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May 13th, 2020

Dear Dr. Bajaj and Dr. Singh,

Enclosed please find the revised version of our manuscript (JoVE61299R1) entitled, "Guided differentiation of mature kidney podocytes from human induced pluripotent stem cells under chemically defined conditions", which we would like you to reconsider for publication as a Video Research Article in *JoVE*. Thank you for giving us the opportunity to respond to the concerns raised by the reviewers.

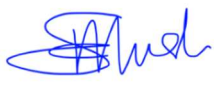
I believe that the prime revisions requested by your editorial team included the word count for our short abstract, the need for additional information for the DU11 human induced pluripotent stem cell line used in the study, the use of commercial language (such as Matrigel and Laminin) in the protocol, and a request to highlight 2.75 pages that identifies the essential steps of the protocol for filming. In our revised version of the manuscript, we have addressed the editorial comments as requested. These revisions include edits to reduce the word limit in the short abstract and ensuring that the long abstract is still within the specified word count; clarification that the DU11 stem cell line was previously derived at Duke University's Stem Cell Core Facility and citing relevant publication on their characterization and use to derive other organ-specific cell types including cardiomyocytes. We have also included a note clarify that our lab routinely tests the cells for mycoplasma (which was found to be negative) and our most recent karyotype analysis confirmed that the DU11 cells were normal. Additionally, we have removed commercial languages and provided relevant information in the Table of Materials per Dr. Bajaj's suggestions during our follow-up emails for clarification on this request. Furthermore, we have highlighted in the revised version of the manuscript the filmable content of the protocol as requested by the JoVE editorial team.

In regards to the more general intimation by Reviewer #3 that other reports that attempted to derive kidney cells from pluripotent stem cells using embryoid body and organoid approaches could have possibly generated podocytes under chemically defined conditions or with high purity (without subpopulation selection) has no basis given that these methods require the use of undefined animal-derived serum and by definition, organoids and embryoid bodies generate highly heterogenous populations that will also include non-kidney cell types (e.g.: skin cells, neurons, and other unidentified cells), thus producing a low yield of the desired cell types and necessitating subpopulation selection or sorting – which typically compromises the viability of the cells. The podocyte differentiation media we created in our protocol were formulated in our lab and are currently not available commercially. The term "chemically-defined" is used to indicate serum-free conditions and the fact that the composition, identity, and concentrations of all the chemicals used are known, such that readers of this manuscript who are interested in adapting the protocol will be able to create their own medium by following the step-wise directions provided in this protocol. The chemical components include soluble and insoluble (adhesion) proteins and small molecules, as well as the E8 fragment of Laminin 511 used as cell culture matrix. To the best of our knowledge, our method for the derivation of podocytes is the first in vitro stem cell differentiation method to produce podocytes with characteristics of the mature and functional phenotype with high yield (>90% yield) without subpopulation selection, genetic manipulation, or need for xenotransplantation to induce lineage specification.

In any case, in response to the reviewer concerns, we now clarify these key points that explain the differences and the limitations of the other differentiation approaches when trying to produce a more specific and specialized cell type such as kidney glomerular podocytes with specificity and efficiency. We have revised the Discussion section to place our work more clearly in an appropriate context.

Given the overall positive responses from most of the reviewers, the importance of these findings in illuminating the biology and mechanisms of human kidney tissue development, as well as providing new tools for nephrotoxicity testing, disease modeling, and drug screening, we hope that you'll be able to make a final decision regarding the publication of this article. To help expedite your review, we have tracked and underlined all changes to the manuscript in the revised version submitted.

Sincerely,



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