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## PEER-REVIEW REPORT

**Name of journal:** *World Journal of Stem Cells*

**Manuscript NO:** 112278

**Title:** Hypoxia facilitates triple-negative breast cancer stem cells enrichment and stemness maintenance through oxidized ataxia telangiectasia mutated-induced one-carbon metabolism

**Provenance and peer review:** Unsolicited manuscript; Externally peer reviewed

**Peer-review model:** Single blind

**Reviewer's code:** 05781374

**Position:** Peer Reviewer

**Academic degree and professional title:** Associate Professor, Professor

**Reviewer's Country/Territory:** China

**Author's Country/Territory:** China

**Manuscript submission date:** 2025-07-22

**Reviewer chosen by:** AI Editor

**Reviewer accepted review:** 2025-07-28 01:59

**Reviewer performed review:** 2025-07-29 12:08

**Review time:** 1 Day and 10 Hours

<b>Content to be reviewed</b>	Are all references related to the topic of the manuscript? <b>Yes</b> Does the manuscript's content fall within the scope of the journal? <b>Yes</b> Does the Abstract contain the contents of each part of the manuscript (IMRaD)? <b>Yes</b> Is there any Key Word that is not included in the manuscript title? <b>No</b>
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Do authors' affiliations correspond to the content of the manuscript? **Yes**

Are the Key Words complete? **Yes**

Is the content of the Introduction adequate? **Yes**

Is the content of the Materials and Methods complete?

**No**

Is the description of the experiments clear and complete? **No**

Are the experimental data presented in the manuscript's biostatistics content reliable? **Yes**

Are the experimental data of the Results true and reliable? **Yes**

Are the quality and resolution of the images up to standard? **No**

Do the selection and design of the figures and tables follow the principles of necessity and clarity? **No**

Is there any duplication between various parts of the manuscript and between the main text and the content presented in the figures and tables? **No**

Are the figures and tables numbered consecutively in the order in which they appear in the manuscript? **Yes**

Is the content of the Discussion reasonable? **Yes**

Is the Conclusion reasonable? **Yes**

Are all references necessary and reasonable? **Yes**

Do authors omit important references? **Yes**

Do authors only cite their own earlier publications? **No**

Is the manuscript's text correct, concise, and clear? **No**

Will the manuscript's content be of interest to readers?  
**Yes**

Are additional experiments needed for the study? **Yes**



	Does the research scope comply with ethics? <b>Yes</b>
<b>Scientific quality</b>	Grade B (Very good)
<b>Novelty of this manuscript</b>	Grade B (Very Good)
<b>Creativity or innovation of this manuscript</b>	Grade B (Very Good)
<b>Scientific significance of the conclusion in this manuscript</b>	Grade B (Very Good)
<b>Language quality</b>	Grade C (Good)
<b>Does this manuscript describe a study of the existing knowledge system?</b>	Yes
<b>Does this manuscript report a revolutionary innovation?</b>	No
<b>Does this manuscript report an unconventional innovation?</b>	No
<b>Conclusion</b>	Major revision
<b>Re-review</b>	No
<b>Peer-reviewer statements</b>	Peer-Review: Anonymous
	Conflicts-of-Interest: No
<b>Are your review comments generated by AI tools?</b>	No

### SPECIFIC COMMENTS TO AUTHORS

This manuscript investigates a novel mechanism by which intratumoral hypoxia promotes enrichment and maintenance of triple-negative breast cancer stem cells (TNBC-CSCs) via oxidation-activated ATM and reprogramming of one-carbon metabolism. The study is timely and addresses an important gap in understanding how tumor microenvironmental cues regulate CSC metabolism and stemness. Strengths include the integration of in vivo and in vitro models, use of both metabolic profiling



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and functional rescue experiments, and the identification of a clear signaling axis (oxidized ATM → c-Myc → SHMT2/MTHFD2). However, the manuscript would benefit from more rigorous quantitative reporting (e.g., fold-changes, n-values), clearer methodological details (oxygen tension settings, replicates), and a more concise framing of the clinical implications. Overall, with these improvements, the work will significantly advance the field of cancer metabolism and CSC biology.

#### Abstract

The opening sentences repeat background that can be shortened. For example, merge “Tumor stem cells...significant challenge” with “Intratumoral hypoxia...solid tumors” into one brief statement.

Quantitative data are absent. Please include key fold-changes (e.g., percentage increase in mammosphere-forming efficiency under hypoxia) and statistical significance to give the reader immediate sense of your major findings.

The phrase “oxidized ATM” is introduced without definition. Consider rephrasing: “hypoxia-induced ROS triggers ATM oxidation (p-ATM) independent of DNA breaks.”

Replace “dryness” with “stemness” – this appears to be a translation error.

#### Introduction

The transition from general TNBC clinical challenges to CSC metabolism is abrupt. Insert a brief paragraph bridging hypoxia, ROS, and metabolic reprogramming before introducing one-carbon metabolism.

Many in-text citations still read “ADDIN EN.CITE.” Please update these to journal style (e.g., “[3,4]”).

End the Introduction with an explicit, single-sentence hypothesis, for example: “We hypothesize that hypoxia-activated, oxidized ATM promotes TNBC-CSC stemness by upregulating c-Myc-mediated MTHFD2/SHMT2 expression and enhancing one-carbon metabolism.”



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## Materials & Methods

**Cell Culture:** Specify exact oxygen tensions (e.g.,  $1.0 \pm 0.1\% \text{ O}_2$ ) and how oxygen was controlled/monitored.

**shRNA Knockdown:** Report knockdown efficiency (mRNA or protein %) for each target.

**Mammosphere Assay:** Clarify how many biological replicates and independent experiments were performed. Indicate how MFE was calculated (e.g.,  $\text{MFE} = \text{number of spheres formed} / \text{number of cells plated} \times 100\%$ ).

**Statistical Analysis:** State which test (e.g., ANOVA with Tukey's post-hoc) was used for multi-group comparisons, and how normality was assessed.

## Results

In Fig 1A-B, please report exact percentages of  $\text{CD44}^+/\text{CD24}^-$  cells (mean  $\pm$  SD) and fold-change in stem gene expression, rather than only saying "significantly increased."

Indicate n-values in each bar graph legend (e.g.,  $n = 3$  independent assays).

Include a non-CSC control (e.g., parental adherent cells) to demonstrate specificity of KU60019 effects.

Present time-course of ATM phosphorylation under hypoxia vs.  $\text{H}_2\text{O}_2$  to reinforce the DNA-damage independence of oxidized ATM.

In Fig 3B-C, provide enrichment scores or p-values for key pathways (serine/glycine vs. purine metabolism).

Explicitly note which metabolites (e.g., 3-phosphoserine, formate) were most altered to strengthen the interpretation.

In your luciferase assays, report fold-activation and whether mutation of c-Myc sites abolished activity.

For ChIP, include input controls and enrichment relative to IgG.

The "one-carbon metabolite backfill" is critical. Please specify which metabolites (glycine, formate, etc.) were used and their concentrations.



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#### Discussion

More critically contrast your findings with existing studies on hypoxia-driven one-carbon metabolism in CSCs (e.g., Samanta & Semenza, 2016).

Acknowledge that mammosphere assays, while informative, do not fully recapitulate in vivo CSC behavior. Suggest future orthotopic xenograft experiments testing ATM or SHMT2 inhibition.

Briefly discuss how ATM inhibitors might synergize with antifolate drugs in TNBC treatment, given the role of one-carbon metabolism.

#### Figures & Tables

Ensure all abbreviations are defined (e.g., "MFE," "p-ATM S1981").

Increase resolution of immunoblots; show full-length blots in Supplementary.

Use consistent symbols (e.g.,  $p < 0.05$ ,  $*p < 0.01$ ) and define them in each legend.



## PEER-REVIEW REPORT

**Name of journal:** *World Journal of Stem Cells*

**Manuscript NO:** 112278

**Title:** Hypoxia facilitates triple-negative breast cancer stem cells enrichment and stemness maintenance through oxidized ataxia telangiectasia mutated-induced one-carbon metabolism

**Provenance and peer review:** Unsolicited manuscript; Externally peer reviewed

**Peer-review model:** Single blind

**Reviewer's code:** 08328525

**Position:** Peer Reviewer

**Academic degree and professional title:** Associate Professor, PhD

**Reviewer's Country/Territory:** Türkiye

**Author's Country/Territory:** China

**Manuscript submission date:** 2025-07-22

**Reviewer chosen by:** Jia-Lin Zhang

**Reviewer accepted review:** 2025-07-31 07:01

**Reviewer performed review:** 2025-08-10 11:23

**Review time:** 10 Days and 4 Hours

<b>Content to be reviewed</b>	Do authors omit important references? <b>Yes</b> Are all references necessary and reasonable? <b>Yes</b> Are all references related to the topic of the manuscript? <b>Yes</b> Do authors only cite their own earlier publications? <b>No</b> Is the manuscript's text correct, concise, and clear? <b>Yes</b> Will the manuscript's content be of interest to readers? <b>Yes</b>
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Are additional experiments needed for the study? **No**

Does the research scope comply with ethics? **Not  
Applicable**

Does the manuscript's content fall within the scope of  
the journal? **Yes**

Is there any Key Word that is not included in the  
manuscript title? **Yes**

Do authors' affiliations correspond to the content of the  
manuscript? **Yes**

Does the Abstract contain the contents of each part of  
the manuscript (IMRaD)? **Yes**

Are the Key Words complete? **Yes**

Is the content of the Introduction adequate? **Yes**

Is the content of the Materials and Methods complete?  
**Yes**

Is the description of the experiments clear and  
complete? **No**

Are the experimental data presented in the  
manuscript's biostatistics content reliable? **Yes**

Are the experimental data of the Results true and  
reliable? **Yes**

Are the quality and resolution of the images up to  
standard? **Yes**

Do the selection and design of the figures and tables  
follow the principles of necessity and clarity? **Yes**

Is there any duplication between various parts of the  
manuscript and between the main text and the content  
presented in the figures and tables? **No**

Are the figures and tables numbered consecutively in  
the order in which they appear in the manuscript? **Yes**



	Is the content of the Discussion reasonable? <b>Yes</b> Is the Conclusion reasonable? <b>Yes</b>
Scientific quality	Grade C (Good)
Novelty of this manuscript	Grade C (Good)
Creativity or innovation of this manuscript	Grade C (Good)
Scientific significance of the conclusion in this manuscript	Grade C (Good)
Language quality	Grade C (Good)
Does this manuscript describe a study of the existing knowledge system?	No
Does this manuscript report a revolutionary innovation?	No
Does this manuscript report an unconventional innovation?	Yes
Conclusion	Minor revision
Re-review	Yes
Peer-reviewer statements	Peer-Review: Anonymous
	Conflicts-of-Interest: No
Are your review comments generated by AI tools?	No

### SPECIFIC COMMENTS TO AUTHORS

This manuscript presents a study on the mammosphere formation efficiency (MFE) of Hs578T and MDA-MB-231 breast cancer cells under hypoxic conditions. The authors use well-established protocols to generate and quantify mammospheres and evaluate self-renewal potential over serial passages. The topic is relevant and timely, particularly for understanding cancer stem-like properties in aggressive breast cancer subtypes.



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The introduction provides a relevant and timely perspective on the challenge of cancer stem cells (CSCs) in the context of triple-negative breast cancer (TNBC). The authors present a clear hypothesis: that hypoxia-induced oxidized ATM promotes stemness via c-Myc-driven metabolic reprogramming, specifically through the one-carbon metabolic enzymes MTHFD2 and SHMT2. This is a potentially novel and mechanistically important area of investigation, especially given the clinical difficulty in treating TNBC. The manuscript addresses a clinically unmet need understanding mechanisms of TNBC recurrence and resistance driven by CSCs. It proposes a novel mechanistic axis (oxidized ATM -Myc → MTHFD2) to understanding hypoxia, DNA damage signaling, and metabolic reprogramming. The methodology is generally well-described, and the manuscript provides valuable data for the field of cancer stem cell research. However, several aspects of the manuscript require clarification or improvement before it is suitable for publication.

-Tumor stem cells (CSC) should be Cancer stem cells (CSCs)

-TNBC-CSCS should be TNBC-CSCs

-Was antibiotic supplementation used? (e.g., penicillin/streptomycin)

-There are unnecessary repetitions.

- Hypoxia conditions should be defined first and then referred to simply as "hypoxia." Hypoxia (1% O<sub>2</sub>) is repeated three times:

- "in hypoxia (1% O<sub>2</sub>)"
- "under hypoxia (1% O<sub>2</sub>)"
- "under hypoxic conditions with an oxygen level of 1%"

-While the source of lentiviral vectors is provided, details about the transduction protocol are missing. Please specify:

- MOI
- Transduction reagents



- Selection method
  - Duration of knockdown verification
- The promoter regions of MTHFD2 and SHMT2 were cloned into pGL3-basic. Please provide:
- The genomic coordinates of the cloned regions.
  - The restriction enzyme sites used for cloning.
  - Whether the sequence was verified by Sanger sequencing.
- Please be consistent with units and formatting (e.g., write “ $\mu\text{g}$ ” instead of “ug”, and “ $\mu\text{L}$ ” instead of “ul” if mentioned).The number of cells or total protein used per assay should be reported.
- The specific incubation time and temperature for colorimetric measurement are not mentioned.
- Spectrophotometer settings, including the wavelength used for absorbance readings, should be stated (typically ~450 nm for this kit).
- The method and software used to generate and fit the standard curve should be clarified.
- Data Normalization and Replicates:
- How were the NADPH/NADP<sup>+</sup> ratios normalized? Per number of cells, total protein content, or other?
  - How many biological and technical replicates were performed per condition?
  - Were results expressed as a ratio or as relative changes compared to a control group?



**RE-REVIEW REPORT OF REVISED MANUSCRIPT**

**Name of journal:** *World Journal of Stem Cells*

**Manuscript NO:** 112278

**Title:** Hypoxia facilitates triple-negative breast cancer stem cells enrichment and stemness maintenance through oxidized ataxia telangiectasia mutated-induced one-carbon metabolism

**Provenance and peer review:** Unsolicited manuscript; Externally peer reviewed

**Peer-review model:** Single blind

**Reviewer’s code:** 05781374

**Position:** Peer Reviewer

**Academic degree and professional title:** Associate Professor, Professor

**Reviewer’s Country/Territory:** China

**Author’s Country/Territory:** China

**Manuscript submission date:** 2025-07-22

**Reviewer chosen by:** Jing-Jie Wang

**Reviewer accepted review:** 2025-10-17 01:37

**Reviewer performed review:** 2025-10-17 02:01

**Review time:** 1 Hour

<b>Content to be reviewed</b>	<p>Does the manuscript’s content fall within the scope of the journal? <b>Yes</b></p> <p>Are the Key Words complete? <b>Yes</b></p> <p>Does the Abstract contain the contents of each part of the manuscript (IMRaD)? <b>No</b></p> <p>Is the content of the Introduction adequate? <b>Yes</b></p> <p>Do authors’ affiliations correspond to the content of the manuscript? <b>Yes</b></p>
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Is the content of the Materials and Methods complete?

**No**

Is there any Key Word that is not included in the manuscript title? **No**

Is the description of the experiments clear and complete? **No**

Are the experimental data presented in the manuscript's biostatistics content reliable? **No**

Are the experimental data of the Results true and reliable? **Yes**

Are the quality and resolution of the images up to standard? **Yes**

Do the selection and design of the figures and tables follow the principles of necessity and clarity? **Yes**

Is there any duplication between various parts of the manuscript and between the main text and the content presented in the figures and tables? **No**

Are the figures and tables numbered consecutively in the order in which they appear in the manuscript? **Yes**

Is the content of the Discussion reasonable? **Yes**

Is the Conclusion reasonable? **No**

Are all references necessary and reasonable? **Yes**

Do authors omit important references? **Yes**

Are all references related to the topic of the manuscript? **Yes**

Do authors only cite their own earlier publications? **No**

Is the manuscript's text correct, concise, and clear? **Yes**

Will the manuscript's content be of interest to readers?  
**Yes**

Are additional experiments needed for the study? **Yes**



	Does the research scope comply with ethics? <b>Yes</b>
<b>Scientific quality</b>	Grade B (Very good)
<b>Novelty of this manuscript</b>	Grade C (Good)
<b>Creativity or innovation of this manuscript</b>	Grade C (Good)
<b>Scientific significance of the conclusion in this manuscript</b>	Grade B (Very Good)
<b>Language quality</b>	Grade B (Very good)
<b>Does this manuscript describe a study of the existing knowledge system?</b>	Yes
<b>Does this manuscript report a revolutionary innovation?</b>	No
<b>Does this manuscript report an unconventional innovation?</b>	No
<b>Conclusion</b>	Major revision
<b>Peer-reviewer statements</b>	Peer-Review: Anonymous
	Conflicts-of-Interest: No
<b>Are your review comments generated by AI tools?</b>	No

### **SPECIFIC COMMENTS TO AUTHORS**

The authors have made a substantial and generally helpful set of revisions: they corrected terminology, supplied many methodological details (oxygen setting, knockdown efficiency, replicate numbers, statistical tests), clarified key assay conditions (NADPH assay incubation and analysis), added pathway statistics and metabolite detail, reported fold-changes and n-values in figures, and added ChIP controls and metabolite “backfill” concentrations. These changes address many of the reviewers’ technical and reporting concerns. However, a few important items remain incompletely addressed or



require additional experimental evidence. These outstanding issues are not all minor editorial points – several touch on mechanistic support and data rigor (controls and functional promoter validation, time-course data, clarity about viral/transfection methods). Because of those remaining gaps I recommend further revision rather than acceptance at this stage.

These items remain concerns and should be resolved before acceptance.

1. Reviewer explicitly asked for non-CSC (parental adherent) controls to demonstrate KU60019 selectivity/effect. The authors did not perform these controls; they justified focusing on CSCs and cited literature. The authors should either provide parental cell data for key readouts (e.g., KU60019 effect on mammosphere formation, CD44<sup>+</sup>/CD24<sup>-</sup> fraction, NADPH/NADP<sup>+</sup>) or present a stronger rationale and additional citations showing why ATM inhibition would be expected to be CSC-selective in the cell lines used. Absent one of these, this remains a substantive limitation.
2. ChIP shows occupancy but does not prove those E-boxes are functionally required for promoter activation. Perform E-box mutagenesis (or at least one key E-box) in the luciferase reporter to show loss of activation; if this is not feasible now, the authors must tone down causal claims that c-Myc directly activates expression via those sites.
3. The assertion that oxidized ATM activation is DNA-damage independent is central. Endpoint data plus literature is suggestive but a short time-course comparing p-ATM and  $\gamma$ H2AX after hypoxia vs H<sub>2</sub>O<sub>2</sub> would be simple and informative. Add a brief time-course experiment (several early time points) showing p-ATM increase without  $\gamma$ H2AX induction under hypoxia, or, if not performed, explicitly label this as a limitation and avoid definitive wording.
4. The response states MOI = 10 and that Lipofectamine 3000 was used as “transduction reagent.” Typically Lipofectamine is used for plasmid transfection; viral transduction uses polybrene or direct infection. This mixing of terms raises concern about exactly how



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gene delivery was performed. Correct and clarify exactly what was done: (a) if lentiviral particles were used, state MOI, presence/absence of polybrene, infection duration, multiplicity, and selection conditions; (b) if plasmid transfection (Lipofectamine) was used, state that and remove “MOI” terminology. This must be unambiguous.

5. Give base-pair positions (GRCh38 coordinates) and primer sequences for the cloned promoter fragments in Methods or Supplementary.

6. Reviewer 2 asked for richer quantitative reporting and transparency. The authors should deposit metabolomics, RNA-seq (if any), and source data (uncropped blots, FCS flow files) in appropriate repositories and include accession numbers in the manuscript.

7. If mutagenesis not done, ensure ChIP data include % input and IgG and show multiple replicates (authors say they did that; verify figures).