DR. RICARDO ROSSELLÓ NEVARES

UNIVERSIDAD DE PUERTO RICO, RECINTO DE CIENCIAS MEDICAS
UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCES CAMPUS
ESCUELA DE MEDICINA, SCHOOL OF MEDICINE

DEPARTAMENTO DE BIOQUIMICA
DEPARTMENT OF BIOCHEMISTRY

July 20th, 2016

Dear Editor

Please find enclosed the entitled manuscript in Word Format (file 24267-Review.*Doc*). Changes are highlighted in the manuscript.

Title: ***Characterization and Genetic Manipulation of primed stem cells into a functional naïve state with ESRRB***

Authors: Ricardo Antonio Rossello, Andreas Pfenning, Jason T Howard, Ute Hochgeschwender

ESPS Manuscript No. **24267**

The manuscript has been improved according to suggestions of reviewers:

Reviewer Major Comment 1: *The major drawback of the manuscript is the misleading use of the word reprogramming. Indeed, cells changed their functional properties however they did not change the overall program, they still have same phenotype and hopefully ES markers expression […]I recommend to substitute wording "reprogramming" for "overexpression" throughout the manuscript including the title.*

Answer: In accordance to the author recommendation, we have changed the word reprogramming for overexpression. We also changed the title to "Characterization and Genetic Manipulation of primed stem cells into a functional naïve state with ESRRB". There was an overall evaluation of the context in which the words were used.

Reviewer Major Comment 2: The title and the concept of "functional" and "non-functional" ES cells is entirely inappropriate. Both of these cell types are pluripotent, that is they can differentiate into all types of tissues, and chimera formation is another potential, which e.g. cannot be tested in human ES cells. The current, acceptable terminology may be "naive" ES cells for those, which can form chimeras and "primed" for those which may not (based on different origin, gene expression profile, etc.)

Answer: This change has been addressed throughout the manuscript and in the title (see previous reviewer)

Reviewer Major Comment 3: *ESRRB is clearly involved in the overexpression of Nanog, thus an appropriate control for "reprogramming" of primed cells should have included Nanog. In the absence of this experiment the exclusive role of ESRRB cannot be appreciated in naive state generation.*

Answer: We are thankful for the reviewer's recommendations in this regard. This was the mayor change we had to make new cassettes and perform new experiments. We were able to

PO BOX 16412, SAN JUAN, PUERTO RICO 00908

repeat the experiment, using a positive control Nanog cassette to overexpress this gene in primed cells. All groups induced with only Nanog overexpression failed to create chimeras. Therefore, we have concluded that ESRRB's effect goes beyond only Nanog.

Reviewer Major Comment 4: "Both ESRRB and ERAS which have been proved to control the functional state of ESCs, are not transcription factor. Then the mechanisms of their effect remain elusive. Discussion about it will be appreciated."

Answer: **Although these two proteins are not transcription factors, they have been implicated in stem cell control. For example, Eras has been identified to provoke tumorogenic growth in stem cells, expressed only in stem cells and silenced in somatic cells due to epigenetic changes. Adding ERas exogenously in a constitutively expressed promoter would overcome this limitation. ESRRB has been implicated as key downstream regulator of self renewal, downstream of GSK-3. Inhibition of GSK-3 has been implicated with supporting mESC state.**

Other minor comments were also assessed:

1) Plain and concise conclusion in abstract
2) Supplemental files assessed; documents were changed to excel files
3) Changes to figures 1, 2, 3 and 4
4) Keeping Table 3 and
5) In addition, Core tip, Comments sections were added.
6) Removal of Pax-3 that was mistakenly labeled
7) Standardizing nomenclature of genes (Upper case initial, lower case afterward; e.g. C-myc)
8) All authors are native English speakers. We have evaluated the grammar of the manuscript as per some recommendations.
9) Clarification of log-fold interpretation

We are grateful for your time and consideration in the publication of this article. Should you have any other questions, please don't hesitate to contact us.

Sincerely,

Ricardo Rossello
Assistant Professor, Metropolitan University, Cupey Puerto Rico
Adjunct Professor, Dept. of Biochemistry, University of Puerto Rico
202-615-2012
ricardo.rossello@upr.edu