Author's response to reviews

**Title:** Mitochondrial Uncouplers Inhibit Hepatic Stellate Cell Activation

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**Author's response to reviews:** see over
Dear Dr. Canbay,

Hereby we resubmit our revised manuscript, entitled "Mitochondrial Uncouplers Inhibit Hepatic Stellate Cell Activation" by Guimarães et al. We were happy to read that there was enthusiasm for the topic and that the manuscript would be considered to be acceptable with a satisfactory revision. All the modifications in the manuscript are underlined and point by point answers to the reviewers are listed below. We thank the reviewers for their suggestions and hope that the manuscript will now fulfill the requirements for publication in BMC Gastroenterology.

Yours sincerely,

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Reviewer: 1

Comments to Author

Guimarães et al. describe the effect of induced mild mitochondrial uncoupling on hepatic stellate cell (HSC) activation and fibrogenic activity. Mitochondrial uncouplers were shown to be capable of inhibiting initial HSC activation, reducing HSC proliferation as well as suppressing TGF-β1 signaling suggesting mitochondrial uncoupling as a new strategy for liver fibrosis therapy. Overall, this is an interesting, well written manuscript with no major weaknesses.

Major Compulsory Revisions:

n/a

Minor Essential Revisions:

1) All data are expressed as mean ± SEM and not mean ± SD as it is claimed in “Statistical analysis” of the “Materials and Methods” section.

2) The reviewer assumes that FCCP and Valinomycin concentrations of 5 µM were employed in the experiments Fig.1, 4 and 5 (similarly to Fig. 2). However, this should be mentioned in the figure legends or “Result” section.

Answer:
We thank the reviewer for her corrections of mistakes in the legends and text. They have now been corrected accordingly.

Discretionary Revisions:

1) As a courtesy to the reader, information about product lengths for each primer pair as well as suppliers of the agents SnPP and TGF-β should be provided.
Answer:
We inserted a new column in the table 1 named “product length” with the size of each amplicon. We also inserted at the section Methods the suppliers of TGF-β and SnPP.

2) Fig. 2C could be confirmed by immunoblot (similarly to Fig. 6B/C). The effect of FCCP and Valinomycin demonstrated in Fig. 6B to a certain amount might be due to unequal loading.

Answer:
We appreciate the suggestion of an additional immunoblot showing the effect of uncouplers on α-SMA levels. Nevertheless, due to technical limitations in the isolation and accumulation of the necessary amount of protein to perform such experiment, we used a different strategy, immunocytochemistry. Additionally we believe that the large difference between the α-SMA fluorescence among groups observed in figure 2C sufficiently represents the effect of mitochondrial uncoupling on α-SMA expression. We also additionally analyzed the protein expression on figure by densitometry, in order to account any unequal loading issues in figure 6B.

Reviewer: 2
Comments to Author

The authors describe the impact of mitochondrial uncoupling on culture-induced HSC activation and the response to TGF-β. This study is of high interest, because the authors show inhibition of profibrogenic features by mitochondrial uncoupling in stellate cells. This knowledge might be useful concerning future therapeutic approaches. The manuscript is well written and the study includes a wide range of sophisticated technologies such as stellate cell preparation from human liver tissues and many others.

There is only a minor objection.
Minor comment:
The reviewer just asks the authors to correct the title of table 1 into:
Table 1: Primer sequences, probes and accession numbers of transcripts, used for RT PCR quantification.
Further, the reviewer suggests that the authors should show this table as a supplemental table.

Answer:
We thank the reviewer for her suggestions. The table is now a supplementary document and the title has been modified.