Reviewer #1 (Comments to the Author (Required)):   
  
Suggested corrections has been done.   
  
  
Reviewer #2 (Comments to the Author (Required)):   
  
The revised manuscript addressed my questions well except question #2. I would suggest the authors specify the normalization method (Fig 2C) in detail in the main text accordingly. 

We added a brief description of the normalization method in the materials and methods section (page 11, paragraph 2).

Moreover, I would suggest the authors validate maybe 2-3 genes of interest by qPCR. For example, the authors could select maybe 2 genes that have been identified to be "actively regulated during the course of the adipocyte differentiation" and maybe another 2 genes that have been identified based on the similarity in expression patterns, and confirm the findings with qPCR. This would help to make the interpretation from the sequencing findings more convincing, and make your overall conclusion "autophagy plays a critical role in removal of some intracellular components and supply of energy sources for lipid biogenesis during adipogenesis" more sound. 

Cell culture, MDI induction and qPCR quantification of representative genes of the distinct autophagy gene patters were conduction. We added a new figure (figure 4) to show the new data and described it in the results section (paragraph 3, page 6). A new table (supplementary table 1) and the methods section (paragraph 3, page 13) were extended to describe the protocols that were used to generate the new data.

I would appreciate if the authors could highlight any changes in the future revised manuscript using a different color. That would be very helpful for the reviewers to track the revisions. Thanks.

We made sure to set the track changes on, so the changes should be highlighted in different colors and referred to by marks.

Reviewer #3 (Comments to the Author (Required)):   
  
There are no comments for the authors.   
  
  
Reviewer #4 (Comments to the Author (Required)):   
  
1. As stated before, a low replicate experiment may not provide a sufficiently detailed view of the differential expression. Although DESeq2 or edgeR can more or less handle with such case, the reliability of the subsequent data analysis is significantly reduced. In this study, only two replicates (not even 3 replicates) were performed for each time point, and therefore validation of these findings with in vitro experiments is required. 

Cell culture, MDI induction and qPCR quantification of representative genes of the distinct autophagy gene patters were conduction. We added a new figure (figure 4) to show the new data and described it in the results section (paragraph 3, page 6). A new table (supplementary table 1) and the methods section (paragraph 3, page 13) were extended to describe the protocols that were used to generate the new data.

In reply to the original issue of sample size, we stated the reasons for using those two particular datasets. Mainly the data quality and the suitability of the experimental design to answer the questions of interest. Secondly, we addressed the disadvantage of the low replicate number by choosing the most suitable statistical methods to our knowledge. Finally, we established the agreement between two independent datasets by showing the correlation of autophagy genes in the two datasets at the corresponding differentiation stages.

We investigated other options, namely finding other datasets and combining data from independent experiments and similar experimental designs. The data quality was the concern with the first approach, and the strong batch effects and technical difficulty was the issue with the second.

It is worth mentioning that we used the differential expression as a proxy to which of the autophagy genes are interesting and worthy investigating. We didn’t attempt to estimate any effect size or use such information in the downstream analysis, which would be consequently compromised by in accurate estimates due to the low sample size.

2. The data provided by this study is limited. Hence, functional analysis of few selected target genes is highly recommended, for instance, experiments showing the effect of at least some of these genes on differentiation. 

In addition to the new data described in reply to point 1, we would like to address these concerns with the following comments:

We deliberately limited the focus of the analysis to be able the few tens of gene sets and pathways and few hundreds of genes involved in autophagy to be able to look closely at the process of interest and the mediating pathways.

Our analysis is concerned with the changes in the expression patterns of autophagy genes during the differentiation of 3T3-L1 cells, establishing relations between these patterns and use the published literature and annotations to give context to some of these changes. The advantage of RNA-seq and other high-throughput technologies, that cannot be replicated by qPCR, is to measure many elements simultaneously and maintaining the relations between these elements. Such relations are used to perform the enrichment analysis and finding patterns.

At the same time, we used a validation dataset to ensure the reliability of these findings. While we don’t expect that to independent datasets would agree completely, the measurements of interest showed high correlations between both dataset. This we believe is a suitable validation approach for these claims.

3. As stated in the rebuttal letter, the majority of the changed genes are highly involved in other biological pathways, such as, differentiation, proliferation and so on. Please provide data showing the importance of autophagy during differentiation by in vitro experiments!

The importance of autophagy in adipocyte differentiation is experimentally established, we referred to a few of these studies in the discussion section. Moreover, while we didn’t make particular claims about individual genes in this study, we discussed the potential involvement of some important autophagy genes in the process of adipocyte differentiation based on patterns observed in the main dataset. In these cases, we referred only to the findings that were experimentally investigated in other published studies.