Point-by-point responses to comments raised by Reviewer 1

In their study "Hepatocyte specific gp130 signalling underlies APAP induced liver injury" Dong et al. show, using two newly generated conditional KO mouse models for gp130 and IL11, that liver damage caused by an acetaminophen overdose can be ameliorated by reducing IL11 signalling via gp130 in hepatocytes.

Overall the study is well designed and thoroughly executed. The authors do make a convincing argument that hepatocytes are the major source of IL-11 secretion upon APAP-induced liver injury. However, the study is lacking in some aspects as the described results are mostly observational and no mechanistic explanation is pursued.

Response: We thank the Reviewer for appreciating our study and for the suggestions.

Major comment:

1. *One very important aspect of APAP-induced liver injury is not at all investigated or discussed, though it might be quite relevant for the observed phenotypes – the role of senescence in liver regeneration after acute liver injury. IL-11 is considered one of the cytokines associated with the senescence-associated secretory phenotype (SASP). Furthermore, IL1beta, IL6 and TNFa, all found to be downregulated in both CKOgp130 and CKOIL11 mice, are part of the SASP. Therefore, this potential angle needs to be investigated and either confirmed or excluded as a potential mechanism for the observed phenotype*

Response: We have previously described in detail the mechanism underlying IL11-induced hepatotoxicity in our Science Translational Medicine paper of 2021 (PMID: 34108253), which we cited. The mechanism described in this manuscript (PMID: 34108253) was summarized in Supplementary Figure 17. We reiterate the legend of this figure here for the reviewer: "*Metabolism of APAP in hepatocytes leads to mitochondrial damage by NAPQI and ROS production, which triggers IL11 expression and IL11 secretion. IL11RA is highly expressed on hepatocytes and secreted IL11 activates an autocrine signaling loop to upregulate NOX4 and generate further ROS. ROS-dependent JNK activation drives hepatocyte death which results in a combination of necrosis and other forms of cell death, while also limiting hepatic regeneration*." This mechanism was confirmed and expanded on further in our subsequent publication in Elife (PMID: 34435951). The pathways underlying this mechanism (ERK, NOX4, JNK) were further demonstrated in the current study. The reviewer is therefore incorrect to assert that the mechanism(s) of IL11 hepatotoxicity has not been addressed. That is not to say that there are potentially minor additional contributions to the overall pathophysiology by factors including (but not limited to) senescence, apoptosis, necroptosis, failure of local stem cell activity, failure of bone marrow stem cell migration, failure of hepatocyte mitosis, excessive or insufficient inflammation or other unknown factors. However, we highlight that the centrally important and accepted mechanism has been described, peer reviewed and published. We point out the current study is built on the published foundational mechanistic observations and goes on to address new and different questions, as posed at the end of the introduction.

2. *It would be easier to interpret the findings if the data weren't shown as individual separate time points but rather as a time course, which would make it easier for the reader to appreciate the differences in eg. Peak damage in the models. Furthermore, it would be equally of interest to the reader to see H&E stainings of the earlier time points and the APAP-induced damage as well as mRNA expression of the investigated pro-inflammatory markers (or SASP markers?).*

Response: In the revision, we now show new H&E staining data at an earlier time point (6 hours post APAP) as Figure 2G and 5G. With regard to the presentation style, we believe this is a subjective point that has not been brought up by other reviewers and is not in keeping with how we would

normally present data from this type of experiment. As mentioned above we do not think profiling the SASP in isolation is informative, given our STM and Elife publications relating to mechanism, see discussion above.

Minor comments:

1. *L85: Reference 24 (Ho et al. Optimized Adeno-Associated Virus 8 Produces 529 Hepatocyte-Specific Cre-Mediated Recombination without Toxicity or Affecting Liver Regeneration. Am. J. Physiol. Gastrointest. 530 Liver Physiol. 2008) describes AAV-MUP-iCre rather than the AAV-Alb-iCre used in this study. A reference showing specificity of AAV-Alb-iCre is needed instead.*

Response: We thank the reviewer for highlighting this typographical error the text should have read "While albumin-driven Cre expression from germline in the mouse is associated with Cre protein in cells beyond hepatocytes (ref 23), AAV8-Cre has hepatocyte-restricted expression in the adult (ref 24) ". This has been corrected in the revision and the reference retained, as is appropriate. We showed the specificity of efficacy of AAV-Alb-iCre in the liver in Figure 1 (CKO^{gp130}) and Figure 4 (CKO^{Il11})

2. *Figure 3H: the H&E image of the CKOgp130 mouse does not seem to be representative of the quantification of the necrotic area. Can the authors choose a more representative image?*

Response: As suggested, we have replaced the H&E image of the CKO^{gp130} in Figure 3H with a more representative example.