**Peer Review File**

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**Reviewer A**

**Comment 1:** In the introduction of the manuscript, it is necessary to clearly indicate the relationship between ANXA9 and tumor-infiltrating immune cells and the role of ANXA9 play in prognosis in LUAD.

**Reply 1:** Thank you for your comments. We made the correction according to the comment.

**Changes in the text:** Page 4, line 94-99

**Comment 2:** What are the biggest advantages and disadvantages of nanocomposites in this study? What is the most difficult technical challenge to overcome? Suggest adding relevant comparative analysis.

**Reply 2:** Recent studies demonstrated that HM had the ability to inhibit tumor cells. However, poor absorption and bioavailability of HM when administered systemically has resulted in inability to realize its therapeutic potential. In the meantime, HM has a wide range of side effect on human body. The biggest advantages were that we provided an effective strategy to deliver this anti-tumor agent effectively and safely. And the disadvantages were that we didn't keep track of nanocomposites dynamically in vivo. I think the most difficult technical challenge is how to continuously track the antitumor effect and metabolism of nanocomposites in vivo. Also, this is one of our main research directions in the future.

**Changes in the text:** Page 4, line 108-110

**Comment 3:** It is recommended to focus on the advances in nanoparticle design that overcome heterogeneous barriers to delivery.

**Reply 3:** Thank you for your comments. Nanoparticles is a new technology in the area of pharmaceutics. Its good target and delayed release effect come from it special physico-chemical property, which makes it become the focus in the study area of pharmaceutics. We will focus on drug high load rate, tumor targeting, low toxicity, visualization, and controlled drug release of nanoparticles in the future.

**Changes in the text:** N/A

**Comment 4:** The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Systemic immune microenvironment and regulatory network analysis in patients with lung adenocarcinoma, PMID: 35116596”. It is recommended to quote this article.

**Reply 4:** Thank you for your comments. We made the correction according to the comment.
**Changes in the text:** Reference No. 16

**Comment 5:** There are many genes that regulate the metastasis in lung adenocarcinoma. Why did the author choose ANXA9 for research? Please describe the reason.
**Reply 5:** Our team are currently investigating the association of Annexins (ANXs) family proteins with tumor bone metastasis. We performed microarray analysis to screen expression profiles of mRNAs in SM and lung adenocarcinoma cancer samples. We found that ANXA9 was significantly upregulated in SM of LUAD and promoted cell proliferation and migration in vitro. Meanwhile, on the base of the first stage of research, we present for the first time, through bioinformatics analysis of TCGA database and assays using clinical specimens, the correlation between LUAD and ANXA9. We also show possible mechanisms by which ANXA9 promotes SM of cancer cells. Finally, we confirmed that ANXA9 plays an oncogenic role in LUAD and accelerated metastatic progression. Hence, ANXA9 may serve as a potential therapeutic target for lung cancer patients.
**Changes in the text:** N/A

**Comment 6:** What is the effect of this study on further treatment and prognosis of lung adenocarcinoma? Please add relevant content to the discussion.
**Reply 6:** Thank you for your comments. We made the correction according to the comment.
**Changes in the text:** Page 17, line 534-539

**Reviewer B**

**Comment 1:** First, the title needs to indicate the research methodology of this study, i.e., in vivo experiment. Please also indicate this in elsewhere of this paper, since the authors did not validate this in human patients.
**Reply 1:** Thank you for your comments. We made the correction according to the comment.
**Changes in the text:** Page 2, line 47 and Page 5, line 127

**Comment 2:** Second, the abstract needs some revisions. The background did not describe the knowledge gap and potential clinical significance of this research focus. The methods need to describe the bioinformatics analysis and how the prognostic role

of ANXA9 was analyzed. The results need to quantify the findings by reporting statistics including HR values, expression levels, and accurate P values. The conclusion needs more detailed comments for the clinical implications in human patients with LUAD.

**Reply 2:** Thank you for your comments. We made the correction according to the comment.

**Changes in the text:** Page 2, the part of abstract

**Comment 3:** Third, in the introduction of the main text, the authors did not explain why ANXA9 was associated with the SM and poor prognosis in LUAD and what the potential clinical significance of this research focus is. The clinical evidence and potential therapeutic mechanisms of HM for LUAD including SM in LUAD should be extensively reviewed.

**Reply 3:** Thank you for your comments. Our team are currently investigating the association of Annexins (ANXs) family proteins with tumor bone metastasis. We performed microarray analysis to screen expression profiles of mRNAs in SM and lung adenocarcinoma cancer samples (*Neoplasma 2019; 66(6): 930–938*). At the same time, our research is innovative, and there is a lack of relevant literature to support the relationship between ANXA9 and SM of LUAD. We explain fewer about this in the introduction. Poor absorption and bioavailability, serious toxicity to the nervous system of HM impeded its clinical application and the further study.

**Changes in the text:** Page 4, line 101-106

**Comment 4:** Fourth, in the methodology of the main text, please have a brief overview of the research procedures of this study and the questions to be answered by these procedures. The authors need to describe the clinical sample and prognosis outcomes including SM in the TCGA database. In statistics, please describe the test of normality of continuous variables, the test of the independent prognostic role of ANXA9, and ensure $P<0.05$ is two-sided.

**Reply 4:** Thank you for your comments. We made the correction according to the comment.

**Changes in the text:** Page 11, the part of Statistical analysis

**Reviewer C**

1. ARRIVE checklist:

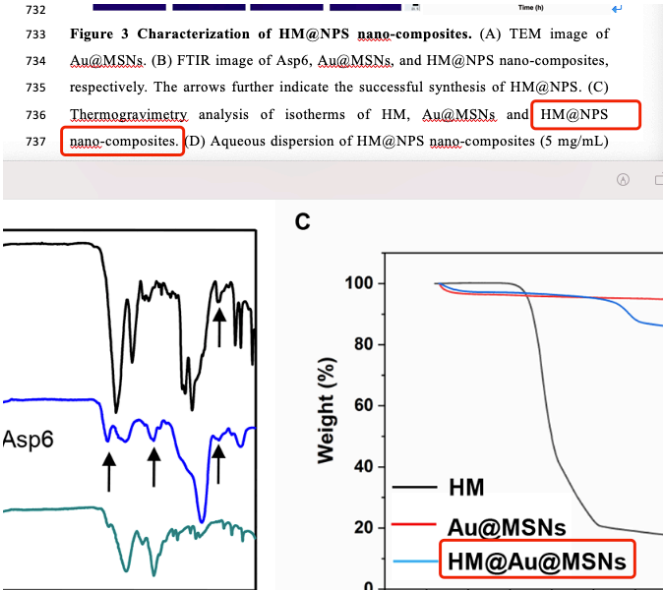
Item 5: We cannot find such information in your paper, please check if blind was used in your study.

| | | | |
|---|---|---|---|
| Blinding | 5 | Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis). | Page 10/line 306-307 |

2. Please indicate staining methods in Figure 2D legends.

**Reply:** Thank you for your comments. We made the correction according to the comment.

3. Figure 3: Please check if the figure matches the legend.

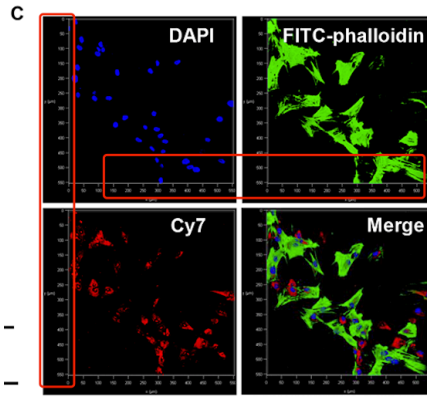



**Reply:** confirmed, thanks.

4. Figure 4:

a. Figure 4A-4B: Please re-edit the figure, it could not be identified.


b. Figure 4C: And the figure was too vague, please send us a higher resolution version.



c. Please indicate the staining method of Figure 4E in figure legends.

**Reply:** Thank you for your comments. We made the correction according to the comment.

d. Figure 4C: please provide the magnification in the legend.

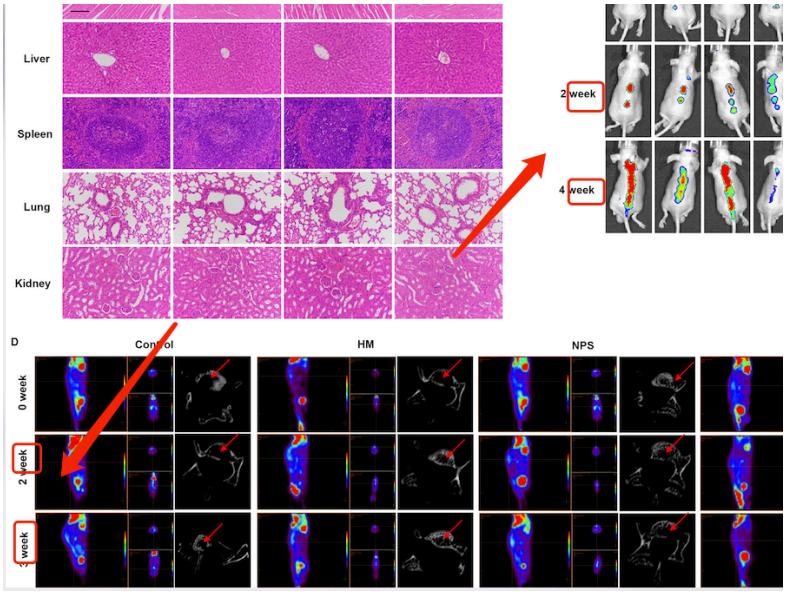
Reply 2: Thank you for your comments. We made the correction according to the comment

Changes in the text: Page 27, line 789

5. Please indicate the staining method of Figure 5B in figure legends.

**Reply:** Thank you for your comments. We made the correction according to the comment.

6. Figure 6: Check if "week" should be "weeks".




**Reply:** Thank you for your comments. We made the correction according to the comment.

7. Please provide the source of the cells.

216 **##Cell transfection** ↵

217 Human lung cancer cell lines (A549) and human lung bronchial epithelial 16HBE

218 cells were routinely cultured in Dulbecco's modified Eagle medium (DMEM) with 10%

219 fetal bovine serum (FBS) and 1% penicillin/streptomycin.↵

Reply 7: Thank you for your comments. We made the correction according to the comment

Changes in the text: Page 7, line 202