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The potential mechanism of MicroRNA involvement in the regulation of muscle development in weaned piglets by tryptophan and its metabolites

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Abstract

Background Muscle development is a key factor influencing the growth performance of piglets. Optimizing this developmental process is crucial for enhancing breeding efficiency and economic profitability. Tryptophan (Trp) is considered one of the key limiting amino acids for weaned piglets, plays an essential role in regulating feed intake, growth, and muscle development. However, the regulatory mechanisms by which Trp and its derivatives influence muscle development in weaned piglets remain unclear.

Methods The aim of this study was to investigate the regulatory pathways and potential mechanisms of Trp and its metabolites on muscle development in weaned piglets. In this study, 10 healthy castrated male piglets, 28 days old and weaned, were selected and randomly assigned to a control group (CON, 0.14% Trp) and a high tryptophan group (HT, 0.35% Trp), with 5 in each group. After a 7-day pre-feeding period, the formal feeding began, and after 28 days, the pigs were slaughtered and the longissimus dorsi muscles was collected for transcriptome sequencing.

Results The results indicated that different dietary Trp levels led to the identification of sixteen differentially expressed microRNAs (DE miRNAs) in the longissimus dorsi muscle of the weaned piglets. Target gene functional enrichment analysis showed that these DE miRNAs are involved in muscle cell proliferation, differentiation, protein deposition, and muscle development through multiple biological pathways. Furthermore, we constructed a protein-protein interaction (PPI) network for the target genes, with the enriched core gene cluster functions associated with cellular proliferation, signaling pathways, hormone release, and muscle development. Finally, qRT-PCR validated the reliability and accuracy of the RNA-seq results, revealing a correlation coefficient of 0.97 between the two methods.

Conclusions This study uncovers the potential mechanisms by which miRNAs participate in the regulation of muscle development in weaned piglets mediated by Trp and its metabolites, providing a theoretical basis and practical guidance for optimizing piglet management and health improvement.

Keywords Tryptophan, Weaned piglet, Muscle development, MicroRNAs, Transcriptome

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Background

The weaning phase is a pivotal and challenging period for piglets in the pork production industry [1]. At this stage, piglets must transition from a diet reliant on milk to one predominantly consisting of solid feed. This transition significantly influences their growth, health, and production capabilities [2]. Notably, muscle development is crucial for the overall growth and meat quality of piglets since it directly impacts their growth rates [3]. The processes of muscle cell proliferation, differentiation, and protein synthesis require effective regulation following weaning to ensure the health and productivity of the animals. Studies have shown that effective nutritional management, suitable growth environments, and robust genetic selection can significantly promote muscle development in piglets and enhance farming profitability [4].

Tryptophan (Trp) is an essential amino acid that serves as a critical component in protein synthesis and plays a significant role in regulating host growth, behavior, immune function, and stress response [5, 6]. Recent studies have highlighted the importance of Trp and its metabolites in animal growth and development, particularly in the regulation of muscle growth [7, 8]. Trp influences muscle development and growth by affecting cellular proliferation, differentiation, and apoptosis through its metabolites, including serotonin (5-HT) and kynurenine (KYN) [9]. Clinical research in humans has indicated that Trp supplementation can effectively counteract age-related muscle loss and atrophy [10], suggesting a direct relationship between Trp and muscle growth. Notably, Trp is considered the second or third limiting amino acid in typical grain-based diets for pigs. Early animal trials have shown that the addition of Trp to diets promotes growth and improves feed efficiency. Further analysis revealed a close correlation between ribosomal activity in muscle cells and Trp levels, indicating that Trp can enhance protein synthesis in muscle tissue [11]. However, in actual production, the nutritional intake of pigs is affected by many factors such as health status, nutritional purity, environmental factors, feeding management and feed palatability, so the daily nutritional intake of piglets may be insufficient [11]. 5-HT, a key metabolite of Trp, stimulates the proliferation of various smooth muscle cells. L6 cells, which are myoblasts derived from rat skeletal muscle, have demonstrated a significant enhancement in glucose transport and absorption efficiency of approximately 50% following treatment with 5-HT in vitro [12]. Additionally, 5-HT enhances the activity of fructose-1,6-bisphosphate in skeletal muscle through phospholipase C, promoting glycolytic reactions and improving glucose utilization in muscles [13]. These findings suggest that 5-HT and its receptors play a crucial regulatory role in glucose metabolism within muscle tissue. At the same time, melatonin, a further metabolite derived from 5-HT, can influence muscle growth and regeneration [14]. Moreover, KYN, the principal metabolite of Trp, has a notable negative impact on muscle growth [15, 16], however, research has confirmed that exercise can facilitate the conversion of KYN into non-neurotoxic kynurenic acid in muscle tissue, thereby mitigating KYN's detrimental effects [17]. This shows that Trp can support piglet adaptation to new conditions after weaning, reduce stress levels, promote gastrointestinal health, and help reduce group aggressive behavior, which is often a problem in intensive rearing [5, 6].

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by recognizing and binding to specific mRNAs, leading to translational repression or degradation. They play a significant role in animal muscle development [18]. Previous studies have identified numerous specific miRNAs associated with muscle development, among which miR-1, miR-206, and miR-133 were the first to be linked to this process. Research exploring the roles of miR-1, miR-206, and miR-133 in cultured myoblasts has revealed that they participate in the regulation of skeletal muscle proliferation and differentiation in distinct yet tissue-specific manners, indicating a mechanism through which they execute differentiation programs [18–20]. With the rapid advancement of high-throughput sequencing technology and bioinformatics, researchers can now efficiently screen and identify miRNAs involved in muscle development [21]. Recent studies increasingly indicate that miRNAs not only regulate the expression of genes associated with Trp metabolism but can also influence their own expression by modulating the activity of metabolic pathways [22]. However, the mechanisms by which miRNAs regulate Trp metabolism in the context of muscle development in weaned piglets remain unclear.

Therefore, building upon prior research, this study investigates the patterns of miRNA expression modulated by varying dietary levels of Trp during the muscle development of weaned piglets. Furthermore, we conducted a functional analysis of miRNA target genes to elucidate the potential pathways through which Trp metabolites influence muscle development. This research enhances our understanding of the role of Trp and its metabolites in muscle development and the regulatory mechanisms of miRNAs, providing new insights for future breeding management and nutritional strategies. By optimizing nutrition for weaned piglets and implementing targeted miRNA interventions, we may significantly improve their growth and feed conversion rates, ultimately leading to more efficient farming outcomes.

Materials and methods

Animal sample collection

In this study, we selected ten 28-day-old weaned, castrated male pigs of mixed breed (Duroc × Landrace × Yorkshire) and divided them into two groups: a control group (CON) and a high tryptophan group (HT), with five pigs per group. The average weight of the piglets in both groups was 6.54 kg. Existing studies have shown that the Trp content in weaned piglet diets recommended by the NRC (2012) is slightly insufficient, and at the same time, factors such as health status of the herd, nutritional purity, environmental factors, and feeding management can lead to lower nutrient intake [8, 23, 24]. However, increasing the Trp content of piglet diets can improve the growth performance and disease resistance of piglets [23]. In addition, based on previous studies we found that diets with an added amount of 0.14% Trp did not cause significant negative effects on weaned piglets [8, 23, 24]. Therefore, we set the level of tryptophan at 0.14% for the CON group and 0.35% for the HT group, i.e., 0.21% tryptophan was added to the diet of the CON group (Sangon Biotechnology Co., Ltd.). The specific composition and nutritional levels of the diet of the control group are shown in Table 1. Each piglet was individually housed in pens measuring 1.5 × 0.5 × 0.8 m, maintained at a controlled temperature of 27 ± 1.2℃ and relative humidity levels between 60% and 70%. The piglets were provided with unlimited access to food and water to ensure optimal growth and welfare. After a 7-day pre-feeding period, they were formally fed for 28 days. At the end of the feeding experiment, three piglets per group with body weights close to the group average were selected for intravenous pentobarbital sodium (50 mg/kg·BW) anesthesia and slaughtered. The longissimus dorsi muscle was then harvested from a location 5 cm laterally from the midline, positioned between the third and fourth to last ribs. The muscle specimens were rapidly frozen in liquid nitrogen and stored at -80℃, where they were kept for subsequent RNA extraction, library preparation, and sequencing.

Table 1 Ingredient composition and nutrient levels of experimental dietary(DM Basis %)

Ingredients	Content[d]
Corn	68.72
Soybean meal	8.07
Corn gluten meal	8.67
Whey	5
Wheat bran	5
Monocalcium phosphate	0.44
Limestone	1
NaCl	0.3
Trace mineral premix [a]	1
Threonine	0.18
Lysine	0.82
Methionine	0.1
Tryptophan [b]	0
Alanine	0.7
Total	100
Composition	
DE (MJ/kg) [c]	14.1
CP (%)	16.2
Ca (%)	0.73
CF (%)	2.6
Available P (%)	0.36
Lysine (%)	1.27
Methionine (%)	0.37
Threonine (%)	0.75
Tryptophan (%)	0.14

[a] Provided the following per kilogram of diet: 1845 IU vitamin A, 200 IU vitamin D, 12 IU vitamin E, 0.48 mg vitamin K, 9.3 mg pantothenic acid, 3.1 mg riboflavin, 0.29 mg folic acid, 29.0 mg niacin, 1.0 mg thiamine, 4.3 mg vitamin B6, 0.05 mg biotin, 0.016 mg vitamin B12, and 0.43 g choline. Provided the following per kilogram of diet: 84 mg Zn, 97 mg Fe, 3.2 mg Mn, 5.2 mg Cu, 0.14 mg I, and 0.259 mg Se

[b] L-tryptophan was purchased from Sangon Biotech (Shanghai, China)

[c] DE was calculated value and others were measured values of a corn and soybean-based diet. Abbreviations: DE, digestible energy; CP, crude protein; CF, crude fiber

[d] CON: Tryptophan content is 0.14%; HT: Tryptophan content is 0.35%. The same below

Total RNA isolation, library construction, and RNA-seq

The total RNA of the longissimus dorsi muscle was extracted by TRIzol (Invitrogen, CA, USA) method, and the RNA samples were strictly controlled. During the assessment of total RNA quality, we systematically utilized agarose gel electrophoresis, NanoDrop spectrophotometry, and Agilent 2100 bioanalyzer to evaluate RNA degradation, contamination, purity, and integrity. Thereafter, library construction was performed using the Hieff NGS® Ultima Dual-mode RNA Library Prep Kit (Yeasen, Shanghai, China), which involved directly ligating adapters to both ends of the Small RNA, reverse transcribing to synthesize cDNA, followed by PCR amplification. The target DNA fragments were separated using PAGE gel electrophoresis, and the cDNA library was obtained through gel extraction. Subsequently, Qubit2.0 and Agilent 2100 were employed to measure the library's concentration and insert size, while qPCR was used to accurately quantify the effective concentration, ensuring the library's quality. Following the validation of library quality, the libraries were pooled according to their effective concentrations and the data requirements for targeted sequencing. The libraries corresponding to six muscle samples were designated as CON-1, CON-2, CON-3, HT-1, HT-2, and HT-3, and subsequently underwent Illumina SE50 sequencing. The sequencing work was delegated to Novogene (Beijing, China).

Raw RNA-seq data processing

The Raw reads obtained from high-throughput sequencing underwent quality control to discard low-quality sequences, resulting in clean reads. During this stage, the Q20, Q30, and GC content of the clean reads were calculated. Sequences with lengths between 18 and 35 nt were selected for further analysis. The filtered sequences were aligned to the *Sus scrofa* reference genome using Bowtie2 and HISAT2 (https://mart.ensembl.org/Sus_scrofa). Successfully aligned sequences were then classified for miRNA types and quantities using the Rfam database. Following this, known and novel miRNAs were identified through alignment with the miRBase database, employing miREvo and mirdeep2 software for identification. Lastly, expression levels for all miRNAs across the samples were statistically analyzed, and expression normalization was achieved using TPM, calculated by the formula: TPM = actual miRNA counts/total counts of clean tags × 10^6 [21].

Analysis of differentially expressed MiRNAs

The analysis of differentially expressed (DE) miRNAs was conducted using DESeq2 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html). DE miRNAs were identified with the thresholds of Padj < 0.05 and|fold change| > 1.2.

DE MiRNA target gene prediction and target gene protein-protein interaction analysis

MiRNAs target gene prediction was conducted using RNAhybrid and Miranda software, followed by the construction of a target relationship network. Subsequently, all target gene information was collected and imported into the STRING database (https://cn.string-db.org/cgi/input?sessionId=bkQlBViDKMCh&input_page_active_form=multiple_identifiers), selecting the species as *Sus scrofa* to retrieve Protein-Protein Interaction (PPI) information. This information was then imported into Cytoscape 3.10.0 to construct the PPI network. Finally, based on the PPI network, gene clusters and their interaction networks were filtered and organized using the MCODE plugin with default parameters in Cytoscape 3.10.0.

Table 2 Primer information for DE MiRNAs

Gene	Primer sequences (5′-3′)	GenBank No.
ssc-miR-363	UUAACGUGCCAUAGGUAGACAUU	MN753044.1
ssc-miR-183	GUGAAUUACCGAAGGGCCAUA	NR_038495.1
ssc-miR-106a	AAAAGUGCUUACAGUGCAG-GUAGC	NR_038481.1
ssc-miR-20b	CAAAGUGCUCACAGUGCAGGUAG	MN753764.1
ssc-miR-128	CGGGGCCGUAGCACUGUCUGA	MN753288.1
ssc-miR-7135-3p	UAGACAGACAGAGAGACUCGUC	MN753285.1
ssc-miR-29a-3p	GCUGGUUUCAUAUGGUG-GUUUAGA	NR_038524.1
ssc-miR-10,390	AUACUACUGACAGACCGCAACCU	NR_162175.1
U6	AACGCTTCACGAATTTGCGT	XM_021068422.1

Functional enrichment analysis of MiRNA host genes and core target genes

The enrichment analyses of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) are essential bioinformatics tools for elucidating the functions and biological processes of gene sets. In this research, GOseq (https://www.bioconductor.org/packages/release/bioc/html/goseq.html) and the Kobas database (http://bioinfo.org/kobas) were utilized to perform GO and KEGG enrichment analyses on the DE miRNAs target genes and the core genes of gene clusters. This allowed for an investigation into the potential mechanisms by which miRNAs participate in the regulation of muscle development. In addition, we performed GO and KEGG analyses through the ClueGO plugin in Cytoscape 3.10.0, investigating biological processes, cellular components, and molecular functions, and exploring the related pathways that these functions may influence. Finally, we utilized the OmicShare Bioinformatics Cloud Platform (https://www.omicshare.com) to perform gene set enrichment analysis (GSEA) on the gene sets associated with the relevant pathways.

qRT-PCR validation of RNA-seq results

To validate the reliability and accuracy of the RNA-seq sequencing results, we randomly selected eight out of the sixteen DE miRNAs for qRT-PCR verification, including four upregulated miRNAs and four downregulated miRNAs. cDNA of the miRNAs was amplified using the 5× All-In-One RT MasterMix [Applied Biological Materials (ABM) Inc., Shanghai, China] kit, followed by qRT-PCR using the Mir-X miRNA qRT-PCR TB Green Kit (Takara, Shanghai, China). Three muscle samples from each group were tested in quadruplicate, with U6 used as the reference gene. The results of the RT-qPCR were calculated using the comparative CT method ($2^{-\Delta\Delta CT}$). Finally, the correlation between the RNA-seq and qRT-PCR results was computed. Table 2 presents the primer sequences for DE miRNAs and U6.

Results

Quality assessment of MiRNA sequencing results

RNA sequencing analysis of cDNA libraries from six muscle samples was conducted using the Illumina SE50 platform, generating a total of 97,327,125 raw reads. After removing low-quality sequences, 96,147,132 clean reads remained, accounting for over 98.78% of the original reads. The error rate for each sample was 0.01%, with Q20 values ranging from 98.92 to 99.39%, Q30 values from 95.81 to 97.56%, and GC content between 46.19% and 48.21% (Table 3). These results indicate that the

Table 3 Filtering statistics for sequencing data

Sample name	Raw reads	Clean reads	Raw bases (G)	Clean bases (G)	Error rate (%)	Q20	Q30	GC content
CON-1	18,975,131	18,743,745	0.949	0.94	0.01%	99.33%	97.53%	46.68%
CON-2	16,870,425	16,702,572	0.844	0.84	0.01%	99.39%	97.56%	47.52%
CON-3	15,304,424	15,069,554	0.765	0.75	0.01%	99.31%	97.30%	47.52%
HT-1	16,083,697	15,928,406	0.804	0.80	0.01%	98.92%	95.81%	48.21%
HT-2	13,517,690	13,350,870	0.676	0.67	0.01%	99.37%	97.44%	46.19%
HT-3	16,575,758	16,351,985	0.829	0.82	0.01%	99.28%	96.91%	46.92%

Table 4 Statistics of known MiRNA comparisons for each sample

Sample name	CON-1	CON-2	CON-3	HT-1	HT-2	HT-3
Total sRNA	18,524,784	16,515,096	14,655,579	15,524,774	13,196,363	16,137,203
Mapped sRNA	17,487,417 (94.40%)	15,433,383 (93.45%)	13,878,862 (94.70%)	14,873,098 (95.80%)	12,432,269 (94.21%)	15,133,502 (93.78%)
"+"Mapped sRNA	11,578,674 (62.50%)	10,282,925 (62.26%)	10,062,591 (68.66%)	10,876,107 (70.06%)	8,209,646 (62.21%)	10,549,462 (65.37%)
"-"Mapped sRNA	5,908,743 (31.90%)	5,150,458 (31.19%)	3,816,271 (26.04%)	3,996,991 (25.75%)	4,222,623 (32.00%)	4,584,040 (28.41%)
Mapped mature	335	328	332	332	317	327
Mapped hairpin	304	302	302	306	294	297
Mapped uniq sRNA	3892	4089	3962	4175	3421	3852
Mapped total sRNA	10,938,723	9,758,589	8,796,516	9,559,428	7,564,203	9,314,951

sequencing data from the six muscle samples meet the requirements for subsequent functional analyses. To further quantify miRNA and identify novel miRNAs, we aligned the length-filtered small RNA (sRNA) to the *Sus scrofa* reference sequence to analyze the distribution of sRNA on the reference sequence. The CON group contained 16,565,153 total reads, while the HT group had 14,952,780 total reads, with mapped sRNA making up 93.78–95.80% of the total reads for each sample. Additionally, the proportion of reads mapped to the same strand of the reference sequence varied from 62.21 to 70.06%, while those mapped to the opposite strand ranged from 25.75 to 32.00%. Subsequently, to identify novel miRNAs and better understand their expression and function within cells, we compared the mapped reads from the reference sequence to sequences within a specified range in miRBase, resulting in detailed information about the matched sRNA, including the secondary structure of known miRNAs, sequences, lengths, and occurrence frequencies. The number of mature miRNA forms matched in each sample ranged from 317 to 335, while precursor miRNAs matched between 294 and 306. The variety of aligned sRNAs was between 3,421 and 4,175, and the total counts of aligned sRNAs varied from 7,564,203 to 10,938,723 (Table 4). This data suggest that the current sequencing results can be utilized for further analysis of the potential mechanisms by which Trp affects muscle development in piglets.

Identification, screening and expression pattern analysis of MiRNAs

We screened sRNA within a specific length range from the clean reads of each sample for subsequent analysis. Generally, the length of sRNAs falls within the range of 18 to 40 nt, with miRNAs primarily clustered around 22 nt. In this study, miRNA types and quantities were identified using the Rfam database. The distribution frequency of miRNAs in the CON group was 60.28% (Fig. 1A), while in the HT group it was 61.69% (Fig. 1B). Figure 1C and D illustrate the classification results of all sRNAs, showing that identified miRNAs accounted for 63.05% of all sRNAs in the CON group and 62.22% in the HT group (Supplementary Table 1). Additionally, the Pearson correlation coefficients between the sample groups were greater than 0.973 (Fig. 1E and Supplementary Table 2), and the PCA results indicated good reproducibility within the groups, with substantial component differences: the first principal component accounted for 47.2% and the second for 15.7% (Fig. 1F). TPM density distribution provided an overall assessment of gene expression patterns across samples, with normalized miRNA expression levels showing remarkable consistency among all samples (Fig. 1G-H and Supplementary Table 3). Taken together, the identification, selection, and expression pattern analysis of miRNAs suggest that the sequencing data obtained are suitable for further functional analysis.

Analysis of DE MiRNAs

To elucidate the potential mechanisms by which miRNAs are involved in Trp regulation of muscle development in weaned piglets, we assessed the expression levels of miRNAs in the longissimus dorsi muscles of each group and performed differential expression analysis. The results indicated that compared to the CON library, the HT library contained 16 DE miRNAs, with 10 miRNAs being upregulated (ssc-miR-9, ssc-miR-9-1, ssc-miR-9843-3p,

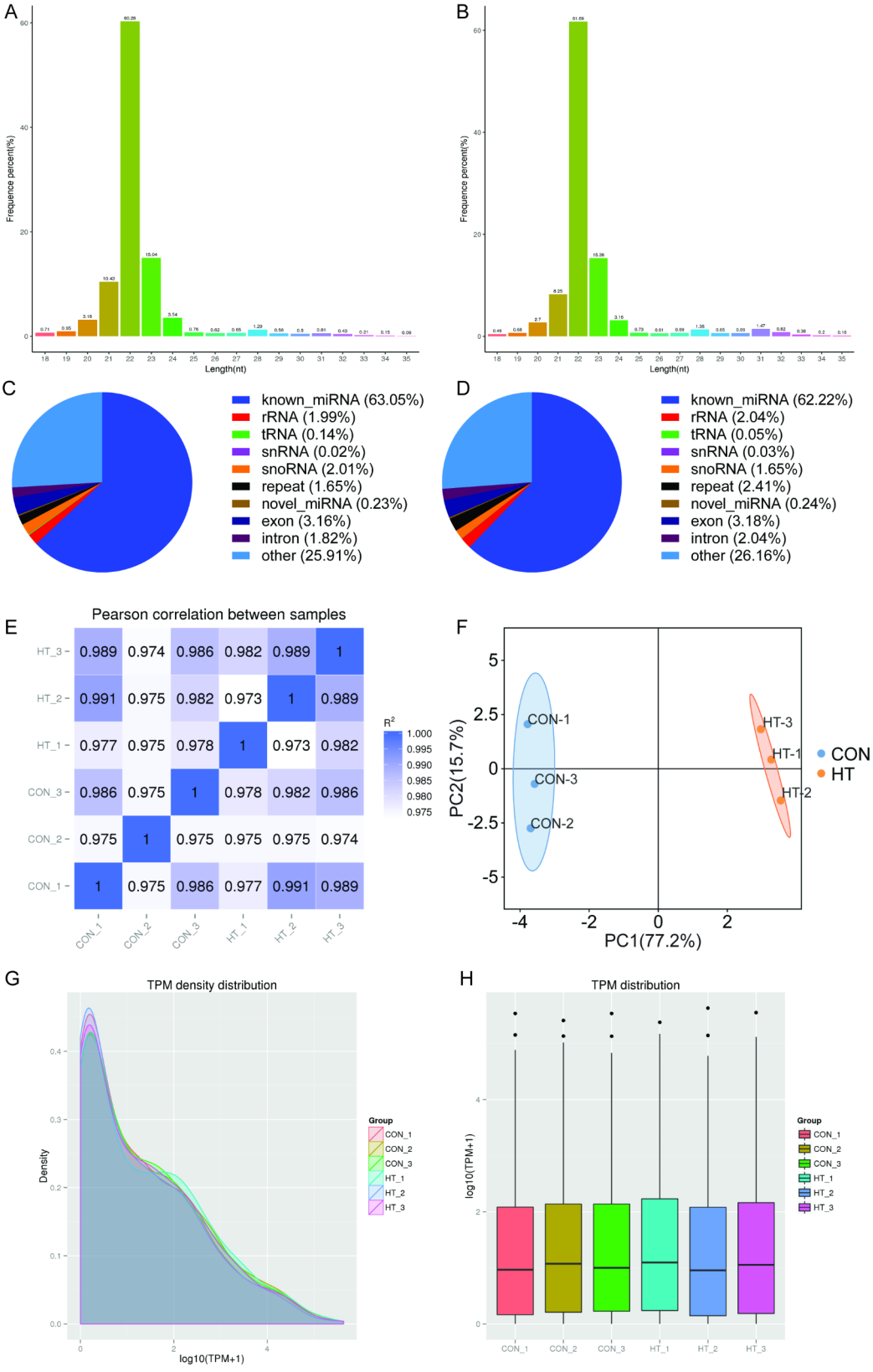

Fig. 1 (See legend on next page.)

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Fig. 1 MiRNA identification, screening and expression pattern analysis in longissimus dorsi muscles ($n = 3$). (**A**-**B**) Statistics of the length distribution of the total sRNA fragments obtained, where the horizontal coordinate is the length of the reads and the vertical coordinate is the proportion of reads of that length. (**C**-**D**) Classification statistics of sRNAs. (**E**) Inter-sample correlation check, where the horizontal and vertical coordinates are log10(TPM + 1) for the samples (R2: square of pearson correlation coefficient; Rho: spearman correlation coefficient; Tau: kendall-tau correlation coefficient). (**F**) Plot of PCA scores for CON and HT. (**G**-**H**) The TPM density distribution enables an overall examination of the gene expression pattern of the samples, where the horizontal coordinate is the log10(TPM + 1) value of the miRNA and the vertical coordinate is the density corresponding to log10(TPM + 1)

ssc-miR-218-5p, ssc-miR-497, ssc-miR-363, ssc-miR-183, novel_80, ssc-miR-106a and ssc-miR-20b) and 6 miRNAs downregulated (ssc-miR-10390, ssc-miR-34c, ssc-miR-29a-3p, ssc-miR-7135-3p, ssc-miR-128 and ssc-miR-182) (Fig. 2A-B and Supplementary Table 4). To further explore the expression patterns of DE miRNAs in the CON and HT groups, we conducted hierarchical clustering analysis and generated a heatmap of DE miRNAs. The results demonstrated significant differences in the expression of these miRNAs between the two groups (Fig. 2C).

Target gene network construction of DE MiRNAs

To further investigate the functions of DE miRNAs in the longissimus dorsi muscles and their potential role in Trp-mediated regulation of muscle development in weaned piglets, we predicted the target genes of DE miRNAs using RNAhybrid and Miranda software, constructing a target interaction network consisting of 16 DE miRNAs, 441 target genes, and 457 nodes. These target genes may play a crucial role in the regulation of muscle development in weaned piglets by DE miRNAs (Fig. 3 and Supplementary Table 5).

Functional enrichment analysis of target genes for DE MiRNAs

The results of GO enrichment analysis for the target genes of DE miRNAs indicated that there are a total of 241 significantly enriched pathways ($P < 0.05$), many of which are related to crucial cellular functions such as cell division, cell proliferation, cell signaling, muscle development, positive regulation of growth, growth factor activity, and response to amino acid starvation (Fig. 4A and Supplementary Table 6). In addition, the KEGG enrichment analysis showed that there are 40 pathways significantly enriched ($P < 0.05$), several of which pertain specifically to muscle development. These pathways encompass cellular metabolism, signaling pathways, the Notch signaling pathway, the PI3K-Akt signaling pathway, the cGMP-PKG signaling pathway, the mTOR signaling pathway, the MAPK signaling pathway, the PPAR signaling pathway, and the thyroid hormone signaling pathway (Fig. 4B and Supplementary Table 7). To further elucidate the role of Trp on its targets, we conducted ClueGO enrichment analysis based on the construction of DE miRNA target genes. The results indicated that the target genes were significantly enriched in 12 pathways ($P < 0.05$), specifically anatomical structure formation involved in morphogenesis, notch signaling pathway, regulation of gene expression, epigenetic, regulation of cell growth, regulation of apoptotic signaling pathway, proteasomal protein catabolic process, positive reguiaton of cell adhesion, negative regulation of protein modification process, muscle cell differentiation, positive reguiaton of MAPK cascade, mitotic cell cycle and small molecule catabolic process (Fig. 4C). GSEA enrichment analysis showed that DE miRNAs were mainly enriched in relevant metabolic pathways (Fig. 4D-F). This suggests that Trp and its metabolites may regulate biological processes such as cell adhesion, proliferation, apoptosis, and differentiation through several key signaling pathways, thereby influencing muscle protein deposition. Additionally, epigenetic factors and protein modifications can also regulate the expression of genes associated with muscle development.

PPI network construction of DE miRNA target genes in longissimus dorsi muscles and its gene cluster acquisition

The PPI network is formed by the interactions between proteins, and constructing this network is essential for systematically studying the mechanisms by which DE miRNAs affect their target genes. In this study, we used the STRING database and Cytoscape 3.10.0 to construct a PPI network for the 411 identified target genes. After filtering out nodes with lower interaction significance, the network was left with 136 nodes and 242 edges. Each node represents a target, with node color intensity and size indicating the strength of interaction—darker colors and larger radii reflect higher degree values and stronger interactions (Fig. 5A and Supplementary Table 8). Following this, we used the MCODE plugin in Cytoscape 3.10.0 to analyze the PPI network, leading to the identification of two major gene clusters and their interaction networks. The first cluster contains 15 genes (Fig. 5B), while the second cluster consists of 10 genes (Fig. 5C). The functions of these two key gene clusters are primarily associated with biological processes including cell proliferation, signaling mechanisms, hormone release, and muscle development (Fig. 5D-E and Supplementary Table 9).

Validation of DE MiRNAs

The qRT-PCR validation of muscle samples indicates that the reliability and accuracy of the sequencing results in

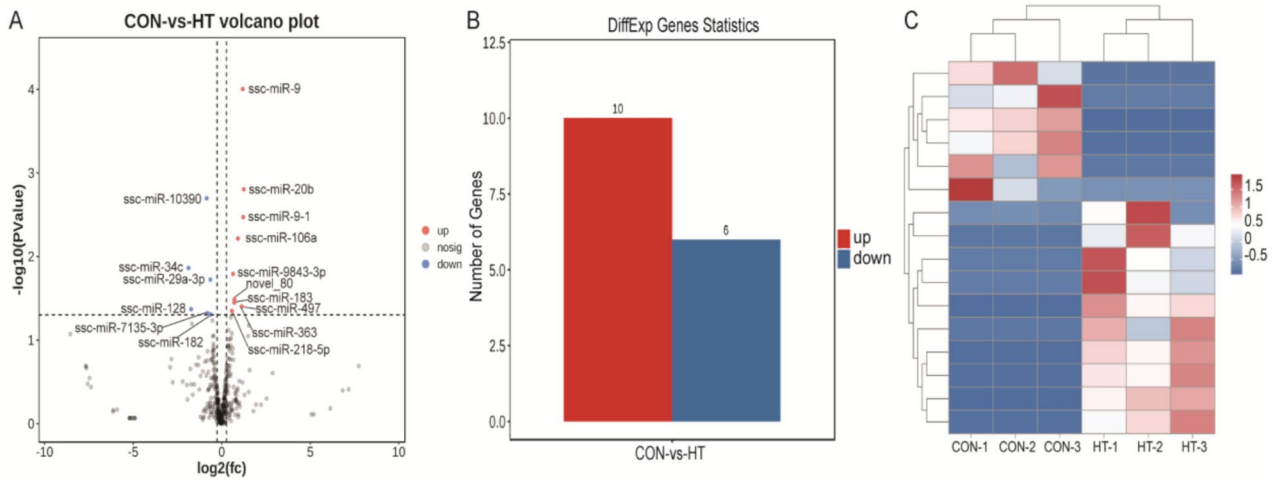

Fig. 2 Distribution and functional enrichment analysis of DE miRNAs in longissimus dorsi muscles ($n=3$). (**A**) Volcano plot of DE miRNAs in muscle samples. (**B**) Number of DE miRNAs in muscle samples. (**C**) Heatmap of DE miRNAs in muscle samples. Up-regulated expression is indicated in red and down-regulated expression is indicated in blue

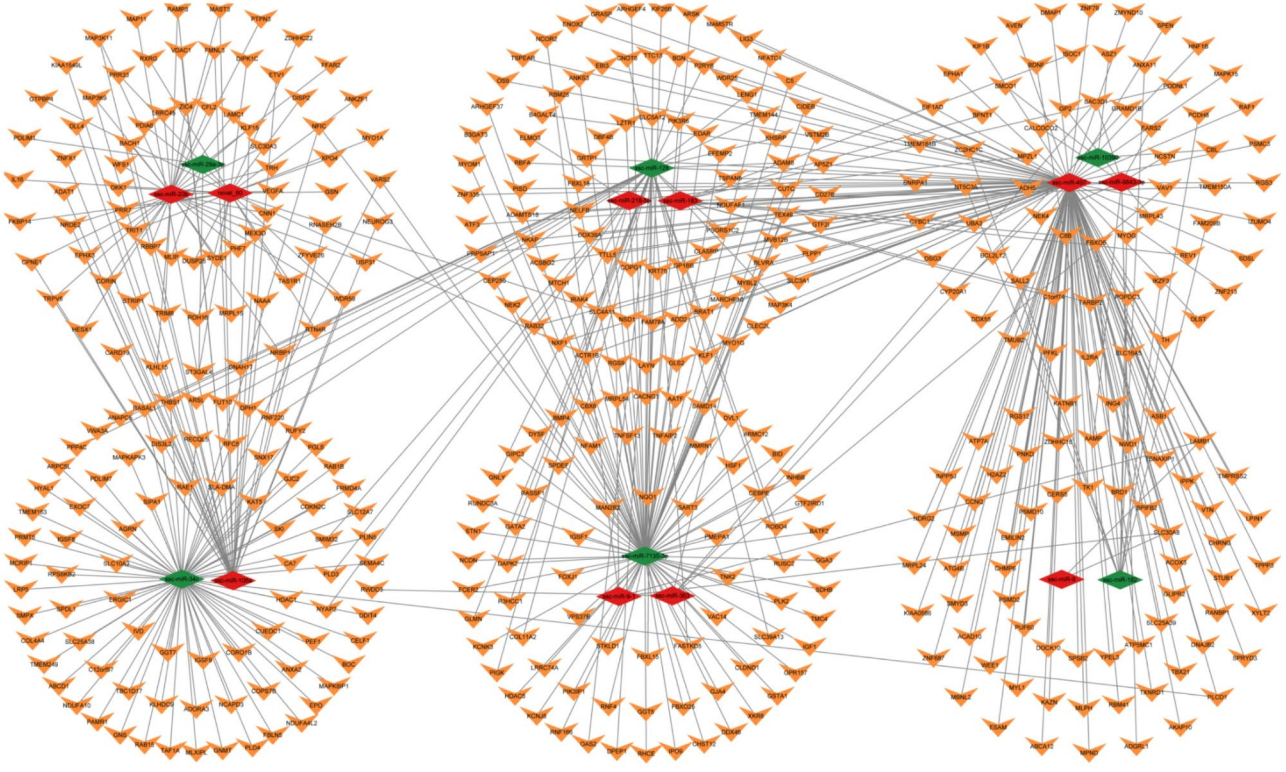

Fig. 3 Target gene network of DE miRNAs in longissimus dorsi muscles ($n=3$). Red diamonds represent up-regulated DE miRNAs, green diamonds represent down-regulated DE miRNAs, and orange cones represent target genes of DE miRNAs. The connecting line between them represents the targeting relationship between DE miRNA and its target gene

this study are quite good, mainly reflected in the fact that the trends of the qRT-PCR data are the same as those of the RNA-seq sequencing data, with minimal differences between the two datasets (Fig. 6A), and a correlation coefficient of 0.97 between them (Fig. 6B).

Potential mechanism of miRNA-mediated trp regulation of muscle development in weaned piglets

We combined the enrichment results of DE miRNAs target genes and the reference pathway map of core gene clusters to reconstruct the potential pathways through which miRNAs mediate the regulation of Trp and its

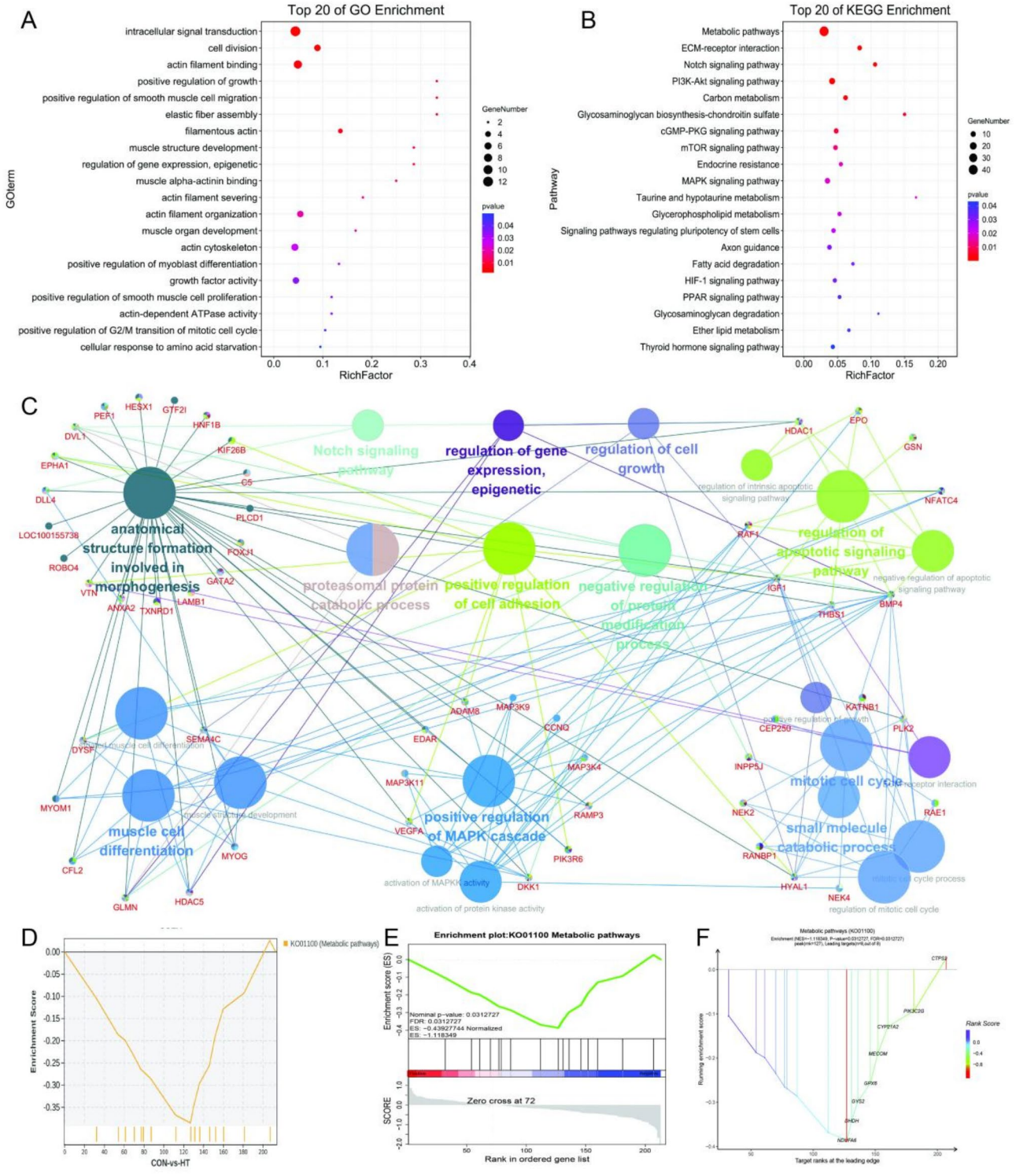

Fig. 4 Functional enrichment analysis of target genes for DE miRNA in longissimus dorsi muscles ($n=3$). (**A**) GO enrichment analysis of DE miRNA target genes (TOP 20). (**B**) KEGG enrichment analysis of DE miRNA target genes (TOP 20). (**C**) Target gene and corresponding pathway interaction networks. Circles of different sizes represent different pathways and corresponding genes, and the connecting lines between the circles represent the connectivity between the pathways and corresponding genes. (**D**-**F**) GSEA enrichment analysis of DE miRNA target genes

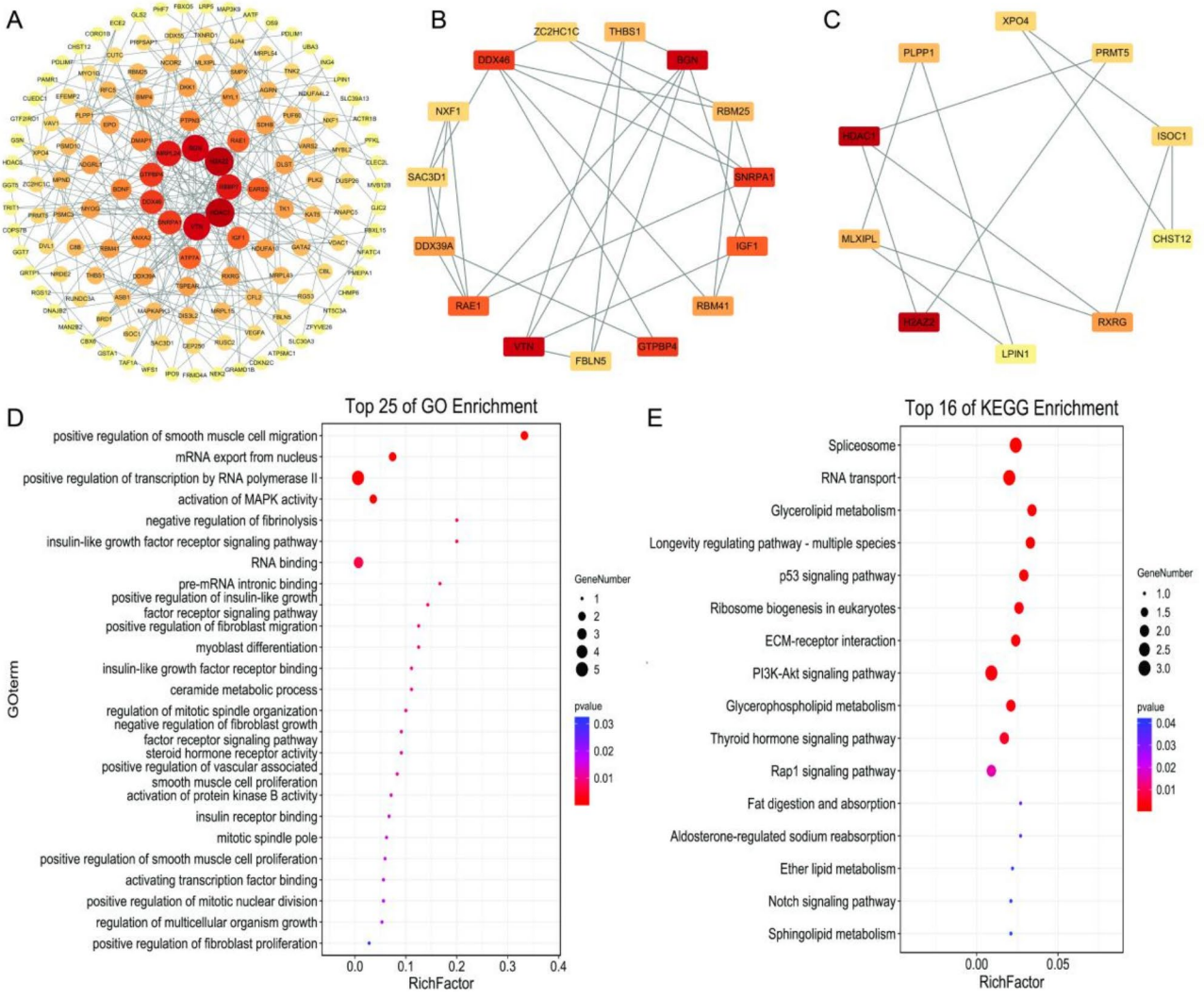

Fig. 5 PPI network construction, gene cluster screening and functional enrichment analysis of DE miRNA target genes in longissimus dorsi muscles ($n=3$). (**A**) DE miRNA target gene PPI network, nodes of different sizes and colors represent different proteins, and the connecting lines indicate the interactions between different proteins; the larger the circle and the darker the color, the higher the degree of connectivity of the protein. (**B**-**C**) Core gene clusters obtained by MCODE plugin screening. (**D**) GO enrichment analysis of core gene clusters (TOP25). (**E**) KEGG enrichment analysis of core gene clusters (TOP16)

metabolites in the muscle development of weaned piglets. The PI3K-AKT, mTOR, cGMP-PKG, MAPK, and North signaling pathways may be the primary biological pathways through which miRNAs are involved in the regulation of Trp and its metabolites in weaned piglet muscle development (Fig. 7).

Discussion

Muscle constitutes the main component of pork, and sufficient muscle mass correlates with increased meat production. The goal of many breeders is to promote the healthy development of piglet muscles through scientific feeding practices and genetic selection. Furthermore, proper muscle development can enhance piglets' resistance to diseases, thereby reducing the incidence of illness, which is vital for decreasing production costs and improving profitability. Healthy muscle development also facilitates normal physical activity in piglets, contributing to better growth performance and overall quality of life. In our earlier research, we discovered that incorporating an appropriate level of Trp into the diets of weaned piglets significantly improved their average daily feed intake, average daily weight gain, longissimus dorsi muscle weight, muscle protein concentration, and muscle fiber density, while also significantly lowering the feed-to-gain ratio [8]. One of the main functions of Trp as an essential amino acid is to participate in protein synthesis. In addition to this, Trp is converted to related metabolites through the 5-HT pathway, the kynurenine pathway and the indole pathway to regulate life processes. Previous

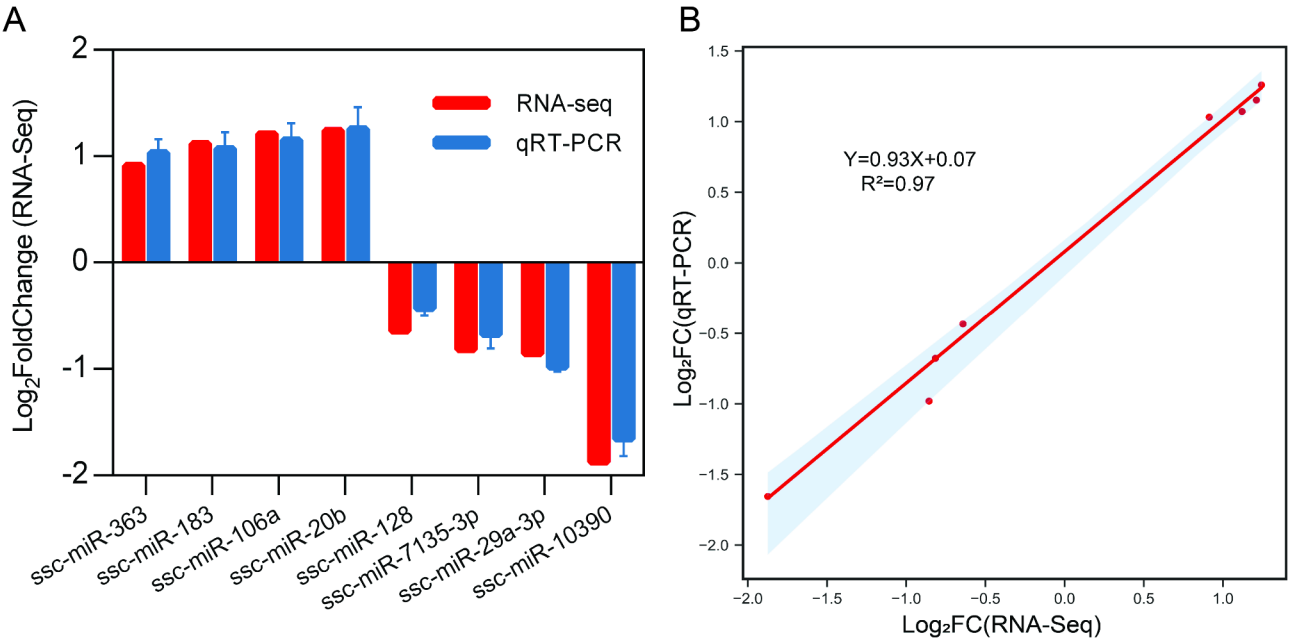

Fig. 6 Validation of DE miRNAs in longissimus dorsi muscles ($n=3$). (**A**) DE miRNAs were validated using qRT-PCR. (**B**) RNA-seq and qRT-PCR correlation analysis of DE miRNAs

studies have demonstrated that Trp and its metabolites are crucial in regulating the proliferation of muscle cells and muscle development in both humans and animals [25]. For example, the addition of rumen-protected Trp to the diets of castrated bulls promotes muscle development by upregulating the expression of MYF6, MyoG, FABP4, and LPL genes in the longissimus dorsi muscle, thereby enhancing the animals' ability to metabolize and transport fat within the muscle [26]. Conversely, a diet lacking in Trp significantly weakens the glycolytic response in skeletal muscle [27], adversely affecting animal weight and muscle development. Furthermore, in vitro experiments have confirmed that low levels of Trp inhibit the proliferation and differentiation of C2C12 myoblasts [28]. This indicates that an appropriate supplementation of Trp positively impacts skeletal muscle growth, development, and metabolism in animals [26, 29, 30]. Additionally, moderate adjustments to dietary nutritional levels can result in differential expression of non-coding RNAs in tissues or cells, which in turn regulates the expression of related genes, influences cell proliferation, differentiation, and function, and affects the stability and translation efficiency of relevant mRNAs [31, 32]. Therefore, building upon our earlier research, we conducted miRNA transcriptome sequencing of the longissimus dorsi muscle in piglets to uncover the potential mechanisms by which miRNAs regulate Trp's influence on the muscle development of weaned piglets.

We observed data discrepancies during the selection and identification of miRNAs from the sequencing data after quality control and alignment. The distribution frequencies of miRNAs in the CON and HT groups were 60.28% and 61.69%, respectively, while the proportion of miRNAs within the sRNA of muscle samples in each group was 63.05% and 62.22%. This variation is primarily attributed to the fact that miRNAs predominantly range from 20 to 24 nt in length, with a peak at 22 nt [33]. As a result, the proportion of miRNAs in the sRNA is slightly elevated. Principal Component Analysis (PCA) indicated that Trp modification in the diets of piglets altered the expression of miRNAs in muscle, suggesting that miRNAs play a regulatory role in Trp-mediated muscle development. Furthermore, the differential expression of miRNAs in muscle samples from different groups corroborated this observation.

MiRNAs are crucial regulatory factors in the process of muscle development, participating in various pathways involved in muscle cell proliferation and muscle growth. Previous studies have identified several key miRNAs, including ssc-miR-363 [34], ssc-miR-128 [34], ssc-miR-29a [34], ssc-miR-182 [8], ssc-miR-34c [8], ssc-miR-497 [34], ssc-miR-20b [35], ssc-miR-183 [35], ssc-miR-9 [35], ssc-miR-9-1 [35], and ssc-miR-106a [35], as important regulators of skeletal muscle development, regeneration, and signal transduction in pigs, which aligns with some of our study's findings. Notably, novel_80 is a previously unreported miRNA whose target genes are involved in cellular signaling, muscle cell proliferation, differentiation, motility regulation, muscle repair, and stress response regulation. Therefore, novel_80 may represent a unique miRNA involved in the role of Trp in the muscle development of weaned piglets, although further in vitro

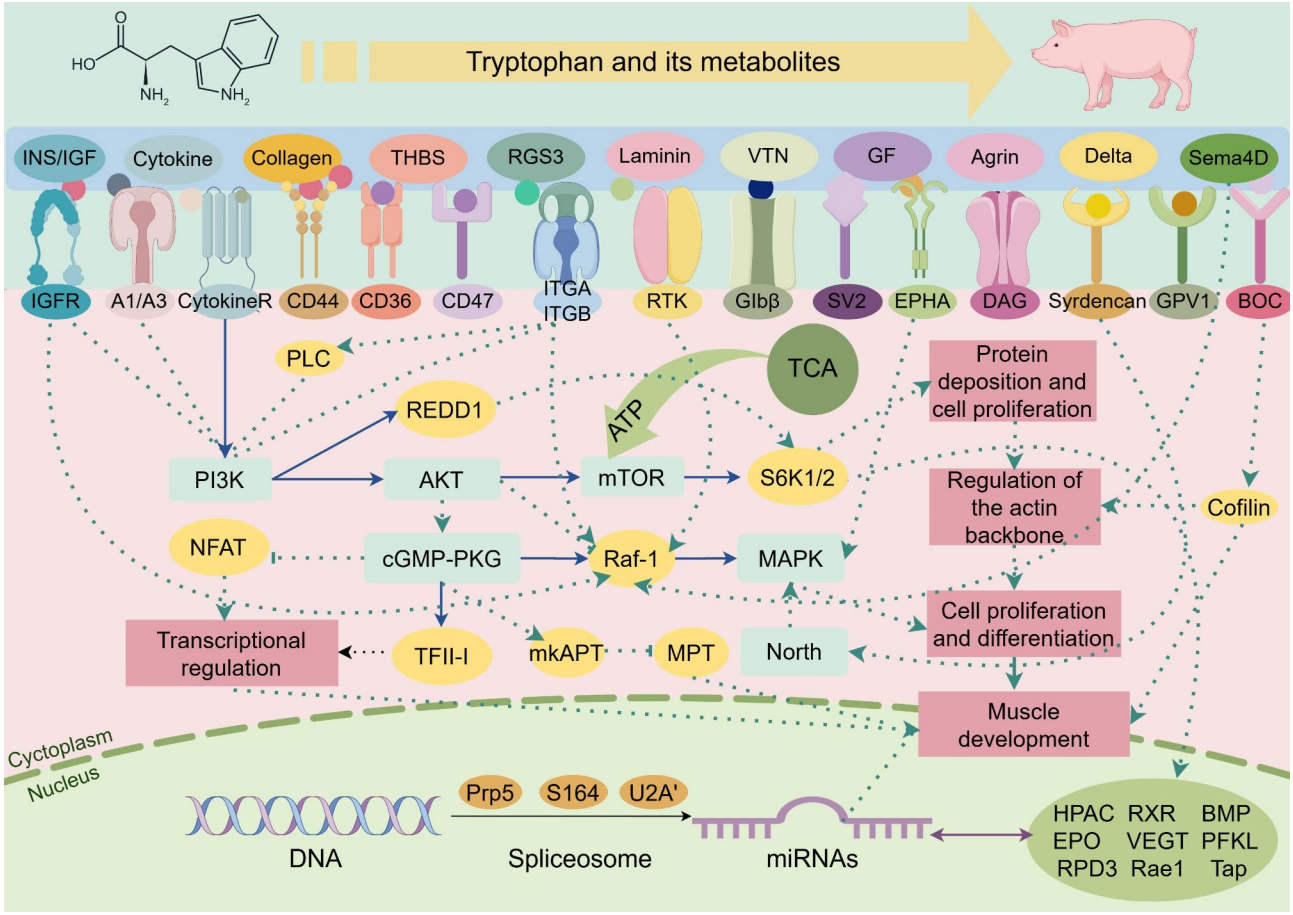

Fig. 7 Potential mechanisms of Trp and its metabolites regulating muscle development in weaned piglets through miRNAs (The solid arrow in the figure represents the direct interaction between receptor and gene pathway, and the dotted line represents the indirect interaction between receptor and gene pathway)

validation is required. Previous studies have indicated that miRNAs can regulate gene expression at the post-transcriptional level and are involved in various biological processes, including cell differentiation, development, metabolism, proliferation, and apoptosis. The mechanisms of miRNA action primarily involve two pathways: first, miRNAs bind to the open reading frame of target mRNAs, forming a double-stranded complex that leads to mRNA degradation; second, miRNAs interact with the 3'UTR of target mRNAs, thereby inhibiting post-transcriptional translation. Additionally, some research suggests that miRNAs can bind to the 5' UTR and coding regions to suppress gene expression, while interactions with the promoter region may induce transcription [18–20]. Building on this, we predicted the targets of 16 DE miRNAs, including novel_80. Among these, myogenin (MyoG), a member of the muscle regulatory factors (MRFs) family, plays a direct role in muscle fiber formation and proliferation [36]. Additionally, POPDC3, a core gene regulating muscle nutrient supply, can participate in intercellular signaling by activating the cGMP-PKG signaling pathway and regulating energy supply within muscle tissue [37]. The construction of the PPI network for all DE miRNA target genes further illustrates the interactions between the corresponding protein molecules. This indicates that DE miRNAs participate in various biological processes such as signal transduction, gene expression regulation, energy and material metabolism, and cell cycle control through their respective pathways [38]. To further refine our analysis and identify key regulatory genes or targeted gene networks, we organized the corresponding gene clusters and their associations, which are primarily related to biological pathways involved in cellular metabolism, material transport, signal transduction, and muscle development. These findings suggest that the DE miRNAs in the longissimus dorsi muscle samples regulate muscle development in piglets through their target genes and the interactions of the associated proteins. Notably, this functional enrichment analysis of DE miRNAs target genes indicates that Trp is involved in the muscle development process of piglets, including the breakdown and utilization of amino acids by myocytes,

suggesting that Trp metabolites play an important role in this series of biological processes.

In the context of extensive genes and data, enrichment analysis can help prioritize genes or gene clusters that demonstrate significant enrichment within a specific biological framework, thereby enhancing the focus and effectiveness of research [39]. In this study, the enrichment of target genes and gene clusters for DE miRNAs indicates that Trp and its metabolites gradually activate the PI3K-AKT, mTOR, cGMP-PKG, MAPK, and North signaling pathways through the signaling actions of multiple membrane receptors, further contributing to muscle cell proliferation, differentiation, protein deposition, actin cytoskeleton composition, and muscle development. Glycerophospholipids and chondroitin sulfate are essential components of membrane structures, and numerous animal studies suggest that they are critical for signaling within and outside the membrane [40–42]. Functional enrichment analysis suggests that Trp and its metabolites may interact with relevant membrane receptors through glycerophospholipid and chondroitin sulfate signaling pathways, thereby activating pathways associated with muscle development. Moreover, ECM-receptor interactions play a vital role in regulating cell phenotype and behavior, primarily facilitated through cell surface receptors, including both direct and indirect interactions mediated by cooperative molecules [43]. Similarly, ClueGO enrichment analysis has confirmed the potential mechanisms by which Trp and its metabolites may regulate muscle development in piglets. Our research has identified that 15 receptor proteins, including IGFR, are involved in Trp's regulation of muscle development, and these receptors can transmit signals through certain genes to relevant pathways. The PI3K-AKT signaling pathway plays a crucial role in muscle development, as it regulates muscle cell growth, proliferation, and survival through various mechanisms. Wang et al. [44] noted that miR-1 can mediate PLAG1's influence on the PI3K-Akt signaling pathway, thereby promoting the proliferation of primary bovine myoblasts. On this basis, our enrichment results suggest that PI3K can directly interact with Regulated in Development and DNA Damage Response 1 (REDD1). REDD1, as a stress-inducible factor, can further regulate Ribosomal Protein S6 Kinase (S6K) [45]. As an effector of mTOR, S6K is primarily involved in various biological processes, including transcription, translation, protein and lipid synthesis, cell growth, and cellular metabolism [46]. This mechanism is similar to how Trp and its metabolites enhance animal growth performance by alleviating stress and regulating biological rhythms [47, 48]. Additionally, our enrichment analysis indicates that AKT indirectly influences the cGMP-PKG pathway, subsequently regulating the MAPK signaling pathway through Raf-1, and participating in gene expression related to the cell cycle, apoptosis, differentiation, and migration. Furthermore, cGMP-PKG also relies on the transcriptional regulatory process supported by TFII-I. Similar studies have elucidated specific interactions between cGMP-PKG and TFII-I [49]. Moreover, research indicates that Syndecan is a key regulator of cellular signaling and biological function [50]. Trp and its metabolites mediate signaling to Notch through Syndecan and regulate gene expression via the MAPK pathway. While Notch signaling is highly conserved among multicellular organisms, its role in determining cell fate during development and maintaining homeostasis in adult tissues is significant and cannot be overlooked [51, 52]. These findings are consistent with the roles of known miRNAs in other biological developmental processes, further underscoring the importance of miRNAs in regulating muscle development in piglets. Relevant research indicates that excessively high levels of Trp in the diets of weaned piglets may adversely affect gut health, growth performance, blood parameters, the immune system, and behavior [53, 54]. Although our study did not observe or address these specific indicators, we advocate for prioritizing appropriate nutritional levels when formulating diets to ensure the health and optimal growth performance of piglets, before considering the economic value of the diet.

Conclusions

Overall, this study demonstrated that the inclusion of Trp in the diet significantly alters the differential expression of miRNAs in the muscles of weaned piglets. These miRNAs participate in various pathways related to muscle development through multiple target genes and their associated proteins. These results indicate that miRNAs-mediated Trp has a potential effect on the muscle development of weaned piglets, which provides new evidence for miRNAs to regulate the muscle development of weaned piglets and lays an important theoretical foundation for subsequent research and application. However, the mechanisms through which Trp metabolites mediate the regulation of miRNAs in the context of muscle development in piglets necessitate further investigation. We anticipate that further feeding trials and data analysis will reveal additional biological mechanisms, thereby providing a more robust basis for the development of the livestock industry.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11424-0 .

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

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Not applicable.

Author contributions
T.H and Z.Y designed the experiment and wrote the manuscript. T.H, Q.C, J.M, and J.L carried out the experiment, organized the data, and carried out statistical analyses. Q.C, H.L, and D.M carried out data visualization. T.H, Q.C, J.M, J.L, H.L, D.M, and Z.Y discussed the manuscript. T.H, H.L, and Z.Y participated in the revision of the manuscript. Z.Y provided financial support. All authors read and approved the final manuscript.

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Data availability
All data generated or analyzed in this study are included in this article and its Supplementary Information files (https://doi.org/10.57760/sciencedb.17409).

Declarations

Ethics approval and consent to participate
The use of piglets in all experiments strictly adhered to the Guidelines for the Use of Animals of the Chinese Committee of the Ministry of Agriculture (Beijing, China) and the permission of the Ethics Committee of the College of Animal Science and Technology of Southwest University (IACUC-20190824-24).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Han X, Hu X, Jin W, Ding S. Dietary nutrition, intestinal microbiota dysbiosis and post-weaning diarrhea in piglets. Anim Nutr. 2024;17:188–207. https://doi.org/10.1016/j.aninu.2023.12.010
2. Hu R, Wu S, Li B, Tan J, Yan J, Wang Y, et al. Dietary ferulic acid and vanillic acid on inflammation, gut barrier function and growth performance in lipopolysaccharide-challenged piglets. Anim Nutr. 2022;8:144–52. https://doi.org/10.1016/j.aninu.2021.06.009
3. Chen L, Zhong Y, Ouyang X, Wang C, Yin L, Huang J, et al. Effects of β-alanine on intestinal development and immune performance of weaned piglets. Anim Nutr. 2023;12:398–408. https://doi.org/10.1016/j.aninu.2022.10.008
4. Yan E, Guo J, Yin J. Nutritional regulation of skeletal muscle energy metabolism, lipid accumulation and meat quality in pigs. Anim Nutr. 2023;14:185–92. https://doi.org/10.1016/j.aninu.2023.04.009
5. Yang L, Chu Z, Liu M, Zou Q, Li J, Liu Q, et al. Amino acid metabolism in immune cells: essential regulators of the effector functions, and promising opportunities to enhance cancer immunotherapy. J Hematol Oncol. 2023;16(1):59–91. https://doi.org/10.1186/s13045-023-01453-1
6. Marcano AJ, Wendy BB, Mark WH, Carlos MI. The role of tryptophan metabolites in musculoskeletal stem cell aging. Int J Mol Sci. 2020;21(18):6670–6670. https://doi.org/10.3390/ijms21186670
7. He T, Li C, Chen Q, Li R, Luo J, Mao J, et al. Combined analysis of LncRNA and mRNA emphasizes the potential role of tryptophan-mediated regulation of muscle development in weaned piglets by LncRNA. J Anim Sci. 2024;102:264–77. https://doi.org/10.1093/jas/skae264
8. He T, Yuan Z, Chen Q, Luo J, Mao J, Tang Z, et al. Circular RNAs mediate the effects of dietary tryptophan on the transformation of muscle fiber types in weaned piglets. J Agric Food Chem. 2024;72(15):8595–605. https://doi.org/10.1021/acs.jafc.4c00762
9. Xue C, Li G, Zheng Q, Gu X, Shi Q, Su Y, et al. Tryptophan metabolism in health and disease. Cell Metabol. 2023;35(8):1304–26. https://doi.org/10.1016/j.cmet.2023.06.004
10. Musumeci G, Imbesi R, Trovato FM, Szychlinska MA, Aiello FC, Buffa P, et al. Importance of serotonin (5-HT) and its precursor l-tryptophan for homeostasis and function of skeletal muscle in rats. A morphological and endocrinological study. Acta Histochem. 2015;117(3):267–74. https://doi.org/10.1016/j.acthis.2015.03.003
11. Giezenaar C, Montoya CA, Kreutz K, Hodgkinson S, Roy NC, Mace LJ, et al. Effects of different protein sources on amino acid absorption and plasma appearance of tryptophan, large neutral amino acids, and tryptophan metabolites in pigs. J Nutr. 2024:1–15. https://doi.org/10.1016/j.tjnut.2024.07.010
12. Hajduch É, Rencurel F, Balendran A, Batty IHC, Downes P, Harinder SH. Serotonin (5-hydroxytryptamine), a novel regulator of glucose transport in rat skeletal muscle. J Biol Chem. 1999;274(19):13563–8. https://doi.org/10.1074/jbc.274.19.13563
13. Xu P, Huang S, Zhang H, Mao C, Zhou XE, Cheng X, et al. Structural insights into the lipid and ligand regulation of serotonin receptors. Nature. 2021;592(7854):469–73. https://doi.org/10.1038/s41586-021-03376-8
14. Zheng KY, Gao B, Wang HJ, He JG, Chen HS, Hu ZL, et al. Melatonin ameliorates depressive-like behaviors in ovariectomized mice by improving tryptophan metabolism via inhibition of gut microbe alistipes inops. Adv Sci. 2024:e2309473–88. https://doi.org/10.1002/advs.202309473
15. Allison DJ, Nederveen JP, Snijders T, Bell KE, Kumbhare D, Phillips SM, et al. Exercise training impacts skeletal muscle gene expression related to the kynurenine pathway. Am J Physiol Cell Physiol. 2019;316(3):C444–8. https://doi.org/10.1152/ajpcell.00448.2018
16. Kaiser H, Yu K, Pandya C, Mendhe B, Isales CM, Lawrence ME, et al. Kynurenine, a tryptophan metabolite that increases with age, induces muscle atrophy and lipid peroxidation. Oxid Med Cell Longev. 2019;2019:1–9. https://doi.org/10.1155/2019/9894238
17. Igor Č, Leandro ZA, Jorge LR, Kynurenines. Tryptophan's metabolites in exercise, inflammation, and mental health. Science. 2017;357(6349):369–75. https://doi.org/10.1126/science.aaf9794
18. Klockner I, Schutt C, Gerhardt T, Boettger T, Braun T. Control of CRK-RAC1 activity by the miR-1/206/133 MiRNA family is essential for neuromuscular junction function. Nat Commun. 2022;13(1):3180–96. https://doi.org/10.1038/s41467-022-30778-7
19. Nariyama M, Mori M, Shimazaki E, Ando H, Ohnuki Y, Abo T, et al. Functions of miR-1 and miR-133a during the postnatal development of masseter and gastrocnemius muscles. Mol Cell Biochem. 2015;407(1–2):17–27. https://doi.org/10.1007/s11010-015-2450-y
20. Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, et al. The muscle-specific MicroRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nat Med. 2007;13(4):486–91. https://doi.org/10.1038/nm1569
21. Chen B, Yue Y, Li J, Yuan C, Guo T, Zhang D, et al. Global DNA methylation, MiRNA, and mRNA profiles in sheep skeletal muscle promoted by hybridization. J Agric Food Chem. 2023;71(41):15398–406. https://doi.org/10.1021/acs.jafc.3c02173
22. Xu F, Ren Y, Teng Y, Mu J, Tang J, Sundaram K, et al. Tryptophan as a new member of RNA-induced silencing complexes prevents colon cancer liver metastasis. Adv Sci. 2024;11(31):e2307937. https://doi.org/10.1002/advs.202307937
23. Hu Z, Li F, Jiang Q, Wang W, Tan B, Tang X, et al. Intestinal tryptophan metabolism in disease prevention and swine production. Anim Nutr. 2023;15:364–74. https://doi.org/10.1016/j.aninu.2023.08.002
24. Miao J, Adewole D, Liu S, Xi P, Yang C, Yin Y. Tryptophan supplementation increases reproduction performance, milk yield, and milk composition in lactating sows and growth performance of their piglets. J Agric Food Chem. 2019;67:5096–104. https://doi.org/10.1021/acs.jafc.9b00446

25. Zhang D, Ning J, Ramprasath T, Yu C, Zheng X, Song P, et al. Kynurenine promotes neonatal heart regeneration by stimulating cardiomyocyte proliferation and cardiac angiogenesis. Nat Commun. 2022;13:6371–82. https://doi.org/10.1038/s41467-022-33734-7
26. Priatno W, Jo YH, Lee HG, Lee JS, Moon J, Lee HG. Dietary supplementation of L-tryptophan increases muscle development, adipose tissue catabolism and fatty acid transportation in the muscles of Hanwoo steers. J Anim Sci Technol. 2020;62(5):595–604. https://doi.org/10.5187/jast.2020.62.5.595
27. Soranobu N, Nobuhiko N, Hiroshi N, Taku M, Yuto K, Kimihiro Y, et al. Low levels of serum tryptophan underlie skeletal muscle atrophy. Nutrients. 2020;12(4):978–93. https://doi.org/10.3390/nu12040978
28. Sanfilippo S, Imbesi RM, Sanfilippo S. Effects of a Tryptophan deficient diet on the morphology of skeletal muscle fibers of the rat. Preliminary observations at neuroendocrinological and submicroscopical levels. Ital J Anat Embryol. 1995;100(1):131–41.
29. Dukes A, Davis C, El Refaey M, Upadhyay S, Mork S, Arounleut P, et al. The aromatic amino acid tryptophan stimulates skeletal muscle IGF1/p70s6k/mTor signaling in vivo and the expression of myogenic genes in vitro. Nutrition. 2015;31(7–8):1018–24. https://doi.org/10.1016/j.nut.2015.02.011
30. Lin FD, Smith TK, Bayley HS. A role for tryptophan in regulation of protein synthesis in porcine muscle. J Nutr. 1988;118(4):445–9. https://doi.org/10.1093/jn/118.4.445
31. He T, Chen Q, Yuan Z, Yang Y, Cao K, Luo J, et al. Effects of maternal high-fat diet on fetal growth, placental nutrient transporters and circular RNA expression profiles. Food Funct. 2023;14(20):9391–406. https://doi.org/10.1039/d3fo02202a
32. He T, Yuan Z, Chen Q, Luo J, Mao J, Yang Y, et al. Circular RNAs mediate the regulation of maternal placental nutrient transport and fetal development by sugar-sweetened beverages. Food Res Int. 2024;193:114856–66. https://doi.org/10.1016/j.foodres.2024.114856
33. Wang L, Nasa SA, Alexandre W, Abigail RW, Paulo CJ, Rachel JP, et al. A MicroRNA linking human positive selection and metabolic disorders. Cell. 2020;183(3):684–701. https://doi.org/10.1016/j.cell.2020.09.017
34. Nielsen M, Hansen JH, Hedegaard J, Nielsen RO, Panitz F, Bendixen C, et al. MicroRNA identity and abundance in porcine skeletal muscles determined by deep sequencing. Anim Genet. 2010;41(2):159–68. https://doi.org/10.1111/j.1365-2052.2009.01981.x
35. Chen T, Xi QY, Ye RS, Cheng X, Qi QE, Wang SB, et al. Exploration of MicroRNAs in porcine milk exosomes. BMC Genomics. 2014;15:100–18. https://doi.org/10.1186/1471-2164-15-100
36. Wang L, Xu Z, Ling D, Li J, Wang Y, Shan T. The regulatory role of dietary factors in skeletal muscle development, regeneration and function. Crit Rev Food Sci Nutr. 2020;62(3):764–82. https://doi.org/10.1080/10408398.2020.1828812
37. Vissing J, Johnson K, Töpf A, Nafissi S, Manera JD, French VM, et al. POPDC3 gene variants associate with a new form of limb girdle muscular dystrophy. Ann Neurol. 2019;86(6):832–43. https://doi.org/10.1002/ana.25620
38. Chen B, Fan W, Liu J, Wu FX. Identifying protein complexes and functional modules–from static PPI networks to dynamic PPI networks. Brief Bioinform. 2014;15(2):177–94. https://doi.org/10.1093/bib/bbt039
39. Gu X, Jin B, Qi Z, Yin X. Identification of potential MicroRNAs and KEGG pathways in denervation muscle atrophy based on meta-analysis. Sci Rep. 2021;11(1):13560–74. https://doi.org/10.1038/s41598-021-92489-1
40. Chen S, Zong G, Wu Q, Yun H, Niu Z, Zheng H, et al. Associations of plasma glycerophospholipid profile with modifiable lifestyles and incident diabetes in middle-aged and older Chinese. Diabetologia. 2022;65(2):315–28. https://doi.org/10.1007/s00125-021-05611-3
41. Chen S, Wu Q, Zhu L, Zong G, Li H, Zheng H, et al. Plasma glycerophospholipid profile, erythrocyte n-3 PUFAs, and metabolic syndrome incidence: a prospective study in Chinese men and women. Am J Clin Nutr. 2021;114(1):143–53. https://doi.org/10.1093/ajcn/nqab050
42. Hsu HC, Ke YL, Lai YH, Hsieh WC, Lin CH, Huang SS, et al. Chondroitin sulfate enhances proliferation and migration via inducing β-catenin and intracellular ROS as well as suppressing metalloproteinases through Akt/NF-κB pathway inhibition in human chondrocytes. J Nutr Health Aging. 2022;26(3):307–13. https://doi.org/10.1007/s12603-022-1752-5
43. Zhang Y, Gu T, Xu S, Wang J, Zhu X. Anti-liver fibrosis role of miRNA-96-5p via targeting FN1 and inhibiting ECM-receptor interaction pathway. Appl Biochem Biotechnol. 2023;195(11):6840–55. https://doi.org/10.1007/s12010-023-04385-1
44. Wang J, Huang Y, Xu J, Yue B, Wen Y, Wang X, et al. Pleomorphic adenoma gene 1 (PLAG1) promotes proliferation and inhibits apoptosis of bovine primary myoblasts through the PI3K-Akt signaling pathway. J Anim Sci. 2022;100(4):98. https://doi.org/10.1093/jas/skac098
45. Tatekoshi Y, Kouzu HS, Shapiro J, Chang H, Jesus AD, Paulina JS, et al. Loss of the RNA-binding protein ZFP36L2 results in Peri-partum cardiomyopathy through dysregulation of the P53-mTOR pathway. Circul Res. 2020;127. https://doi.org/10.1161/res.127.suppl_1.263
46. Liu Q, Meng Q, Ding Y, Jiang J, Kang C, Yuan L, et al. The unfixed light pattern contributes to depressive-like behaviors in male mice. Chemosphere. 2023;339:139680–139680. https://doi.org/10.1042/bj20110892
47. Liu Q, Meng Q, Ding Y, Jiang J, Kang C, Yuan L, et al. The unfixed light pattern contributes to depressive-like behaviors in male mice. Chemosphere. 2023;339:139680–139680. https://doi.org/10.1016/j.chemosphere.2023.139680
48. Petrus P P, Cervantes M, Samad M, Satō T, Chao A, Sato S, et al. Tryptophan metabolism is a physiological integrator regulating circadian rhythms. Mol Metabolism. 2022;101556(101556). https://doi.org/10.1016/j.molmet.2022.101556
49. Angela LM, Brooke DP, Luciana LA, Annika JC, David LM, Anne MV, et al. TFII-I-mediated polymerase pausing antagonizes GLI2 induction by TGFβ. Nucleic Acids Res. 2020;48(13):7169–81. https://doi.org/10.1093/nar/gkaa476
50. Lu R. Syndecan-2 identifies and regulates HSCs. Blood. 2022;139(2):153–4. https://doi.org/10.1182/blood.2021014116
51. Sana SH, Markus J, Jacqueline T, Lena W, Thomas L, Viola N, et al. Endothelial notch signaling controls insulin transport in muscle. EMBO Mol Med. 2020;12:e09271–87. https://doi.org/10.15252/emmm.201809271
52. Ito Y, Mari Y, Takuya Y, Katsuya H, Takeshi N, Takahiko S. The reciprocal regulation between mitochondrial-associated membranes and notch signaling in skeletal muscle atrophy. eLife. 2023;12:RP89381–99. https://doi.org/10.7554/elife.89381.3
53. Tossou MC, Liu H, Bai M, Chen S, Cai Y, Duraipandiyan V, et al. Effect of high dietary tryptophan on intestinal morphology and tight junction protein of weaned pig. Biomed Res Int. 2016:2912418–23. https://doi.org/10.1155/2016/2912418
54. Li YZ, Kerr BJ, Kidd MT, Gonyou HW. Use of supplementary tryptophan to modify the behavior of pigs. J Anim Sci. 2006;84(1):212–20. https://doi.org/10.2527/2006.841212x

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