Point-by-point responses to the reviewer's comments

We sincerely appreciate the chance to revise our manuscript. We would like to thank the reviewers for the constructive comments, which were very helpful for improving the manuscript and our understanding. We have carefully addressed all the reviewers' comments as described in the rebuttal letter and revised the manuscript accordingly. We hope that the manuscript is now acceptable for your reconsideration. Detailed point-by-point responses are provided below, with the reviewers' comments indicated in italics.

Reviewer #1

The author investigated that the mechanism of PINK1/DRP1 pathway in intestinal I/R injury. The Resluts of this study seemed very interested. However, I think several problems in this manuscript.

Major comments

1、The author wrote "thinking" which should be written in discussion in "Introduction" or "Results" . ie, Our findings demonstrate that PINK1 is a protective regulator on mitochondrial quality control and apoptosis inhibition in the model of intestinal I/R injury, which may provide a potential therapeutic target on intestinal I/R injury.(Introduction), These results suggest that I/R decreased mitochondrial fission related regulators p-DRP1 Ser637 and PINK1. However, the mechanism of mitochondrial fission in intestinal I/R injury is still unclear.(Result 1), Previous studies have revealed that excessive mitochondrial fission can lead to cellular apoptosis and tissue injury under I/R condition in liver, brain and kidney[13-15]. However, whether mitochondrial fission participates in intestinal I/R injury is uncovered. Thus, we founded intestinal I/R model (45-min ischemia and 4-hour reperfusion) in mice, which were pretreated with mdivi-1, a mitochondrial division inhibitor as mentioned above[31]. (Result 2), The imbalance of mitochondrial morphology is an important reason that can cause apoptosis and cell death under

stress[32]. Thus we suppose that mdivi-1 may prevent intestinal I/R injury through regulating mitochondrial homeostasis. (Result 3). I think pure results should be written in Result, not authors idea. These should be wrriten in Discussion.

Response:

Thank you for your comments on our manuscript. We have corrected the sentences in the revised manuscript, as indicated in red (page 10, lines 15-19; page 13, lines 10-13).

2、Regarding in vivo, how about survival among each groups ? The suthor should state about survival.

Response:

Thank you for your comment. We performed an additional 24-hour overall survival study (page 10, lines 26-29), and the results are listed at the end of the manuscript (Supplementary Figure S1, C).

3、In general, lung injury (SIRDS) derived from I/R injury of intestin is well known. How about lung injury of mice?

Response:

Thank you for your advice. We collected the lung tissue and evaluated the histological changes by H&E staining (page 10, lines 20-23; Supplementary Figure S1A, B)

Minor comments

1、In introduction, line 5, IECs should be stated by full spell (first time).

Response:

We apologize for our mistake. We have fully defined “IECs” as “intestinal epithelial

cells" and marked this change in red (page 3, line 20)

Reviewer #2

The authors clearly demonstrate the role played by mitochondrial dynamics in the events associated with damage induced after ischemia / reperfusion in intestinal cells. They used two models (in vivo and in vitro). Particularly they shed light on the mechanism that involves mitochondrial fission after PINK1-mediated phosphorylation of the DRP1 protein. These events are directly related to ROS levels of mitochondrial origin and from there, with the triggering of cell apoptosis. In this regard, they offer evidence on the role of PINK1 as a protective regulator of mitochondrial function and its potential use as a therapeutic target in lesions associated with ischemia injury.

Response:

The revised manuscript has been proofread by *American Journal Experts,* and the certificate has been submitted.

Reviewer #3

This manuscript has investigated the involvement of PINK and Drp1 in I/R using a mice model. The data presented are of good quality, and the data interpretation are fairly accurate. The only concern is the language. The manuscript can be significantly improved by a more careful editing.

Response:

The revised manuscript has been proofread by *American Journal Experts,* and the certificate has been submitted.