Dear editors:

The authors appreciate your and the reviewers' serious and professional evaluation of our manuscript (**Manuscript NO:** 53842) entitled "**Folic acid attenuates high-fat diet-induced steatohepatitis via deacetylase SIRT1-dependent restoration of PPARα**". Those comments are all valuable and helpful for revising and improving our paper. Based on their suggestions, we have seriously revised the manuscript. The point-by-point responses follow. Our responses to the Editor and Reviewers are highlighted in **blue words.**

Minor points1: In patients with NAFLD, lower percentage of *Bacteroidetes* and higher levels of *Prevotella* and *Porphyromonas* species compared to healthy controls are reported. Therefore, it would be better that not only diversity of gut microbiota but also changes in the specific bacterial species could be analyzed.

We deeply appreciate the reviewer's suggestion. In our present study, we also analyzed the relative abundance of gut microbiota from different levels. As shown in Figure 1A, lower abundance of Bacteroidetes was detected in HFD group, and folic acid administration could partially increase levels of Bacteroidetes. Compared with HFD group, an increased several genera such as Pseudomonadaceae and Leptotrichiaceae were observed (Figure 1B). This part of results indicated us that folic acid could also change the structure and abundance of gut microbiota which may contribute to the improvement of NAFLD.

Changes in the text:

(See page 14, line8-12): Besides, compared to control group, lower abundance of Bacteroidetes was detected in HFD group, and folic acid administration could partially increase levels of Bacteroidetes. Compared with HFD group, an

increased several genera such as Pseudomonadaceae and Leptotrichiaceae were observed (data not shown).

Minor points 2: In the group of folic acid, serum levels of fasting blood glucose were significantly decreased compared with high-fat group. Because diabetes mellitus is associated with fatty liver, the role of amelioration of glucose metabolism in the preferable effect for NAFLD should be discussed.

Thanks for your suggestion. Significantly lower fasting blood glucose (FBG) levels in group of folic acid in present study indicated us that folic acid may play a role in glucose metabolism in metabolic disease including NAFLD. Studies showed that chronic folic acid deficiency induced glucose metabolism disorders [1]. Folic acid treatment decreased glucose levels in diabetic rat models [2]. Administration of folic acid improved insulin resistance through altering the DNA methylation profile in high-fat diet mice [3]. All the results indicated us that folic acid had a protective effect in glucose metabolism under NAFLD conditions. And we will aim to further research related mechanism of folic acid in host glucose metabolism in future studies.

Changes in the text:

(See page 16, line 4-11) Significantly lower FBG levels in folic acid group indicated us that folic acid may play a role in glucose metabolism in metabolic disease including NAFLD. Studies showed that chronic folic acid deficiency induced glucose metabolism disorder [31]. Folic acid treatment decreased glucose levels in diabetic rat model [32]. Administration of folic acid improved insulin resistance through altering the DNA methylation profile in high-fat diet mice [33]. This indicated us that folic acid could improve glucose metabolism in NASH conditions, and specific mechanisms need further research.

Taken together, we believe that the quality of the revised version is greatly improved, and we look forward to your final positive decision.

References

1 **Zhao M**, Yuan MM, Yuan L, Huang LL, Liao JH, Yu XL, Su C, Chen YH, Yang YY, Yu H, Xu DX. Chronic folate deficiency induces glucose and lipid metabolism disorders and subsequent cognitive dysfunction in mice. *PloS one* 2018; **13**(8): e0202910-e0202910 [PMID: 30153273 DOI: 10.1371/journal.pone.0202910]

2 **Mutavdzin S**, Gopcevic K, Stankovic S, Jakovljevic Uzelac J, Labudovic Borovic M, Djuric D. The Effects of Folic Acid Administration on Cardiac Oxidative Stress and Cardiovascular Biomarkers in Diabetic Rats. *Oxid Med Cell Longev* 2019; **2019**: 1342549-1342549 [PMID: 31308875 DOI: 10.1155/2019/1342549]

3 **Li W**, Tang R, Ma F, Ouyang S, Liu Z, Wu J. Folic acid supplementation alters the DNA methylation profile and improves insulin resistance in high-fat-diet-fed mice. *J Nutr Biochem* 2018; **59**: 76-83 [PMID: 29986310 DOI: 10.1016/j.jnutbio.2018.05.010]

Figure Legend

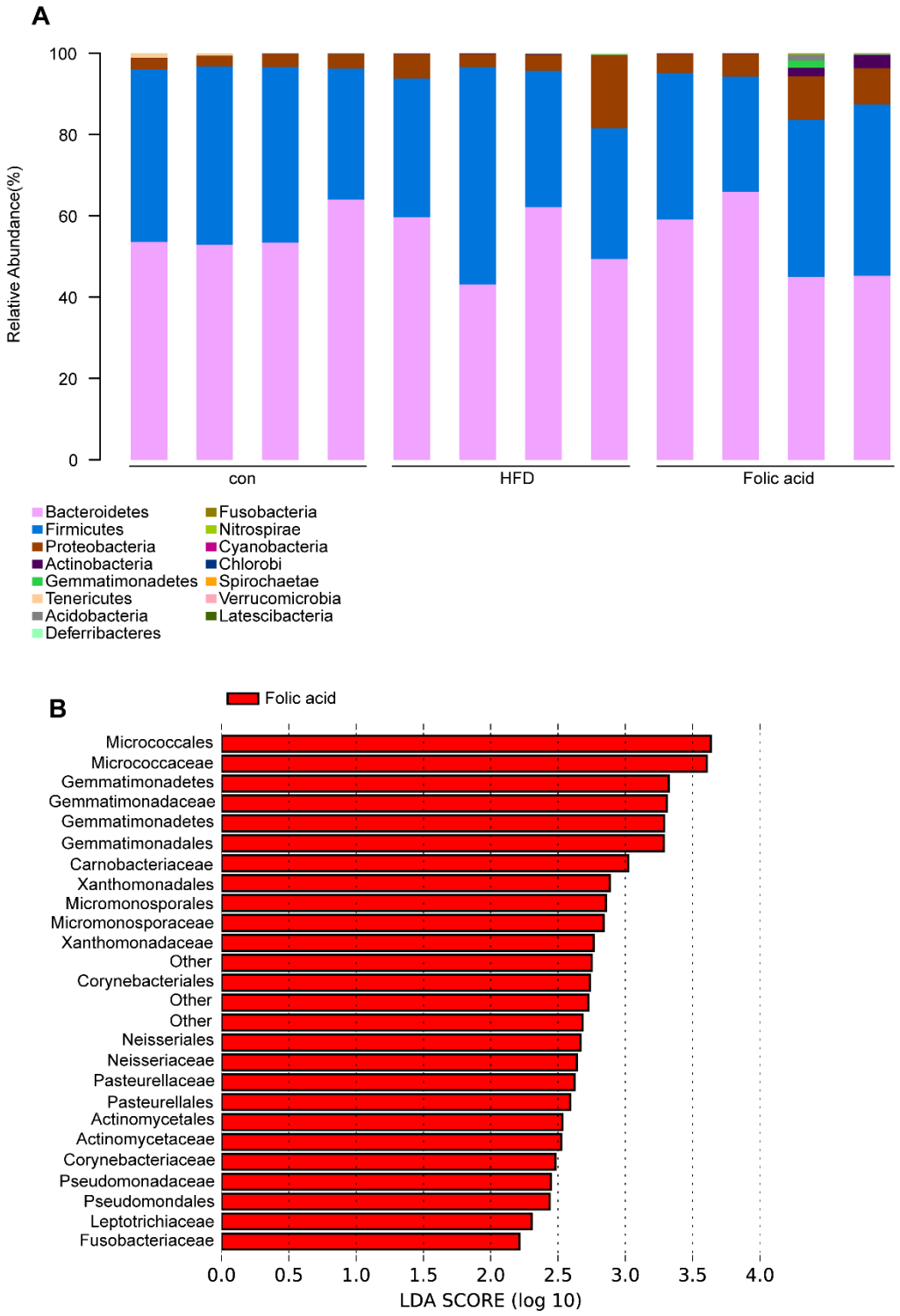

Figure 1 Relative abundance of each group A: relative abundance at phylum level; B: Linear discriminant analysis coupled with effect size measurements analysis(n=4)