Screening of lncRNA Profiles During Intramuscular Adipogenic Differentiation Inlongissimus Dorsi and Semitendinosus Muscles in Pigs

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Abstract

Background: Intramuscular fat content is an important factor that determines meat quality in pigs. In recent years, epigenetic regulation has increasingly studied the physiological model of intramuscular fat. Although long non-coding RNAs play essential roles in various biological processes, their role in intramuscular fat deposition in pigs remains largely unknown.

Results: In this study, intramuscular preadipocytes in the *longissimus dorsi* and *semitendinosus* of Large White pigs were isolated and induced into adipogenic differentiation *in vitro*. High-throughput RNA-seq was then carried out to estimate the expression of lncRNAs at 0, 2, and 8 days post-differentiation. At this stage, 2135 lncRNAs, including 575 novel lncRNAs, were identified. lncRNAs are shorter, less expressed, and less conserved RNAs than protein-coding mRNAs. KEGG analysis showed that the differentially expressed lncRNAs were more common in pathways that are closely involved with adipogenesis and lipid metabolism. Of these, lnc_000368, a previously undescribed lncRNA, was found to gradually increase during the adipogenic process. qRT-PCR and a western blot revealed that the knockdown of lnc_000368 by siRNA significantly repressed the expression of adipogenic genes (PPARγ, aP2, CEBPβ) and lipolytic genes (ATGL and HSL). As a result, lipid accumulation in porcine intramuscular adipocytes was impaired by the silencing of lnc_000368, as indicated by Oil Red O staining.

Conclusions: Overall, our study identified a genome-wide lncRNA profile related to porcine intramuscular fat deposition, and the results suggest that lnc_000368 is a potential target gene that might be targeted in pig breeding in the future.

1 Background

Pork occupies an important part in the human diet as an important dietary protein source [1]. The intramuscular fat (IMF) content has been shown to be positively correlated with pork quality, as high intramuscular fat content can significantly improve the flavor and tenderness of the pork [2]. However, in the last decades, extensive breeding selection aimed at increasing lean mass has resulted in low IMF and worsening flavor, and thus increasing IMF has become an important concern in the pig breeding industry [3].

Long non-coding RNAs (lncRNAs) are a type of non-coding RNA that are over 200 nt in length, and are involved in various biological processes [4, 5], such as adipose deposition [6]. It has been reported that the differentiation of adipocytes is affected by the natural antisense transcript of adiponectin, which forms a duplex with sense mRNA to inhibit the translation of adiponectin [7]. Moreover, PU.1 AS lncRNA has also been found to promote adipogenic differentiation in porcine intramuscular adipocytes [8]. Furthermore, lncRNAs were differentially expressed in pig breeds with differing IMF contents [9]. These studies indicate that lncRNAs may be critical modulators in porcine IMF accumulation, thus potentially providing novel breeding selection markers that select for improved pork quality. Our previous study showed that the intramuscular adipocytes in *longissimus dorsi* muscles possessed greater lipid-forming

ability than those in *semitendinosus* muscles [10]. In the present study, we isolated intramuscular preadipocytes in *longissimus dorsi* and *semitendinosus* muscles, performed high-throughput lncRNA sequencing of these cells, and aimed to identify the common lncRNAs that regulate porcine intramuscular adipogenic differentiation.

2 Methods

2.1 Cell culture & transfection

Newborn male Large White piglets (3 days old) were procured from the experimental piglet of Northwest A&F University (Yangling, China). All animals were maintained on a 12:12-h light cycle, and feeding conditions and slaughter methods were in accordance with national animal welfare regulations. In this study, all experiments on animals were approved by the Experimental Animal Management Committee of Northwest A & F University and comply with animal welfare regulations.

Intramuscular preadipocytes were isolated from the *longissimus dorsi* (LD) and *semitendinosus* (SD) muscles of these piglets using the method previously described by [10]. To summarize, LD and SD muscles were quickly excised, rinsed twice in sterile pre-cooled phosphate-buffered saline (PBS), and then cut into 1 mm^3. Muscle fragments were incubated in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12; Hyclone, Logan, UT, USA) containing 0.1% I type collagenase (270 U/mg; Gibco, Carlsbad, CA) for 1.5 hours in a 37 °C water bath, with continuous shaking. The products were then sequentially passed through a 70 mesh and then a 200 mesh to obtain single cells. The cells were seeded in a dish with DMEM/F12 medium containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA). After two hours, we changed the medium to keep only adherent cells.

When cells reached 70–80% density, Lipofectamine® RNAiMAX Reagent (Thermo Fisher, Waltham, MA, USA) mixed with siRNA (Rib-bio, Guangzhou, China) was used for transfection. When cells got to 100% confluence, a mixture containing 10% FBS, 5 µg/mL (872 nM) insulin, 1 µM dexamethasone, and 0.5 mM isobutyl methylxanthine (IBMX, Sigma – Aldrich, St. Louis, MO) was used to induce adipogenic differentiation. Two days later, a DMEM/F12 medium containing 10% FBS and 5 µg/mL (872 nM) insulin was changed to maintain differentiation. Cells at 0, 2, and 8 days of adipogenic differentiation were harvested for RNA-seq.

2.2 RNA extraction and transcriptome sequencing

Trizol (Takara Bio, Otsu, Japan) was used to extract total RNA as per the manufacturer's instructions. The concentration of RNA was measured using NanoDrop 2000 (Thermo Fisher, Waltham, MA, USA), and the RNA was then stored in a -80°C refrigerator for storage.

The mRNA fragment was then reverse transcribed into first-strand cDNA using reverse transcriptase (Takara Bio, Otsu, Japan) and random primers. The RNA template was then removed, and double-stranded cDNA was produced. End repair, poly-A tail processing, adapto r ligation and cDNA purification

and enrichment were then performed. Sequencing was carried out using an Illumina HiSeq 2500 sequencing system, with 100 bp paired-end sequencing.

Next, raw data were measured by each internal script in the fastq format. Moreover, with the strict screening criteria, the clean data from raw data were collected. Synchronously, the related indicators of clean data were calculated, including Q20, Q30, and GC content. The high quality clean data were used for further downstream analyses.

Scripture (beta2) [11] and Cufflinks (v2.1.1) [12] were used to combine the mapping metrics of each sample. Scripture was run with default parameters, while Cufflinks was run with 'min-frags-per-transfrag = 0' and '--library-type', while other parameters set to default. Coding-Non-Coding Index (CNCI), Coding Potential Calculator (CPC) and Pfam-scan were used to predict the encoding ability of novel lncRNA.

2.3 Quantification of gene expression level

Cuffdiff (V2.1.1) was used to calculate the abundance of lncRNA. The FPKM of lncRNA was calculated by summing the FPKM of the transcripts in each sample genome. The Cuffdiff program was used to perform statistical modelling based on a negative binomial distribution to determine differential expression in digital transcripts of gene expression data. Statistical results were considered differential expressed when $P^* < 0.05$.

2.4 Bioinformatic analysis

PhyloFit was used to compute phylogenetic models for conserved and non-conserved regions between species, and HMM transition parameters were set to phyloP to compute a set of conservation scores for lncRNA and coding genes [13].

For *cis*-acting lncRNA, coding genes 10 kb/100 kb upstream and downstrea m of each lncRNA were searched for functional analysis of lncRNA. Meanwhile, for *trans*-acting lncRNA, the correlation between lncRNA and the coding gene was calculated using a custom script; these genes are considered as target genes of lncRNA predicted by position. KOBAS software (http://kobas.cbi.pku.edu.cn) was used to perform KEGG pathway enrichment analysis for each target gene predicted by lncRNA.

2.5 Real-time quantitative PCR

Approximately 500 ng of RNA was reverse transcribed using the PrimeScript RT Enzyme Mix (Takara Bio, Otsu, Japan) system to produce cDNA. Real-time quantitative PCR was performed on ABI StepOne Plus using the SYBR Premix Ex Taq™ system (Vazyme Biotech, Nanjing, China). The relative expression level of target genes was evaluated by the $2^{-\Delta\Delta Ct}$ method, with β-actin acting as an internal reference gene. Sequences for all primers are shown in Table 1.

Table 1
Primer sequences for real-time qPCR

Gene	Primer Sequences
ALDBSSCT0000011643	F:CGCTTTGCTTCTTCTAGCCC
	R:GTTCATTAGCTTGGTTTGCTGC
ALDBSSCT0000004851	F:CGGCGGGTGACATTCTAAG
	R:GGCCAATCCGGCTCGC
LNC_000368	F:CGGCCAAGACTAACCAAGGG
	R:TGCCCACTGTCACCCTAACT
LNC_000359	F:GCCATGAGGAAGCCAAACTG
	R:TAAAGTTGGTGCGGGTGTCA
LNC_000170	F:TTTCTGGAATGCCCGCTCTG
	R:TGGGGTGGTTGATTGTAGCC
LNC_000076	F:TTTCTGGAATGCCCGCTCTG
	R:TGGGGTGGTTGATTGTAGCC
LNC_000108	F:GACAAGCTCCCAGACACCTC
	R:GGCTACAGATGAGGTCAGCC
β-actin	F:GGACTTCGAGCAGGAGATGG
	R:AGGAAGGAGGGCTGGAAGAG
PPARγ	F:AGGACTACCAAAGTGCCATCAAA
	R:GAGGCTTTATCCCCACAGACAC
aP2	F:GAGCACCATAACCTTAGATGGA
	R:AAATTCTGGTAGCCGTGACA
C/EBPβ	F:GCACAGCGACGAGTACAAGA
	R:TATGCTGCGTCTCCAGGTTG
ATGL	F:CCTCATTCCACCTGCTCTCC
	R:GTGATGGTGCTCTTGAGTTCGT
HSL	F:CACTGACTGCTGACCCCAAG
	R:TCCTCACTGTCCTGTCCTTCAC

2.6 RNA-FISH

Firstly, the back subcutaneous fat pad was removed and fixed in 4% polyformaldehyde. Tissues were cut into 3 μm sections using a freezing microtome (Leica, CM1950). After washing the sample with PBS, 200 μl of the pre-hybrid solution was added and kept at 37°C for 30 min. Next, the hybridization solution containing 2.5 μl of lncRNA FISH Probe Mix (Invitrogen™) (or 'internal reference FISH probe') was added to the mixture and incubated overnight at 37 °C in the dark. The slices were then washed with PBS 3 times, 5 min per wash. DAPI was added to stain the nuclei. Preparation complete, the fluorescence was observed under a fluorescence microscope (Nikon, TE2000-S). The specific FISH probe sequence designed based on the lnc_000368 sequence was 5⊠-DIG-GCCACCCAACCCCAGACCACAGCCTAC-DIG-3⊠.

2.7 Oil Red O staining

Porcine primary intramuscular preadipocytes were washed 3 times with pre-cooled PBS, fixed in 4% paraformaldehyde for 30 min, and then washed with PBS 3 times. Cells were incubated in filtered 0.5% Oil Red O working solution for 30 min; the Oil Red O solution was then discarded. Cells were washed 3 times with PBS and observed under a microscope (Olympus, CKX53). Isopropanol was then used to extract cellular triglycerides, and the absorbance was measured at a wavelength of 510 nm for use in further quantitative analysis.

2.8 Western Blot

Polyacrylamide gels were used to separate and mark proteins of different sizes. The proteins were then transferred to a PVDF membrane. Next, the membrane was soaked in 5% skim milk for 2 hours, and then incubated with primary antibodies overnight (PPARγ, Abcam, ab59256; HSL, #2435, CST; aP2, sc-18661, Santa Cruz; ATGL, #2138, CST; CEBP/β, sc-7962, Santa Cruz; β-actin, KM9001T, Sungene). Dilute the antibody to the manufacturer's recommended concentration. After incubation, the membrane was washed 3 times with TBST solution, and secondary antibodies (Goat Anti-Mouse IgG, Boster, BA1038; Goat Anti-Rabbit IgG, Boster, BA1039) were added. Finally, the western blots were exposed to the Bio-Rad imaging system.

2.9 Statistical analysis

Each experiment was repeated at least three times independently, and representative results are shown. Differences between groups were assessed for significance with the t test, and the results are presented as mean ± SEM. $P^* < 0.05$ was considered statistically significant. $P * < 0.01$ is considered to have a very significant relationship.

3 Results

3.1 Characteristics of lncRNA expressed in porcine intramuscular adipocytes

To profile the lncRNA expressed during adipogenic differentiation in porcine intramuscular adipocytes, high-throughput RNA-seq was used to screen the transcriptome at 0, 2, and 8 days after differentiation.

From this, 707,853,440 raw reads were identified by RNA-seq, and 664,015,984 clean reads (66.41 GB, 0.01% error rate) remained after excluding low-quality data. The cleaned data were then compared to the pig genome (tax ID: 9823) to search for potential lncRNAs (Fig. 1A). In total, 2135 lncRNAs, including 575 novel lncRNAs, were identified using 3 different coding potential prediction software (CNCI, CPC and Pfam-scan) (Fig. 1B). Compared with protein-coding mRNAs obtained in the RNA-seq, lncRNAs were generally shorter (Fig. 1C), contained fewer exons (Fig. 1D), and had significantly lower ORF numbers (Fig. 1E). The novel lncRNAs identified in our study consisted of 500 lincRNAs and 75 antisense lncRNAs (Fig. 1F). The expression of lncRNAs was also much lower than that of mRNAs (Fig. 1G). Lastly, a phastCons analysis revealed that the lncRNAs were less conserved than mRNAs (Fig. 1H).

3.2 Screening and identification of differentially expressed genes (DEGs) during intramuscular adipogenic differentiation

In intramuscular adipocytes isolated from *longissimus dorsal*, 199 lncRNAs were found to be differentially expressed between 0 and 2 days after adipogenic induction (77 up-regulated, 122 down-regulated). There were 98 up-regulated and 45 down-regulated lncRNAs in well-differentiated intramuscular adipocytes (8 days after differentiation) when compared with preadipocytes (0 days after differentiation). From the 2nd to the 8th day after adipogenic differentiation, there were 144 up-regulated and 43 down-regulated lncRNAs (Fig. 2A).

With regards to the intramuscular adipocytes derived from *semitendinosus*, 350 differentially expressed lncRNAs were identified in total. There were 78 up-regulated and 89 down-regulated lncRNA in adipocytes of 2 days after adipogenic differentiation compared with preadipocytes (day 0), and 115 ascending and 51 descending lncRNAs in lipid-laden adipocytes (8 days after induction) in comparison with preadipocytes. Moreover, 132 lncRNAs increased and 64 lncRNAs decreased between 2 and 8 days after differentiation (Fig. 2B).

Heat maps were made of differentially expressed lncRNAs of interest (Fig. 2C).

To further validate the expression of some differentially expressed lncRNAs in RNA-seq, 2 annotated (ALDBSSCT0000011643, ALDBSSCT0000004851) and 5 novel lncRNAs of interest (lnc_000368, lnc _000359, lnc _000170, lnc _000076, lnc _000108) were selected, and their expression patterns were further confirmed by RT-qPCR (Fig. 2D). Overall, the expression patterns of these lncRNAs are mostly consistent with the results of RNA-seq.

3.3 KEGG analysis of differentially expressed lncRNAs during intramuscular adipogenic differentiation

KEGG analysis of target genes predicted by lncRNAs. KEGG analysis results show, on day 0 and day 2 of LD differentiation, KEGG analysis enriched signal pathways such as Ras signaling pathway, NF-kappa B

signaling pathway (Fig. 3A), and on day 0 and day 8 enriched signal pathways such as Fatty acid metabolism, Fatty acid biosynthesis (Fig. 3B). The KEGG analysis on day 0 and day 2 of SD differentiation enriched signal pathways such as NF-kappa B signaling pathway (Fig. 3C), and on day 0 and day 8 enriched signal pathways such as PPAR signaling pathway and Insulin signaling pathway (Fig. 3D). Among the significantly enriched pathways, fat deposition related pathways such as PI3K-AKT, adipocytokine signaling, PPAR signaling pathway, Toll-like receptor signaling pathway, and Jak-STAT signaling pathway, were up-regulated, indicating that lncRNAs likely participated in intramuscular fat deposition through these pathways.

3.4 Characterization of lnc_000368

Lnc_000368 is an intergenic lncRNA located on pig Chr 2, 143856229−143907415, and is composed of two exons (Fig. 4A). The concentration of lnc_000368 gradually increased during intramuscular adipogenic differentiation in both *longissimus dorsal* and *semitendinosus*, as shown in the RNA-seq and RT-qPCR results (Fig. 2D). In order to explore the expression pattern of lnc_000368 in pig growth and development, we tested the tissues of Large White pigs at different ages. Further RT-qPCR analysis showed that lnc_000368 was highly enriched in back fat pads in 180-day-old Large White pigs (Fig. 4B), indicating that lnc_000368 is potentially involved in adipogenesis. The expression of lnc_000368 in *longissimus dorsal* and *semitendinosus* muscles reached a peak at 30 and 90 days, respectively (Fig. 4C, D). By predicting target genes based on their location in the genome, interaction protein analysis was performed using Cytoscape software (Fig. 4E). Among these potential targets, TGFB1 (Transforming Growth Factor Beta 1) and GNAS (Guanine Nucleotide Binding Protein, Alpha) have been demonstrated to be involved in adipogenesis [14, 15]. RNA-FISH showed that lnc_000368 was distributed in both the nucleus and the cytoplasm in the back subcutaneous fat pads of 90-day-old pigs (Fig. 4F).

3.5 Lnc_000368 promotes intramuscular adipocyte differentiation

To explore the role lnc_00368 potentially plays during intramuscular adipogenesis, siRNAs targeting lnc_00368 were designed and transfected into intramuscular preadipocytes isolated from *longissimus dorsal*. The well-differentiated cells were harvested and subjected to further analysis at day 8 after adipogenic differentiation. Results showed that lnc_00368 siRNAs effectively reduced the expression of lnc_000368 (Fig. 5A). Adipogenic transcription factors such as PPARγ, CEBPβ, and aP2, were significantly repressed by lnc_000368 siRNA at the mRNA level, while the key enzymes of lipolysis, ATGL and HSL, were down-regulated (Fig. 5B). The expression of these marker genes at the protein level was reduced (Fig. 5C, D). Oil Red O staining revealed that lipid accumulation was significantly reduced upon siRNA transfection (Fig. 5F). Collectively, our data showed that lnc_000368 is a potential enhancer for intramuscular adipogenesis.

4 Discussion

Although lncRNAs have gained increasing attention in recent years, understanding of how lncRNA affects intramuscular fat deposition in pigs is still limited due to the interspecies specificity of lncRNA, even though intramuscular fat is an economically important feature in determining meat quality [2]. Past studies have shown that lncRNAs are associated with fat deposition [16], and thus we proposed that lncRNAs may also be involved in the regulation of intramuscular fat deposition in pigs. An interesting characteristic some studies have found is that even under the same differentiation conditions, intramuscular adipocytes in *longissimus dorsi* are bigger and contain more lipid droplets than in *semitendinosus* [10]. Resultantly, the intramuscular fat content in *longissimus dorsi* is higher than in *semitendinosus* in adult pigs [17, 18]. For this reason, in this study we selected intramuscular adipocytes from *longissimus dorsi* and *semitendinosus* muscles of Large White pigs for lncRNA-sequencing, aiming to identify the key lncRNAs that may be involved in the regulation of intramuscular adipocyte differentiation.

In this study, a total of 2135 lncRNAs, including 575 novel lncRNAs, were identified. Compared with protein-coding mRNAs, the number of exons and ORFs of lncRNAs were significantly lower in lncRNAs. In terms of conservation between species, lncRNAs were also far less conserved than mRNAs. The lncRNAs obtained through RNA-seq were roughly the same in terms of length, number of exons, and conservation, as those found in previous studies on porcine adipose tissues [19] and intramuscular fat [20]. KEGG analysis showed that the target genes of these lncRNAs were enriched in some classical signaling pathways related to fat formation, inflammation, and growth. This suggests that these differentially expressed lncRNAs may be involved in regulating the adipogenesis of intramuscular adipocytes.

Of the lncRNAs, lnc_000368 was gradually upregulated in intramuscular adipogenic differentiation in both *longissimus dorsi* and *semitendinosus* muscles; thus, we speculate that it may promote intramuscular adipocyte differentiation. Lnc_000368 was highly expressed in the adipose tissue of pigs, and additional analysis indicated that the knockdown of lnc_000368 might significantly repress lipid accumulation and reduce the expression of adipogenesis-related genes, such as PPARγ, CEBPβ, and aP2. Among them, PPARγ is a very important regulator of adipogenesis. In previous studies, PPARγ was considered to be almost the most critical factor that determines adipocytes differentiation [21, 22]. This suggests that lnc_000368 may be a novel enhancer of intramuscular adipogenesis.

In order to explore the underlying mechanism, cellular localization of lnc_000368 was detected using a FISH probe, and results showed that lnc_000368 was distributed in both the nucleus and cytoplasm. It illustrates that nuclear lncRNAs may participate in cellular activity through transcriptional regulation, dose compensation effect, enhancer regulation [23]. Cytoplasmic lncRNA may be involved in regulating mRNA stability, regulating mRNA translation, and acting as a competitive endogenous RNA or a precursor of microRNA [24]. Considering the cellular distribution, lnc_000368 may be a part of any of the mechanisms discussed above. Furthermore, among the target genes predicted by genomic position of lnc_000368, TGFB1 (Transforming Growth Factor Beta 1) [25] and GNAS (Guanine Nucleotide Binding Protein, Alpha) [26] have the potential to regulate adipocyte differentiation. Thus lnc_000368 likely

regulates the differentiation of intramuscular adipocytes by regulating these two genes. However, the specific mechanism of action requires further research.

In summary, our study screened out regulatory lncRNAs during the differentiation of porcine intramuscular adipocytes using RNA-sEq. With regards to intramuscular fat accumulation, adipocyte proliferation and differentiation are two important factors that contribute to lipid deposition [27]. In previous studies, lncRNAs were found to exert a regulatory role in the proliferation of porcine intramuscular adipocytes [9]. Our study showed that lncRNA may also modulate the adipogenic process of intramuscular adipocytes. Both of these regulatory lncRNAs could potentially be used as genetic markers in pig breeding.

5 Conclusions

In this study, we screened a group of lncRNAs that may be involved in the regulation of porcine intramuscular adipocyte differentiation, and performed qRT-PCR verification and KEGG analysis on them. From this we carried out a detailed analysis of lnc_000368, and then silenced in porcine intramuscular adipocytes, and found that the differentiation ability of the cells was reduced. Therefore, we concluded that lnc_000368 is a lncRNA that promotes the differentiation of porcine intramuscular adipocytes.

Abbreviations

IMF
intramuscular fat
LncRNA
long non-coding RNA
PPARγ
Peroxisome Proliferator Activated Receptor Gamma
aP2
Adipocyte-Type Fatty Acid-Binding Protein
CEBPβ
CCAAT Enhancer Binding Protein Beta

Declarations

Ethics approval and consent to participate

In this study, all experiments were approved by the Management Committee of Northwest A & F University and comply with animal welfare regulations.

Consent for publication

All authors agreed to publish.

Availability of data and material

All the data generated in this study are authentic and reliable, and the original sequencing data can be obtained by contacting the corresponding author.

Competing interests

The authors declare that no competing financial interests exist.

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Authors' contributions

XW wrote this paper and analyzed the data. YP and MX performed the cell culture and cell-based analysis. XC provided some methods. XS and GY gave critical suggestions about the experiment design and manuscript preparation. XL designed the experiment and revised the manuscript. All authors have read and approved the manuscript.

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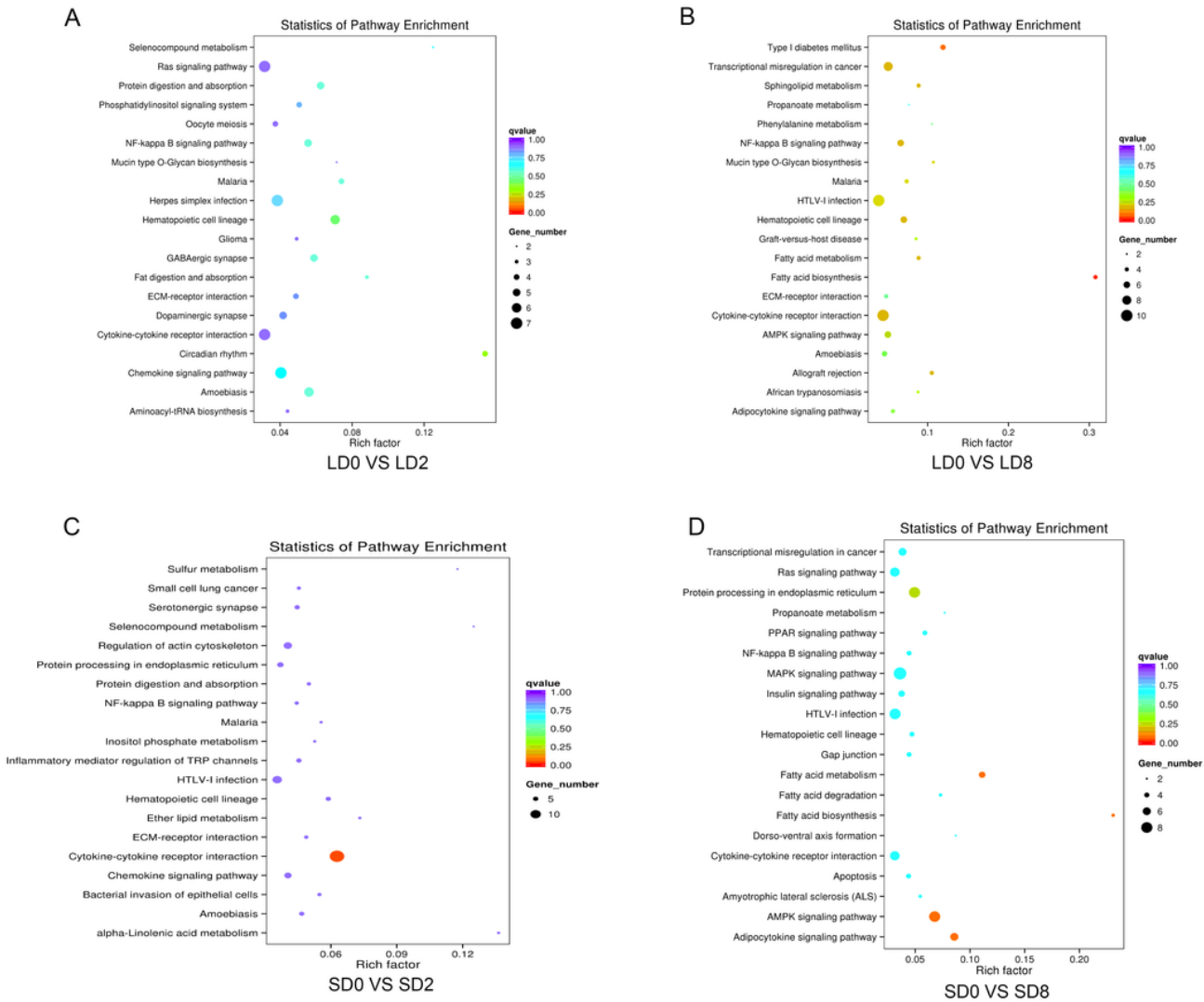
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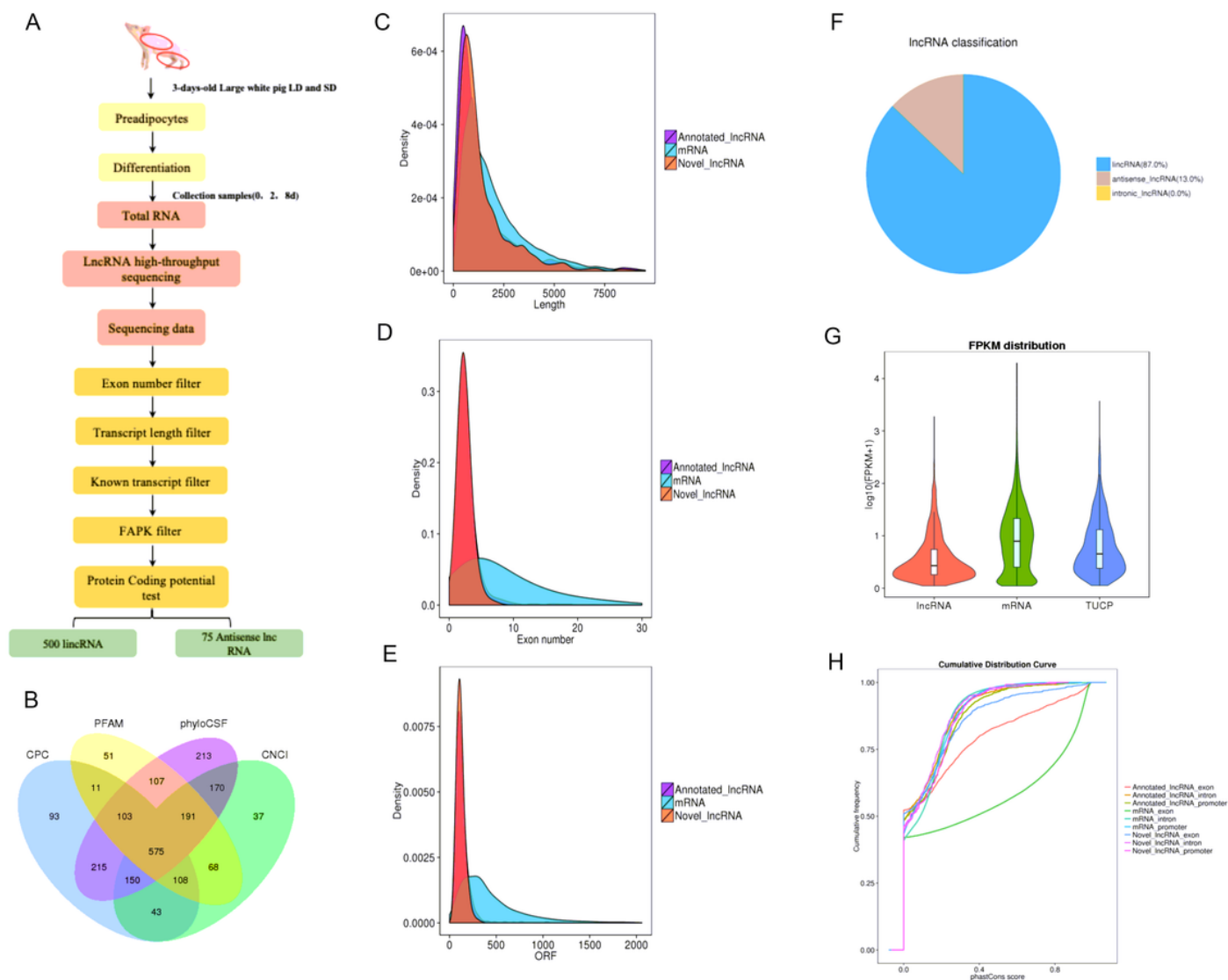
Figure 1

A general overview of porcine intramuscular fat lncRNA sequencing. (A) The process of sampling and sequencing the internal muscles of pigs. See the Materials and Methods section for details. (B) The coding potential of the lncRNA obtained by sequencing and the different databases were compared. The analysis of the coding potential of candidate lncRNAs was performed using four conventional methods (CPC, PFAM, phyloGSF and CNCI), and 575 lncRNAs were screened for follow-up analysis. (C) Transcription length distribution of mRNA and lncRNA. (D) Distribution of exon numbers of mRNA and lncRNA. (E) Distribution of ORF numbers of genes and lncRNA. (F) The classification of lncRNA obtained by sequencing, consisting of 87% of lncRNA and 13% of antisense lncRNA. (G) Expression levels of mRNA and lncRNA (represented by log10 (FPKM + 1). (H) Conservative analysis of mRNA and lncRNA.
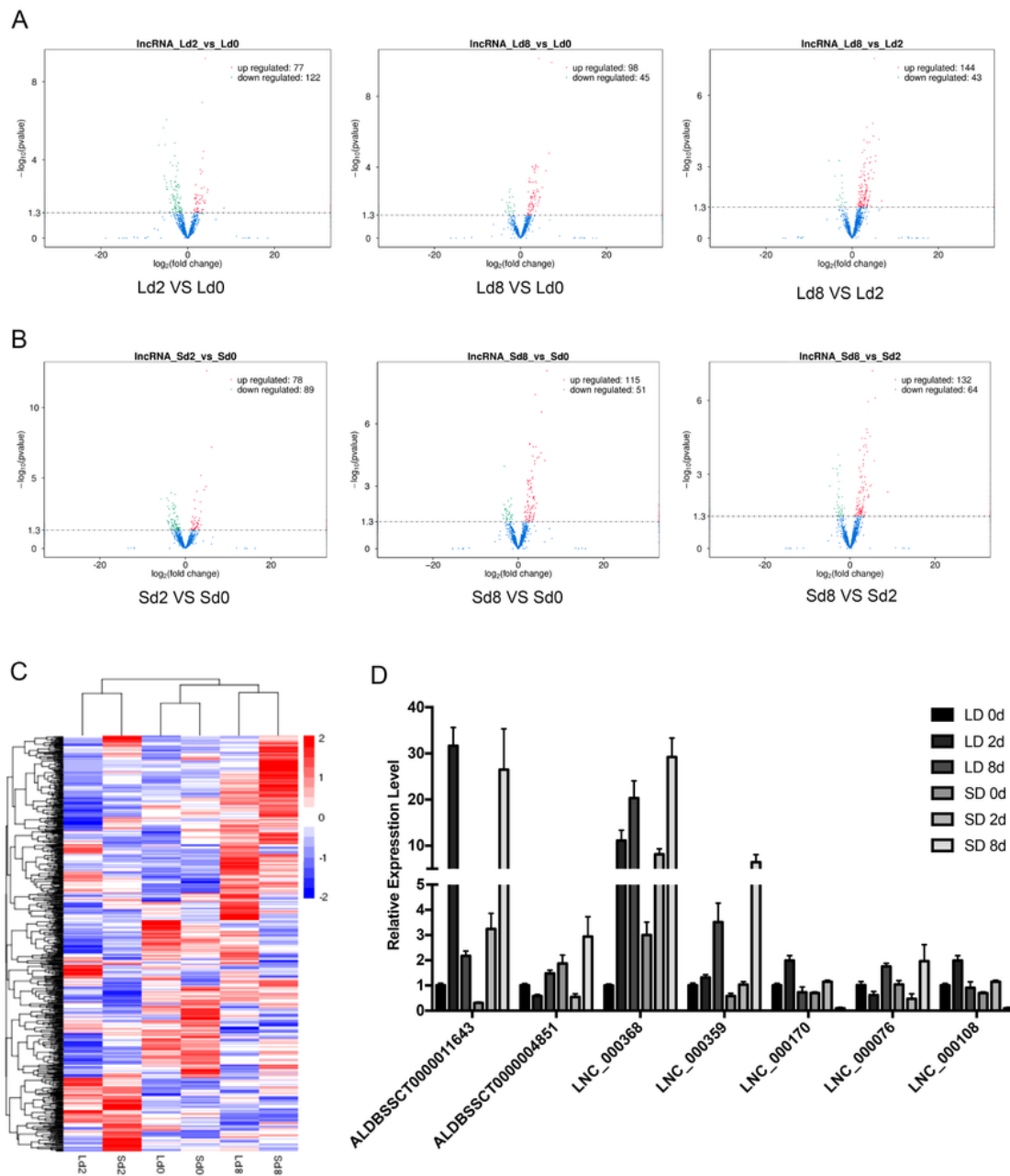
Figure 2

Expression levels of differentially expressed lncRNA in the differentiation of two intramuscular adipocytes. (A) Distribution of expression of lncRNA in the LD muscle. (B) Distribution of expression of lncRNA in the semitendinosus. (C) Heat map showing the differential distribution of presence in the differentiation of two different intramuscular adipocytes. (D) Detection of the relative expression levels of

lncRNA using a comparative cycle threshold (ΔCT) method, with β-actin as an internal reference gene. The data are shown as the mean ± SEM (n = 3).

Figure 3

KEGG analysis of DEGs. (A) Enrichment pathway within the LD muscle at day 0 and day 2. (B) Enrichment pathway within the LD muscle at day 0 and day 8. (C) Enrichment pathway within the SD muscle at day 0 and day 2. (D) Enrichment pathway within the SD muscle day 0 and day 8.
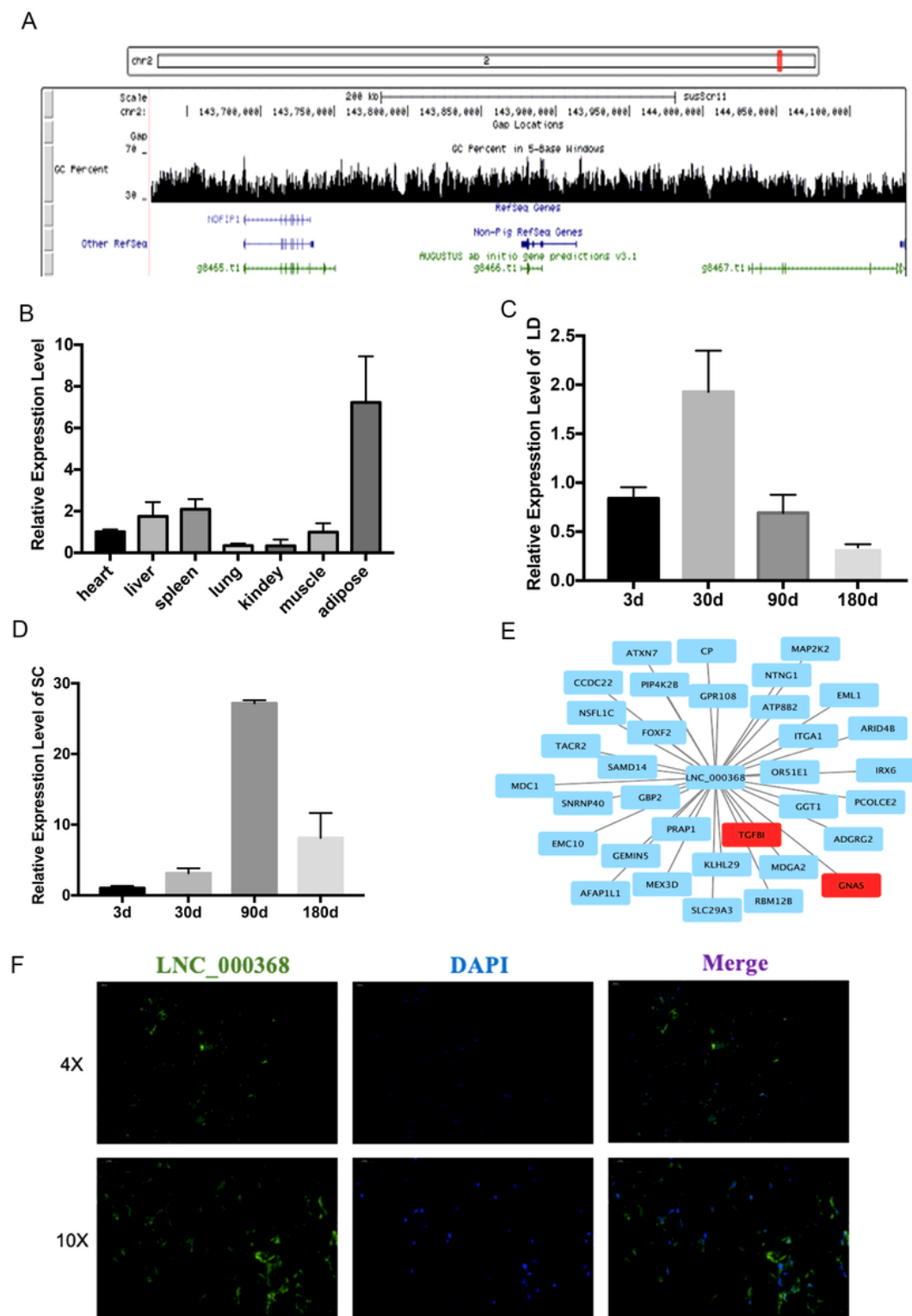
Figure 4

The spatiotemporal distribution of lnc_000368. (A) Schematic diagram of the transcription structure of lnc_000368. (B) 180-day-old pigs were tested for expression of lnc_000368 in different tissues. (C) The expression level of lnc_000368 was detected in the different day-old pigs. (D) The expression level of lnc_000368 was detected in the subcutaneous adipose tissue of pigs of different ages. (E) Cystoscope software was used to visualize the predicted protein product of lnc_000368. (F) FISH photographs of the

adipose tissue of 90-day-old pigs, the scale bar in the picture are 300 μm and 100 μm. The data are shown as the mean ± SEM (n = 3).
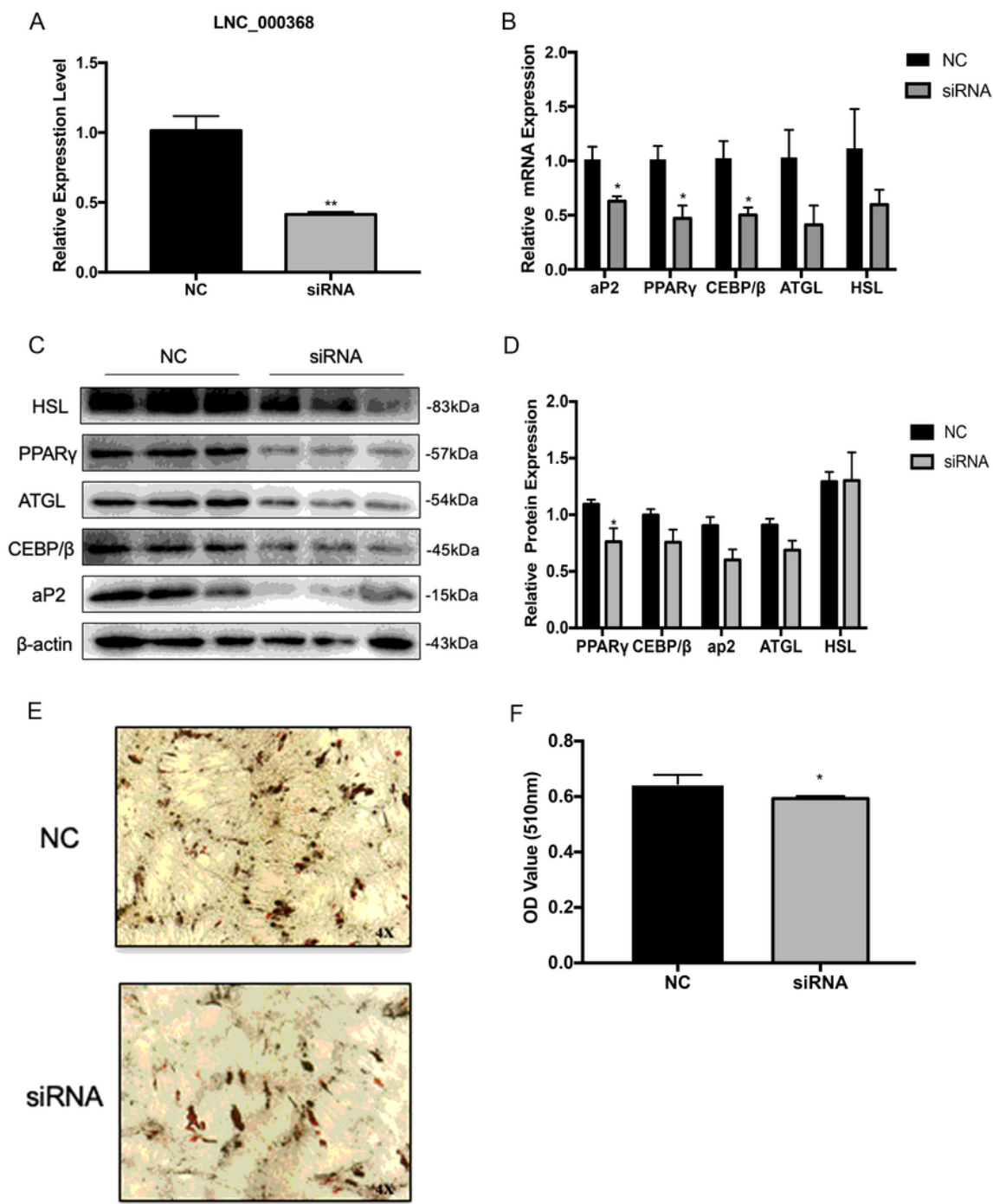

Figure 5

Lnc_000368 promotes intramuscular adipocyte differentiation. (A) Lnc_000368 was detected by transfecting cells with siRNA for 48 hours, thereby successfully interfering with the expression of lnc_000368. (B) qRT-PCR detection of intramuscular adipocytes induced by transfection 8 days after

transfection (C, D) Western blot analysis of intramuscular adipocytes induced by transfection 8 days after transfection. (E, F) The formation of lipid droplets in the intramuscular adipocytes after 8 days was detected by Oil Red O staining. The scale bar in the picture is 300 μm. The data are shown as the mean ± SEM (n = 3); *P < 0.05.

Supplementary Files

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

- AdditionalFile10lncRNAmRNAcolocationresults.xls
- AdditionalFile9Sd8vsSd0.EnrichedKEGGPathwaytop20ALL.xls
- AdditionalFile8Sd2vsSd0.EnrichedKEGGPathwaytop20ALL.xls
- AdditionalFile7Ld8vsLd0.EnrichedKEGGPathwaytop20ALL.xls
- AdditionalFile6Ld2vsLd0.EnrichedKEGGPathwaytop20ALL.xls
- AdditionalFile5lncRNASd8vsSd0.DEGALL.xls
- AdditionalFile4lncRNASd2vsSd0.DEGALL.xls
- AdditionalFile3lncRNALd8vsLd0.DEGALL.xls
- AdditionalFile2lncRNALd2vsLd0.DEGALL.xls
- AdditionalFile1NovellncRNA.fa