

Identification of Genes related to Growth and Lipid Deposition from Transcriptome Profiles of Wujin Pigs and Landrace Pigs Muscle Tissue

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Abstract

Intramuscular Fat (IMF) content is an important trait closely related to meat quality, which is highly variable among pig breeds from diverse genetic backgrounds. This study identifies and compares the differential expression of functional genes associated with muscle growth and fat deposition. High-throughput sequencing has become a powerful technique for analyzing the whole transcription profiles of organisms. We adopted RNA sequencing to detect transcriptome in the longissimus dorsi muscle of Wujin pigs (a Chinese indigenous breed) and Landrace pigs (a western lean-type breed) with different IMF content. For the Wujin and Landrace pig libraries, over 6.6 and 7.2 million clean reads were generated by transcriptome sequencing, respectively. A total of 682 Differentially Expressed Genes (DEGs) were identified in our study ($|\log_2FC| > 1$, $p < 0.05$), with 296 up-regulated and 386 down-regulated genes in Wujin pigs compared with Landrace pigs. The Gene Ontology analysis revealed that DEGs were significantly associated with processes such as cholesterol import, chylomicron remnant clearance, and cholesterol efflux. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis revealed that DEGs were significantly enriched in pathways related to the biosynthesis of unsaturated fatty acids, ErbB (Erythroblastic Oncogene B) signaling pathway, metabolic pathways, and oxidative phosphorylation. The key genes, namely PDP1 (Pyruvate Dehydrogenase Phosphatase Catalytic Subunit 1), EGR1 (Early Growth Response 1), IL6R (Interleukin 6 Receptor), and ATF3 (Activating Transcription Factor 3) exhibit correlations with muscle growth, while TMEM182 (Transmembrane Protein 182), AGL (Amylo-Alpha-1, 6-Glucosidase, 4-Alpha-Glucanotransferase), and ADCY9 (Adenylate Cyclase 9) exhibit correlations with lipid deposition. The Protein-Protein Interaction (PPI) network with 418 nodes and 863 edges was constructed and 4 modules were extracted from the entire network. In summary, this study identified candidate genes and putative signaling pathways and provided useful information to further investigation of the mechanism of muscle growth and lipid deposition in pigs.

Keywords: Pigs; Longissimus muscle; Transcriptomics; Lipid deposition; Candidate gene

Abbreviations: IMF: Intramuscular Fat; DEGs: Differentially Expressed Genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; ErbB: Erythroblastic Oncogene B; PDP1: Pyruvate Dehydrogenase Phosphatase Catalytic Subunit 1; EGR1: Early Growth Response 1; IL6R: Interleukin 6 Receptor; ATF3: Activating Transcription Factor 3; TMEM182: Transmembrane Protein 182; AGL: Amylo-Alpha-1, 6-Glucosidase, 4-Alpha-Glucanotransferase; ADCY9: Adenylate Cyclase 9; PPI: Protein-Protein Interaction; SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid; GC: Gas Chromatography; GO: Gene Ontology; STRING: Search Tool for the Retrieval of Interacting Genes; MCODE: Molecular Complex Detection; FC: Fold Change; SEM: Standard Errors of the Means; NDUFV1: NADH Ubiquinone Oxidoreductase Core Subunit V1; NDUFB9: NADH Ubiquinone Oxidoreductase Subunit B9; UQCRC1: Ubiquinol-Cytochrome C Reductase Core Protein 1; NDUFA10: NADH Ubiquinone Oxidoreductase Subunit A10; NDUFS2: NADH Ubiquinone Oxidoreductase Core Subunit S2; ATP5MC1: ATP Synthase Membrane Subunit C Locus 1; NDUFS6: NADH Ubiquinone Oxidoreductase Subunit S6; COX4I1: Cytochrome C Oxidase Subunit 4I1; TUFM: Tu Translation Elongation Factor, Mitochondrial; NDUFB8: NADH Ubiquinone Oxidoreductase Subunit B8

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Introduction

As is widely recognized, Western pig breeds have the characteristics of high growth rate and high lean meat percentage. However, Chinese pigs own the high IMF content and excellent meat quality [1,2]. In recent years, consumers have increasingly turned their focus towards pork from indigenous breeds due to its succulence and distinctive flavor, paralleling the advancement of the national economy. In contrast to introduced breeds, indigenous breeds exhibit certain drawbacks, including lower percentages of lean meat and reduced water-holding capacity [3]. The Wujin pig, a native Chinese fat-type breed, is renowned for its superior meat quality and comparatively elevated IMF content [4-6]. The Landrace pig, a lean-type breed, is distinguished by its rapid growth rate and high proportion of lean meat [7]. Therefore, these two pig breeds offer valuable models with varying lipid deposition capacities to illuminate the fundamental mechanisms underlying intramuscular fat deposition in both adipose and lean pigs.

Traits related to fatness, such as back fat thickness and IMF content, exhibit positive correlations with meat tenderness, juiciness, and flavor [8]. These traits hold economic significance in pig breeding as they can exert an influence on meat quality and carcass composition. Chinese indigenous pigs, celebrated for their rich meaty flavor, typically possess intramuscular fat content exceeding 5%, in contrast to approximately 2% in imported commercial pigs [9]. Nonetheless, traits related to growth and meat quality are intricate quantitative characteristics influenced by numerous interacting genes. Transcriptome profiling offers an effective means to detect novel and less abundant transcripts.

Several RNA-seq studies have been conducted on muscle tissues from various animal species, including hen [10], cattle [11], large yellow croaker (*Larimichthys crocea*) [12], grass carp [13], goat [14], and black muscovy duck [15]. These studies have contributed to a more comprehensive understanding of the mRNA transcriptome in animal muscles. Transcriptome profiling enables the simultaneous measurement of differential gene expression in a specific tissue, making it a powerful tool for identifying genes associated with different phenotypes [2]. Our previous findings have suggested that the mechanism behind the elevated IMF content in fatty pigs may be attributed to their heightened lipogenesis and fatty acid transport capacity, while displaying a lower lipolysis capacity. However, research on the screening of genes related to muscle growth and intramuscular fat deposition through high-throughput sequencing technology is still limited, and the precise mechanisms governing muscle growth and fat deposition in both fat and lean pigs remain unclear.

In this study, we generated transcriptome profiles of muscle tissue in the Chinese indigenous pig breeds (Wujin pigs) and the introduced pig breeds (Landrace pigs) and conducted a comparative study of different breeds of pigs, and finally identified the functional genes and the regulation networks that control muscle growth and fat deposition in pigs.

Materials and Methods

Animal care

The experiments were performed according to the ARRIVE guidelines (<https://arriveguidelines.org>) and approved by the Ethics Committee of Experimental Animal of Yunnan Agricultural University (Approval Code: 201603017, Approval Date: 14 March 2016).

Animals and sampling

A total of three male Wujin pigs and three male Landrace pigs of the same batch with similar body weight were randomly selected as the study object raised in the pig-breeding institute of the Yunnan Academy of Animal Husbandry and Veterinary Sciences. Pigs of the same breed come from the same maternal parent and parity. They were raised under a standardized feeding regimen with free access to water. Once the pigs reached a body weight of 100 kg, they were transported to the Yunnan Agricultural Center Meats Laboratory and slaughtered following electrical stunning. Muscle tissues from the longissimus dorsi were collected from each animal. A portion was preserved at -20 °C for IMF content analysis, while the remainder was frozen in liquid nitrogen for total RNA extraction.

Determination of intramuscular fat content

The IMF content of muscle samples was determined after extraction of crude fat using Soxhlet Extraction (SZF-06A, Shanghai Xinjia Electronic Co., Ltd., Shanghai, China) with petroleum ether (boiling temperature range: 60 °C to 90 °C). Three replications were conducted for each sample.

Determination of fatty acid content

Intramuscular fat was extracted through the method of Bligh. The extract was used for Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA), and Polyunsaturated Fatty Acid (PUFA) measurements. The SFAs, MUFA, and PUFA were analyzed *via* Gas Chromatography (GC).

Transcriptome sample preparation for sequencing

Total RNA was extracted from the longissimus muscle of pigs using the RNA Simple Total RNA kit (TIANGEN, Beijing, China). Sequencing libraries were prepared with the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB, Ipswich, USA), following the manufacturer's instructions.

Clustering and sequencing

The indexing and clustering of the samples were carried out using a cBot Cluster Generation System, employing the TruSeq PE Cluster Kit v3-cBot-HS (Illumina), following the manufacturer's guidelines. Subsequently, the prepared libraries were sequenced on an Illumina HiSeq platform, producing 100 bp paired end reads.

Mapping and assembling of reads to the reference genome

The quality filtered fastq files were mapped to the *Sus scrofa* reference genome build 11.1. Top Hat (version 2.1.0.) and HTSeq

(version 0.6.1) were used to count the read numbers mapped to each gene. RPKM of each gene was calculated based on the length of the gene and read counts mapped to this gene.

Differential expression analysis

Prior to differential gene expression analysis, for each sequenced library, the read counts were adjusted by edgeR program package through one scaling normalized factor. Differential expression analysis of two conditions was performed using the DESeq R package (1.12.0). The p values were adjusted using the Benjamini and Hochberg method. Corrected p value of 0.05 and log₂ (fold change) of 1 were set as the threshold for significant differential gene expression.

Functional analysis of differentially expressed genes

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the Goseq R package, in which gene length bias was corrected. GO terms with P-value less than 0.05 were considered significantly enriched by differentially expressed genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource by genome sequencing and other high-throughput experimental technologies. KEGG was used to understand the advanced functions and utility of biological systems.

Protein-protein interaction network (PPI) construction and module analysis

The protein-protein interaction network of the DEGs was constructed based on the Search Tool for the Retrieval of Interacting

Genes (STRING) database (STRING, version 9.1, <http://string91.embl.de/>) [16], and predicted by the use of Cytoscape (version 3.0; <http://cytoscape.org/>). The hub genes were then selected with a connectivity degree 10 after calculating the degree of each node. Module analysis of the PPI network was performed with the parameters of minimum size > 4 and minimum density < 0.05 using Molecular Complex Detection (MCODE) [17].

Data analysis

The acquired data were initially processed in an Excel spreadsheet and analyzed using one-way ANOVA in SPSS 25.0. The results are presented in tables as means with pooled standard errors of the means (SEM). Multiple comparisons of means were conducted using LSD and Duncan methodologies, with significance determined based on P-values. Extremely significant differences were indicated by P < 0.01, significant differences by P < 0.05, and non-significant differences by P > 0.05.

Results

Saturated fatty acid and intramuscular fat content

The results of the SFA, MUFA, PUFA, and IMF content for the two pig breeds are presented in Table 1. Wujin pigs exhibited higher IMF levels compared to Landrace pigs (P < 0.05), while they showed lower SFA and PUFA levels than Landrace pigs (P < 0.05). These results suggest that the muscle tissues the muscle tissues are suitable for identifying genes associated with muscle growth and lipid deposition.

Table 1: Fatty acid and intramuscular fat content of the longissimus dorsi muscle in two pig breeds.

Pig Breed	SFA	MUFA	PUFA	IMF
Wujin pigs	40.20±0.68*	45.80±0.74	14.00±0.30*	6.58±0.13*
Landrace pigs	44.44±0.73*	45.04±0.96	10.52±0.12*	3.90±0.10*

Note: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; IMF, Intramuscular fat. SFA: C14:0, C16:0, C18:0, C20:0, MUFA: C16:1, C18:1, PUFA: C18:2, C18:3, C20:4, C20:5, C22:5. Data are expressed as means ± SEM and values in the same row with asterisks differ significantly (* P<0.05, ** P<0.01).

High-throughput sequencing and read mapping

In this study, a total of six libraries in muscle tissue were established by high-throughput RNA sequencing. The number of original reads for Wujin pigs was 6,795,774, and Landrace pigs was 7,229,998. After quality control of the original reads with Q20, sequence duplication level, and GC content, there was 6,589,139

clean reads for Wujin pigs, accounting for 99.18% of the original reads, and 7,171,030 clean reads for Landrace pigs, accounting for 99.18%. The samples were of good quality, and the percentage of reads aligned to the unique location of the reference genome was 80.82% to 94% among the clean reads, and the unique matched reads were 85.16% and 74.64% (Table 2). In summary, the sequencing data was qualified for the subsequent data analysis.

Table 2: Summary of sequencing reads aligned with the *Sus scrofa* genome and annotated genes.

Index	Wujin Pigs	Landrace Pigs
Total reads	6795774	7229998
Clean reads	6589139	7171030
Clean ratio (%)	99.18	96.96
Q30 (%)	94.28	95.95
Total mapped reads	6194011 (94%)	5795933 (80.82%)

Multiple mapped reads	582511 (8.84%)	443548 (6.19%)
Unique matched reads	5611500 (85.16%)	5352385 (74.64%)

The detected genes were distributed in all chromosomal regions, and the genome coverage was plotted along the chromosome based on the expression of genes. If the FPKM values of all the detected

genes were divided into five intervals, less than 0.1, 0.1 to 0.3, 0.3 to 3.75, 3.75 to 15, 15 to 60 and more than 60, the distribution of the FPKM of the genes was shown to be similar (Figure 1).

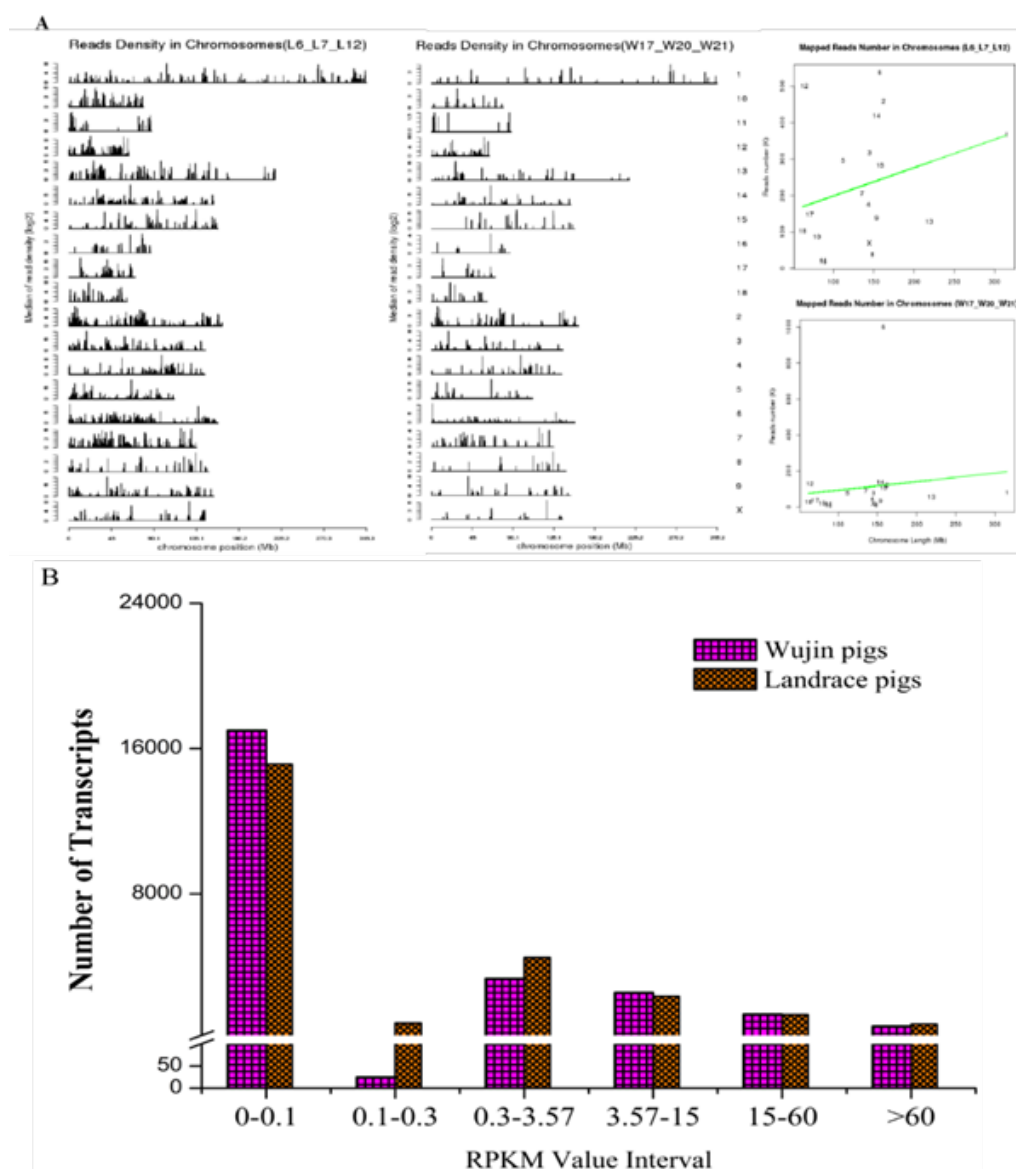


Figure 1: Summary of RNA sequence (RNA-Seq) mapping data.

A. Reads density in chromosome and mapped reads number in chromosome.

B. The number of detected genes with different expression levels against the range of fragments per kilobase of exon length million mapped reads (FPKM) values.

Differentially expressed genes analysis

With $P < 0.05$ and $|\log_2 FC| > 1$ as the threshold, a total of 682 DEGs were identified. Among them, we identified 296 up-regulated genes and 386 down-regulated genes when comparing Wujin and Landrace pigs (Figure 2). The most significant 15 up-regulated

differentially expressed genes in Wujin pigs and Landrace pigs (Table S1). The most significant 15 down-regulated differentially expressed genes in Wujin pigs and Landrace pigs (Table S2). A number of differentially expressed genes were highly expressed in the muscle tissues of both groups.

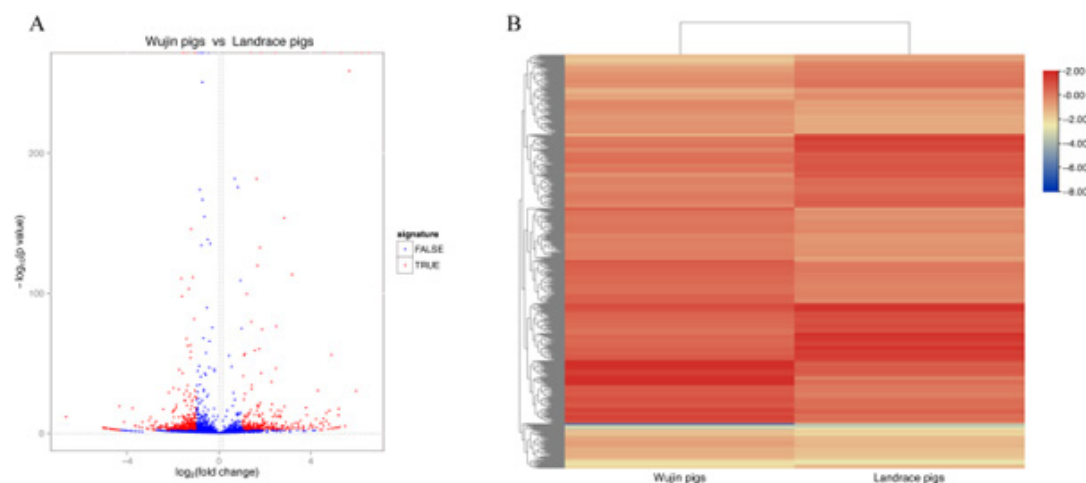


Figure 2: Preliminary analysis of transcriptome profiles.

- A. Volcano plot of genes differentially expressed between Wujin and Landrace pigs. The y-axis corresponds to the mean expression value of \log_{10} (P-value), and the x-axis displays the \log_2 (FC) value. The red dots represent the significantly expressed genes ($FDR < 0.05$), the blue dots represent the genes whose expression levels did not reach statistical significance.
- B. Cluster heat map of DEGs. Each row represents a gene. Different colors indicate the expression levels of genes in pigs. The redder, the higher the expression level; the bluer, the lower the expression level.

Table S1: List of the most significant 15 up-regulated differentially expressed genes in Wujin pigs and Landrace pigs.

Ensembl ID	Log2(FC)	P-Value	Q-Value	Gene	Up/Down
ENSSSCG000000248	6.46621845	-	-		Up
ENSSSCG000000251	6.16152432	-	-		Up
ENSSSCG000000278	5.90235497	2.02E-32	9.18E-31		Up
ENSSSCG000000240	5.89685517	-	-		Up
ENSSSCG000000279	5.59606129	9.32E-262	1.69E-259		Up
ENSSSCG000000251	5.42792127	1.07E-06	9.54E-06		Up
ENSSSCG000000213	5.24651401	-	-		Up
ENSSSCG000000228	5.18963692	3.96E-06	3.13E-05	<i>CRYBG3</i>	Up
ENSSSCG000000210	5.14899493	9.82E-20	3.04E-18	<i>NRIP1</i>	Up
ENSSSCG000000152	5.13518914	4.75E-15	1.09E-13		Up
ENSSSCG000000150	5.10599317	1.41E-05	9.67E-05	<i>ZC3H12C</i>	Up
ENSSSCG000000069	5.01971192	1.46E-05	9.96E-05		Up
ENSSSCG000000217	4.91334809	5.26E-05	0.000305		Up
ENSSSCG000000038	4.82706684	5.50E-05	0.000315	<i>JAK1</i>	Up
ENSSSCG000000067	4.82706684	5.50E-05	0.000315	<i>TENT5C</i>	Up

Note: “-” represents one or two groups’ reads count were 0.

Table S2: List of the most significant 15 down-regulated differentially expressed genes in Wujin pigs and Landrace pigs.

Ensembl ID	Log2(FC)	P-Value	Q-Value	Gene	Up/Down
ENSSSCG0000001227	-4.73013744	0.00024292	0.00119969	<i>TIMP1</i>	Down
ENSSSCG0000001375	-4.73013744	0.00024292	0.00119969	<i>YJU2B</i>	Down
ENSSSCG0000001375	-4.78672097	0.00017633	0.00091	<i>SLC15A3</i>	Down
ENSSSCG0000001780	-4.78672097	0.00017633	0.00091	<i>TIMM22</i>	Down
ENSSSCG0000002191	-4.78672097	0.00017633	0.00091	<i>HIPK4</i>	Down
ENSSSCG0000000685	-4.84116876	0.00012817	0.00068607		Down
ENSSSCG0000000003	-4.94426225	6.80E-05	0.00038215	<i>TSPO</i>	Down

ENSSSCG0000001291	-4.99317185	4.96E-05	0.00029025	<i>RPS6KB2</i>	Down
ENSSSCG0000001295	-4.99317185	4.96E-05	0.00029025	<i>SF3B2</i>	Down
ENSSSCG0000002905	-4.99317185	4.96E-05	0.00029025	<i>MRPL55</i>	Down
ENSSSCG0000000591	-5.04047756	3.63E-05	0.00021919	<i>SHARPIN</i>	Down
ENSSSCG0000002823	-5.04047756	3.63E-05	0.00021919		Down
ENSSSCG0000002328	-5.08628125	2.65E-05	0.00016616		Down
ENSSSCG0000002292	-5.13067537	1.94E-05	0.00012688		Down
ENSSSCG0000000894	-6.74449274	9.38E-14	1.95E-12	<i>ALB</i>	Down

Note: “-” represents one or two groups’ reads count were 0.

In the 682 DEGs, three genes, TMEM182, AGL, and ADCY9 exhibited prominent differential expression with $P < 0.001$ and $|\log_2FC| > 1$ that are related to lipid deposition (Table 3). Four

genes, PDP1, EGR1, IL6R, ATF3 and exhibited prominent differential expression with $P < 0.001$ and $|\log_2FC| > 1$ that are related to muscle growth (Table 4).

Table 3: Differentially expressed genes with $|\log_2FC| > 1$ and $P < 0.001$ that are related to lipid deposition.

Gene ID	Gene Name	Wujin Pig RPKM	Landrace pig RPKM	\log_2FC	P-Value	Up/Down Regulation
ENSSSCG00000008150	<i>TMEM182</i>	25.58	7.23	1.76	0.0002	up
ENSSSCG00000006872	<i>AGL</i>	6.68	1.78	1.84	0.0001	up
ENSSSCG00000007950	<i>ADCY9</i>	48.94	20.75	1.17	0.0003	up

Table 4: Differentially expressed genes with $|\log_2FC| > 1$ and $P < 0.001$ that are related to muscle growth.

Gene ID	Gene Name	Wujin Pig RPKM	Landrace Pig RPKM	\log_2FC	P-Value	Up/Down Regulation
ENSSSCG00000006107	<i>PDP1</i>	9.55	0.64	3.83	0.0001	up
ENSSSCG00000014336	<i>EGR1</i>	11.07	1.63	2.7	0	up
ENSSSCG00000027628	<i>IL6R</i>	8.3	2.06	1.94	0	up
ENSSSCG00000015595	<i>ATF3</i>	379.31	103.72	1.8	0	up

GO annotation and enrichment analysis of differentially expressed genes

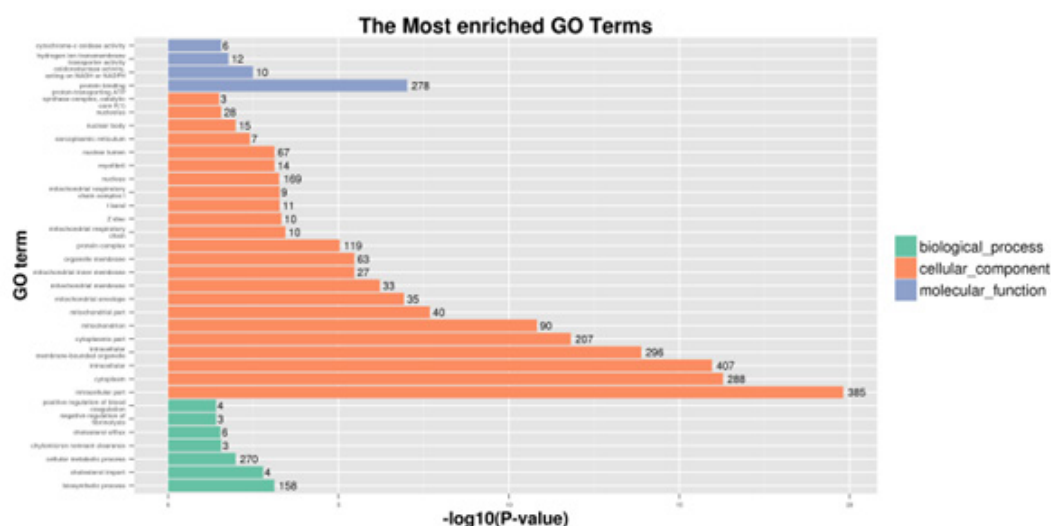


Figure 3: GO term enrichment analysis of Differentially Expressed Genes (DEGs). Brown column, violet column and cyan column meant Cellular Component (CC), Molecular Function (MF), and Biological Process (BP), respectively.

To further elucidate the functional roles of the 682 DEGs, GO term enrichment analysis was performed to search for significantly overrepresented categories. A total of 496 terms were significantly enriched in the three categories ($P < 0.05$), including biological process, cellular component, and molecular function (Table S3). The 34 top terms were obtained by GO enrichment, including 7 terms

for biological process, 23 terms for cell component, and 4 terms for molecular function and were further analyzed to determine the associated regulatory functions. Three terms were related to lipid metabolism, including cholesterol import, chylomicron remnant clearance, and cholesterol efflux (Figure 3).

Table S3: GO term enrichment of differentially expressed genes.

GO_Accession	Description	Term_Type	pValue
GO:0044424	intracellular part	cellular_component	1.07E-24
GO:0005737	cytoplasm	cellular_component	7.28E-21
GO:0005622	intracellular	cellular_component	2.29E-20
GO:0043231	intracellular membrane-bounded organelle	cellular_component	7.27E-18
GO:0044444	cytoplasmic part	cellular_component	1.06E-15
GO:0005739	mitochondrion	cellular_component	1.15E-14
GO:0044429	mitochondrial part	cellular_component	1.88E-11
GO:0005515	protein binding	molecular_function	1.01E-10
GO:0005740	mitochondrial envelope	cellular_component	1.33E-10
GO:0031966	mitochondrial membrane	cellular_component	7.25E-10
GO:0005743	mitochondrial inner membrane	cellular_component	4.67E-09
GO:0031090	organelle membrane	cellular_component	4.79E-09
GO:0043234	protein complex	cellular_component	1.44E-08
GO:0005746	mitochondrial respiratory chain	cellular_component	0.00000073
GO:0030018	Z disc	cellular_component	0.000000987
GO:0031674	I band	cellular_component	0.00000116
GO:0005747	I band	cellular_component	0.00000126
GO:0005634	nucleus	cellular_component	0.00000135
GO:0030016	myofibril	cellular_component	0.00000205
GO:0031981	nuclear lumen	cellular_component	0.00000209
GO:0009058	biosynthetic process	biological_process	0.00000218
GO:0070508	cholesterol import	biological_process	0.00000513
GO:0016651	oxidoreductase activity, acting on NADH or	molecular_function	0.0000106
GO:0016529	sarcoplasmic reticulum	cellular_component	0.0000133
GO:0016604	nuclear body	cellular_component	0.0000399
GO:0044237	cellular metabolic process	biological_process	0.000041
GO:0015078	hydrogen ion transmembrane transporter activity	molecular_function	0.0000675
GO:0005730	nucleolus	cellular_component	0.000122
GO:0034382	chylomicron remnant clearance	biological_process	0.0001231
GO:0004129	cytochrome-c oxidase activity	molecular_function	0.0001301
GO:0033344	cholesterol efflux	biological_process	0.000138
GO:0045261	proton-transporting ATP synthase complex,catalytic core F(1)	cellular_component	0.0001537
GO:0051918	negative regulation of fibrinolysis	biological_process	0.0001986
GO:0030194	positive regulation of blood coagulation	biological_process	0.0001999
GO:0042802	identical protein binding	molecular_function	0.0002851
GO:0005523	tropomyosin binding	molecular_function	0.0003157

GO:0016525	negative regulation of angiogenesis	biological_process	0.0003599
GO:0034366	spherical high-density lipoprotein particle	cellular_component	0.0005387
GO:0032488	Cdc42 protein signal transduction	biological_process	0.0005589
GO:0008203	cholesterol metabolic process	biological_process	0.0006154
GO:0034447	very-low-density lipoprotein particle clearance	biological_process	0.0006164
GO:0034364	high-density lipoprotein particle	cellular_component	0.000747
GO:0005654	nucleoplasm	cellular_component	0.0007547
GO:0045892	negative regulation of transcription, DNA-	biological_process	0.0008626
GO:0050136	NADH dehydrogenase (quinone) activity	molecular_function	0.0008739
GO:0006641	triglyceride metabolic process	biological_process	0.0009296
GO:0015986	ATP synthesis coupled proton transport	biological_process	0.0009606
GO:0016051	carbohydrate biosynthetic process	biological_process	0.0009632
GO:0008134	transcription factor binding	molecular_function	0.0009654
GO:0034115	negative regulation of heterotypic cell-cell	biological_process	0.0009868
GO:0050840	extracellular matrix binding	molecular_function	0.0010214
GO:0034384	high-density lipoprotein particle clearance	biological_process	0.0010222
GO:0008152	metabolic process	biological_process	0.0010372
GO:0010761	fibroblast migration	biological_process	0.0010398
GO:0003954	NADH dehydrogenase activity	molecular_function	0.0010455
GO:0030518	steroid hormone receptor signaling pathway	biological_process	0.0010839
GO:0008307	structural constituent of muscle	molecular_function	0.0011474
GO:0042632	cholesterol homeostasis	biological_process	0.0011655
GO:0004367	glycerol-3-phosphate dehydrogenase [NAD+]	molecular_function	0.001223
GO:0046168	glycerol-3-phosphate catabolic process	biological_process	0.001223
GO:0003714	transcription corepressor activity	molecular_function	0.0012293
GO:0008202	steroid metabolic process	biological_process	0.0012567
GO:0019684	photosynthesis, light reaction	biological_process	0.0013268
GO:0051787	misfolded protein binding	molecular_function	0.0013543
GO:0003712	transcription cofactor activity	molecular_function	0.0013577
GO:0006807	nitrogen compound metabolic process	biological_process	0.0013776
GO:0005753	mitochondrial proton-transporting ATP synthase complex	cellular_component	0.0013815
GO:0031032	actomyosin structure organization	biological_process	0.0014014
GO:0009299	mRNA transcription	biological_process	0.0014647
GO:0071813	lipoprotein particle binding	molecular_function	0.0014742
GO:0006596	polyamine biosynthetic process	biological_process	0.0014793
GO:0007162	negative regulation of cell adhesion	biological_process	0.0017696
GO:0016607	nuclear speck	cellular_component	0.0018891
GO:0043499	eukaryotic cell surface binding	molecular_function	0.0020409

GO:0002039	p53 binding	molecular_function	0.0020572
GO:0005977	glycogen metabolic process	biological_process	0.0020867
GO:0042777	plasma membrane ATP synthesis coupled proton transport	biological_process	0.0021223
GO:0007005	mitochondrion organization	biological_process	0.002182
GO:0005976	polysaccharide metabolic process	biological_process	0.0023724
GO:0033700	phospholipid efflux	biological_process	0.0024098
GO:0070653	high-density lipoprotein particle receptor binding	molecular_function	0.0024843
GO:0007010	cytoskeleton organization	biological_process	0.0024862
GO:0001568	blood vessel development	biological_process	0.0025313
GO:0034363	intermediate-density lipoprotein particle	cellular_component	0.0025757
GO:0017166	vinculin binding	molecular_function	0.0025848
GO:0005783	endoplasmic reticulum	cellular_component	0.0025968
GO:0034375	high-density lipoprotein particle remodeling	biological_process	0.0026835
GO:0030301	cholesterol transport	biological_process	0.0027037
GO:0034614	cellular response to reactive oxygen species	biological_process	0.0028612
GO:0043065	positive regulation of apoptosis	biological_process	0.0028781
GO:0060620	regulation of cholesterol import	biological_process	0.0028794
GO:0034361	very-low-density lipoprotein particle	cellular_component	0.0029047
GO:0008035	high-density lipoprotein particle binding	molecular_function	0.0029422
GO:0046966	thyroid hormone receptor binding	molecular_function	0.0029723
GO:0010916	negative regulation of very-low-density lipoprotein particle clearance	biological_process	0.0030567
GO:0044267	cellular protein metabolic process	biological_process	0.0030961
GO:0006366	transcription from RNA polymerase II promoter	biological_process	0.0031003
GO:0043534	blood vessel endothelial cell migration	biological_process	0.003191
GO:0030195	negative regulation of blood coagulation	biological_process	0.0033018
GO:0050790	regulation of catalytic activity	biological_process	0.0034185
GO:0042809	vitamin D receptor binding	molecular_function	0.0034341
GO:0035264	multicellular organism growth	biological_process	0.0034461
GO:0030423	targeting of mRNA for destruction involved in RNA interference	biological_process	0.0034715
GO:0033017	sarcoplasmic reticulum membrane	cellular_component	0.0036067
GO:0071816	tail-anchored membrane protein insertion into ER membrane	biological_process	0.0036286
GO:0071818	BAT3 complex	cellular_component	0.0036286
GO:0008092	cytoskeletal protein binding	molecular_function	0.0036306
GO:0032682	negative regulation of chemokine production	biological_process	0.0036724
GO:0071986	Ragulator complex	cellular_component	0.0036847
GO:0003746	translation elongation factor activity	molecular_function	0.0037159

GO:0009331	glycerol-3-phosphate dehydrogenase complex	cellular_component	0.0037664
GO:2000147	positive regulation of cell motility	biological_process	0.0037856
GO:0001525	angiogenesis	biological_process	0.0038177
GO:0001944	vasculature development	biological_process	0.0038484
GO:0033629	negative regulation of cell adhesion mediated by integrin	biological_process	0.0039973
GO:0007517	muscle organ development	biological_process	0.0040295
GO:0016070	RNA metabolic process	biological_process	0.0041193
GO:0018026	peptidyl-lysine monomethylation	biological_process	0.0041422
GO:0018027	peptidyl-lysine dimethylation	biological_process	0.0041446
GO:0010811	positive regulation of cell-substrate adhesion	biological_process	0.004149
GO:0072358	cardiovascular system development	biological_process	0.004401
GO:0016011	dystroglycan complex	cellular_component	0.0045247
GO:0010874	regulation of cholesterol efflux	biological_process	0.0045657
GO:0043498	cell surface binding	molecular_function	0.0046268
GO:0006139	nucleobase-containing compound metabolic	biological_process	0.004899
GO:0010596	negative regulation of endothelial cell migration	biological_process	0.0049726
GO:0097067	cellular response to thyroid hormone stimulus	biological_process	0.0049949
GO:0043691	reverse cholesterol transport	biological_process	0.00502
GO:0071013	catalytic step 2 spliceosome	cellular_component	0.0050258
GO:0016477	cell migration	biological_process	0.0050379
GO:0001937	negative regulation of endothelial cell	biological_process	0.0052894
GO:0006817	phosphate ion transport	biological_process	0.0053352
GO:0008180	signalosome	cellular_component	0.0053397
GO:0016491	oxidoreductase activity	molecular_function	0.0054185
GO:0046889	positive regulation of lipid biosynthetic process	biological_process	0.0055355
GO:0030155	regulation of cell adhesion	biological_process	0.0055678
GO:0030521	androgen receptor signaling pathway	biological_process	0.0056508
GO:0043085	positive regulation of catalytic activity	biological_process	0.0056594
GO:0005604	basement membrane	cellular_component	0.0056751
GO:0030520	estrogen receptor signaling pathway	biological_process	0.0056786
GO:0008137	NADH dehydrogenase (ubiquinone) activity	molecular_function	0.0056848
GO:0008201	heparin binding	molecular_function	0.0059363
GO:0000288	nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	biological_process	0.0060145
GO:0006006	glucose metabolic process	biological_process	0.0060242
GO:0007263	nitric oxide mediated signal transduction	biological_process	0.0060899
GO:0043537	negative regulation of blood vessel endothelial cell migration	biological_process	0.0062393

GO:0090181	regulation of cholesterol metabolic process	biological_process	0.0063398
GO:0010468	regulation of gene expression	biological_process	0.0064534
GO:0090207	regulation of triglyceride metabolic process	biological_process	0.0065484
GO:0031593	polyubiquitin binding	molecular_function	0.0065842
GO:0010181	FMN binding	molecular_function	0.0066315
GO:0070328	triglyceride homeostasis	biological_process	0.0067852
GO:0001913	T cell mediated cytotoxicity	biological_process	0.0069458
GO:0030334	regulation of cell migration	biological_process	0.0069494
GO:0030193	regulation of blood coagulation	biological_process	0.0069654
GO:0035166	post-embryonic hemopoiesis	biological_process	0.0069951
GO:0055088	lipid homeostasis	biological_process	0.0070297
GO:0018076	N-terminal peptidyl-lysine acetylation	biological_process	0.0071193
GO:0006094	gluconeogenesis	biological_process	0.0071279
GO:0019934	cGMP-mediated signaling	biological_process	0.0072684
GO:0070326	very-low-density lipoprotein particle receptor	molecular_function	0.0072684
GO:0008610	lipid biosynthetic process	biological_process	0.0072853
GO:0010757	negative regulation of plasminogen activation	biological_process	0.0073054
GO:0006412	translation	biological_process	0.0074363
GO:0016010	dystrophin-associated glycoprotein complex	cellular_component	0.0075858
GO:0042789	mRNA transcription from RNA polymerase II promoter	biological_process	0.0076109
GO:0000015	phosphopyruvate hydratase complex	cellular_component	0.007674
GO:0004634	phosphopyruvate hydratase activity	molecular_function	0.007674
GO:0000085	G2 phase of mitotic cell cycle	biological_process	0.007743
GO:0051856	adhesion to symbiont	biological_process	0.0080824
GO:0001085	RNA polymerase II transcription factor binding	molecular_function	0.008176
GO:0005665	DNA-directed RNA polymerase II, core complex	cellular_component	0.0083343
GO:0003924	GTPase activity	molecular_function	0.0083715
GO:0051291	protein heterooligomerization	biological_process	0.0083875
GO:0031638	zymogen activation	biological_process	0.0084982
GO:0045121	membrane raft	cellular_component	0.008545
GO:0043236	laminin binding	molecular_function	0.0086114
GO:0016922	ligand-dependent nuclear receptor binding	molecular_function	0.0088229
GO:0000122	negative regulation of transcription from RNA polymerase II promoter	biological_process	0.0089626
GO:2001020	regulation of response to DNA damage stimulus	biological_process	0.0092859
GO:0016874	ligase activity	molecular_function	0.0093681
GO:0001667	ameboidal cell migration	biological_process	0.0094898
GO:0046933	hydrogen ion transporting ATP synthase activity, rotational mechanism	molecular_function	0.0095466

GO:0005681	spliceosomal complex	cellular_component	0.0101762
GO:0030008	TRAPP complex	cellular_component	0.010293
GO:0051000	positive regulation of nitric-oxide synthase	biological_process	0.0107814
GO:0072593	reactive oxygen species metabolic process	biological_process	0.0109245
GO:0046983	protein dimerization activity	molecular_function	0.0110109
GO:0030176	integral to endoplasmic reticulum membrane	cellular_component	0.0111393
GO:0042383	sarcolemma	cellular_component	0.011213
GO:0031398	positive regulation of protein ubiquitination	biological_process	0.0112277
GO:0042981	regulation of apoptosis	biological_process	0.011228
GO:0051287	NAD binding	molecular_function	0.0112321
GO:0006388	tRNA splicing, via endonucleolytic cleavage and ligation	biological_process	0.0112941
GO:0007262	STAT protein import into nucleus	biological_process	0.0113028
GO:0006979	response to oxidative stress	biological_process	0.0114497
GO:0043426	MRF binding	molecular_function	0.0115197
GO:0035257	nuclear hormone receptor binding	molecular_function	0.0116093
GO:0046961	proton-transporting ATPase activity, rotational mechanism	molecular_function	0.0120069
GO:0032403	protein complex binding	molecular_function	0.0120092
GO:0042405	nuclear inclusion body	cellular_component	0.0120985
GO:0045717	negative regulation of fatty acid biosynthetic	biological_process	0.0121174
GO:0033628	regulation of cell adhesion mediated by integrin	biological_process	0.0121941
GO:0042627	chylomicron	cellular_component	0.0122062
GO:0042288	MHC class I protein binding	molecular_function	0.0123426
GO:0006351	transcription, DNA-dependent	biological_process	0.0123895
GO:0022408	negative regulation of cell-cell adhesion	biological_process	0.0124257
GO:2000060	positive regulation of protein ubiquitination involved in ubiquitin-dependent protein catabolic	biological_process	0.01246
GO:0007528	neuromuscular junction development	biological_process	0.0127109
GO:0003824	catalytic activity	molecular_function	0.0127129
GO:0030239	myofibril assembly	biological_process	0.0127461
GO:0030510	regulation of BMP signaling pathway	biological_process	0.0127539
GO:0045604	regulation of epidermal cell differentiation	biological_process	0.01285
GO:0010033	response to organic substance	biological_process	0.0132296
GO:0034380	high-density lipoprotein particle assembly	biological_process	0.0133361
GO:0006357	regulation of transcription from RNA polymerase II promoter	biological_process	0.0135412
GO:0042803	protein homodimerization activity	molecular_function	0.0135761
GO:0008652	cellular amino acid biosynthetic process	biological_process	0.0136327

GO:0005789	endoplasmic reticulum membrane	cellular_component	0.0140089
GO:0012501	programmed cell death	biological_process	0.0141661
GO:0070887	cellular response to chemical stimulus	biological_process	0.014453
GO:0005539	glycosaminoglycan binding	molecular_function	0.0149645
GO:0016567	protein ubiquitination	biological_process	0.0151551
GO:0030036	actin cytoskeleton organization	biological_process	0.0151833
GO:0006869	lipid transport	biological_process	0.0153033
GO:0005856	cytoskeleton	cellular_component	0.0154144
GO:0015992	proton transport	biological_process	0.015435
GO:0008219	cell death	biological_process	0.015681
GO:0001885	endothelial cell development	biological_process	0.0157105
GO:0001935	endothelial cell proliferation	biological_process	0.0160497
GO:0007029	endoplasmic reticulum organization	biological_process	0.0162018
GO:0051646	mitochondrion localization	biological_process	0.0162091
GO:0006707	cholesterol catabolic process	biological_process	0.0162196
GO:0006367	transcription initiation from RNA polymerase II promoter	biological_process	0.0163217
GO:0030522	intracellular receptor mediated signaling pathway	biological_process	0.0164917
GO:0006464	protein modification process	biological_process	0.016689
GO:0051788	response to misfolded protein	biological_process	0.0168283
GO:0006072	glycerol-3-phosphate metabolic process	biological_process	0.0169703
GO:0032436	positive regulation of proteasomal ubiquitin dependent protein catabolic process	biological_process	0.0174579
GO:0034504	protein localization to nucleus	biological_process	0.0177514
GO:0033178	proton-transporting two-sector ATPase complex, catalytic domain	cellular_component	0.0179041
GO:0044212	transcription regulatory region DNA binding	molecular_function	0.0180351
GO:0042176	regulation of protein catabolic process	biological_process	0.0182289
GO:0033148	positive regulation of estrogen receptor signaling pathway	biological_process	0.0185113
GO:0006352	transcription initiation, DNA-dependent	biological_process	0.0185821
GO:0042692	muscle cell differentiation	biological_process	0.0187662
GO:0051341	regulation of oxidoreductase activity	biological_process	0.0188408
GO:0006915	apoptosis	biological_process	0.0189245
GO:0034599	cellular response to oxidative stress	biological_process	0.0189969
GO:0051393	alpha-actinin binding	molecular_function	0.0192148
GO:0045600	positive regulation of fat cell differentiation	biological_process	0.0195125
GO:0010872	regulation of cholesterol esterification	biological_process	0.0195549
GO:0030335	positive regulation of cell migration	biological_process	0.0197869
GO:0000287	magnesium ion binding	molecular_function	0.0199799

GO:0051353	positive regulation of oxidoreductase activity	biological_process	0.0201221
GO:0006096	glycolysis	biological_process	0.0201568
GO:0006754	ATP biosynthetic process	biological_process	0.0201918
GO:0030513	positive regulation of BMP signaling pathway	biological_process	0.0205082
GO:0046782	regulation of viral transcription	biological_process	0.02064
GO:0040014	regulation of multicellular organism growth	biological_process	0.0207986
GO:0042773	ATP synthesis coupled electron transport	biological_process	0.0210145
GO:0045214	sarcomere organization	biological_process	0.0211127
GO:0015914	phospholipid transport	biological_process	0.0213612
GO:0006633	fatty acid biosynthetic process	biological_process	0.0216208
GO:0051493	regulation of cytoskeleton organization	biological_process	0.022039
GO:0050662	coenzyme binding	molecular_function	0.0221708
GO:0030336	negative regulation of cell migration	biological_process	0.0223201
GO:0016469	proton-transporting two-sector ATPase complex	cellular_component	0.0229318
GO:0015030	Cajal body	cellular_component	0.023227
GO:0048741	skeletal muscle fiber development	biological_process	0.0233403
GO:0030509	BMP signaling pathway	biological_process	0.0233993
GO:0006457	protein folding	biological_process	0.0234742
GO:0046324	regulation of glucose import	biological_process	0.0238026
GO:0014704	intercalated disc	cellular_component	0.0242046
GO:0030529	ribonucleoprotein complex	cellular_component	0.024519
GO:0008354	germ cell migration	biological_process	0.0247832
GO:0005978	glycogen biosynthetic process	biological_process	0.0248165
GO:0045765	regulation of angiogenesis	biological_process	0.0248728
GO:0032008	positive regulation of TOR signaling cascade	biological_process	0.0250313
GO:0007566	embryo implantation	biological_process	0.0251716
GO:0008253	5'-nucleotidase activity	molecular_function	0.0253165
GO:0005801	cis-Golgi network	cellular_component	0.0253424
GO:0006338	chromatin remodeling	biological_process	0.0253684
GO:0016209	antioxidant activity	molecular_function	0.0256616
GO:0043923	positive regulation by host of viral transcription	biological_process	0.0256783
GO:0016829	lyase activity	molecular_function	0.0258003
GO:0006695	cholesterol biosynthetic process	biological_process	0.0260098
GO:0009117	nucleotide metabolic process	biological_process	0.0266601
GO:0033619	membrane protein proteolysis	biological_process	0.0266851
GO:0008024	positive transcription elongation factor complex b	cellular_component	0.0273931
GO:0008969	phosphohistidine phosphatase activity	molecular_function	0.0277314
GO:0035971	peptidyl-histidine dephosphorylation	biological_process	0.0277314

GO:2000984	negative regulation of ATP citrate synthase	biological_process	0.0277314
GO:0048133	male germ-line stem cell division	biological_process	0.0278319
GO:0003713	transcription coactivator activity	molecular_function	0.0287704
GO:0061036	positive regulation of cartilage development	biological_process	0.0287925
GO:0034191	apolipoprotein A-I receptor binding	molecular_function	0.0288344
GO:0050713	negative regulation of interleukin-1 beta secretion	biological_process	0.0288344
GO:0060354	negative regulation of cell adhesion molecule production	biological_process	0.0288344
GO:0090026	positive regulation of monocyte chemotaxis	biological_process	0.029078
GO:0032805	positive regulation of low-density lipoprotein particle receptor catabolic process	biological_process	0.0296671
GO:0042159	lipoprotein catabolic process	biological_process	0.0296671
GO:0046911	metal chelating activity	molecular_function	0.0296671
GO:0030911	TPR domain binding	molecular_function	0.0297809
GO:0006642	triglyceride mobilization	biological_process	0.0299183
GO:0010897	negative regulation of triglyceride catabolic	biological_process	0.0299183
GO:0010987	negative regulation of high-density lipoprotein particle clearance	biological_process	0.0299183
GO:0060538	skeletal muscle organ development	biological_process	0.0300553
GO:0046331	lateral inhibition	biological_process	0.0303131
GO:0061106	negative regulation of stomach neuroendocrine cell differentiation	biological_process	0.0303131
GO:0071820	N-box binding	molecular_function	0.0303131
GO:0072141	renal interstitial cell development	biological_process	0.0303131
GO:2000227	negative regulation of pancreatic A cell	biological_process	0.0303131
GO:2000974	negative regulation of pro-B cell differentiation	biological_process	0.0303131
GO:0071796	K6-linked polyubiquitin binding	molecular_function	0.030375
GO:0001967	suckling behavior	biological_process	0.0308437
GO:0035087	siRNA loading onto RISC involved in RNA interference	biological_process	0.0311164
GO:0051736	ATP-dependent polyribonucleotide 5'-hydroxyl kinase activity	molecular_function	0.0311164
GO:0002041	intussusceptive angiogenesis	biological_process	0.0315323
GO:0016783	sulfurtransferase activity	molecular_function	0.0317795
GO:0004853	uroporphyrinogen decarboxylase activity	molecular_function	0.0318475
GO:2001141	regulation of RNA biosynthetic process	biological_process	0.0320215
GO:0004313	[acyl-carrier-protein] S-acetyltransferase activity	molecular_function	0.0321019
GO:0004316	3-oxoacyl-[acyl-carrier-protein] reductase (NADPH) activity	molecular_function	0.0321019
GO:0004317	3-hydroxypalmitoyl-[acyl-carrier-protein] dehydratase activity	molecular_function	0.0321019

GO:0004319	enoyl-[acyl-carrier-protein] reductase (NADPH, B-specific) activity	molecular_function	0.0321019
GO:0004320	oleoyl-[acyl-carrier-protein] hydrolase activity	molecular_function	0.0321019
GO:0016295	myristoyl-[acyl-carrier-protein] hydrolase activity	molecular_function	0.0321019
GO:0016296	palmitoyl-[acyl-carrier-protein] hydrolase activity	molecular_function	0.0321019
GO:0016297	acyl-[acyl-carrier-protein] hydrolase activity	molecular_function	0.0321019
GO:0034450	ubiquitin-ubiquitin ligase activity	molecular_function	0.0322331
GO:0034383	low-density lipoprotein particle clearance	biological_process	0.0324788
GO:0060213	positive regulation of nuclear- transcribed mRNA poly(A) tail shortening	biological_process	0.0326581
GO:0006461	protein complex assembly	biological_process	0.0327404
GO:0043034	costamere	cellular_component	0.0329226
GO:0045444	fat cell differentiation	biological_process	0.0329391
GO:0045454	cell redox homeostasis	biological_process	0.0330439
GO:0030262	apoptotic nuclear change	biological_process	0.0333183
GO:0045665	negative regulation of neuron differentiation	biological_process	0.0333778
GO:0016322	neuron remodeling	biological_process	0.0337986
GO:0010212	response to ionizing radiation	biological_process	0.0338149
GO:0003881	CDP-diacylglycerol-inositol 3-phosphatidyltransferase activity	molecular_function	0.0339858
GO:0001010	sequence-specific DNA binding transcription factor recruiting transcription factor activity	molecular_function	0.0340387
GO:2000342	negative regulation of chemokine (C-X-C motif) ligand 2 production	biological_process	0.0340387
GO:0051131	chaperone-mediated protein complex assembly	biological_process	0.0341034
GO:0090096	positive regulation of metanephric cap mesenchymal cell proliferation	biological_process	0.0343912
GO:0043254	regulation of protein complex assembly	biological_process	0.0345241
GO:0035280	miRNA loading onto RISC involved in gene silencing by miRNA	biological_process	0.0345243
GO:0001078	RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in negative regulation of transcription	molecular_function	0.0351025
GO:0045893	positive regulation of transcription, DNA-	biological_process	0.0351818
GO:0045725	positive regulation of glycogen biosynthetic	biological_process	0.035207
GO:0016605	PML body	cellular_component	0.0352199
GO:0017127	cholesterol transporter activity	molecular_function	0.035224
GO:0031647	regulation of protein stability	biological_process	0.0353462
GO:0004586	ornithine decarboxylase activity	molecular_function	0.0353879

GO:0071310	cellular response to organic substance	biological_process	0.0354304
GO:0044324	regulation of transcription involved in anterior/posterior axis specification	biological_process	0.0355557
GO:2000016	negative regulation of determination of dorsal	biological_process	0.0355557
GO:0032439	endosome localization	biological_process	0.0355742
GO:0035491	positive regulation of leukotriene production involved in inflammatory response	biological_process	0.0356754
GO:0061044	negative regulation of vascular wound healing	biological_process	0.0356754
GO:2000098	negative regulation of smooth muscle cell-matrix adhesion	biological_process	0.0356754
GO:0001849	complement component C1q binding	molecular_function	0.0358919
GO:0035499	carnosine biosynthetic process	biological_process	0.0358928
GO:0047730	carnosine synthase activity	molecular_function	0.0358928
GO:0008483	transaminase activity	molecular_function	0.0358938
GO:0034394	protein localization at cell surface	biological_process	0.0359605
GO:2000008	regulation of protein localization at cell surface	biological_process	0.0362491
GO:0051258	protein polymerization	biological_process	0.036412
GO:0006184	GTP catabolic process	biological_process	0.0365314
GO:0005829	cytosol	cellular_component	0.0366309
GO:0032006	regulation of TOR signaling cascade	biological_process	0.0366917
GO:0030023	extracellular matrix constituent conferring	molecular_function	0.0368971
GO:0031748	D1 dopamine receptor binding	molecular_function	0.0369168
GO:0004308	exo-alpha-sialidase activity	molecular_function	0.0369884
GO:0010309	acireductone dioxygenase [iron(II)-requiring]	molecular_function	0.0370766
GO:0035994	response to muscle stretch	biological_process	0.0372028
GO:0018012	N-terminal peptidyl-alanine trimethylation	biological_process	0.0372723
GO:0018016	N-terminal peptidyl-proline dimethylation	biological_process	0.0372723
GO:0035572	N-terminal peptidyl-serine dimethylation	biological_process	0.0372723
GO:0035573	N-terminal peptidyl-serine trimethylation	biological_process	0.0372723
GO:2000277	positive regulation of oxidative phosphorylation uncoupler activity	biological_process	0.0375732
GO:0070885	negative regulation of calcineurin-NFAT signaling pathway	biological_process	0.0375941
GO:2000504	positive regulation of blood vessel remodeling	biological_process	0.0376207
GO:0032463	negative regulation of protein	biological_process	0.0376268
GO:0042583	chromaffin granule	cellular_component	0.0376268
GO:0004631	phosphomevalonate kinase activity	molecular_function	0.0376618
GO:0005055	laminin receptor activity	molecular_function	0.037666

GO:0030387	fructosamine-3-kinase activity	molecular_function	0.0376739
GO:0030389	fructosamine metabolic process	biological_process	0.0376739
GO:0000244	assembly of spliceosomal tri-snRNP	biological_process	0.0376748
GO:0002543	activation of blood coagulation via clotting	biological_process	0.0376748
GO:0002581	negative regulation of antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	biological_process	0.0376748
GO:0002605	negative regulation of dendritic cell antigen processing and presentation	biological_process	0.0376748
GO:0003360	brainstem development	biological_process	0.0376748
GO:0003972	RNA ligase (ATP) activity	molecular_function	0.0376748
GO:0004014	adenosylmethionine decarboxylase activity	molecular_function	0.0376748
GO:0004081	bis(5'-nucleosyl)-tetraphosphatase (asymmetrical) activity	molecular_function	0.0376748
GO:0004133	glycogen debranching enzyme activity	molecular_function	0.0376748
GO:0004134	4-alpha-glucanotransferase activity	molecular_function	0.0376748
GO:0004135	amylase-1,6-glucosidase activity	molecular_function	0.0376748
GO:0004164	diphthine synthase activity	molecular_function	0.0376748
GO:0004347	glucose-6-phosphate isomerase activity	molecular_function	0.0376748
GO:0004349	glutamate 5-kinase activity	molecular_function	0.0376748
GO:0004350	glutamate-5-semialdehyde dehydrogenase	molecular_function	0.0376748
GO:0004824	lysine-tRNA ligase activity	molecular_function	0.0376748
GO:0005290	L-histidine transmembrane transporter activity	molecular_function	0.0376748
GO:0005785	signal recognition particle receptor complex	cellular_component	0.0376748
GO:0005969	serine-pyruvate aminotransferase complex	cellular_component	0.0376748
GO:0006430	lysyl-tRNA aminoacylation	biological_process	0.0376748
GO:0008295	spermidine biosynthetic process	biological_process	0.0376748
GO:0008750	NAD(P)+ transhydrogenase (AB-specific) activity	molecular_function	0.0376748
GO:0008796	bis(5'-nucleosyl)-tetraphosphatase activity	molecular_function	0.0376748
GO:0009279	cell outer membrane	cellular_component	0.0376748
GO:0010751	negative regulation of nitric oxide mediated signal transduction	biological_process	0.0376748
GO:0010754	negative regulation of cGMP-mediated signaling	biological_process	0.0376748
GO:0010825	positive regulation of centrosome duplication	biological_process	0.0376748
GO:0010899	regulation of phosphatidylcholine catabolic	biological_process	0.0376748
GO:0014801	longitudinal sarcoplasmic reticulum	cellular_component	0.0376748

GO:0015817	histidine transport	biological_process	0.0376748
GO:0016401	palmitoyl-CoA oxidase activity	molecular_function	0.0376748
GO:0017064	fatty acid amide hydrolase activity	molecular_function	0.0376748
GO:0019836	hemolysis by symbiont of host erythrocytes	biological_process	0.0376748
GO:0021682	nerve maturation	biological_process	0.0376748
GO:0030108	HLA-A specific activating MHC class I receptor activity	molecular_function	0.0376748
GO:0031100	organ regeneration	biological_process	0.0376748
GO:0032427	GBD domain binding	molecular_function	0.0376748
GO:0032534	regulation of microvillus assembly	biological_process	0.0376748
GO:0036033	mediator complex binding	molecular_function	0.0376748
GO:0042272	nuclear RNA export factor complex	cellular_component	0.0376748
GO:0042413	carnitine catabolic process	biological_process	0.0376748
GO:0042978	ornithine decarboxylase activator activity	molecular_function	0.0376748
GO:0043111	replication fork arrest	biological_process	0.0376748
GO:0044302	dentate gyrus mossy fiber	cellular_component	0.0376748
GO:0044354	macropinosome	cellular_component	0.0376748
GO:0046079	dUMP catabolic process	biological_process	0.0376748
GO:0046322	negative regulation of fatty acid oxidation	biological_process	0.0376748
GO:0046814	virion attachment, binding of host cell surface coreceptor	biological_process	0.0376748
GO:0047276	N-acetyllactosaminide 3-alpha galactosyltransferase activity	molecular_function	0.0376748
GO:0047312	L-phenylalanine:pyruvate aminotransferase	molecular_function	0.0376748
GO:0047945	L-glutamine:pyruvate aminotransferase activity	molecular_function	0.0376748
GO:0051463	negative regulation of cortisol secretion	biological_process	0.0376748
GO:0060125	negative regulation of growth hormone secretion	biological_process	0.0376748
GO:0060668	regulation of branching involved in salivary gland morphogenesis by extracellular matrix-epithelial cell signaling	biological_process	0.0376748
GO:0060837	blood vessel endothelial cell differentiation	biological_process	0.0376748
GO:0070506	high-density lipoprotein particle receptor activity	molecular_function	0.0376748
GO:0070990	snRNP binding	molecular_function	0.0376748
GO:0097045	phosphatidylserine exposure on blood platelet	biological_process	0.0376748
GO:0004820	glycine-tRNA ligase activity	molecular_function	0.0376748
GO:0006426	glycyl-tRNA aminoacylation	biological_process	0.0376748
GO:0008705	methionine synthase activity	molecular_function	0.0376748
GO:0015966	diadenosine tetraphosphate biosynthetic process	biological_process	0.0376748
GO:0004903	growth hormone receptor activity	molecular_function	0.0376748

GO:0060244	negative regulation of cell proliferation involved in contact inhibition	biological_process	0.0376748
GO:0070195	growth hormone receptor complex	cellular_component	0.0376748
GO:0006975	DNA damage induced protein phosphorylation	biological_process	0.0376748
GO:0014701	junctional sarcoplasmic reticulum membrane	cellular_component	0.0376748
GO:0007624	ultradian rhythm	biological_process	0.0376748
GO:0016199	axon midline choice point recognition	biological_process	0.0376748
GO:2000195	negative regulation of female gonad development	biological_process	0.0376748
GO:0070742	C2H2 zinc finger domain binding	molecular_function	0.0377027
GO:0043488	regulation of mRNA stability	biological_process	0.0379497
GO:0016831	carboxy-lyase activity	molecular_function	0.0384208
GO:0016012	sarcoglycan complex	cellular_component	0.0384868
GO:0016126	sterol biosynthetic process	biological_process	0.0390428
GO:0072546	ER membrane protein complex	cellular_component	0.0392941
GO:0006337	nucleosome disassembly	biological_process	0.0393974
GO:0003779	actin binding	molecular_function	0.0397867
GO:0043393	regulation of protein binding	biological_process	0.0400108
GO:0031116	positive regulation of microtubule polymerization	biological_process	0.0401797
GO:0006913	nucleocytoplasmic transport	biological_process	0.040916
GO:0046332	SMAD binding	molecular_function	0.0410673
GO:0030169	low-density lipoprotein particle binding	molecular_function	0.041238
GO:0044319	wound healing, spreading of cells	biological_process	0.0412883
GO:0008242	omega peptidase activity	molecular_function	0.0415166
GO:0001968	fibronectin binding	molecular_function	0.0415407
GO:0005815	microtubule organizing center	cellular_component	0.0419165
GO:0016514	SWI/SNF complex	cellular_component	0.0419678
GO:0002839	positive regulation of immune response to tumor	biological_process	0.0423847
GO:0021903	rostrocaudal neural tube patterning	biological_process	0.0431246
GO:0002011	morphogenesis of an epithelial sheet	biological_process	0.0433509
GO:0005581	collagen	cellular_component	0.0441293
GO:0006629	lipid metabolic process	biological_process	0.0445507
GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	biological_process	0.0450683
GO:0042157	lipoprotein metabolic process	biological_process	0.0465469
GO:0001102	RNA polymerase II activating transcription factor binding	molecular_function	0.0469261
GO:0009062	fatty acid catabolic process	biological_process	0.0470206
GO:0000413	protein peptidyl-prolyl isomerization	biological_process	0.0470508
GO:0003755	peptidyl-prolyl cis-trans isomerase activity	molecular_function	0.0470508
GO:0034329	cell junction assembly	biological_process	0.0473038

GO:0005813	centrosome	cellular_component	0.0476146
GO:0000266	mitochondrial fission	biological_process	0.0476537
GO:0017069	snRNA binding	molecular_function	0.0477835
GO:0060053	neurofilament cytoskeleton	cellular_component	0.0487999
GO:0050860	negative regulation of T cell receptor signaling pathway	biological_process	0.0488189
GO:0006402	mRNA catabolic process	biological_process	0.0495531

Kyoto encyclopedia of genes and genomes enrichment for functional analysis of differentially expressed genes

To identify the pathways these DEGs involved, we integrated the 682 DEGs into the KEGG pathway database, and a total of 185 pathways were enriched (Table S4). KEGG pathway enrichment

analysis showed that the DEGs were statistically significantly enriched in 11 pathways ($P < 0.05$) (Figure 4). The pathways associated with muscle growth and lipid deposition include biosynthesis of unsaturated fatty acids, ErbB signaling pathway, metabolic pathways, and oxidative phosphorylation.

Table S4: KEGG pathways enrichment of differentially expressed genes.

Pathways	Id	P-Value
Parkinson's disease	ssc05012	6.87E-10
Oxidative phosphorylation	ssc00190	7.79E-09
Alzheimer's disease	ssc05010	4.07E-08
Huntington's disease	ssc05016	5.31E-07
Arginine and proline metabolism	ssc00330	0.001381844
Metabolic pathways	ssc01100	0.005716269
ErbB signaling pathway	ssc04012	0.007914644
Cardiac muscle contraction	ssc04260	0.02127572
Alanine, aspartate and glutamate metabolism	ssc00250	0.026916743
Biosynthesis of unsaturated fatty acids	ssc01040	0.037077945
Bacterial invasion of epithelial cells	ssc05100	0.046510544
Nicotinate and nicotinamide metabolism	ssc00760	0.063759788
Cysteine and methionine metabolism	ssc00270	0.070735243
Bladder cancer	ssc05219	0.070735243
Insulin signaling pathway	ssc04910	0.076205916
Regulation of actin cytoskeleton	ssc04810	0.087807249
Gap junction	ssc04540	0.089015969
Glycolysis / Gluconeogenesis	ssc00010	0.090370432
Focal adhesion	ssc04510	0.091163843
Circadian rhythm-mammal	ssc04710	0.094230871
Sulfur relay system	ssc04122	0.094230871
TGF-beta signaling pathway	ssc04350	0.109035475
Selenocompound metabolism	ssc00450	0.113354041
Terpenoid backbone biosynthesis	ssc00900	0.113354041
Renal cell carcinoma	ssc05211	0.113855709
Purine metabolism	ssc00230	0.116740868
PPAR signaling pathway	ssc03320	0.131153704
Fructose and mannose metabolism	ssc00051	0.14975294
Endometrial cancer	ssc05213	0.15854135
Dilated cardiomyopathy	ssc05414	0.172277975
Hypertrophic Cardiomyopathy (HCM)	ssc05410	0.172277975
Renin-angiotensin system	ssc04614	0.175340579
Chronic myeloid leukemia	ssc05220	0.178310329

Axon guidance	ssc04360	0.189073196
Prion diseases	ssc05020	0.19400562
Starch and sucrose metabolism	ssc00500	0.209414418
Viral myocarditis	ssc05416	0.220101799
Pyruvate metabolism	ssc00620	0.225080448
Ribosome	ssc03010	0.227559949
Glycerophospholipid metabolism	ssc00564	0.230990654
Dorso-ventral axis formation	ssc04320	0.241205595
Fc gamma R-mediated phagocytosis	ssc04666	0.253206829
Fat digestion and absorption	ssc04975	0.25702104
Vitamin digestion and absorption	ssc04977	0.263477555
Phagosome	ssc04145	0.264642782
Aminoacyl-tRNA biosynthesis	ssc00970	0.283963093
Colorectal cancer	ssc05210	0.283963093
Pentose phosphate pathway	ssc00030	0.28575366
Glycosaminoglycan biosynthesis - chondroitin sulfate	ssc00532	0.28575366
Nitrogen metabolism	ssc00910	0.28575366
Galactose metabolism	ssc00052	0.28575366
Type II diabetes mellitus	ssc04930	0.289516389
Thyroid cancer	ssc05216	0.307956712
Adherens junction	ssc04520	0.310832219
Amyotrophic Lateral Sclerosis (ALS)	ssc05014	0.322282
Protein export	ssc03060	0.330018542
Folate biosynthesis	ssc00790	0.345404824
Glycosphingolipid biosynthesis - ganglio series	ssc00604	0.345404824
RNA degradation	ssc03018	0.351508265
HTLV-I infection	ssc05166	0.35221846
Fatty acid metabolism	ssc00071	0.355061648
Peroxisome	ssc04146	0.365094952
MAPK signaling pathway	ssc04010	0.367589136
Citrate cycle (TCA cycle)	ssc00020	0.373486323
Glutathione metabolism	ssc00480	0.387627964
Vascular smooth muscle contraction	ssc04270	0.393420034
Acute myeloid leukemia	ssc05221	0.403767903
Amino sugar and nucleotide sugar metabolism	ssc00520	0.403767903
Cholinergic synapse	ssc04725	0.405248162
Arrhythmogenic Right Ventricular Cardiomyopathy (ARV)	ssc05412	0.405709871
Leukocyte transendothelial migration	ssc04670	0.414368993
Glioma	ssc05214	0.419782147
Spliceosome	ssc03040	0.424984239
Other glycan degradation	ssc00511	0.454150421
Glycerolipid metabolism	ssc00561	0.466873464
Salmonella infection	ssc05132	0.472060428
Basal transcription factors	ssc03022	0.476328492
Protein processing in endoplasmic reticulum	ssc04141	0.480436288
Gastric acid secretion	ssc04971	0.48219569
Non-homologous end-joining	ssc03450	0.486229812

Carbohydrate digestion and absorption	ssc04973	0.495655265
Notch signaling pathway	ssc04330	0.495655265
ECM-receptor interaction	ssc04512	0.497843598
Pyrimidine metabolism	ssc00240	0.497843598
Legionellosis	ssc05134	0.512189762
GABAergic synapse	ssc04727	0.512189762
alpha-Linolenic acid metabolism	ssc00592	0.516426212
One carbon pool by folate	ssc00670	0.516426212
Melanogenesis	ssc04916	0.535456049
Cell cycle	ssc04110	0.563873393
Pentose and glucuronate interconversions	ssc00040	0.571605148
Pancreatic cancer	ssc05212	0.582871653
Melanoma	ssc05218	0.582871653
Prostate cancer	ssc05215	0.583299209
Neurotrophin signaling pathway	ssc04722	0.595091819
p53 signaling pathway	ssc04115	0.596204921
Chemokine signaling pathway	ssc04062	0.606346147
Protein digestion and absorption	ssc04974	0.609257148
Phenylalanine metabolism	ssc00360	0.620495045
RIG-I-like receptor signaling pathway	ssc04622	0.622024563
Pathways in cancer	ssc05200	0.62879929
Non-small cell lung cancer	ssc05223	0.632976209
Retrograde endocannabinoid signaling	ssc04723	0.658592841
Long-term potentiation	ssc04720	0.662385211
Fatty acid elongation	ssc00062	0.663811872
Maturity onset diabetes of the young	ssc04950	0.663811872
Glycosaminoglycan biosynthesis - heparan sulfate	ssc00534	0.663811872
GnRH signaling pathway	ssc04912	0.670199745
Wnt signaling pathway	ssc04310	0.671848788
Leishmaniasis	ssc05140	0.681514851
Pertussis	ssc05133	0.681514851
Jak-STAT signaling pathway	ssc04630	0.683117043
Glycosphingolipid biosynthesis - lacto and neolacto seri	ssc00601	0.683581603
Proteasome	ssc03050	0.689868861
RNA polymerase	ssc03020	0.702190185
Transcriptional misregulation in cancer	ssc05202	0.706661879
Progesterone-mediated oocyte maturation	ssc04914	0.713718207
RNA transport	ssc03013	0.714646429
N-Glycan biosynthesis	ssc00510	0.715483352
Pancreatic secretion	ssc04972	0.723877547
Taste transduction	ssc04742	0.736192372
Small cell lung cancer	ssc05222	0.743348278
Long-term depression	ssc04730	0.750552518
Bile secretion	ssc04976	0.761387188
Glutamatergic synapse	ssc04724	0.761709844
Calcium signaling pathway	ssc04020	0.769989031

Porphyrin and chlorophyll metabolism	ssc00860	0.780065018
Chagas disease (American trypanosomiasis)	ssc05142	0.782302998
Malaria	ssc05144	0.791472967
Ubiquitin mediated proteolysis	ssc04120	0.796571851
Tyrosine metabolism	ssc00350	0.805185214
Sphingolipid metabolism	ssc00600	0.816649287
Regulation of autophagy	ssc04140	0.816649287
B cell receptor signaling pathway	ssc04662	0.818160768
Serotonergic synapse	ssc04726	0.824721207
Amoebiasis	ssc05146	0.824721207
Salivary secretion	ssc04970	0.826353067
Aldosterone-regulated sodium reabsorption	ssc04960	0.827439569
Lysine degradation	ssc00310	0.827439569
Herpes simplex infection	ssc05168	0.82774627
Cocaine addiction	ssc05030	0.837595613
Tryptophan metabolism	ssc00380	0.837595613
African trypanosomiasis	ssc05143	0.847154654
Adipocytokine signaling pathway	ssc04920	0.848972209
mRNA surveillance pathway	ssc03015	0.848972209
Endocytosis	ssc04144	0.860832957
Hepatitis C	ssc05160	0.866989286
Valine, leucine and isoleucine degradation	ssc00280	0.872590109
T cell receptor signaling pathway	ssc04660	0.872923662
Graft-versus-host disease	ssc05332	0.880091685
Vasopressin-regulated water reabsorption	ssc04962	0.880091685
Antigen processing and presentation	ssc04612	0.880778932
Fanconi anemia pathway	ssc03460	0.887152127
Lysosome	ssc04142	0.896728729
Basal cell carcinoma	ssc05217	0.900051727
Osteoclast differentiation	ssc04380	0.905071694
Allograft rejection	ssc05330	0.905938232
Hematopoietic cell lineage	ssc04640	0.914993702
mTOR signaling pathway	ssc04150	0.921600339
Type I diabetes mellitus	ssc04940	0.92621914
Toxoplasmosis	ssc05145	0.926566428
VEGF signaling pathway	ssc04370	0.930566165
Drug metabolism - cytochrome P450	ssc00982	0.934657384
Intestinal immune network for IgA production	ssc04672	0.938507833
Inositol phosphate metabolism	ssc00562	0.938507833
Oocyte meiosis	ssc04114	0.939823876
Metabolism of xenobiotics by cytochrome P450	ssc00980	0.942131663
Cytosolic DNA-sensing pathway	ssc04623	0.942131663
Natural killer cell mediated cytotoxicity	ssc04650	0.942751499
Toll-like receptor signaling pathway	ssc04620	0.942751499
Arachidonic acid metabolism	ssc00590	0.945542197
Autoimmune thyroid disease	ssc05320	0.948751972
Dopaminergic synapse	ssc04728	0.950742982

Fc epsilon RI signaling pathway	ssc04664	0.951772793
Influenza A	ssc05164	0.953464752
Rheumatoid arthritis	ssc05323	0.955466701
Tuberculosis	ssc05152	0.957133356
Cell Adhesion Molecules (CAMs)	ssc04514	0.965526488
Phosphatidylinositol signaling system	ssc04070	0.966508231
Ribosome biogenesis in eukaryotes	ssc03008	0.968483465
Cytokine-cytokine receptor interaction	ssc04060	0.97118963
Tight junction	ssc04530	0.971856601
Complement and coagulation cascades	ssc04610	0.973738045
Neuroactive ligand-receptor interaction	ssc04080	0.988253026
Measles	ssc05162	0.998751564

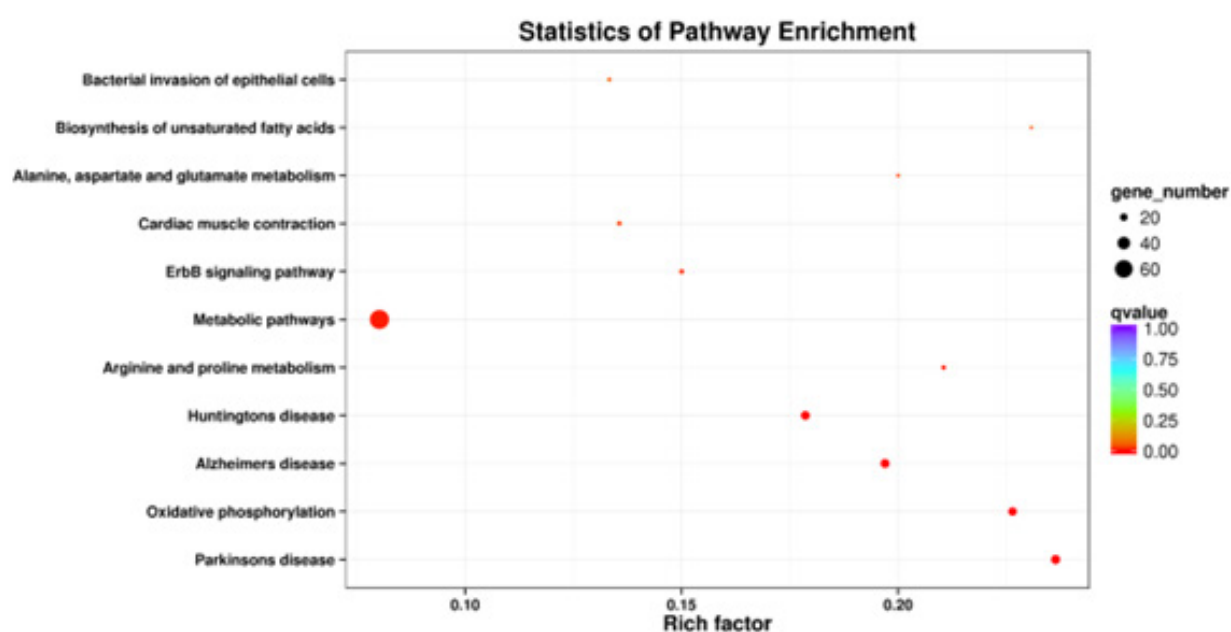


Figure 4: The diagrams for the KEGG pathway enrichment degree of DEGs. The abscissa indicates the value of rich factors (the ratio of annotated DEGs to all genes of the enriched pathway); the ordinate indicates the pathways enriched. The P-value of each term is represented by the color depth. The number of DEGs is indicated by the size of the circle. Note that the complete list of bioinformatics analysis results is shown in Supplementary Tables.

PPI network construction and hub gene identification

After STRING analysis of the DEGs, the PPI network was constructed with 418 nodes and 863 interactions (Figure 5). After being visualized by Cytoscape the connectivity degree of each node was calculated. NDUFV1 (NADH: Ubiquinone Oxidoreductase Core Subunit V1), NDUFB9 (NADH: Ubiquinone Oxidoreductase Subunit B9), UQCRC1 (Ubiquinol-Cytochrome C Reductase Core Protein 1), NDUFA10 (NADH: Ubiquinone Oxidoreductase Subunit A10), NDUFS2 (NADH: Ubiquinone Oxidoreductase Core Subunit S2), ATP5MC1 (ATP Synthase Membrane Subunit C Locus 1), NDUFS6 (NADH: Ubiquinone Oxidoreductase Subunit S6), COX4I1 (Cytochrome C Oxidase Subunit 4I1), TUFM (Tu Translation

Elongation Factor, Mitochondrial), and NDUFB8 (NADH: Ubiquinone Oxidoreductase Subunit B8) were the top 10 hub genes with the closest connections to other nodes (Figure 6A). The whole PPI network was analyzed by MCODE (Figure 6B). A total of 12 modules were identified within the PPI network. Among these, four modules (Modules 1-4) with both MCODE score > 5 and nodes > 5 were further selected for functional analysis. The pathway enrichment analysis revealed that DEGs in module 1 were involved in cellular respiration and energy production and indirectly affect muscle growth. The genes in module 1 were up regulated in Landrace pigs muscle tissue.

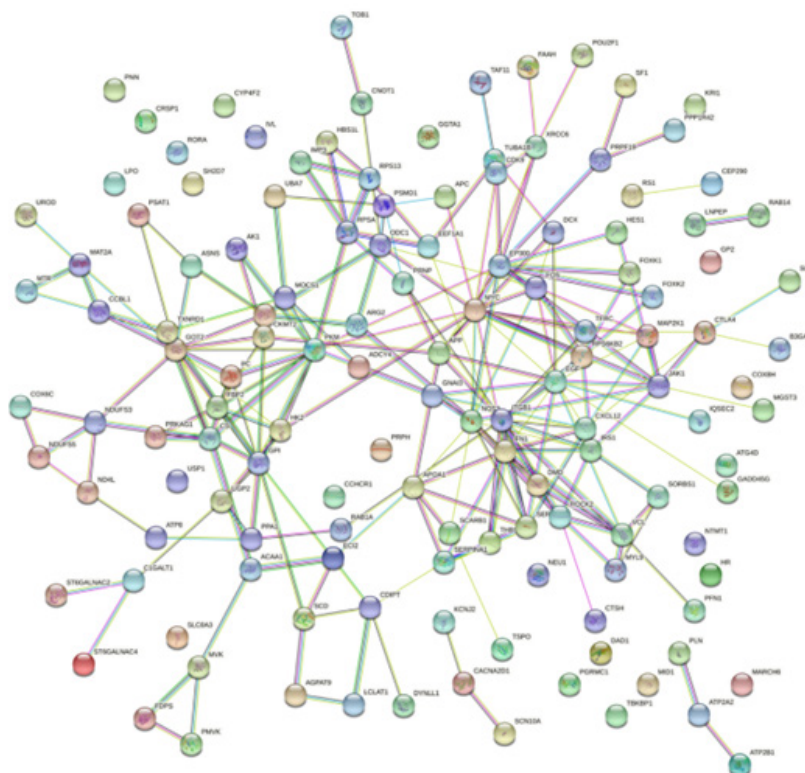


Figure 5: PPI network construction.

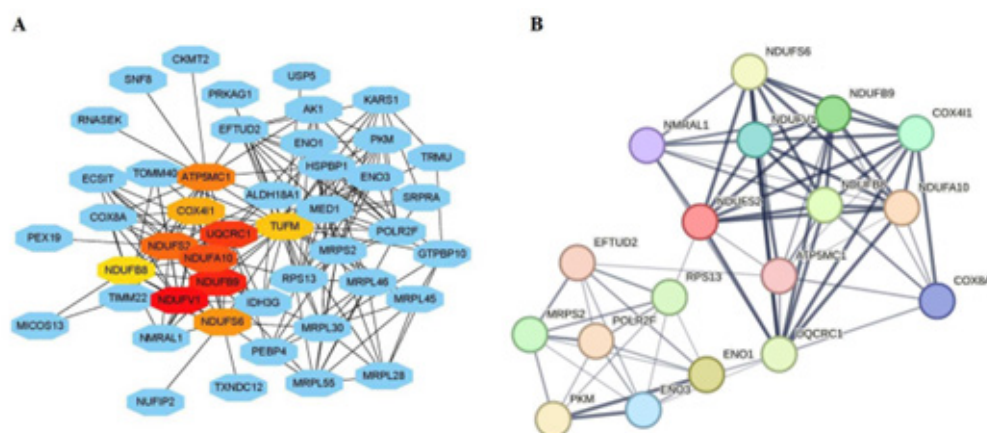


Figure 6: Hub gene identification.

A. Top hub gene identification *via* cytohubba.
B. Module 1 identification *via* MCODE.

Discussion

In this study, the longissimus dorsi muscle samples from two pig breeds, comprising a Chinese breed (Wujin pigs) and an introduced breed (Landrace pigs), were used to compare the differences of the transcriptome's profiles. We identified 682 DEGs that were mainly enriched in GO terms associated with cholesterol import, chylomicron remnant clearance, cholesterol efflux, and regulation of the lipid metabolic process, etc. In previous studies, the amount of DEG in pig muscle tissue is slightly different based on transcriptome analysis [18-21]. It is speculated that the reason

is that the experimental design or screening threshold is different. In the present study, we selected a fat-type pig breed (Wujin pigs) known for its characteristics of high lipid deposition and slow body growth and contrasted it with a lean-type pig breed (Landrace pigs) known for its traits of low lipid deposition and fast growth. And SFA, PUFA and IMF content measured in this study (Table 1) confirmed the phenotype difference between the breeds. The three genes associated with muscle growth, as well as the four genes linked to fat deposition, which we have identified, have also been previously documented in earlier studies [22,23].

Fat deposition in adipose is a complex metabolic process involving many genes, including coding and no coding genes [24]. In this study, GO and KEGG analysis results of DEGs showed that Wujin pig muscle had strong lipid deposition capacity. Among them, cholesterol import, chylomicron remnant clearance, cholesterol efflux, and biosynthesis of unsaturated fatty acids were significantly enriched. This may also further explain the characteristics of the high intramuscular fat content characteristics of Wujin pigs. Our previous report indicated that the mechanism of higher IMF content in fatty pig breeds may be due to the higher expression of lipogenic genes and fatty acids transporting genes and the lower expression of lipid catabolic genes in muscle tissue [25]. This conclusion is repeated in this study. In our data, the key lipogenic genes such as ATF3 and EGR1 had higher expression levels in the group with high-IMF content, which may be the reason for the stronger lipid deposition capacity in the Chinese pigs than the introduced pigs.

In this study, the construction of the PPI network and the identification of the hub genes were carried out for the differential expression genes. Ten hub genes were screened, including DUF8B, TUFM, NDUFB9, ATP5MC1, NDUFS2, NDUFA10, NDUFS6, NDUFV1, COX4I1, UQCRC1. The hub genes are primarily associated with the downregulation of the mitochondrial respiratory chain and energy metabolism. Muscle growth and maintenance require a lot of energy, and mitochondria play an important role in energy production in cells [26,27]. The proteins encoded by these genes are involved in the mitochondrial respiratory chain and energy expenditure, which is critical for providing sufficient energy to support the growth, repair, and function of the harvested muscle tissue [28].

ATF3 is a stress-induced transcription factor that plays vital roles in modulating metabolism, immunity, and oncogenesis [29]. The expression of ATF3 gene can be regulated by nutrients and metabolic signals, such as insulin and glucocorticoids. These metabolic signals play an important role in fat deposition and muscle growth. Therefore, the expression of ATF3 gene may have some indirect connection with fat deposition and muscle growth process. There are research reports that PDP1 may function as part of a larger protein/DNA complex that interacts with Myocyte Enhancer Factor 2 (MEF2) to regulate transcription of *Drosophila* muscle genes [30]. However, the functions of these candidate genes remain to be further investigated.

Conclusion

In conclusion, this study did a transcriptomic analysis in the longissimus dorsi muscle tissue of Wujin pigs and Landrace pigs. In this study, we identified 682 DEGs potentially associated with crucial pathways of muscle growth and lipid deposition, such as cholesterol import, chylomicron remnant clearance, cholesterol efflux, biosynthesis of unsaturated fatty acids, the ErbB signaling pathway, and oxidative phosphorylation. And 3 and 4 of the DEGs were related closely to muscle growth and lipid metabolism, respectively. In summary, this study presents several candidate genes for porcine muscle growth and lipid deposition and provides a basis for future research on the molecular.

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References

1. Tao X, Kong FJ, Liang Y, Yang XM, Yang YK, et al. (2023) Screening of candidate genes related to differences in growth and development between Chinese indigenous and Western pig breeds. *Physiol Genomics* 55(3): 147-153.
2. Xu H, Huang Y, Li WZ, Yang Mh, Ge Cr, et al. (2014) Muscle biological characteristics of differentially expressed genes in Wujin and Landrace pigs. *Journal of Integrative Agriculture* 13(10): 2236-2242.
3. Wang Z, Li Q, Chamba Y, Zhang B, Shang P, et al. (2015) Identification of genes related to growth and lipid deposition from Transcriptome profiles of pig muscle tissue. *PLoS One* 10(10): e0141138.
4. Ge CR, Zhao SM, Zhang X, Lai H, Li CQ, et al. (2008) Effects of different dietary protein levels on the meat quality of Wujin Pigs. *Acta Veterinaria et Zootechnica Sinica* 39: 1692-1700.
5. Zhao SM, Ren LJ, Chen L, Zhang X, Cheng ML, et al. (2009) Differential expression of lipid metabolism related genes in porcine muscle tissue leading to different intramuscular fat deposition. *Lipids* 44(11): 1029-1037.
6. Zhang X, Zhao SM, Ge CR, Lai H, Li CQ, et al. (2008) Effects of dietary energy levels on growth performance and carcass composition of Wujin pigs. *Chinese Journal of Animal Nutrition* 20: 489-500.
7. Sahoo SS, Bodenreider O, Rutter JL, Skinner KJ, Sheth AP (2008) An ontology-driven semantic mashup of gene and biological pathway information: application to the domain of nicotine dependence. *J Biomed Inform* 41(5): 752-765.
8. Hausman GJ, Dodson MV, Ajuwon K, Azain M, Barnes KM, et al. (2009) Board-invited review: the biology and regulation of preadipocytes and adipocytes in meat animals. *J Anim Sci* 87(4): 1218-1246.
9. He J, Wu XL, Zeng Q, Li H, Ma H, et al. (2020) Genomic mating as sustainable breeding for Chinese indigenous Ningxiang pigs. *PLoS One* 15(8): e0236629.
10. Wang Y, Jia X, Hsieh JCF, Monson MS, Zhang J, et al. (2021) Transcriptome response of liver and muscle in heat-stressed laying hens. *Genes* 12(2): 255.
11. Wei X, Zhu Y, Zhao X, Zhao Y, Jing Y, et al. (2022) Transcriptome profiling of mRNAs in muscle tissue of Pinan cattle and Nanyang cattle. *Gene* 825: 146435.
12. Wei Z, Zhou H, Zhang Y, Zhang Q, Zhang W, Mai K (2018) Integrative analysis of transcriptomics and metabolomics profiling on flesh quality of large yellow croaker *Larimichthys crocea* fed a diet with hydroxyproline supplementation. *British Journal of Nutrition* 119(4): 359-367.
13. Fu B, Xie J, Kaneko G, Wang G, Yang H, et al. (2022) MicroRNA-dependent regulation of targeted mRNAs for improved muscle texture in crisp grass carp fed with broad bean. *Food Res Int* 155: 111071.
14. Shen JY, Zhen HM, Li L, Zhang YT, Wang JQ, et al. (2022) Identification and characterization of circular RNAs in Longissimus dorsi muscle tissue from two goat breeds using RNA-Seq. *Mol Genet Genomics* 297(3): 817-831.
15. Hu Z, Cao J, Liu G, Zhang H, Liu X (2020) Comparative transcriptome profiling of skeletal muscle from black muscovy duck at different growth stages using RNA-seq. *Genes (Basel)* 11(10): 1228.
16. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, et al. (2013) STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res* 41(Database issue): D808-815.

17. Xie R, Li B, Jia L, Li Y (2022) Identification of core genes and pathways in melanoma metastasis via bioinformatics analysis. *Int J Mol Sci* 23(2): 794.
18. Ghosh M, Sodhi SS, Song KD, Kim JH, Mongre RK, et al. (2015) Evaluation of body growth and immunity-related differentially expressed genes through deep RNA sequencing in the piglets of Jeju native pig and Berkshire. *Anim Genet* 46(3): 255-264.
19. Jing L, Hou Y, Wu H, Miao Y, Li X, et al. (2015) Transcriptome analysis of mRNA and miRNA in skeletal muscle indicates an important network for differential Residual Feed Intake in pigs. *Sci Rep* 5: 11953.
20. Ropk K, Zukowski K, Eckert R, Gurgul A, Piorkowska K, et al. (2014) Comprehensive analysis of the whole transcriptomes from two different pig breeds using RNA-Seq method. *Anim Genet* 45(5): 674-684.
21. Sodhi SS, Song KD, Ghosh M, Sharma N, Lee SJ, et al. (2014) Comparative transcriptomic analysis by RNA-seq to discern differential expression of genes in liver and muscle tissues of adult Berkshire and Jeju native pig. *Gene* 546(2): 233-242.
22. Rautureau Y, Deschambault V, Higgins ME, Rivas D, Mecteau M, et al. (2018) ADCY9 (Adenylate Cyclase Type 9) Inactivation protects from atherosclerosis only in the absence of CETP (Cholesteryl Ester Transfer Protein). *Circulation* 138(16): 1677-1692.
23. Zhao X, Mo D, Li A, Gong W, Xiao S, et al. (2011) Comparative analyses by sequencing of transcriptomes during skeletal muscle development between pig breeds differing in muscle growth rate and fatness. *PLoS One* 6(5): e19774.
24. Xing K, Wang K, Ao H, Chen S, Tan Z, et al. (2019) Comparative adipose transcriptome analysis digs out genes related to fat deposition in two pig breeds. *Sci Rep* 9(1): 12925.
25. Zhao SM, Wan QH, Cheng ML, Huang Y, Li WZ, et al. (2009) Effect of monoclonal antibody on expression of lipid metabolism related genes in porcine adipocytes. *Comp Biochem Physiol B Biochem Mol Biol* 154(4): 449-454.
26. Boengler K, Kosiol M, Mayr M, Schulz R, Rohrbach S (2017) Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. *J Cachexia Sarcopenia Muscle* 8(3): 349-369.
27. Goodpaster BH, Sparks LM (2017) Metabolic flexibility in health and disease. *Cell Metab* 25(5): 1027-1036.
28. Kummer E, Ban N (2021) Mechanisms and regulation of protein synthesis in mitochondria. *Nat Rev Mol Cell Biol* 22(5): 307-325.
29. Ku HC, Cheng CF (2020) Master regulator activating transcription factor 3 (ATF₃) in metabolic homeostasis and cancer. *Front Endocrinol (Lausanne)* 11: 556.
30. Asensio C, Muzzin P, Rohner F (2004) Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. *Int J Obes Relat Metab Disord* 28(Suppl 4): S45-52.