The point-by-point responses to the reviewers' comments

Responses to comments from the Referee 1

The manuscript starts with the observation that FEN1 protein levels are strongly reduced upon H2O2 treatment in different cancer cell lines. This occurs independently of YY levels, what pushed the authors to look for alternative regulation factors. Bioinformatic analysis of the 5'UTR region of FEN1 gene reveals the existence of potential G4 forming sequences, among which number 4 is selected and confirmed. A mutant in this sequence is also crated for further experiments, but it is not used later on in vivo. Further in vitro experiments demonstrate that H2O2 treatment of the existence of 8oxoG instead of G affects the stability of the G4 structure. G4 destabilization by TMPyP4 decreased FEN1 protein levels. A search for proteins that interact with this G4 identifies several RNA binding factors, among which hnRNPA1 was confirmed. Up to here, the manuscript is well conducted and nice. However, hnRNPA1 knockdown still allows FEN1 downregulation upon H2O2 treatment (Figure 5B) and the effect on DNA damage seems independent on oxidative stress.

Thus, in general, the manuscript could be interesting at the level of FEN1 expression regulation but the consequences of the loss of this regulation or the mechanism need further investigation

Thank you very much for the positive comments.

Specific comments that need to be addressed:

- The manuscript starts with the observation that FEN1 protein levels are strongly reduced upon H2O2 treatment in different cancer cell lines. However, it has been previously reported that FEN1 levels decrease in HeLa cells upon oxidative damage. Have the authors tested HeLa cells themselves? Can they reproduce published data?

Thank you for the comment. In this manuscript, we found that FEN1 was downregulated under oxidative stress in different cancer cell lines. However, as we mentioned in Page 13 line358, our previous study has showed that oxidative stress upregulated FEN1 expression in HeLa cells (Ma Z, et al. *Redox Biol.* 2020; 37: 101653). Considering the oxidative stress condition is different, we re-examined FEN1 expression in HeLa cells according to the oxidative stress conditions in this manuscript. The data showed that FEN1 was significant upregulated under oxidative stress, which confirmed our previous work (Fig 1. for review only). The inconsistent result indicates there may be other mechanisms involved in FEN1 expression regulation in HeLa cells.

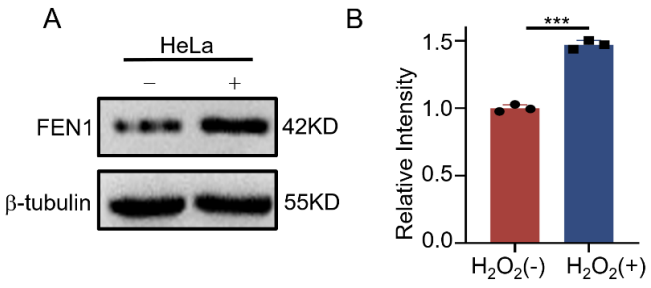


Figure 1. FEN1 was downregulated under oxidative stress in HeLa cells. (**A**) HeLa cells were treated with 300 μM H_2O_2 for 2 h and then recovered for 4 h. Western blot was used to measure FEN1 protein levels and β-tubulin was used as a loading control. (**B**) Quantitative analysis of

panel (A). The data represent the mean ± SD of three independent experiments. Student's t-test, ***$p < 0.001$.

-the authors claim a regulation at the level of mRNA since the model is that the mRNA stability is reduced but the current version of the manuscript show only western blots. Northern blots and/or RT-qPCR experiments are required.
Thank you for your good suggestion. We have added RT-qPCR experimental data in the revised manuscript. (Figures 1C, 3G, 3J and 3M)

-why is rPQS3 not selected? The profile in Figure 2C is quite similar to rPQS4
Thank you for the comment. As shown in figures 2C, rPQS3 and rPQS4 are indeed very similar. Since rPQS4 has a higher peak at 265 nm, it can form a more typical parallel G quadruplex and the structure will be more stable. Of course, we do not rule out the effect of rPQS3 on the expression of FEN1, and we will conduct further research on rPQS3 in the future.

-what would be the effect of G4 stabilization by pyridostatin? Are the authors able to detect increased FEN1 expression upon oxidative stress in this case?
We sincerely appreciate the valuable comments. To further clarify the role of FEN1 rG4, we applied Western blot to detect FEN1expression in A549 and HeLa cells after treatment with pyridostatin. Now, the WB data is added in the Figures. (Figures 3J, 3K, 3L, 3M, 3N and 3O)

-figure 5A. the correlation coefficient is rather low and I am not sure that this adds much to the paper at this point. Moreover, why is only breast cancer analyzed and not other tumors?
Thank you for pointing out the issue. We have added the relevance of hnRNAP1 and FEN1 in other tumors in the revised manuscript. (Figures 5A-5D)

-figure 5E, isn't significant the increase of gH2AX foci significant in sh hnRNPA1 cells (untreated with H2O2)? Has this been reported before? This should be discussed. In other words, the increase of damage seems independent on H2O2.
Yes. In sh-hnRNPA1 cells, the foci numbers of γH2AX were significantly increased and the P value was 0.000006. So far, there were no reports we read showing that hnRNPA1 knockdown increased the level of γH2AX. Figure 5F and 5H showed that knockdown of hnRNPA1 lead to downregulation of FEN1, which might further lead to DNA replication stress. Therefore, the increased level of γH2AX may be partly due to the enhanced DNA replication stress. We have added this discussion to the new manuscript. (Page 13 line 390)
In figures 5G and 5H, the foci numbers of γH2AX were dramatically increased after hnRNPA1 knockdown combined with H_2O_2 treatment, which revealed oxidative stress exacerbated the effect of hnRNPA1 knockdown-induced γH2AX levels.

Other minor comments:
-the statistic used in each panel is not clear or indicated in the figure legends.
Thank you for your reminder. Based on your comments, we have added the statistics used in each panel in the figure legends. (Page 4 line 162, Page 5 line 190, Page 7 line 240, Page 10 line 284, Page 11, line316, Page 12, line 340)

-Redundancy in line 51: In the BER system...in BER.

Thank you. We have removed redundant words. (Page 2 line 52)

-line 55: word missing?: hypomethylation of FEN1 *promoter?*

We sincerely thank the reviewer for careful reading. As suggested by the reviewer, we have corrected the " hypomethylation of FEN1" into "hypomethylation of FEN1 promoter". (Page 2 line 55)

-line 63: word missing?: the key *role* of FEN1

Thank you. We have corrected the " the key of FEN1" into " the key role of FEN1". (Page 2 line 63)

-line 315: n missing: knockdow*n*

Thanks for your correction. The typo is now revised. (Page 12 line 327)