Dear editor:

We are grateful for the opportunity to revise our manuscript with the manuscript ID: JoVE59016. We deeply appreciate reviewers’ time, efforts, and helpful remarks on our manuscript. We have revised the manuscript based on the reviewers’ suggestions and editor’s detailed comments, and include a separate document with point-by-point responses to the comments, questions, and concerns of the reviewers’. All changes in the manuscript are in red and underlined. We hope that you, the reviewers, and the editorial board now feel our paper is worthy of publication in Journal of Visualized Experiments.

Sincerely yours

Yung-Chia Chen

Associate Professor

Kaohsiung Medical University

Editorial comments

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

A: A: Thanks for reviewer’s suggestion! We have done our best to correct all spelling or grammar errors in the manuscript.

1. Please include at least one paragraph of text to explain the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures.

A: We have revised the section of representative results according to editor’s comment. Please see the following text.

**REPRESENTATIVE RESULTS:**

Using the procedure described here, primary cultured adrenal cells could be distinguished under phase-contrast microscope (figure 1A). To further confirm the vesicles within the cytoplasm are lipid droplets, the immunofluorescence staining of adipose-differentiated related protein (ADRP) can be conducted on Day-3 cultures (figure 1B). Cells were grown on the glass coverslips. The cells were washed with phosphate buffered saline (PBS) three times and fixed with 4% paraformaldehyde. After washing three times with PBS, cells were blocked and permeabilized with 0.1% Triton X-100 in 5% nonfat milk. The primary ADRP antibody was diluted (1:50) in 5% nonfat milk and was incubated overnight. After 3 times of PBS washing, cells were incubated with goat anti-rabbit Alexa Flour 488 secondary antibodies (1:100). Repeat the washing steps, the cells were then mounted with Prolong Gold antifade mounting medium and were examined under the fluorescence microscope. Moreover, the hormone-producing ability of the rat adrenal cells can be determined by corticosterone assay. Stimulation of ACTH on rat adrenal cells could be served as a positive control (figure 1C). The collected media were diluted and assayed for corticosterone content according to the procedure described by chen et al. (2015)11.

1. Please remove all headers from Representative Results and Discussion.

A: Thanks for the kindly suggestion! The headers from representative results and discussion have been removed.

1. Please section you protocol steps. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary.

A: Thanks for the kindly suggestion! We have revised the protocol section according to the editor’s comments.