Peer Review File

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Response to Reviewer A's comments.

1) **Comment 1**: In abstract, the aim of this work was not in consistent with the conclusion, which should be revised by "We successfully generated an AT2 cell-specific LCMR1 conditional knockout mouse model and further revealed the crucial role of LCMR1 in maintaining AT2 cell homeostasis."

   Reply 1: We thank the reviewer for this suggestion. We have revised the conclusion in the abstract as advised.

   Changes in the text: We have modified our text as advised (see Page 3, line 62-64).

2) **Comment 2**: In keywords, format of words should be abbreviated or spelled in full consistently.

   Reply 2: We thank the reviewer for this comment. We have deleted the full name of LCMR1 (lung cancer metastasis-related protein 1) and demonstrated the abbreviated format of all the keywords consistently.

   Changes in the text: We have modified our text as advised (see Page 3, line 6).

3) **Comment 3**: LCMR1 was found in tumor cell lines, but authors failed to illustrate the reason why they discussed the potential role of LCMR1 in AT2 cells, which need detailed introduction.

   Reply 3: We thank the reviewer for this comment. As we mentioned in the introduction part, LCMR1 was a novel gene that was originally identified and registered by our team in 2002 (Genbank accession number: AY148462). The gene function study of LCMR1 has been the focus of our team since it was identified. Our previous work mainly concentrated on its role in lung cancer and were mostly in vitro experiments. We seek to develop deeper understanding of the functions of LCMR1 in normal organs and tissues in vivo. As respiratory doctors, we firstly chose to investigate the role of LCMR1 in normal lung tissues via gene knockout mouse models. Sftpc-CreER mice are the most commonly used inducible lung-specific Cre mouse models, which mediates gene deletion specifically in AT2 cells after tamoxifen induction. We hope to conduct more researches on the role of LCMR1 in other organs and tissues in the future.

4) **Comment 4**: Several remarks should be highlighted. For example, the white arrow in Figure 2B is better to be replaced by yellow. Correspondingly, magnification by transmission electron microscopy should be displayed in figure legend. Colours of each histogram in this paper should be obvious, rather than unnoticeable ones. Additionally, it is necessary to display each repeated mouse with scatter plots in

bar charts.

Reply 4: We thank the reviewer for these remarks. We have replaced the the white arrow by yellow in Figure 2B, and displayed the magnification by transmission electron microscopy in the figure legend (see Page 23, line 716-717). We have also revised the colors of histograms in which black color represents $LCMR1^{flox/flox}$ mice and red color represents $LCMR1^{\Delta AT2}$ mice. Scatter plots were displayed in the corresponding colors.

Changes in the text: We have modified our text as advised (see Page 23, line 716-717; Figure 1-6 revised).

5) **Comment 5**: The authors should further demonstrate the reason why they detected these markers in BALF to evaluate LCMR1 mediated AT2 cell homeostasis. Of note, authors should make it clear that these variations in BALF were contributed to AT2 cell rather than other reasons.

Reply 5: We thank the reviewer for this comment. We found inflammatory cell infiltration and edema in the lung tissues of $LCMR1^{\Delta AT2}$ mice through H&E staining. We further detected inflammatory cells and cytokines in BALF to verify the existence of enhanced inflammatory response in the lung tissues of $LCMR1^{\Delta AT2}$ mice. TNF-α, IL-1β, IL-6 and IL-10 were typical inflammatory cytokines, and could change obviously with lung inflammation and injury. We detected these cytokines to demonstrate the inflammatory response of the whole lung, not only the homeostasis of AT2 cells.

Response to Reviewer B's comments.

1) **Comment 1**: The fifth section of results showed contrary expression of SP-D, but there was no explanation about the confusing result. Please give a reasonable explanation and add relative experiments to confirm your explanation.

Reply 1: We thank the reviewer for this comment. We tried to explain the significantly increased expression of SP-D in the discussion part (see Page 16, line 503-509). One possible cause may be that SP-D can also be synthesized and secreted by Clara cells and macrophages. We found increased number of macrophages in $LCMR1^{\Delta AT2}$ mice compared with $LCMR1^{flox/flox}$ mice. Additionally, the changes in the lung tissues of $LCMR1^{\Delta AT2}$ mice produced a phenotype with features resembling lung injury. It was previously reported that SP-D expression was increased in the lung tissues of animals with acute lung injury. We hope these contents can answer your question.

2) **Comment 2**: There were some non-standard writing problems in the text. For example, the gene name should be written in italics like LCMR1.

Reply 2: We thank the reviewer for this comment. We have revised the font of all

the gene names to be italics in the main document and supplementary material.

Changes in the text: We have modified our text as advised, which were marked by annotations on the right.

3) **Comment 3**: The testing markers firstly showed in the article should be followed by their signification to indicate the results and help readers to understand.

Reply 3: We thank the reviewer for this comment. We mentioned the signification of some markers we detected in the original manuscript. For example, we wrote a sentence to explain the role of α-SMA as a marker of the myofibroblasts and listed relevant literature (see Page 11, line 333). Therefore, the expression of α-SMA can indicate the level of fibrosis. We also explained the reason to analyze the expressions of SP-A, SP-B, SP-C, and SP-D through a sentence (see Page 13, line 399-400) as these surfactant proteins are synthesized and secreted by AT2 cells.

4) **Comment 4**: All the reagents and kits should be added with item number.

Reply 4: We thank the reviewer for this comment. We have added the item numbers of all the reagents and kits in the Methods part, which were shown after the brand in red.

Changes in the text: We added some contents as advised in the Methods part.

5) **Comment 5**: There were some mistakes in grammar and the subtitle should be summed up succinctly. The authors should summarize the data properly.

Reply 5: We thank the reviewer for these comments. We have double-checked the entire article for gramma mistakes. According to the author instructions of the journal, the word limit of a running title is no more than 60 characters including spaces, and we have tried our best to include the gene name, research method, species and cells, and the resultant phenotype in the title.