We greatly appreciate the constructive and helpful comments of the editor and reviewers. We have extensively revised our manuscript according to the various concerns. The point-by-point responses are outlined below:

**RESPONSES TO THE EDITORIAL COMMENTS**

**Comment 1:** Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

**Response 1:** Thank you. We have thoroughly proofread our manuscript to ensure that there are no spelling or grammar issues.

**Comment 2:** Please revise lines 43-47, 50-52, 57-58, 61-62, 94-97 to avoid previously published text.

**Response 2:** We have extensively revised these sections of the manuscript.

**Comment 3:** Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or.docx file to your editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

**Response 3:** Thank you. We have reached out to the journal to obtain copyright permission (please see attached e-mail). We have cited the figures appropriately.

**Comment 4:** Figure 2: Please define the error bars in the figure legend. Please explain what the blue arrow represents.

**Response 4:** The definition of the error bars has been added to the figure legend (SEM ± Mean) for Figure 2. Also, an explanation for the blue arrow has been included in the figure as the beginning of low dose angiotensin II infusion.

**Comment 5:** Please remove the embedded figure(s) from the manuscript.

**Response 5:** Done

**Comment 6:** Keywords: Please provide at least 6 keywords or phrases.

**Response 6:** We have included 6 keywords: “Inflammation, Adoptive Transfer, Dendritic Cell Isolation, Dendritic Cells, Hypertension, Immune Cell Sorting, Flow Cytometry” to the resubmission of the manuscript.

**Comment 7:** Abstract: Please do not include references here.

**Response 7:** Done

**Comment 8:** JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. 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. You may use the generic term followed by “(see table of materials)” to draw readers’ attention to specific commercial names. Example of commercial sounding language in your maniscrpt are: Miltenyi Biotec, using trypan blue exclusion, QuadroMACs, autoMACs, PrecisionGlide, Becton Dickson, NORM-JECT, Henke-Sass Wolf etc.

**Response 8:** We apologize for this oversight. We removed all the commercial sounding language from the revised manuscript.

**Comment 9:** Please provide more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. 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. See examples below. Please specify the age, gender and strain of mice.

**Response 9:** We have included the age (10-12 weeks old), gender (male), and strain (C57bl/6) to the revised manuscript. In the revised manuscript, we have added more details to the mechanical dissociation steps, and noted another protocol that will work without using automated equipment.

**Comment 10:** Please specify the volume of RPMI 1640 medium used.

**Response 10:** We have specified the volume of RPMI 1640 medium used.

**Comment 11:** Please specify the rotation speed.

**Response 11:** Rotation speed is 20 rpm. This piece of information has been added to the revised manuscript.

**Comment 12:** Is supernatant aspirated after centrifugation? Please specify.

**Response 12:** The supernatant is aspirated after centrifugation. This has been added to the revised manuscript.

**Comment 13:** Please describe how to use trypan blue exclusion.

**Response 13:** A step by step protocol for trypan blue exclusion has been added to the revised manuscript.

**Comment 14:** Table of Equipment and Materials: Please sort the items in alphabetical order according to the Name of Material/Equipment. Please remove trademark (™) and registered (®) symbols form the Table of Equipment and Materials.

**Response 14:** Done.

**RESPONSES TO REVIEWER 1**

**Comment 1:** Does it make sense to isolate DCs from the kidney, since DCs are abundantly found in the kidney?

**Response 1:** Although there are resident DCs found in the kidney, isolation of these DCs for adoptive transfer is not feasible because of the small numbers. We find that there are approximately 4 x 106 DCs in the spleen. Due to the abundance of DCs in the spleen, we optimized our protocol to isolate DCs from this secondary lymphoid organ.

**Comment 2:** If possible, a diagram illustrating the angle of retro-orbital injection may be helpful. Depending on the institution, the mice may require daily monitoring for 3-4 days following implantation of osmotic minipump.

**Response 2:** Thank you. We have added a diagram illustrating the angle of retro-orbital injection. Please see Figure 1 of the revised manuscript. We have also added this important note to the protocol to advise readers of the need to monitor their animals for multiple days after implantation of the osmotic minipumps (Section 6.5).

**Comment 3:** In the discussion, the authors could mention that the use of additional DC markers could allow one to parse different subtypes of DCs.

**Response 3:** We agree. Therefore, we have added a sentence in the discussion to advise readers that this protocol could be extremely helpful in identifying DC subsets depending on what surface expression markers are used during the flow cytometry step.

**Comment 4:** As the goal of this method is to transfer cells from one animal to another, the efficiency of this transfer should be evaluated. It is imperative to measure the rate of reconstitution of these cells I the recipient animals to not only show that these cells are engrafting at a reasonable rate, but also to show that the rate of engraftment does not different between control or high salt cells.

**Response 4:** Thank you. We agree that it is important to evaluate engraftment after adoptive transfer. We have added this to the discussion. In prior studies, we adoptively transferred DCs from mice transgenic for Enhanced Green Fluorescent protein into naïve recipient mice. We then performed flow cytometry of various tissues in the recipient mice ten days later and found that these cells predominantly accumulate in the spleen of recipient mice, and to a lesser extent in the kidney and aorta. This was increased if the donor mouse was treated with angiotensin II. Based on this finding, we predict that the rate of engraftment will be higher in high salt treated DCs.

**Comment 5**: The purity of 70% is worrisome. As alluded to by the authors, FACS sorting allows for an extremely high purity isolation and previously published work from this journal showed that cDC isolation from the lung using FACS reached a purity over 95%. It would be useful for a magnetic bead approach due to the coast and equipment availability. Work by Malosse and Henri showed that standard density centrifugation using Histopaque resulted in a dendritic cell purity of 70% from the kidney and after microbead isolation, a DC purity of 86%. As such, the purity of 70% does not appear to be an improvement.

**Response 5:** In the revised manuscript (Figure 3), we have added flow cytometric analysis on how to obtain a highly pure (92%) population of CD11c+ cells using microbeads. Also, in Figure 3, we have demonstrated the purity of the manufacture’s protocol, and subsequently troubleshot the protocol to demonstrate to readers how to obtain a highly pure cell population. Please see the note under section 4.5 of the revised manuscript.

**RESPONSES TO REVIEWER 4**

**Comment 1:** While a complete protocol, there are sections, notable 3&4, that are essentially a retelling of a kit/supplier protocol. While ‘isolation’ itself is key to the usefulness, detailing what is otherwise in an instruction manual is not terribly useful. If the authors have troubleshot the provided supplier methods, they should clearly state so.

**Response 1:** We have extensively revised and troubleshot the manufacture’s protocol to obtain a highly pure population of CD11c+ cells. Please see response to reviewer 3 comment 5.

**Comment 2:** The resulting data are impressive as a realm of the laboratory’s work. Why, however, spleen-derived mature dendritic cells are a necessary part of the protocol should be clearly elaborated. Classical immunologic methods of isolating primary blood monocytes or bone marrow and then driving differentiation are well established (though admittedly much more tedious). Likewise, why not isolated DCs of any source from salt-treated mice? Is the immune phenotype and response of these CD11c+ splenocytes (a low % population only drive hypertension due to *ex vivo* salt + FBS components?

**Response 2:** Thank you. Your comment is well taken that there are classical immunologic methods of isolating primary blood monocytes or bone marrow and then driving differentiation are well established. We do not observe a salt-induced activation of DCs generated ex vivo using cytokine cocktails. In fact, we paradoxically observe that high salt reduces the activation status of DCs generated in this manner. This may be attributed the fact that the cytokine cocktail including GM-CSF and IL-4 as well as prolonged in vitro culturing may maximally activate the DCs.

**Comment 3:** 1.3. Is this basal RPMI or supplemented?

**Response 3:** We have added our formulation of the RPMI 1640 medium that we routinely use in the laboratory for dendritic cell culture media in the revised manuscript (Section 1).

**Comment 4:** 2.1. enzymes should be in activity units so as to compare across suppliers. Or list a specific supplier (even Roche, for example, lists an activity range).

**Response 4:** The enzymes that were used for digestion of tissues are now included in activity units in the revised manuscript.

**Comment 5:** what is a c-tube?

**Response 5:** A c-tube is a specific tube supplied by a manufacture (see table of materials) that is used for mechanical digestion to dissociate the spleens.

**Comment 6:** what is a semiautomated homogenizer, a specific, necessary device? Does mincing work?

**Response 6:** A semiautomated homogenizer is a device used for mechanical digestion to dissociate splenocytes into a single cell suspension (see table of materials and methods). While it is quick, convenient and gentle preventing excessive cell death, it can be replaced by smashing the spleen through a 40 µm filter and thoroughly rinsing with RPMI media. We have added this alternative to the revised manuscript.

**Comment 7:** is the resuspension volume dependent on the cell count?

**Response 7:** The resuspension volume is dependent on cell count. One cannot exceed 108 cells per 400 μl. We have added a “note” to the revised manuscript (Section 3.3).

**Comment 8:** Throughout: 4C or on ice make a difference?

**Response 8:** Most of the 4°C temperatures in the protocol are during centrifugation and prolonged incubation. Staining with cell surface markers for flow cytometric analysis is done on ice. We have added notes on where 4°C is required in the revised manuscript.

**Comment 9:** is this protocol specific to this kit? Miltenyi has multiple options/methods for using the same reagents

**Response 9:** We agree. We use this CD11c Microbead kit specifically for isolating CD11c+ cells from murine mice. There could be another option/method for using this specific kit, but this is the protocol that has been optimized in our laboratory.

**Comment 10:** 4.6. a second cell count… this one makes sense, so the first was to normalize cell density?

**Response 10:** The first cell count was to ensure that the resuspension volume was adequate prior to incubating with the CD11c microbeads.

**Comment 11:** 6.2. Is a 2 mL tube necessary or a standard 1.6 mL microcentrifuge tube sufficient? Should be based on volume used or is the extra space necessary for something?

**Response 11:** Thank you for this comment. This is based on volume, and the extra space is not needed. A standard 1.6 mL microcentrifuge tube is sufficient.

**Comment 12:** 6.3. do the authors use PBS? That P is for phosphate, not physiological

**Response 12:** In our laboratory we use PBS. We apologize for this oversight in the previous manuscript and we have corrected the “P’ for phosphate.

**Comment 13:** 6.5. note to see the discussion, or note here that i.v. works fine. The reviewer would argue that i.v. is easier and more reproducible than retro-orbital, but that is a preference. As such, detailing the injection method is perhaps not necessary as the authors later note that this should only be done by a skilled surgeon.

**Response 13:** We agree that I.V. will work fine for adoptive transfer of immune cells. We have added a note that the readers can also do I.V. injection (section 6.6). Furthermore, this has been added to the discussion in the revised manuscript, that there are multiple options of adoptively transferring immune cells to a recipient animal.

**Comment 14:** 7.2. does the lean:total mass ratio matter for Ang II application; i.e. must the mice be a certain age and/or weight range for these numbers to apply.

**Response 14:** We typically study mice at 12 weeks of age. Mice younger than this age tend to have a low yield of splenocytes and DCs. The weight of the mice is considered when calculating the dose of angiotensin II.

**Comment 15:** 8.4. betadine application would disinfect but it does not directly ‘promote wound healing’

**Response 15:** Thank you. This has been corrected in the revised manuscript.

**Comment 16:** 9. Say at 14 days, but dosing the minipump did not, in section 7, not a time frame for delivery

**Response 16:** The osmotic minipump used in our Ang II model (model 2002; Alzet) are 14-day osmotic minipumps. This important piece of information has been added to the revised manuscript.

**Comment 17:** 10. Centrifuge speed increased to 350g (from 300g in earlier parts) necessary?

**Response 17:** Thank you. We routinely use 300-350g. Thus, the increase to 350g is not necessary. We have updated the manuscript to include the range for consistence.

**Comment 18:** 10.2 cell viability stain: 1 uL of any stain would not follow this protocol. Many dyes, DAPI, PI, would never be incubated 15 minutes and would be concentration-dependent. Adjust for or not specific recommended protocol).

**Response 18:** We agree with the reviewer and have added a note for the specific cell viability stain that we use in our laboratory.