Dear Editor in Chief,

Thank you for giving us the opportunity to revise our manuscript. Yours and the reviewers comments definitely helped to describe the technique more clear.

We addressed all comments below and in the manuscript.

With Kind regards,

Daniel Schock-Kusch

**Editorial comments**  
Changes to be made by the Author(s):  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Done  
2. Please label/number the institutional affiliation of each author sequentially. Lorenzo Ressel should be followed by 3 not 4.

This has been corrected.  
3. Please revise the Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

This has been completed.  
4. Please define all abbreviations before use.

This has been completed.  
5. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

This has been completed.  
6. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s;

etc.

This has been completed.  
7. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

This has been included.  
8. 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. For example: MediBeacon GmbH, Fresenius Kabi, Kendall, Leukosilk, Veet, Nair, etc.

This has been completed.  
9. 1.1-1.9: The Protocol should contain only action items that direct the reader to do something. Please move the material and equipment information to the Materials Table.

This has been completed.  
10. 2.1: How many times are considered a few times?

This has been added.  
11. 2.2.1: Please specify the sex, age, and strain of mouse.

This has been added to the figure legends where relevant, but not to the protocol section since different strains of mice can be used for this procedure. This is also discussed in the introduction and discussion sections.

12. Please revise the protocol to contain only action items that direct the reader to do something. The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.”  
13. 2.2.2, 2.2.3, 4.1, 4.5, 6.4.1, 6.4.2, 6.7, 7.3, 8.1, 8.5, etc.: Please write in the imperative tense in complete sentences.

The protocol has been edited to the imperative tense.  
14. Please add more details to your protocol steps. 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.

More information has been added to the protocol  
15. 4.1: Please describe how.

See response below  
16. 4.3: Is this step repetitive to step 4.1?

Yes, step 4.1 was intended to introduce the hair removal steps. This has been edited for clarity.   
17. 4.2, 6.1: Please mention the concentration of isoflurane and how proper anesthetization is confirmed. Are the mice anesthetized both during shaving and placement of the transdermal GFR monitor? Please clarify.

This information has been added.  
18. A schematic showing the attachment of the device on the animal’s back may be helpful.

We have added some photographs showing the shaving and device placement steps (new Fig 1)  
19. 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.

This has been completed.  
20. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

This has been completed.  
21. Representative Results: Please elaborate the Figures more.

We have elaborated more about the figures in the text.  
22. Figures 2 and 3: Please define the error bars in the figure legend.

This information has been included.  
23. Figure legends: Please shorten the figure legends of Figures 1 and 4. The Discussion of the Figures should be placed in the Representative Results. Details of the methodology should not be in the Figure Legends, but rather the Protocol.

The figure legends have been shortened and some information has been moved to the main text.   
24. Discussion: Please also discuss any limitations of the technique.

More information on the limitations of the technique has been added to the discussion.  
25. 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.

This has been included now.  
  
**Reviewers' comments**  
  
**Reviewer #1**  
  
Major Concerns

198-200: I cannot agree to this point. Our experience shows, that „tight" is not beneficial at all. Rather the tape has to be attached firmly to the skin/ fur. For us it is absolute imperative to not achieve firm attachment by wrapping the tape around the torso tightly (which might be intuitive to do). The adhesiveness and fixation of the imager is not a function of the tightness of the tape-wrap. We try to press the tape to the skin/fur and thereby strictly following the the skin's circumference. In our experience the mice react very sensitive to even seemingly minor restrictions around the chest. Only a mere adhesive-mediated attachment without tightening the device around the animal results in "homeostatic" conditions during the recording period, without altered and hectic moving patterns, most likely resulting in blood pressure artifacts. Also, we advise the mobilization of the upper limbs and shoulders after mounting the stripes, by gently stretching the upper limbs of the animal. This usually results in a slight (though to the animal significant) re-positioning of the adhesive stripes on the torso, which later grants more freedom of movement (in the sense of less restriction).

We thank the reviewer for his/her comments on this point and agree that the tape should not be wrapped tightly around the animal. We have re-worded the instructions accordingly to avoid misunderstanding.

249-252: Figure 2 seems redundant in light of figure 3. I would recommend to rather translate the t ½ values of figure 3 to GFR as well and show both parameters together, as done for fig 2.

We disagree that this figure is redundant, as we are showing two very different time points in IRI injury – the new figure 3 shows early, acute injury, and figure 4 shows late, chronic injury. We have now edited the figures and legends to make the difference clear, and we have also switched figures 3 and 4 so that the acute data is shown before the chronic data. Additionally, we have included both half-life and GFR graphs for both figures.   
  
258-260: Although we use semi-quantitative tubular injury scoring ourselves, the method lacks sensitivity (and reliability). The by far most sensitive parameter of kidney injury is protein expression or mRNA transcription of Havcr1 (aka Tim1, Kim1). A good correlation with this parameter would strengthen the point, that transcutaneous GFR measurement is in fact reflecting kidney health.  
We would like to thank the reviewer for this comment, however while we agree that Kim1 mRNA and protein are considered valuable markers of renal injury, many (including ourselves) still believe that histopathology is the gold standard for assessing kidney damage. While we accept that the two assays are complementary, we are not aware of any publication which suggests that Kim-1 supersedes the value of histopathology to assess the severity of renal injury. Furthermore, papers that evaluate Kim-1 still use histopathology as their gold standard, for example [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2744478/](https://na01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fpmc%2Farticles%2FPMC2744478%2F&data=02%7C01%7Clauren.scarfe%40vanderbilt.edu%7Ce378dee438514f43e58a08d5d77785ea%7Cba5a7f39e3be4ab3b45067fa80faecad%7C0%7C0%7C636651830715924204&sdata=sYT2YYjJMAuN0FeUXeNYIDEOqnNfFmC22zUsKNOECw8%3D&reserved=0). The reviewer should note that histopathology scoring was performed by a qualified ECVP Board Certified Veterinary pathologist who was blinded to the treatment of the animal, and as such we have confidence that the scoring is as objective and as accurate as possible.

On the contrary, discussing the supremacy of GFR over SCr and BUN (poorer correlation) is pointless, since the field is well aware of the fact, that both parameters are poor biomarkers for kidney function and health. Their predominance in the field is only due to cost benefits in the clinic. On the contrary, investigating the correlation of transdermal GFR assessment and Scr (Jaffe reaction and/ or HPLC) and BUN, respectively, would be more interesting. In my opinion, this would bring greater impact to the authors manuscript, as well, since Scr and BUN are still widely used in preclinical settings, where GFR would be way more appropriate (and available thanks to the authors magnificent invention). Showing correlations of t ½ vs Scr and t ½ vs BUN will point out, that those biomarkers are obsolete, especially in translational kidney research.

This is a very good point and we thank the reviewer for suggesting this. We have changed the correlation graphs such that tubular injury, serum creatinine and BUN are each correlated with FITC-sinistrin half-life and include discussion about this in the manuscript.   
  
307-317: The point made regarding dermal pigmentation and measurement sensitivity is indeed a FAQ and is very well addressed. Nevertheless, the complete lack of a (short) comparison with other available methods to measure GFR is surprising and weakens the manuscript's potential impact.  
We have now included a short comparison of pros and cons of the technique to other methods.

Minor Concerns

160-161: The patch size is not specified beforehand and a cut off is only meaningful if rat-sized patches are used. The smaller (round-edged) patches available from MediBeacon fit perfectly for mice. Here, further cut off is not beneficial. Inside-out-folded adhesive stripes can be used to attach the battery to the device, if no excess patch material is available (as done in our lab without any problems).

We have now edited the text to describe both types of patches.

169-191: For operators with narcosis chambers I would suggest the following approach: First, assemble the device, attach the battery, check if it's working and remove the backing from the adhesive patch. Put it upside down on the table to keep it ready. Ready the adhesive tape by laying it out adhesive side up on the table next to the device. Only then anesthetize the animal. Upon removal of the mouse from the chamber, wipe the skin with ethanol and dry quickly with another cotton swab. Attach the fully assembled imager to the mouse before moving mouse and imager to the adhesive tape. Fixate the tape to the mouse. This approach proved to be extremely fast by simultaneously greatly reducing the anesthesia time (and hence depth of anesthesia and occurrence of associated artefacts).

We thank the reviewer for this description of their method, however we do not agree that this is a significantly faster way of performing the protocol. Our protocol instructs to prepare the device, anaesthetize the mouse, attach the battery, then attach the device, so the only difference to the protocol described by the reviewer above is when the battery is attached. It only takes a few seconds to attach the battery so doing this before anaesthetizing the mouse does not reduce overall anesthesia time.  
  
193-196: Left-right confusion? 194: "right" instead of "left"?

We thank the reviewer for pointing out this mistake, and it has now been corrected. Further information has also been added to clarify the right/left instructions.

221-226: We advise beginners to utilize isoflurane narcosis for this step as well. This is in favor of prolonged battery survival.

We have included an alternative step to describe anesthetizing the mouse for device removal, however we would like to point out that this does not affect battery survival. In fact it will actually take longer to remove the device the mouse, as you have to wait for it to fall asleep and in this time the mouse will be jumping around the chamber and could get a paw stuck in the battery wires, thus possibly damaging the wires. Also, the second anesthesia session in a short space of time may affect the wellbeing of the mouse, especially if it is already suffering from kidney injury. We have taught a number of beginners to remove the device on a conscious mouse and it is not very difficult.  
  
Material table examples incomplete. A complete list might be of great interest for establishing the method anew.

We have now included this.  
  
Low picture quality.

We have now included higher quality images.  
  
**Reviewer #2**

Minor Concerns  
The reviewer has few suggestions, which are mostly minor nature that will potentially improve this work:

Introduction part lacks general aim of the presented manuscript. Adding a sentence providing rational for the need for described protocol would be helpful for the reader to follow the story.

Additional information has been added at the end of the introduction.

Protocol, part 4 (remove hair from the mouse) - what if the skin (after removing hair) appears irritated or wounded? Authors should consider adding a sentence commenting on the condition of the skin.

Additional information has been added to address this point.

Protocol, part 4 (FITC-Sinistrin injection) - Author need carefully describe the light sensitivity of prepared injection solution.

This is already addressed in the protocol in step 1.1 – lines 126-127 of the original version, lines 109-110 of the new version.

Representative results - Authors present GFR results obtained from BALB/c mice. What about other commonly used mouse strains (C57BL6 or 129)? Addition of a comment about other stains would be helpful for potential readers.

We have included a comment on this and referred to a list of publications.

-Representative results - Authors may consider adding a table with reference/control values for GFR in mice. That would greatly help researchers to interpret their data.

We agree with the reviewer that such a table would be helpful to the reader, however it is difficult to provide reference values that will account for all of the variabilities in mice (age, gender, strain etc.). Furthermore, we have already provided the readers with a small amount of reference values in figures 2 and 3 (fig 3 and 4 in new version). We have now included some more references to direct the reader to published literature for additional data.   
  
**Reviewer #3**

Minor Concerns

1) In item 6.8 in the protocol, the authors suggest collecting a background reading in anesthetized mice. Considering that a correct baseline reading is critical to obtain an accurate GFR measurement, is it possible the anesthesia might modify the level of this baseline reading? Although acquiring a baseline reading in conscious animals will probably have more artifacts, it might be more accurate and representative of the actual GFR measurement.

We thank the reviewer for this suggestion, however it is not possible to wake the animal up for the baseline measurement. This is because of the restraint that would be required to perform an IV tail injection in a conscious mouse – the mouse won’t fit in the restrainer with the device attached, and the pressure on the device during restraint would disrupt the readings. Additionally, a new algorithm has been developed to correct for these shifts in the baseline reading, this is now mentioned in the discussion.

2) For Figures 3 and 4, authors should include the actual GFR value besides the FITC-sinistrin half-life as it is more physiologically relevant.

This has now been included for Figures 3 and 4. Figure 5 still uses FITC sinistrin half-life for clarity of presentation, since half-life is directly proportional to the severity of injury, while GFR is inversely correlated with injury.

3) For Figure 4, it would be interesting to include a correction GFR vs. BUN or GFR vs. SCr as they are directly related.

This is an excellent idea, and has now been changed per the reviewer’s suggestion.