Universitätsklinikum Düsseldorf

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European Hypertension Excellence Centre

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Rebuttle document manuscript JoVE58542

Dear Dr. Bajaj,

Thank you for editorial review of the manuscript. Below you will find the Point by Point reply:

Editorial comments:

1. The editor has formatted the manuscript as per the journal's style. Please retain the same.
Response: Thank you, we have retained the format.

2. Please address all the specific comments marked in the manuscript.

2.1 We cannot have commercial terms in the manuscript. Please use a generic term instead. Also, commercial terms cannot be a keyword. All commercial terms should be sufficiently referenced in the Table of Materials. Maybe --- magnetic beads?
Response: Thank you for pointing this out. We have changed all the terms "dynabeads" to magnetic beads.

2.2. Introduction, first sentence: Citation?
Response: We have included a citation (Jefferson J.A. et al.; Podocyte Biology for the Bedside, American Journal of Kidney Disease 2011).

2.3. Protocol point 1.4: Magnetic beads?
Response: Thank you. We have changed dynabeads to magnetic beads accordingly.

2.4 Protocol point 2.7: Is this the same as vena cava?
Response: Thank you for this comment. We have changed cava vein into vena cava.

2.5. Protocol point 2.8: Ligation how- with a sterile suture? If yes what size?
Response: Thank you for pointing this out. The ligation is performed with a silk thread, size 4-0 to 6-0. We have added this information to the sentence.

2.6 Protocol 3.2: How do you ensure that the syringe is bubble free- any extra precaution which needs to be taken during changing? – Please include it as a note here.
Response: Thank you for your comment. We have included a note accordingly.

2.7 Protocol point 4.1: How much PBSCM?
Response: Thank you. We have added 15 mL PBSCM to point 4.1.

2.8 Protocol point 4.4: Into a new tube right?
Response: Thank you for this point. The digested tissue is centrifuged in a 50 mL tube. The pellet is then transferred into a new 2 mL tube. We have added the term "new" to point 4.4.

2.9 Protocol point 5.1: How do you ensure that only glomeruli gets attached to the magnetic beads?
Response: Thank you for this well taken point. As the magnetic beads embolize the glomeruli, they will not be found in the tubular system. However, tubular structures and cell debris might be still attached to embolized glomeruli before washing. Remaining attached tubular structures and cell debris will be lost during the washing procedure. Free magnetic beads will however attach to the magnet but will not confound future analysis as they do not contain any tissue/cells.

2.10. Protocol point 5.2: Provide the step number from below. -5.3?
Response: We added 5.3 accordingly.

2.11 Protocol point 5.3: Include a note stating what kind of structures will be observed under the microscope?
Response: Thank you for this comment. We have included a not indicating the structures that can be observed under the microscope.

2.12. Representative Results: Magnetic beads.
Response: We have changed dynabeads to magnetic beads.

2.13. Figure legends, Figure 1: Please include a scale bar for C and D in the figure as well.
Response: Thank you. We have included a scale bar in figures 1C and 1D.

2.14 Figure legends, Figure 3: For the IP blots please show equal loading control blot as well.
Response: Thank you for pointing this out. For figure 3B the nephrin staining (IP:nephrin, WB:nephrin) is the loading control for the IP. For figures 3A and 3C, IPs performed with

streptavidin beads show no staining for actin. The lysis buffer contains ATP so that actin is released from its adapters. In addition, actin does not precipitate with streptavidin agarose beads as it is an intracellular protein. Other cell surface markers will not be stained in control animals, as they have not been perfused with biotin. For the biotin perfused animal, commercially available antibodies against Neph1 and GLEPP1 did not work in our hands and this experiment. To show equal loading of the gel, we performed an amido black staining. As you can see from the additional figure for the editor, the running front shows equal loading. However, the sensitivity of the staining seems to be not enough to show all of the proteins on the blot.

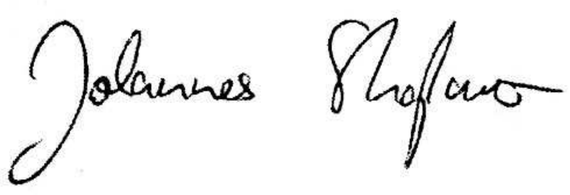
2.1.5 Figure legends, figure 4: Please include a scale bar for panel D.
Response: We have included a scale bar in the figure and figure legend.

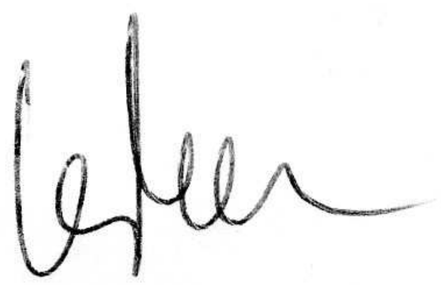
2.1.6 Figure legends, figure 5: Please include a scale bar for panel D.
Response: We have included a scale bar in the figure and figure legend.

2.1.7 Discussion: 3x magnetic beads?
Response: We have changed dynabeads to magnetic beads

3. After editing, please ensure that the highlight is no more than 2.75 pages including heading and spacings.
Response: Thank you for this helpful comment. We have changed the highlighted part accordingly.

Yours Sincerely,





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