**Re: Manuscript # JoVE50183 Revision**

**Evaluation of muscle function of the extensor digitorum longus muscle ex vivo and tibialis anterior muscle in situ in mice**

(Comments from the reviewers are highlighted in blue color)

***Responses to concerns raised by Reviewer 1***

*Summary:* This manuscript describes a method in evaluating the EDL muscle contractile and passive force properties Ex vivo. In addition the method of measuring TA muscle contractility is described. An example of mdx muscle is used to demonstrate both techniques. This is a well written and important methods manuscript that will be of interest to those in the muscle research field.

Response: We thank the reviewer for the thoughtful and encouraging comments on our study. We have addressed all the concerns in the following responses.

*Major Concerns:* None  
  
*Minor Concerns:*1. The authors set the temperature to 30 degree when measuring force in EDL muscles. Why is it 30 degree rather than 37 degree?

Response: A study by Faulkner and Segal (1985), showed that the EDL muscle yields the maximum and most stable isometric force at 30oC 1. In the revised manuscript, we have included the reference in the method text.

2. No statistic information was mentioned.

Response: In the revised manuscript, we have included statistic information for data analysis.

***Responses to concerns raised by Reviewer 2***

*Summary:* This is very interesting and novel method to measure muscle force ex vivo. The authors described very interesting and useful physiological assays to measure mechanical properties in EDL and TA muscles in mice. I recommend for publication with minor revisions.

Response: We thank the reviewer for the positive feedback. We have addressed all comments and revised the manuscript accordingly.

*Major Concerns:* There is no major concern.  
  
*Minor Concerns:*1. Page 3; "Turn on the circulating water-bath and adjust the temperature to 30ºC."  
Please add rationale why it is set to 30C.

Response: In the revised manuscript, we have included a reference in the text method to show that the optimal temperature for EDL isometric for in an ex-vivo preparation is 30oC 1.

2. Page 3; PSI; Please spell out the abbreviation.

Response: In the revised manuscript, we defined the PSI in the method text.

3. Page 3; anesthetic cocktail. Please specify the content of the cocktail.

Response: The drugs used in the anesthetic cocktail are defined in the materials and equipment table (Table 1). We had referred the table to the sentence.

4. Please add methods for statistical analysis.

Response: In the revised manuscript, we defined the methods for the statistical analysis.

5. Results; mdx; Italic font should be used.

Response: We have revised the “mdx” word accordingly.

6. Figure 4E Please provide error bars.

Response: The standard error of the passive stress mean value is very small comparing to the stress value. That is why it is hard to differentiate between the error bar and the point marker at each strain. This indicates that the variability between samples is reduced and it confirms the precision of our experiment procedure.

**References**

1 Segal, S. S. & Faulkner, J. A. Temperature-dependent physiological stability of rat skeletal muscle in vitro. *Am J Physiol* **248**, C265-270. (1985).