Dear Drs. Dsouza and Troyer,

Thank you for the useful and detailed comment to our original draft. We were happy to hear that both Reviewers agree with the timeliness and usefulness of publishing a protocol for ChIPseq optimized towards the use for brown adipose tissue studies. In addition to proofreading the manuscript and checking for correct use of metric units and abbreviations, we have now edited the manuscript in response to both the Editorial and Reviewers’ comments as detailed below. Please let us know if there is anything else we should edit/improve upon. We look forward to working with you towards a final written and filmed version of the manuscript.

With our best regards,

Valentina Perissi and colleagues

**Editorial Comments:**  
  
• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.  
  
• **Introduction:** Please expand your Introduction to include the following: The advantages over alternative techniques with applicable references to previous studies; Description of the context of the technique in the wider body of literature; Information that can help readers to determine if the method is appropriate for their application.

Introduction has been edited according to these guidelines.

• **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 add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) 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. Some examples:

1) 1.1: What is the source of the BAT? How was it extracted? Please add a note with a reference to mention this. What is the size/weight of 1 BAT pad?  
2) 1.4: What is the composition of the lysis buffer?  
3) 1.5: Mention sonication frequency and amplitude.  
4) 1.5: What is the composition of the ChI dilution buffer? What is the V/cm for the electrophoresis? Mention gel%.  
5) 1.7: Which antibody? Mention concentration.  
6) Convert all centrifuge speeds to g (e.g. in 1.4, 1.5, 2.2, 2.3).  
7) 2.2: Please reference Table 1.  
8) Please apply the above ideas to add additional missing details to sections 3-6.

Thanks for all these useful suggestions that have now been incorporated in the revised manuscript.

• **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.  
5) Please bear in mind that software steps without a graphical user interface/calculations/ command line scripting (such as section 6) cannot be filmed. Please exclude section 6 from your highlights.

Text has been highlighted in the revised manuscript

• **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: modifications and troubleshooting, limitations of the technique, significance with respect to existing methods, future applications and critical steps within the protocol.

Discussion has been edited according to these guidelines.

• **Figure/Table Legends:**  
1) Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.  
2) Please remove the figure/table legends from the figure files and place them directly below the Representative Results text.

Figure/Legends have been edited according to these guidelines.  
  
• **Commercial Language:**JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Advence Stainless Balls, Advence Bullet Blender, Diogenode Bioraptor Sonicator, Diagenode, Durapore, SYBR, Illumina  
1) Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.  
2) Please also check Figure 2.  
We have removed the commercial language from the text. For clarity we have left in the text the catalog numbers of specific reagents used as requested by one of the Reviewers. If needed, those as well can be removed as they are also included in the updated Table of Materials

• **Table of Materials:**Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as sonicator, buffers, antibodies, etc.  
Table of Materials has been edited according to these guidelines.

• Please define all abbreviations at first use.  
  
• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.  
  
• 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]."

Figure 2 and 3 are original. Figure 1 is adapted from Cardamone et al., Molecular Cell, 2018. As stated in the CellPress editorial policies webpage (<https://www.cell.com/trends/editorial-policies>) authors’ rights include “Prepare other derivative works that extend the article into book-length form or otherwise re-use portions or excerpts in other works, with full acknowledgment of its original publication in the journal”.

**Comments from Peer-Reviewers:**  
  
**Reviewer #1:**  
Manuscript Summary:  
This manuscript described a protocol to perform ChIP-seq from Brown Adipose Tissue (BAT) isolated from mouse. There is no doubt that this protocol was suitable to map both histone modifications and non-histone profiles.  
  
Major Concerns:  
1. In figure 1, while it was expected to see the decrease of GPS2 binding on the known target, but I was confused by the increases of both PolII (usually active mark) and H3K9me3 (usually repressive mark), could the authors explain more about this?

We agree this was an unexpected finding. We fear that discussing the results of that specific study here would detract from the general focus on the technique per se. To address the Reviewer’s comment and potentially other readers’ questions about these findings we have now referred to the original manuscript where we discuss at length the accumulation of inactive POL2 on repressed promoters.

2. I almost don't see any catalog# in here, the authors should include them to all the reagents (e.g. antibody).

Thanks for pointing this out. We have now included catalog# for all reagents used.

3. It would be better to have time frames for the experiments, and important notes & warnings for certain details.

We agree it was important to include information about time frames for the different steps of the protocol, which we have now included.   
  
Minor Concerns:  
1. Line 23, the authors mentioned that "Commonly used in ChIP assays are samples derived from in vitro cell culture of immortalized cell…", actually there are tons of ChIP-seq paper for the in vivo studies.

We apologize for the misunderstanding, we were specifically referring to mechanistic studies in the brown adipose tissue. We have now edited the text accordingly.

2. Line 91 & line 140, used 50ul in 1000ul lysates as an input was too high, especially the authors used this to make the standard curve with [1/10](http://airmail.calendar/2019-01-10%2012:00:00%20EST), 1/100 and 1/1000 (better to have at least 1/5000).

Thanks for pointing this out as a source of possible misunderstanding. We have now clarified that we keep 1% of the lysates as input. Performing 3 ChIPs from each BAT pad means 30ul input from 3000ul lysates not 1000ul. We also commented about the fact that further dilutions might be needed to capture the linear range.

3. Line 147, "4 DNA from ChIP (or input control)" should be "3ul DNA from ChIP (or input control)".

Sorry, it looked like the math was wrong because we had not properly explained that we use 1ul of pre-mixed primers rather than 1ul each. Thanks for pointing this out. We have edited the text to clarify.

4. Line 155, "…If more than one peak is visualized, this is indicative of multiple, non-specific amplicons…", no necessary correct, it depends how the peaks look like, they may indicate uncompleted denature DNA.

This is absolutely correct, we have edited the text to highlight that multiple peaks are problematic and alternative primers should be designed.

5. Y axis in figure 1, usually "Signal relative to input%".

Corrected as suggested in the figure  
  
  
**Reviewer #2:**  
Manuscript Summary:  
The manuscript "Chromatin Immunoprecipitation (ChIP) from Murine Brown Adipose Tissue" of Cardamone et al describes the method for detecting in vivo epigenetic modifications and transcriptional regulators binding in chromatin isolated from brown adipose tissue (BAT). Further, authors describe how to combine the method with qPCR and then next-gen sequencing in order to describe genome-wide chromatin composition in the tissue of interest.  
Although ChIP and ChIP-seq methods are present for over a decade in the literature, majority of works and methods describe the use of this extremely useful technology in isolated cells or in immortalized cell lines. It is also very well understood that ChIP on the material isolated directly from the animal/tissue is very challenging and the method has to be optimized for each tissue separately. Therefore, this manuscript describes precisely how ChIP can be performed on adipose tissue.  
  
Major Concerns:  
no major concerns were noted  
  
Minor Concerns:  
The method is very well described. Scientists are provided stepwise protocol of execution of the technique, including a short discussion of the potential modifications. In fact, in our opinion the discussion is the part that should be expanded. There are several methods of chromatin immunoprecipitation used in the field and this manuscript does not discuss several options that could be used or whether their method has particular advantage in the case of BAT.

Thanks for acknowledging the usefulness of this protocol, we have revised the text accordingly to your suggestions and expanded the discussion about the steps we have optimized to include a justification of the experimental choice made in this regard. We hope this can be helpful if one wants to further optimize or adapt the protocol to similar samples (i.e. other adipose tissue depots or lipid-laden tissues)

In particular:  
-Line 94 - authors suggest to use 2ug of the antibody of interest per immunoprecipitation. Although it is very often the case, several antibodies are not quantified by ug. It should be at list mentioned that each specific new antibody amount should be optimized.

Yes, we agree, antibody optimization is a critical component of any ChIP/ChIPseq protocol. We have edited the text to clarify the indication about the amount of antibody and to highlight the need for optimization

-Line 100 - Authors suggest use of Protein A beads slurry. Use of magnetic beads and eventual superiority of authors' method above magnetic beads should be explained. We believe also that as an example catalog number of used beads should be given for readers.

Thanks for this comment, we have included in the Discussion an explanation for this choice.

-Line 114 - Phenol/chloroform extraction is a suggested method of DNA isolation. It should be noted that there are several other methods of DNA extraction available on the marked and why (if it is the case) authors prefers this method above others.

Same as above.

-Line 146-7 - numbers do not add up - 5+1+1+4 is not 10…

As written in response to Reviewer #1, we apologize for the confusion. It looked like the math was wrong because we had not properly explained that we use 1ul of pre-mixed primers rather than 1ul each. Thanks for pointing this out. We have edited the text to clarify.