Integrative Transcriptome Analysis Define Novel lncRNA Potential Regulation Function through SLC22A7 in Yak Liver

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Abstract

Background: The yak (*Bos grunniens*) is a crucial resource to supply meat and milk to the people localized in Qinghai-Tibetan plateau area. To identify lncRNAs regulating metabolism in yak, this work adopted transcriptome method to simultaneously profile mRNAs and lncRNAs of liver in yak under three representative age (**LD**: **L**iver 1 **D**ay, **LM**: **L**iver 15 **M**onths, **LY**: **L**iver 5 **Y**ears) conditions.

Result: Of 288 differentially expressed lncRNAs, function-oriented selection yield 88 regulated metabolically related lncRNAs that were differentially expressed at least two age conditions. These lncRNAs predicted by lncRNA-mRNA correlation analysis to function in various aspects of metabolism. Selected regulations of liver metabolically related lncRNAs were further verified by qRT-PCR. Furthermore, one novel LncRNA were selected to validate its function and result showed it potentially regulated SLC22A7, a well-known organic anion transporter.

Conclusion: Combining high throughput RNA-seq screening screens, bioinformatics predictions, lncRNA-mRNA correlation analysis and qRT-PCR analysis, this study supports that a class of lncRNAs function as important metabolic regulators and establishes a foundation for further investigating the role of lncRNAs in yak.

1 Introduction

The yak (*Bos grunniens*) lives throughout the Qinghai-Tibetan plateau in western China at high altitudes where harsh and variable plateau climate exist including low humidity, temperature, and oxygen levels; strong winds and ultraviolet radiation. The yak has adapted to thrive under these harsh conditions, where few other livestock could survive. The yak is a crucial resource to supply meat and milk to the people localized in Qinghai-Tibetan plateau area. For these reasons, the yak is one of the most critical domestic animals for the 6.5 million Tibetans [1]. Based on the fact that the yak represents the primary source of meat and milk for the people of this region, it is imperative to understand the genetic makeup of the yak and how this can influence its development.

While, metabolic process is one of the most basic activity which is related to all fundamental biological and physiological activities in all animals [2]. Liver, the most important metabolic organs, engage in continuous dialogs to regulate overall metabolic balance by endocrine factors. And it is also a complex digestive gland in ruminants including yak and plays a critical role in the metabolism of substances. More importantly, during the developmental period, liver plays a critical role [3–5]. Dorland et al. previously reported the period of transition from new born to young cattle involved considerable metabolic adaptation in dairy cows and the liver [3]. Therefore, in the present study, the liver of new born (1 day), young yak (15 months) and mature yak (5 years) were chosen to be sampled.

Although a lot of work has been done in studying the regulation of individual metabolic pathways in recent years, there are still quite a few unknow fields in the complicated regulatory networks that control

metabolic physiology. It remains difficult to develop useful treatment strategy against metabolic disorders such as diabetes and obesity [6–8]. Nearly two-thirds of transcripts are noncoding RNAs, mainly from regions previously considered as junk gene [9]. Among the noncoding RNAs identified so far, lncRNA is the longest and most difficult to understand, which are transcripts of 200 nt or longer that lack protein coding potential. LncRNAs have been recognized in all model organisms [10], and their number has been increasing continuously [11–13]. It has been shown that lncRNAs have an influence on several aspects of cellular function, including chromatin modification and transcription regulation, RNA stability, and translational control [14–15]. Evolution studies have shown that more than 1,000 lncRNAs may have conservative functions in mammals [16]. There were also many reports in bovine indicates lncRNAs function in embryo development [17], skeletal muscle [18] and mammary glands [19]. In mouse, *in silico* method had been used to identify functional lncRNAs in metabolic process [20]. However, it is still unknown fields in ruminant animals like bovine or yak.

Therefore, in order to systemically illustrate the importance of lncRNAs in the homeostasis of metabolism of yak, this work combined a high throughput RNA-seq screening of lncRNAs transcripts from liver of yak in diverse ages to establish a comprehensive workflow to determine functional lncRNAs in metabolic regulation. LncRNA-mRNA network analysis is used to predict lncRNAs function, and qRT-PCR analysis connects lncRNAs with specific metabolic pathways. In conclusion, our research supports lncRNAs as a critical composition of metabolism and provides a fundation for systemically identifying and predicting functional lncRNAs that regulate the homeostasis of metabolism.

2 Material And Methods

2.1 Animal

Totally nine Maiwa yaks from Yak Technology Park (Hongyuan County of Sichuan Province in China) at diverse age conditions (1 day, 15 months and 5 years old) were used in this study and each developmental age stage had three yaks. The experimental animals were healthy and under the same management. Yaks were weighing 10.55 ~ 14.24 kg, 96.38 ~ 101.37 kg and 240.73 ~ 296.36 kg. All yaks were stunned with a captive bolt pistol (Cash 8000 Model Stunner, 0.22 calibre, 4.5 grain cartridge) to ameliorate the suffering of the animals prior to their humane killing, following which exsanguination via a transverse incision of the neck was carried out in the slaughterhouse. Then, the liver tissues were excised immediately and rapidly stored in liquid nitrogen until RNA isolation. Yaks with different age were established for profiling the transcriptomes in liver tissue.

2.2 RNA quantification and qualification

RNAiso Plus (TaKaRa, Japan) was used to extract total liver tissue RNA. The extent of RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA). RNA integrity was assessed

using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). If the $OD_{260}/_{280}$ value of total liver tissue RNA is between 1.8 ~ 2.2, the quality inspection is qualified (Supplementary Table 1) and the next analysis can be carried out.

2.3 Library preparation for high-throughput sequencing

A total amount of 3 μg RNA per sample was used as input material for the RNA sample preparations. A chain-specific library was constructed by removing rRNA, that was, sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) [21]. Briefly, mRNAs were purified from total RNA using poly-T oligo-attached magnetic beads. First strand cDNA was synthesized using random hexamer primer and RNase H. Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. The purified double-stranded cDNA was subjected to end repair, was added poly-A tail, and was connected to a sequencing adapter. In order to select cDNA fragments of preferentially 150 ~ 200 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then 3μL USER enzymes (NEB, USA) were used to degrade the second strand of U-containing cDNA, and perform PCR amplification to obtain the library. After the library was constructed, Qubit2.0 Fluorometer (Invitrogen, USA) was used for preliminary quantification. PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system. qRT-PCR (Bio-Rad, USA) was used to accurately quantify the effective concentration of the library to ensure library quality. The library preparations were sequenced on an Illumina Hiseq platform and 150 bp paired-end reads were generated.

2.4 Transcriptome sequencing data analysis

The quality-controlled Clean Reads was compared with the yak reference genome (BosGru_v2.0: ftp://ftp.ensembl.org/pub/release-97/fasta/bos_mutus/) quickly and accurately by the HISAT2 v2.0.5 software (http://ccb.jhu.edu/software/hisat2) to obtain Mapping Reads for subsequent analysis [22]. At the same time, the comparison results were evaluated for quality. By analyzing the different regions and chromosome distributions of Mapping Reads in the reference genome, to obtain the comparison efficiency and mapping information about Mapping Reads for each sample [23]. The new transcripts for Mapping Reads were assembled and quantified by StringTie (1.3.3b) software [24]. Sequencing depth and gene length were corrected using FPKM [25]. Differential expression analysis was performed using the DESeq2 R package (1.10.1). DESeq2 provide statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. Genes with an adjusted padj < 0.05 found by DESeq2 were assigned as differentially expressed [26].

2.5 Functional enrichment

ClusterProfiler (3.4.4) software was used to perform GO function enrichment analysis and KEGG pathway enrichment analysis on differential gene sets, to predict the biological processes and functions that they may participate in, and to make corresponding classification and statistics [27]. All lncRNAs were used for target gene prediction, that was, the target genes of lncRNAs were predicted by the positional relationship

(co-location) and expression correlation (co-expression) of lncRNAs and protein-coding genes [28]. Then, functional enrichment analysis (GO / KEGG) was performed on the target genes of differential lncRNAs to predict the main function of lncRNAs.

2.6 shRNA knockdown in AML12 cell lines

We designed shRNA targeted TCONS_00098792 by Invitrogen software (https://rnaidesigner.invitrogen.com/rnaiexpress/), with a scramble shRNA as control. The shRNA sequence was Top 5'-CACCGCACCGACAATGACGACAACACGAATGTTGTCGTCATTGTCGGTGC and Bottom 5'-AAAAGCACCGACAATGACGACAACATTCGTGTTGTCGTCATTGTCGGTGC – 3'. The shRNAs oligonucleotides were ligated into pLKOpuro.1 plasmid and then were co-transfected with V-SVG and Δ8.2 into HEK293T cells seeded in 10 cm dish. After 48 h, the lentivirus was collected and filtered by 0.45 μm filter to remove the cellular debris. The AML12 cell line (20%~30%) was infected by shRNA or scramble lentivirus, and then the stable expressing shRNA AML12 cell line were selected by puromycin (1 μg/mL). The SLC22A7 protein expression was identified with Anti-SLC22A7/OAT2 antibody - C-terminal (ab191018) as the first antibody by WB.

2.7 Real-time fluorescence quantitative PCR

Measurement of gene expression with qRT-PCR has been applied in our studies. Briefly, total RNA was extracted from liver tissue of each group using a RNAiso Plus (TaKaRa, Japan). RNA was complete without degradation, good quality and high purity, which meet the requirements of subsequent experiments. qRT-PCR was performed in a total volume of 10 μL containing 5.2 μL of TB Green™ Premix Ex Taq™ (TaKaRa, Japan), 1 μL of cDNA, 0.8 μL of each primer (10 mM) and 2.2 μL of double-distilled water. The reaction condition used were as follows: 95°C for 3 min, followed by 39 cycles of 95°C for 10 s, Tm for 20 s and 72°C for 20 s, with the dissolution curve increasing from 0.5°C to 95°C every 5 s. The assays were performed on a real-time fluorescence quantitative PCR System (Bio-Rad, USA). For each sample, the cycle threshold (CT) values were obtained from three replicates. β-actin mRNA was employed as an internal reference. The primers used for amplification of target and internal reference genes were presented in Supplementary Table 2. The relative expression levels of target genes were analyzed using the $2^{-\Delta\Delta CT}$ method.

2.8 Data analysis

All data are presented as means ± SEM. Comparisons were made by 2-tailed Student's t tests. Values of $P < 0.05$ were considered significant.

3 Result

3.1 Dynamic regulation of lncRNAs and mRNAs in liver under different age conditions

Both mRNA and lncRNA transcripts were detected in our study, current expression profile could compare the transcripts of lncRNAs and mRNAs at different developmental age stages in the yak liver. Hierarchical cluster analysis was performed on all expressed transcripts, which showed the mRNAs expression profiles clearly divide all samples into three different groups according to age stages (1 day, 15 months, 5 years) and these samples were closely clustered together in each group of different developmental age stages (Fig. 1A, top left). But what is interesting to us is that an almost identical sample aggregation pattern showed in the result of lncRNAs (Fig. 1A, top right), which indicates that expression profiles of lncRNAs could be used as a signal indicating similar to those of protein-encoded mRNAs. Using the RNA-seq method, 35216 mRNAs and 10073 lncRNAs transcripts were detected from transcripts in the yak livers at three different developmental age stages (LD: Liver 1 Day, LM: Liver 15 Months, LY: Liver 5 Years) (Fig. 2B).

In addition, all samples were divided into diverse groups for both mRNAs and lncRNAs through PCAs analysis on all regulated transcripts (Fig. 1C), which indicate that regulated lncRNAs and mRNAs transcripts might coordinate related biological processes. Furthermore, to determine their functional connections, analysis of the network with lncRNA-mRNA through related analysis of samples were conducted. 433 mRNAs and 152 lncRNAs were identified to be regulated by LD versus LM, 412 mRNAs and 160 lncRNAs by LD versus LY, as well as 263 mRNAs and 66 lncRNAs by LM versus LY (Fig. 1D), suggesting that their expression levels were strictly controlled by age conditions of the yak.

3.2 Functional analysis of differentially expressed genes (DEGs) in each age condition of yak liver

At LD versus LM, the DEGs of many functional Gene ontology (GO) categories enriched for metabolism, ion binding, developmental process and so on. While in KEGG pathway analysis, DEGs were also enriched for metabolism, biosynthesis, ECM-receptor interaction and so on (Fig. 2A). At LD versus LY, there were functional GO categories enriched for metabolism, tissue remodeling and developmental process and so on. While in the KEGG pathway analysis, DEGs were also enriched for terms like metabolism, biosynthesis, PI3K-Akt signaling pathway and so on (Fig. 2B). Similar functional GO categories of metabolism, biosynthetic process and oxidation reduction process and so on were enriched at LM versus LY. While in the KEGG pathway analysis, DEGs were also enriched for terms like metabolism, biosynthesis, focal adhesion and so on (Fig. 2C). All these results suggest metabolism related function were developed during the aged process of yak (Fig. 2).

3.3 Functional prediction of metabolically related differentially expressed lncRNAs by lncRNA-mRNA co-expression correlation under at least two age conditions

By correlation of lncRNA-mRNA expression interaction network, dynamically regulated lncRNAs were predicted their function at different developmental ages. 88 non-redundant lncRNAs regulated at least

two of the three age conditions were selected from the totally 288 regulated differentially expressed lncRNAs (Fig. 1D). Furthermore, to detect the specific function of these metabolically associated lncRNAs as example, six metabolically related lncRNAs were selected for further analysis (Fig. 3).

Taking six lncRNAs as examples to make the functional analysis, correlation analysis predicts their role in metabolism or cell differentiation. TCONS_00098792 and XLOC_045379 were predicted to be related with fat single organism metabolic process (Fig. 3A and 3B), and XLOC_021536 and XLOC_041441 associated with collagen metabolic processes and protein metabolic processes in liver, respectively (Fig. 3C and 3D). Moreover, TCONS_00032537 and XLOC_183608 have been shown to be enriched in cell proliferation and transport (Fig. 3E and 3F). These results indicate that the established co-expression network can effectively predict the potential metabolic functions of age-regulated and metabolically sensitive lncRNAs. In addition, the dynamic regulation of several randomly selected lncRNAs under different age conditions was confirmed by qRT-PCR, proving the feasibility of this method (Fig. 4).

3.4 Functional validation of differentially expressed lncRNA indicates Novel LncRNA potentially regulating SLC22A7

To further determine if this integrative transcriptome analysis is sufficient to identify lncRNAs that function as metabolic regulators, we tested the specific metabolic function for one lncRNA (TCONS_00098792) in hepatic AML12 cell line in mouse. Firstly, both RNA-sequence and QPCR data were analyzed showing the expression of TCONS_00098792 and its target genes, which indicated the SLC22A7 were up regulated when LncRNA expressed low (Fig. 5A, B). To specifically examine role of TCONS_00098792, we knocked it down in mouse AML12 cell line by administrating shRNA, which were tested functionally effective (Figure C). SLC22A7 were found to be increased at both mRNA and protein levels in the AML12 cell line of LncRNA knockdown compared with those of control (Figs. 4D, E). Taken together, these results indicate that the novel LncRNA potentially perform its function by regulating SLC22A7.

4 Discussion

Integrative lncRNAs function related to metabolic were selected using high-throughput RNA-seq screening, and a workflow for the discovery and characterization of functional lncRNAs were established in yak at different ages. The data obtained in this experiment support that lncRNAs was a crucial component of metabolism. Both the expression profile of lncRNAs and RNAs have change coordinately according to different developmental ages including 1 day, 15 months, and 5 years. Moreover, groups of metabolism relative lncRNAs were regulated in liver by ages, and their expression often changes significantly in yaks at diverse ages, supporting their potential biological significance.

Identifying lncRNAs regulating metabolic and finding its functional properties is still a hard task in animals and only a few lncRNAs could regulate metabolism were reported for now [29–32]. Moreover, it is impossible to directly determine the role of lncRNAs in metabolic process, for the reason of its non-coding feature. In order to get over this difficulty, this article build a functional lncRNAs detection method by

interactive analysis of lncRNAs in liver of yaks at different ages. This integrated approach efficiently reduce 288 age-adjusted lncRNAs to 88 putative metabolic lncRNAs which is differentially expressed at least in two age stages.

Only a few previous published papers showed that lncRNAs play a critical role in regulating metabolic pathway in mice [29–32], and certainly there is no report on the function of lncRNAs in yak. In order to understand the processes of yak liver metabolism in depth, more lncRNAs need to be discovered and characterized, and confirming the workflow of lncRNAs could speed up the selection and characterization of metabolic lncRNAs, which offered more perspective for the complex metabolism networks.

Making out the impact of lncRNAs in metabolic pathways at different developmental ages could enhance the unknown fields of complex metabolic physiology. Comparative genomics method had been widely used in revealing potential functions of novel protein-coding genes based on information of homologous genes or protein motifs, but has proved to be noneffective in finding the function of lncRNAs [33]. For most lncRNAs have not been functionally studied, and more importantly, lncRNAs were much more conservative than mRNAs, even between closely related model organisms [34]. Since most lncRNAs are unique to different animals, it is difficult to infer their role according to the sequence matching or evolutionary records [35].

For it is hard to predict functions for lncRNAs, it becomes very difficult to discover and characterize lncRNAs that regulate metabolism. Since metabolism is essential for almost all the biological processes, all organisms, any controlling with vital metabolic pathways is achieved usually through reconnecting metabolic fluxes. Therefore, a strict functional measurement is needed to determine critical points in metabolic regulation. To check the function of lncRNA further in animal models, it is firstly necessary to correctly infer the functional information of lncRNAs. In addition, the results of this experiment can also be beneficial to design targeted and detection methods to determine the unique metabolism in specific lncRNA, which is usually suppressed in complex interaction or compensation situation.

To further determine if this integrative transcriptome analysis is sufficient to identify lncRNAs that function as metabolic regulators, we tested the specific metabolic function for one lncRNA (TCONS_00098792) in mouse hepatic AML12 cell line. These *in vitro* results indicate that this novel LncRNA perform its function by regulating SLC22A7 (Fig. 5). It is reported that SLC22A7 is a facilitative transporter of cGMP, which mediates a host of cellular responses to various stimuli, resulting in the regulation of many critical physiologic functions [36]. Others also reported that SLC22A7 is a sodium-independent multi-specific organic anion/dimethyldicarboxylate exchanger [37]. Moreover, SLC22A7 expressed highly in liver tissue and play critical role in liver [38]. Taken together, our result showed a novel LncRNA potentially play its role by regulating SLC22A7 in yak liver, which also indicate this efficient method to predict metabolic lncRNAs could significantly accelerate the identification of important lncRNAs metabolic regulators.

5 Conclusions

The present study has illustrated the expression of metabolism-related lncRNAs in different ages of yak and lay a foundation for future finding and characterizing metabolism relative lncRNAs. One Novel lncRNA were proved to perform its function by regulating SLC22A7. This study could provide useful information for revealing the metabolic functions of lncRNAs in yak liver.

Abbreviations

RNA: Ribonucleic acid; lncRNAs: Long noncoding RNAs; mRNAs: Messenger RNAs; LD: Liver 1 day; LM: Liver 15 months; LY: Liver 5 years; PCR: Polymerase chain reaction; qRT-PCR: Real-time fluorescence quantitative PCR; RNA-seq: RNA sequence; DNA: Deoxyribonucleic acid; cDNA: Complementary DNA; RNase H: M-MuLV reverse transcriptase; FPKM: Fragments per kilobase of transcript sequence per millions base pairs sequenced; padj: Corrected p-value for multiple hypothesis test; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; PCAs: Principal-component analysis; ECM: Extracellular matrix; PI3K-Akt: Phosphatidylinositide 3-kinases-serine/threonine kinase

Declarations

Acknowledgements

Not applicable.

Authors' contributions

LW and WX conceived and designed this study. FF performed experiments, analyzed data. WX wrote the manuscript. FF and JG collected sample collection. LW and XL contributed to the revisions. All authors reviewed and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

All animal experiments were conducted according to the regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised in June 2004) and approved by the Institution Animal Care and Use Committee in the Southwest Minzu University, Chengdu, China.

Consent for publication

All of the authors have approved the final version of the manuscript, agree with this submission to Journal of Animal Science and Biotechnology.

Conflict of interests

The authors declare that there is no conflict of interest in the research reported.

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Figures

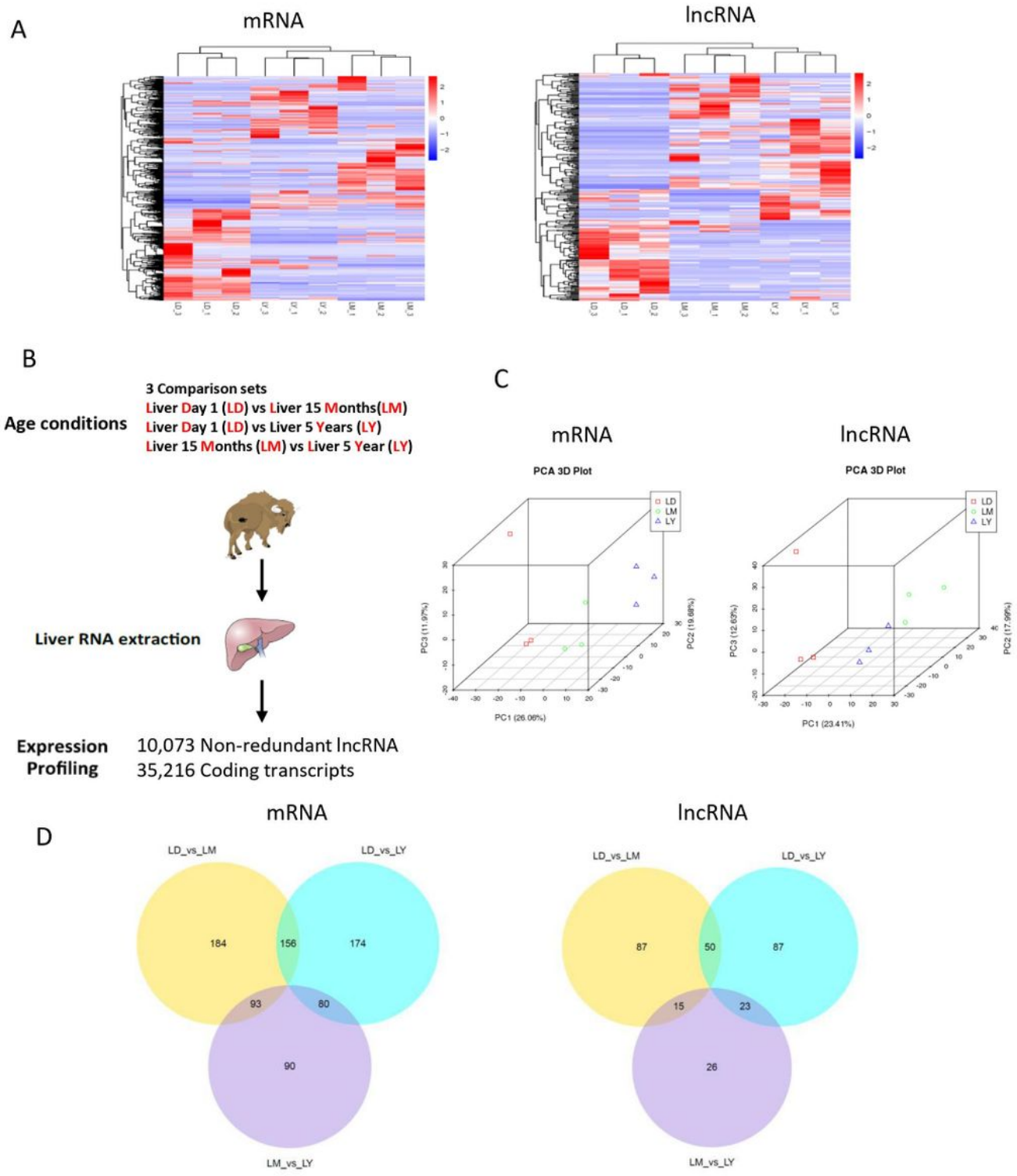


Figure 1

Dynamic Regulation of LncRNAs and mRNAs in Key Metabolic Organs under Multiple Age Conditions (A) Hierarchical clustering (top) and PCAs (bottom) of differentially expressed mRNAs (left) and lncRNAs (right) in yak livers at different age conditions. The differentially expressed genes were defined by one-way ANOVA to be significantly different. (B) Experimental outline. Each metabolic condition contains three yak, and the expression profiles of mRNAs or lncRNAs from 9 liver samples were analyzed. (C) PCAs (bottom) of differentially expressed mRNAs (left) and lncRNAs (right) in yak livers. (D) Numerical distribution of age-specifically and commonly regulated mRNAs (left) and lncRNAs (right) in liver.

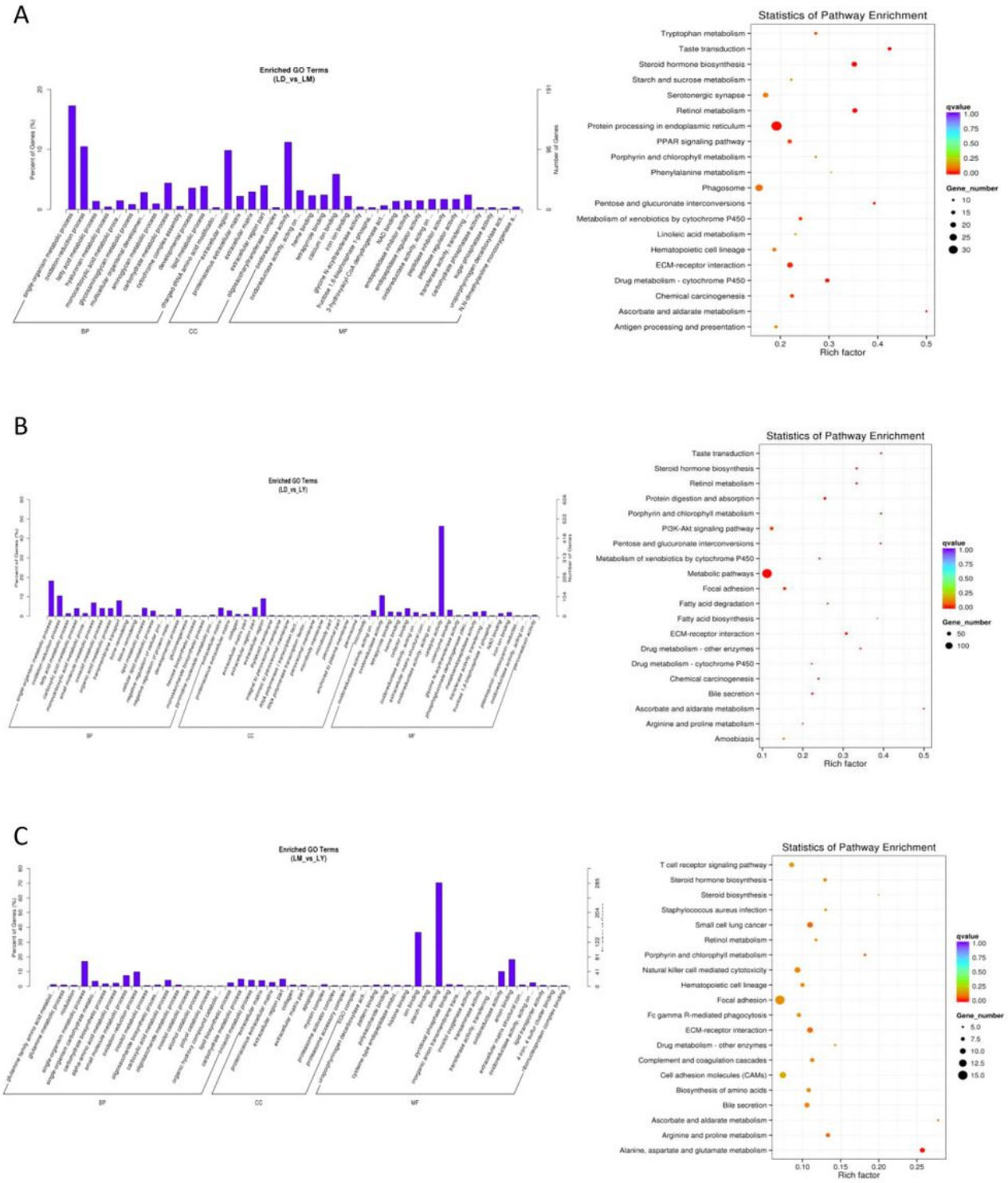


Figure 2

gene ontology (GO) and KEGG pathway terms for differentially expressed mRNAs in each age condition in liver (A) Gene ontology (left) and KEGG pathway(right) terms for differentially expressed mRNAs of LD vs LM in yak liver. (B) Gene ontology (left) and KEGG pathway(right) terms for differentially expressed mRNAs of LD vs LY in yak liver. (C) Gene ontology (left) and KEGG pathway(right) terms for differentially expressed mRNAs of LM vs LY in yak liver.

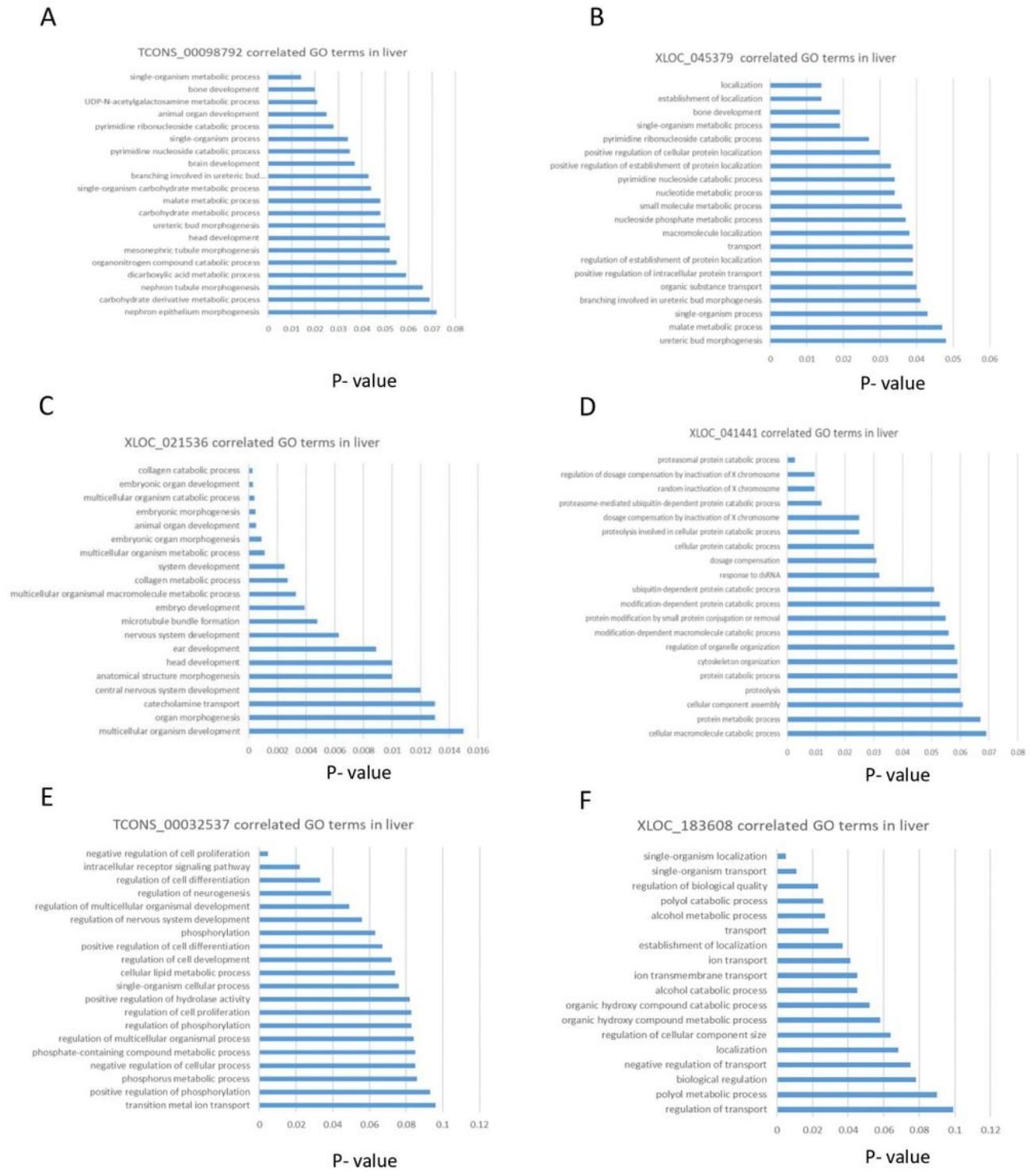
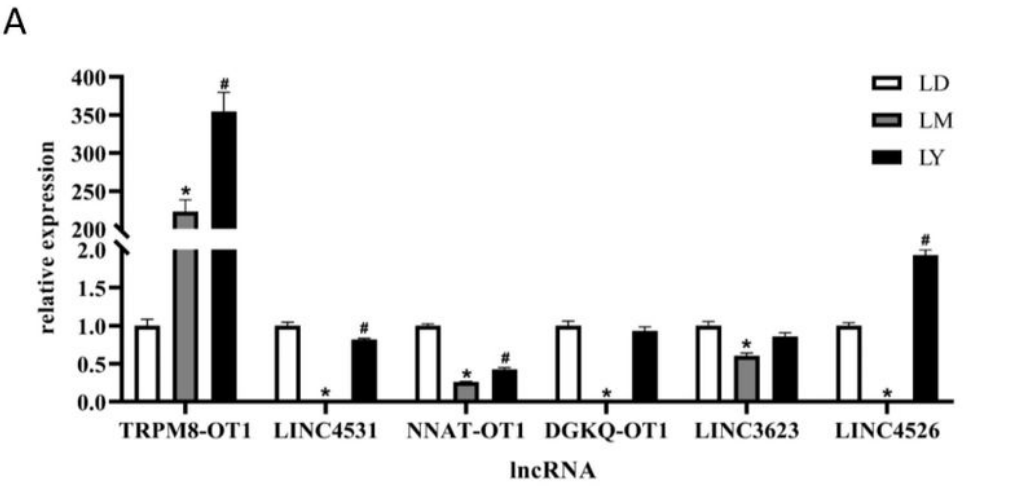
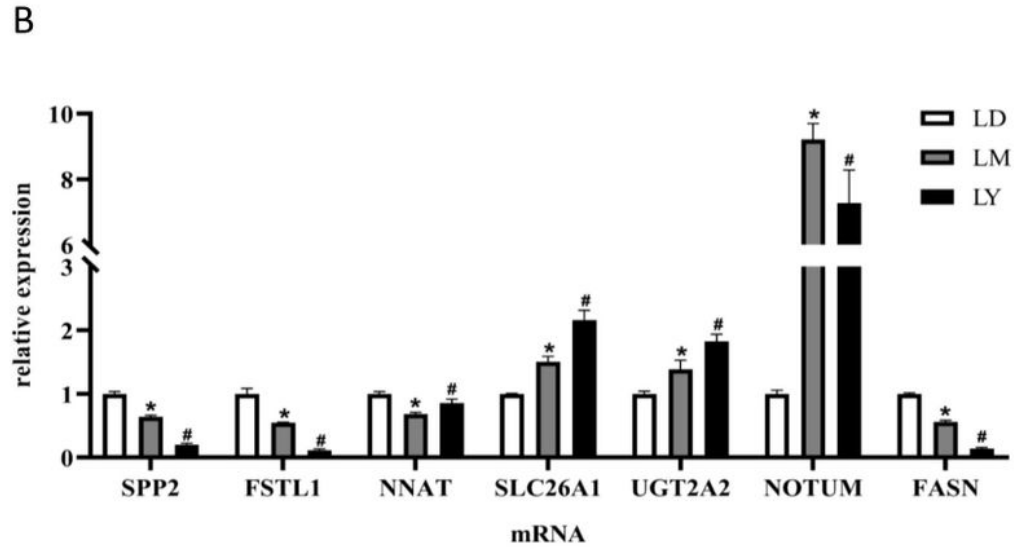


Figure 3

Functional Prediction of Metabolically related Differentially expressed LncRNAs by LncRNA-mRNA Co-expression Correlation under at least two Age Conditions (A) Gene ontology terms for differentially expressed lncRNA TCONS_00098792 in yak liver. (B) Gene ontology terms for differentially expressed lncRNA XLOC_045379 in yak liver. (C)Gene ontology terms for differentially expressed lncRNA XLOC_021536 in yak liver. (D)Gene ontology terms for differentially expressed lncRNA XLOC_041441 in

yak liver. (E) Gene ontology terms for differentially expressed lncRNA TCONS_00032537 in yak liver. (F) Gene ontology terms for differentially expressed lncRNA XLOC_183608 in yak liver.





C

mRNA

FPKM value	Correlation between RNA-seq and FPKM
0.5<FPKM<10	r = 0.896
10<FPKM<100	r = 0.742
FPKM >100	r = 0.974
FPKM >0.5 (all)	r = 0.98

lncRNA

FPKM value	Correlation between RNA-seq and FPKM
0.001<FPKM<1	r = 0.877
FPKM>1	r = 0.982
FPKM >0.001 (all)	r = 0.983

Figure 4

qPCR validation of selected Differentially expressed LncRNAs and mRNAs (A) Validation of lncRNA RNA-seq results by quantitative real-time PCR analysis. Expression levels of 6 lncRNAs were quantified. Error bars are SEM, n=4. *P<0.05 (LD vs LM), # p<0.05 (LD vs LY). (B) Validation of mRNA RNA-seq results by

quantitative real-time PCR analysis. Expression levels of 7 mRNAs were quantified. Error bars are SEM, n=4. *P<0.05 (LD vs LM), # p<0.05 (LD vs LY). (C) Pearson correlation of log2-transformd fold changes between FPKM and RNA-seq for differentially expressed mRNA(left) and lncRNAs(right).

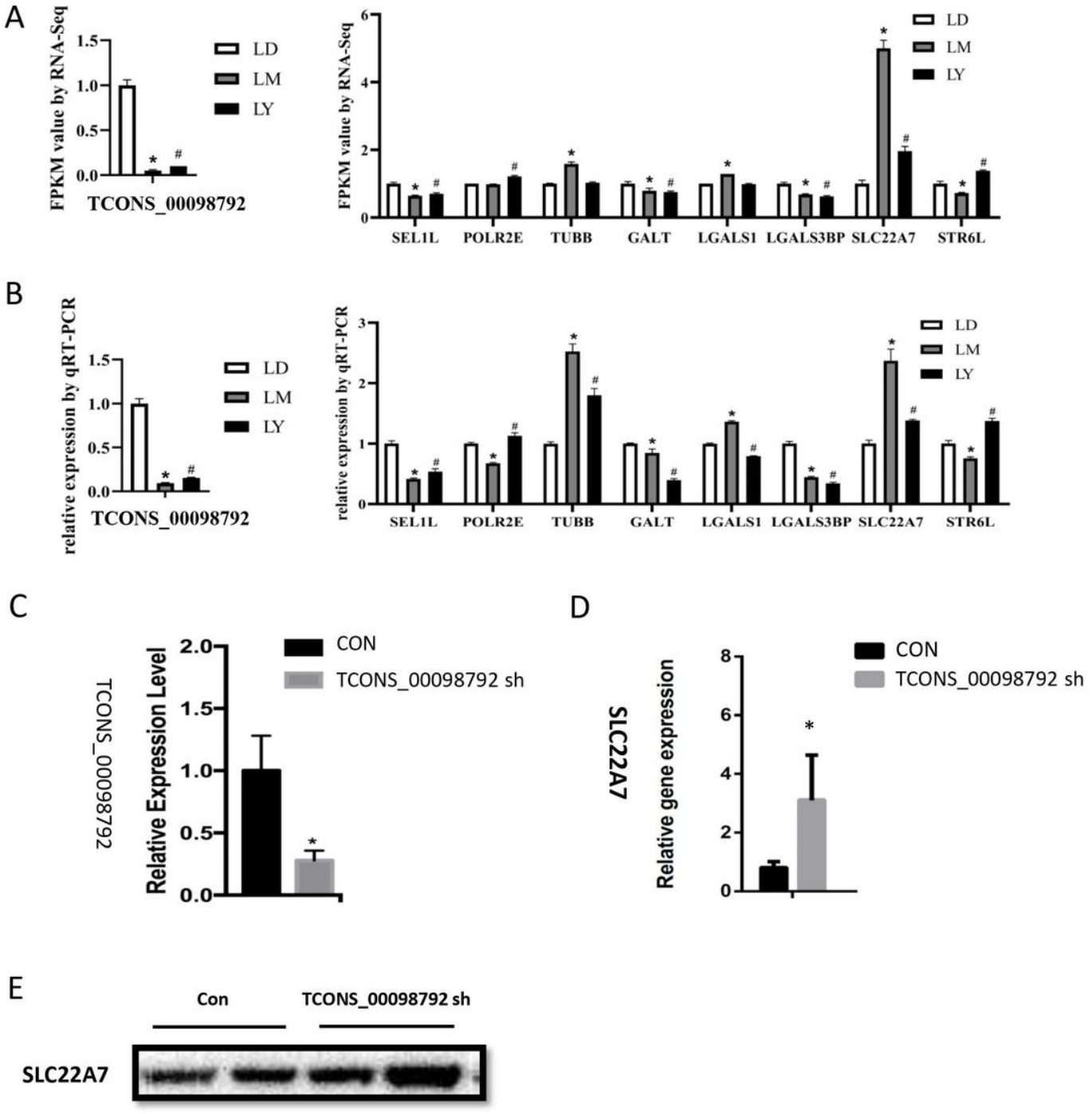


Figure 5

Functional validation of Novel LncRNA in AML12 cell line of mouse (A) RNA-seq result of expression level of TCONS_00098792 and its target genes; (B) QPCR result of expression level of TCONS_00098792 and its target genes; (C) Knockdown of TCONS_00098792 in AML12 cell line; (D) QPCR result of SLC22A7 in

control and TCONS_00098792 knockdown group; (E) Western blot of SLC22A7 in control and TCONS_00098792 knockdown group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable2021.4.25.pdf