

### **Reviewer A**

The authors identified overlapping DEGs from GSE144274 and GSE243193 and performed functional analysis to explore the underlying mechanisms. They successfully identified seven key genes (ACTA2, HLA-DRA, HLA-A, PECAM1, HLA-C, IRF1, and CD74) that differentiate patients with PAH from healthy individuals.

#### Major comments:

The authors should mention detailed reasons or justification for the selection of the datasets (GSE144274 and GSE243193).

**Reply 1:** Thanks for the splendid comment. We appreciate your suggestion to provide a detailed justification for the selection of the datasets GSE144274 and GSE243193.

**Changes in the text:** In our revised manuscript, we will include a comprehensive explanation of the criteria we used for selecting these datasets. (See Page 10, line 303-307)

#### Minor comments:

The authors should revise the quality of the figures: Figure 2, 3, and 4.

**Reply 2:** Thanks for the attention to detail and understand the importance of clear and high-quality visuals in conveying our research findings. In response to your comments, we will revise Figures 2, 3, and 4 to enhance their quality. We aim to ensure that the figures effectively illustrate our results and support the overall narrative of the manuscript.

**Changes in the text:** This revision will include improving the resolution, adjusting the color contrast for better visibility, and ensuring that all labels and legends are clear and easily readable. (See figure 2-4).

### **Reviewer B**

This is a nicely written study of the genes involved in pulmonary arterial hypertension. Target genes were identified from patients with PAH. IRF1 was selected and then tested in rat and mice models of PAH, demonstrating higher expression and that inhibition with 10HDAA could reduce PAH. The study is well conducted, the results are interesting and the paper is well written.

1. How did you specifically choose IRF1 as your target. Is it simply the availability and cost effectiveness of 10HDAA that made you choose it? Perhaps you could elaborate on this and also on 10HDAA in the discussion.

**Reply 1:** Thanks for the in-depth comments. While the availability and cost-effectiveness of 10-HDAA are indeed factors that influenced our choice, our decision was primarily driven by the biological relevance of IRF1 in the context of our study. In the revised manuscript, we will expand the discussion to provide a more detailed rationale for selecting IRF1.

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Changes in the text: IRF1 was selected as our target based on its critical role in immune response and gene regulation. Previous studies have highlighted its significance in programmed cell death of endothelial cells and smooth muscle cells, which in part aligns with the objectives of our research. (See Page 11, line 331-334)

2. It's not entirely clear to me what function the rat model serves. Is it just further validation of the role of IRF1 in a different model? You could not have also administered 10HDAA to a group of these rats?

Reply 2: Thank you for your thoughtful comments regarding the role of the rat model in our study. We appreciate your inquiry about its function and the potential for administering 10-HDAA to mice instead of rats.

The rat model was employed to provide further validation of the role of IRF1 in a different biological context. Specifically, we aimed to investigate pulmonary arterial hypertension (PAH) and its typical animal models are MCT rats and HySu mice, but their modeling principles are different. By utilizing MCT model, we sought to confirm our findings from previous studies and to explore how IRF1 influences PAH.

Regarding the administration of 10-HDAA to a group of rats, we acknowledge that this is a valid point. While our current study did not include this approach, we recognize the potential benefits of such an experiment in further elucidating the effects of IRF1 in vivo. However, rats usually weigh ten times that of mice. The dosage of 10-HDAA required by gavage in rats is too much to afford, making it difficult to proceed with the experiment.

Changes in the text: In the revised manuscript, we will clarify the rationale behind our choice of model and discuss the feasibility and implications of administering 10-HDAA to the rat subjects. (See Page 11, line 336-340)

3. Lines 257 to 263 compare the PAH+10HDAA to the PAH model. Can you also compare 10HDAA to the control group? It seems like there may be only a partial effect of the 10HDAA in preventing pulmonary hypertension.

Reply 3: We apologize for this oversight. We are grateful for your suggestion to also include a comparison of the 10-HDAA group to the control group. We agree that comparing the effects of 10-HDAA to the control group would provide valuable insights into its potential role in preventing pulmonary hypertension.

Changes in the text: In our revised manuscript, we will include this comparison and analyze the outcomes to better illustrate the effects of 10HDAA in the context of pulmonary hypertension. (See Page 10, line 292-304)

4. Do lines 130 - 133 apply to both animal models or just the rats? A clearer IRB statement for the whole study should be included in the methods.

Reply 4: Thank you once again for your insightful comments. To clarify, the content in lines 130-133 pertains specifically to the both animal models used in our study. We are committed to enhancing the clarity of our manuscript based on your suggestions.

Changes in the text: We have modified our text as advised.(See Page 6, line 157-159)

**Reviewer C**

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1. keywords

“bioinformatic analysis” is a keyword while it can not be found in the main text. Please check and revise.

Reply 1: Thank you for your valuable feedback regarding the use of the keyword "bioinformatic analysis." We noticed that “biomarker” is also a keyword cannot be found in the main text. We will review the manuscript and ensure that the revised term is appropriately integrated into the main text. Necessary revisions will be made to provide clarity and context for this keyword.

Changes in the text: We have modified our text as advised. (See Page 2 line 60)

2. Should “rats” here be “mice”?

146 **##A Sugen 5416- and hypoxia-induced PAH mouse model**  
147   
148 ~~C57BL/6J male mice (8 to 10 weeks old, weighing 20–25 mg) were obtained from~~  
149 ~~the Soochow University Laboratory Animal Center (SULAC). Using simple~~  
150 ~~randomization method, we divided the mice into a control group (n=12), hypoxia plus~~  
151 ~~Sugen 5416 group (HySu; n=12), and HySu plus 10-hydroxydecanoic acid (10-HDAA;~~  
152 ~~Sanying, Wuhan, Hubei, China) group (HySu + 10-HDAA; n=6). Mice from both the~~  
153 ~~PAH and PAH + 10-HDAA groups were raised in a hypoxic environment (10% O<sub>2</sub>) and~~  
154 ~~received a subcutaneous injection of Sugen 5416 (20 mg/kg; MedChem Express,~~  
155 ~~Monmouth Junction, NJ, USA) per week for 4 weeks. The control group received~~  
156 ~~injections of 5% saline with the same volume as that of Sugen 5416 applied each week~~  
157 ~~in the PAH group. Mice from the PAH + 10-HDAA group received a 5-week intragastric~~  
158 ~~administration with 100 mg/kg of 10-HDAA once a day at 1 week before Sugen 5416~~  
159 ~~injection. [The breeding and experimental procedures involving rats were approved by](#)~~  
160 ~~[the Animal Ethics Committee of the Fourth Affiliated Hospital of Soochow University](#)~~  
161 ~~[\(Lunshen No. 230148\).](#)~~  
162 

a. We will revise the text to replace "rats" with "mice" as appropriate. The sentence will be combined as follows: “Experiments were performed under a project license (Lunshen No. 230148) granted by the Animal Ethics Committee of the Fourth Affiliated Hospital of Soochow University and in compliance with Soochow University guidelines for the care and use of animals.”

2. 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.

*Other studies suggest that IRF1 may play an inhibitory role in the regeneration of the endothelium (22).*

*Furthermore, recent studies have shown that inhibiting IRF1 expression can reduce the abnormal proliferation of smooth muscle cells (24)*

*Previous studies have highlighted its significance in programmed cell death of endothelial cells and smooth muscle cells, which in part aligns with the objectives of our research (21)*

Reply 4: We will revise the text to ensure clarity and proper citation. We will ensure that all

references are numbered consecutively in the order in which they are first mentioned in the text. Changes in the text: We have modified our text as advised. (See Page 11 line 341, line 344 and line 348)

3. Should “Figure 3F” here be “Figure 4F”? Please check and revise.

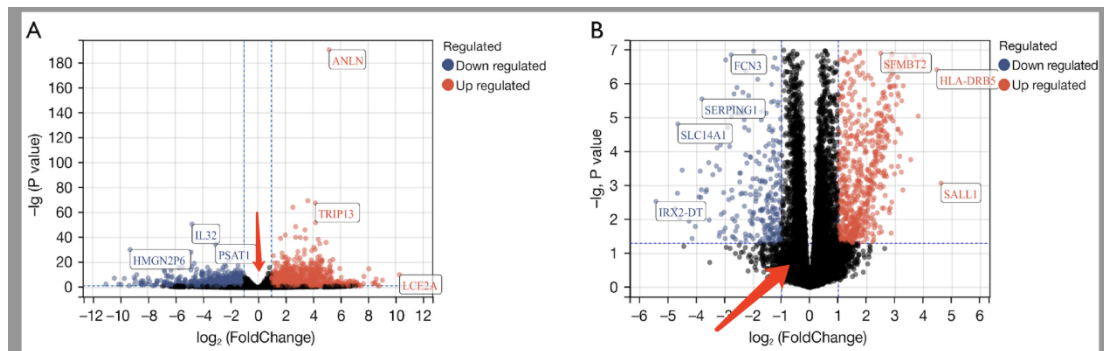
262 Cytoscape. The top 10 key genes identified by the three algorithms (degree, MNC, and  
 263 MCC) in CytoHubba and the principal module from MCODE were intersected (Figure  
 264 4B-4E). A Venn diagram from the three algorithms is presented in Figure 3F. Seven key  
 265 hub genes were identified: *ACTA2*, *HLA-DRA*, *HLA-A*, *PECAMI1*, *HLA-C*, *IRF1*, and  
 266 *CD74*.

Reply: Thank you for your careful review of our manuscript. We checked the reference to “Figure 3F” and confirmed it should indeed be “Figure 4F.”

Changes in the text: We have modified our link in the text as advised. (See Page 9 line 269).

4. Fig 2A, 2B

Please also explain the meaning of the black area (pointed) in the legend or Figure 2.



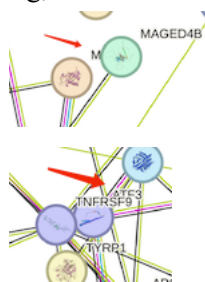
Reply: Thank you for your valuable feedback regarding Figure 2. We will revise the figure to include a clear explanation of the meaning of the black area that is pointed out. This addition will help enhance the clarity of the figure and ensure that readers understand its significance within the context of our study.

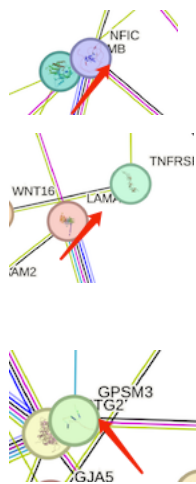
Changes in the figure: We have modified our figure 2 in the text as advised. (See Page 17 line 517-518 and Figure-2 revised.png)

5. Figure 4

Figure 4A is incomplete as some words are covered. Please revise.

Eg,



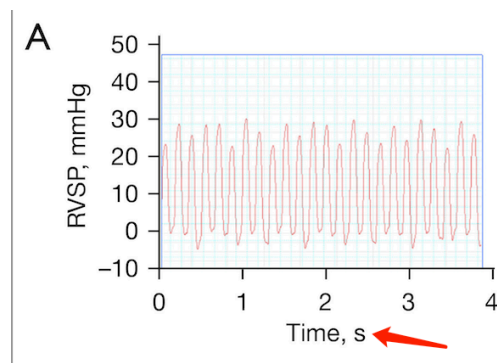


Reply: Thank you for your feedback regarding Figure 4A. We will revise the figure to include a clear text display of the genes that is pointed out.

Changes in the figure: We have modified our figure 4A in the text as advised. (See Figure-4 revised R2.png).

6. Fig 5, fig 7

Please explain the meaning of “s” in the legend.

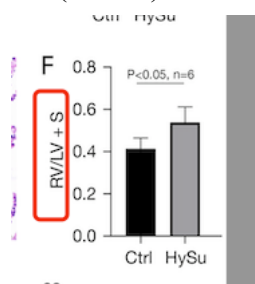


Reply: Thank you for your insightful feedback regarding the figure legend. We will clarify the meaning of "s" in the legend. The “s” here means seconds.

Changes in the text: We have modified our figure legend as advised. (See Page 20 line 560 and Page 22 line 584).

7. Fig 5F, 5L, 7J

RV/(LV + S) in the figure legend while it is RV/LV + S in the mentioned figures. Please unify.



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Reply: Thank you for your valuable feedback regarding the discrepancies in the figure legends and the figures themselves. We will ensure that the notation for  $RV/(LV + S)$  in the figure legend is unified with the representation  $RV/LV + S$  in Figures 5F, 5L, and 7J.

Changes in the text: We have modified our figure legend as advised. (See Page 20 line 550, 554, 557 and Page 22 line 580, 582).