Dear Reviewer,

Thank you for your kind consideration for publication of our manuscript entitled “The synergistic anti-tumor activity of EZH2 inhibitor SHR2554 and HDAC inhibitor chidamide through ORC1 reduction of DNA replication process in diffuse large B cell lymphoma” in Cancers. We deeply appreciate your insightful comments and helpful suggestions. We have made revisions accordingly and responded all the comments point-by-point as follows.

1. In simple summary: it says twice wide-type?? Should be wild-type?

   The Simple Summary section was updated according to the comment of reviewer. (Paragraph 1, Page 1)

2. In figure 2 parts a/b and c/d seem very similar, could c and d be moved to the supplemental data?

   The different drug administration methods were used in Figure 2. The DLBCL cells were spontaneously treated with EZH2 inhibitor and HDAC inhibitor to analyzed synergistic effects in Figure 2a&b. On the other hand, the sequential drug administration of EZH2 inhibitor and HDAC inhibitor was used in Figure 2c&d. All these results indicated that the co-administration of these epigenetic inhibitors could induce synergistic effects in different drug administration methods. The related information was updated in Paragraph 3, Page 8.

3. The gene expression data in figure 4: there is quite a difference in U2392 and SUDHL6 in gene expression in numbers but also in GO’s. Are there also genes that are changed that they have in common? Of course this is only data from 2 individual cell lines, can there be much emphasis on such minimal results?

   The gene ontology (GO) and gene set enrichment analysis (GSEA) were performed based on RNA-Seq data to investigate the mechanism underlying the related pathway regulated by combined treatment of EZH2 and HDAC inhibitor. Significant enrichment of DNA replication process was observed in both EZH2 wild-type and mutant tumor cells which was consistent with the results of cell proliferation and cell cycle in Figure 2 and 3. Since the GO and GSEA analysis illustrated that the DNA replication process related ORC1 expression was decreased in both U2932 and SU-DHL-6 cells, the mRNA expression of ORC1 were next analyzed by real-time PCR. As shown in Figure 4d, the results from qPCR analysis indicated that the combination treatment significantly decreased ORC1 expression in both EZH2 wild-type and mutant tumor cells. To further illustrate the possible anti-tumor mechanisms of combination treatment, the ORC1 shRNA were transfected into both U2932 and SU-DHL-6 cells. The knockdown of ORC1 expression suppressed proliferation of tumor cells, which indicated ORC1 expression is critical to the survival of DLBCL cells (Figure 4e&f). Interestingly, the similar proliferation inhibitory effect was observed in ORC1 shRNA and combination treatment, which

indicating that ORC1 expression was essential for DLBCL tumor cells and suppression of ORC1 in DNA replication process may contribute to the synergistic anti-tumor effect after co-administration of SHR2554 and HBI8000. The related information was updated in Paragraph 1, Page 14.

4. Why was pcr done for ORC1, it is not clear why ORC1 was picked as a representative of what including shRNA knock downs. Please make clear why ORC1 was picked, based on which results!

Significant enrichment of DNA replication process was observed in both EZH2 wild-type and mutant tumor cells. Since the GO and GSEA analysis illustrated that the DNA replication process related ORC1 expression was decreased in both U2932 and SU-DHL-6 cells, the mRNA expression of ORC1 were next analyzed by real-time PCR. As shown in Figure 4D, the results from qPCR analysis indicated that the combination treatment significantly decreased ORC1 expression in both EZH2 wild-type and mutant tumor cells. The origin recognition complex (ORC) plays an important role in the initiation of DNA replication. ORC1 is the largest component subunit of ORC protein and determines the biological activity of DNA replication initiation sites and cell proliferation. Thus, the ORC1 was pick up to further illustrate the possible anti-tumor mechanisms of combination treatment. The related information was updated in Paragraph 1, Page 14.