Reviewer #1: Very interesting study. After a minor revision, it can be accepted for publication. The discussion should be revised, there may differences in response between HCC cell lines and the human tumors in situ, as suggested by the expression of the staining also in non-tumor adjacent tissue.

Response to Reviewer #1: We argued that The Dbx2 expression of HCC cell lines was in accordance with human tumors in situ. As figure 1D showed, Dbx2 expression was found in both non-tumor adjacent tissue and tumor tissue. However, Dbx2 expression of tumor was obviously higher than non-tumor adjacent tissue. In HCC cell lines, as figure 1F showed, Dbx2 expression stayed in higher level in most of HCC cell lines, but the SMMC-7721 cell lines had a similar or lower expression of Dbx2 than normal hepatoma epithelial LO2 cells.

Reviewer #2: Congratulate the authors. Very interesting study about the Dbx2 performs a tumor-promoting function in HCC via regulating the Shh-Gli1 pathway. The study was well designed and the manuscript is well organized. Only some very minor language polishing should take attention. Thank you.

Response to Reviewer #2: Thank you so much for your positive comments. We have corrected manuscript by native speakers of English

Reviewer #3: I have read with great interest the manuscript entitled “Dbx2 performs a tumor-promoting function in HCC via regulating the Shh-Gli1 pathway”, submitted to the World Journal of Gastroenterology. In this basic study the authors investigate the role of Dbx2 in regulating the HCC cell proliferation, apoptosis and cell migration. Additionally, they correlated the immunohistochemical expression of this marker in 76 surgically resected HCC with clinical and pathological features of the tumor. First, I would like to congratulate the authors for the extensive work performed and for the investigating the expression of Dbx2 in human tissue, apart from HCC cell lines. The methodology employed for the experiments is scientifically sound and meaningful, and the manuscript well written and reads well. However, I have some concerns

mainly regarding over-interpretation of the data. Major comments: 1. Major concern is over-interpretation of the data. Many statements throughout the manuscript are based on the finding that overexpression of Dbx2 is associated with tumor growth/ large tumor size on the surgically resected HCC. For example, this affirmation can be found: - At the introduction, in the results section, “resected HCC tissues compared to that in matched adjacent non-tumorous tissues and clinically correlated with large tumor size” - At the result, “We speculate that Dbx2 may participate in the development of HCC.” - At the discussion, “the first evidence of aberrant upregulation of Dbx2 in HCC tissues and indicate the clinical significance of Dbx2 in HCC pathogenesis” The data provided to support those statements are presented in table 2. Examining carefully the results, the occurrence of male sex was higher in Dbx2 high group. However, the whole cohort of patients were predominantly constituted of men, thus attributing this difference to Dbx2 may be at least debatable. This should be addressed or discussed. More worryingly, the interpretation of the second significant difference between groups, regarding tumor size, should be re-considered. The “Dbx2 Low group” had the majority of patients with a tumor <5cm, however in “Dbx2 High” group the number of patients with a tumor greater or smaller than 5cm was basically equal. Therefore, higher expression of Dbx2 was not really associated with large tumor size and this data does not support those statements above. Additionally, Dbx2 was positive in 40.79% of the cases in adjacent NON-tumor tissues. Although this rate was higher in tumors, this figure is relevant, and in accordance, it should be at least discussed in the manuscript. From figure 1E, approximately 38% of the tumor tissue had a similar or lower expression of Dbx2 than the adjacent non-tumor tissue. 2. The response to ectopic expression of Dbx2 varies between different HCC cell lines, as seen in Figure 5A. Please comment on this. Minor comments: 1. In accordance with the previous comments, please clarify along the manuscript that whereas the mechanistical findings observed suggest that Dbx2 works in HCC lines, further studies should look how it would affect an in vivo human tumor. For example, the first phrase of the second paragraph in the discussion says “knockdown of Dbx2 inhibited HCC proliferation”. Please consider discussing that

there may differences in response between HCC cell lines and the human tumors in situ, as suggested by the expression of the staining also in non-tumor adjacent tissue. 2. The title should be amended accordingly, as “Dbx2 performs a tumor-promoting function in HCC CELL LINES via regulating the Shh-Gli1 pathway”

Response to Reviewer #3: Major comments:

1. Major concern is over-interpretation of the data. Many statements throughout the manuscript are based on the finding that overexpression of Dbx2 is associated with tumor growth/ large tumor size on the surgically resected HCC. For example, this affirmation can be found: At the introduction, in the results section, “resected HCC tissues compared to that in matched adjacent non-tumorous tissues and clinically correlated with large tumor size”. At the result, “We speculate that Dbx2 may participate in the development of HCC.” At the discussion, “the first evidence of aberrant upregulation of Dbx2 in HCC tissues and indicate the clinical significance of Dbx2 in HCC pathogenesis” The data provided to support those statements are presented in table 2.

   Response: In this study, higher Dbx2 expression were found in 5 HCC cell lines and 76 surgically resected HCC tissues compared to that in matched adjacent non-tumorous tissues. Expression of Dbx2 may be correlated with tumor size. Based on our data, we estimated that Dbx2 expression might be related to T stage of HCC and poor prognosis. Compared to non-tumorous tissues, aberrant upregulation of Dbx2 was found in HCC tissues in our study. Dbx2 might be another maker of HCC in the future.

2. Examining carefully the results, the occurrence of male sex was higher in Dbx2 high group. However, the whole cohort of patients were predominantly constituted of men, thus attributing this difference to Dbx2 may be at least debatable. This should be addressed or discussed.

Response: Due to specimen collection, there were data deviation in our study, we will increase the sample size next step.

3. More worryingly, the interpretation of the second significant difference between groups, regarding tumor size, should be re-considered. The "Dbx2 Low group" had the majority of patients with a tumor <5cm, however in "Dbx2 High" group the number of patients with a tumor greater or smaller than 5cm was basically equal. Therefore, higher expression of Dbx2 was not really associated with large tumor size and this data does not support those statements above.

   Response: These differences between the two groups of patients could have biased, we will increase the sample size to confirm that higher expression of Dbx2 was associated with large tumor size or not. We will discuss this group biased in our discussion.

4. Additionally, Dbx2 was positive in 40.79% of the cases in adjacent NON-tumor tissues. Although this rate was higher in tumors, this figure is relevant, and in accordance, it should be at least discussed in the manuscript. From figure 1E, approximately 38% of the tumor tissue had a similar or lower expression of Dbx2 than the adjacent non-tumor tissue.

   Response: Dbx2 expression of tumors than adjacent NON-tumor tissues as was higher in tumors as figure 1D showed，however, Dbx2 was positive in 40.79% of the cases in adjacent NON-tumor tissues, it may confer that Dbx2 expression was related with tumor development. From figure 1E, approximately 38% of the tumor tissue had a similar or lower expression of Dbx2 than the adjacent non-tumor tissue. The response to ectopic expression of Dbx2 varies between different HCC cell lines, as seen in Figure 5A. We argued that Dbx2 expression might be not specific.

5. The response to ectopic expression of Dbx2 varies between different HCC cell lines, as seen in Figure 5A. Please comment on this.

Response: Our data clearly showed that Dbx2 interacted with Shh in SMMC-7721 and Huh7 cell lines. Loss of Dbx2 resulted in significant repression of the Shh pathway during short-term Shh stimulation in HepG2 and Huh7 cells.

Minor comments:

6. In accordance with the previous comments, please clarify along the manuscript that whereas the mechanistical findings observed suggest that Dbx2 works in HCC lines, further studies should look how it would affect an in vivo human tumor. For example, the first phrase of the second paragraph in the discussion says "knockdown of Dbx2 inhibited HCC proliferation". Please consider discussing that there may differences in response between HCC cell lines and the human tumors in situ, as suggested by the expression of the staining also in non-tumor adjacent tissue.

   Response: Thank you so much for your constructive comments. We have revised our manuscript

7. The title should be amended accordingly, as "Dbx2 performs a tumor-promoting function in HCC CELL LINES via regulating the Shh-Gli1 pathway"

   Response: We have made correction according to your excellent comments.