Reviewer's report

Title: Absence of Chloride Intracellular Channel 4 (CLIC4) Predisposes to Acute Kidney Injury But Has Minimal Impact on Recovery

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Reviewer: Mark Dockrell

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Minor Essential Revisions

There are some statements in the text that require citations such as the sentence in the first paragraph of the Background starting “Toxic or ischemic injury to the kidney tubules triggers…; and the sentence in the results section “Absence of CLIC4 has been previously shown to impair angiogenesis; CLIC4 has been implicated in the intracellular tubulogenesis of endothelial cells and is present in both glomerular and peritubular endothelial cells in the kidney”

Some statements in the results require better supporting evidence or more data. “Identically treated, stained, and imaged sections from Clic4 null mice served as the negative control and showed no significant signal with the CLIC4 antibody (not shown).” In view of the extensive staining for CLIC 4, even apparently in the lumen of the tubule, I think the authors should make this negative control available for inspection.

The authors also state that “In epithelial cells, CLIC4 (red) is prominent in a subset of the proximal tubules,” from what I can see of the image CLIC4 appears in all the proximal tubules, perhaps the authors could present an image with proximal tubules negative for CLIC4.

The authors report nuclear staining for CLIC4 in the proximal tubule, I found it difficult to discern specific nuclear staining from the image, can the authors indicate a prominent example?

Page 15 the authors state “Baseline characteristics of the mice are shown in table 1.” I presume they mean table 2.

Page 16 the authors state “…kidney injury with higher average and medium blood urea at 48 hours…” should this be median blood urea?

Later on Page 16 the authors state “With loss of the brush border, LTA staining of is less prominent but enough residual staining remains to easily identify the proximal tubule.” In the image I have I could not detect any LTA staining, could the authors provide a better image?
When discussing severity of renal injury as determined by BUN the authors appear to select a value of 200 on some occasions and 100 on others; it would be useful if the authors justified the values they were choosing.

On page 17 the authors state “mice with greater initial injury had higher BUN at 21 Days”, I thought they were defining degree of injury by BUN level.

I couldn’t see where the authors explain precisely how they arrived at the percentage fibrosis, this should be explained clearly.

Discretionary Revisions

I am uncertain whether the experiments carried out can justify the conclusion that “Taken together, the data do not support a model similar to that of the keratinocytes in which a substantial fraction of CLIC4 is targeted to the nucleus where it significantly potentiates TGF# signaling.” The method that was used would not necessarily detect whether CLIC4 increased the duration of phosphor- Smad expression in the cell nucleus. The question would be do you see a more rapid decline in p-Smad expression in the absence of CLIC4 and from what I could see there was no decline over the period investigated. It would seem very possible that nuclear membrane chloride channels could regulate nuclear activity.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests’