Re-submission of the revised manuscript (Manuscript NO: 46577), entitled “The role and mechanism of circ-PRKCI in hepatocellular carcinoma”.

Dear Dr. Lian-Sheng Ma,

Thank you for your editorial efforts for our manuscript. Per your instructions for resubmission, we are submitting the revised manuscript of the above article. We also thank very much the anonymous reviewer for the constructive comments to strengthen this manuscript.

The detailed responses on a point-by-point basis are described below and the reviewer’s critiques have been accommodated fully in various parts of the revised version (shown in BLUE color).

We hope that you and the reviewer will now find the paper suitable for publication in World Journal of Gastroenterology.

Sincerely yours,
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Reviewer #1: The author showed the role and mechanism of circ-PRKCI and E2E7 in HCC. The work is well written and interested. However, I think the authors should consider several revisions.

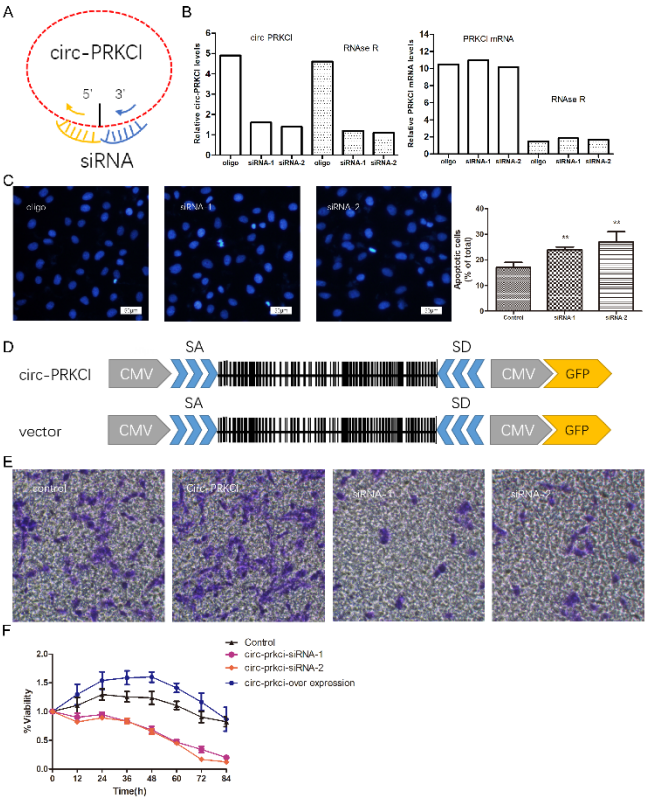
Major #1. In general, lymph nodes metastases of HCC is not so much. I think these were so much in this study. Is there any relation between lymph nodes metastases and circ-PRKCI/E2E7?

Reply: Thank you for your review. Circ-PRKCI produced from the *PRKCI* gene at 3q26.2 amplicon. It has been reported that amplification of PRKCI is associated with lymph node metastasis in esophageal squamous cell carcinoma.

#2. The authors should perform MTT-assay by using circ-PRKCI knock out or down, and over expression.

Reply: Thank you for your constructive comment. The MTT-assay has been supplemented. And the result showed that when circ-PRKCI knocked out, cell proliferation decreased, while increased after circ-PRKCI overexpression. It been showed in Figure 3F.

In the revised Figure 3:

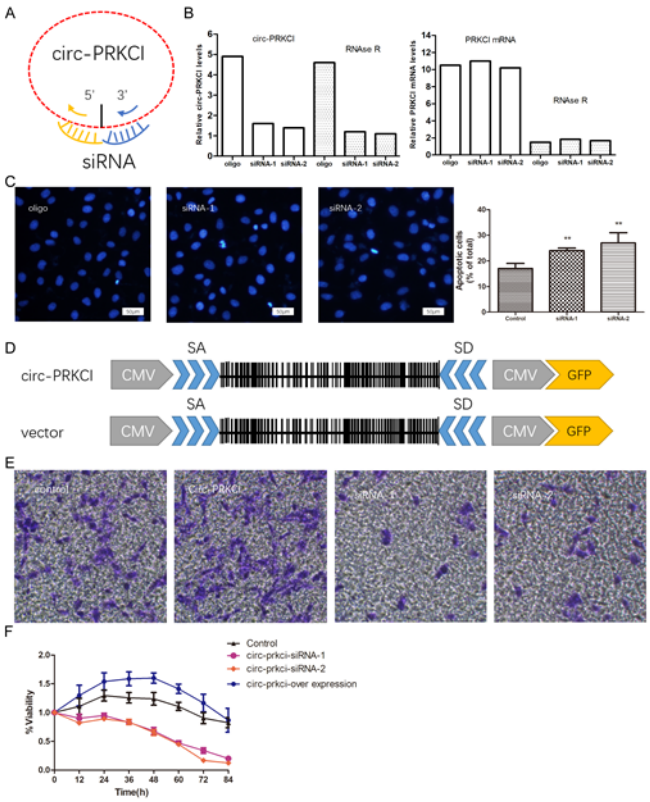


#3. Regarding Figure 3C, the authors showed representable photos about the apoptosis. I recommend that the authors should digitize and compare by statistics (ex: apoptotic index, etc…).

Reply: Thank you for your constructive comment. We have added a picture into

Figure 3C to presented statistical significance.

In the revised Figure 3:



#4. Regarding Figure 5, the authors should state the "reference value" of High or Low. The authors also should show relapse-free survival. Does "Survival" mean only cancer related death or overall? It should be stated.

Reply: Thank you for your careful review. A reference line has been represented in Figure 5. And the survival analysis was performed on the survival data downloaded from TCGA database. However, there was no data on the survival of patients without recurrence in the database. So, we cannot show relapse-free survival. In addition, "Survival" means only cancer related death. It has been supplemented in Figure 5.

In the revised Figure 5:

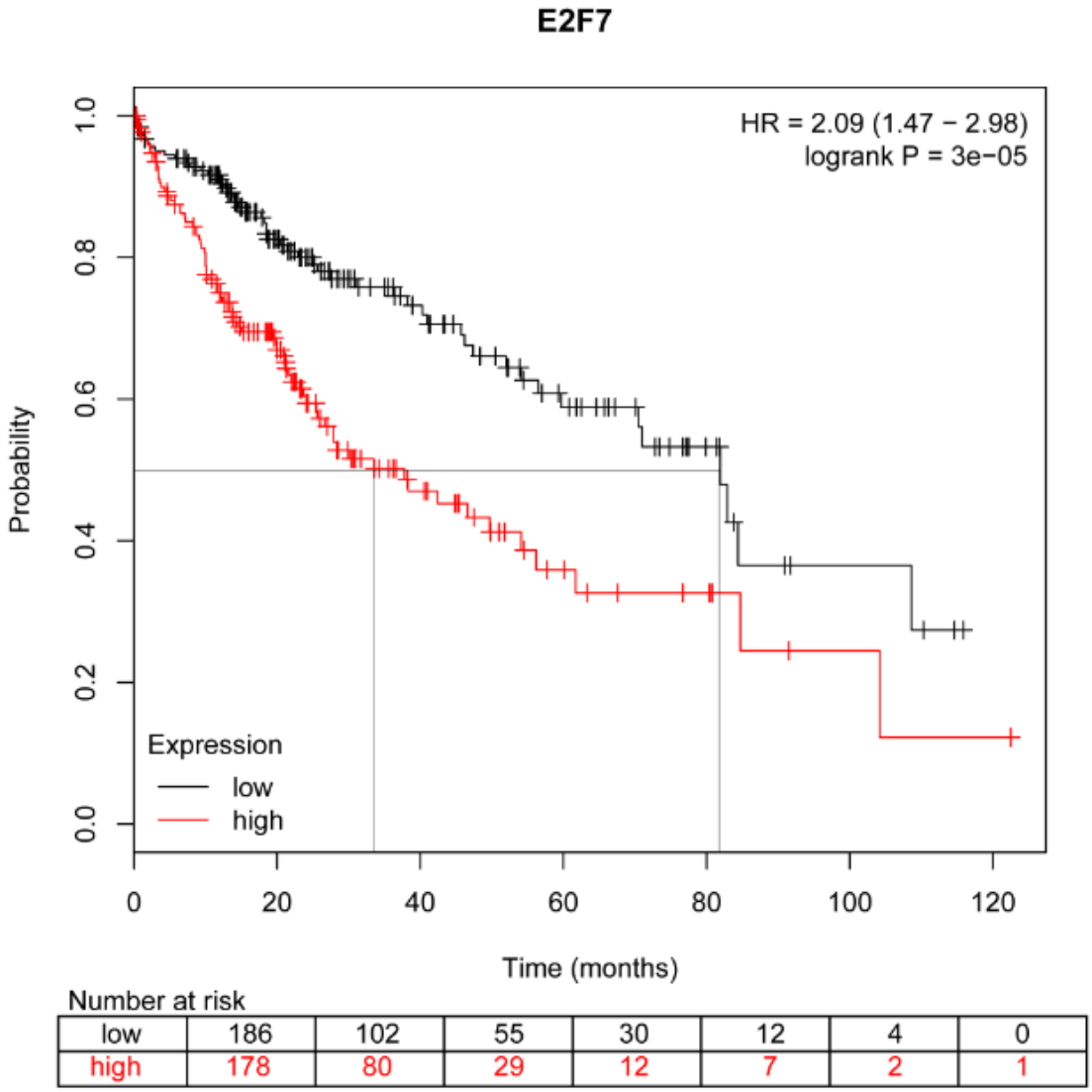


Figure 5 E2F7 affects survival rate of patients with liver cancer. The higher the expression level of E2F7, the lower the survival rate of patients.

* "survival" means only cancer related death.

#5. In Table 2, I recommend the authors had better to add portal venous invasion or hepatic venous invasion.

Reply: Thank you for your useful suggestion. In Table 2, we have supplemented "Portal venous invasion"

In the revised Table 2:

Table 2. Distribution of characteristics in HCC patients and control subjects

Variables	Patients (n=20)		Controls(n=20)		P^a
	n	%	n	%	
Age (years, mean ± SD)	63.5±5.9		60.3±7.8		0.68

Weight (kg, mean ± SD)	68.2±6.3		67.1±8.5		0.81
Gender					
Male	11	55.0	12	60.0	0.79
Female	9	45.0	8	40.0	
Depth of invasion					
T1/T2	7	35.0			
T3/T4	13	65.0			
Lymph node metastasis					
N0	11	55.0			
N1/N2/N3	9	45.0			
Distant metastasis					
M0	8	40.0			
M1	12	60.0			
TNM stage					
I/II	6	30.0			
III/IV	14	70.0			
Portal venous invasion					
Yes	9	45.0			
No	11	55.0			

[a] Independent-Samples T Test and Two-sided χ^2 test for selected variables distributions between cases and controls.

Minor #1. I think the explanation about circ-PRKCI is insufficient. I recommend more detail explanation about circ-PRKCI in “Introduction”.

Reply: Thank you for your constructive comment. According to it, we have tried our best to add more information about circ-PRKCI. However, few studies focus on circ-PRKCI, so the information is limited.

In the revised INTRODUCTION section:

“…Circ-PRKCI produced from the PRKCI gene at 3q26.2 amplicon. It has been verified that down-regulation of circ-PRKCI can inhibit the expression of PLCB1, a target of miR-1324, to inhibit the cell migration and proliferation of congenital Hirschsprung's disease [10]. In lung adenocarcinoma, circ-PRKCI acts as a sponge of miRNA-545 and miRNA-589, and eliminates their inhibitory effects on proto-oncogene transcription factor E2F7 [11]. In esophageal squamous cell carcinoma, circ-PRKCI can sponge miR-3680-3p to regulate AKT3 expression [12]. ….”

Reviewer #2: The manuscript of "The role and mechanism of circ-PRKCI in hepatocellular carcinoma" by Qi et al try to explore the role and mechanism of circ-PRKCI in HCC, it's very interesting and useful. It may be as a new biomarker for diagnosis of HCC or other digestive system tumors. This manuscript can be accepted after some minor revision:

1 the authors test many digestive system tumor cells, but just present some results in HCC, are there obvious difference? Why?

Reply: Thank you for your careful review. The expression level of circ-PRKCI in multiple digestive system tumor cells were present in Figure 2C. It was observed that the expression level in all tumor cells is significantly higher than that in control. And it in HCC cells is the highest. In addition, the relevant content has been supplemented in the manuscript.

In the revised *Expression characteristics of circ-PRKCI in hepatocellular carcinoma* section:

"…It was observed that the expression levels in different tumor cell lines is significantly higher than that in normal cells, and it is highest in HCC cell lines HepG2 and Hep3B (Figure 2C), signifying circ-PRKCI is generally highly expressed in digestive system tumors. …"

2 Please improve image resolution.

Reply: Thank you for your careful review. According to it, the resolution of all images has been improved.

3 Some grammar problems need to be solved.

Reply: Thank you for your careful review. We have improved the resolution of all images to ensure clarity. In addition, we have checked the whole manuscript to correct grammatical errors.