Reviewer 3

Comments and Suggestions for Authors

Overall, this is a straightforward study of the effects of a PI3-K inhibitor, alpelisib (alp), in suppressing viability and proliferation of PTEN-haploinsufficient lipoma, either alone or in combination with mTOR inhibitor, rapamycin.

➢ Thank you for your comments.

Please refer to the following comments when revising the manuscript:

Table 1. Can the authors discuss the status of the remaining PTEN allele? Especially for LipPD1. Can PTEN protein be detected to qualify the use of the term haploinsufficient lipoma? How can the authors be sure that the remaining “wild-type” alleles are not inactivated through methylation, promoter deletion, etc? It will be very informative if a Western blot including all three LipPD cell lysates analyzed for PTEN, and other signaling molecules, if feasible.

➢ Thank you for this interesting question. In a previous study, we analysed PTEN and AKT phosphorylation in the LipPD cell cultures. While we actually cannot exclude the possibility of PTEN promoter inactivation, we showed a reduction, but no absence of PTEN mRNA and/or protein as well as increased AKT phosphorylation compared to PTEN wildtype controls. Specifically for LipPD1 we found that PTEN levels were reduced by approximately 50% compared to PTEN wildtype preadipocytes. The reference to this study by Kässner et al. is found in the manuscript (#18) and we additionally referred to it in the Material and Methods section (page 12, lines 354-358).

Fig. 5 Only minimal apoptotic cell death (<10%) in alp treated LipPD1 cells. Have the authors investigated other mechanisms of cell death such as autophagy, or necroptosis?

➢ We thank the reviewer for this suggestion, which was also mentioned by reviewers 1 and 2. To confirm our negative results from apoptosis assays, we performed LDH cytotoxicity assays which were found to be negative as well. We did not observe any rise in LDH during alpelisib treatment, thus concluding that the changes we observe in the WST-1 cell viability assay are mainly caused by inhibition of proliferation. We restructured Figure 5, added the results (Figure 5 b and page 6, lines 146-148 and supplementary Figure S5) and discussed them (page 11, lines 280-281).

Fig. 6 and 7. There appears to be some discrepancy between the >90% reduction in pS6/S6 levels in LipPD1 cells at 10 μM alp and the mere 50% drop in the fraction of pS6 positive cells by IF.

➢ While the Western blots were performed after 24 h alpelisib treatment, the immunofluorescence staining was done after 48 h. Moreover, we assume that the observed differences are seen due to methodological differences between Western blots and immunofluorescence staining. While in Western blots quantification is based on the level of pS6/S6 of all cells, for the immunofluorescence we only counted green cells above a certain threshold using Celleste Image Analysis Software (Thermo Fisher Scientific) software for analysis. We accordingly added a passage to the methods section (page 14, line 417).