Reviewer’s report

Title: Expression of components of the urothelial cholinergic system in bladder and cultivated primary urothelial cells of the pig

Version: 3 Date: 26 Jun 2019

Reviewer: Reviewer 2

Reviewer's report:

"PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses one or several testable research questions? (Brief or other article types: is there a clear objective?)
Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?
No - there are minor issues

EXECUTION - Are the experiments and analyses performed with sufficient technical rigor to allow confidence in the results?
No - there are minor issues

STATISTICS - Is the use of statistics in the manuscript appropriate?
N/A - there are no statistics in this study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?
Yes - the author's interpretation is reasonable

OVERALL MANUSCRIPT POTENTIAL - Has the author addressed your concerns sufficiently for you to now recommend the work as a technically sound contribution? If not, can further revisions be made to make the work technically sound?
Probably - with minor revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: The authors have indicated in the response letter that IHC on the additional targets requested is not possible at the present time due to technical issues (requirement to test and optimize human antibodies on porcine tissues).

REQUESTED REVISIONS:
I would request the authors to state in the manuscript explicitly why IHC validation of the additional targets was not performed (in addition, please also state which targets were attempted
but not successful)."

**Please confirm that you have included your review in the ‘Comments to Author’ box?**

As a minimum standard, please include a few sentences that outline what you think are the authors’ hypothesis/objectives, their main results, and the conclusions drawn. Your report should constructively instruct authors on how they can strengthen their paper to the point where it may be acceptable for publication, or provide detailed reasons as to why the manuscript does not fulfill our criteria for consideration. Please supply appropriate evidence using examples from the manuscript to substantiate your comments. Please break your comments into two bulleted or numbered sections: major and minor comments.

Please note that we may not be able to use your review if no comments are provided.

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**Are the methods appropriate and well described to allow independent reproduction of experiments?**

Please state in the ‘Comments to Authors’ box below what you think are the strengths and weaknesses of the methods (study design, data collection, and data analysis), and what is required, if anything, to improve the quality of reporting

No

**Does the work include the necessary controls?**

If not, please explain in the ‘Comments to Author’ box below.

No

**Are you able to assess the statistics?**

- Are the statistical test(s) used in this study appropriate and well described?
- Is the exact sample size (n) reported for each experimental group/condition (as a number, not a range)?
- Are the description of any error bars and probability values appropriate?
- Are all error bars defined in the corresponding figure legends?
- Has a sample size calculation been included, or a description and rationale about how sample sizes were chosen?
Please can you confirm which of the following statements apply to your statistical assessment of the manuscript (Please include details of what the authors need to address in the ‘Comments to Author’ box):

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**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in the ‘Comments to Author’ box below.

Yes

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable

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No

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