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**Multi-omics Profiling Reveals the Interplay Between Collagen Structure and
Lipid Metabolism Underlying Meat Quality in Songlei black pig**

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23

24 **Abstract:**

25 Meat quality is a complex trait influenced by multiple genetic and metabolic factors.

26 This study systematically characterized the meat quality of Songlei black pig (SL), a

27 Chinese crossbred line with excellent meat quality, by comparing it with commercial

28 Duroc × Landrace × Yorkshire (DLY) pigs (n = 40 per group) using multi-omics

29 approaches. Compared with the DLY group, the SL group exhibited significantly

30 superior meat quality traits, including higher intramuscular fat content, better

31 marbling and meat redness, lower shear force and collagen content ($p < 0.01$), as well

32 as richer essential amino acids, healthy fatty acids, and zinc. Multi-omics analyses

33 identified 946 differentially expressed genes and 821 differential metabolites.

34 *COL1A1*, *COL1A2*, *COL3A1*, *SPPI*, and *TLR4* were downregulated in SL and

35 positively correlated with shear force. Histamine and lysophosphatidylcholine (LPC

36 18:3) were associated with intramuscular fat and meat color. These molecules may

37 serve as candidate biomarkers associated with pork tenderness and fat deposition.

38 Together, these findings provide a molecular basis for marker-assisted breeding of

39 high-quality pork.

40 **Keywords:** Songlei black pig, Duroc × Landrace × Yorkshire, Meat quality traits,

41 Transcriptomics, Metabolomics, Collagen metabolism

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47 1. Introduction

48 Global population growth and rising living standards are driving an increasing
49 demand for protein, with growing emphasis on high-quality animal-derived sources.
50 In China, pork is a primary dietary protein source [1]. Consumer preferences have
51 shifted from quantity to quality. Meat eating quality is influenced by multiple factors,
52 including breed, nutrition, and management. Among these, breed is a fundamental
53 determinant [2]. Currently, commercial breeds such as the Duroc × Landrace ×
54 Yorkshire cross dominate the pork market due to their high growth efficiency and lean
55 meat yield. However, intensive selection for these traits has often compromised meat
56 flavor and juiciness [3]. By contrast, China's indigenous pig breeds are known for
57 superior meat quality, including higher intramuscular fat content and richer flavor
58 [4,5]. Their slower growth rate, however, limits large-scale commercial application.
59 Therefore, crossbreeding that combines the meat quality of local breeds with the
60 growth efficiency of commercial lines represents a promising strategy for high-quality
61 pork production.

The Songlei black pig (SL) is a synthetic line currently under development. It was established by crossing Songliao black pigs with Leixiang pigs, and is currently in its second generation of selection. This breeding strategy combines the growth performance of the Songliao parent with the superior meat quality of the Leixiang parent. The SL exhibits high intramuscular fat content, tender meat, and desirable flavor, making it a valuable genetic resource for premium pork production. We hypothesized that the superior meat quality of SL pork, particularly its tenderness and intramuscular fat deposition, is associated with coordinated changes in extracellular matrix and lipid metabolic pathways, and that integrating transcriptomic and metabolomic profiling can reveal the key molecular networks underlying these traits. However, a systematic and quantitative comparison of meat quality between the SL and the widely recognized DLY has not yet been reported. Specifically, the meat quality advantages of the Songlei black pig lack comprehensive quantitative validation, and the underlying molecular mechanisms remain unclear. This knowledge gap limits international recognition and hinders further commercial utilization of this breed. Therefore, systematic investigation is urgently needed to elucidate the regulatory pathways and molecular networks contributing to these quality traits, which would provide essential baseline data for future breeding programs and commercial promotion.

Recently, high-throughput technologies like transcriptomics and metabolomics have become powerful tools for understanding the molecular networks behind complex meat traits [6–8]. Therefore, this study aimed to systematically compare the

meat quality of SL and DLY by integrating phenotypic analysis with multi-omics approaches. We measured various indicators, including meat quality traits, and amino acid and fatty acid compositions. Transcriptomic and metabolomic profiles were also generated and integrated. Although multi-omics approaches have been applied to other pig breeds, the specific molecular networks underlying meat quality in the Songlei black pig—a recently developed breed in its second generation of systematic selection—remain largely unexplored. The novelty of this study lies in: (1) the first comprehensive multi-omics characterization of meat quality in the Songlei black pig, including systematic comparisons of meat quality traits, amino acid and fatty acid compositions with the commercial DLY breed; (2) the identification of collagen-related genes (COL1A1, COL1A2, COL3A1, and COL5A1) as hub regulators associated with tenderness; and (3) the discovery of coordinated gene–metabolite networks linking collagen metabolism and lipid signaling, providing candidate biomarkers for future breeding programs. This work provides important molecular insights for the precise utilization of local pig genetic resources in breeding superior meat-type lines.

2. Materials and Methods

2.1. Animals and Sample Collection

Animal experiments were conducted at Feimasi Animal Husbandry Co., Ltd. (Changchun, China) in strict accordance with guidelines approved by the Experimental Animal Ethics Committee (IACUC) of Jilin Agricultural University

(Approval No. 2025 06 18 001). A total of 80 healthy pigs (40 Songlei black pig, SL; 40 Duroc × Landrace × Yorkshire pigs, DLY) were randomly selected from the same farm. All pigs were reared under identical conditions and fed the same diets. Pigs were slaughtered at a target live weight of approximately 120 kg, which represents the standard commercial slaughter weight in the local market. The average age at slaughter was approximately 270 days for SL pigs and 210 days for DLY pigs. All pigs were slaughtered in May. After 12 h of fasting with free access to water, pigs were slaughtered by electrical stunning, exsanguinated, dehaired, eviscerated and split down the midline in compliance with the Chinese guidelines (Science and Technology Ministry of China, 2006). Carcasses were chilled at 4°C immediately postmortem. Longissimus dorsi muscle samples for molecular analyses were collected within 1 h postmortem, snap-frozen in liquid nitrogen, and stored at −80°C until further use. These conditions prevent bacterial growth and associated histamine production, ensuring that the detected metabolites reflect endogenous levels rather than postmortem spoilage artifacts. For meat quality measurements, the left carcass side was held at 4°C for 24 h postmortem prior to sample collection. Longissimus dorsi muscle samples were collected from the left side of each carcass and a 20 cm segment positioned anterior to the third-to-last thoracic vertebra was excised and used for subsequent analyses. For molecular analyses, tissue aliquots were rapidly frozen in liquid nitrogen and stored at −80 °C until further use. For the multi-omics analyses, six Longissimus dorsi muscle samples per breed with intramuscular fat content closest to the breed-specific mean were selected from the 40 individuals per breed for

integrated analyses of transcriptomics, metabolomics, amino acid composition, and fatty acid profile. For multi-omics analyses, $n = 6$ per group—a sample size consistent with standard practice in pig meat transcriptomic and metabolomic studies, and sufficient to detect biologically meaningful differences [6,9,10]. Samples were selected based on IMF content closest to the breed mean, ensuring representation of typical phenotypes while minimizing inter-individual variation and avoiding extreme outliers.

2.2. Meat Quality

Meat quality traits of the longissimus dorsi muscle were measured according to the Chinese agricultural industry standard NY/T 821-2019 (Technical code of practice for pork quality assessment), including color, marbling, pH, drip loss, pressing loss, shear force, and intramuscular fat (IMF) content. Meat color score and marbling score were assessed subjectively at 45 min post-mortem under natural light using the National Pork Producers Council (NPPC) standards. Objective color was measured using a portable colorimeter (MiniScan EZ 4500, HunterLab, USA) at both 45 min and 24 h post-mortem. The colorimeter was calibrated with a white plate and black trap, and measurements were performed using illuminant D₆₅, a 10° standard observer, and a 25 mm aperture. Color parameters were recorded as L^* (lightness), a^* (redness), and b^* (yellowness). According to NY/T 821-2019, the 45 min measurement is recorded as color_{45min}, and the 24 h measurement as color_{24h}. The pH was determined at the same time points using a pH meter (Bulader, Beijing, China), recorded as pH_{45min}.

and pH_{24h}, respectively, following the standard's terminology. Marbling score was assessed at 24 h post-mortem. All color and pH measurements were performed in triplicate per sample, and the average was used for analysis. Drip loss was determined by suspending a meat sample (15 × 30 × 15 mm) at 4 °C for 48 h and calculating the percentage weight loss. Pressing loss was determined using a meat water-holding capacity tester (Bulader, Beijing, China). A cylindrical meat sample (diameter: 2.5 cm; thickness: 1 cm) was placed between two layers of filter paper. A 35 kg weight was then applied to the sample for 5 min. The weight of the sample was measured before and after pressing, and the pressing loss was calculated as the percentage of weight loss. Shear force was measured by cooking samples to a final core temperature of 75 °C, then removing a 1 cm round strip for shear force (Bulader, Beijing, China). Intramuscular fat content was determined by the Soxhlet extraction method.

2.3. Determination of Amino acid and Fatty acid Composition

Amino acid composition was determined by liquid chromatography-tandem mass spectrometry. Muscle sample (0.5 g) was hydrolyzed with 10 mL of 6 mol/L HCl at 110°C for 24 h. The hydrolysate was cooled, filtered, diluted with water, vortexed, centrifuged at 3000 rpm for 10 min, and filtered through a 0.22 µm membrane filter prior to analysis. Separation was performed on an Agilent XDB-C18 column (100 mm × 2.1 mm, 1.7 µm) using an Agilent 1290-6470 LC-MS/MS system. Column temperature: 35°C; flow rate: 0.4 mL/min; injection volume: 2 µL. Mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in methanol–acetonitrile

(1:4, v/v). Gradient: 0–6 min, 5–95% B; 6–8 min, 95% B; 8–8.01 min, 95–5% B; 8.01–10 min, 5% B. Mass spectrometry was performed using electrospray ionization in positive mode with multiple reaction monitoring. Source parameters: drying gas temperature 250°C; drying gas flow 7 L/min; nebulizer pressure 30 psi; sheath gas temperature 325°C; sheath gas flow 11 L/min; capillary voltage 3500 V; nozzle voltage 0 V. Amino acid reference standards (National Institutes for Food and Drug Control, China) were used for external standard calibration. Results are expressed as g/100 g muscle tissue.

Fatty acid composition was determined by gas chromatography-mass spectrometry. Freeze-dried muscle (0.5 g) was extracted with petroleum ether under ultrasonication for 20 min [11]. The extract was filtered and methylated using hexane–diethyl ether (2:1, v/v) and 1.0 mol/L KOH in methanol. The organic phase was collected, diluted with hexane, filtered (0.45 µm), and analyzed by GC-MS. Separation was performed on a SH-Rt-25660 column (100 m × 0.25 mm × 0.20 µm; Shimadzu) using a Shimadzu GCMS-TQ8040 NX. Injection port: 240°C; split ratio: 10:1; column flow: 1.3 mL/min; ion source: 230°C; interface: 250°C; solvent delay: 10 min. A 37-component FAME standard mixture (Shanghai ANPEL, Cat. No. 65900100) was used for external standard calibration. Results are expressed as mg/100 g muscle tissue.

2.4. Determination of Muscle Nutrition Composition

Moisture content was determined by oven drying at 105 °C to constant weight.

Crude protein content was measured using the Kjeldahl method with a Haineng K9840 Kjeldahl analyzer (Haineng, China). Samples were digested in concentrated sulfuric acid with catalysts, and the released ammonia was distilled and titrated with standardized hydrochloric acid. A blank determination was included for correction [12]. Mineral elements, including calcium, iron, zinc, and selenium, were quantified by inductively coupled plasma mass spectrometry using an Agilent 7800 ICP-MS (Agilent Technologies, USA). Approximately 0.5 g of each sample was digested with nitric acid in a microwave digestion system. After digestion, samples were diluted and analyzed. Calibration was performed with mixed standard solutions, in which germanium and scandium served as internal standards. Meanwhile, 10% nitric acid was used as the procedural blank [13].

2.5. RNA Extraction, Sequencing and Transcriptome Data Analysis

Total RNA sequencing was performed by Novogene Co., Ltd. (Beijing, China). Strand-specific RNA-seq libraries were generated from six biological replicates per group following rigorous RNA quality assessment and rRNA depletion. Libraries were prepared through RNA purification, end repair, A-tailing, adapter ligation, and PCR amplification, and subsequently sequenced on an Illumina platform. Raw reads were processed using fastp (v0.23.2) to remove adapter sequences, reads containing more than 10% ambiguous bases, and low-quality reads [14]. Clean reads were aligned to the pig reference genome (Sscrofa11.1) using HISAT2 (v2.2.1) [15].

210 Transcript assembly and gene expression quantification were conducted with String
 211 Tie, and differential expression analysis was performed using DESeq2 (v1.20) [16].
 212 Genes with $|\log_2\text{FoldChange}| > 1$ and $\text{padj} < 0.05$ were defined as differentially
 213 expressed genes (DEGs). Functional enrichment analyses of DEGs were conducted
 214 using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)
 215 databases.

216 2.6. Quantitative Real-Time PCR (qRT-PCR) Validation of DEGs

217 Ten DEGs (*COL3A1*, *COL1A2*, *COL1A1*, *COL5A1*, *SPP1*, *TLR4*, *THBS1*, *CCN2*,
 218 *ARG2*, *EGR1*) were selected for RT-qPCR validation. Four biological replicates were
 219 analyzed per group, each measured in triplicate. Total RNA was extracted from the
 220 same samples used for RNA sequencing. Primers were designed using NCBI
 221 Primer-BLAST, and *GAPDH* served as the internal reference and primer sequences
 222 are provided in (Table S1). Gene expression differences between groups were
 223 assessed using GraphPad Prism 10.

224 2.7. Untargeted Metabolome Analysis

225 Untargeted metabolomic profiling was performed by Novogene Inc. (Beijing,
 226 China) using six Longissimus dorsi muscle samples per group. For sample
 227 preparation, approximately 20 mg of tissue was weighed and extracted with 400 μL of
 228 methanol-water (7:3, v/v) containing an internal standard. The mixture was shaken at
 229 1500 rpm for 5 min, incubated on ice for 15 min, and centrifuged at 12,000 rpm for 10
 230 min at 4°C. A 300 μL aliquot of supernatant was collected and stored at -20°C for 30

min. The solution was then centrifuged again at 12,000 rpm for 3 min at 4°C, and 200
μL of the supernatant was transferred for LC-MS/MS analysis. A pooled quality
control sample was prepared by mixing equal volumes of each sample and was
injected at regular intervals throughout the run. Chromatographic separation was
performed on a Waters ACQUITY UPLC BEH Amide column (2.1 mm × 50 mm, 1.7
μm). The mobile phase consisted of solvent A (25 mmol/L ammonium
acetate-ammonia solution, pH 9.75) and solvent B (acetonitrile). The autosampler
temperature was maintained at 4°C, and the injection volume was 2 μL. Mass
spectrometry data were acquired in information-dependent acquisition mode using an
Orbitrap Exploris 120 mass spectrometer controlled by Xcalibur software (version
4.3), operating in both positive and negative ion modes. The ESI source parameters
were as follows: sheath gas flow rate, 50 Arb; auxiliary gas flow rate, 15 Arb;
capillary temperature, 320°C; MS1 resolution, 60,000; MS2 resolution, 15,000;
normalized stepped collision energies of 20, 30, and 40 eV; spray voltage of 3.8 kV
(positive) and −3.4 kV (negative). Raw data were processed using XCMS for peak
detection, alignment, and retention time correction [17]. Metabolites were annotated
by comparison with reference standards and public databases. Metabolite
identification confidence was classified according to the metabolomics standards
initiative guidelines [18]. In brief, metabolites confirmed by comparison with
authentic reference standards were assigned to MSI Level 1 (identified metabolites).
Metabolites annotated by matching MS/MS spectra to the Novogene in-house
database, the Human Metabolome Database, and the Kyoto Encyclopedia of Genes

and Genomes were assigned to MSI Level 2 (putatively annotated compounds). The differential metabolites reported in this study were primarily identified at MSI Level 2, with a subset validated at Level 1 using reference standards. PCA and PLS-DA were performed to evaluate sample clustering and to identify differential metabolites between groups. The screening criteria were $|\log_2FC| \geq 0.58$, variable importance in projection (VIP) ≥ 1 , and p -value < 0.05 . Volcano plots and heatmaps were used for visualization. Functional annotation and pathway enrichment analyses were conducted using the KEGG and HMDB databases, with $p < 0.05$ as the significance threshold [19].

2.8. Comprehensive Integrative Analysis of Transcriptome and Metabolome Data

To explore potential associations between differentially expressed genes and differential metabolites, transcriptomic and metabolomic data were integrated based on shared KEGG pathways. A protein-protein interaction network was constructed using the STRING database (v12.0), and key hub genes were identified from the network using Cytoscape (v3.10.2). Pearson correlation analysis was performed to evaluate associations between genes and metabolites. Prior to analysis, gene expression data (FPKM) and metabolite peak areas were $\log_2$ -transformed to improve normality and comparability. Correlation coefficients (r) were calculated for each gene-metabolite pair across all 12 samples, with $|r| > 0.8$ and $p < 0.05$ considered significant. The results were visualized using heatmaps and Sankey diagrams. To further validate the candidate molecules, correlations between key genes/metabolites

and major meat quality traits (IMF content, shear force, and pH) were also analyzed using Pearson correlation. All correlation analyses were performed without multiple testing correction, as this study was exploratory and intended to generate hypotheses for future validation. All statistical tests were two-tailed.

2.9. Statistical Analysis

Data analysis and graphing were performed using Microsoft Excel and GraphPad Prism 10 software packages [20,21]. Results are presented as mean $\pm$ standard deviation. Group differences were assessed by Student's t-test, with $p < 0.05$ considered significant and $p < 0.01$ highly significant. Correlation analyses were performed in RStudio and visualized as heatmaps.

3. Results

3.1. Comparison of Meat Quality Traits

Comparison of meat quality traits between DLY and SL demonstrated significantly improved meat quality traits (Figure 1, Table S2). Results showed that meat color score, marbling score, pH_{45min} and pH_{24h}, intramuscular fat content, and the $a^*_{45\text{ min}}$ were all significantly higher in SL ($p < 0.01$). In contrast, the $L^*_{45\text{ min}}$, $b^*_{45\text{ min}}$ and shear force were significantly lower in SL ($p < 0.01$). These findings demonstrate significant differences in meat quality traits between SL and DLY breeds.

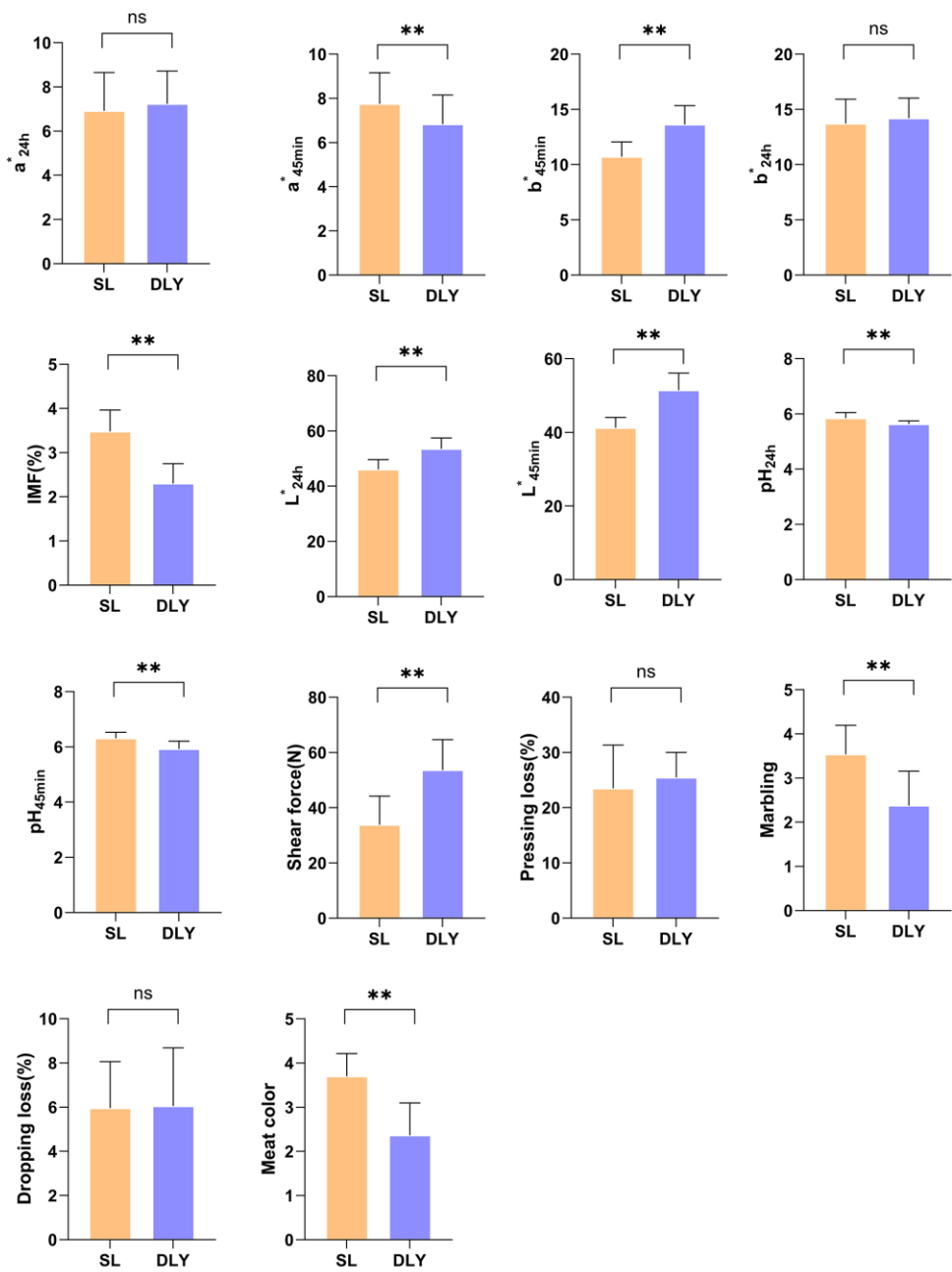


Figure 1. Comparison of meat quality traits between Songlei black pig (SL) and Duroc × Landrace × Yorkshire pigs (DLY). Statistical significance is indicated as ns ($p > 0.05$) and ** ($p < 0.01$).

3.2. Comparison of Amino Acid 、 Fatty Acid and Nutritional Composition

Profiles of Longissimus Dorsi Muscle

Compared with DLY, SL contained significantly higher concentrations of aspartic acid, glutamic acid, glycine, alanine, and arginine ($p < 0.01$). Furthermore, the essential amino acids and the flavor amino acids were significantly higher in SL ($p < 0.05$) (Table 1). SL also exhibited significantly elevated levels of several fatty acids relative to DLY ($p < 0.05$), including 17-heptadecenoic acid, oleic acid, linoleic acid, octadecatrienoic acid, α -linolenic acid, cis-11,14-eicosadienoic acid, γ -linolenic acid and Eicosapentaenoic acid (EPA). Accordingly, the total concentrations of monounsaturated fatty acids, polyunsaturated fatty acids, and total fatty acids were significantly higher in SL ($p < 0.05$) (Table 2). In addition, analysis of general nutritional composition revealed that fat and zinc contents were significantly higher in SL than in DLY ($p < 0.01$) (Table S3). Collectively, These findings confirm that SL muscle possesses a more favourable amino acid, fatty acid, and nutritional composition.

Table 1. Profile of amino acid concentration (g/100g) in longissimus dorsi muscle muscle of SL and DLY breeds^a

Amino acids	Abbreviations	SL	DLY	P
Aspartic acid	Asp	2.59±0.06 ^A	2.43±0.05 ^B	0.001
Threonine	Thr	3.15±0.05	3.07±0.10	0.093
Serine	Ser	1.41±0.05 ^a	1.35±0.04 ^b	0.033
Glutamic acid	Glu	5.38±0.23 ^A	4.87±0.16 ^B	0.001

Proline	Pro	2.56±0.15	2.39±0.11	0.056
Glycine	Gly	1.85±0.06 ^A	1.67±0.05 ^B	0.000
Alanine	Ala	2.30±0.08 ^A	2.07±0.06 ^B	0.000
L-valine	Val	3.95±0.08	3.83±0.13	0.078
Methionine	Met	2.38±0.04	2.31±0.10	0.139
Isoleucine	Ile	3.52±0.85 ^a	3.32±0.15 ^b	0.017
Leucine	Leu	5.35±0.08 ^a	5.17±0.14 ^b	0.019
Tyrosine	Tyr	2.73±0.05	2.73±0.12	0.987
Phenylalanine	Phe	3.35±0.05	3.28±0.10	0.156
Histidine	His	2.13±0.10 ^a	1.91±0.21 ^b	0.042
Lysine	Lys	6.49±0.19	6.27±0.22	0.089
Arginine	Arg	2.97±0.08 ^A	2.77±0.11 ^B	0.005
Cystine	Cys	3.24±0.80	3.46±1.26	0.727
Essential amino acids	EAA	28.19±0.50 ^a	27.24±0.89 ^b	0.044
Non-essential amino acids	NEAA	27.14±1.23	25.64±1.24	0.061
Flavor amino acids	FAA	18.19±0.44 ^A	17.04±0.49 ^B	0.002

Total amino acids	TAA	55.34±1.57 ^a	52.89±1.49 ^b	0.019
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^aNote: EAAs: sum of essential amino acids; NEAAs: sum of nonessential amino acids; FAAs: sum of flavor amino acids; TAAs: sum of total amino acids. SL: Songlei black pigs; DLY: Duroc × Landrace × Yorkshire pigs. Values with different lowercase letters indicate significant differences ($p < 0.05$), whereas different uppercase letters indicate highly significant differences ($p < 0.01$). The following table is the same.

Table2. Profile of fatty acid concentration (mg/100 g) in Longissimus dorsi muscles of SL and DLY breeds^b

Fatty acids	Abbreviations	SL	DLY	P
Butyric acid	C4:0	0.05±0.01	0.05±0.02	0.868
Hexanoic acid	C6:0	0.05±0.02	0.04±0.03	0.461
Octanoic acid	C8:0	0.29±0.10	0.22±0.13	0.330
Decanoic acid	C10:0	2.80±0.87	2.31±1.41	0.488
Undecanoic acid	C11:0	0.05±0.06	0.03±0.07	0.688
Lauric acid	C12:0	2.41±0.83	1.60±0.94	0.144
Tridecanoic acid	C13:0	0.02±0.08	0.02±0.01	0.513
Myristic acid	C14:0	32.28±9.49	22.28±11.97	0.140
Myristoleic acid	C14:1n5	0.83±0.31	0.71±0.41	0.588
Pentadecanoic acid	C15:0	0.40±0.15	0.31±0.18	0.387

Palmitic acid	C16:0	226.30±44.75	192.50±62.01	0.116
Palmitoleic acid	C16:1n7	66.62±14.79	59.42±30.48	0.614
Heptadecanoic acid	C17:0	4.09±1.78	2.49±1.28	0.105
17-Heptadecenoic acid	C17:1n7	3.73±1.63 ^a	1.35±1.43 ^b	0.022
Stearic acid	C18:0	154.14±40.61	103.67±44.02	0.066
Trans-oleic acid	C18:1n9t	3.54±1.22	3.42±2.83	0.927
Oleic acid	C18:1n9	223.54±83.73 ^a	117.25±55.58 ^b	0.027
Trans-linoleic acid	C18:2n6t	0.11±0.04	0.09±0.05	0.555
Linoleic acid	C18:2n6c	114.98±47.85 ^a	63.98±23.10 ^b	0.041
Arachidic acid	C20:0	6.22±1.93	3.38±2.64	0.059
Octadecatrienoic acid	C18:3n6	0.98±0.53 ^a	0.39±0.19 ^b	0.028
Cis-11-eicosenoic acid	C20:1	19.68±5.71	13.81±7.69	0.164
α-linolenic acid	C18:3n3	7.08±3.91 ^a	3.21±1.45 ^b	0.046
Heneicosanoic acid	C21:0	0.04±0.01	0.03±0.02	0.419
cis-11,14-eicosadienoic acid	C20:2	10.02±5.28 ^a	4.23±1.84 ^b	0.030
Behenic acid	C22:0	0.14±0.13	0.13±0.10	0.894

γ -linolenic acid	C20:3n6	2.43 $\pm$ 1.14 ^a	1.18 $\pm$ 0.55 ^b	0.036
Erucic acid	C22:1n9	0.28 $\pm$ 0.09	0.22 $\pm$ 0.13	0.376
Eicosatrienoic acid	C20:3n3	0.89 $\pm$ 0.45	0.55 $\pm$ 0.28	0.149
Arachidonic acid	C20:4n6	7.14 $\pm$ 2.64	5.04 $\pm$ 2.10	0.158
Tricosanoic acid	C23:0	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.380
Cis-13,16- docosadienoic acid	C22:2n6	0.07 $\pm$ 0.06	0.04 $\pm$ 0.02	0.297
Lignoceric acid	C24:0	0.05 $\pm$ 0.04	0.05 $\pm$ 0.03	0.709
EPA	C20:5n3	0.11 $\pm$ 0.04 ^a	0.06 $\pm$ 0.04 ^b	0.046
Nervonic acid	C24:1n9	0.06 $\pm$ 0.02	0.06 $\pm$ 0.02	0.553
DHA	C22:6n3	0.12 $\pm$ 0.07	0.10 $\pm$ 0.05	0.467
Saturated fatty acid	SFA	429.33 $\pm$ 99.49	309.12 $\pm$ 124.03	0.094
Unsaturated fatty acid	UFA	462.21 $\pm$ 149.91 ^a	275.09 $\pm$ 113.39 ^b	0.035
Monounsaturated fatty acid	MUFA	318.28 $\pm$ 99.85 ^a	196.23 $\pm$ 86.17 ^b	0.047
Polyunsaturated fatty acid	PUFA	143.93 $\pm$ 61.69 ^a	78.87 $\pm$ 28.17 ^b	0.042
Total fatty acid	TFA	891.54 $\pm$ 245.47 ^a	584.21 $\pm$ 231.66 ^b	0.050

^bNote: SFA: sum of saturated fatty acids; UFA: sum of unsaturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; TFA : sum of total fatty acids. SL: Songlei black pigs; DLY: Duroc × Landrace × Yorkshire pigs.

3.3. Transcriptome Summary Results, Comparative and Functional Analysis of Longissimus Dorsi Muscle

Whole-transcriptome sequencing of 12 samples yielded 168.08 Gb of raw data. After quality control, 164.45 Gb of clean data were retained, with Q30 scores ranging from 95.91% to 96.59% and an average GC content of 45.01% (Table S4). The overall mapping rates to the Sscrofa11.1 reference genome ranged from 94.89% to 96.18% across all samples, with comparable values between SL and DLY groups (Table S4). A total of 19,193 genes were expressed. Comparative analysis identified 946 differentially expressed genes (DEGs), of which 318 were upregulated and 628 were downregulated in SL relative to DLY (Figure 2A, Table S5). Hierarchical clustering analysis based on the expression profiles of all DEGs clearly distinguished the two breeds, with all SL samples clustering together and separate from the DLY samples (Figure 2B). Both principal component analysis (PCA) and hierarchical clustering based on these DEGs clearly separated the two breeds (Figure S1A, Figure 2A), confirming distinct transcriptomic profiles. The expression trends of ten selected DEGs were validated by qRT-PCR. Results were consistent with the RNA-seq data, confirming the reliability of the transcriptomic findings (Figure 3). Functional Gene

Ontology (GO) and KEGG pathway enrichment analyses were performed based on the differentially expressed genes (Tables S6, 7). The top 20 enriched GO terms are shown in (Figure 2C). Enriched biological processes included response to oxygen-containing compounds (GO:1901700). Within cellular components, plasma membrane protein complex (GO:0098797) was prominent. For molecular function, calcium ion binding (GO:0005509) was significantly enriched (Figure S1B). KEGG analysis identified 35 significantly enriched pathways ($p < 0.05$), such as TGF-beta signaling pathway, HIF-1 signaling pathway, FoxO signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, and ECM-receptor interaction, etc. (Figure 2D, Table S7). These results indicate substantial transcriptional differences between SL and DLY muscle, particularly KEGG pathways are crucial for the differentiation of meat quality.

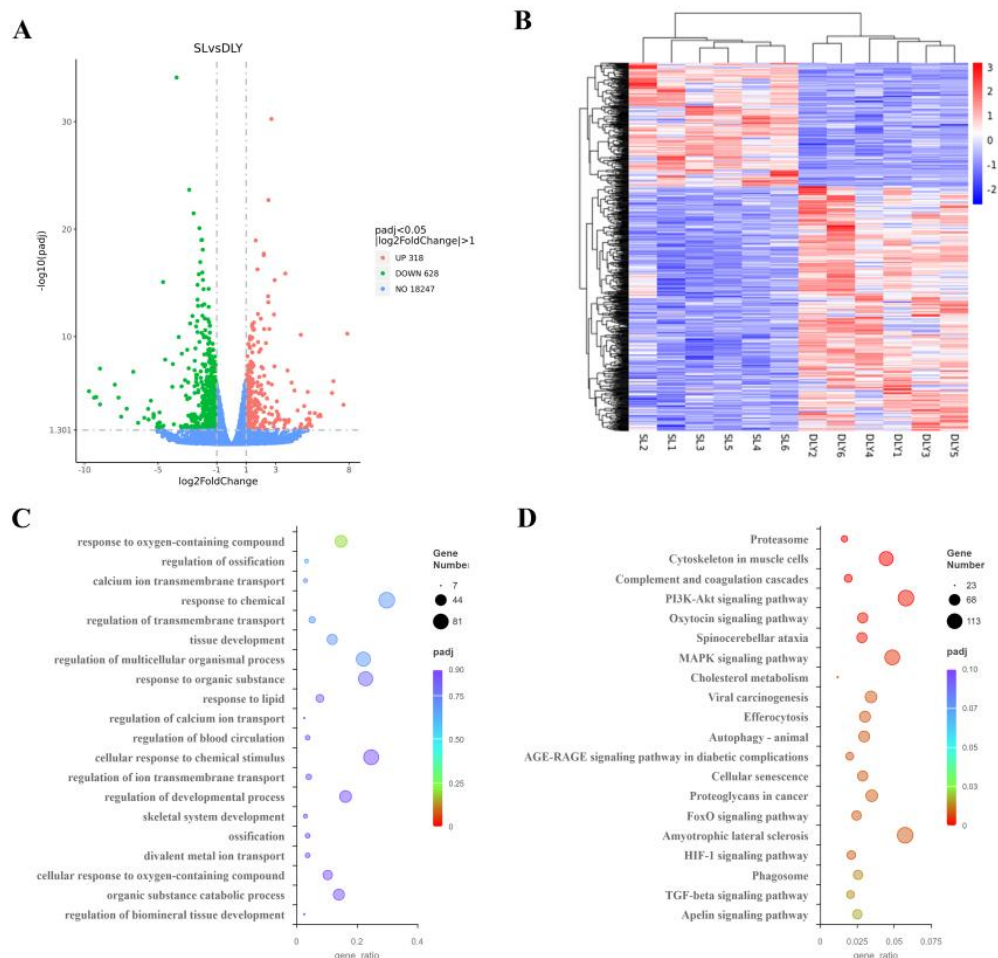


Figure 2. Comprehensive transcriptomic profiling with functional enrichment of differentially expressed genes between Songlei black pig (SL) and Duroc × Landrace × Yorkshire pigs (DLY). (A) Volcano plot of DEGs. (B) Clustered heatmap of both SL and DLY. (C) Bubble plot of Gene Ontology enrichment. (D) Bubble plot of KEGG pathway enrichment.

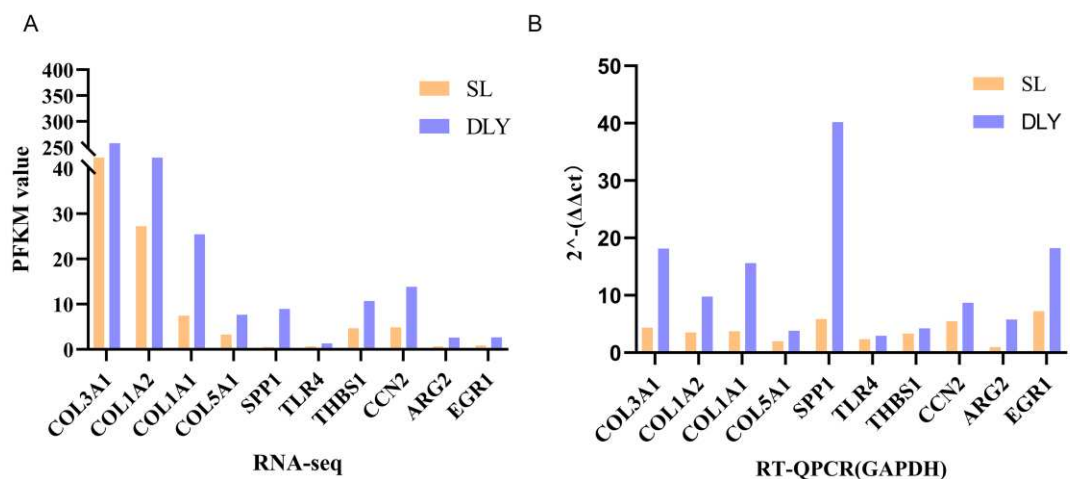


Figure 3. Validation of gene expression in the longissimus dorsi muscle. (A) Transcript abundance of selected target genes measured by RNA sequencing. (B) Relative mRNA expression determined by RT qPCR and normalized to GAPDH.

3.4. Comparative and Functional Analysis of Untargeted Metabolites Analysis in Longissimus Dorsi Muscle

Non-targeted metabolomic analysis of longissimus dorsi muscle samples was conducted using UHPLC-MS/MS. A total of 2,911 metabolites were detected (1,487 in negative and 1,424 in positive ion mode) (Table S8). Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) showed clear separation between SL and DLY groups (Figure 4A, B). Based on $|\log_2\text{FoldChange}| \geq 0.58$, variable importance in projection (VIP) > 1.0 , and $p\text{value} < 0.05$, we identified 821 significantly differential metabolites (DEMs), of which 502 were upregulated and 319 were downregulated in SL (Figure 4C). Functional KEGG pathway enrichment analysis of the differential metabolites revealed significant enrichment in nine

pathways ($p < 0.05$), including tryptophan metabolism, citrate cycle (TCA cycle), efferocytosis, choline metabolism in cancer, histidine metabolism, glycerophospholipid metabolism, aldosterone synthesis and secretion, cortisol synthesis and secretion, and Cushing syndrome (Figure 4D, Table S9). Based on Human Metabolome Database (HMDB) annotation, these metabolites were predominantly classified as lipids and lipids-like molecules, organic acids and derivatives, organoheterocyclic compounds, and benzenoids (Table S10, Figure S2). These findings indicate pronounced metabolic differences between SL and DLY muscle, particularly in pathways related to lipid metabolism and energy regulation.

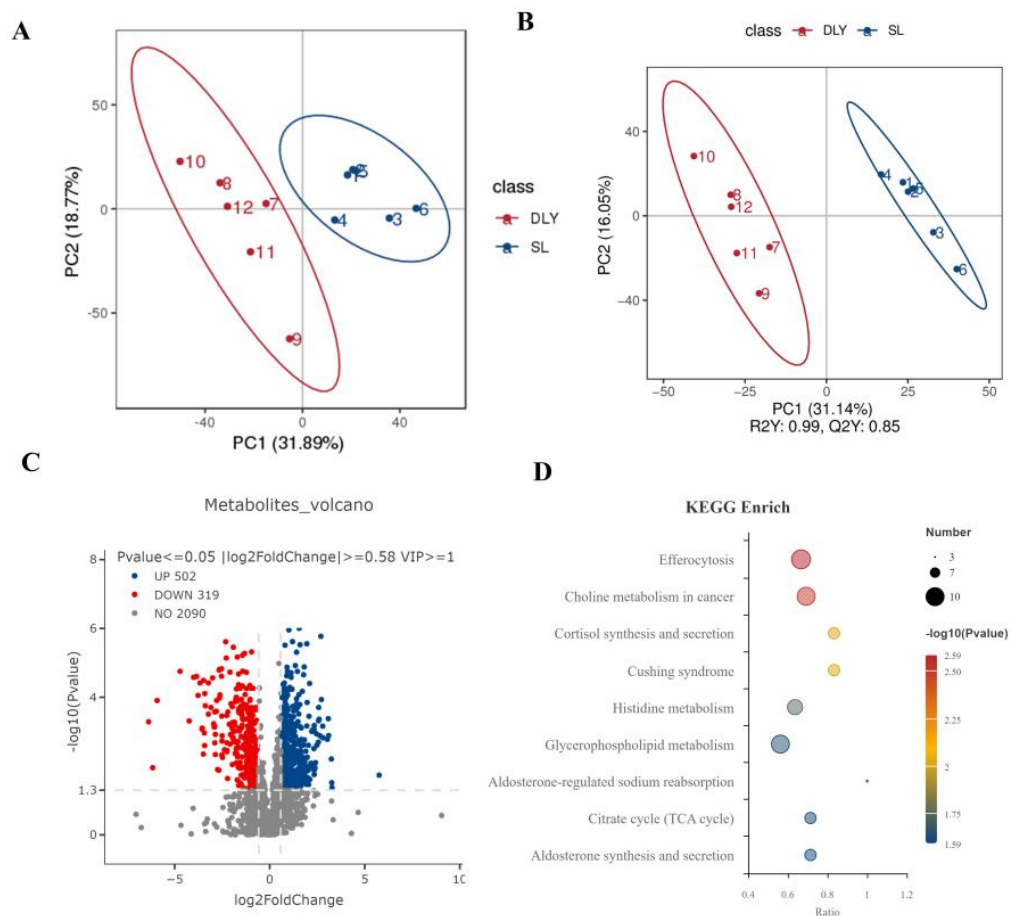


Figure 4. Comparative analysis of longissimus dorsi muscle metabolites and functional analysis of Songlei black pig (SL) and Duroc $\times$ Landrace $\times$ Yorkshire pigs (DLY). (A) principal component analysis (PCA) expressed metabolites of longissimus dorsi muscle between SL and DLY. (B) PLS-DA analysis of expressed metabolites. (C) Volcano graph of expressed up-regulated and down-regulated metabolites. (D) functional KEGG enrichment pathway analysis of DEM.

3.5. Transcriptome and Metabolome Integration Analysis

Integrated analysis of differentially expressed genes (DEGs) and differentially expressed metabolites (DEMs) revealed substantial overlap in enriched KEGG pathways between the two datasets. A total of 113 shared pathways were identified for metabolites detected in negative ion mode and 69 shared pathways for those detected in positive ion mode (Table S11, Figure S3).

Among these, pathways that were significantly enriched by DEGs and are closely related to meat quality were prioritized, including the HIF-1 signaling pathway, Apelin signaling pathway, propanoate metabolism, arginine biosynthesis, protein digestion and absorption, cholesterol metabolism, ovarian steroidogenesis, beta-alanine metabolism, parathyroid hormone synthesis, secretion and action, and biosynthesis of amino acids. Together with efferocytosis, which was significantly enriched in both transcriptomic and metabolomic datasets, a total of 11 pathways were selected for further analysis (Figure 5A). A protein–protein interaction network was constructed based on the genes enriched in these 11 pathways, resulting in a network

of 68 interacting genes (Figure 5B). Key genes identified within this network included *COL3A1*, *COL1A2*, *COL1A1*, *COL5A1*, *SPP1*, *TLR4*, *THBS1*, *CCN2*, *ARG2*, and *EGR1*, which are potentially associated with meat quality traits. Ten representative metabolites mapped to these gene-enriched pathways were identified, including α -ketoglutaric acid, cAMP, LysoPC (18:1/0:0), LPC 18:3, histamine, L-leucine, L-methionine, L-histidine, L-tyrosine, and calcidiol. Pearson correlation analysis showed that histamine was negatively correlated with *COL3A1*, *COL1A2*, *COL1A1*, *COL5A1*, *TLR4*, and *EGR1*. L-histidine showed negative correlations with *COL3A1*, *COL1A2*, *COL5A1*, and *EGR1*. In contrast, LPC 18:3 exhibited positive correlations with *COL3A1*, *COL1A2*, *TLR4*, *ARG2*, and *EGR1*, while cAMP was positively correlated with *COL3A1*, *CCN2*, and *EGR1* ($|r| > 0.8$, $p < 0.05$) (Table S12, Figure 6A). These integrative findings suggest that collagen-related genes and specific metabolites form a coordinated regulatory network that may underlie the superior meat quality observed in SL pigs.

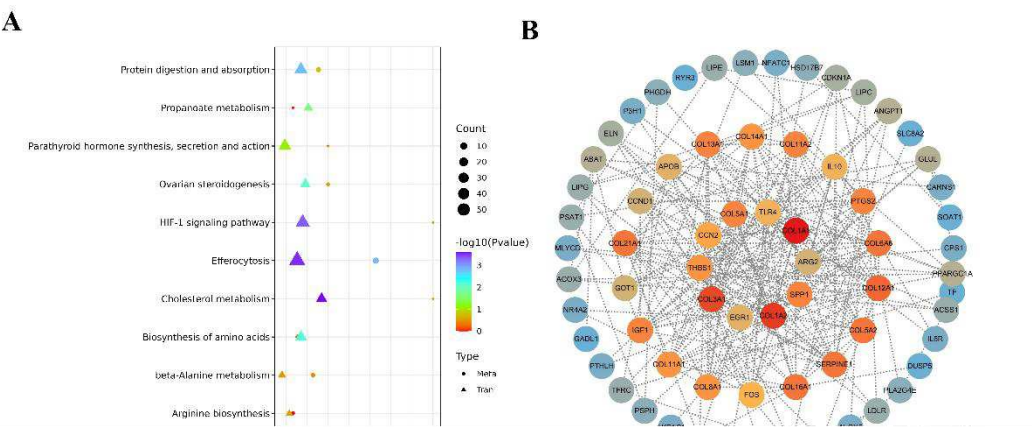


Figure 5. Joint analysis of transcriptome and metabolome. (A) Common KEGG pathways enriched by DEGs and DEMs. (B) Protein-protein interaction (PPI) analysis of DEGs expressed in similar KEGG pathways of transcriptome and metabolome.

3.6. Correlation analysis of meat quality traits with key genes and metabolites

Correlation analyses of meat quality traits with key differentially expressed genes and metabolites identified several significant associations ($|r| > 0.8$, $p < 0.05$). These molecules can be broadly grouped into functional categories related to extracellular matrix/collagen, lipid metabolism, and immune/amino acid signaling. Intramuscular fat content was positively correlated with histamine and L-histidine, but negatively correlated with *COL3A1*, *TLR4*, calcidiol, and LPC 18:3. For L_{45min} , positive correlations were observed with ARG2, EGR1, SPP1, TLR4, and LPC 18:3, while negative correlations were found with L-Tyrosine, histamine, L-histidine and L-methionine. Marbling score was positively associated with L-histidine, but negatively with calcidiol, and LPC 18:3. Meat color showed a positive correlation with L-methionine, and L-Histidine, negative correlations with *TLR4*, *COL3A1*, *EGR1*, LPC 18:3, cAMP, and Calcidiol. Shear force was positively correlated with *COL3A1*, TLR4, LPC 18:3, and alpha-ketoglutaric acid, whereas it was negatively correlated with L-histidine. For b_{45min} , positive correlations were found with *COL3A1*, *COL1A2*, *COL5A1*, *CCN2*, *EGR1*, *THBS1*, LPC 18:3, and cAMP, and a negative correlation with L-histidine. Finally, pH_{45min} was positively correlated with L-histidine (Table S12, Figure 6B). Our results suggest that histamine and LPC 18:3 were

significantly correlated with multiple pork quality traits and may serve as potential biomarkers. Meanwhile, collagen family genes (*COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*) and *TLR4* were identified as core genes associated with meat quality. Overall, these genes and metabolites form interconnected molecular networks that are closely associated with the formation of important traits such as intramuscular fat, tenderness, and meat color.

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Figure 6. Correlation analysis. (A) Genes and metabolites correlation heat map. (B) Meat quality traits with key genes and metabolites heat map. Statistical significance is indicated by asterisks: * = $p < 0.05$, ** = $p < 0.01$.

4. Discussion

Pork is a key source of high-quality protein. Its eating quality depends on multiple traits, including color, marbling, tenderness, flavor, and nutritional value. Commonly used indicators for pork quality assessment include meat color, marbling score, shear force, and intramuscular fat content of the longissimus dorsi muscle [22]. Commercial crossbred pigs, such as Duroc × Landrace × Yorkshire (DLY), dominate global production due to their efficient growth and high lean meat yield. However, these pigs exhibit low intramuscular fat and pale meat color, which limits their ability

to meet the growing consumer demand for premium pork [23]. Therefore, a key strategy for high-quality pork production is to introduce superior meat quality traits from indigenous breeds into modern breeding populations, while maintaining production efficiency. In this study, we performed a comprehensive multi-omics comparison between Songlei black pig (SL) and DLY pigs to characterize the differences in meat quality phenotypes, nutritional composition, and molecular characteristics. We confirmed the multidimensional meat quality superiority of SL pigs and identified potential molecular markers.

This study first established the significant sensory advantages of SL pork. SL pork showed higher subjective color scores and objective redness (a^* value), indicating better color acceptability to consumers. High redness is often associated with a higher proportion of oxidative muscle fibers, which are rich in mitochondria and tend to accumulate neutral lipids [24]. Consistent with this, SL pigs had significantly higher intramuscular fat content than DLY pigs. Increased intramuscular fat directly improves juiciness, flavor intensity, and overall palatability. Meanwhile, SL pork showed significantly lower shear force than DLY pork, reflecting superior tenderness. Notably, no significant differences were found between the two groups in drip loss, or pressing loss. This suggests that the higher fat content in SL pork does not compromise water-holding capacity, nor does it indicate stress-related quality defects.

Beyond sensory quality, nutritional composition determines the health value and consumer preference for pork. Indigenous Chinese pig breeds have been widely reported to exhibit favorable fatty acid and amino acid profiles [9], and our study systematically confirmed this in Songlei black pig. In terms of amino acids, total essential amino acid content was significantly higher in SL pork than in DLY pork. Notably, umami-related amino acids—aspartic acid, glutamic acid, glycine, alanine, and arginine—were all significantly enriched in SL pork. These compounds contribute strongly to the umami taste and indicate that SL pork has a richer and more complex flavor profile.

Regarding fatty acids, SL pork showed a distinct profile. Total monounsaturated fatty acids and polyunsaturated fatty acids were significantly higher than in DLY pork, with marked increases in oleic acid, linoleic acid, α -linolenic acid, and eicosapentaenoic acid (EPA). Oleic acid is widely recognized for its cardiovascular benefits through improved blood lipid profiles. Linoleic acid is an essential fatty acid and a precursor for various bioactive lipids [25]. α -Linolenic acid, a characteristic fatty acid in indigenous breeds, serves as a flavor precursor and can be converted into DHA, supporting neural development and anti-inflammatory functions [26]. EPA is well known for its anti-inflammatory and cardioprotective effects [27,28]. This unique combination of fatty acids gives SL pork both improved eating quality and added health value.

For mineral content, SL pork had significantly higher zinc levels than DLY pork ($p < 0.01$). Zinc is an essential cofactor for antioxidant enzymes and enzymes involved in protein metabolism. In meat, it helps stabilize cell membrane structure and influences postmortem tenderization efficiency [29,30]. Therefore, SL pork may serve as a naturally zinc-rich food and provide a high-quality dietary source of zinc.

To explore the molecular basis of these phenotypic advantages, we performed integrated transcriptomic and metabolomic analyses. A total of 946 differentially expressed genes were identified. KEGG enrichment analysis showed significant enrichment in pathways such as TGF- β , HIF-1, FoxO, PI3K-Akt, MAPK, and ECM-receptor signaling pathways. While these pathways have been widely reported in meat quality studies of other pig breeds, the downstream gene expression patterns in SL pigs were distinctive—most notably, the general downregulation of collagen family genes. This clearly distinguishes SL from DLY pigs and provides direct molecular evidence for their low collagen content and high tenderness. Metabolomic analysis identified 821 differentially abundant metabolites, enriched in pathways including the tricarboxylic acid cycle, glycerophospholipid metabolism, and histidine metabolism. Lipids and lipid-like molecules comprised the largest category of differential metabolites, consistent with the marked differences in fatty acid profiles.

Protein-protein interaction network analysis highlighted several collagen genes—*COL1A1*, *COL1A2*, *COL3A1*, and *COL5A1*—as central hubs. These genes encode the main structural components of the muscle extracellular matrix (ECM).

521 Their down-regulation in SL is correlated with the observed lower collagen content
522 and improved tenderness. Consistent with this association, reduced expression of
523 these genes likely decreases the synthesis and cross-linking of collagen fibers,
524 weakening the ECM's mechanical strength and reducing its restriction on myofibrils.
525 This may facilitate easier myofibril fragmentation during post-mortem aging,
526 enhancing tenderness [4,31,32]. However, this interpretation is based on correlative
527 evidence, and the causal relationship between COL gene downregulation and
528 tenderness improvement remains to be experimentally validated. The TGF- β /Smad
529 pathway, which was enriched among the DEGs, has been established as an upstream
530 regulator of collagen gene expression in other species [33]. Its enrichment, together
531 with the consistent downregulation of collagen genes, raises the possibility that this
532 pathway may be involved in the distinct ECM phenotype of SL muscle. Nevertheless,
533 as TGF- β protein activity was not directly measured in this study, this interpretation
534 should be considered preliminary and hypothesis-generating. Gene-metabolite
535 correlation analysis further revealed two functionally relevant pairs. Histamine
536 showed a strong negative correlation with multiple core collagen genes. Although
537 histamine is typically considered an indicator of meat spoilage [34–36], it is worth
538 noting that all samples in this study were collected within 1 h postmortem and
539 immediately snap-frozen, conditions that effectively prevent bacterial proliferation
540 and postmortem histamine accumulation. The histamine detected therefore likely
541 reflects endogenous levels rather than spoilage artifacts. The inverse correlation
542 between histamine and collagen genes raises an intriguing but unproven hypothesis:

that endogenous histamine may be associated with collagen gene expression under physiological conditions. However, this interpretation is based solely on correlative evidence, and functional validation—for example, treating fibroblasts with histamine and measuring COL1A1/1A2/3A1 expression—would be required to establish any causal link. Until such data are available, this association should be regarded as hypothesis-generating rather than conclusive. Conversely, LPC 18:3 was positively correlated with *COL1A1*, *TLR4*, *ARG2*, and *EGRI*. LPC is a bioactive signaling molecule [37]. *TLR4* activation can influence the inflammatory microenvironment and regulate protein degradation and tenderization [38]. *ARG2* is involved in polyamine synthesis and affects fibroblast activity and fat deposition[39]. *EGRI* is a transcription factor that promotes adipocyte differentiation [40]. The co-expression pattern of LPC 18:3 with these genes suggests that this metabolite associated with pathways involved in lipid signaling, inflammatory regulation, and extracellular matrix remodeling.

Through integrated multi-omics and phenotypic association analysis, we identified a set of molecular markers strongly correlated with meat quality traits. These markers are associated with the differences between SL and DLY pigs and show consistent and strong correlations with multiple meat quality indicators—including intramuscular fat, shear force, and meat color. As such, they may hold potential for use in breeding selection or early screening, although this requires further validation in independent populations.

Collagen is the main structural component of the muscle connective tissue matrix, and its content and cross-linking status directly determine pork tenderness. In this study, *COL1A1*, *COL1A2*, and *COL3A1* were consistently downregulated in SL muscle and showed strong positive correlations with shear force ($|r| > 0.8$, $p < 0.01$). This relationship was validated by both RNA-seq and qRT-PCR, indicating that collagen gene expression levels can directly reflect the mechanical strength of the extracellular matrix. The role of *COL1A1* and *COL3A1* in determining meat tenderness is biologically plausible, as these genes encode major structural components of the muscle ECM, and their expression levels directly influence collagen content and cross-linking, which in turn affect shear force [41,42]. *SPPI* and *TLR4*, both involved in ECM remodeling, were co-downregulated with the core collagen genes, suggesting a global suppression of fibroblast activation programs in SL muscle. From a breeding perspective, expression levels of *COL1A1*, *COL1A2*, and *COL3A1* in longissimus dorsi muscle biopsies—or their protein products in circulation—could serve as early predictors of tenderness potential before slaughter. Given that collagen content shows moderate heritability in pigs, these genes could eventually be suitable targets for marker-assisted selection, provided their predictive value is validated in independent cohorts and their genetic basis is resolved through GWAS or eQTL mapping [43].

L-Histidine is an essential amino acid and a precursor for carnosine and histamine. It contributes to muscle buffering capacity and antioxidant status [44,45]. In this study, L-histidine was significantly enriched in SL muscle. It showed positive

correlations with IMF content and marbling score, and negative correlations with shear force and L* value. These associations suggest that L-histidine may integrate lipid metabolism and meat quality traits. Previous studies have shown that histidine and related dipeptides can improve lipid metabolism and reduce oxidative stress in adipose tissue, and L-histidine serves as a precursor for carnosine, a key dipeptide in this process [46]. The association between L-histidine and IMF in our study is consistent with these known metabolic functions. However, the positive correlation with IMF does not imply causation. Instead, it may reflect a link between amino acid availability and muscle fat deposition. The negative correlation with shear force may be related to its antioxidant function, which could reduce protein oxidation during postmortem aging. Given its ease of quantification, L-histidine content in longissimus dorsi muscle or serum could serve as a low-cost, high-throughput phenotypic marker for IMF and tenderness.

LPC 18:3 is a bioactive glycerophospholipid derived from α -linolenic acid metabolism. Lysophosphatidylcholines are known to act as bioactive signaling molecules involved in inflammatory regulation and tissue remodeling [37]. In contrast to L-histidine, LPC 18:3 was downregulated in SL muscle and negatively correlated with IMF, marbling, and redness, while positively correlated with shear force and lightness. This inverse relationship positions LPC 18:3 as a negative marker of superior meat quality. The elevated LPC 18:3 levels in DLY muscle may reflect phospholipid remodeling associated with rapid glycolytic metabolism and a lean phenotype. Its positive correlation with *COL1A1*, *TLR4*, and *ARG2* further suggests

that LPC 18:3 is associated with pathways involved in ECM remodeling and adipocyte–fibroblast crosstalk. The dual association of LPC 18:3—negatively correlated with IMF and meat color, yet positively correlated with COL1A1 and TLR4—may be explained by its role as a pro-inflammatory signaling molecule. Lysophosphatidylcholines are known to activate Toll-like receptor 4 (TLR4) signaling, which can promote inflammatory responses and, in turn, stimulate fibroblast activity and extracellular matrix remodeling, including collagen synthesis [37,38]. In this context, elevated LPC 18:3 in DLY muscle may contribute to a more pro-fibrotic microenvironment, favoring collagen deposition and thereby reducing tenderness, while simultaneously suppressing adipogenic differentiation. This interpretation is consistent with the observed positive correlation between LPC 18:3 and COL1A1/TLR4, and with its negative correlation with IMF and meat color. However, this proposed link remains speculative in the absence of direct functional evidence, and targeted experiments—such as treating fibroblasts or adipocytes with LPC 18:3 and measuring TLR4/COL1A1 expression—would be necessary to validate this hypothesis. In practice, LPC 18:3 could be incorporated into a multi-marker index—along with collagen gene expression and L-histidine—for early stratification of meat quality potential in breeding stock.

Overall, the molecular markers identified in this study are not isolated, but rather form an interactive network linking ECM structure, lipid metabolism, and amino acid homeostasis. Their strong correlations with multiple meat quality traits provide empirical support for their use as composite biomarkers. It should be noted, however,

that correlation analysis reveals associations between traits but does not imply causation [41,42]. Future work should validate the predictive performance of these candidate markers in independent populations and across different muscle types, develop rapid detection methods suitable for industrial-scale screening, and explore the genetic basis of differential collagen gene expression—such as SNPs or eQTLs. Incorporating such integrated markers into routine breeding programs may accelerate genetic improvement of meat quality traits without compromising growth efficiency, which is a central goal in the breeding of premium pig breeds such as Songlei black pig.

While the identified biomarkers show strong associative evidence, several limitations should be addressed before their implementation in breeding programs. First, the functions of the differentially expressed genes and metabolites have not yet been validated by in vitro or in vivo experiments. Although correlation analyses suggest potential regulatory axes—such as histamine with collagen genes, and LPC 18:3 with *TLR4*—the causal relationships require confirmation through fibroblast intervention assays or gene-edited animal models. Second, all samples were collected from the same farm and at the same slaughter weight. While this minimized environmental variation, it does not allow assessment of genotype-by-environment interactions on marker stability. Third, this study only analyzed the longissimus dorsi muscle. Whether the meat quality traits and molecular markers identified are applicable to other premium cuts—such as the psoas major or semimembranosus—remains to be determined. Fourth, the genetic basis of collagen gene

downregulation—whether due to cis-regulatory variation or differences in upstream transcription factor activity—is not yet clear. Future integration of genome-wide association studies and expression quantitative trait locus mapping will help translate these candidate markers into tools directly applicable in breeding. Importantly, the correlative nature of our multi-omics analyses does not imply direct causal relationships. Although we observed consistent associations between collagen gene downregulation and improved tenderness, and between histamine levels and IMF deposition, these findings are based on correlation analyses that cannot distinguish cause from effect. The associations identified in this study should be viewed as hypothesis-generating rather than functionally validated. Future studies employing fibroblast-specific knockout models, CRISPR-Cas9-edited cell lines, or targeted intervention experiments are needed to establish definitive causal relationships between collagen gene expression, metabolite profiles, and meat quality traits. We acknowledge that the multi-omics analyses were performed on a subset of six individuals per breed selected based on IMF content closest to the breed mean. While this selection strategy was designed to minimize inter-individual variation and to capture breed-typical molecular profiles, it may not fully reflect the range of phenotypic variation present within each breed. Future studies with larger multi-omics cohorts would be valuable to validate the generalizability of our findings.

5. Conclusion

This study demonstrates that Songlei black pig exhibit superior meat quality compared with the commercial DLY cross, characterized by improved appearance, tenderness, intramuscular fat, and favorable fatty acid and amino acid composition. These differences are associated with coordinated transcriptomic and metabolomic features, including reduced expression of collagen-related genes and distinct lipid metabolic patterns. Key metabolites such as histamine and LPC 18:3 were correlated with multiple meat quality traits, whereas histamine showed an inverse correlation with collagen gene expression that warrants further investigation. The findings highlight the SL breed as a valuable genetic resource and provide molecular markers relevant to meat quality improvement. For translational application, the identified markers—particularly COL1A1/COL3A1 expression and LPC 18:3 levels—hold potential for early screening in breeding programs, as they are measurable in muscle biopsies or serum and show consistent correlations with tenderness and IMF. However, routine implementation would require the development of cost-effective, high-throughput detection methods (e.g., qPCR-based assays for collagen genes or targeted LC-MS/MS for LPC 18:3), as well as validation across independent pig populations and different muscle types to confirm their predictive value and generalizability. Future studies should also assess whether these markers are breed-specific or applicable to a wider range of genetic backgrounds before they can be integrated into routine marker-assisted selection.

Supplementary Information

692 Table S1: Primers for qRT-PCR of longissimus dorsi muscle;

693 Table S2: Comparison of Meat Quality Traits between SL and DLY Pigs;

694 Table S3: Determination nutritional content of SL and DLY Pigs;

695 Table S4: Sequencing data evaluation statistics;

696 Table S5: Differentially expressed genes in longissimus dorsi muscle;

697 Table S6: GO enrichment analysis of differentially expressed genes in SL and

698 DLY Pigs;

699 Table S7: KEGG enrichment analysis of differentially expressed genes in SL and

700 DLY Pigs;

701 Table S8: Differentially expressed metabolites in longissimus dorsi muscle;

702 Table S9: KEGG pathway enrichment of differentially metabolites in SL and

703 DLY Pigs;

704 Table S10: HMDB classification annotation;

705 Table S11: Common KEGG Pathways Enriched by DEGs and DEMs;

706 Table S12: Correlation analysis of genes and metabolites;

707 Table S13: Correlation analysis of meat quality traits with key genes and

708 metabolites;

709 Figure S1 Comprehensive transcriptomic profiling with functional enrichment of

710 differentially expressed genes between Songlei Black pigs (SL) and Duroc ×

Landrace × Yorkshire pigs (DLY). (A) Principal component analysis. (B) Circular plot of Gene Ontology enrichment;

Figure S2: HMDB classification of differential metabolites. (A) Positive ion mode. (B) Negative ion mode. Pie charts show superclass distribution based on annotated metabolite counts;

Figure S3: Joint analysis of transcriptome and metabolome. (A) Joint analysis of negative ion mode DEMs and DEGs expressed in similar pathways. (B) Joint analysis of positive ion mode DEMs and DEGs expressed in similar pathways.

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Not applicable.

Authors' contributions

Q.Z. and S.-M.Z. conceptualized the research; Q.Z., J.-B.Z., and J.X. designed the methodology; Q.Z., J.-B.Z., J.X., Y.-P.Z., Y.-X., S.T.K., Y.-L.Z., M.L., and Z.-Y.Z. collected the samples; Q.Z., J.-B.Z., J.X., Y.-P.Z., Y.-X., and S.T.K. conducted data analysis and software programming; Q.Z., J.-B.Z., and J.X. performed the literature search; Q.Z. wrote the original draft; S.T.K., Y.Z., and S.-M.Z. reviewed and edited the manuscript; Y.Z. and S.-M.Z. provided funding and administered the project; W.-S.S., L.P., and Y.Z. supervised the study. All authors reviewed and approved the final manuscript.

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733 Data availability

734 The RNA-Seq raw data have been deposited in the Genome Sequence Archive of
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736 Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences, under
737 accession number CRA037333.

738 Declarations

739 Ethics approval and consent to participate

740 All animal procedures were performed in accordance with the guidelines
741 approved by the Institutional Animal Care and Use Committee (IACUC) of Jilin
742 Agricultural University. The study protocol was approved by the IACUC under
743 approval number (2025 06 18 001). Informed consent was obtained from the farm
744 owner for the collection of all samples used in this study.

745 Consent for publication

746 Not applicable.

747 Competing interests

748 The authors declare no competing interests.

749 Clinical trial number

750 Not applicable.

751 Consent to Participate

752 Not applicable.

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