Dear Dr. Troyer and *JOVE* Editorial Staff:

Below please find our point-by-point response to reviewer/editorial comments.

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please ensure that all specific details (e.g. button clicks for software actions, numerical values for settings, etc) have been added to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
1) 2.1: Mention animal strain and age.

We thank for your comments and we apologize for not mentioning details of animals used in the study. The details of used animals are now in the text.

2) 2.5, 2.10, 2.11, : Convert speed to x g.

We thank for your comments and we acknowledge that gravity is more appropriate than RPM for centrifugal force. We changed the unit into x g.

• **Protocol Numbering:**
1) Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary.

We apologize for not properly using the suggested numbering. We corrected this error.

2) All steps should be lined up at the left margin with no indentations.

We apologize for the incorrect formatting. We corrected the formatting accordingly.

3) Please add a one-line space after each protocol step.

We corrected the formatting accordingly.

• **Protocol Highlight:** Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow

from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.

We have highlighted the protocol section according to the suggested guidelines.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

We appreciate these comments and we revised the discussion section appropriately.

• **Figures:** Remove the text “Cho_Figure #”

Figure text has been removed as requested.

• **References:** Please spell out journal names.

We apologize for this formatting error. We now corrected journal names in the References section.

• **Table of Materials:** Please sort in alphabetical order.

The materials are now all sorted in alphabetical order.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Comments from Peer-Reviewers:

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

In this protocol, Cho and Doles present a method for isolating Sca1+ adipocyte progenitor cells that can be used for demanding downstream applications such as single cell-RNA sequencing.

General Comments:

1. The choice of Sca1 as a marker to broadly identify adipocyte progenitor cells (APCs) is appropriate as Sca1 is a common marker of mesenchymal stem cells and us a surface marker for the various currently identified subpopulation of APCs.

2. This protocol is well-described and follows standard steps for obtaining adipose tissue (here, epididymal fat).

3. The rationale for using flow cytometric sorting to obtain the viable cells is well-supported.

4. The authors note that the protocol is specific for SCA1+ APCs only.

We greatly appreciate these positive comments.

Major Concerns:

1. It would be helpful if the authors indicate whether this approach is also applicable to other fat pads, particularly inguinal and brown fat since those depots are often targeted for analysis.

We appreciate these comments, and we acknowledge that discussing other adipose tissues will be helpful for readers in this field. Other adipose tissues, such as inguinal fat pad and brown adipose tissue, have also been similarly dissociated with collagenase to isolate adipose progenitor cells, so the present protocol can likely be applied to other adipose tissues. The text has been revised to discuss this issue.

2. Section 3, step 6: This is the first mention of "PI" in the protocol. The addition of propidium iodide to determine cell viability should be specifically included in an earlier step. Alternatively, if DAPI alone is being used to eliminate nonviable cells, please delete mention of propidium iodide throughout the text (in this step, under Representative Results, Figure 2 and Fig. 2 legend, Name of Material/Equipment).

We appreciate your comments, and we apologize for making any confusion by not clearly describing the procedures. “PI” in step 3.6 was typo, and it is now corrected to “DAPI”, as DAPI was used to deplete nonviable cells in FACS. However, PI was also used in our protocol to further evaluate cell viability after cell sorting (Figure 2). Because DAPI was already used for cell sorting to isolate viable cells during cell sorting, PI was alternatively used for following assessment of viability.

3. Figure 3. How is percent single cells determined? This is important to clarify for the reader/viewer since the highlighted downstream application is single-cell sequencing.

We thank for your comments, and we apologize for not clearly explaining it. The percentage of each population was defined as percentage of each gated population in Figure 2 out of total cells. Especially, the single cell gated population is shown in the third panel in Figure 2. The text is now revised accordingly.

4. For the uninitiated in flow cytometry, it will be helpful to explain how aggregated cells are identified, when present.

We appreciate this comment, and it is now revised in the text in step 3.7.

Minor Concerns:
Section 2, step 7. In the absence of a rotating incubator at 37C, what might the reader substitute?

In the absence of a rotating incubator, we note that a regular incubator can be used with occasional agitation. This alternative is noted in the revised text.

Reviewer #2:
Manuscript Summary:
The author provides straightforward protocol to isolate adipose progenitor cells as defined by Sca1+/CD31-/CD45-/Ter119-. As adipocyte progenitors have a critical role in adipose tissue remodeling and are key to the health of adipose tissue, this work is of general interest. A strength of this protocol is that it can be modified to the interest of labs using alternative surface markers to isolate the APCs.

Major Concerns:
I don't have major concerns

Minor Concerns:
1. Typically, when adipose tissue progenitor cells are isolated, there is functional characterization of the cells. For example, they are transplanted into mice to become adipose tissue. Can the author refer to their previous work or other work to give context as to why they selected these markers and the functional characterization of sorted cells?

We greatly appreciate these comments. The protocol can be further applied to sort heterogeneous subpopulations within adipose progenitor cells. We note that we performed such analyses in a previously published study (now mentioned/cited in the Introduction section).

2. Please provide sorting scheme (even if simple) of the FACS experiment

We appreciate this comment and we now provide details of the FACS experiment in step 3.6.

3. Can the authors provide an estimate of the number of APCs. isolated from fat pads from 4 mice?

We thank for this comment. Typically 50,000 – 100,000 cells can be sorted, and this is described in step 3.6.