

## Peer Review File

Article information: <https://dx.doi.org/10.21037/jtd-24-188>

### Reviewer A

The paper titled “The role and mechanism of PDZ binding kinase in hypobaric and hypoxic acute lung injury” is interesting. Overexpression of PBK inhibits the expression of p53 and activates SIRT1-PINK1 axis mediated mitochondrial autophagy to alleviate HHALI. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The analysis of inflammatory factors in this study is less. If cytokine profiling is chosen to predict HHALI, is it more meaningful? Is it more likely to analyze and predict markers of HHALI? Suggest adding relevant content.

**Reply:** We have modified our text as advised (see line 374-381).

2) How does mitochondrial autophagy affect acute lung injury and macrophage polarization to overcome injury? Suggest adding relevant content.

**Reply:** We have modified our text as advised (see line 431-438).

3) It is recommended to mark the observation position with arrows in results of H&E stain.

**Reply:** We have modified our text as advised (see fig 1 and fig 3).

4) This study is entirely an animal experiment, and it is recommended to extract human samples for further validation.

**Reply:** The issue of sample collection has been considered by our research team. However, this study primarily focuses on acute lung injury under low-pressure and low-oxygen conditions from high-altitude regions, and the collection of lung tissue from these injuries is subject to several ethical limitations, making sampling challenging.

5) What is the complex interplay between autophagy and oxidative stress in the development of lung disease? Suggest adding relevant content.

**Reply:** We have modified our text as advised (see line 417-421).

6) The number of samples in this study is too small, and a large sample study should be added for verification.

**Reply:** Establishing a model takes a long time, approximately 1-2 months, and currently there are certain difficulties in establishing the model.

7) How to understand the time relationship between hypobaric and hypoxic and injury?

How to distinguish HHALI from patients with other causes? Suggest adding relevant content in the discussion.

**Reply:** We have modified our text as advised (see line 341-344).

## **Reviewer B**

1. It is 'Beas-2b' in the elsewhere of paper. Please unify.

198 When the **Beas-2B** cell line was cultured to 70% of

We have modified our text as advised.

### **2. Reference/citations**

- a. Citation (14) is missing in the main text. Reference 14 should be cited consecutively between references 13 and 15. Please check and revise.

82 damage (12). HH induces molecular changes associated with oxidative stress, inflammation,  
83 and protein kinase activation (13).  
84 PDZ-binding kinase (PBK), also known as T-lymphokine-activated killer cell-originated  
85 protein kinase (TOPK), is a serine/threonine kinase that participates in cell cycle regulation and  
86 mitotic progression (15). It is predominantly expressed in actively proliferating cells,

- b. The authors mentioned “studies...”, while only one reference was cited. Change “Studies” to “A study” or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

We have modified our text as advised (line 86).

*Several studies have indicated that there is a complex cascade relationship between p53 and SIRT1, which interact with and mutually influence each other during mitochondrial autophagy and cell apoptosis processes (46).*

- c. Please confirm if citations are needed in this sentence, as you mentioned “Multiple studies”.

We have modified our text as advised (line 410-411).

*Multiple studies point to the complex cascade relationship between p53 and SIRT1.*

We have modified our text as advised (line 456).

3. When using **abbreviations** in table/figure or table/figure description, please mention the entire expression in a footnote below the corresponding table/figure. **Please check and**

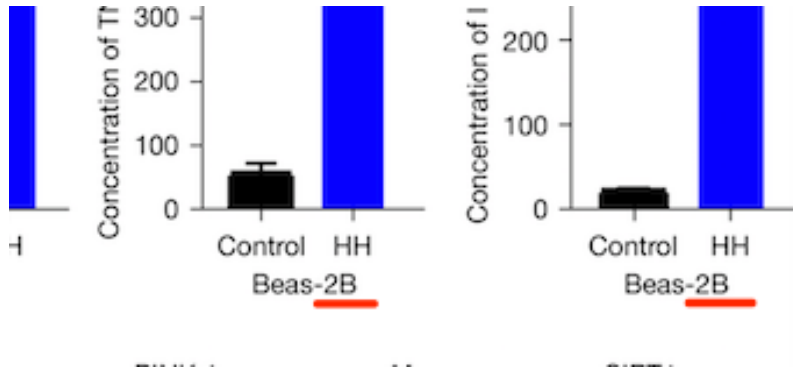
revise.

Such as: HH, W/D, PBK, PDZ, PTNE, (in figure 1); PBK, PDZ, AAV5, LV-NC, (in figure 2); etc.

We have modified our text as advised.

#### 4. Figure 1

a. It is 'Beas-2b' in the paper. Please unify.



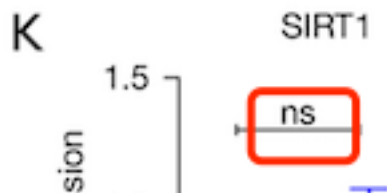
We have modified our text as advised.

b. No “\*, \*\*” in figure 1, but you explained them in the figure legend. Please check and revise.

651 the HH group and control group of Beas-2b cells. \* P<0.05, \*\* P<0.01, \*\*\* P<0.001. ←

We have modified our text as advised.

c. Please explain 'ns' in the figure legend.



We have modified our text as advised.

#### 5. Figure 2

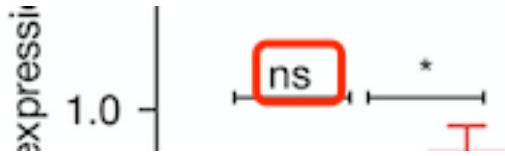
a. No “\*\*\*” in figure 2, but you explained it in the figure legend. Please check and revise.

658

659 **\*\* P<0.01, \*\*\* P<0.001.** ←

We have modified our text as advised.

b. Please explain 'ns' in the figure legend.



We have modified our text as advised.

### 6. Figure 3

No “\*, \*\*” in figure 3, but you explained them in the figure legend. Please check and revise.

663 staining, lung injury scoring, and W/D ratio (wet/dry ratio) of Balb/c mice. \* P<0.05, \*\* P<0.01,  
664 \*\*\* P<0.001 ↵

We have modified our text as advised.

### 7. Figure 5

No “\*, \*\*” in figure 5, but you explained them in the figure legend. Please check and revise.

675 dismutase) in the Balb/c mouse lung homogenates. \* P<0.05, \*\* P<0.01, \*\*\* P<0.001  
676 ↵

We have modified our text as advised.

### 8. Figure 6

No “\*, \*\*” in figure, but you explained them in the figure legend. Please check and revise.

681 and statistical analysis was performed based on the green-red fluorescence ratio. \* P<0.05, \*\*  
682 P<0.01, \*\*\* P<0.001. ↵

We have modified our text as advised.

### 9. Figure 7

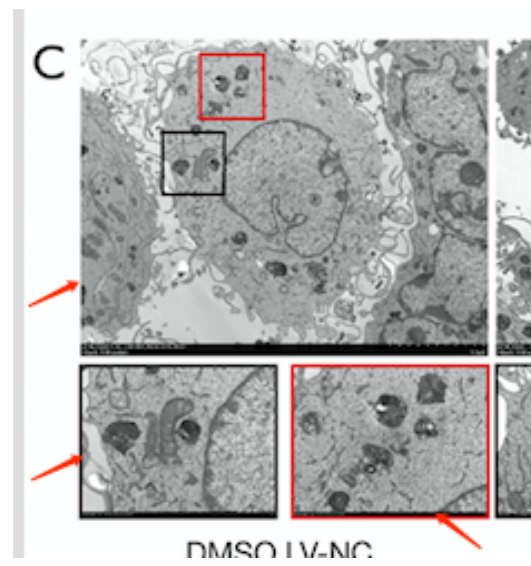
a. No “\*\*\*” in figure, but you explained it in the figure legend. Please check and revise.

691 morphology indicated by the black box  
692 \*\* P<0.01, \*\*\* P<0.001. ↵  
693 ↵

We have modified our text as advised.

b. Please indicate the **observation method, and magnification** of these three different sizes of

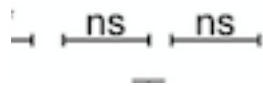
cell maps in figure legend.



We have modified our text as advised.

### 10. Figure 8

Please explain 'ns' in the figure legend.



We have modified our text as advised.