Reviewer's report

Title: Reduced transient receptor potential vanilloid 2 expression in alveolar macrophages causes COPD in mice through impaired phagocytic activity

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Reviewer: Karthik Suresh

Reviewer's report:

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In this manuscript, Masubuchi, et al. describe the presence of decreased TRPV2 expression and impaired phagocytosis in macrophages from CSE mice and also report decreased phagocytosis in macrophages from TRPV2 -/- mice. These are interesting findings, but I do have a few questions/comments re: presentation of the immunofluorescence data. Importantly, I think evidence of increased COPD severity in TRPV2 -/- mice would need to be shown before the claim that TRPV2 contributes to CSE pathobiology can be made (see point 6, below)

1. Figure 1: The Immunofluorescence images are not clear. Higher magnification images with counterstain (DAPI or Hoechst) are needed.

2. Figure 2: Please indicate molecular weight. Please provide a full length gel showing the other bands (if any) that were present on the gel. Also, the B-actin loading control is not uniform, suggesting that less protein may have been loaded in the 24h lane column.

3. Figure 1 and Figure 4: Not all F4/80 cells seem to also express TRPV2. What % of F4/80 cells were TRPV2+. This can be done 2 ways: if the authors are able to perform flow cytometry, gating on TRPV2 followed by F4/80+/ would be useful. If not, counting of % positive cells by a blinded investigator on immunofluorescence should be done. Conversely, does TRPV2 stain cells that are F4/80 negative, and if so, what could these cell types be?

4. Can FITC-dextran internalization be imaged? It would be more compelling to show images of internalized FITC-dextran in WT and siTRPV2 or CS-exposed cells to show that the amount of phagocytosed dextran is lower.
5. TRPV4 levels: What were the % macrophages in the cell diffs of BAL performed in CSE mice. Were the cells from the BAL sorted prior to analysis. If not, it is clear that the reduction of TRPV2 levels could be attributed to decreased macrophage TRPV2 levels since other cell types are present in the BAL. If unsorted BAL cells were used, the authors will need to state this in the discussion and couch the discussion with appropriate caveats re: the BAL data.

6. Discussion: "evidence indicating that development of smoke-induced COPD is partly attributed to reduced phagocytosis". This claim seems premature. First, do TRPV2-/- mice develop worsened smoke-induced OLD (as assessed by histology scores of emphysema, pulmonary function testing). Ideally, this should be done in mice that are only deficient for TRPV2 in macrophages, but at a minimum, data on COPD development/severity in TRPV2-/- mice are needed to substantiate the claim that this channel plays a role in COPD pathobiology.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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