Comments from the editor

57： “The medium containing with DMEM” does not make sense. I suppose authors used DMEM supplemented with FBS et ac. Please change. Also please specify the manufacturer of materials used in the study.

Reply：We have revised this paragraph: 3T3-L1 cells were obtained from Prof. Shulin Yang (Institute of Animal sciences, Chinese Academy of Agricultural Sciences). Medium supplemented with DMEM (Gibco, 11965), 10% FBS (Gibco, 12483) and 1% penicillin-streptomycin (Gibco, 15240) (maintaining medium) was used to culture 3T3-L1 cells. Cultures were conducted with 1×106 cells/well in 6-well cell culture plates. These cells were grown until confluence (day 0), then differentiation was induced by adding 1 mM insulin (Sigma I5523), 0.25 mM dexamethasone (Sigma D175), and 0.5 mM 3-isobutyl-1-methylxanthine (Sigma 15879) in the maintaining medium. After 4 days, cells were cultured in maintaining medium with 1 mM insulin. On day 10, the fully differentiated adipocytes were used for Oil Red O (Sigma, O1391) staining. Cells at day 0 (D0) and day 10 (D10) were collected and stored at -80℃ for subsequent experiments (L55-64).

Suggestion from the editor: please rephrase the expression “Medium supplemented with DMEM (Gibco, 11965),” to “DMEM supplemented with”.

128: “301 metabolites were up”: readers will understand what the authors mean, but this is not a proper expression. Same as the rest of this paragraph. Authors could use increased and decreased instead of up and down.

Reply：Thank you very much. We have revised them: Based on VIP values and relative abundance, we identified 454 differentially expressed MS2 metabolites between D10 and D0, among which 301 were up-regulated and 153 were down-regulated (Figure 3A). These differentially expressed metabolites included 214 glycerophospholipids (GPs), 152 glycerolipids (GLs), 73 sphingolipids (SPs), ten fatty acyls (FAs), three sterol lipids (ST), and two prenol lipids (PLs) (Figure 3B, Table S3). Among the 214 differentially expressed GPs, there were 50 PCs (26 decreased, 24 increased), 36 LPCs (16 decreased, 20 increased), 29 EtherPEs (17 down, 12 increased), 23 PEs (four decreased, 19 increased), 17 PIs (one decreased,16 increased), 11 EtherPCs (eight decreased, threee increased), 11 LNAPEs (one decreased, ten increased) and nine LPEs (two decreased, seven increased). Among the 152 differentially abundant GLs, there were 66 TGs (2 decreased, 64 increased), 27 EtherTGs (four decreased, 23 increased), 25 DGs (all increased), and 23 EtherMGDGs (18 decreased, five up). The 73 differentially expressed SPs included 28 SMs (16 decreased, 12 up), 12 Cer_NS (three decreased, nine up), nine

HexCer_NS (four decreased, five increased) and eight SHexCer (five decreased, three increased) (L135-147).

Suggestion from the editor: Some of “decreased” and “increase” genes are still expressed as “down” and “up”, respectively. Please unify the notation. Also, please unify the notation of numbers to Arabic numerals or English notation.

Reviewer: Hui Wang

2. According to Figure 4, the biochemical pathway enrichment for differently expressed lipids indicates minor effects on regulation of lipolysis in adipocytes. But in contrast, authors wrote from line 145-148 that differently regulated lipids molecules in adipocytes related to" regulation of lipolysis in adipocytes". If lipolysis process indeed changes significantly in mature adipocytes, did the authors quantify the lipolysis between preadipocytes and mature adipocytes? or the genes expression for lipolysis?

Reply：Thank you very much. This pathway is enriched from our differentially expressed lipids (Figure 4), and the *p*-value of this pathway is 0.0025 (Table S6), which showed that this pathway changes significantly in mature adipocytes. Through KEGG pathway analysis of differentially expressed genes, we also enriched the differentially expressed genes in this pathway (Figure 7 and Table S6). We examined several genes in this pathway, such as *Lipe*, *Pnpla2*, *Plin1*, *Pnpla2* and *Fabp4* (Figure 10). These genes are all significantly changed during adipogenesis.

Suggestion from the editor: Please show how to quantify the significance and show deviation if possible.

3. Figure 6 was confusing to distinguish qPCR data from RNAseq data. In addition, statistical analysis is missing from this Figure.

Reply：Thank you very much. We have modified the picture, and a more detailed explanation is given in the figure legend: Bar graph (blue) showing results from qRT-PCR (left ordinate). The line chart represents the results from RNA-seq analysis (right ordinate in red) (L424-425).

Suggestion from the editor: Please show how to quantify the significance and show what bars mean (for example, standard deviations or standard errors), in the figure legend.

4. Figure 7: KEGG enrichment results shows the pathway enrichment showed the thermogenesis pathway. This is very interesting, did the author checked the KEGG, what caused the significant increases in thermogenesis?

Reply：There are 133 differential expressed genes in thermogenesis pathway, and 12 genes (*Plin1*, *Cox7a1*, *Lipe*, *Pnpla2*, *Acsl1*, *Cyc1*, *Uqcrfs1*, *Uqcr11, mt-Co1, Cpt1a, and*

Creb3l1) with a fold change > 5 and FPKM > 1 among these gene. In this study, we examined the expression of *Plin1, Lipe, Pnpla2* and *Acsl* by RT-qPCR, they were all significantly changed during adipogenesis.

Suggestion from the editor: Please make sure that you describe the above statements in the text.