April 7th, 2021

Dear Editors of JoVE

Please find enclosed the second revised version of the Manuscript JoVE62209 "Differentiation of porcine induced pluripotent stem cells (piPSCs) into neural progenitor cells (NPCs)", submitted to the Journal of Visualized Experiments (JoVE).

The revised version of the manuscript highlights the changes performed in yellow, and the point-by-point answer to reviewers is included in this response letter.

Editorial comments:

1. Some additional details are needed in the protocol. Please see the comments in the attached manuscript.

Answer:

Most of the details are related to culture conditions and passaging routine. In order to better clarify these points, we have included an initial sentence:

All procedures involving cellular culture and incubations are performed in a controlled atmosphere (38.5ºC and 20% CO_2 in air, maximum humidity). Cellular passaging was performed by 5 min incubation with dissociation reagent and cells recovered after centrifugation (300G).

Also, we highlight that one of the questions raised is about the details about cellular dissociation procedure. As we were advised to remove commercial names such as Accutase and TryPle express, the only possibility to better detail these procedures are to include a more general protocol. We are willing to adequate again if needed.

2. Please revise the discussion to include limitations and critical steps of the protocol.

Answer:

We have included the information as follows:

The lack of generation of bonafide piPSCs still hampers its full potential in regenerative medicine, although a great number of studies have already been reported[18, 19] on the generation and use of these cells. An important question to be addressed is the limitation of the exogenous expression of pluripotency factors, often due to the use of integrative systems used to reprogram somatic cells in this species. The transgene expression may

lead to a possible incomplete differentiation *in vitro*, and thus, functional and terminally differentiated neurons are still a drawback to be surpassed.

We hope to have appropriately addressed all questions raised, and we are willing to make any other corrections if needed.

Sincerely yours,

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