

A transcriptomic analysis reveals novel patterns of gene expression during 3T3-L1 adipocyte differentiation

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Abstract

Background: Obesity is characterized by increased adipose tissue mass that results from increased fat cell size (hypertrophy) and number (hyperplasia). The molecular mechanisms that govern the regulation and differentiation of adipocytes play a critical role for better understanding of the pathological mechanism of obesity. However, the mechanism of adipocyte differentiation is still unclear.

Objective: The present study aims to compare the gene expression changes during adipocyte differentiation in the transcriptomic level, which may help to better understand the mechanism of adipocyte differentiation.

Methods: RNA sequencing technology, GO and KEGG analysis and quantitative RT-PCR method were used in this study.

Results: A lot of genes were up- or down-regulated between each two stages of 3T3-L1 adipocyte. GO and KEGG analysis revealed that lipid metabolism and oxidation-reduction reaction were mainly involved in the whole process of adipocyte differentiation. Moreover, decreased immune response and cell cycle, adhesion were occurred in the late phase of adipocyte differentiation, which were demonstrated by divergent expression pattern analysis. In addition, quantitative RT-PCR results demonstrated that the mRNA expression level of Trpv4, Trpm4, Trpm5 and Trpm7 were significantly decreased in the differentiated adipocytes. On the other hand, the mRNA expression level of Trpv1, Trpv2, Trpv6 and Trpc1 were significantly increased.

Conclusions: These data presents the description of transcription profile changes in adipocyte differentiation and provides an in-depth analysis of the possible mechanisms of adipocyte differentiation. These data offer new insight into the understanding of the mechanisms of adipocyte differentiation.

Background

The prevalence of obesity has been recognized as a serious global health problem [1]. Obesity is believed to be a result from an imbalance between energy intake and energy expenditure [2] and which is also a serious health problem that is implicated in various diseases including type II diabetes, hypertension, coronary heart diseases, and cancer [3, 4]. Thus, obesity has received considerable attention as a major health hazard [5]. Statistical analysis suggested that 36.5% of adults were obese in the United States during 2011–2014 [6, 7]. In addition, obesity has reached epidemic proportions in most developed countries of the world with 30–40% of adults being obese [8], and its frequency continues to increase at an alarming rate in developing countries [9]. Therefore, these developments require urgent strategies for the prevention and reversal of obesity and related metabolic diseases.

Obesity is characterized by increased adipose tissue mass that results from increased fat cell size (hypertrophy) and number (hyperplasia), suggesting the major contribution of adipocytes in obesity [10].

Adipocyte is highly specialized cells that play a key role in energy homeostasis [11]. Adipocyte hypertrophy and hyperplasia occurs in different levels: molecular level, subcellular level and cellular function level. The changes of adipocyte are usually accompanied by transcriptional changes as well. Therefore, the molecular mechanisms that govern the regulation and differentiation of adipocytes could be the major issue for better understanding of the pathological mechanism of obesity. To date, several papers have already reported the strong correlations between obesity and transcriptional changes in human adipocytes [12–14]. However, the full expression profile in transcriptome level that correlated to the hypertrophy and hyperplasia of adipocyte, which is important for better understanding the mechanisms of adipocyte differentiation, is still unclear.

Calcium signaling in adipocyte differentiation is relatively little known, despite its suggested importance [15, 16]. Transient receptor potential (TRP) ion channels are a major class of Ca^{2+} -permeable channels, most of which are non-selective Ca^{2+} -permeable cation channels [17]. TRP channels have six transmembrane (TM) domains (TM1 to TM6) and a pore loop between TM5 and TM6 with both N- and C-termini in the cytosol [18]. The TRP channel superfamily is now classified into six subfamilies in mammals: TRPV (Vanilloid), TRPC (Canonical), TRPM (Melastatin), TRPML (Mucolipin), TRPP (Polycystin) and TRPA (Ankyrin). TRP channels are unique cellular sensors characterized by promiscuous activation mechanisms, including thermal and mechanical activation [19]. The main signaling pathway which TRP channels involved are derived by channel activation-induced calcium influx and triggered $[\text{Ca}^{2+}]_i$. To date, several TRP channels have been reported to be involved in the physiological functions of adipocytes [20–24].

Therefore, the present study is aimed to analyze the transcriptomic changes during adipocyte differentiation in a 3T3-L1 cell line, which is important for further understanding the molecular mechanisms of adipocyte differentiation. This study would provide a comprehensive understanding at the transcriptome level during adipocyte differentiation. The transcriptomic profile in 3T3-L1 adipocytes from different differentiation stages was examined using RNAseq technology, gene ontology (GO) and KEGG analysis. Our data revealed that a lot of genes were up- or down- regulated during adipocyte differentiation. Moreover, most of the gene expression changes were occurred in the first four days (early phase) of adipocyte differentiation. GO and KEGG analysis demonstrated that these altered genes were mainly involved in metabolic process, lipid metabolism and oxidation-reduction process. In addition, quantitative PCR results indicated that several TRP channel mRNA expression were altered during adipocyte differentiation.

Materials And Methods

Cell culture and differentiation

Murine 3T3-L1 preadipocytes were plated in 6-well or 12-well plate and cultured in a complete medium, Dulbecco's Modified Eagle Medium (DMEM) with higher glucose levels (Gibco) supplemented with 10% fetal bovine serum (Gibco, South American origin), 2 mM L-glutamine and 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$

streptomycin. Grow the cells to 95–97% confluence in complete medium. To induce differentiation, induction medium including complete medium plus white differentiation cocktail (2 µg/ml dexamethasone, 0.5 mM IBMX, 10 µg/ml insulin) were added at day 0. After 2 days induction, change medium to maintenance medium (a complete medium with 10 µg/ml insulin). After additional 2 days (day 4), change to fresh maintenance medium for additional 4 days. 8 days after adding the induction medium, cells are fully matured to differentiated adipocytes.

Total RNA extraction

Cells were collected and pooled from each stage (Pre-, 4-day differentiated and 8-day differentiated adipocytes) in tubes with Trizol reagent (Sigma-Aldrich, St. Louis, MO, USA). RNA isolation was followed by chloroform extraction and isopropanol precipitation. The extracted RNA was approximately 1 µg and stored at – 80 °C deep freezer until use.

RNA sequence and data analysis

Samples ($n = 3$ in each group) were prepared by a mixture of the RNA from different stage of adipocytes. The preparation of the cDNA library from each sample and the sequencing was performed by Beijing Genomics Institute (BGI, Shenzhen, China). The cDNA originating from the RNA fragments were paired and sequenced using the high throughput sequencing platform of Illumina HiSeq.3000 and 6 G raw data per sample were obtained on average. The sequencing reads which containing low-quality, adaptor-polluted and high content of unknown base (N) reads, were removed. Clean reads are then mapped to reference using HISAT/Bowtie2 tool [25, 26]. Genes expression level is quantified by a software package called RSEM [27]. Based on the gene expression level, we identified the differentially expression genes (DEGs) between groups using NOIseq [28] and PossionDis [29] algorithms. The NIH Database for Annotation, Visualization and Integrated Discovery (DAVID), which uses a modified Fisher's exact test followed by Benjamini-Hochberg multiple hypothesis testing correction, was used to perform gene functional annotation clustering using *Mus musculus* as background, and default options and annotation categories. Significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were identified using a hypergeometric test and Benjamini–Hochberg FDR correction by KOBAS 3.0, a web server for gene/protein functional annotation (Annotate module) and functional gene set enrichment (Enrichment module) [30, 31]. The sequencing results have been submitted to the Gene Expression Omnibus (GEO) database and assigned the accession number as GSE129957.

Quantitative real-time RT-PCR

Mouse gene copy numbers were determined by quantitative RT-PCR using SYBR Green MASTER Mix (Invitrogen) following the manufacturer's protocol. Data were collected during each extension phase of the PCR reaction and analyzed using ABI-7700 SDS software (Applied Biosystems, Foster City, CA, USA). The results were standardized for comparison by measuring levels of 36B4 mRNA in each sample. The primer sequence information was referenced from previous papers [32, 33].

Oil red O staining

Oil red O staining was performed using oil red O dye (Sigma, St. Louis, USA). In brief, the adipocytes were fixed with 4% formalin and incubated at room temperature for at least 1 hr. After fixation, cells were washed twice with purified water and then washed with 60% isopropanol at RT for 5 min. The cells were dried completely at room temperature, and oil red O solution was added and then incubated at room temperature for 10 min. Oil red O solution was removed by addition of purified water, and the cells were washed 4 times with purified water. Images were acquired using a microscope (Olympus, Tokyo, Japan).

Statistical analysis

An empirical Bayesian analysis was performed to shrink the dispersions towards a consensus value, effectively borrowing information between genes [34, 35]. Differential expression was assessed for each gene using an exact test analogous to Fisher's exact test [34, 36]. Genes with a q-value lower than 0.05 and with a fold change greater than 2 were considered differentially expressed. All data were represented as means \pm SEM. Statistical analysis was performed with one-way ANOVA followed by multiple t-tests with Bonferroni correction using Origin 8.5 software. Only two-tailed P values less than 0.05 were considered to be significantly different.

Results

The dramatic morphological changes and lipid droplets increases were occurred during 3T3-L1 adipocyte differentiation

First of all, the schematic of 3T3-L1 adipocytes during differentiation was shown (Fig. 1A). The representative phase contrast (Fig. 1B) and Oil red O staining (Fig. 1C) images of 3T3-L1 cells at pre-, 4-day-differentiated, 8-day-differentiated adipocyte were shown as well. The results demonstrated that the obvious morphological changes and lipid droplets increases were occurred during 3T3-L1 adipocyte differentiation, suggested the differentiation of 3T3-L1 adipocytes was carried out successfully.

Transcripts regulated in 3T3-L1 adipocyte during differentiation

The transcriptome data was generated from different stage of 3T3-L1 adipocyte using RNA-seq technology. Table 1 briefly summarized the information of sequencing data from each sample, averagely generating 24 Mb clean reads after filtering low quality. Clean reads are mapped to reference. Each of the data sets contained 24 Mb reads and a mapping rate of 92–93%. Moreover, we counted the number of identified expressed genes and calculated its proportion and distribution to total gene number in database of each sample as Figure S1A. The correlation of gene expression level among samples is a key criterion to test whether the experiments are reliable and whether the samples chosen are reasonable. Principal component analysis method was performed to assess the gene expression level. 3T3-L1 adipocytes from three different differentiation stages were dramatically separated from each stage

(Figure S1B). We also calculated the correlation value between each two samples based on normalized expression result and draw correlation heatmap (Figure S1C).

Table 1
The summary of raw RNA sequencing data set.

Sample	Total Raw Reads (Mb)	Total Clean Reads (Mb)	Clean Reads Q20 (%)	Clean Reads Q30 (%)	Clean Reads Ratio (%)	Total Mapping Ratio
3T3-L1-Day 0-1	24	24	96.67	88.04	99.34	0.9325
3T3-L1-Day 0-2	21	21	96.62	87.74	99.54	0.9343
3T3-L1-Day 0-3	24	24	96.44	87.61	99.53	0.9272
3T3-L1-Day 4 - 1	24	24	96.58	87.66	99.62	0.9357
3T3-L1-Day 4 - 2	24	24	96.47	87.38	99.49	0.9324
3T3-L1-Day 4 - 3	24	24	96.14	86.75	99.19	0.9283
3T3-L1-Day 8 - 1	24	24	95.73	85.62	99.6	0.9293
3T3-L1-Day 8 - 2	24	24	96.43	87.1	99.67	0.9338
3T3-L1-Day 8 - 3	24	24	95.47	85.28	99.53	0.9207
It showed the summary of RNA sequencing data of 9 samples, including Raw Reads Number, Clean Reads Number, Clean Data Rate, Mapped Rate, and percentage of clean reads as well as Q20 (Phred quality scores Q) and Q30.						

DEGs screening is aimed to find out DEGs between groups and perform further functional analysis on them. The representative distributions of genes up- or down-regulated between each two stages were shown in the volcanos as Fig. 2A, B and C. The red dots represent the up-regulated genes, while the green dots represent the down-regulated genes. Our results revealed that there are a lot of genes were up- or down- regulated between each two stages. A venn diagram presents the number of DEGs that are unique or shared in each comparison (Fig. 2D).

Gene ontology analysis of the differential genes

To better understand the associated functions of DEGs in 3T3-L1 adipocytes during differentiation, GO analysis was used to perform an enrichment analysis and classifications (Fig. 3). GO analysis identified enriched biological processes associated with “lipid metabolic process”, “oxidation – reduction process” and “metabolic process”, indicating that a strong metabolic process were occurred from either adipocyte differentiation day 0 to day 4 (early phase, Fig. 3A) or adipocyte differentiation day 0 to day 8 (whole process, Fig. 3B). On the other hand, the biological process associated with “cell adhesion” was mainly involved in adipocyte differentiation day 4 to day 8 (late phase, Fig. 3C). Identified enriched cellular component terms associated with “membrane”, “extracellular region” and “extracellular space”, suggesting multifarious cellular components were involved in adipocyte differentiation (Fig. 3A, B and C). Enriched molecular functions were defined associated with “oxidoreductase activity”, “catalytic activity”, “protein homodimerization activity”, “calcium ion binding” and “hormone activity”, implying that oxidation – reduction process and intracellular signaling transduction are the major molecular function during adipocyte differentiation (Fig. 3A, B and C).

Analysis of important KEGG pathways

We next used the differential genes for KEGG pathway enrichment using KOBAS as previous reported [30, 31]. The differential genes were significantly enriched in the classifications of “metabolic pathways” either in the early phase (Fig. 4A) or the whole process of adipocyte differentiation (Fig. 4B). In addition to the “metabolic pathways”, the “HIF – 1 signaling pathway”, “PI3K – Akt signaling pathway”, “MAPK signaling pathway” were also enriched in the late phase of adipocyte differentiation (Fig. 4C). These results suggested that metabolic pathway is the major process involved in adipocyte differentiation.

Divergent gene expression patterns during 3T3-L1 adipocyte differentiation

In order to determine the elaborate expression patterns of these differential genes, a hierarchical clustering was performed. DEGs were partitioned mainly into 8 distinct expression patterns as shown in Fig. 5A. Profile 2 included the largest number of genes (602 genes), indicating that many genes expressed with the model of first increase. 513 genes were first decrease (profile 7), 202 genes were expressed with a steady increase (profile 3) and decrease (profile 8) (Fig. 5A). Then, the DEGs in 8 profiles were used to generate a heatmap using the FPKM values (Fig. 5B). Moreover, the profiles presented different GO functions by gene ontology and most increased expression of genes (profile 1, 2, 3 and 4) during adipocyte differentiation were mainly responsible for “metabolic process”, “lipid metabolic process”, “oxidation-reduction process”, and “fat cell differentiation”, suggesting that the metabolism function was dramatically enhanced during the differentiation process of 3T3-L1 adipocytes. On the other hand, the decreased expression of genes (profile 5, 6, 7 and 8) during adipocyte differentiation were mainly involved in “immune system process”, “defense response to virus”, “cell cycle” and “cell adhesion”, demonstrating the decreased immune response and cell cycle during the differentiation process. These

results suggested the increased lipid metabolism and decreased immune function, cell migration during adipocyte differentiation.

Validation of TRP channels by quantitative real-time PCR

Calcium signaling in adipocyte differentiation is still unknown. Most of the TRP channels are calcium-permeable cation channels. As shown in Fig. 6A, a transcription heatmap of the mRNA expression of all TRP channels which have been detected in adipocyte was generated. Moreover, we validated several interested TRP channel mRNA expression levels using quantitative RT-PCR. Our results demonstrated that the mRNA expression level of *Trpv4*, *Trpm4*, *Trpm5* and *Trpm7* were significantly decreased in the differentiated adipocytes. On the other hand, the mRNA expression level of *Trpv1*, *Trpv2*, *Trpv6* and *Trpc1* were significantly increased, compared with pre-adipocytes (Fig. 6B).

Discussion

Obesity is characterized by increased adipose tissue mass that results from increased fat cell size (hypertrophy) and number (hyperplasia), suggesting that the main contributor to obesity is an adipose tissue [10]. Hypertrophy and hyperplasia are two possible growth mechanisms of adipose tissue. Hypertrophy (energy storage) occurs prior to hyperplasia to meet the need for additional fat storage capacity in the progression of obesity [37]. Hyperplastic growth (adipocyte differentiation) appears at early stage in adipose tissue development and late stage of obesity [38, 39]. Therefore, understanding the molecular mechanisms of hypertrophy and hyperplasia for modulation of adipocyte has been the subject of intense investigation, which could help to find novel approaches for preventing and combating human obesity. In the present study, we provided several lines of evidences that many genes were up- or down-regulated during adipocyte differentiation. Most of the gene expression changes were occurred in the early four days of differentiation. GO and KEGG analysis demonstrated that these altered genes were mainly involved in metabolic process, lipid metabolism and oxidation-reduction process. Moreover, our results demonstrated the mRNA expression levels of several TRP channels were altered during adipocyte differentiation, suggested that these TRP channels might be involved in adipocyte differentiation.

Transcriptome changes result the proteome alteration in cells, which subsequently affect the molecular and cellular functions. 3T3-L1 cells are a model cell line which could be induced from pre-adipocytes (fibroblast-like cells) to the differentiated adipocytes (round cells with lipid droplets) (Fig. 1). 3T3-L1 adipocyte differentiation was conducted in a Petri dish in vitro, this allows us to investigate the mechanism of adipocyte differentiation. Therefore, we performed RNA sequencing experiment to detect the transcriptomic changes during adipocyte differentiation. As shown in Fig. 1, we chose pre-adipocytes, 4-day differentiated adipocytes (middle stage) and 8-day differentiated adipocytes (matured adipocytes) for RNA sequencing. The morphological changes in early phase of adipocyte differentiation were dramatically. Lipid droplets were already observed in 4-day-differentiated adipocytes. The lipid droplets and cell size were further enlarged in the late phase of adipocyte differentiation (Fig. 1). In parallel with the morphological changes, the RNAseq results showed that there were 1295 genes up-regulated, 1114

genes down-regulated in the early phase of adipocyte differentiation. Moreover, there were 523 genes up-regulated, 325 genes down-regulated in the late phase of adipocyte differentiation (Fig. 2). Taken together, our results revealed that the major gene expressional and functional changes might occur in the early phase of adipocyte differentiation, suggested that the early 4 days differentiation might play a decisive role in adipocyte differentiation.

GO analysis demonstrated that the DEGs during the whole process of adipocyte differentiation were significantly enriched in the classifications of "lipid metabolic process", "oxidation – reduction process", "metabolic process", and "oxidoreductase activity" (Fig. 3). KEGG analysis revealed that the differential genes were significantly involved in the classifications of "metabolic pathways" (Fig. 4). Moreover, the DEGs during the late phase of adipocyte differentiation were enriched in the classification of "cell adhesion", "hormone activity", "metabolic pathways", "HIF – 1 signaling pathway" and "PI3K – Akt signaling pathway". These results revealed that the metabolic and oxidation-reduction process were the major process during adipocyte differentiation, especially in the early 4 days of differentiation. On the other hand, signaling pathways changes and cell adhesion, proliferation were mainly happened in the late phase of adipocyte differentiation. Our results suggested that lipid metabolism and oxidation – reduction reaction are the major processes in the early phase of adipocyte differentiation. In addition to the lipid metabolism, cell aging and signaling pathway are mainly involved in the late phase of adipocyte differentiation. These results were in coincidence with the morphological changes which we observed during adipocyte differentiation.

Birsoy K et al. has compared the gene expression of adipogenesis in vivo and in vitro using 3T3-L1 cells in culture [14]. The results demonstrated that 3T3-L1 adipocyte differentiation in culture share similar expression patterns with the development of WAT in vivo, provided direct evidences that differentiation of adipocyte in culture recapitulates many of the transcriptional programs that are functional during development of WAT in vivo. Moreover, a transcriptome analysis of adipose tissue from pigs revealed DEGs related to adipose growth, lipid metabolism, extracellular matrix and immune response [40]. Jiang MK et al. performed RNA sequencing during adipogenesis using the primary cultured brown adipocyte, they found 6668 DEGs during adipogenesis but without GO and KEGG analysis [41]. Our present study examined the transcriptional profile changes during adipocyte differentiation using a 3T3-L1 cell line. We performed KEGG, Go analysis and hierarchical clustering for the first time, demonstrated the cellular functions during adipocyte differentiation are phase-dependent, although lipid metabolism and metabolic process are involved throughout the whole process of adipocyte differentiation.

RNA sequencing results revealed 8 divergent gene expression patterns during adipocyte differentiation. We therefore performed a hierarchical clustering to generate a heatmap of the 8 divergent gene expression patterns (Fig. 5). The most significant patterns were profile 2 and 7, which are first increase and decrease, respectively. GO analysis revealed that these 2 patterns were mainly involve an increased metabolism ability, decreased immune response and cellular functions. Moreover, increased fat cell differentiation and decreased mRNA transcriptomic function were also enriched in profile 2 and 7. These

results clearly demonstrated the distinct expression patterns involve different cellular functions, which further revealed the differentiation mechanisms of adipocyte.

To date, calcium signaling is poorly known in adipocyte differentiation. Most of the TRP channels are calcium-permeable channels. Bishnoi M et al. reported that TRPV1, TRPV3, TRPM8, TRPC4, TRPC6 were differentially expressed in preadipocytes and adipocytes [32]. We previously reported that *Trpv1* and *Trpv3* mRNA were significantly decreased, whereas *Trpv2* and *Trpv4* mRNA were significantly increased in WAT of either db/db or diet-induced obesity mice [42]. It has been reported that TRPV1 and TRPV3 were significantly decreased in WAT of obesity mice, and involved in adipogenesis of WAT [20, 43]. TRPV2 is up-regulated in matured brown adipocyte and involved in brown adipocyte differentiation [21, 44]. Moreover, TRPC1 regulates brown adipose tissue activity in a PPAR γ -dependent manner [45]. TRPV4 is decreased in differentiated adipocyte, and involved in the regulation of adipose oxidative metabolism, inflammation, and energy homeostasis [22]. TRPM4, but not TRPM5, has been reported to be required for adipogenesis [46]. In addition, TRPM7 has been reported to be involved in osteogenic differentiation of mesenchymal stromal cells through osterix pathway [47]. Our present results demonstrated that the mRNA expression levels of *Trpv4*, *Trpm4*, *Trpm5* and *Trpm7* were significantly decreased in the differentiated adipocytes. On the other hand, the mRNA expression levels of *Trpv1*, *Trpv2*, *Trpv6* and *Trpc1* were significantly increased, compared with pre-adipocytes (Fig. 6). Taken together, our results suggested that these altered TRP channels, including TRPV1, TRPV2, TRPV4, TRPV6, TRPM4, TRPM5 and TRPM7 and TRPC1, might be involved in adipocyte differentiation.

Conclusions

This study presents the description of transcription profile changes in adipocyte differentiation and provides an in-depth analysis of the possible mechanisms of adipocyte differentiation. Our data demonstrated that adipocyte differentiation mainly involves a metabolism process. The decreased immune responses and cell cycle were occurred during the differentiation of adipocyte. In addition, the altered TRP channels, including TRPV1, TRPV2, TRPV4, TRPV6, TRPM4, TRPM5 and TRPM7 and TRPC1, might be involved in adipocyte differentiation. This study offer new insight into the understanding of the mechanisms of adipocyte differentiation.

Abbreviations

DMEM: Dulbecco's Modified Eagle Medium

DEGs: differentially expression genes

DAVID: Database for Annotation, Visualization and Integrated Discovery

GO: gene ontology

Gene Expression Omnibus (GEO)

KEGG: Kyoto Encyclopedia of Genes and Genomes

TRP channels: Transient receptor potential channels

TRPV: TRP Vanilloid

Declarations

Ethical Approval and Consent to participate

All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of Shenzhen University.

Consent for publication

The paper has been read and approved by all authors for publication.

Availability of data and materials

The sequencing results have been submitted to the Gene Expression Omnibus (GEO) database and assigned the accession number as GSE129957.

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Author contributions

The authors' contributions were as follows: W Sun, S Tang and T Zhu were responsible for the concept and design of the study; W Sun, Z Yu, S Yang, C Jiang, Y Kou and X Liao were involved with experimental and analytical aspects of the manuscript; W Sun, S Tang and T Zhu performed data interpretation, presentation and writing of the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1: The summary of raw RNA sequencing data set.

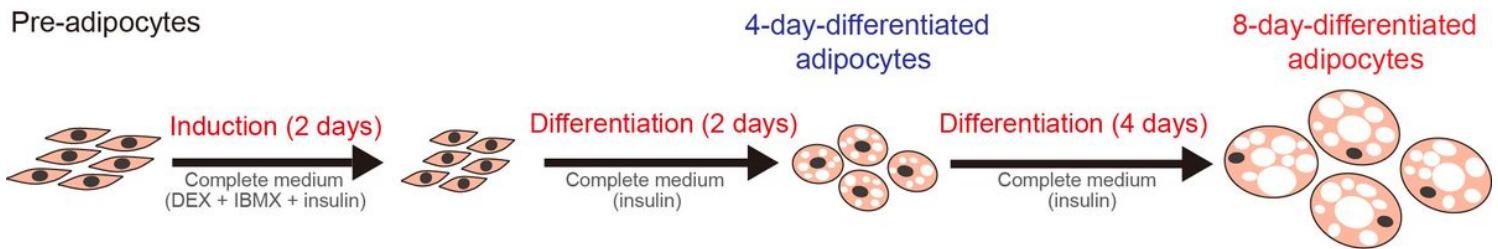
Sample	Total Raw Reads (Mb)	Total Clean Reads (Mb)	Clean Reads Q20 (%)	Clean Reads Q30 (%)	Clean Reads Ratio (%)	Total Mapping Ratio
3T3-L1-Day 0-1	24	24	96.67	88.04	99.34	0.9325
3T3-L1-Day 0-2	21	21	96.62	87.74	99.54	0.9343
3T3-L1-Day 0-3	24	24	96.44	87.61	99.53	0.9272
3T3-L1-Day 4-1	24	24	96.58	87.66	99.62	0.9357
3T3-L1-Day 4-2	24	24	96.47	87.38	99.49	0.9324
3T3-L1-Day 4-3	24	24	96.14	86.75	99.19	0.9283
3T3-L1-Day 8-1	24	24	95.73	85.62	99.6	0.9293
3T3-L1-Day 8-2	24	24	96.43	87.1	99.67	0.9338
3T3-L1-Day 8-3	24	24	95.47	85.28	99.53	0.9207

It showed the summary of RNA sequencing data of 9 samples, including Raw Reads Number, Clean Reads Number, Clean Data Rate, Mapped Rate, and percentage of clean reads as well as Q20 (Phred quality scores Q) and Q30.

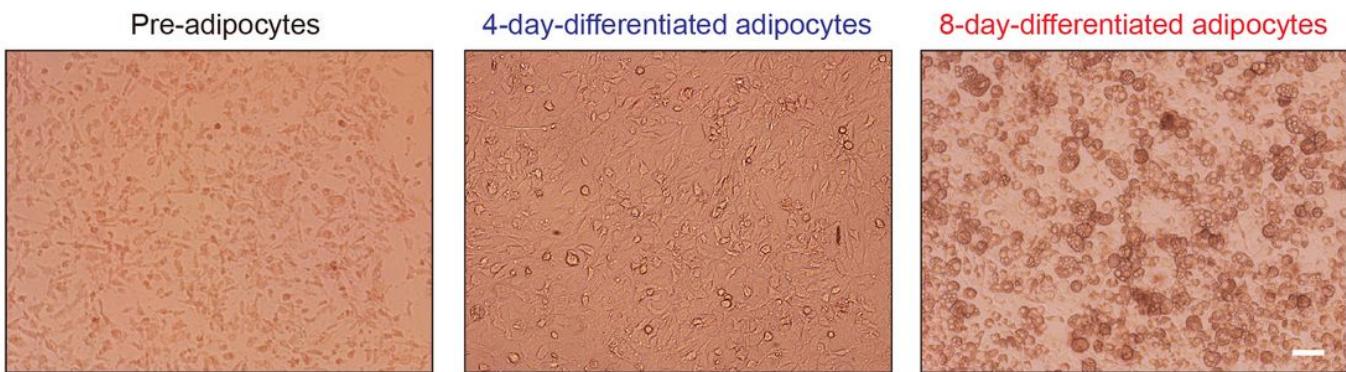
Figures

Figure 1

A



B



C

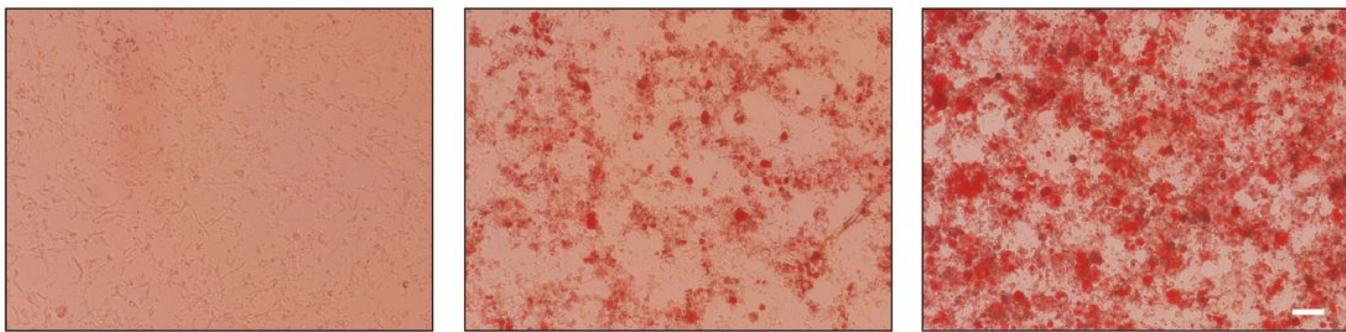
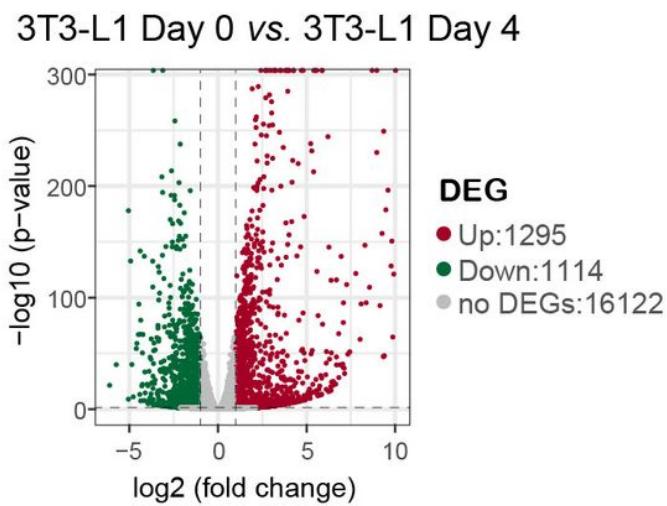


Figure 1

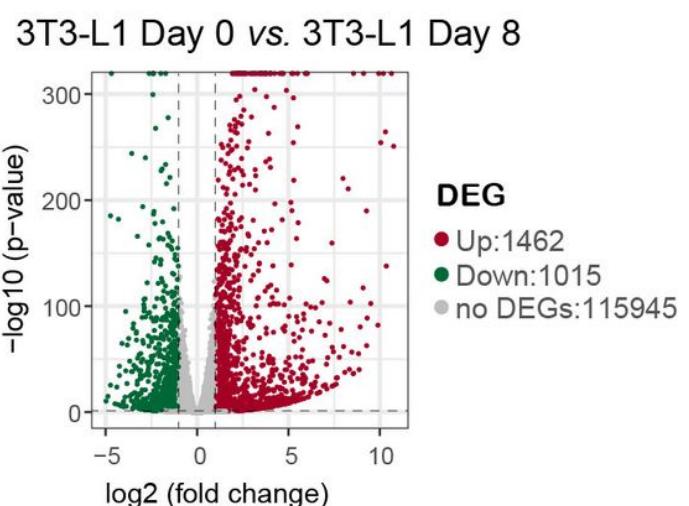
The schematic of the differentiation of 3T3-L1 adipocytes A Induction of 3T3-L1 adipocyte differentiation by DEX and IBMX initially results in cell growth. B and C The representative phase contrast (B) and Oil Red O staining (C) images of 3T3-L1 cells at pre-adipocyte, 4-day-differentiated, 8-day-differentiated adipocyte stage. Scale bar: 100 μ m.

Figure 2

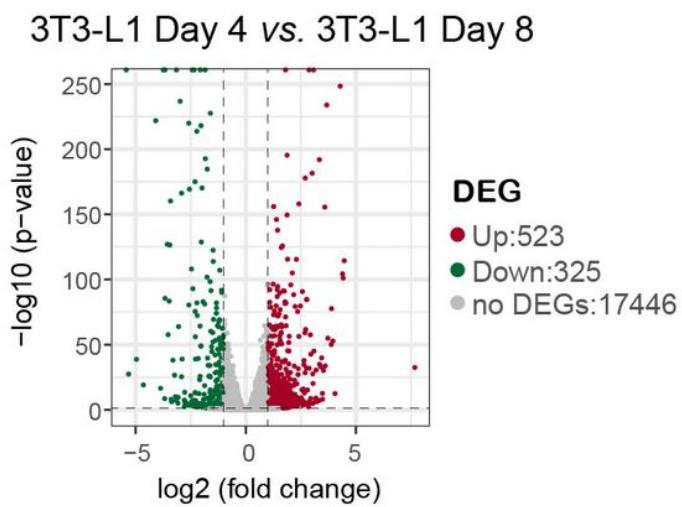
A



B



C



D

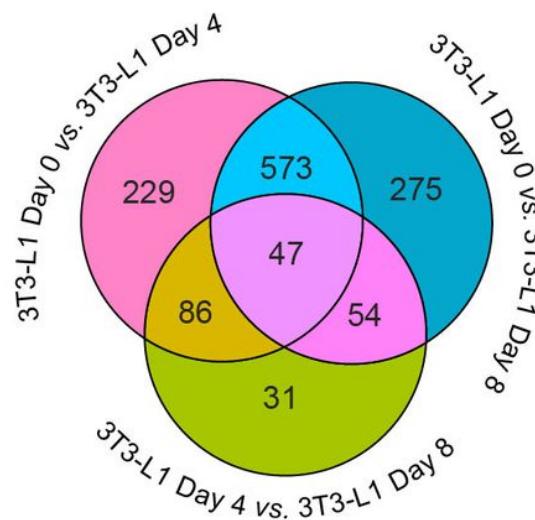


Figure 2

A number of differentially expressed genes in 3T3-L1 adipocyte from different stages A, B and C Volcano plot for the samples with mRNA expression differences. Log2 (fold change) is plotted as the abscissa and log10 (Corrected P Value) is plotted as the ordinate. Significantly up-regulated genes are indicated in red and down-regulated genes are indicated in green. D A Venn diagram presents the number of differentially expressed genes (DEGs) that are unique or shared in every paired group.

Figure 3

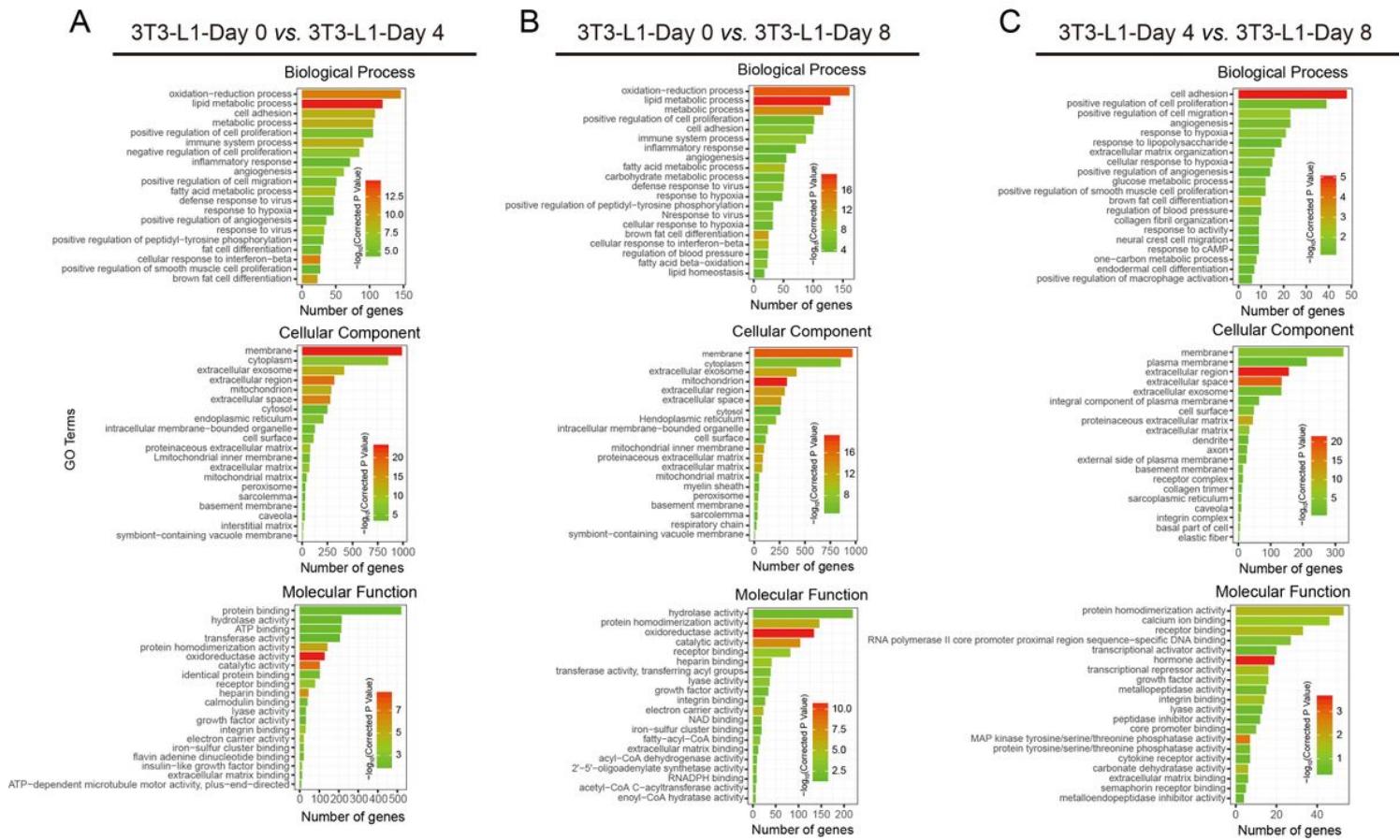
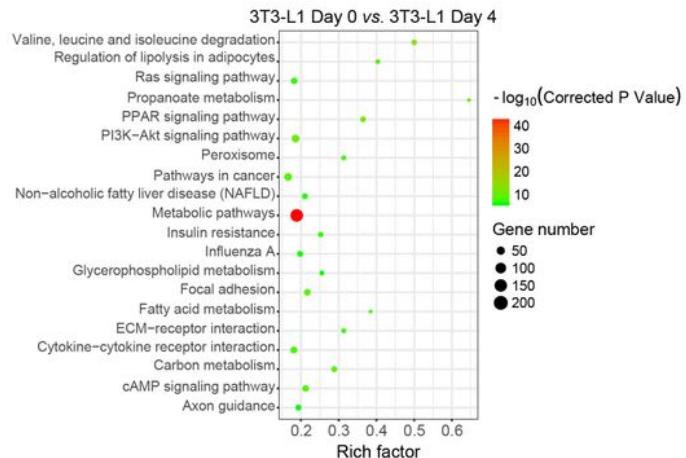


Figure 3

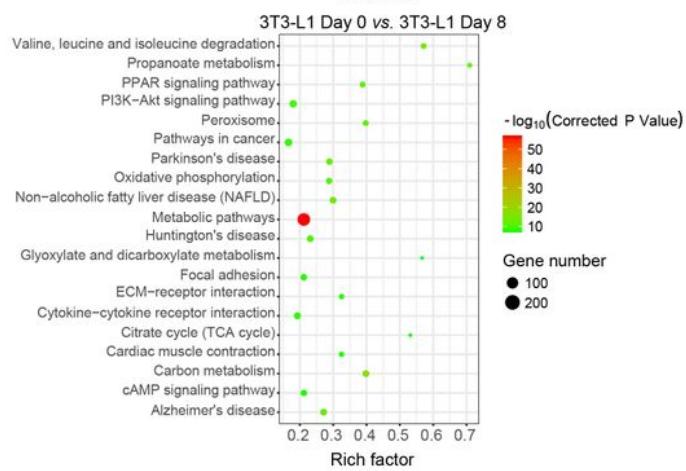
Functional analyses of DEGs by Gene Ontology classifications A, B and C. The comparison of Gene Ontology (GO) enrichment. It showed the top 20 significantly enriched GO terms including biological process, cellular component and molecular function. The enriched gene number as the abscissa and GO terms is plotted as the ordinate.

Figure 4

A



B



C

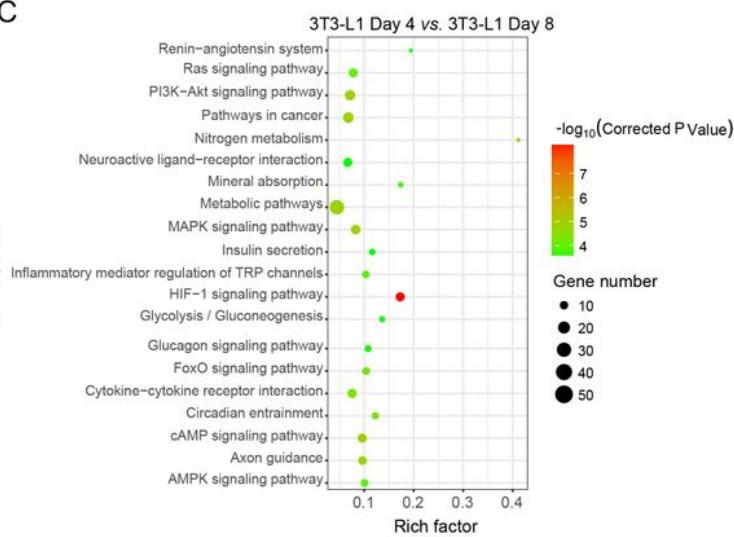


Figure 4

KEGG classifications of DEGs in 3T3-L1 adipocyte from different stages A B and C The comparison of pathway enrichment in 3T3-L1 adipocyte from different stages. It showed the top 20 significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The rich factor as the abscissa and KEGG terms is plotted as the ordinate.

Figure 5

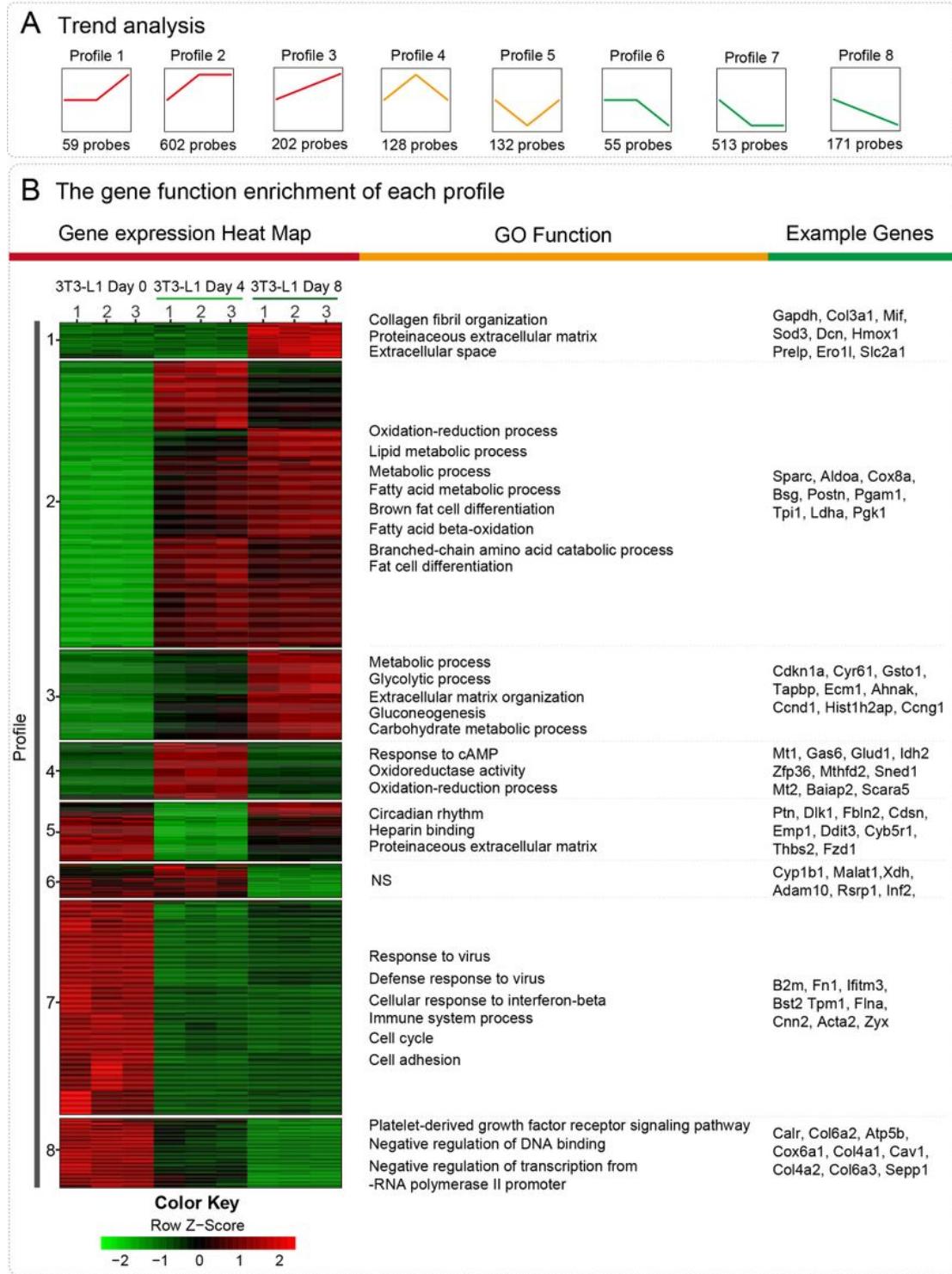


Figure 5

Trends analysis of DEGs in 3T3-L1 adipocyte from different stages A A sketch map of the cluster analysis of DEGs. The number at the bottom of each cluster represents the number of DEGs in the cluster. B A transcription heatmap with K-means of the mRNA expression in 3T3-L1 adipocyte from different stages and GO classification of each cluster was shown. Color annotation of heatmap is shown in the bottom color scale. Up-regulated or down-regulated genes are presented by red or green bars.

Figure 6

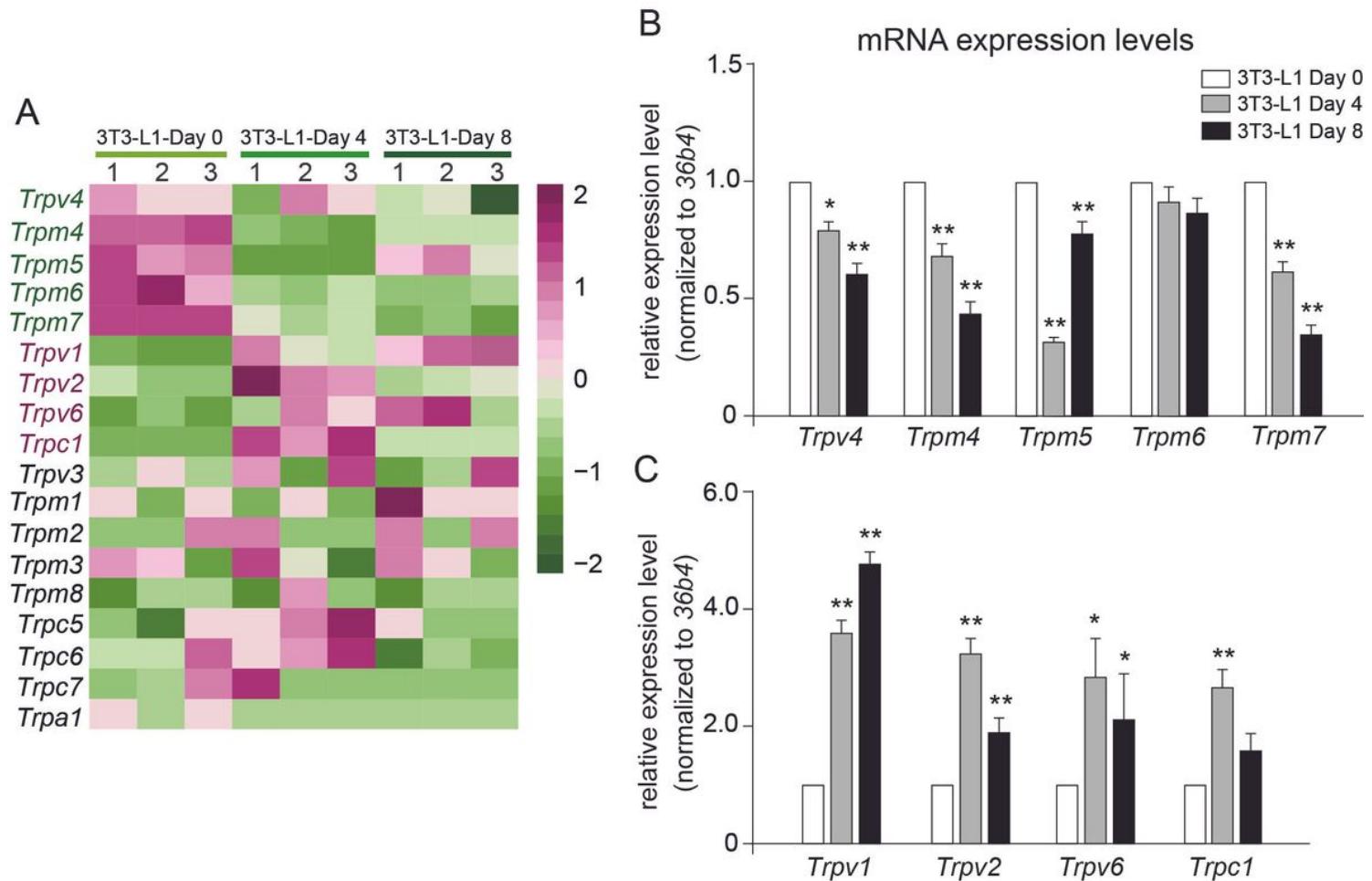


Figure 6

Validation of TRP channels by quantitative real-time PCR A A transcription heatmap of the mRNA expression of TRP channels in 3T3-L1 adipocytes. Color scale is shown in the right side. Up-regulated or down-regulated genes are presented by red or green bars. B The mRNA expression levels of Trpv4, Trpm4, Trpm5, Trpm6, Trpm7, Trpv1, Trpv2, Trpv6, Trpc1, Trpm8 and Trpa1 in 3T3-L1 adipocytes from different differentiation stage. Mean \pm SEM, n = 8, ** P < 0.01. One-way ANOVA followed by 2-tailed t-test with Bonferroni correction.

Supplementary Files

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