Dear editor,

We appreciate the reviewer and your serious and professional evaluation of our manuscript (Manuscript NO: 56608). Based on these suggestions, we have addressed each of the concerns that were raised by the reviewer and revised the manuscript accordingly. This resulted in some changes in text of the manuscript. For your convenience, the following is point to point response to the reviewers' comments. Corresponding changes made in the manuscript are marked with red in the paper.

In addition, in order to improve the language quality of the manuscript, it has been edited at American Journal Experts. We have tried our best to revise our manuscript according to the comments.

Response to Reviewers' comments:

Reviewer #1

Specific Comments to Authors: The title of the manuscript is appropriate to the subject matter discussed in the paper. The authors have used the TCGA HCC dataset to find out the expression level of PLK1 and BIRC5 in a total of 374 HCC data sets. Authors found that both PKL1 and BRIC5 overexpressed in a subset of HCC data set. They discovered that co-expression of these proteins is frequently detected in HCC with p53 mutation, which also correlated with poor clinical outcome. They went on to use inhibitors of either PKL1 or BRIC5 to target Huh7 carrying Y220C mutation effectively, but HepG2 cell with WTp53 was not affected. Specific comments:

1. What is the expression status of these proteins in HCC tumors having wild type p53?

Response: Thank you very much for the positive comments on our work and all suggestions for improvement. PLK1 is a direct target for the p53 transcription factor, which binds to the PLK1 promoter to suppress its expression[1]. In response to DNA damage, wild-type p53 but not mutant p53 suppresses PLK1 expression in an E2F1-dependent manner[2]. Therefore, PLK1 expression is suppressed in cells with wild-type p53, and PLK1 is upregulated in cells with mutated p53 that lead to the loss or inactivation of p53. A similar scenario may also apply to the p53-BIRC5 axis, since wild-type but not mutant p53 also transcriptionally represses BIRC5 expression to promote p53-dependent cell apoptosis[3-5]. BIRC5 overexpression negatively regulates the expression of wild-type p53[6]. We have included these in third paragraph of the discussion, lines 4 to 9, and lines 11 to 14 (Page 12).

2. HuH7 carries a p53 mutation. The mutant p53 is not inactive in HuH7 cells

(http://dx.doi.org/10.1128/JVI.00729-15).

Response: Thanks for pointing this out. It has been shown that HuH7 cells harbor a homozygous p53 mutation (Y220C), which is a destabilizing mutation that results in partial DNA-binding activity compared to wild-type p53[7]. This type of mutant p53 can be pharmaceutically reactivated and functionally rescued by p53 activators such as ARP-246[8,9]. We have included these in third paragraph of the discussion, lines 29 to 33 (Page 13).

3. Do these proteins have an inhibitory effect on p53 function? Inhibition of either of these proteins may activate p53 function and induce apoptosis.

Response: Great point. Functioning within a negative regulatory feedback loop, PLK1 negatively regulates p53 transcriptional activation via physical interaction and through the phosphorylation of PLK1[10]. BIRC5 overexpression negatively regulates the expression of wild-type p53[6]. Inhibition of PLK1 using small molecules such as GSK461364A and volasertib was previously shown to have differential anti-tumor activity and cell apoptosis based on p53 mutation status[11-14]. Inhibition of BIRC5 by YM155 leads to decreased BIRC5 expression and increased expression of PUMA, a direct target of p53, which results in cell apoptosis[15]. Indeed, this phenomenon is observed in Huh7 cells with mutated p53 and in HepG2 cells with wild-type p53.We have included these in third paragraph of the discussion, lines 9 to 11, lines 13 to 14, and lines 23 to 29, (Page 12 and 13).

4. Protein-protein interaction between these protein and wild type p53 should be done using immunoprecipitation and Western blotting.

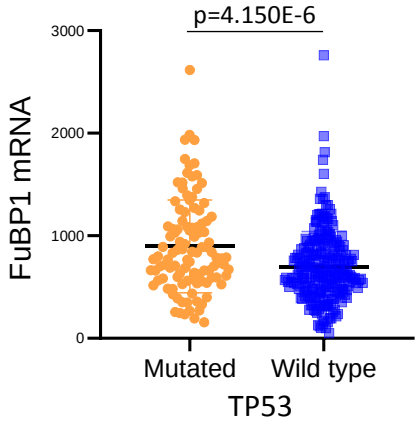
Response: Thanks for the comment. BIRC5 and PLK1 interact during mitosis, and PLK1 phosphorylates BIRC5 at serine 20[16]. In turn, BIRC5 regulates PLK1 localization to the kinetochore for the recruitment and dynamic localization of the BIRC5-containing chromosomal passenger complex (CPC) during cell division throughout mitosis[17,18]. However, no direct interaction between BIRC5 and p53 has been reported yet. Altogether, these results may explain why HCC cells with mutated p53 express higher levels of PLK1 and BIRC5 and suggest that PLK1 and BIRC5 work coordinately in contributing to cancer malignancies in cancer cells with p53 mutations. We have included these in third paragraph of the discussion, lines 14 to 22 (Page 12 and 13).

5. The status of other proteins such as FuBP1 which is overexpressed in 80 % of HCC tumors with CHC background, and strongly inhibits p53 function.

Response: Thanks for the comments on FuBP1. We did not detect any significant correlation between FUBP1 expression and that of either PLK1 or BIRC5, although FuBP1

is overexpressed in 80% of HCC tumors with chronic hepatitis C (CHC)[19]. FuBP1 is overexpressed in 80% of HCC tumors with CHC[19] and has been shown to inhibit p53 function[7]. However, we did not detect any significant correlation between FUBP1 expression with that of either PLK1 or BIRC5, indicating that FUBP1 is not functionally correlated with PLK1 and BIRC5 in contributing to HCC malignancy. We have included these in lines 5-8 on page 9 of the results section, Figure 1B (top row, two panels in the right) and third paragraph of the discussion, lines 33 to 37 (Page 13).

However, when we found that FUBP1 expression is significantly higher in HCC tumors with p53 mutants, compared to those with p53 mutants (data below), suggesting that FUBP1 may play a role in PLK1/BIRC5-independent manner in HCC tumors. Therefore, we did not include the data in our revised manuscript.



Response to Editors' comments:

1. I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

Response: Thanks for your suggestion. We have uploaded the copies of the approved grant application forms.

2. I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor. In consideration of color-blind readers, please avoid using red and green for contrast in vector graphics or images.

Response: Thanks for the tip. We have revised the figure panels 5F-5I, 6C, 7A-C and 8C-D accordingly. We have uploaded all figures.

3. I found the authors did not write the "article highlight" section. Please write the "article highlights" section at the end of the main text.

Response: Thanks for the tip. We have added the part accordingly.

4. The Institutional Review Board Approval Form was not applicable. The authors need to fill out the ARRIVE checklist with page numbers.

Response: Thank for your suggestions. We have submitted the 2 related documents according to the requirement.

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