Reviewer's report

**Title:** NPC1L1 knockout protects against colitis-associated tumorigenesis in mice

**Version:** 4  **Date:** 22 November 2014

**Reviewer:** D. Brent Brent Polk

**Reviewer's report:**

**Major Compulsory Revisions:**

1. The main conclusion of the paper that NPC1L1-/- strongly protects against colitis-associated tumorigenesis should be tempered. The data suggest that this is a mild effect.

2. The conclusion that the tumors were smaller in the NPC1L1-/- mice is not supported by the data since there do not appear to be any significant differences in the size distribution of the tumors. Furthermore, there was no difference in the number of tumors in the 18 week group.

3. It is unclear how the authors define malignant vs. non-malignant or how the malignant/tumor ratio was determined (Fig 2D), please clarify.

4. Since inflammation plays a major role in promoting tumorigenesis in the AOM/DSS model, the role of NPC1L1 in regulating inflammatory severity following DSS alone should be specifically evaluated in more detail than that shown in Fig 3C. Also, what was the treatment of the mice included in this panel? The inflammatory markers in Fig 3D are non-specific and not helpful to evaluate inflammation.

5. The conclusion that tumorigenesis is p53-independent is not strongly supported by the data, and previous studies with this model have already established that it is primarily beta catenin/Wnt driven. Please revise.

**Minor Essential Revisions:**

1. The data in Fig 1C is also included in Fig 1D-G; this is repetitive and unnecessary.

2. The axis labels in Fig 1D-G are confusing. Please label the x axis on each and clarify in the legend.

3. Please comment on whether the WT and NPC1L1-/- mice were littermates, and also whether all experimental mice received AOM injections at the same time from the same lot. There can be substantial variability in AOM/DSS results between litters and also with different lots/ages of AOM.

4. The role of plasma lipid changes in tumorigenesis are unclear given that there were no changes in plasma lipids in the 20 week group, which was the group used to analyze histological tumor changes. Please discuss.

5. The Western blot data for beta catenin in Fig 4 is unconvincing and difficult to interpret. Densitometry should be included and immunostaining of tumors would
be helpful.

Discretionary Revisions

1. The authors should comment on whether the NPC1L1 antagonist, ezetimibe would show similar results to those of the NPC1L1-/-.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.