Author's response to reviews

Title: Podocyte specific knock out of selenoproteins does not enhance nephropathy in streptozotocin diabetic C57BL/6 mice

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Author's response to reviews: see over
On behalf of all authors, I am pleased to have the opportunity to resubmit manuscript 1026614180177015. Podocyte specific knock out of selenoproteins does not enhance nephropathy in streptozotocin diabetic C57BL/6 mice, by Marsha N Blauwkamp, Jingcheng Yu, MaryLee A Schin, Kathleen A Burke, Marla J Berry, Bradley A Carlson, Frank C Brosius III and Ronald J Koenig. Our responses to the comments of the reviewers are as follows:

Referee 1
Major Compulsory Revisions
In the revised ms, we have assessed oxidative stress by IHC using an anti-nitrotyrosine antibody (Fig 5). Nitrotyrosine immunostaining was not increased in podocytes from KO versus control mice. Thus, at least by this measure, the loss of selenoproteins did not increase podocyte oxidative stress.

Minor Essential Revisions
1. The reviewer asks whether expression of Cre itself in podocytes might be toxic. This seems extremely unlikely because published studies indicate no glomerular abnormalities in podocin-Cre mice, now referenced in the revised manuscript and specifically stated in the first paragraph of Methods. We did not assess the phenotype of podocin-Cre;TrspL/+ mice and do not have kidneys saved from these mice to do so.

2. Thank you for catching the error. Min has been changed to sec.

3. The initial imaging under the microscope was at 20X, but the images were analyzed at a final magnification of 150X by Metamorph software. This is now clarified in Methods, Histology and Morphometry.

4. Fig 1 has been re-done so that the sizes of panels A-D are equal.

Discretionary Revisions
We appreciate the suggestion of evaluating endothelial selenoproteins in future studies.

Referee 2
The reviewer asks whether there is evidence for a compensatory increase in the expression of antioxidant enzymes when podocyte selenoproteins are knocked out. To assess this, we attempted to perform IHC for catalase (Abcam Ab1877), Mn-SOD/SOD2 (Millipore 06-984), and NQO1 (Abcam ab34173). Podocyte staining for catalase and SOD2 was not consistently above that for normal IgG under multiple conditions for the IHC. Podocyte staining for NQO1 was clearly detectable, and was similar in the KO and control podocytes. Thus, at least for this antioxidant enzyme, there is no compensatory increase in the KO mice. The NQO1 staining is shown in Figure 6.

Referee 3
1. We have now evaluated oxidative stress by IHC for nitrotyrosine. See reply to Referee 1.

With these changes we hope that our manuscript is acceptable for publication, and we look forward to hearing from you.
Sincerely,
Ron Koenig