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July 02, 2020

Dear Dr. DSouza,

We are happy to submit our revised manuscript (JoVE61542) titled “*Nuclei isolation from fresh frozen brain tumors for single-nucleus RNA-seq and ATAC-seq*” by Narayanan *et al.*

We thank the reviewers and the editor for their insightful suggestions. We have addressed all the editorial and reviewer comments. This involved addition of new analysis (Figure 4) that was requested. We believe the changes have substantially improved the manuscript. We thank you for your enthusiasm and for your interest in our work.

In the revised version of the manuscript, we incorporated the following major edits:

1. We included a computational comparison of snRNA-seq data obtained using the protocol described in the manuscript to four publicly available snRNA-seq datasets (Figure 4). These results are now added to the Representative Results section and show that our protocol generates high-quality data when compared to recently published snRNA-seq datasets.

2. We extended the Discussion section to include further details about the protocol, along with potential issues and considerations (e.g. clumping, amount/quality of starting material, and processing/storage times).

3. We updated the Protocol section to include additional quality control steps (Steps 1.1.7 and 3.1.3) and notes.

We hope the manuscript is now suitable for publication and look forward to hearing from you.

Sincerely,

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