Reviewer 1 （Number ID：03659753）：
To authors
The authors examined the relationship among parp1-sirt1-HMGB1 release and the mechanism of HMGB1 release from H2O2-injured hepatocytes related to sirt1 functional inhibition. I think this manuscript will be interesting and contain important information. However, some contents of the present study are unclear and so confusable. There are some problems as following:
1. In Figure 4 and 5, HMGB1 protein levels should be shown. In these Figures, the authors examined the relationships between Sirt1 expression and HMGB1 acetylation. However, I cannot accurately understand the relationships between HMGB1 and Sirt1 expression in each condition.
In Figure 5A, HMGB1 expression will be increased by EX527 treatment. However, in Figure 5D, HMGB1 expression is not different in any conditions, and only HMGB1 acetylation is increased by sh-Sirt1. Moreover, I cannot confirm the decrease of Sirt1 expression in H2O2 treatment, such as in Figure4A. These points should be explained more clearly.
Replay:
Because there are many data in Fig 5,we just focused on the acetylated HMGB1 change after cells treated with sirt1 inhibitor EX527 In Figure both 5A and 5D.
HMGB1 is one of substrate of sirt1 which catalyzes HMGB1 deacetylation to keep it in nucleus. Once, sirt1 inhibited by EX527, HMGB1 exists in the form of hyperacetylation which is easier to translocate from nucleus to cytoplasm or finally releases to outside. In Fig5, sirt1 suppression leads to hyperacetylated HMGB1 increases.

2. In the last paragraph in Results section, the authors concluded that Sirt1 negatively regulated Parp1. However, the authors also mentioned “Parp1-Sirt1-HMGB1” pathway is crucial for HMGB1 acetylation and release. After all, what is the first alteration in H2O2 treatment? Is the acetylation of Parp1 or decrease of Sirt1 activity? These points are so important and should be shown correctly.
Replay: H2O2, a highly diffusive molecule, can easily cross plasma membrane and cause a series of events, it is difficult to discriminate which event is the first.
PARP1 and sirt1 are functional interaction inhibition, and both of them are NAD^+-dependent enzymes. PARP1 over activation leading to NAD^+ depletion, Which can inhibited SIRT1 activity, on the contrary, SIRT1 suppressed parp1 by deacetylation.

3. In the Introduction section, the authors hypothesize that “Sirt1 activity suppression leads to HMGB1 deacetylation” or “DNA damage triggers the cascade reaction of parp1–sirt1–HMGB1 deacetylation”. However, this is inconsistent with your results in this study.

Replay: sorry, this is a writing mistake, and it is corrected in manuscript.

4. In Figure 2A, 2D, or 6A, the authors mentioned elevated LDH release, HMGB1 concentration, and NAD+ content are restored to nearly normal levels at 24 hr. However, why such results occur? At least the possible explanation should be mentioned in discussion section. Moreover, the data at 24hr should be added in Figure 6A.

Replay: when cell exposed in H_2O_2, the plasma membrane permeability decreased, meanwhile the plasma membrane fluidity attenuated. These changes resulted in preventing H_2O_2 from entering the cells further, and conferring a higher resistance to oxidative stress. As a result, cells can be adapt to the external H_2O_2 and restore.

Yes, the data at 24hr was added in Figure 6A.

5. The contents of Method section should be explained in more detail. For example, time course of H2O2 treatment should be described in Figure 2B, 2C, 6B, or 6C. In the immunoprecipitation assay, the control samples such as IP by IgG should be shown. For examples, in Figure 1E, it is so confusable that the protein level is different between IB Parp1 in IP Parp1 samples and Parp1. Images in Figure 1C or 3A should be shown in high magnification, because I cannot confirm the alterations.

Replay: the results of IgG of IP was showed in Figs.

The experiments of IP were done again.

We can upload the separate file for figure 1C or figure 3A.

6. In “H2O2-induced SIRT1 decrease leading to HMGB1 release” paragraph of Results section, the contents of manuscript and Figures are different. They should be corrected.

Replay: thanks. it is corrected in manuscript.

7. There will be many description errors. English proofreading should be carefully performed again.

Replay: yes, I sincerely appreciate you for carefully examination my manuscript.

Reviewer 2 （Number ID：00646291）：

1、“HMGB1 acts outside the cell as a damage-associated molecular pattern (DAMP) molecule, triggering the sterile inflammatory response which then becomes amplified by cytokines and chemokines.” The sentence is not clear and should be rewritten. What is sterile inflammatory response?

Replay: the first paragraph is rewritten again.

2、 “In the present study, we hypothesize that HMGB1 release in H2O2-injured hepatocytes is regulated by DNA-damage-mediated parp1 activation, which causes NAD+ over-depletion followed by sirt1 activity suppression, leading to HMGB1 deacetylation and finally release.” If SIRT1 activity is suppressed HMGB1 should be hypeacetylated and not deacetylated. Further details for the HMGB1 in the culture medium should be provided.
Replay: sorry it’s a writing mistake and is corrected in manuscript.

3、 In figure 1F increased Parp1 mRNA levels are shown in HFD/etOH treated mice, whereas the Parp1 protein levels seem to be the same in both control and HFD/etOH treated mice. Authors should discuss this discrepancy.
Replay: In Fig1E from top to bottom, the first band is parp1 protein levels and the third band is its internal standard GAPDH. The parp1 expression increased in HFD/etOH treated mice.
in Fig1F, the result is about mRNA of Parp1 which increased in HFD/etOH treated mice.
So, the results both mRNA and protein of parp1 are identical.

4、“Together, these findings suggest that cultured hepatocytes are injured by H2O2 as a consequence of HMGB1 translocation from the nucleus to the cytoplasm and then released to medium with the concomitantly elevated proportion of the acetylated form.” This conclusion should be rewritten as the results shown in the figure 3 do not suggest that hepatocytes are injured by H2O2 as a consequence of HMGB1 translocation from the nucleus to the cytoplasm. The only conclusion that can be derived from the results shown in figure 3 is that hypeacetylation of HMGB1 coincides with cytoplasmic localization of this protein.
Replay: this conclusion comes from the results of both Fig 2 and Fig 3, not just from Fig3. The results of Fig 2 showed that hepatocytes are injured by H2O2 and released to medium.

5、“Furthermore, the sirt1 enzyme activity in control group was 3.28±0.14 nmoL/mg/min, but was 3.06±0.13 nmoL/mg/min, 3.12±0.02 nmoL/mg/min and 085±0.05 nmoL/mg/min in H2O2 treated groups respectively (Fig4C).” Figure 4C does not show sirt1 enzymatic activity.
Replay: it is corrected in manuscript.

6、“Cells were treated with 0.1μM SRT1720 (an activator of sirt1) for 4h, the HMGB1 contents in medium significantly decreased to 15.6±2.87ng/mL compared with 61.09±9.86 ng/mL in H2O2 treatment alone (Fig4D). Figure 4D does not show HMGB1 content in the medium.
Replay: it is corrected in manuscript.

7、In the figure 5A the total HMGB1 protein levels increase in EX527 cells and

not only the hyperacetylated HMGB1 protein levels. What is the reason for that Sirt1 protein levels decrease in cells treated with EX527?

Replay: ex527 is a selective inhibitor of sirt1. In our experiments of this manuscript, it can suppresses sirt1 protein levels. This result was reported by others.
several references about ex527 inhibits sirt1 proteins were attached here:

(1). Sirtuin-1 (SIRT1) stimulates growth-plate chondrogenesis by attenuating the PERK–eIF-2α–CHOP pathway in the unfolded protein response. **J Biol Chem. 2018 Jun 1; 293(22): 8614–8625. doi: 10.1074/jbc.M117.809822**

(2). Sirtuin inhibitors, EX527 and AGK2, suppress cell migration by inhibiting HSF1 protein stability. ONCOLOGY REPORTS 35: 235-242, 2016.

(3). Melatonin attenuates acute kidney ischemia/reperfusion injury in diabetic rats by activation of the SIRT1/Nrf2/HO-1 signaling pathway. Bioscience Reports (2019) 39 BSR20181614 https://doi.org/10.1042/BSR20181614.

8.“The NAD+ levels decreased from 0.5h and reached ~50% inhibition at 8h, then restored 24h after cells were treated with H2O2 (Fig6A).” The 24h time point is not shown in figure 6A.
Replay: the data at 24h was added in Figure 6A.

Reviewer 3 （Number ID：03766580）
The present basic research study has been nicely designed and executed.
Replay：I sincerely thank you for your review.