Dear Phillip,

Thank you for handling our manuscript (JoVE61230) on our isolation protocol for nuclei from adipocytes for single cell RNA-sequencing. As you can see below, all the editorial as well as referees' comments have been addressed. I believe that the manuscript is ready to move on to video production. Please feel free to contact me if you have any questions.

Sincerely,
Kosaku

Kosaku Shinoda, Ph.D.
Assistant Professor, Albert Einstein Medicine

Editorial comments:

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

We carefully proofread the manuscript for spelling or grammatical errors.

E-2. Please define all abbreviations before use, e.g., SVF, t-SNE.

We have defined all the abbreviations including SVF (Stromal Vascular Fraction) and t-SNE (t-Distributed Stochastic Neighbor Embedding).

E-3 JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please limit the use of commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

We have removed all commercial language from main text.

E-4. Please provide at least 6 key words or phrases in a separate section in the manuscript.

We have provided 6 keywords on page 1.

E-5. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

Animal care and experimentation were performed according to procedures approved by the Institutional Animal Care and Use Committee at the Albert Einstein College of Medicine. This statement is included in page 3 line 78.

E-6. For each protocol step/substep, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

E-6A. Please provide more detail or a reference about collecting adipose tissue.

The detailed protocol for collecting adipose tissue has been published in JoVE in 2013 (Aune et al.). We have included the citation.

E-6B. 'More digestion buffer' is vague; do you mean the entire volume prepared in 1.1? Also, how strong is the shaking?

The recommended amount of buffer is about half of the prepared digestion buffer (10ml is prepared for 5 mice). This information has been added in page 3 line 109.

E-6C. How exactly do you wash the sample? Is that step 3.3?

This information has been added in page 3 line 123.

E-7. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, or .pdf file (4 files in total).

This has been corrected.

E-8. Figures 3 and 4 do not appear to be cited correctly in the Results section.

This has been corrected.

E-9. Figure 4B, C: Please explain the red coloring, e.g., with a scale.

mRNA level is indicated as follows: white (no expression) and red (high expression). This is added to the legend. We also have included scale for Figure 4B.

E-10. Please do not abbreviate journal titles.

This has been corrected.

E-11 Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

We have included all the materials and equipment used, including sorter, in the Table of Materials.

Reviewers 1's comments:

R1-1 This is a very detailed, well-written protocol of isolating nuclei from adipose tissue. Isolating high quality, pure nuclei from adipose tissue has been a barrier for single cell sequencing analysis of adipocyte in vivo. The current manuscript provides a practical protocol to address this unmet need, and should generate strong interest to the adipocyte field.

We appreciate the reviewer's comment.

Reviewer 2's comments:

R2-1 - Well done manuscript, very useful protocol for isolation of Nuclei from adipose tissue.

We appreciate the reviewer's comment.

R2-2 - Even though floating adipocytes fraction by centrifuge, still many of the cell contaminated-endothelial, immuncells etc.Because recently other paper reported single cell analysis from adipose tissue (eLife 2019;8:e49501 doi: 10.7554/eLife.49501), I re-analyzed with RAW data and found much of the cell types-adipocytes, endothelialcells, immunecells ... like this. Figure4- I would request to add the anotation using canonical marker(endothelial, adipocytes, immunecells etc...) . Otherwise the authors can not say subpopulations of adipocytes exists.

We have added the annotation using canonical markers in Figure 4.

R2-3 - In this protocol, it is not clear to filter/how collect toplayer with 40um filter. Using cell filter 40um and then centrifuge to correct top layer? Please clarify this protocol.

This is now clarified in 3.2 (page 4 line 122).