Rebuttal relating to the editorial and reviewer comments regarding:

**Real-Time Pressure-Volume Analysis of Acute Myocardial Infarction in Mice**

by Michel *et al*.

MS: 57621 R0 112917

We would like to thank the editor for the cordial invitation to resubmit our revised manuscript. Furthermore, we would like to express our thanks to the editor and the referees for the thorough evaluation of our manuscript and their important comments. We have carefully considered their recommendations and incorporated the changes proposed in the manuscript.

In the following we respond in detail to the questions:

**Editorial comments:**

**1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.**

The manuscript was thoroughly proofread.

**2. Please provide an email address for each author on the first page.**

We added all email addresses to the first page (l. 11-13).

**3. Please rephrase the Long Abstract to more clearly state the goal of the protocol in one paragraph with 150-300 word. Please attention that in the final form, Long Abstract will be used as the Abstract. The Short Abstract will be used as Highlight for the databases.**

The headlines used in the abstract (Introduction, Methods,…) were removed. The abstract was rephrased and shortened to more clearly state the goal of the protocol (l. 26-46).

**4. Please use SI units, e.g. please use “µL” instead of “µl”. Please leave a white space between the values and the units.**

We checked the manuscript for correct use of SI units and corrected if necessary. We added white spaces (l. 305, 307, Figures).

**5. Please define all abbreviations before use.**

We checked all abbreviations and added definitions when necessary.

**6. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.**

We removed all commercial language and modified the comments/description section to fit with the terms used in the manuscript (l. 96-98).

**7. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.**

All text in the protocol section is now written in the imperative tense or as a “Note” (l. 132, 147, 151, 159, 165, 190, 201, 205)

**8. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. The Protocol steps should contain only 2-3 actions per step and a maximum of 4 sentences per step.**

Steps exceeding 3-4 sentences have been divided up into several smaller steps

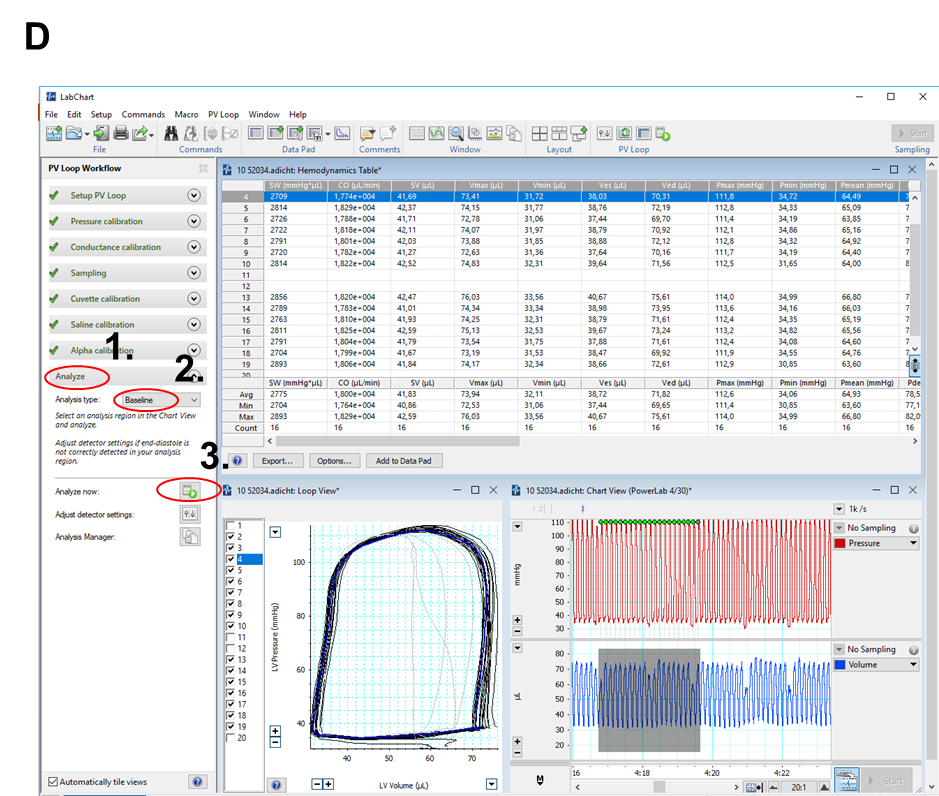
**(l. 110-116, 120-172, 176-200, 203-228, 232-257).**

**9. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.**

The protocol was checked for proper description of the steps. We added a reference to published material that provides additional information and schematic images of the procedure (l. 190).

**10. For steps that involve software or analyzing tools, please make sure to provide all the details such as “click this”, “select that”, “observe this”, etc. Please mention all the steps that are necessary to execute the action item. Please provide details so a reader may replicate your analysis including buttons clicked, inputs, screenshots, etc. This is the level of detail we’re looking for. Please keep in mind that software steps without a graphical user interface cannot be filmed.**

We added more details to steps involving the software (6.1). We added a screenshot of the software (Figure 3 D) and encircled the buttons that need to be activated (l. 232-235).



**11. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).**

Personal pronouns were replaced with alternatives (l. 22, 116).

**12. Please leave a blank line between all protocol steps as well as Notes.**

Done.

**13. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.**

The numbering has been adjusted. We removed dashes (l. 78-257 and 6.4, l. 247-257).

**14. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution. Please mention the age, gender and strain of the animals.**

The paragraph has been moved to the beginning of the protocol. Age, gender and strain of the mice have been added (l. 79-83).

**15. Protocol: 2.2 (currently 2.b): Please avoid using any personal pronouns.**

We changed “you” to “towards the investigator” (l. 116).

**16. Protocol: 3.1 (3.a): Please clearly describe and explain each action. Please include all the instruments used.**

We added details and used instruments (l. 120-121).

**17. Protocol: 3.2 (3.b): How is each action done? Using what?**

We added details on how the preparation is done using a forceps (l. 126-130).

**18. Protocol: 3.6 (3.f): Please use the imperative tense for all the sentences of the protocol steps. Please move the discussion to the Discussion section.**

Imperative tense was inserted and the last part has been re-edited to fit the “Protocol” section (l. 144-157).

**19. Protocol: Text after 5: If that is a note, please indicate it as a Note.**

Indicated as “Note” (l. 203-205).

**20. Protocol: 5.2 (5.b), 5.3 (5.c): Please use the imperative tense for all the sentences of the protocol steps. Please move the discussion to the Discussion section.**

Imperative tense was inserted. The part describing changes caused by ion concentration that did not fit the “protocol” section was changed and moved to “Discussion” (l. 210-223, l. 326-330).

**21. Protocol: 6.1 (6.a) Please clearly describe each action in the imperative tense. If using a software or analyzing tool, please include all the buttons clicked, or refer to appropriate references.**

We added details to the descriptions and inserted a screenshot (l. 232-235, Figure 3 D).

**22. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.**

We consider steps 3, 4 and 6 to be the most important for the protocol and suitable as filmable content. We uploaded one version with highlighted protocol for filming and one version with highlighted changes since revision (l. 118-199, 232-257).

**23. Please upload each Figure individually to your Editorial Manager account as a .png, .pdf, or a .tiff file. Please combine all panels of one figure into a single image file.**

Figures were uploaded as separate files for each figure combining all panels of a single figure.

**24. Figure 2: Please define and add the scale bars.**

Scale bars were added (Figure 2).

**25. Figure 3: (A) and (B): please add the values and units for the horizontal axis. (C) Please use “L” instead of “l”.**

Values and units have been added. “l” was corrected (Figure 3 A+B).

**26. Figure 4: (A) and (C): please define the horizontal axis. (C) Please use “L” instead of “l”. (H) please define each axis.**

Horizontal axis defined (A+C), “l” corrected (Figure 4 A+C).

**27. Figure 5: Please use “L” instead of “l”. (C): please include the values for each axis.**

“l” corrected, values added (Figure 5 C).

**28. If you are reusing figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [AUTHOR] et al.[REFERENCE]”.**

We did not reuse any figures.

**29. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please list all the materials, equipment, instrument, and software used in your work.**

We double-checked the table of supplies.

**Reviewers' comments:**

**Reviewer #1: Manuscript Summary: The manuscript describes a very technical procedure for monitoring real time cardiac function during acute myocardial ischemia and reperfusion. This is a very important advancement in establishing relevant animal models to test therapeutic interventions that may translate to humans. Being able to capture real time changes in hemodynamics and cardiac function is necessary in order to make these models relevant for human interventions. This research group are recognized experts in vascular biology and cardiology and are very well published in this area. This manuscript is well suited for JoVE due to technical and skillful procedures required for this model. Visualization of the procedure is necessary for others to replicate their teachings**

We thank the reviewer for her/his comments.

**Major Concerns: none**

**Minor Concerns:**

**It would be helpful to include an optimal time of stabilization and data collection prior to the LCA Ligation.**

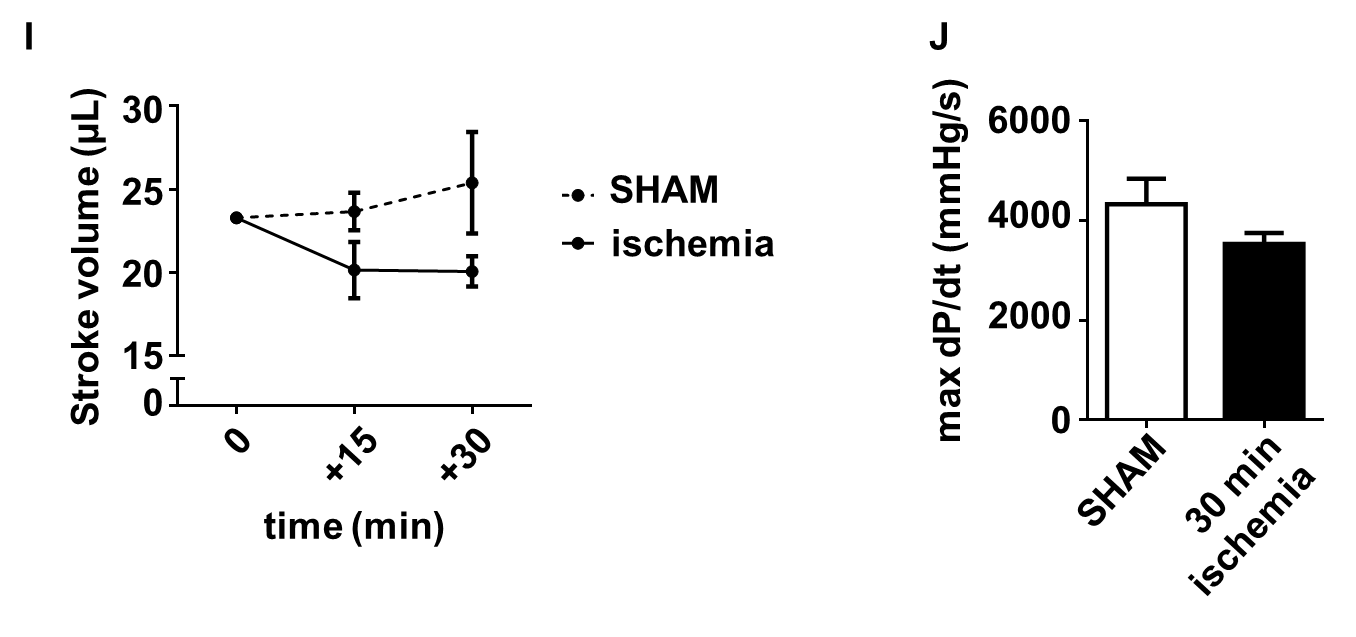
We thank the reviewer for raising this point. We added a description for optimal data collection after opening of the pericardium before LCA ligation (l. 181-183)

**How much time is necessary and does this vary from one surgical technician to the next based on skill.**

We added details on this topic (Step 4.3 “Note): I/R surgery should be performed within 5 min independently from the investigating operator (l. 190-191).

**Showing changes in SHAM operated animals would be helpful to demonstrate that the acute changes during ischemia reperfusion are indeed that profound rather than loss of function from catheter implantation and anaesthesia over the course of the experimental protocol.**

We thank the reviewer for this important issue. We added figures showing SHAM operated animals (Figure 4 I+J) and referred to them in the “results” section stating that SHAM did not induce LV systolic or diastolic dysfunction (l. 280-281).



**Reviewer #2: Manuscript Summary:**

**The manuscript "Real-Time Invasive Hemodynamic Imaging of Acute Myocardial Infarction in Mice" by Michel et al. describes the use of conductance catheter technology to monitor the pressure/volume (PV) relation in mice during and after coronary occlusion. This animal study was very well planned and well executed by a laboratory that is widely-recognized for their work in myocardial ischemia as well as advanced laboratory methods for the study of myocardial ischemia. As such, the work reported here represents a valuable contribution to the cardiovascular research community (in general) and to JoVE (in particular).**

**Overall, the manuscript is extremely well-written, particularly considering that English may not be a first language for the authors. Only upon careful analysis can one detect a few typos or grammar issues. However, these issues are quite minor and could easily be overlooked.**

**Thus the manuscript could readily be accepted in its current form should the authors elect not to incorporate the minor suggestions recommended below.**

We thank the reviewer for her/his comments.

**Major Concerns: NONE**

**Minor Concerns:**

**Page 1, line 3 (Title): The authors might consider rephrasing the title to make it somewhat more precise. In particular, the majority of readers will equate "hemodynamics" with blood pressure monitoring and may therefore miss the fact that a far more sophisticated PV analysis is being conducted here. Further, the word "imaging" might be confused with "medical imaging," which of course is not the subject of this manuscript. Thus, one might prefer a title such as: "Real-Time Invasive Monitoring of Pressure-Volume Relationship during Acute Myocardial Infarction in Mice." Alternatively, one might consider: "Real-Time Conductance Monitoring of Pressure-Volume Relationship during Acute Myocardial Infarction in Mice" or "Real-Time Pressure-Volume Analysis of Acute Myocardial Infarction in Mice."**

We thank the reviewer for this important issue. We changed the title to “Real-Time Pressure-Volume Analysis of Acute Myocardial Infarction in Mice” (l. 3).

**Page 1, line 44: To be consistent with the reperfusion period specified later in the manuscript, recommend changing "20 minutes" to "10 minutes". Alternatively "10-20 minutes" could be specified throughout.**

We apologize for the mistake. We changed the reperfusion period to 20 min in the protocol section (l. 198).

**Page 3, lines 123-129: Steps (d) and (e) under "Cardiac Catheterization" contain elements that appear to be duplicated and overall read a bit less smoothly than the rest of this most excellent description of a complex surgical procedure. One might consider adopting something akin to the following:**

We thank the reviewer for these helpful remarks. We adapted the changes to the manuscript.

**d. Perform a wedge-shaped incision 1 mm proximal to the cranial knot to open the vessel with micro scissors. A small drop of blood will indicate proper execution of this step. Insert the catheter carefully for 10 mm. Stretching the incision with forceps can make this process easier. Start recording of catheter data.**

l. 144-151.

**e. Extract the vascular clamp. Add 1-2 drops of saline to the incision to facilitate catheter movement. Continue introducing the catheter for approximately another 10 mm. After passing the proximal knot with the sensor tip, fasten the knot carefully to prevent blood reflux alongside the thinner parts of the catheter.**

l. 153-157.

**In considering Steps (d) and (e), please note that the following sentence should either be removed or clarified: "The size of the sensor tip of the catheter should reflux of blood." This sentence seemed difficult to interpret, so maybe a line of text is missing here? Or perhaps what was meant was: "The sensor at the tip of the catheter should become refluxed with blood."**

We apologize for the mistake. The word “prevent” was missing – we corrected the sentence to “The size of the sensor at the tip of the catheter prevents reflux of blood when extracting the vascular clamp.” (l. 159-160).

**Page 3, lines 123-129: The last sentence in step (e) seems to suggest that the proximal knot should be fastened tightly before the catheter reaches the LV. Perhaps the ligature is tightened just enough to prevent blood reflux and this point, then fastened more tightly in step (f) after the proper placement is optimized by the volumetric reading from the conductance catheter? If so, please clarify.**

We thank the reviewer for this issue. We added details to this step: “…fasten the knot carefully just enough to prevent blood reflux alongside the thinner parts of the catheter without impairing catheter movement.” (l. 155-157) […] “Fasten the proximal knot more tightly to prevent catheter movement.” (l. 172).

**Page 4, line 146: Under I/R surgery, it may be helpful to specify the location along the LCA where the suture is placed relative to the left auricle.**

We thank the reviewer for this comment. We specified the location of the LCA and the location of the suture (4.3; l. 185-188).

**Page 4, line 147: Under I/R surgery, a "small silicon tube" is specified, whereas Fig 2B indicates a polyethylene tube. Perhaps a soft silicon tube might offer advantages over a hard PE tube, but please consider making the figure consistent with the text.**

We changed the description in the figure to “silicon tube” (Figure 2 B).

**Page 4, line 157: Please consider "to ensure valid results".**

Done (l. 206).

**Page 5, line 187: Rather than "to avoid falsification by artefacts," please consider "to avoid sampling error".**

Done (l. 244).

**Page 5, line 211: Rather than "characteristically," please consider "characteristic".**

Done (l. 263).

**Page 5, line 215: Rather than "incision," please consider "excursion".**

Done (l. 267).

**Page 5, line 216: Rather than "paling," please consider "blanching".**

Done (l. 268).

**Page 6, line 235: Rather than "situs," please consider "sites" or "procedures".**

Done (“Procedures”) (l. 288).

**Page 6, line 235: Rather than "commune," please consider "common".**

Done (l. 288-289).

**Page 6, line 236: With regard to the "silicon tube," please refer to the comment for line 147, above.**

Done (“silicon”) (l. 289).

**Page 6, line 238: Rather than "incision," please consider "excursion".**

Done (l. 291).

**Page 6, lines 257-260: In order to be technically correct, it should probably be acknowledged that echo and MRI typically yield the parameters of EF, stroke volume and cardiac output. Please consider rephrasing to something like:**

**"In addition to the LV volumetric parameters typically obtained by echocardiography or MRI (chamber volumes, EF, stroke volume and cardiac output), PV analysis yields a more complete profile of LV function by simultaneously providing measures of LV systolic performance (contractility dP/dt, stroke work) and LV compliance (-dP/dt, Tau) as a parameter for diastolic function."**

We thank the reviewer for this remark and adapted the suggestion (l. 313-317).

**Page 7, line 272: Mortality is a relative term, but please consider deleting "very" since the in-hospital mortality rate associated with a coronary event (<7%) is actually pretty low as compared with other maladies such as pancreatic cancer.**

“Very” has been deleted (l. 338-339).

**Page 7, lines 275-277: Please consider deleting the following sentence since it rather undercuts the last sentence in the discussion (that occurs directly afterwards): "Differences in coronary anatomy and hemodynamic compensation of LCA occlusion are therefore possible limitations for transmission of the acquired data on patient's care." This is because the "differences in coronary anatomy and hemodynamic compensation of LCA occlusion" are probably very minor as compared to differences in the location of the coronary occlusion. This relates to the comment raised above regarding line 146. The mortality due to coronary occlusion is directly related to the area at risk, and patients with a complete occlusion of the left main will experience far more LV dysfunction (and higher acute mortality) than patients with a more distal coronary occlusion. Thus it may be prudent to explore more proximal ligations in mice (say proximal enough to incur 7% mortality) before concluding that a mouse is somehow more resistant to hemodynamic impairment than a human. Overall, one might argue that it is too early to make such a comparison given that so few hemodynamic studies have been conducted during MI in mice, or in humans. As approximately 20-fold more researchers have access to Millar pressure catheters as compared to Millar PV conductance catheters, a related (and perhaps more appropriate) subject for discussion might be the relative merits of monitoring LV hemodynamics (pressure only) versus the more elegant and comprehensive PV analysis described in this manuscript.**

We thank the reviewer for this important issue. We deleted the sentence as suggested. We added a new paragraph to the discussion describing the advantages of simultaneous acquisition of pressure and volume data compared to pressure data alone (l. 332-336).

We expended the paragraph concerning LCA ligation in mice compared to LCA occlusions in humans mentioning that this effect could be due to more proximal occlusions in humans (l. 342-343).