Author's response to reviews

Title: The Deleted in Liver Cancer 1 (Dlc1) tumor suppressor is haploinsufficient for mammary gland development and epithelial cell polarity

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Author's response to reviews: see over
Dear Editor:

We have uploaded our revised manuscript # MS: 176609294159241 entitled: “The Deleted in Liver Cancer 1 (Dlc1) tumor suppressor is haploinsufficient for mammary gland development and epithelial cell polarity.” Detailed answers to the reviewers’ comments are given below. Thanks to the reviewers for their helpful suggestions that have helped to improve the manuscript.

Yours sincerely

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Answers to Reviewers Comments:
Reviewer -1

Comment -1
n 1. In fig7, the upper panel, the bands of activated rhoA are not clear enough, they should show a more clear figure.

We agree that the Fig. 7 submitted with the manuscript was not a clear figure. A better Western is presented, which supports the interpretation of increased RhoA activity in the heterozygous Dlc1gt/+ trapped mice.

Comment- 2

n The statistics in fig7, are there any difference between WT and Dlc1gt/+ without serum treatment? They have not shown it. and the statistics seem not consistent with upper panel in active Rho.

We repeated the experiment several times to increase the statistical significance. Using the Students T test, we found that there is statistically significant increase of RhoA activity in the DLC-1 gt/+ primary cells compared with wild type cells with and without serum treatment. This is given in the Figure legend.

Reviewer -2

Comment -1

n In order to investigate how Dlc1 affects mammary gland, the authors need to use mammary lineage markers, cell proliferation and apoptosis markers to characterize the Dlc1 GT/+ mammary gland.
We agree with the suggestion of the Reviewer and we have incorporated a new Fig 5 in the manuscript consisting of mammary glands stained with Cytokeratins 14, and 18. We did not see any visible differences between the Dlc1 or wild type mice. We also have incorporated Cleaved Caspase -3 and Ki67 markers to characterize the mammary gland and acinar structures. These can be found in Fig 5 & 7. In agreement with others (Jechlinger M, Podsypanina K, Varmus H: Genes Dev 2009, 23: 1677-1688), we did not find increased apoptosis during lumen formation in acinar structure formation in vitro. No significant increase in Ki67 staining was found in mammary tissues of Dlc1 gt/+ mice compared to WT mice.

Comment- 2

- Does Dlc1 GT/+ mouse form hyperplasia or mammary tumor?
- These mice do not show any hyperplasia or mammary tumors, indicating that Dlc1 loss is insufficient for tumor formation. A sentence stating this fact was added to the manuscript.

Comment -3

- The Dlc1 GT/+ cells do not form lumen in 3D culture, this may be due to inhibition of apoptosis, and may not be due to lost polarity. The authors need to perform staining with both apoptotic and polarity markers to confirm their conclusions
- As suggested by the Reviewer, we have included polarity markers E-cadherin, Beta-catenin and alpha-6 integrin (Fig 6). We found these to be affected in the acinar structures with filled up lumen in the mammary epithelial cells from DLC-1 gt/+ mice unlike wildtype. But when we used cleaved caspase -3 to stain the 3D acinar structures as such we did not see evidence of apoptosis (Fig 7). We used Etoposide as a positive control where we could see the Cleaved Caspase-3 in the acinar structures. These results suggest that as such filling up of the luminal space in the DLC-1 gt/+ acinar structures are not due to defects in apoptosis and support the idea that a defect in epithelial polarity is responsible.

Comment -4

- The quality of Figure 7A is not good. Please provide a better quality figure.
- We agree with the Reviewer and have therefore incorporated a clear representative blot picture of Figure 7A.

Minor Comment

Comment -5

- In order to connect Dlc1 function to RhoA, the authors may need to use dominant negative and constitutively active form of Rho and perform 3D culture experiments or provide evidence from the literature to connect D1c1 and RhoA.
In our previous publication on the Dlc1gt mice, (Sabbir et al 2010 BMC Biology), we showed that embryo cells from homozygous Dlc1^{gt/gt} mice showed elevated RhoA activity. As well others have published that Dlc1 is a RhoGap for RhoA in vitro. We have added statements to this effect in the manuscript.