**Editorial comments (in email):**

1. The editor has formatted the manuscript as per the journal's style. Please retain the same. Please rephrase the title to be crisp and do not use abbreviations.

Done.

2. Please address specific comments marked in the manuscript.

Done.

3. Please do not have a paragraph of texts in the protocol section. Please have numbered steps only.

Okay. This has been changed.

4. Please specific sex, age, the strain of the animal in use.

This is already described in the first line of the “Protocol” (“male C57BL/6 mice, aged 12 weeks”).

5. Once the protocol is edited, please highlight 2.75 pages of text including heading and spacing which will be used for filming purpose.

We have highlighted the relevant text the final edited Word file (58146\_R2\_RE\_highlighted). We have also cut and paste the highlighted text into another Word file (58146\_R2\_RE\_script).

6. Figure 2: Please use the correct degree symbol. Please leave a single space between number and units.

The degree symbol has been corrected.

**Specific comments in the document:**

* Please make the title more crisp. Please do not use abbreviations in the title.

This has been changed.

* Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”
* Please reword this better.
* Please use American English throughout. .. this has been converted to one. Please check.

These points have been addressed/changed in the text.

* ECMO murine model?

Yes. We have added the word “murine” now.

Due to abundance of what kind of genetically modified mice?

We would argue that this is self-explanatory and, in any case, should not be covered in the Abstract. There are hundreds of molecular pathways that could be investigated with knockout mice and dozens of disease/conditions that could investigated with geneticall altered mice. We cover these ideas briefly in the last paragraph of the Discussion.

* Please revise the Introduction to include all of the following:

a) A clear statement of the overall goal of this method

b) The rationale behind the development and/or use of this technique

c) The advantages over alternative techniques with applicable references to previous studies

d) A description of the context of the technique in the wider body of literature

e) Information to help readers to determine whether the method is appropriate for their application

All of these aspects are included in the Introduction.

* What kind of model? Mice model?

No. We have now clarified this was in patients.

* Shouldn’t this come after describing what ECMO is at the first place?

We have now added a sentence beforehand to define what ECMO is.

* The sentence before this says no mouse model is ECMO is described in literature. This one says due to the availability of huge number of genetically modified mouse strains… There seem to a link missing between the two.

We have now clarified, “Due to the availability of a huge number of genetically modified mouse strains, having a mouse ECMO model would allow the molecular mechanisms involved in ECMO-related complications to be investigated.”

* We cannot have paragraphs of text in the protocol section. Please convert this to a numbered step and rewrite in imperative tense. Alternatively this detail can be moved to the introduction section as well.

Okay, but we did this in our last JOVE paper as well (Madrahimov et al 2017). We have now moved some of this text into the Introduction. Other details were requested by one reviewer but basically we just want to say that many components of the circuit are as described in our last paper (which we have now referenced the text moved to the Introduction).

Please do not use commercial terms in the manuscript. Please refer to the commercial term in the table of materials and use generic term instead.

We only added the commercial names of the solution at the request of one of the reviewers. We’ll take it out again.

How do you check this – by toes pinch? Please mention the same.

We already mentioned this is the text (“check pedal withdrawal and pain reflexes”) but we have now moved these details into the same sentence so you can’t miss it.

Please mention this step right where the step is happening. Please write the protocol in the way you are performing the experiment.

Okay, the steps have now been rearranged.

* As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

Yes, we have covered all of these topics. The Discussion was originally 6 paragraphs but due to the comments of the scientific reviewers, a new paragraph was added to address their comments.