Reviewer’s report

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

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Reviewer: Robert Eferl

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Summary
The authors induced CRCs with AOM/DSS in NPCL1-/- mice and observed reduced tumorigenesis and malignant tumor progression in comparison to control mice. NPCL1 protein is not expressed in CRCs and the colonic mucosa pointing to a non-tumor cell autonomous mechanism for reduced tumor formation. Several potential mechanisms such as reduced serum lipid levels, reduced colonic inflammation after AOM/DSS and reduced activation of the oncoprotein Jun in the colon mucosa are suggested.

Major compulsory revisions
1. It seems that the authors have used separate mouse colonies (wt and NPCL1-/-) for their analysis. Although both were C57/BL6, this approach cannot be recommended because AOM/DSS-induced CRC formation is influenced by many modifier genes. Reasonable data can only be obtained with littermates, derived from heterozygous NPCL1+/- breedings that are kept in the same cage. This would account for a genetic drift of separate colonies and the influence of commensal bacteria on CRC formation. The tumor data including histopathological evaluation of low grade, high grade and invasive tumors have to be reproduced with littermate controls.

2. The protein expression data in Figure 4 are interesting but should be verified by IHC-stainings of tumors and adjacent tissue. Tumors from AOM/DSS-treated mice are difficult to isolate since they are not pedunculated like that of ApcMin mice. Therefore, contamination with adjacent non-tumor tissue cannot be excluded and IHC is recommended.

Minor essential revisions
1. Proofreading by a native speaker is essential.
2. Page 8, 9 and discussion: replace ß-cantenin for ß-catenin.
3. Supplementary Figure 1 A-E is not referred to in the manuscript text. It remains also unclear why the parameters in Figure 1A-E have been measured. Does the length of the small intestine influence CRC formation? A brief explanation would be helpful.
4. It seems that the livers of NPCL1-/- mice shown in Supplementary Figure 1F are consistently bigger than those of wt mice although measured weight values in the Figure legend and the bar diagram in Supplementary Figure 1D claim the
opposite. How can this be explained?

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

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

Declaration of competing interests: no competing interests