We would like to thank the reviewers for all the valuable comments to improve the manuscript. We have addressed all the comments as shown below.

Reviewer #1: This is an interesting study. However, the effect of DLL4 silencing is not clearly elucidated requiring further explanation. Moreover, the conclusion in this regard is confusing "intrinsically suppresses HBV replication" as mentioned in the abstract conclusion. while in results and discussion it is mentioned "Unexpectedly, increased viral replication was observed in HepG2.2.15 upon DLL4 silencing in vivo"

Answer: We agreed with the reviewer's comments and added the following to the text:

Regarding the effect of DLL4 silencing, the following as added "The effects of DLL4 silencing in HCC cell line was observed at two main levels, one was the effect on tumour growth and the other was the effect on viral replication."

Regarding the "intrinsic effect" of DLL4 on promoting HBV viral replication, we added the following to the discussion "How does DLL4 silencing promote HBV viral replication? There are two possibilities, the intrinsic and/or the extrinsic effect. If silencing DLL4 creates environment that are friendly to viral replication such as inducing less anti-viral cytokines IFNα/β or promoting skewed helper T cell polarization, this is considered an extrinsic effect. In contrast, what we observed is that there was no significant difference in IFNα/β level between control and tumour with silenced DLL4 and the mice lacked adaptive immune response. Thus, we concluded that the viral replication promoting effect must be intrinsic, namely the intracellular environment with reducing DLL4 level allows the virus to replicate better. Currently, there is no evidence that this effect is dependent upon reducing Notch signalling.**"**

Reviewer#2

Comment 1." The authors investigated the tumour suppressing effect of DLL4 silenced HCC cell line using HepB expressing cell line in vitro and increased HBV viral production that is not consistent previous result of authors'. Is it applicable to another cell lines, non-hepB expressing cell line? Virus can intervene for the promotion or inhibition of the carcinoma."

Answer: The reviewer correctly pointed out the discrepancy of our previous report on the effect of DLL4 silencing on HBV replication in vitro and our current finding in in vivo. This is an unexpected finding and we explained this in the discussion as "Unexpectedly, increased viral replication was observed in HepG2.2.15 upon DLL4 silencing *in vivo*. We previously reported that silencing *DLL4* in HepG2.2.15 *in vitro* did not alter viral replication[22]. This discrepancy highlights the more complex multi-cellular interactions *in vivo*.".

The mechanism how DLL4 regulates HBV viral replication is currently not know. We added the following to the text discussing this point: "If hypoxia and cellular stress due to defective angiogenesis caused by DLL4 silencing is the cause for enhancing viral replication, it is speculated that silencing DLL4 expression may promote viral replication in other cell types as well. Whether increased HBV replication is the cause for reduced tumour growth is not determine."

Comment 2. "Recently a lot of result of gene set affecting on the cancer cells were reported, like TCGA data published last year (Cell, July 2017). That is very intensive result using HCC tumour tissues that is collected globally. There are strong gene characteristics, copy number, RNA expression, microRNAs, methylation etc. genomic characterization related HCC. Notch or DLL is not enlisted in the significant genes in that study. Reviewer would recommend the result is to be validated or compared by the result of other investigators including TCGA data, and put the authors' view in the discussion.

Answer: Thank you for the insightful comments. We agreed with the reviewer on this point and added the following to the discussion:

"Recent comprehensive and integrative genomic characterization of hepatocellular carcinoma was performed on various HCC of different etiologies. Although Notch receptors/ligands and the associated signalling molecules did not stand out as the prime mutated genes, the core Notch signalling was one of the pathways in HCC with enriched frequencies of functionally impactful mutations (ranked as No. 71)[42]. In HCV-related HCC, the Notch tumour signature genes (activation and deregulation) were found in 31.8% of patients (n=91), suggesting a partial role of Notch signalling in promoting HCC in HCV infection[16]. This analysis result highlights the fact that Notch signalling may be involved in certain, but not all, subsets of HCC. In addition, it is not known whether HCC arising from other non-viral infection causes but DLL4 has been linked to liver fibrosis and non-alcoholic steatohepatitis pathogenesis[43]."

References

16 **Villanueva A**, Alsinet C, Yanger K, Hoshida Y, Zong Y, Toffanin S, Rodriguez-Carunchio L, Sole M, Thung S, Stanger BZ, Llovet JM. Notch signaling is activated in

human hepatocellular carcinoma and induces tumor formation in mice. *Gastroenterology* 2012; **143**(6): 1660-1669 e1667 [PMID: 22974708 PMCID: 3505826 DOI: 10.1053/j.gastro.2012.09.002]
42 **Cancer Genome Atlas Research Network**. Electronic address wbe, Cancer Genome Atlas Research N. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017; **169**(7): 1327-1341 e1323 [PMID: 28622513 PMCID: PMC5680778 DOI: 10.1016/j.cell.2017.05.046]
43 **Kawaguchi K**, Honda M, Kaneko S. The Role of Notch Signaling in Liver Diseases: Contribution to Development and Cancer. *Int J Cancer Clin Res* 2017; **4**(1) [DOI: 10.23937/2378-3419/1410079]

Reviewer#3

Comment 1. "Some studies stated that high level of dll4 may inhibit the growth and metastasis of HCC; which may be different than your findings. So; you should give clear explanation to the readers and add this in discussion."

Answer: We have addressed this comment by revised manuscript in discussion as "However, there were studies suggested that the high level of DLL4 associated with inhibition of tumour growth and metastasis in HCC [35, 36]. Notch receptors have been suggested to play a role in both oncogenes and tumour-suppressor gene in different cell types [37, 38]. We hypothesized that DLL4 may act as an oncogenic in the initiation stage of tumour development and then act as a tumour suppressor in the late stage for inhibiting tumour metastasis depend on the DLL4 isoform or other tumour microenvironments. However, the dual function of DLL4 as tumour-suppressor and oncogenicity associated their isoform need to be further clarified."

References

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Comment 2. "Do you think these results could be applied to other etiologies of HCC?: This should be clarified in discussion."

Answer: Thank you for the comment. We discussed the possibility that Notch/Dll4 may be involved in HCC of other etiologies as follows:

"In HCV-related HCC, the Notch tumour signature genes (activation and deregulation) were found in 31.8% of patients (n=91), suggesting a partial role of Notch signalling in promoting HCC in HCV infection[16]. This analysis result highlights the fact that Notch signalling may be involved in certain, but not all, subsets of HCC. In addition, it is not known whether HCC arising from other non-viral infection causes but DLL4 has been linked to liver fibrosis and non-alcoholic steatohepatitis pathogenesis[43]."

References

16 **Villanueva A**, Alsinet C, Yanger K, Hoshida Y, Zong Y, Toffanin S, Rodriguez-Carunchio L, Sole M, Thung S, Stanger BZ, Llovet JM. Notch signaling is activated in human hepatocellular carcinoma and induces tumor formation in mice. *Gastroenterology* 2012; **143**(6): 1660-1669 e1667 [PMID: 22974708 PMCID: 3505826 DOI: 10.1053/j.gastro.2012.09.002]

43 **Kawaguchi K**, Honda M, Kaneko S. The Role of Notch Signaling in Liver Diseases: Contribution to Development and Cancer. *Int J Cancer Clin Res* 2017; **4**(1) [DOI: 10.23937/2378-3419/1410079]

Reviewer #4

The authors examined the DLL4 signal on HCC tumour growth and VEGF/VEGFR2 expression on HCC tumour by employing HCC xenograft of mouse models made by HepG2.2.15 or shDLL4 HepG2.2.15 cell line subcutaneous injection. The overall experimental design and performance are well conducted. The results from the studies are convincing and interesting. The data are properly presented.

Answer: Thank you for the comment about our research.