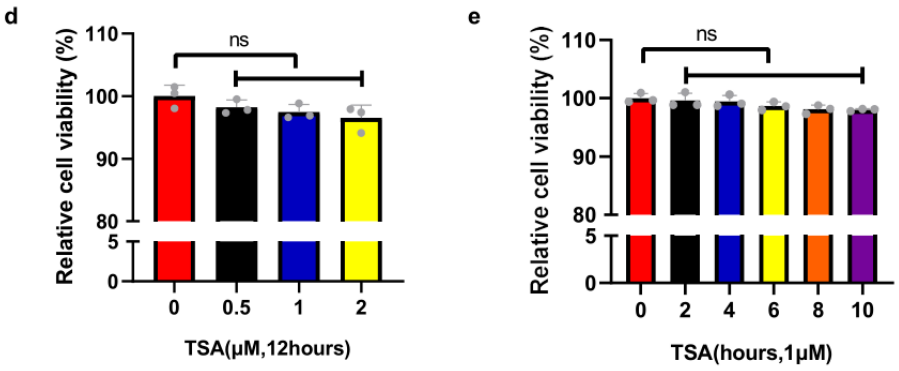
v Figures 1D: please provide cell viability data with increasing time and concentration of TSA. The decrease in PSAT1 may be due to dead cells.

Response:

According to your advice, we detected the cell viability with increasing time and concentration of TSA. Supplementary Fig. 1d and 1e showed that the cells could tolerate TSA treatment well under these conditions.

(page 7, lines 112-114)

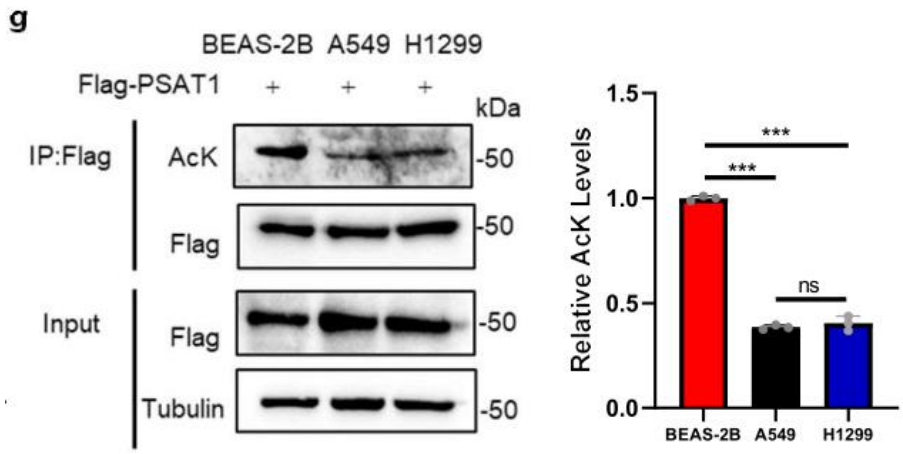
The cell viability was also determined under these conditions and Supplementary Fig. 1d, e showed that these treatments did not affect the cell viability significantly.



v Figure 1G: Needs densitometry and statistical analysis. The difference between A549 and H1299 with BEAS does not appear to be significant. Moreover, was it a difference between two lung cancer cells?

Response:

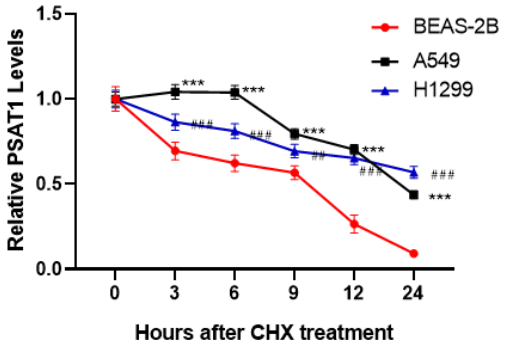
According to your advice, we made statistical analysis on PSAT1 acetylation between A549 and H1299 with BEAS-2B based on the results of three independent experiments. Fig. 1g showed that the acetylation levels of PSAT1 in A549 and H1299 cells were significantly lower than that in BEAS-2B cells, but there were no differences between A549 and H1299 cells.



v Figure 1H (particularly H1299) does not support this conclusion: "We next examined the degradation rates of PSAT1 in these cells and found that the degradation of PSAT1 was attenuated in LUAD cells (Figure 1H), suggesting that acetylation affected PSAT1 protein stability."

Response:

In Fig. 1i, we made statistical analysis and the result showed that the degradation rates of PSAT1 in BEAS-2B cells were faster than that in LUAD cells.



v Figure 1I: please provide the rationale for the doses of MG132 and chloroquine being 20 and 10 micromolar? Does chloroquine at 10 micromolar effectively inhibit autophagy?

Response:

The doses of MG132 and chloroquine used in our study were based on previous studies [1-3]. In Supplementary Fig. 1g, we measured the autophagy inhibition effect of CQ (10 μM) by testing the autophagy-related proteins expression levels.

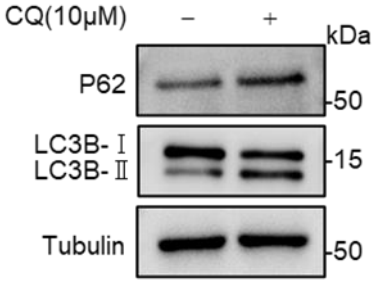
Ref. 1> Liu, Xiangguo et al. "Cellular FLICE-inhibitory protein down-regulation contributes to celecoxib-induced apoptosis in human lung cancer cells." Cancer research vol. 66,23 (2006): 11115-9.

Ref. 2> Datta, Satabdi et al. "Autophagy inhibition with chloroquine reverts paclitaxel resistance and attenuates metastatic potential in human nonsmall lung adenocarcinoma A549 cells via ROS mediated modulation of β-catenin pathway." Apoptosis vol. 24,5-6 (2019): 414-433.

Ref. 3> Lu, Yu-Ting et al. "Sulfuretin protects hepatic cells through regulation of ROS levels and autophagic flux." Acta pharmacologica Sinica vol. 40,7 (2019): 908-918.

(page 8, lines 135-137)

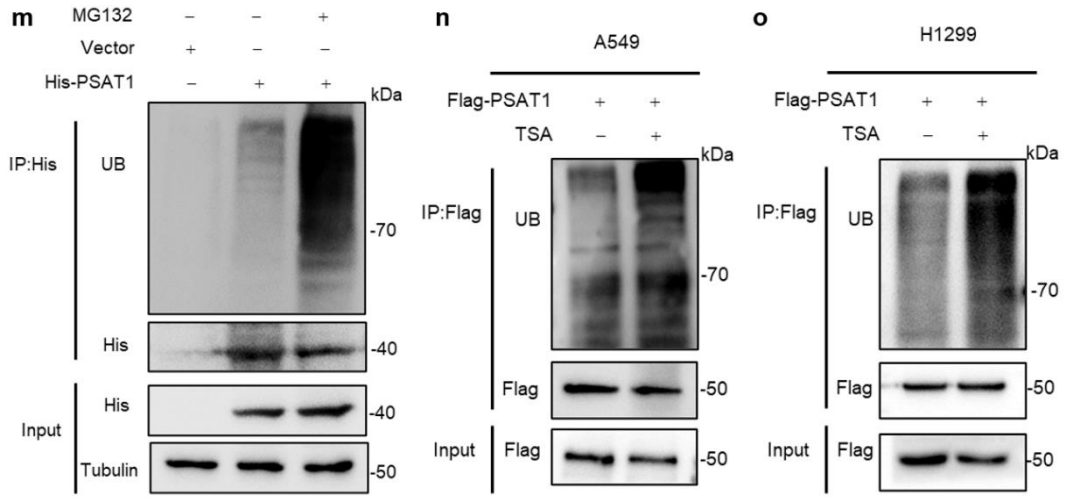
The treatment efficiency of CQ was pre-tested using the autophagy-related proteins by western blot (Supplementary Fig. 1g).



v Figures 1K-1M: the quality of Western blots are poor.

Response:

According to your advice, we performed these experiments again. The clearer results could be seen in Fig .1m-o.



v Is the effect of HDAC7 on PSAT1 exclusive? What is its effect on some other proteins (e.g. PHGDH, ASNS, ATF4, etc) involved in amino acid metabolism?

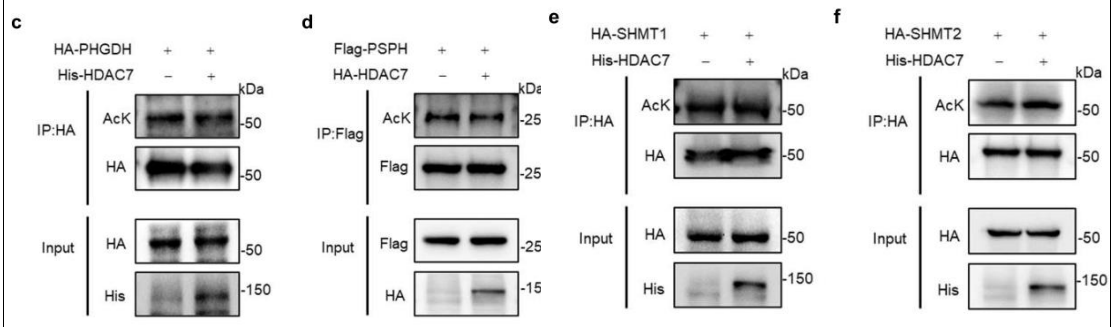
Response:

In the revised manuscript, we also detected the effects of HDAC7 on the acetylation of other enzymes in serine metabolism. The results showed that HDAC7 did not

affected the acetylation of other enzymes in serine metabolism like PHGDH, PSPH and SHMT1/2 (Supplementary Fig. 2c-f). Thus, the deacetylation induced by HDAC7 was unique for PSAT1 in serine metabolism.

(page 9, lines 160-164)

Then we detected the deacetylation efficiencies of some other proteins involved in serine synthesis by HDAC7 overexpression. Supplementary Fig. 2c-f showed that HDAC7 did not affect the acetylation levels of PHGDH, PSPH or serine hydroxymethyltransferase1/2 (SHMT1/2).



v Please explain the process of choosing USP14 from your mass spec data "we performed mass spectrometry analysis and identified USP14 as a putative PSAT1-interacting protein (Supplemental Table 1)."

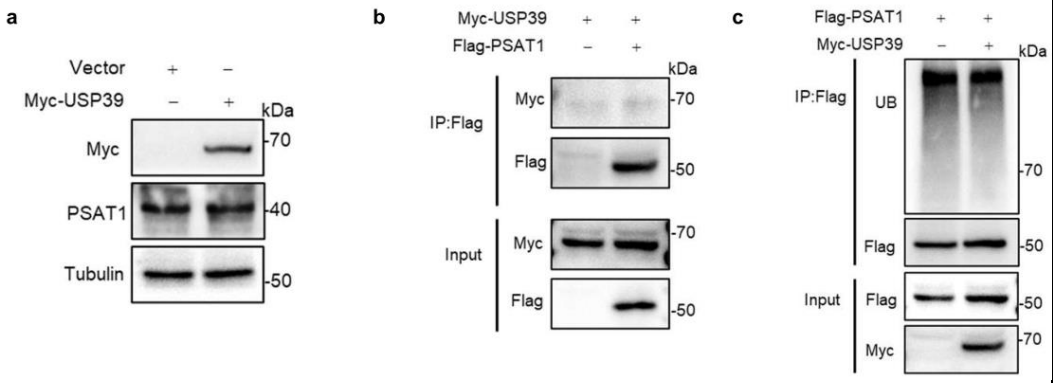
Response:

In our mass spectrometry data, we found two deubiquitinases USP14 and USP39. We demonstrated that USP39 did not interact with PSAT1 and regulate the protein or ubiquitination levels of PSAT1. However, USP14 interacted with PSAT1 and regulated its protein stability. Thus, we selected USP14 for further study. We have added these results in our revised manuscript.

(page 10, lines 190-197)

To explore the potential E3-ligases or deubiquitinases for PSAT1, we performed mass spectrometry analysis. Ultimately, two proteins of DUBs, named USP39 and USP14, were identified as a putative PSAT1-interacting protein (Supplementary data 1). Supplementary Fig. 3a-c showed USP39 could not interact with PSAT1 and regulate the protein or ubiquitination levels of PSAT1.

Supplementary Fig. 3



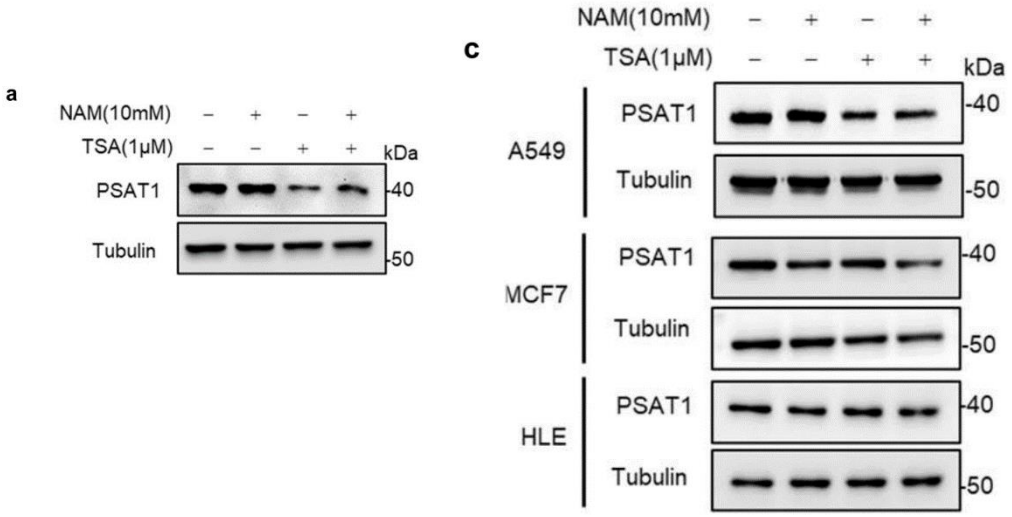
v Is the HDAC7-USP14-PSAT1 axis exclusive for lung adenocarcinoma or is it a universal pathway in several cancer cells?

Response:

According to your advice, we detected the effects of NAM and TSA in LUAD cells (H1299 and A549), breast cancer cells (MCF7) and hepatocellular carcinoma cells (HLE). Fig. 1a and Supplementary Fig. 1c showed that TSA treatment only decreased PSAT1 expression in LUAD cells, but not in breast cancer and liver cancer cells. These results indicated that HDAC7 did not regulate the expression of PSAT1 in other cancer cells, at least in breast cancer and liver cancer cells. Thus, we think that the HDAC7-USP14-PSAT1 axis is unique for LUAD cells.

(page 6, lines 99-104)

The protein expression of PSAT1 was significantly decreased when treating cells with TSA but not NAM in lung cancer cells, however these effects were not observed in breast cancer cell (MCF7) or hepatocellular carcinoma cell (HLE) (Fig. 1a and Supplementary Fig. 1c), suggesting that the phenomenon was specific to LUAD cells.



Minor issues

v The source of Serine is more complicated than "Serine can be obtained from various sources: it can be taken up directly from the extracellular environment, derived from intracellular protein via autophagy, or synthesized de novo via the serine synthesis pathway (SSP)." Please see Hameed KM et al. Front Oncol. 2024 Apr 16;14:1326754.

Response:

According to your advice, we have improved the description of this section.

Ref. 1> Hameed, Kanwal M et al. "Dual targeting of glutamine and serine metabolism in acute myeloid leukemia." Frontiers in oncology vol. 14 1326754. 16 Apr. 2024, doi:10.3389/fonc.2024.1326754

(page 3, lines 46-49)

Serine can be obtained from various sources: it can be taken up directly from the extracellular environment, derived from intracellular protein via autophagy,

produced by glycine conversion or synthesized de novo via the serine synthesis pathway (SSP).

ν All abbreviations should be defined when used for the first time: PSAT1 (line 68), EGFR (line 81), ROS (line 82), GSK3 (line 84), ATF4 (line 89), NRF2 (line 90), LUAD (line 104), HDAC7 (line 106), USP14 (line 106), UBE4B (line 108), HDAC (line 123)

Response:

According to your advice, all abbreviations was defined when used for the first time.

ν This is sentence does not appear to be scientific: "PSAT1 is closely associated with the occurrence, development, treatment, and prognosis of various tumors." How PSAT1 is associated with treatment?!

Response:

There are currently no reports proving that PSAT1 is associated with treatment. We have deleted "treatment" in this sentence.

ν What does this sentence mean "Acetylation plays an important role in cancer metabolism." One can argue that methylation, sulfation, phosphorylation, etc. plays an important role in cancer metabolism and other hallmarks of cancer. Please refrain from general statements in the article that do not provide any specific information.

Response:

According to your advice, we have corrected similar statements in the revised manuscript.

ν Figures 6J, 6K are not readable.

Response:

Thank you for your advice, we made appropriate changes to make the figures clearer. (Fig. 6j, 6k and Supplementary Fig. 5).

v The authors should provide comments in Discussion on how this discovery can be exploited for treating patients with NSCLC and potentially other cancers?

Response:

According to your advice, we have provided the related comments in Discussion part of the revised manuscript.

(page 26-27, lines 520-534)

Given that PSAT1 is a key enzyme in de novo serine synthesis, which provides important cellular metabolic precursors for biosynthetic metabolism, we examined the effects of PSAT1 acetylation on the serine synthesis pathway. In our study, we found that acetylation of PSAT1 on Lysine 51 regulates serine metabolism and proliferation of LUAD cells. Importantly, the chemotherapy drug DDP could increase PSAT1 expression in LUAD cells by decreasing its acetylation on K51 and enhancing its protein stability. DDP is a DNA-damaging agent. We found PSAT1 could regulate nucleotides synthesis which was required for DNA biosynthesis. Knocking down PSAT1 increased the sensitivity of LUAD cells to DDP. However, treating LUAD cells with PTX, another chemotherapy drugs targeting the microtubule cytoskeleton, did not show this effect. These results indicated that inhibiting the function of PSAT1 is an effective strategy to enhance the therapeutic effect of chemotherapy drugs for DNA damage.

Reviewer #2 (Remarks to the Author):

The manuscript by Dr Han and colleagues explores the post-translational regulation by acetylation and its interplay with ubiquitination of the metabolic enzyme PSAT1 in the context of lung adenocarcinoma. The authors show that PSAT1 acetylation is linked to its protein stability. They identified that PSAT1 acetylation promotes its ubiquitination by the E3 ligase UBE4B, leading to its proteasomal degradation. On the other side of this post-translational regulation mechanism, HDAC7 deacetylates PSAT1 promoting the recruitment of USP14, that then deubiquitinates PSAT1 resulting to the

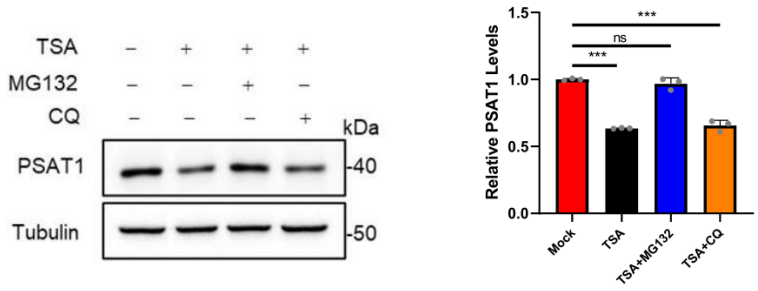
increase of its protein stability. This stabilization of PSAT1 seems to have an effect in the serine metabolism and cell proliferation of lung cancer cells. Overall, this is a convincing work, the manuscript is clear and well-written, the experiments are sound, the authors provide the necessary controls and their conclusions are not overstated.

I have the following comments:

1. Line 157: MG132 treatment also reversed the decreased expression of PSAT1 caused by TSA treatment (Figure 1J). This is not reflected clearly in the provided blot, should provide a quantification graph.

Response:

Thank you very much for your comments on our manuscript. According to your advice, we made statistical analysis based on the results of three independent experiments. The result showed that MG132 treatment reversed the decreased expression of PSAT1 caused by TSA treatment (Fig.1l).



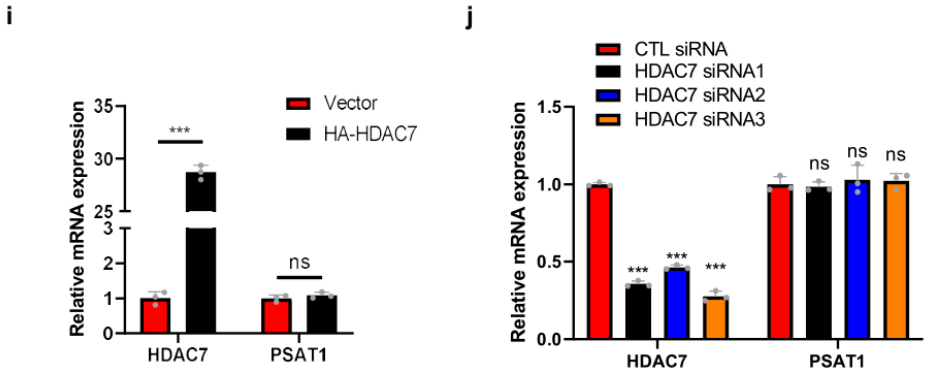
2. To explore the role of HDAC7 in PSAT1 protein expression, we overexpressed HDAC7 in H1299 cells and PSAT1 expression was examined. The result showed that the protein expression of PSAT1 increased significantly (Figure 2M). To further validate the above results, we transfected the HDAC7 specific siRNAs into H1299 cells followed by dectecting PSAT1 expression. Knocking down HDAC7 reduced PSAT1 expression (Figure 2N). What is the effect of overexpression/knock down of HDAC7 on the mRNA levels of PSAT1?

Response:

According to your advice, we detected the mRNA level of PSAT1 in cells with HDAC7 overexpression/knockdown. Supplementary Fig. 2i and 2j showed that HDAC7 overexpression/knockdown did not affected the mRNA level of PSAT1.

(page 9, lines 175-176)

Supplementary Fig. 2i, j showed HDAC7 did not affect the transcription of PSAT1.



3. Figure 6A showed that the acetylation level of PSAT1-K51R was reduced compared with PSAT1-WT. The blot provided here is not very convincing. An additional in vitro acetylation assay using a recombinant wt and mutant PSAT1 might be a good option for a stronger clarification. Also, an immunofluorescence experiment showing the localization of the PSAT1 wt and mutants should be added as a supplementary figure.

Response:

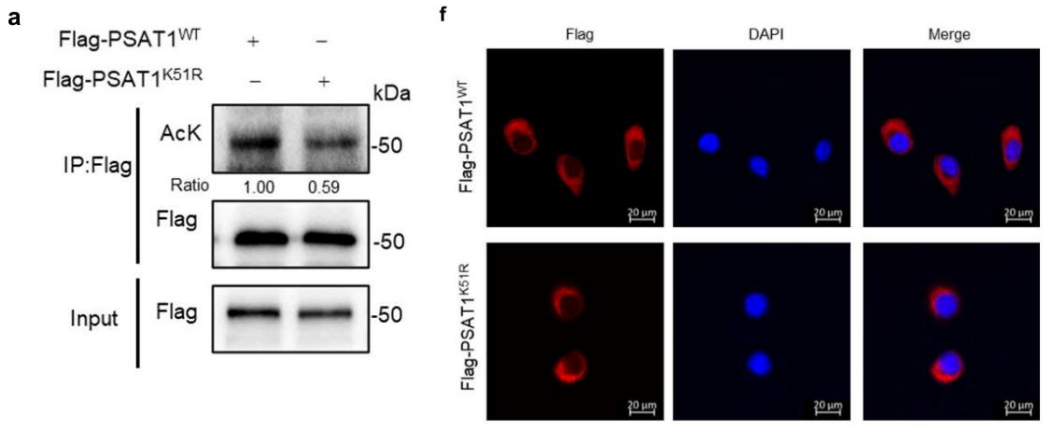
According to your advice, we performed this experiment again and the intensity of protein acetylation was analyzed. The clearer results could be seen in Fig. 6a.

In our study, we did not investigate the acetyltransferase of PSAT1. Thus, it is difficult for us to perform in vitro acetylation assay using the recombinant PSAT1-WT and PSAT1-K51R.

We also detected the localization of PSAT1-WT and PSAT1-K51R using immunofluorescence. Supplementary Fig. 4f showed that both PSAT1-WT and PSAT1-K51R located in cytoplasm and no significant changes were observed.

(page 16, lines 324-327)

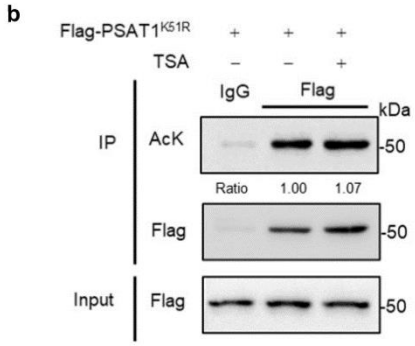
The immunofluorescence assay was performed and Supplementary Fig. 4f demonstrated that the localization of PSAT1-WT and PSAT1-K51R was consistent, both located in the cytoplasm.



4. Neither TSA treatment or HDAC7 overexpression affected the acetylation of PSAT1-K51R, indicating that PSAT1-K51R might be the main acetylation site (Figure 6B and C). In Figure 6B seems like there is a slight increase in the acetylation levels of PSAT1-K51R?

Response:

According to your advice, we performed the experiment in Fig.6b again and analyzed the intensity of protein acetylation. The results showed that the acetylation levels of PSAT1 remained almost unchanged in Fig. 6b.



5. In text corrections:

- Line 188:… dectecting PSAT1 expression. Knocking down HDAC7 reduced PSAT1…. (detecting)

- Line 250: … detected the ubiquCitin chain types using antibodies specific for…. (ubiquitin)

- Line 408:… and EV4A-C showed that overexpressing UBE4B reduced the protein… (Figure S4A-C)

- Line 426:… and EV4F revealed that acetylation affected the ubiquitination of… (Figure S4F)

- Figures 6J and 6K are unreadable: Should get bigger sized letters

Response:

Thank you for your advice, we made appropriate changes to make the figures clearer. (Fig. 6j, 6k and Supplementary Fig. 5). Meanwhile, according to your advice, we have made these corrections in the revised manuscript.

Reviewer #3 (Remarks to the Author):

Overall, the manuscript by Liu, Han, et al is a very interesting, comprehensive (dense) and well-written piece of work. The authors are very methodical in their experiments and present multiple lines of evidence for most conclusions. I have very few minor issues and no real major issues with this work.

Minor concerns:

-The only grammatical error i found is on line 250 "ubiquCitin".

Response: Thank you very much for your comments on our manuscript. We have corrected this error in our revised manuscript.

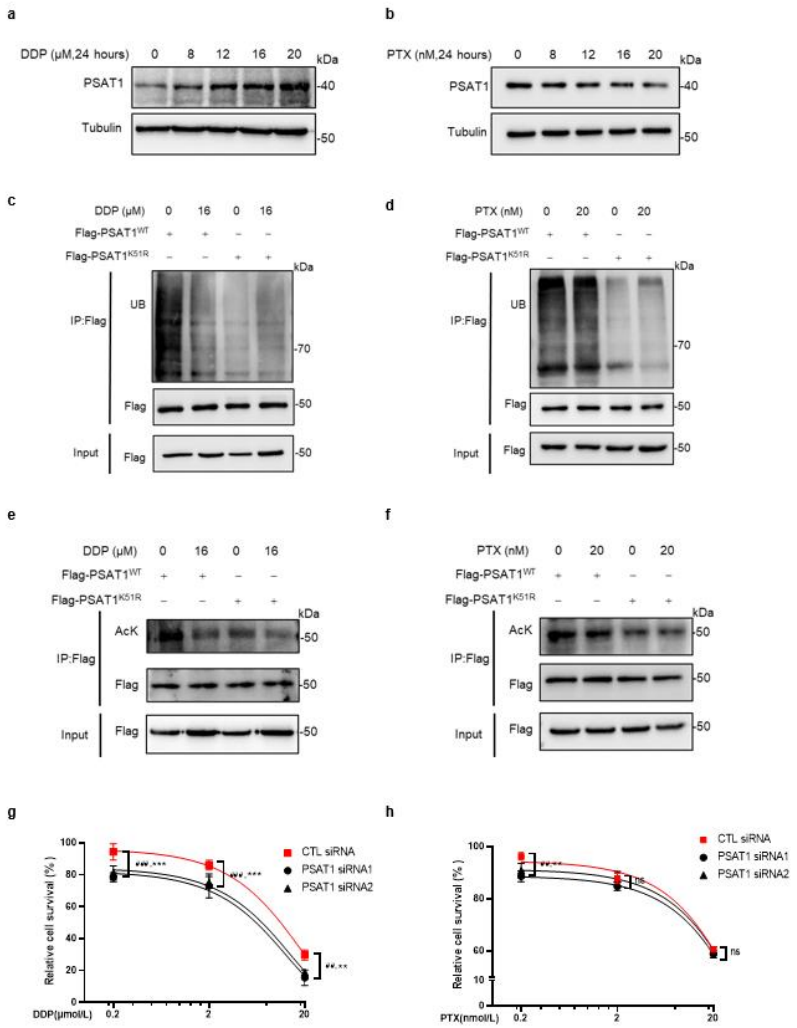
-I feel that the cisplatin portion of the work takes away from the overall mechanistic and biochemical theme of the work. all the cisplatin work is buried in supplemental and

I feel that if it was removed it wouldn't diminish the work.

Response:

Thank you for your advice. Clarifying the relationship between PSAT1 and lung cancer treatment is of great significance. Chemotherapy is one of the main treatment methods for lung adenocarcinoma, and platinum-based drugs combined with paclitaxel are commonly used first-line chemotherapy drugs. To preliminarily clarify the clinical significance of PSAT1 acetylation, we chose cisplatin and paclitaxel to test their effects on PSAT1 acetylation, and detected the effects of PSAT1 on drugs sensitivity. We believe that this may provide potential assistance for the clinical treatment of lung adenocarcinoma, so we retained these results in our manuscript.

Supplementary Fig. 7



Major-ish concern:

-My only slight major concern is that, with the exception of some very early experiments, all work is done in a single cell line. the authors do present 2 other cell lines in figure 1, however the rigor and reproducibility of the work would be strengthened by showing a few other very key findings in another cell line.

Response:

According to your advice, we repeated the key experiments in A549 cell lines (Supplementary Figures). Similar results were observed in both H1299 and A549 cell lines.