Dear Dr. Wang,

In this letter we address the review's comments about our manuscript. Entitled "Detection of hyper-conserved regions in hepatitis B virus X gene potentially useful for gene therapy" (ID 38396). First of all, thanks for the comments and the reviews. We changed the abbreviation as suggested in the comments, and we wrote the highlights field. Moreover, we changed the Figure 1 legend, hoping that this is acceptable. In addition, we have a question about table 1. We think that its format correct according the guidelines, in the contrary, please, indicate us what changes should be performed.

Follow, you will find the answers to reviewers.

We hope that in this present form you will find our manuscript suitable for publishing in *World Journal of Gastroenteroloy* and look forward your response.

Sincerely,

Francisco Rodriguez-Frias, PhD

1) They identified two highly conserved regions. As they discussed, in vitro functional studies should be important to evaluate the potential usefulness of these domains as targets for siRNA-based antiviral gene therapy. However, the experimental study may be included in the next paper. I would like to know whether they consider "these two domains are equivalent" or "one of the domains can be more promising than the other one".

As commented in the manuscript (page 15), it has been reported that HBx translation initiate at multiple sites (Treinin M et al. Mol. Cell. Biol. 1987), and the first hyper-conserved region (nt 1255-1286 in the non-coding gene portion), although it is maintained in all viral transcripts, may not be included in HBX transcripts. Consequently, a siRNA system targeting this region could optimally interfere with the other viral proteins but not with HBx expression. Differently, the second hyper-conserved region (nt 1519-1603), within the

coding- *HBX* portion, should be maintained during HBV genome transcription, including all *HBX* transcripts, being a more promising candidate for siRNA-based therapy.

2) They used various/heterogeneous clinical samples and detected two conserved regions. However, HBx protein is suggested to relate to hepato-carcinogenesis. Did the NGS analysis provide any common variant among the patients who had hepatocellular carcinoma? (Did they find any mutation as a candidate for carcinogenesis-associated sequence in HBx region?)

By analyzing the viral quasispecies variants, we did not find any known mutation associated with HCC (Ali et al. World J Gastroenterol 2014, Kim H et al. World j Gastroenterol 2014). Moreover, this study included only 3/27 HCC patients, which is a relatively small sample size to relate new HBX gene mutations with the appearance of liver cancer. This interesting suggestion will be explored in the future evolution of this study with a higher number of HCC patients.