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: Stuart H Yuspa

Reviewer's report:

The authors address the function of CLIC4, highly expressed in renal proximal tubules, in normal renal physiology and in response to injury induced by folic acid. Comparing kidneys of wildtype and CLIC4 KO mice, the authors conclude that CLIC4 loss produces a mild renal insufficiency, proteinuria thought to be of glomerular origin, increased susceptibility to acute toxicity but no deficiency in repair after toxicity. In fact, although not specifically discussed, the absence of CLIC4 seems to improve repair from toxicity since the initial toxic response is greater but repair is equal. The authors should comment on this possibility. There are very few mechanistic insights produced in the report except that TGFbeta function appears intact in the CLIC4 KO repairing kidneys (although with slightly reduced signaling). Nevertheless the authors have provided a reasonable beginning for further explorations. The authors could improve the presentation in this report with the following considerations:

1. The immunostaining would be strengthened by including panels from KO kidneys to confirm the specificity of the antibody for CLIC4. This is a pretty routine presentation of these kinds of data.
2. Additional function tests on the glomeruli would help to understand why there is proteinuria. Dextran filtration would confirm leakage.
3. Because CLICs are a very close family of proteins, it would be important to determine if compensation occurs after renal injury by upregulation of another CLIC such as CLIC1 or CLIC5. This would be very helpful in understanding the major points of the paper. It would be helpful in understanding the basal status as well.
4. The pSmad results in Fig 13 appear to be normalized to GAPDH. However, they would be more accurately portrayed normalized to total Smad. Without knowing the total Smad content the pSmad measurements are just an estimate. In fact the most useful way of presenting these data would be using nuclear extracts where the pSmads function but this may be difficult from a tissue homogenate. Because of these deficiencies the authors may want to modify their last sentences with some caveats.
5. There are still a number of unsolved mysteries from these mice. Why are the kidneys and body size smaller, why are there fewer glomeruli, again the question of proteinuria with normal BUN, and most interesting is why they heal so well? The question of compensation from other CLIC proteins seems very important in
this paper.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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.