Author’s response to reviews

Title: Functional consequences of enhanced expression of STIM1 and Orai1 in Huh-7 hepatocellular carcinoma tumor-initiating cells

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Authors’ responses to Editorial Comments:

Editorial Comment 1: Any manuscript submitted to a BioMed Central journal must be original and the manuscript, or substantial parts of it, must not be under consideration by any other journal. We note that the current submission contains some textual overlap with other previously published works. Please re-write these sentences and phrases in your own words to ensure no overlap. There is some overlap in the Methods section; please ensure that you summarize the methods and cite the source.

We recommend that authors use an anti-plagiarism software, such as turnitin or any other free ones, to access any overlap, and reduce it.

Authors’ response: The whole document was checked by using Turnitin antiplagiarism program based on the editorial comment and overlapping lines. We were also concerned about long phrases taken word-by-word from our own laboratory protocols published earlier (Selli C, Erac Y, Tosun M. Simultaneous measurement of cytosolic and mitochondrial calcium levels: observations in TRPC1-silenced hepatocellular carcinoma cells. J Pharmacol Toxicol Methods. 2015: 72:29-34). In order to avoid possible overlaps that would possibly give an impression of self-plagiarism only the related reference was cited and all other detailed calcium monitoring protocol was deleted. We appreciate editorial suggestion leading us to correct of this inadvertent mistake.

Editorial Comment 2: If human cell lines are used, authors are strongly encouraged to include the following information in their manuscript:
• The source of the cell line, including when and from where it was obtained
• Whether the cell line has recently been authenticated and by what method
• Whether the cell line has recently been tested for mycoplasma contamination

Further information is available from the International Cell Line Authentication Committee (ICLAC). We recommend that authors check the NCBI database for misidentification and contamination of human cell lines. Please include the sources of all cell lines and their catalogue numbers.

Authors’ response: Human hepatocellular carcinoma cell lines (Huh-7) were provided by our collaborator Dr. Ozturk (Izmir Biomedicine and Genome Center). The cells were originally from Dr. Jack Wands Laboratory at Massachusetts General Hospital, Boston, MA. The first authenticity analysis was performed at DNA Sequencing & Analysis Shared Resource (University of Colorado Cancer Center) via DNA profiling which utilize Applied Biosystem’s Identifier kit (PN 4322288) as described in Method section. The authenticity was reconfirmed by Idexx Bioresearch Company (Germany) before using in our study (Please find the attached original report within additional files). These were performed prior to using the cell in our experimental protocols In addition to this authentication analysis, we routinely check each vials for potential mycoplasma contamination by using Mycoplasma detection Kit (MycoAlert, Lonza) as mentioned in the Method-Cell Culture section in original submission. The first paragraph was rephrased accordingly.

Editorial Comment 3: Please confirm whether any of the cell lines required ethics approval for their use and include a statement in the Ethics approval and consent to participate section in the Declarations.

Authors’ response: No ethical approval is needed as the cell lines used in our study are commercially available cells originally collected from human samples.

Editorial Comment 4: Please include a List of Abbreviations to follow the Conclusions section.

Authors’ response: Abbreviations were added following the conclusion section

Editorial Comment 5: In the Funding section of the Declarations please indicate the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript. If no specific funding was received for this study, please clearly indicate this in the Funding section.
Authors’ response: The funding governmental institution provided only financial support as described in the related section.

Editorial Comment 6: Please note if you wish to acknowledge someone by their full name in the Acknowledgements, please ensure you have obtained permission from them to so do.

Authors’ response: Permissions were provided by asking them individually via e-mail.

Editorial Comment 7: At this stage, please upload your proofread manuscript as a single, final, clean version that does not contain any tracked changes, comments, highlights, strikethrough or text in different colours. All relevant tables and figures should also be clean versions. Figures (and additional files) should remain uploaded as separate files. Should you wish to respond to these revision requests, please include the information in the designated input box only.

P.S.

Authors’ request: Would it be possible to make a minor change in the title in order to emphasize the technical manipulation implying that cancer cells may have more of these proteins?

Original: “Functional consequences of STIM1 and Orai1 overexpression in Huh-7 hepatocellular carcinoma tumor-initiating cells”

Alternative: “Functional consequences of enhanced expression of STIM1 and Orai1 in Huh-7 hepatocellular carcinoma tumor-initiating cells”

Thanks and best regards