Response to Reviewer 2 Comments

Thank you for your comments concerning our manuscript entitled ‘Compound Prescription Xiaoyaosan Improves Depressive-Like Behaviors of Chronically Stressed Mice’ (JoVE58276). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. The comments were all valuable and very helpful for revising and improving our paper and the important guiding significance of our research. We have carefully considered the comments and addressed all of your concerns. We are sorry for the language mistakes in our manuscript. Now we have studied comments carefully and have made correction which we hope meet with approval, and we will search for language polishing by a native speaker if the contents of our manuscript are suitable for publication. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the comments are as flowing:

**Point 1:** The depression animal model established by chronic unpredictable mild stress has been widely accepted in the field, and numerous studies have been published regarding the effect of Xiaoyaosan on depression both in human and animal model, including the works of the authors themselves. What is new finding of the present manuscript?

**Response 1:** Thank you for your thoughtful concern about our manuscript. The purpose of this protocol is to exhibit the methods in the article *Involvement of Normalized Glial Fibrillary Acidic Protein Expression in the Hippocampi in Antidepressant-Like Effects of Xiaoyaosan on Chronically Stressed Mice* by Dr. Xiu-Fang Ding, et al. We hope our manuscript could explain the methodological part of the article mentioned here in a more comprehensive way, and meet with approval from JoVE, then this protocol would be recorded in the form of video.

**Point 2:** The data presented in this manuscript are concerning the preventive effect of Xiaoyaosan on the development of depression, which has little, if any, clinical relevance, since it is hard to image a health people taking a medicine for an unpredictable long time to prevent a potential depression.

**Response 2:** Thank you for your valuable advice. A health people taking a medicine for an unpredictable long time to prevent a potential depression is not suitable in clinical practice. But the approach of prophylactic treatment by medicine to study the mechanism of action is widely used in many animal experiments (Liu X J, Zhou Y Z, Li Z F, et al. *Anti-depressant effects of Xiaoyaosan on rat model of chronic unpredictable mild stress: a plasma metabonomics study based on NMR spectroscopy* [J]. Journal of Pharmacy & Pharmacology, 2012, 64(4):578-588; Ramakers J D, Verstege M I, Thuijls G, et al. *The PPARγ Agonist Rosiglitazone Impairs Colonic Inflammation in Mice with Experimental Colitis* [J]. Journal of Clinical Immunology, 2007, 27(3):275-283; Yue N, Huang H, Zhu X, et al. *Activation of P2X7 receptor and NLRP3 inflammasome assembly in hippocampal glial cells mediates chronic stress-induced depressive-like behaviors* [J]. Journal of Neuroinflammation, 2017, 14(1):102).

Xiaoyaosan, as an herbal prescription, has been used to treat mental disorders for a long time. So, this protocol described the establishment of mouse depressive model and evaluated the preventive and treatment effect of Xiaoyaosan to the depressive-like behaviors of chronically stressed mice.

**Point 3:** More importantly, the authors stated that "The protocol and results demonstrated in this paper originates from the article "Involvement of Normalized Glial Fibrillary Acidic Protein Expression in the Hippocampi in Antidepressant-Like Effects of Xiaoyaosan on Chronically Stressed Mice by Dr. Xiu-Fang Ding, et al." ( line 188-190, and line 277-280). The paper the authors referred to has been published, as ref.13 in the list of references of this manuscript. Therefore, the data of the present manuscript, nevertheless, should not be considered for publication again.

**Response 3:** Thank you very much for your concern about the use of previous data that have been published in our manuscript. The purpose of this protocol is to exhibit the methods in the article *Involvement of Normalized Glial Fibrillary Acidic Protein Expression in the Hippocampi in Antidepressant-Like Effects of Xiaoyaosan on Chronically Stressed Mice* by Dr. Xiu-Fang Ding, et al, we have obtained explicit copyright permission to reuse data from this previous publication. We could present the corresponding results in the manuscript if cited.

Numerous irregular expressions and formats exist throughout the manuscript, in addition to the errors in grammar, which the reviewer will not detail given the concerns above.

**Response:** Thank you very much to point out these issues in our manuscript. We have careful rechecked and corrected the language problems in our manuscript. We hope our revised manuscript could meet publication requirements, and we will seek for the professional polishing. Thank you so much!