Response to Editor and Reviewer Comments

The main corrections in the paper and the responses to the editor and reviewer comments are as follows:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: This study demonstrated that Polygonum multiflorum (PM), a traditional herbal medicine in China, might have beneficial effects on patients with metabolic dysfunction-associated fatty liver disease, probably through restoring mitochondrial dysfunction. The authors also identified 8 PM-derived monomers which could be the candidates to have such effects, by UHPLC/MS. Their methods of both in vitro and in vivo studies were well designed and scientific. However, there might be several issues to be addressed for strengthening the impact of this manuscript. Major points:

1) The reviewer is concerned about less or absence of dose-dependency, especially in vitro study using L02 cells, as compared with in vivo study. For example, Fig. 1-K, Complex-II; Fig. 5-F, Na+-K+ ATPase; Fig. 5-G, Ca2+-Ma2+ ATPase; Fig. 7-I, Ca2+-Ma2+ ATPase. Please add some comments on these data.

Response: As described by the review, less or absence of dose-dependency of PM extract and their ingredient in this study. It is clearly noted that the effects of PM extract and their ingredients on the assayed indicators did not show dose-dependent manners, which was similar to many other herbal medicines (*Front Pharmacol, 2017, 8: 786; J Ethnopharmacol, 2019, 237: 20–27; Biomed Pharmacother, 2019, 112: 108715;……*). This may be attributed to the complex constituents and pharmacological mechanism of the herbs (e.g., a mode of action of multi-compound, multi-pathway and multi-target (*PLoS One, 2014, 9 (5): e95004*)). Besides, it is very likely that the PM extract and their ingredient dosages used in our study were not in the range of displaying dose-

dependence. This has been added to this manuscript (Line 604-610).

2) As the authors described that even MME (live mitochondrial extract of MOD group) had remarkable lowering effects on the cell levels of TC with dose-dependency (Fig. 5-B). If the authors have any UHPLC/MS data of MME, those informations would be useful to understand its mechanism. Please add some comments on this interesting finding.

Response: As described by the review, MME (live mitochondrial extract of MOD group) had remarkable lowering effects on the cell levels of TC with dose-dependency (Fig. 5-B). However, MME treatment did not reverse the other indexes, such as TG, GSH, SOD, Na^{+}-K^{+} ATPase, Ca^{2+}-Ma^{2+} ATPase, and pathological changes induced by fat emulsion, except for the significantly-reversed TC level. These have been described in the manuscript (Lines 484-486). Thus, MME has not effects on L02 adipocytes induced by fat emulsion when all experimental results were comprehensively considered.

3) According to Table 3, the author's description of "spleen index showed a downward trend" in line 549, seemed to be wrong. The SD value of HMG was too big, as compared with that of other groups.

Response: Thank you very much for the comments from reviewer. The description of spleen index in table 3 has been modified (Lines 519-520).

Minor points: 1) The authors should add some comments on Limitations of this study, at the end of Discussion section.

Response: Thank you very much for the comments from reviewer. The comments on limitations of this study have been supplemented at the end of Discussion section in our manuscript (Lines 610-613).

Reviewer 2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: In this manuscript, Li and colleages assessed the role of Polygonum multiflorum (PM) in regulating HFD-induced MAFLD. The authors report that PM improves the mitochondrial ultrastructure and prevented oxidative stress and energy metabolism disorder in liver mitochondria to mitigate fat emulsion-induced cellular steatosis and HFD-induced MAFLD. This manuscript writing still needs to be strengthened. The presented data are clear and most conclusions are solid and sound. The used methods are largely adequate. But there remains some criticism.

1.Mitochondria undergo constant mitochondrial fission and fusion, mitochondrial biogenesis, and mitophagy, which coordinately control mitochondrial morphology, quantity, quality, turnover, and inheritance. Do the authors know whether PM regulates which process in mitochondria and thus protects the mitochondrial ultrastructure.

Response: Thank you very much for the comments from reviewer. As a basic study, we did not thoroughly study which process PM regulated which process in mitochondria at the molecular level, which is worthy of further study. The mechanism of how PM protected mitochondrial ultrastructure may be related to mitochondrial oxidative stress and energy metabolism, which was presented in Fig 4 in the manuscript. The results indicated that PM enhanced energy metabolism and reduced oxidative stress levels to protect mitochondrial structure and functions and further to relief MAFLD. These have been presented in the manuscript.

2.All figure does not use Western Blot and qPCR to strengthen the reliability of the data, it is recommended to detect markers of MAFLD in vivo and in vitro at least.

Response: It is a good idea to included Western Blot and qPCR to strengthen the reliability of the data. However, the Western Blot and qPCR had not been

provided in the present study, which will be met in the further investigation. The reasons are as follows: 1) We first carried out mitochondrial pharmacology and pharmacochemistry studies, which was the overall design of this study. A large number of liver samples have been used in the extraction of liver mitochondrial samples for mitochondrial pharmacology and pharmacochemistry research. The large consumption of liver samples led to the deficiency for analysis of Western Blot and qPCR for further research. 2) Western Blot and qPCR may be detected using the liver samples that can be obtained from another repeated animal experiments. However, 12 consecutive weeks will be consumed to finish the animal experiments. Moreover, this procedure is labor-intensive and expensive. Thus, the animal experiments are not repeatedly performed to obtain liver samples.

3.The clarity of electron microscopy results is difficult to distinguish whether it is mitochondria, lysosomes or other organelles. It is recommended to count mitochondrial parameters (Mitochondrial length, diameter and area) from electron microscopy images.

Response: Thank you very much for the comments from reviewer. A clear image of TEM of mitochondria ultra-structure has been provided. In this TEM image, the mitochondrial morphology can be clearly observed (Fig.4A).