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Integrative multi-omics analysis identifies C-type lectin CNVs as functional targets for breeding mastitis-resistant dairy goats

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Abstract

Mastitis poses a critical challenge to dairy goat production, impacting animal welfare, milk yield, and the microbiological safety of dairy products. This study employed a multi-omics approach to explore the genetic basis of mastitis resistance in Saanen goats, focusing on identifying functional copy number variants (CNVs) and their roles in immune regulation. A genome-wide scan identified 62,860 CNVs, with 115 exhibiting strong selection signals. Among these, two adjacent CNVs overlapping with the C-type lectin genes *LOC108636060* and *LOC108636138* were noteworthy due to their high deletion frequencies in the population of Saanen goats. Functional investigations involving lipopolysaccharide (LPS)-challenged goat mammary epithelial cells (GMECs) demonstrated that the overexpression of these two C-type lectin genes significantly potentiated NF-κB signaling pathway activation. This was accompanied by the marked upregulation of key inflammatory mediators, including *CD14*, *CXCL8*, and *PTGS2*. Integrated metabolomic analysis further validated their involvement in modulating immune-associated metabolic responses, such as alterations in lipid metabolism, amino acid derivatives, and inflammatory signaling molecules. These findings indicate that *LOC108636060* and *LOC108636138* could contribute to mastitis resistance through the regulation of innate immune pathways, with critical roles in orchestrating inflammatory responses. Their functional relevance suggests these genes may serve as promising candidates for selective breeding programs. Incorporating these markers into breeding strategies could facilitate the improvement of udder health, bolster mastitis resistance, and contribute to the overall safety of milk products in dairy goat production.

Keywords Dairy goat, Copy number variant, C-type lectin, Mastitis resistance, Milk safety, Innate immunity

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Introduction

Goat milk has gained growing consumer attention worldwide due to its favorable nutritional profile, digestibility, and hypoallergenic properties compared to cow milk [1, 2]. Originally indigenous to Switzerland, the Saanen breed is renowned for its superior lactational performance. Its global dissemination has facilitated extensive environmental adaptation, notably within China, where sustained selective breeding programs have culminated in the development of distinct regional populations, such as the Tangshan dairy goat [3, 4].

However, the intensification of dairy goat farming has led to a higher prevalence of intramammary infections, particularly mastitis, which remains a major threat to milk production, quality, and safety [5]. Mastitis leads to increased somatic cell counts, microbial contamination, and enzymatic alterations in milk, raising concerns for dairy product hygiene and consumer health [6]. Evidence suggests that under intensive management conditions, Saanen goats may exhibit a higher incidence of mastitis compared to certain indigenous breeds, potentially due to the physiological demands associated with high milk production [7]. The disease is typically caused by bacterial pathogens such as *Staphylococcus aureus*, non-aureus *Staphylococci*, *Streptococcus* spp., and *Escherichia coli* [8–11]. Notably, *E. coli* acts as a major environmental pathogen, triggering inflammation through binding to Toll-like receptor 4 (*TLR4*), which activates MAPK and NF- κ B pathways [12, 13].

A key mediator of *E. coli*-induced inflammation is lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, which stimulates host immune responses through LBP-CD14-mediated signaling, resulting in elevated production of pro-inflammatory cytokines such as *IL-6*, *IL-8*, and *TNF- α* [14–17]. These immune cascades not only influence mammary gland pathology but also directly affect milk composition, microbiological safety, and shelf-life [18]. Thus, LPS-induced in vitro models have become widely used in inflammation studies involving mammary epithelial cells and hepatocytes [19–21].

C-type lectin receptors (CLRs), a family of carbohydrate-recognizing innate immune receptors, play a pivotal role in pathogen recognition and modulation of inflammatory responses, including biofilm inhibition and phagocytic clearance of mastitis-causing bacteria [22–24]. Specific CLRs such as *DNGR-1* (*CLEC9A*), Dectin-1, and Dectin-2 are known to regulate cytokine production and neutrophil recruitment, and are involved in bacterial, fungal, and sterile inflammatory responses [25, 26]. Importantly, CLRs can both support immune defense and be exploited by pathogens for immune evasion [27], making them important molecular targets for understanding host-pathogen interactions. Immune activation

is an energetically demanding process requiring rapid metabolic reprogramming [28]. Beyond the well-characterized Toll-like receptor (TLR) pathways, C-type lectin receptors (CLRs) have emerged as pivotal regulators of this metabolic switch. CLR engagement triggers intracellular cascades, such as the Syk-mTOR axis, which directly modulate glycolytic flux to meet the immediate biosynthetic demands for defense proteins and reactive oxygen species [29].

Copy number variations (CNVs), structural genomic changes affecting gene dosage, are key contributors to phenotypic diversity and disease resistance in livestock, often exerting greater functional effects than single nucleotide polymorphisms (SNPs) [30, 31]. CNVs have been implicated in immune function, pathogen defense, and environmental adaptation, influencing traits such as reproductive efficiency and lactation resilience under stress conditions [32–34].

This study aims to identify CNVs and candidate genes associated with mastitis resistance in Saanen goats through an integration of whole-genome scanning, LPS-challenged mammary epithelial cell models, and multi-omics analysis. By characterizing the immunogenetic mechanisms underlying mastitis susceptibility, our findings establish a theoretical foundation for molecular breeding initiatives designed to enhance animal health and milk safety, thereby supporting food security within the dairy goat industry.

Materials and methods

Genome-wide identification of CNVs and selection signal analysis

A total of 261 public goat whole-genome sequencing datasets were retrieved from the NCBI Sequence Read Archive database (<https://www.ncbi.nlm.nih.gov/genbank/>). The cohort comprised 41 dairy goats and 220 non-dairy goats, with all datasets maintaining an average sequencing depth of approximately 10 $\times$ coverage. Detailed accession information is provided in Table S1. Raw data were processed using Trimmomatic v0.36 [35] to remove adapter sequences and obtain high-quality clean reads (HQRs). The HQRs were subsequently mapped to the goat reference genome (ARS1 https://www.ncbi.nlm.nih.gov/assembly/GCF_001704415.1/) by the Burrows–Wheeler Aligner v0.7.17 (BWA-MEM algorithm) with the maximal exact matches (MEM) “mem” option. Potential PCR duplicates were removed using the Picard software (<http://broadinstitute.github.io/picard>). CNVs were identified using the CNVcaller software [36]. 800 bp was optimized to ensure that each bin contained an adequate number of mapped reads to accurately estimate copy number, given our average sequencing depth of 10 $\times$ coverage sequencing data. Those with a silhouette score > 0.65 and minor allelic frequency > 0.05 were

retained to prevent possible false positives. Genome-wide selection signal analysis was also conducted using V_{ST} and F_{ST} parameters. Annotation of candidate genes from the intersection of the top 1% of CNV regions ranked by V_{ST} and F_{ST} using ANNOVAR with the GFF3 Annotation Database. Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, were conducted on candidate genes using KOBAS v3.0 (<http://kobas.cbi.pku.edu.cn/kobas3>), with significance defined as a corrected p -value ≤ 0.05 .

Culture of goat mammary epithelial cells and establishment of stable transfection cell lines via lentiviral vectors

Primary goat mammary epithelial cells (GMECs, MZ-3311) were purchased from Ningbo Mingzhou Biology. The cells were cultured in a humidified environment at 37 °C with 5% CO₂ in a growth medium supplemented with Dulbecco's modified Eagle's medium (Gibco, USA, C11330500BT), 10% fetal bovine serum (Gibco, USA, A3161001C), and 1% penicillin/streptomycin (Invitrogen, USA, 15140122). Immunofluorescence staining of Cytokeratin 8 and Cytokeratin 18 was performed to identify GMECs purity [37] (Figure S1), and ck-8, ck-18 monoclonal rabbit antibodies were provided by ABclonal (China, A23133, A1022). The coding sequences of two CTL candidate genes were synthesized based on the GenBank reference sequences (XM_018049113 and XM_018048496). The final plasmid (HBLV-b-LOC108636060/LOC108636138-3xflag-ZsGreen-PURO) was constructed by Hanbio Co., Ltd (China).

Once the cells reached 70–80% confluence, polybrene (4 µg/ml) was added as a infection enhancer, and lentiviral transfection was conducted using the half-volume method. Fluorescence efficiency was observed 48 h post-transfection using a fluorescence microscope. To determine the optimal conditions, a range of multiplicities of infection (MOI: 30, 60, 90, and 120) and polybrene concentrations (3, 4, 5, 6, 7, and 8 mg/L) were evaluated. Transduction efficiency was assessed via fluorescence microscopy at 48 h post-infection. Subsequently, stable cell lines were established through a 7-day selection period using complete medium supplemented with puromycin and pyriformide.

Establishment of LPS-induced inflammatory model and integrated multi-omics analysis

The GMEC samples for RNA-seq included the *LOC108636060* overexpressing (*LOC108636060*-AD) and its no-load control (*LOC108636060*-NC), as well as the *LOC108636138* overexpressing (*LOC108636138*-AD) and its no-load control (*LOC108636138*-NC). Three biological replicates were prepared for each group. To

induce inflammation, GMECs were treated with varying concentrations of LPS (1, 5, 10, or 20 µg/mL) and incubated at 37 °C for 12 h. The mRNA expression of the inflammatory factor *IL6* was assessed to confirm successful establishment of the LPS-induced inflammation model in GMECs. For RNA-seq and metabolomic analysis, the LPS-induced inflammation model included the *LOC108636060* overexpression group (LPS-*LOC108636060*-AD) and its control (LPS-*LOC108636060*-NC), as well as the LPS-*LOC108636138*-AD and LPS-*LOC108636138*-NC groups. Each group was run in parallel with three biological replicates.

Total RNA was extracted from the cells using RNAiso Plus (Takara, Japan, 9109). RNA-seq libraries were prepared using the NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB, USA), and sequencing was conducted on the Illumina NovaSeq 6000 platform (Illumina, USA). Quality control of the raw data (PRJNA1066899) was performed using SeqPrep (<https://github.com/jstjohn/SeqPrep>) and Sickle (<https://github.com/najoshi/sickle>) under default settings to obtain clean reads. These clean reads were then aligned to the reference genome (ARS1) using HISAT2 v2.2.4.0 (https://cloud.biohpc.swmed.edu/index.php/s/hisat2-240-Linux_x86_64/download). Gene-level expression was quantified using RSEM (v1.3.1) [38], which provides robust estimates for both transcript and gene expression. To ensure statistical rigor in differential expression analysis, we extracted the raw expected counts generated by RSEM. Differentially expressed genes (DEGs) were identified using DESeq2 (Love et al., 2014) with a significance threshold set at false discovery rate (FDR) < 0.05 and $|\log_2 \text{fold change}| \geq 1$.

After sample collection, specimens were rapidly frozen in liquid nitrogen for 5 min, thawed on ice for an additional 5 min, and then subjected to a 2-minute oscillation. To ensure complete protein precipitation, samples were incubated at -20 °C for 30 min, followed by centrifugation at 12,000×g for 10 min at 4 °C. The supernatant (300 µL) was collected and subjected to a second centrifugation at 12,000×g for 3 min at 4 °C to remove any remaining precipitates before LC-MS/MS analysis.

Metabolomic analysis was performed using a liquid chromatography (LC)-ESI-MS/MS system, which included an ultrahigh-performance LC (ExionLC AD, USA) and a mass spectrometer (QTRAP®, USA). The initial mass spectrometry data were processed using Analyst v1.6.3 (Framingham, USA) and MultiQuant v3.0.3, analyzing a total of 367 metabolites across all samples. Metabolites with variable importance (VIP) values ≥ 1 , fold change ≥ 2 , and fold change ≤ 0.5 in the OPLS-DA projection were identified as significantly differentially abundant metabolites (SDMs) between groups. Functional annotation of SDMs was conducted using the KEGG compound database (<http://www.kegg.jp/kegg>

/compound/). Correlation analysis between genes and metabolites was performed using the Pearson correlation coefficient, with significance defined as $R > 0.80$ and $p\text{-value} < 0.05$.

Quantitative real-time PCR (qRT-PCR) validation

The accuracy of bioinformatics analysis of the RNA-seq data was validated using a CFX96-Touch™ Real-Time PCR Detection System (Bio-Rad, USA). RNA was reverse transcribed to cDNA using the First-Strand cDNA Synthesis Kit (Takara Bio, RR047A, China). Detailed information regarding the candidate genes is provided in Table S2. The qRT-PCR reaction mixture consisted of 10 μL of 2×SYBR Green PCR Master Mix (Takara Bio, China), 20 ng of cDNA, 0.5 μL (10 mmol/L) of each primer, and ddH₂O to reach a final volume of 20 μL. The qRT-PCR amplification protocol was set at 95 °C for 3 min, followed by 40 cycles at 95 °C for 10 s and 55 °C for 30 s. Each sample was tested in triplicate within each run, with a maximum allowable standard deviation of 0.2 for the threshold cycle. Each qRT-PCR experiment was repeated

at least three times. The expression of the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene served as the normalization control, and relative gene expression levels were calculated using the 2^{−ΔΔCt} method. Statistical significance was assessed using g SPSS v19.0, with significance set at $P < 0.05$.

Results

Genome-wide selection signal analysis of dairy goats and nondairy goats

A total of 62,860 autosomal CNVs were identified across the cohort, with lengths primarily ranging from 1,999 bp to 2,602,999 bp. By filtering the top 1% of signals based on $F_{ST} \geq 0.1267$, $V_{ST} \geq 0.0779$ (Fig. 1A), we identified 115 candidate CNVs and 12 potential target genes, including *SLC2A13*, *BMPR1B*, and *DDHD1* (Table S3). KEGG pathway analysis indicated that only three candidate genes (*SEMA3C*, *BMPR1B*, and *CACNA2D1*) were enriched in 13 KEGG pathways (Table S4) (Fig. 1B), such as the hypertrophic cardiomyopathy, mitogen-activated protein kinase signaling pathway, and oxytocin signaling

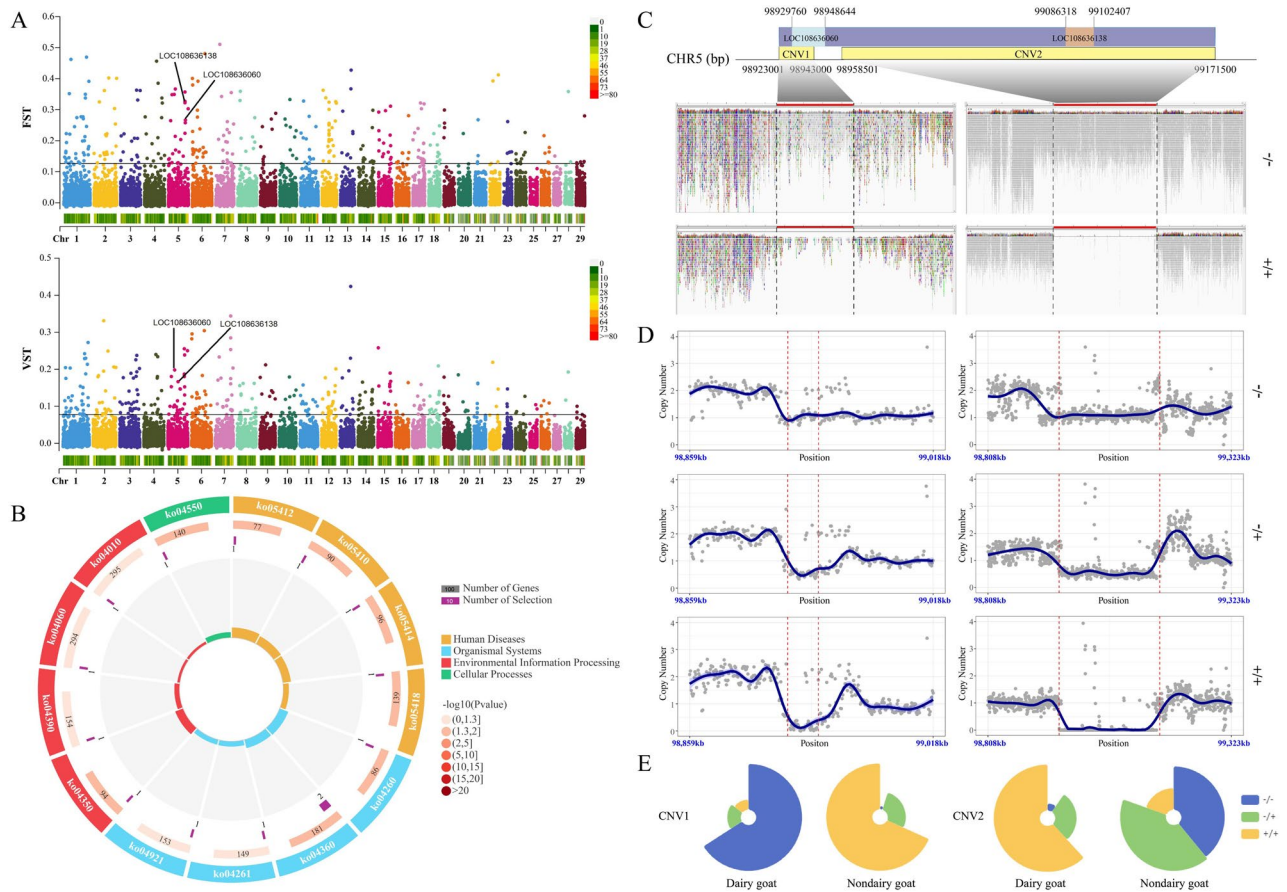


Fig. 1 Genome-wide CNVs selective sweep of dairy goat and HSCs genotype verification. **(A)** Manhattan plot showing the CNV-based selection signals of dairy goat vs. nondairy goat with F_{ST} and V_{ST} . **(B)** KEGG functional enrichment circular diagram of candidate genes. **(C)** Physical location of two candidate HSCs (CNV1 and CNV2) on CHR5 and reads distribution of their homozygous genotypes observed by IGV software. **(D)** Genomic alignment coverage of different genotypes of CNV1 and CNV2 with short-reads. **(E)** Genotype frequency of CNV1 and CNV2 in dairy and nondairy goat

pathway. Notably, two adjacent high-signal CNVs (CNV1: CHR5_98923001–98943000, CNV2: CHR5_98958501–99171500) on chromosome 5 resulted in the deletion of two CTL genes: *LOC108636060* (D11-like member of the CTL domain family) and *LOC108636138* (Killer cell lectin-like receptor (*KLR*) subfamily B member 1B allele A, *KLRB1B-A*) (Fig. 1C), as determined using the Integrative Genome Viewer. The average read coverage of the different high-signal CNVs genotypes are illustrated in Fig. 1D. Specifically, 15% and 68% homozygous deletion genotypes of both CNVs were observed in dairy goats, respectively (Fig. 1E).

Transcriptome analysis of overexpressed candidate genes in GMECs

At an MOI of 90, the observed fluorescence intensity was maximized, and the GMECs demonstrated robust growth (Fig. 2A). Forty-eight hours after lentiviral

transfection, the expression levels of *LOC108636060*-AD and *LOC108636138*-AD were significantly higher than those in the control group (p -value < 0.01; Fig. 2B), indicating successful establishment of stable transfection in cells carrying the target genes.

Transcriptomic analysis revealed distinct gene expression profiles between the CTL-gene deletion and control groups. Specifically, 36 DEGs (e.g., *ITGB3*, *TNFSF18*, and *DDIT4*) were identified between the *LOC108636060*-AD and *LOC1086360*-NC groups (Fig. 2C). Functional enrichment analysis indicated that 12 of these DEGs were significantly enriched across 50 KEGG pathways, with top-ranked terms including platelet activation and the PI3K/Akt signaling pathway (Fig. 2D). Furthermore, 34 DEGs were mapped to 421 GO terms, highlighting biological processes such as CX3C chemokine binding and the positive regulation of NF- κ B transcription factor activity (Fig. 2E). In the comparison between the

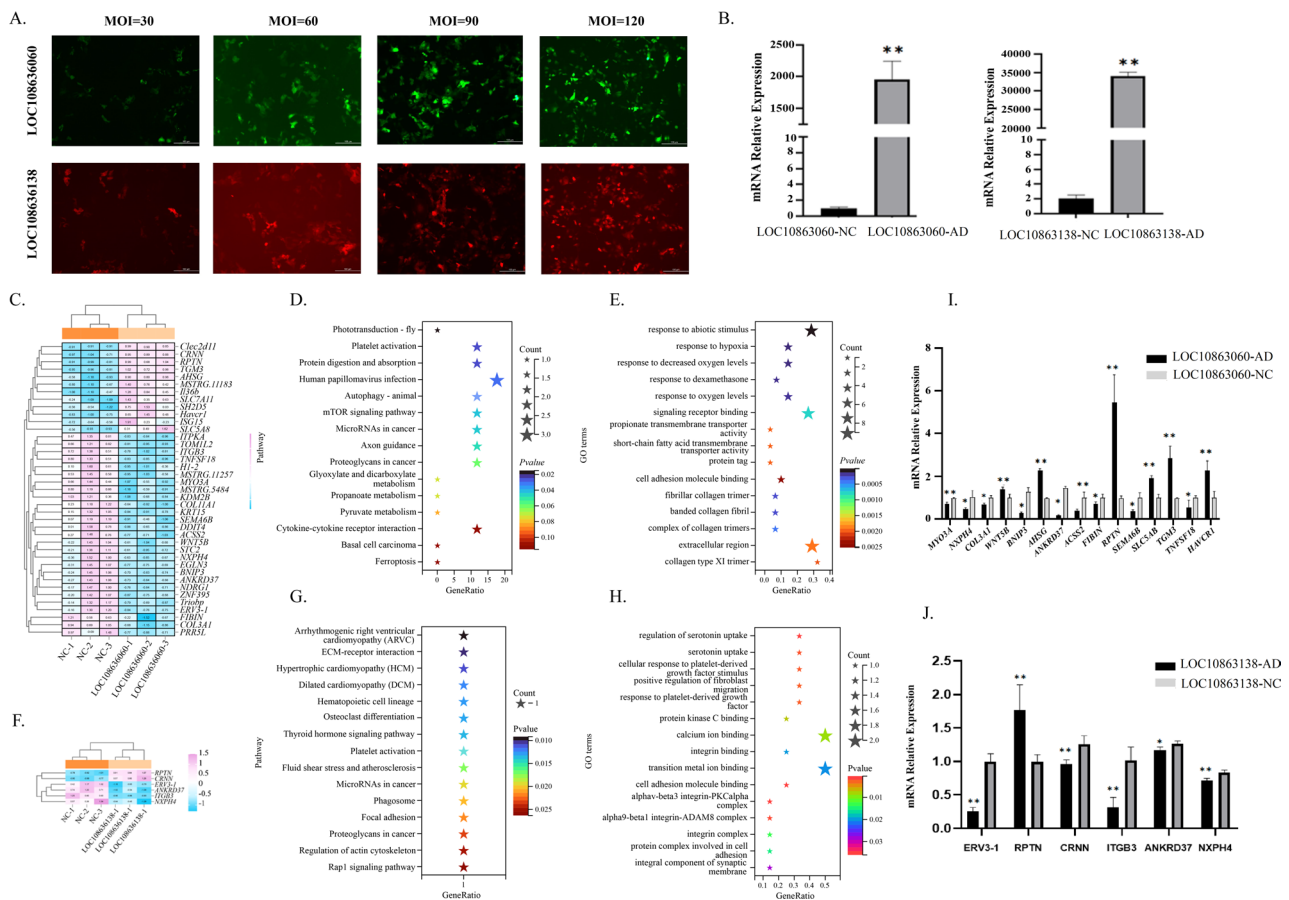


Fig. 2 Transcriptomic regulated network of overexpressed candidate genes in GMECs. **(A)** Infection efficiencies at different multiplicities of infection (MOI: 30, 60, 90, and 120). **(B)** mRNA expression of *LOC108636060* and *LOC108636138* following transfection with overexpressed candidate genes. **(C)** Heatmap visualization of DEGs in samples transfected with overexpressed *LOC108636060* in comparison to that of control. **(D)** The top 15 KEGG Pathways of DEGs in *LOC108636060*-AD versus *LOC108636060*-NC group. **(E)** The top 15 GO terms of DEGs in *LOC108636060*-AD versus *LOC108636060*-NC group. **(F)** Heatmap visualization of DEGs in samples transfected with overexpressed *LOC108636138*. **(G)** The top 15 KEGG Pathways of DEGs in *LOC108636138*-AD versus *LOC108636138*-NC group. **(H)** The top 15 GO terms of DEGs in *LOC108636138*-AD versus *LOC108636138*-NC group. **(I)** mRNA expression of DEGs in samples transfected with overexpressed *LOC108636060* and control. **(J)** mRNA expression of DEGs in samples transfected with overexpressed *LOC108636138* and control. Data are mean $\pm$ SEM ($n=6$). *: p -value < 0.05; **: p -value < 0.01 vs. control

LOC108636138-AD and LOC108636138-NC groups, six DEGs were identified, including *ERV3-1*, *CRNN*, and *ITGB3* (Fig. 2F, Table S5). These genes were annotated with 105 GO terms, covering functions related to breast development and immunity, such as platelet-derived growth factor receptor binding and the positive regulation of T-cell activation. The top 15 GO terms and KEGG pathways are illustrated in Fig. 2G and H, respectively. Notably, *ITGB3* was enriched in 18 KEGG pathways, including immune-related pathways like platelet activation and PI3K-Akt signaling. Lastly, RT-qPCR results (Fig. 2I and J) demonstrate concordance in five out of six genes (83%) and 14 out of 15 genes (93%), supporting the reliability of the transcriptomic data in this study.

Transcriptome analysis of overexpressed candidate genes in LPS-induced GMECs

IL-6 mRNA expression increased in GMECs with increasing LPS concentrations, with 5 $\mu\text{g}/\text{ml}$ identified as the optimal concentration for inducing inflammation without significantly affecting cell viability (Fig. 3A).

Transcriptomic profiling identified 908 DEGs in the LPS-LOC108636138-AD vs. LPS-LOC108636138-NC groups (Fig. 3B), which were enriched in 306 pathways. The top 15 highly enriched KEGG pathways (Fig. 3C) including immune system-related pathways such as cell adhesion molecules, Th17 cell differentiation, and antigen processing and presentation, as well as other pathways, such as NF- κB signaling pathway and inflammatory mediator regulation of TRP channels. Further enrichment analysis revealed that upregulated genes were predominantly associated with Toll-like receptor, IL-17, Th17, NF- κB , PI3K-Akt, and MAPK signaling pathways (Table S6), indicating widespread activation of inflammation and repair-related cascades upon *LOC108636138* overexpression under LPS stimulation. Several key inflammatory mediators, including *CXCL8*, *IL6*, *TGFB2*, and *CD14*, were markedly upregulated, suggesting a central role for *LOC108636138* in amplifying inflammatory responses. In parallel, downregulated DEGs (*CLDN7*, *CDH1*, *PARD6G*) were significantly enriched in pathways related to epithelial structural integrity, including tight

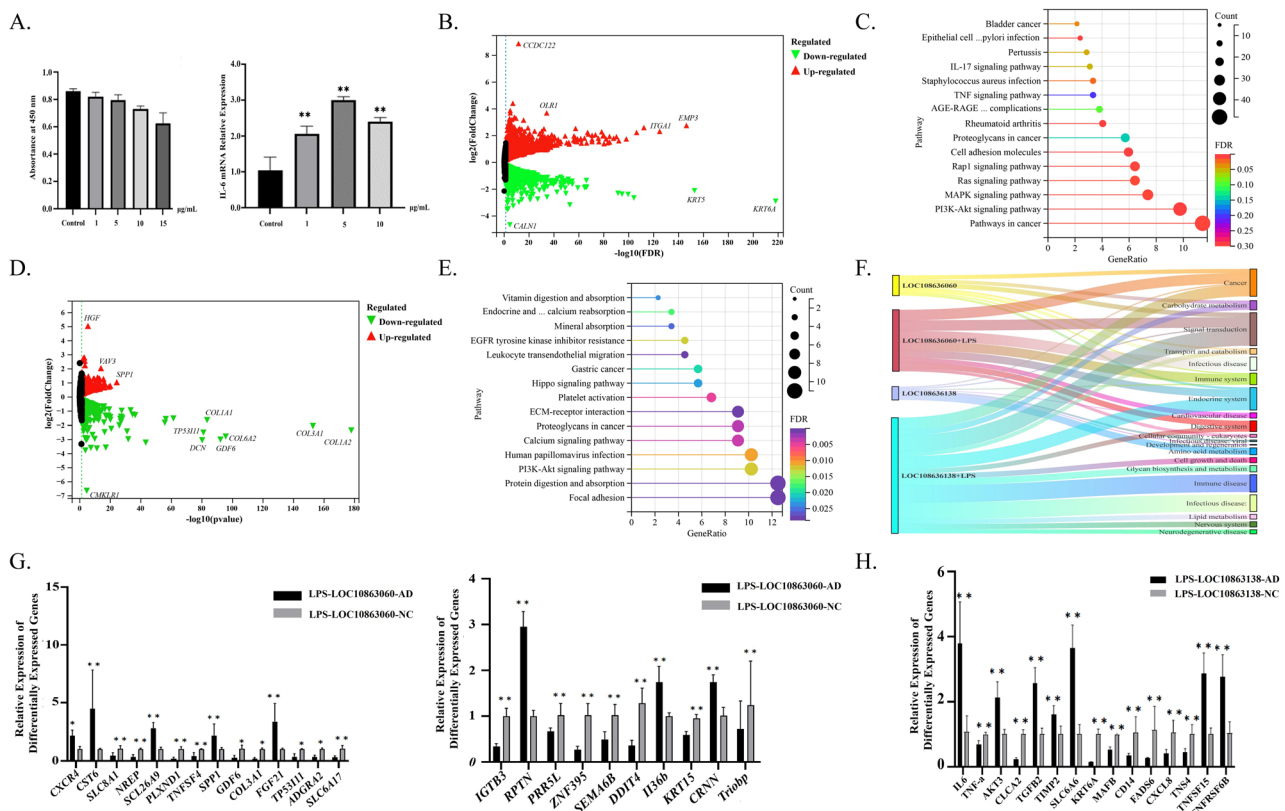


Fig. 3 Transcriptomic regulated network of overexpressed candidate genes in LPS-induced GMECs. **(A)** Cell viability assay measured by CCK-8 Kit and *IL-6* mRNA expression increased with increasing LPS concentration in GMECs. **(B)**Volcano plot clustering of DEGs in samples transfected with overexpressed *LOC108636060* in LPS-induced GMECs. **(C)** The top 15 KEGG Pathways of DEGs in LPS-LOC108636060-AD versus LPS-LOC108636060-NC group. **(D)** Volcano plot clustering of DEGs in samples transfected with overexpressed *LOC108636138* in LPS-induced GMECs. **(E)**The top 15 KEGG Pathways of DEGs in LPS-LOC108636138-AD versus LPS-LOC108636138-NC group. **(F)** Comparative analysis of the regulatory expression of candidate genes between the LPS-GMEC model and normal GMECs model. **(G)** mRNA expression of DEGs in samples with LPS-induced GMECs transfected with overexpressed *LOC108636060* or control and *LOC108636138* or control. Data are mean $\pm$ SEM ($n=6$). *: p -value < 0.05 ; **: p -value < 0.01 vs. control

junction, adherens junction, and cell adhesion molecules (Table S6).

Additionally, 176 DEGs (Fig. 3D) were identified between the LPS-*LOC108636060*-AD and LPS-*LOC108636060*-NC groups, enriching 164 KEGG pathways, including chemokine signaling pathways, IgA production in the intestinal immune network, and T-cell receptor signaling pathways. The top 15 highly enriched KEGG pathways for this group are presented in Fig. 3E. Furthermore, selected immune-related DEGs were validated by RT-qPCR, and the results were consistent with the RNA-seq data (Fig. 3G and H).

Among the identified DEGs, 49 genes were significantly upregulated. Functional enrichment analysis demonstrated that these genes were primarily associated with chemokine signaling, including *CXCR4* and *NCF1*, as well as specialized immune-receptor pathways (T-cell, NK-cell, and B-cell receptor signaling). Furthermore, these genes were significantly enriched in key inflammatory and metabolic cascades, such as the mTOR and Wnt signaling pathways (Table S7). In addition, genes related to cell migration and tissue repair, including *HGF* and *WNT11*, were also upregulated, suggesting a potential role in damage-responsive signaling. In contrast, we found a series of downregulated genes were significantly enriched in pathways related to extracellular matrix organization, collagen structural components (e.g., *COL1A1*, *COL6A2*), focal adhesion, and epithelial polarity regulation, including TGF- β signaling pathways (Table S7). These results suggest that *LOC108636060* is associated with transcriptional suppression of barrier maintenance-related genes, potentially facilitating increased immune cell infiltration under inflammatory conditions.

Comparative analysis of gene regulatory expression between LPS-challenged and control GMECs (Fig. 3F) revealed that both *LOC108636060* and *LOC108636138* exert potent immunomodulatory effects under inflammatory conditions. In the basal state, these genes are associated with a limited set of pathways involved in signal transduction, immunity, and infectious disease. Upon LPS stimulation, however, both genes exhibit a significant expansion in pathway associations, reflecting enhanced regulatory capacity. Specifically, *LOC108636060* showed increased enrichment in pathways related to systemic physiological stress, including digestion, cell growth or death, and cardiovascular function. In contrast, *LOC108636138* was more strongly associated with pathways related to neurodegenerative diseases, lipid and amino acid metabolism, and immune disorders, indicating a potential role in chronic disease modulation and metabolic adaptation. Together, these results highlight distinct yet complementary regulatory patterns of the two genes under inflammatory stimulation.

Metabolomic analysis of overexpressed candidate genes in LPS-induced GMECs

In the LPS-*LOC108636138*-AD vs. LPS-*LOC108636138*-NC comparison, 162 SDMs were identified (Fig. 4A, Table S8), among which 27 SDMs were enriched in 63 KEGG pathways (Fig. 4B). Enriched pathways included necroptosis, platelet activation, TRP channel regulation by inflammatory mediators, and various lipid signaling pathways. Notably, the *LOC108636138* overexpression led to a broad accumulation of membrane-associated lipids, including phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), glycerophospholipids, and arachidonic acid, with enrichment in GPI-anchor biosynthesis, sphingolipid metabolism, and ABC transporter pathways. Additionally, metabolites such as S-methyl-L-cysteine and N-(1-Deoxy-1-fructosyl) phenylalanine were elevated, indicating activation of antioxidant responses and membrane remodeling.

Additionally, 70 SDMs were identified between the LPS-*LOC108636060*-AD vs. LPS-*LOC108636060*-NC comparison (Fig. 4C, Table S9), with 40 SDMs enriched in 46 KEGG pathways (Fig. 4D). Metabolomic profiling revealed a pronounced depletion of various carbohydrates and their derivatives in the overexpression group. Conversely, 50 metabolites were significantly upregulated, notably including lactate, linoleoyl glycine, glutamyl-threonine, deoxycholic acid, and arachidonoyl glycine-compounds intrinsically linked to inflammatory and immune signaling. Pathway analysis highlighted significant disturbances in lipid, amino acid, and carboxylic acid metabolism. Furthermore, we observed a concurrent reduction in vitamin D3 and thiamine levels, suggesting that *LOC108636060* overexpression may disrupt micro-nutrient homeostasis under inflammatory stress.

Correlation analysis of genes and metabolites affected by two CTL genes overexpressed in LPS-GMEC models

In each comparison group, the changes in all differentially expressed metabolites (DEMs) and DEGs are displayed in a nine-quadrant plot. The DEMs and DEGs in quadrants 3 and 7 were positively correlated, whereas those in quadrants 1 and 9 were negatively correlated (Fig. 4E and F). Compared with the LPS-*LOC108636060*-AD vs. LPS-*LOC108636060*-NC group, the LPS-*LOC108636138* vs. LPS-*LOC108636138*-NC group exhibited significantly more DEMs and DEGs, indicating that *LOC108636138* overexpression activated more downstream genes and metabolites in the inflammatory state. To investigate the relationship between DEMs and DEGs, Pearson correlation analysis was conducted, revealing that numerous DEGs were significantly correlated with SDMs in LPS-GMECs overexpressing *LOC108636060* (Table S10). For instance, 7 DEGs, including *COL6A2*, *GDF6*, and *NID2*, were positively correlated with L-lactate. Similarly, 10

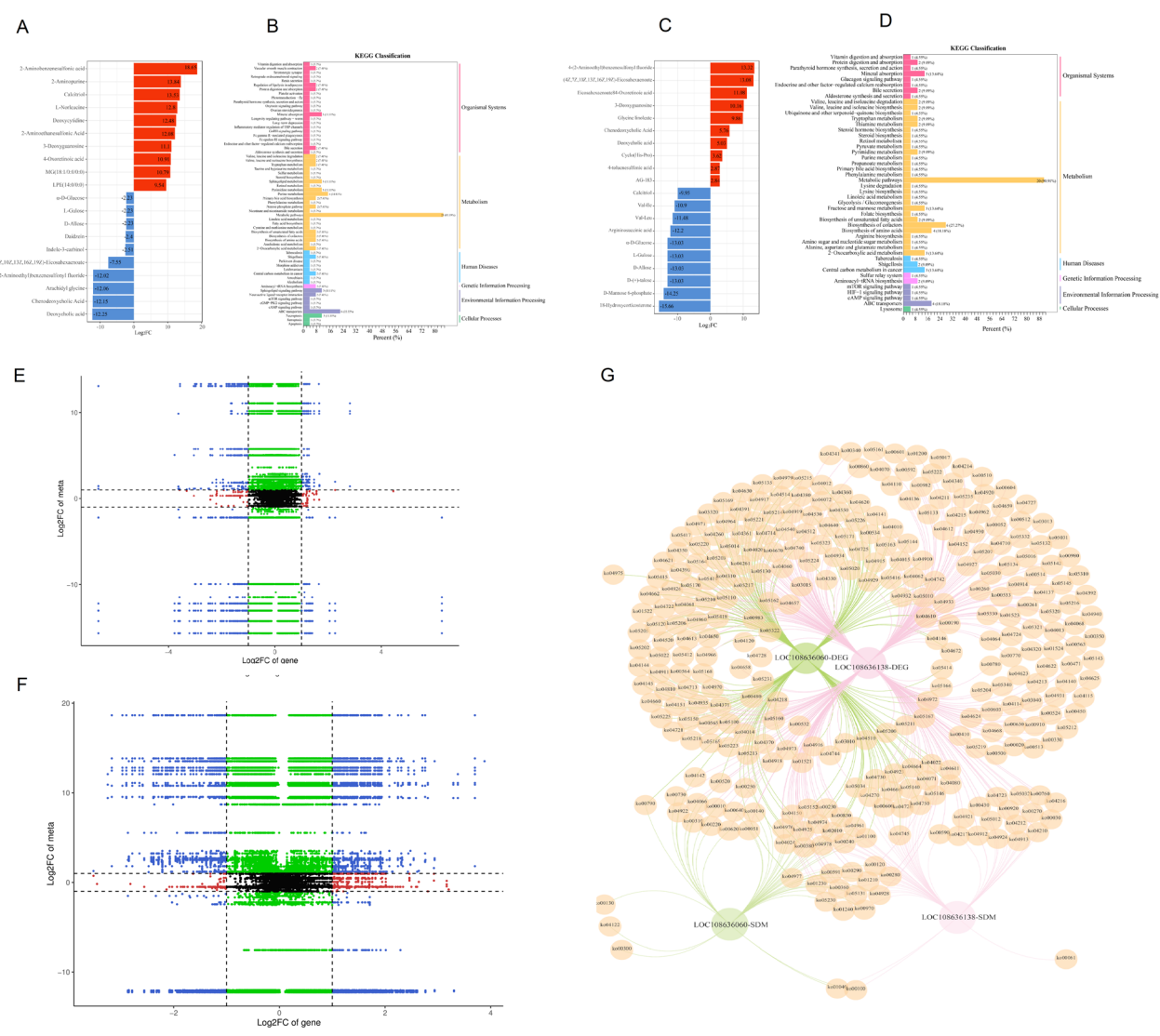


Fig. 4 Metabolomic regulated network of overexpressed candidate genes in LPS-induced GMECs. **(A)** The top 10 up-regulated and down-regulated SDMs with overexpressed *LOC108636060* in LPS-induced GMECs. **(B)** The Pathways classification of SDMs in LPS-LOC108636060-AD versus LPS-LOC108636060-NC group. **(C)** The top 10 up-regulated and down-regulated SDMs with overexpressed *LOC108636138* in LPS-induced GMECs. **(D)** The Pathways classification of SDMs in LPS-LOC108636138-AD versus LPS-LOC108636138-NC group. **(E)** The nine-quadrant plot of LPS-LOC108636060-AD versus LPS-LOC108636060-NC group. **(F)** The nine-quadrant plot of LPS-LOC108636138-AD versus LPS-LOC108636138-NC group. **(G)** The venn network of KEGG pathways of DEGs and SDMs in LPS-GMECs overexpressing *LOC108636060* or *LOC108636138*

DEGs, such as *ADAM28*, *FER1L6*, and *KLK12* were positively correlated with 2-aminobenzenesulfonic acid. Additionally, 38 DEGs, including *COL11A1*, *ATP6V0D2*, and *COL5A3*, were positively correlated with chenodeoxycholic acid, whereas 118 DEGs were positively correlated with thiamine.

To further understand that DEGs and DEMs co-regulate the inflammatory response in this cellular model, shared pathway enrichment analysis was performed. This analysis yielded 19 interacting pathways, including mineral absorption, vitamin digestion, and mTOR signaling pathways, which were enriched by combined DEGs and SDMs in LPS-GMECs overexpressing *LOC108636060*.

Similarly, a larger number of correlated pairs were observed between DEGs and SDMs in LOC108636138-LPS-GMECs (Table S11), with 151 DEGs positively correlated with L-leucine and 431 DEGs positively correlated with chenodeoxycholic acid. Notably, immune-regulation pathways were prominently enriched among the 57 interacting pathways identified in LPS-treated GMECs overexpressing *LOC108636138*.

LOC108636060 overexpression modulated multiple transcription-metabolism co-enrichment pathways, including cAMP signaling, mTOR, retinol metabolism, calcium reabsorption, and aldosterone synthesis (Fig. 4G). Within this group, the structural gene *COL6A2*

showed a significant positive correlation ($P=0.0015$) with lactate and deoxycholic acid levels, illustrating the regulatory connectivity between immune chemotaxis, bile acid signaling, and local metabolic stress.

Discussion

Mastitis remains a primary constraint on dairy goat productivity and milk quality. Beyond its role in lactation, the mammary epithelium acts as a critical immune barrier, directly modulating bacterial defense and safeguarding product safety [8, 39]. In this study, we identified two CNVs within the C-type lectin gene region, specifically *LOC108636060* and *LOC108636138*, which exhibit signatures of positive selection in dairy goats. Functional validation via lentiviral-mediated overexpression demonstrated that these genes significantly modulate the LPS-induced inflammatory response in mammary epithelial cells. Our findings indicate that these genes act as positive regulators of the inflammatory cascade, suggesting their potential utility as candidate markers for selective breeding programs focused on improving mastitis resistance and maintaining milk quality.

C-type lectins (CTLs) are well-known as highly conserved immune-related molecules that are widely distributed across various species. They play important roles in maintaining immune homeostasis, recognizing pathogens, regulating allergic responses, and activating the complement system [22, 40]. Despite these advancements, the genetic variation of CTLs in dairy goats and their potential role in conferring resistance to mastitis remain largely unexplored. In the present study, homology analysis revealed that the goat genes *LOC108636060* and *LOC108636138* are most closely related to human *CLEC2D* and *KLRB1*, respectively [41, 42]. *CLEC2D* gene is a C-type lectin-like molecule interacting with human *CD161* receptor expressed by natural killer cells and subsets of T cells. *KLRB1* encodes *CD161*, a major lectin-like receptor expressed on NK cells, which binds to carbohydrate termini and N-Acetyl-D-lactosamine [43–45]. Upon activation, *KLRB1* enhances T cell proliferation and supports early interactions between NK cells and bone marrow-derived cells. This interaction promotes the release of pro-inflammatory cytokines such as *CXCL8* [46]. These findings suggest that the inflammatory mediators regulated by these genes may directly influence local inflammation within the mammary gland, which in turn determines the levels of bacterial load and inflammatory cytokines in milk.

The findings of this study demonstrate that *LOC108636060* acts as a central orchestrator of the inflammatory response in GMECs. By integrating transcriptomic and metabolomic datasets, we identified a complex immune-metabolic axis that governs host

defense and tissue homeostasis during LPS-induced inflammatory stress.

The results indicate that *LOC108636060* exerts a dual regulatory influence on the inflammatory microenvironment. Under basal conditions, the modulation of genes such as *TNFSF18* and *WNT5B* suggests a homeostatic role [47, 48]. Upon LPS stimulation, *LOC108636060* overexpression shifts the cellular state toward an activated phenotype, characterized by the upregulation of *CXCR4* and *SPP1* [49–51]. These markers are indicative of enhanced immune cell trafficking and remodeling of the inflammatory microenvironment, consistent with rapid defense mobilization against bacterial pathogens.

Under LPS-induced inflammatory stress, *LOC108636060* overexpression triggers a comprehensive reconfiguration of both immune and metabolic landscapes in GMECs. The concurrent enrichment of chemokine, TLR, and B cell receptor signaling pathways, alongside the PI3K-Akt cascade, points to a highly coordinated immune-metabolic regulatory network. These findings suggest that *LOC108636060* acts as a pivotal regulatory hub in GMECs, facilitating the integration of metabolic reprogramming with inflammatory mobilization—a process critical for mounting an effective response to bacterial pathogens [52–55]. Ultimately, this coordination suggests that *LOC108636060* plays a dual role in bridging innate inflammatory responses with adaptive immune signaling during mammary infection.

Metabolomic analysis revealed that *LOC108636060* overexpression induces a distinct metabolic reprogramming in GMECs under inflammatory stress, characterized by reduced thiamine levels alongside elevated lactate and carnitine. Thiamine depletion, often associated with altered mitochondrial function and oxidative stress regulation, may reflect a trade-off between host inflammatory mobilization and micronutrient availability [54, 56–58]. Conversely, the accumulation of lactate and carnitine likely serves as a compensatory anti-inflammatory mechanism. Lactate has been shown to dampen TLR4- NF- κ B signaling via *GPR81*, while carnitine supports mitochondrial efficiency and antimicrobial defense [59, 60]. Beyond intracellular homeostasis, these metabolic shifts have potential implications for milk composition and quality. The increase in lactate and carnitine is consistent with enhanced antimicrobial activity against common mastitis-associated pathogens, such as *Staphylococcus aureus* and *Escherichia coli* [61, 62]. Together, these findings suggest that *LOC108636060* facilitates a coordinated immune-metabolic response that balances inflammatory defense with the maintenance of mammary tissue integrity and milk functionality.

Under non-LPS stimulated conditions, overexpression of *LOC108636138* in GMECs primarily affected several DEGs related to immune regulation and cellular

development, such as *ITGB3*, *ERV3-1*, and *CRNN*. These genes are primarily enriched in pathways governing PI3K-Akt signaling, cell proliferation, and T-cell activation [63–66]. The functional convergence of these DEGs suggests that *LOC108636138* contributes to mammary gland maintenance by fine-tuning epithelial barrier function and extracellular matrix remodeling. Specifically, the regulation of pathways related to epithelial proliferation and immune-cell interaction implies a proactive role in supporting mammary structural integrity and modulating immune-cell activity in the absence of exogenous pathogens. These results indicate that *LOC108636138* may function as a physiological regulator that promotes mammary gland homeostasis, thereby safeguarding udder health during the lactation cycle.

Under LPS stimulation, *LOC108636138* overexpression triggers a robust immune response, characterized by the upregulation of pro-inflammatory mediators such as *CXCL8*, *IL6*, and *CD14*, alongside the activation of the NF- κ B, MAPK, and PI3K-Akt signaling cascades [67–72]. While these pathways are central to mounting an effective defense against bacterial pathogens, our data also highlight a potential feedback mechanism. In parallel, we observed the downregulation of genes critical for junctional stability, including *CLDN7*, *CDH1*, and *PARD6G*. This suppression points to a compromise in epithelial cohesion and barrier integrity [73–75]. In summary, *LOC108636138* serves as a dynamic regulator whose function shifts according to the inflammatory microenvironment. While basal expression supports structural maintenance and immune surveillance, LPS-induced upregulation coordinates an acute immune response that, while transiently altering barrier permeability, ultimately serves to restrict and mitigate pathological inflammation. These findings support the role of *LOC108636138* as a pivotal regulator in maintaining mammary gland homeostasis under stress.

At the metabolic level, *LOC108636138* overexpression under LPS stimulation induces a distinctive metabolite profile, characterized by the upregulation of taurine, L-leucine, and arachidonic acid. Taurine, a natural immunomodulatory molecule, plays important roles in scavenging free radicals, stabilizing membrane structures, and exerting anti-inflammatory effects. Its metabolite, taurine chloramine, alleviates LPS-induced inflammation by reducing the release of inflammatory cytokines such as IL-6 and TNF- α [76, 77]. As a natural antioxidant and inflammation regulator, taurine upregulation helps suppress excessive release of pro-inflammatory mediators in the mammary gland [78]. Taurine and related metabolites have been shown to mitigate inflammation and cellular damage by reducing oxidative stress [79], limiting pathogen-induced inflammatory responses through inhibition of NF- κ B and MAPK signaling pathways,

thereby protecting the integrity of the blood-milk barrier [80]. L-leucine, an essential amino acid, not only regulates mTOR signaling but also enhances T cell responses by promoting IL-2 production [81]. Abnormal leucine metabolism may delay mammary tissue repair and influence the composition and activity of immune factors in milk via modulation of inflammatory mediators in extracellular vesicles, indirectly impacting milk safety and processing stability [82]. Arachidonic acid and its metabolites are involved in the synthesis of prostaglandins and leukotrienes. Although they amplify signaling in acute inflammation, dysregulated arachidonic acid metabolism can exacerbate tissue damage and immune imbalance [83]. Indeed, while the role of milk components in inflammatory pathological processes remains controversial [84], extensive studies indicate that metabolic disturbances during mastitis not only affect immune regulation but also cause fluctuations in certain lipid metabolites in milk. These changes may induce oxidative stress and fatty acid autooxidation, leading to milk flavor defects and potential toxic compound formation [85, 86]. In conclusion, *LOC108636138* functions as an integrated regulator that attenuates pathological inflammation while supporting tissue stability and the functional properties of milk during mammary gland infection.

This study systematically elucidated the transcriptional and metabolic regulatory roles of *LOC108636060* and *LOC108636138* in mammary epithelial cells. Their dual regulation of inflammatory factors, immune receptors, and key metabolic pathways highlights their potential to maintain mammary immune homeostasis. These findings provide novel insights into the connection between mastitis development and milk quality variation. While not yet definitive markers for milk safety, *LOC108636060* and *LOC108636138* show promising potential as molecular breeding targets to enhance mastitis resistance in dairy goats. While several limitations must be acknowledged. First, the use of a primary GMEC model, while effective for mechanistic dissection, lacks the systemic integration of the mammary gland within a multisystemic milieu, where endocrine and immunological interactions often modulate cellular responses. Consequently, our findings regarding transcriptomic and metabolic shifts serve as foundational hypotheses rather than definitive physiological conclusions. Future research should prioritize in vivo validation, utilizing gene-editing models such as CRISPR/Cas9-modified dairy goats to clarify the role of *LOC108636138* and *LOC108636060* in mastitis resistance and milk quality.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42523-026-00559-4>.

Supplementary Material 1
 Supplementary Material 2
 Supplementary Material 3
 Supplementary Material 4
 Supplementary Material 5
 Supplementary Material 6
 Supplementary Material 7
 Supplementary Material 8
 Supplementary Material 9
 Supplementary Material 10
 Supplementary Material 11
 Supplementary Material 12

Author contributions

Ying Yuan: Conceptualization, Methodology, Investigation, Data Curation, Writing - Original Draft. He Cong: Investigation, Data Analysis, Validation. Tao Ren: Methodology, Data Collection, Preliminary Analysis. Peng-Cheng Ruan: Investigation, Method Optimization, Discussion. Lu Xu: Data Processing, Algorithm Implementation. Bai-Gao Yang: Validation, Data Analysis, Visualization. Hao-yuan Zhang: Literature Review, Background Analysis. Ceccobelli Simon: International Collaboration, Methodological Guidance. Zi-Xuan Wang: Data Organization. Yan-Long Liu: Parameter Optimization, Method Improvement. Kaushalendra: Interdisciplinary Collaboration, Theoretical Guidance. Yan-Guo Han: Supervision, Project Administration. Yan Zeng: Writing - Review & Editing, Discussion. Hong Miao: Technical Support, Equipment Management. De-Jun Xu: Project Coordination, Resource Integration. Guang-xin E: Conceptualization, Framework Design, Final Review. All authors reviewed the manuscript.

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Data availability

The raw individual participant data included in this project can be download from NCBI ([<https://www.ncbi.nlm.nih.gov/>] (<https://www.ncbi.nlm.nih.gov/>)). The bulk RNA-seq data generated from study are available from SRA database (PRJNA1066899).

Declarations

Ethics approval and consent to participate

The experiment was conducted at the Center for Experimental Animals at Southwest University (SWU) in accordance with the ARRIVE guidelines and relevant institutional regulations, and was approved by the Ethical Committee of SWU (Permit Number: IACUC-20200715-03).

Competing interests

The authors declare no competing interests.

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