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# Multi-organ comparative transcriptomic study on short- term and long-term hypoxia adaptation in cattle

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# Abstract

Highland Tibetan cattle have evolved unique hypoxic adaptation capabilities from their lowland ancestors; however, the mechanisms that distinguish short-term acclimatization from long-term genetic adaptation remain poorly understood. In this study, we integrated hematological assays and multi-organ transcriptomic analyses across three cattle groups: highland Tibetan cattle (representing long-term genetic adaptation), highland-acclimatized Holstein cattle (representing short-term acclimatization), and lowland Holstein cattle (serving as controls). Hematologically, highland-acclimatized Holsteins displayed elevated red blood cell count, hemoglobin level, and blood viscosity, which constitutes a short-term compensatory stress response to hypoxia. In contrast, Tibetan cattle maintained lower and more stable hematological parameters, reflecting a blunted adaptive strategy shaped by long-term natural selection. Transcriptomic analysis revealed tissue-specific hypoxic response patterns: the heart underwent metabolic reprogramming to support sustained hypoxic adaptation, the liver shifted its functional focus from metabolic compensation to the regulation of immune homeostasis, and the lung exhibited limited transcriptional changes associated with enhanced oxygen exchange efficiency. Immunohistochemical validation demonstrated that GNB1, a key hypoxia-responsive gene, mediates hypoxic adaptation through angiogenesis regulated by the PI3K-AKT pathway. Furthermore, the immune-related gene SLAMF7 was significantly upregulated in the liver of Tibetan cattle, and its recombinant protein exhibited broad-spectrum antibacterial activity, highlighting its critical role in long-term immune adaptation. Collectively, our findings clarify the dynamic transition from short-term physiological stress responses to long-term genetic adaptation in cattle under high-altitude hypoxic conditions, providing novel insights into the hypoxic adaptation mechanisms of ruminants.


# Background

High-altitude environments—characterized by reduced pressure of oxygen, decreased ambient temperature, and elevated ultraviolet radiation—present substantial challenges to organism habitation. To adapt to such extreme environmental conditions, acclimatized newcomers undergo physiological modifications to restore homeostasis, such as regulated blood physiology, remodeled energy metabolism, and enhanced pulmonary oxygenation capacity[1]. For non-adapted low-altitude cattle (e.g., Holstein cattle), exposure to hypoxia environment triggers a cascade of effects, including diminished blood oxygen saturation, cardiopulmonary edema, and metabolic dysfunction. In contrast, native highlanders (e.g., Tibetan cattle) have evolved genetic adaptations through thousands of years evolutionary processes, which improve their viability and reproductive success[2–7]. These two distinct adaptive strategies are designated as short-term acclimatization (STA) and long-term adaptation (LTA), respectively. Notably, certain STA-mediated changes that confer short-term advantages can exhibit long-term detrimental effects. For instance, elevated hemoglobin concentrations enhance oxygen-carrying capacity in the short term, yet they increase the risk of chronic mountain sickness due to their association with heightened blood viscosity[8.9]. Investigations into the two distinct adaptive strategies—short-term acclimatization (STA) and long-term adaptation (LTA)—facilitate the recognition of the

diversity insights into high-altitude adaptation, thereby advancing the understanding of intervention strategies for altitude- or hypoxia-related illnesses.

Transcriptomic studies have revealed that the heart and lungs are key organs exhibiting adaptive transcriptional changes in yaks at different altitudes. Notably, in lung tissue, 90% of the differentially expressed genes (DEGs) showed a non-linear relationship with altitude[10]. Another study reported that DEGs identified in the lungs of yaks from different altitudes were primarily enriched in immune response and cell cycle pathways, whereas DEGs between yaks and low-altitude Zaosheng cattle were mainly enriched in long-chain fatty acid metabolism and protein processing[11]. Beyond the heart and lungs, brain tissue of yaks showed more genes enriched in hypoxia-adaptive pathways compared to Sanjiang cattle, a low-altitude breed, with the cerebellum being particularly sensitive to hypoxia. RNA-seq analysis of lung type II cells from Tibetan and Landrace pigs revealed that DEGs were predominantly involved in glycolysis, aldosterone synthesis and secretion, potentially explaining the superior hypoxic adaptation of Tibetan pigs[12]. Furthermore, DEGs in heart tissues between Tibetan and Yorkshire pigs were mainly associated with HIF signaling and cardiovascular development pathways, while those in lung tissues were linked to HIF signaling, immune response, gas transport, and vascular development[13]. A comparative transcriptomic study of the chorioallantois membrane in Tibetan chicken and low-altitude Huaixiang chicken embryos showed that DEGs were enriched in HIF signaling, angiogenesis and morphogenesis, blood circulation, and the renin-angiotensin system[14]. RNA-seq analysis of heart tissues from Tibetan sheep at different altitudes indicated that DEGs were primarily associated with the PI3K-Akt, Wnt, and PPAR signaling pathways, and involved in regulating mitochondrial dynamics and function[15]. However, the binary comparison model of low-altitude population vs. high-altitude population used in existing studies cannot accurately reflect the dynamic diversity strategies of species adapting to high altitudes.

In this study, to comprehensively characterize the molecular and phenotypic changes underlying short-term hypoxic acclimatization and long-term hypoxic adaptation, we integrated hematological physiological assays, hemorheological measurements, and multi-organ transcriptomic profiling to identify key genetic drivers of hypoxia adaptation. Specifically, we aimed to assess the dynamic regulatory patterns of bovine gene expression signatures associated with acclimatization and adaptation, while clarifying the corresponding phenotypic plasticity and stable adaptive traits at the hematological and hemorheological levels. By revealing the key molecular mechanisms of plateau adaptation, this research aims to provide a theoretical foundation for the selection and improvement of livestock breeds with enhanced tolerance to high-altitude environments.

## Materials and Methods

# Animal rearing and sample preparation

Three experimental groups were designed including two native groups and one immigration group. Specifically, about native groups, we reared lowland cattle (Holstein breed) in Jinnan (36°40 N', 117°01'E, altitude 100m) and highland cattle (Tibetan breed) in Lhasa (29°42'E, 91°65'N, altitude 3500m), China. In addition, the immigration group, we raised lowland cattle (Holstein breed) in Lhasa (29°42'E, 91°65'N, altitude 3500m). We collected anticoagulant whole blood form the resting state of cattle to preform blood biochemical and hemorheology test. The tissue samples were obtained from commercially operated slaughterhouses in city of Lhasa and Jinan, China. Prior to sample collection, we obtained written informed consent from the management of the slaughterhouses, authorizing us to collect residual tissue samples (after routine slaughter for commercial purposes) for experimental use. The tissue samples were frozen in liquid nitrogen and stored in 10% neutral formalin solution. The procedures for animal care were approved by the Animal Welfare Committee of Shandong Academy of Agricultural Sciences, and all experiments were conducted in accordance with approved relevant guidelines and regulations.

## Sequencing, Assembly, and Data Filtering

Three oxygen-sensitive tissues (heart, lung, and liver) were collected to perform RNA-sequencing. The standard TRIzol method (Invitrogen) was employed for the isolation of total RNA from these tissues. RNA sequencing was performed on the HiSeqTM 4000 sequencing platform. A total of 51 libraries were built with average sequencing deep > 10×using the Illumina HiSeqTM 4000 platform. Read mapping could be accurately compared to the cattle reference genome (ARS-UCD1.2) by using HISAT2 software.

## Mapping and annotation of sequencing reads

Raw RNA-seq reads were filtering by removing the following type of reads: (1) more than half of the bases was less than 10; (2) contained more than two Ns; (3) less than 20 in length. After removal of the adapters, the remaining clean reads were aligned to the reference genome (Sscrofa11.0) (http://ftp.ensembl.org/pub/release-72/fasta/sus_scrofa/dna/) HISAT2 v2.0.4 (http://www.ccb.jhu.edu/software/hisat). Then, gene expression was estimated using edgeR (Robinson MD et al, 2010).

## Functional enrichment analysis

The DEGs of up-regulated and down-regulated were classified by GO and KEGG using DAVID online software (https://david.ncifcrf.gov/). The GO terms used were LC, CC and MF, and KEGG pathways with *P*-values<0.01 were considered extremely significant enriched.

## Weighted Gene Co-Expression Network Analysis (WGCNA)

WGCNA was conducted to identify gene modules associated with hypoxic response. The WGCNA package in R was utilized to construct a gene co-expression network, and key modules linked to hypoxia were identified for further exploration. This analysis facilitated the understanding of gene interactions and regulatory pathways involved in the cellular response to hypoxic stress.

# Immunohistochemical

Immunohistochemistry detection of the *GNB1* protein expression in three tissues were performed on 5μm thick paraffin-embedded tissue sections. Slides were incubated at 3% $H_2O_2$ to quench endogenous peroxidase, then boiled for 15min in citrate buffer, rinsed with PBS and processed for staining. After pre-incubation with normal rabbit serum, slides were incubated overnight at 4℃ with rabbit monoclonal antibody for *GNB1*. After PBS wash, the sections were incubated with rabbit-anti-mouse serum (Dako Corp, Glostrup, Denmark) 37 ℃ for 30 min, washed and further incubated with rabbit (mouse) strept avidin-biotin complex (SABC) 37 ℃ for 30 min. The chromogen reaction was allowed to take place in the dark, using 0.025% 3,3-diaminobenzidine (Biogenex, San Ramon, CA, USA). Subsequently, sides were counterstained with Mayer's hematoxylin and visualized and photographed under a light microscope. *GNB1* showed an anti-dilution release of 1:100. Four fields were randomly selected to take pictures in each section under Leica microscope (×400). Image-pro6.0 Image analysis (Media Cybernetics Inc., USA) was performed on the software to determine the brown-yellow particles of mean cumulative optical density (intergrated optical dendity, IOD).

## Protein analysis

For SDS-PAGE, samples were run on a 12% polyacrylamide gel and stained with coomassie brilliant blue. For Western blot analysis, proteins from SDS gel were transferred to an PVDF membrane by semi-dry blotting. The PVDF membrane were incubated with 1,000-fold diluted mouse antibody against His tag for 2 h and then incubated with 2,000-fold diluted HRP-labeled goat anti-mouse IgG for 2 h after washing with phosphate buffer. Signals were visualized by incubation with ECL chemiluminescent substrate and development by X-ray developer. Protein concentrations were determined by using a Bio-Rad Protein Assay (Bio-Rad) in triplicate against a bovine serum albumin (BSA) standard.

## Inhibitory effect of recombinant protein SLAMF7 on bacterial survival

The effects of recombinant protein SLAMF7 on survival of S. aureus (ATCC) 29213 and E. coli (ATCC) 35218 were examined by agar gel diffusion method. For agar gel diffusion, bacteria for lawn seeding were grown in liquid LB media. Inocula were spread on solid agar LB plates. Standard paper discs containing 10 μL of recombinant SLAMF7 were placed on the surface of the agar under sterile condition. Instead of the recombinant protein, penicillin and H2O were used as the positive and negative controls, respectively. The plates were then cultured overnight at 37°C. The inhibitory effects were observed on

the basis of the inhibitory zone. Similar agar gel diffusion examinations were performed using E. coli instead of S. aureus. For E. coli, kanamycin and H2O were used as the positive and negative controls, respectively.

## Results

# Study design and Sample Clustering

The present study was designed to dissect the molecular mechanisms underlying short-term hypoxic acclimatization and long-term genetic adaptation in cattle, with three experimental groups established to simulate distinct adaptive states (Fig. 1). Specifically, lowland Holstein cattle (Group L) were native to lowland environments (Jinan, China; altitude 100m) and reared as the lowland control group. Highland native Tibetan cattle (Group H) were indigenous to high-altitude habitats (Lhasa, China; altitude 3500m) and represented the long-term hypoxia-adapted group. Highland acclimatized Holstein cattle (Group LTH) were derived from lowland Holstein cattle (same genetic background as Group L) transplanted to the aforementioned high-altitude site (Lhasa, China; altitude 3500m) and reared for 6 months to achieve short-term hypoxic acclimatization.

To evaluate the overall similarity and divergence of gene expression profiles among the three experimental groups, principal component analysis (PCA) was performed on the normalized RNA-seq expression matrices of the collected tissues. The analysis was conducted by using R (v4.3.0), with visualization implemented via the ggplot2 package. The first two principal components (PC1 and PC2) collectively explained 49.09% to 56.49% of the total gene expression variance across heart, lung, and liver tissues, providing sufficient discriminatory power to distinguish samples based on their transcriptional profiles. Notably, across all three tissues, samples from the same group clustered tightly —reflecting high reproducibility of the transcriptomic data—whereas samples from different groups exhibited distinct, non-overlapping separation (Fig. 1). No outlier samples were identified in any of the PCA plots, further confirming the high quality of the gene expression data and the rationality of the experimental grouping. These results validated that the transcriptional differences observed among groups were primarily driven by hypoxic adaptive states (short-term acclimatization vs. long-term genetic adaptation) rather than technical variation.

# Analysis of hematological physiological indicator for hypoxia adaptation in cattle

Venous blood samples were collected from three experimental groups for the determination of hematological and hemorheological indicators. Hematological analysis revealed that after Holstein cattle were immigrated to the highland environment, the red blood cell count (RBC) and hemoglobin concentration (HGB) were extremely significantly increased, while hematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) exhibited a significant decrease (Fig. 2). These results indicated that lowland Holstein cattle enhance hematopoietic capacity to drive adaptive

responses to hypoxic high-altitude conditions, and the phenotypic plasticity of these hematological traits is primarily induced by hypoxic stress. In contrast, under high-altitude conditions, Tibetan cattle displayed markedly lower RBC and HGB levels compared with highland-reared Holstein cattle, demonstrating that Tibetan cattle have evolved genetic mechanisms for a blunted hypoxic stress response through long-term adaptive evolution. Additionally, no significant differences were observed in mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) among the three groups (Fig. 2), suggesting that cattle do not achieve high-altitude hypoxic adaptation by modulating red blood cell morphology. Consistent with the hematological results, hemorheological assays showed that highland-reared Holstein cattle had an extremely significant elevation in fibrinogen (FIB), plasma viscosity (PV) and Casson viscosity (CV) following high-altitude exposure (Fig. 3), which indicated that hypoxic stress induced enhanced blood coagulation capacity and increased blood viscosity in Holstein cattle. In contrast, Tibetan cattle exhibited lower blood viscosity and faster blood flow velocity under high-altitude conditions, which constitutes a key physiological mechanism underlying their high-altitude adaptation. Notably, no significant difference in red blood cell aggregation index was detected across the groups, implying that alterations in oxygen concentration do not affect red blood cell aggregability in cattle (Fig. 3).

Figure 3. The differences in hemorheological indicators among the low-altitude Holstein cattle group(L), the high-altitude Xizang cattle group（H）, and the high-altitude Holstein cattle group（LTH）.（A）Red blood cell number,（B）hemoglobin,（C）hematocrit,（D）mean hematocrit volume,（E）average erythrocyte hemoglobin content,（F）average erythrocyte hemoglobin content and red blood cell volume distribution width（G）are presented as indicators of blood physiology. Breeds are indicated by different symbols, while environments are indicated by different colors. Error bars show one standard error based on the binomial distribution. P values determined by a T test of independence.（A）fibrinogen,（B）plasma viscosity,（C）casson viscosity,（D）erythrocyte aggregation index,（E）whole blood undercutting relative index,（F）whole blood tangent relative index and whole blood high incision relative index（G）are presented as indicators of hemorheology phenotype. Breeds are indicated by different symbols, while environments are indicated by different colors. Error bars show one standard error based on the binomial distribution. P values determined by a T test of independence.

**Identification of differentially expressed genes in short-term or long-term hypoxia adaptation in cattle**

Differentially expressed genes (DEGs) linked to short-term acclimatization (STA) were identified by comparing native lowland Holstein cattle with highland-acclimatized Holstein cattle—as these groups share an identical genetic background but inhabit distinct altitudes, the observed DEGs are driven by short-term hypoxic environmental stimuli; conversely, DEGs related to long-term adaptation (LTA) were defined by comparing highland-acclimatized Holstein cattle with native highland Tibetan cattle, reflecting gene expression alterations from long-term high-altitude exposure and inherent genetic adaptation. Under the threshold of false discovery rate (FDR) < 0.05 and P < 0.01, 1000, 324, and 2075 DEGs were detected in the heart, lung, and liver (respectively) for STA, while 1109, 247, and 729 DEGs were identified in the same tissues for LTA. Notably, the lung exhibited fewer DEGs in both acclimatization and

adaptation compared to the heart and liver, highlighting distinct organ-specific hypoxia sensitivity (with the lung showing relatively lower responsiveness); interestingly, the liver harbored fewer DEGs in LTA than in STA (contrasting the heart's slight increase in LTA-associated DEGs), indicating that the liver may have established a stable transcriptional regulatory state during long-term hypoxic adaptation, reducing the need for extensive gene expression remodeling (Fig. 4).

Figure 4. The volcano plot of significantly differential expressed genes. The red dots represented up-regulated genes and blue dots represented down-regulated genes, respectively; Orange module represented short-term high-altitude acclimatization (lowland Holstein cattle VS highland Holstein cattle) and blue module represented long-term high-altitude adaptation (highland Holstein cattle VS highland Tibetan cattle).

# Key transcription genes during hypoxia stress identified by WGCNA

Weighted gene co-expression network analysis (WGCNA) was performed to characterize tissue-specific gene networks associated with short-term hypoxic acclimatization and long-term genetic adaptation in cattle. To construct a robust scale-free network, the soft-thresholding power β = 7 was chosen, at which the scale-free topology fit index reached > 0.6 and relatively high connectivity was maintained (Fig. 5A-B). The characteristic values of different modules were significantly correlated with oxygen stimulation, indicating that the genes within these modules may regulate the adaptation of cattle to high-altitude hypoxia through a synergistic effect(Fig. 5C). Specifically, in heart tissue, modules MElightblue3 and MEskyblue exhibited positive correlations with long-term adapted Tibetan cattle, while showing negative correlations with short-term acclimatized Holstein cattle (Fig. 1D). The distinct divergence suggests that the MElightblue3 and MEskyblue module likely mediates taxon-specific adaptive mechanisms underpinning the long-term high-altitude adaptation of native Tibetan cattle, with potential involvement of genes that regulate cellular homeostasis in hypoxic environments. Similar phenomena also occur in the liver, MEdarkturquoise modules showed the strongest positive correlation with Tibetan cattle(correlation coefficient 0.91), indicating a core role in long-term adaptation (Fig. 5E). In lung tissue, multiple modules (MEorange, MEskyblue1, MEcyan) were positively correlated with H and negatively correlated with LTH. Furthermore, the MEwhite module was prominently positively correlated with LTH, highlighting its central role in acute pulmonary hypoxic response (Fig. 5F). Collectively, these results uncovered conserved co-expression networks in heart and lung for long-term hypoxia adaptation, alongside tissue-specific modules for short-term acclimatization, providing a foundation for subsequent hub gene identification and functional validation.

# Functional enrichment of DEGs in biological pathways and gene ontologies in multiple oxygen-sensitive organs

To prioritize candidate biological pathways and processes involved in STA and LTA, we conducted gene set enrichment analysis (GSEA) on genes annotated to the DEGs. We observed that DEGs associated

with STA were significantly enriched in metabolic processes across both heart and liver tissues. Specifically, metabolic processes included organic substance metabolism, macromolecule metabolism, and nitrogen compound metabolism—functional categories that are central to regulating energy supply and cellular homeostasis under hypoxic stress (Fig. 6A-B). Notably, while this metabolic enrichment pattern was conserved between the two organs, the magnitude of enrichment was more pronounced in the heart: ~1,137 heart-derived STA DEGs mapped to metabolic processes (enrichment score = 11.3%), compared to ~ 890 liver-derived STA DEGs (enrichment score = 9.1%). This observation collectively suggests that the heart tissue prioritizes metabolic reprogramming as its primary adaptive strategy to cope with hypoxic stress—an evolutionarily favorable tactic, given the heart's irreplaceable role in maintaining systemic oxygen delivery and its high energy demand even under oxygen limitation. Compared to STA, the LTA DEGs categories were enriched in immune system process and hemopoiesis pathways in liver, indicating the multiple roles of liver tissue in environmental adaptation (Fig. 6C). This finding is in line with the transcriptomic study of yaks at high altitudes, in which the expression levels of calcium channels decrease whereas innate immunity becomes more active[16.17]. In addition, the lung organ exhibits a different pattern from the other two organs, especially in the pattern of long-term adaptation, LTA DEGs in lung were enriched in cell circulation, including nucleic acid metabolic process, RNA metabolic process and nucleobase-containing compound metabolic process, suggesting that cell circulation play a major role in LTA. This result aligns with observations from the patterns of pulmonary arterial hypertension, caused by excessive proliferation of lung cells[18].

# Validation of key pathway genes expression for hypoxia stimulation

The G protein subunit beta 1 (GNB1) gene encodes the β subunit of heterotrimeric G proteins, which are composed of α, β and γ subunits. G proteins modulate multiple blood pressure regulatory systems including the nervous system, renin-angiotensin system and endothelin system by mediating downstream receptor-coupled signaling[19.20]. WGCNA results revealed that GNB1 was localized in the significant modules across all three cattle groups. KEGG pathway enrichment analysis further identified GNB1 as a component of the PI3K-AKT signaling pathway (Fig. 7A), which modulates angiogenesis and thereby contributes to high-altitude hypoxic adaptation in cattle. To validate the protein-level expression pattern of GNB1—a key hypoxia-responsive gene and core regulatory factor of the oxygen-sensing pathway—immunohistochemical (IHC) staining was performed to detect GNB1 expression in the heart and lung tissues of cattle from the three experimental groups. The results demonstrated significant intergroup differences in both the localization and expression levels of GNB1-positive signals. In heart tissue (Fig. 7B-D), GNB1-positive signals were localized in the cytoplasm and nucleus of cardiomyocytes. Group L exhibited weak GNB1 expression with sparse positive granules. Following short-term high-altitude acclimatization, Group LTH showed a significant enhancement in GNB1 expression, which was 3.13-fold higher than that in Group L ($P < 0.01$). Group H displayed moderate GNB1 expression, which was significantly lower than Group LTH ($P < 0.05$) but significantly higher than Group L. In lung tissue, GNB1-positive signals were predominantly distributed in alveolar epithelial cells, bronchial epithelial cells

and vascular endothelial cells (Fig. 7E-G), with an expression pattern consistent with that in heart tissue. Group L had the weakest GNB1 expression. Group LTH showed a dramatic upregulation of GNB1 expression, which was 4.29-fold higher than that in Group L ($P < 0.001$). Group H exhibited moderate GNB1 expression, which was significantly reduced compared with Group LTH ($P < 0.01$) but significantly higher than Group L ($P < 0.05$). Notably, GNB1-positive cells in Group H were concentrated at the alveolar-capillary interface, whereas the positive signals in Group LTH showed a diffuse distribution pattern.

**SLAMF7 enhances antimicrobial immunity as a key mediator of high-altitude adaptation in cattle**

To further clarify the immune-related mechanisms underlying short- and long-term high-altitude adaptation, we performed expression validation and functional characterization of the candidate gene signaling lymphocyte activation molecule family 7 (SLAMF7). Western blot analysis demonstrated that SLAMF7 protein expression was significantly elevated in the liver tissue of native highland Tibetan cattle (H group) compared to both lowland Holstein cattle (L group) and highland-acclimatized Holstein cattle (LTH group), with the lowest expression levels detected in the LTH group (Fig. 8A). Subsequently, the recombinant SLAMF7 protein was successfully expressed in BL21 Escherichia coli strain and purified to homogeneity, yielding a band of approximately 33.65 kDa that matched the predicted molecular weight (Fig. 8B). Functional assays via the agar diffusion method revealed that recombinant SLAMF7 exhibited antibacterial activity against both Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative), as evidenced by clear inhibition zones (Fig. 8C). Collectively, these findings suggest that SLAMF7 serves as a key functional gene mediating long-term high-altitude adaptation in cattle. The upregulated expression and direct antimicrobial activity collectively enhance immune defense and antibacterial capacity in highland environments, thereby representing a critical adaptive strategy for survival under hypoxic stress.

Figure 8 Expression validation and functional characterization of SLAMF7. (A) Western blot analysis of SLAMF7 protein expression in liver tissues from three cattle groups. (B) SDS-PAGE analysis of prokaryotic expression and purification of recombinant SLAMF7 protein. Lane M: protein molecular weight marker; Lanes 11: purified recombinant SLAMF7 protein (~ 35 kDa, consistent with the predicted molecular weight (C) Antimicrobial activity assay of recombinant SLAMF7 protein against Staphylococcus aureus (left) and Escherichia coli (right) using the agar diffusion method. The positive control (ampicillin) and negative control (PBS) were set as references.

# Discussion

In this study we use a unique experimental design to delineate physiological and molecular adaptations attributable to short-term high-altitude exposure and those resulting from long-term high-altitude exposure. The hematological system serves as the foundation for oxygen transport and maintenance of internal environmental homeostasis in organisms, and alterations in this system can directly reflect the adaptive responses of animals to hypoxic environments. The results of this study demonstrated that after lowland Holstein cattle were translocated to high-altitude regions, their red blood cell count (RBC)

and hemoglobin concentration (HGB) were elevated, while hematocrit (HCT) and mean corpuscular volume (MCV) were reduced. These changes indicated that Holstein cattle can rapidly modulate hematological parameters to enhance oxygen-carrying capacity upon high-altitude exposure; to a certain extent, they also alleviate circulatory pressure caused by increased blood viscosity by reducing red blood cell volume. This represents a typical short-term compensatory response to hypoxic exposure, with strong phenotypic plasticity of hematological traits. In contrast, native highland Tibetan cattle exhibited lower RBC and HGB levels compared with highland-acclimatized Holstein cattle, and adopted a distinct hematological adaptation strategy that differed from the short-term stress response of Holstein cattle. This adaptive characteristic is consistent with that of other high-altitude-adapted species, including Tibetan human populations and Tibetan antelopes[21-23]. The core of this long-term adaptive strategy is not the enhancement of hematopoietic function, but the achievement of a relatively stable oxygen transport state through improved oxygen utilization efficiency, optimized oxygen binding and release processes, and reduced circulatory system burden, all shaped by long-term natural selection. Therefore, Tibetan cattle do not require an increased number of red blood cells to cope with hypoxia, and have evolved a long-term adaptive strategy that maintains effective oxygen supply at a lower hematological cost[24-27]. Hemorheological results further supported this conclusion. Highland-acclimatized Holstein cattle showed elevated fibrinogen (FIB), plasma viscosity (PV) and Casson viscosity (CV) levels, indicating that short-term hypoxic exposure induces significant alterations in hemorheological properties in addition to enhanced hematopoiesis. This hemorheological remodeling can strengthen the stress response capacity of the organism in the short term, but sustained alterations may increase circulatory resistance and the risk of cardiovascular dysfunction. In comparison, Tibetan cattle maintained lower blood viscosity under high-altitude conditions, a hemorheological trait formed during long-term adaptation that facilitates blood flow and tissue perfusion. No significant difference in red blood cell aggregation index was observed among the three cattle groups, suggesting that cattle do not achieve hematological adaptation to high-altitude hypoxia by regulating red blood cell aggregability. This finding is inconsistent with the results of some studies on yaks, indicating that the hematological adaptive pathways to hypoxia exhibit certain species specificity among different ruminants[2]. From a hematological perspective, the identified transition process from short-term phenotypic plasticity to long-term genetic adaptation in cattle under high-altitude hypoxic conditions provides a hematological basis for understanding the divergent hypoxic responses among different cattle breeds.

On molecular level, our findings revealed distinct tissue-specific transcriptional regulation in the heart, liver, and lung during hypoxic adaptation, which arises from the functional specializations of these organs in systemic physiology. As the primary organ responsible for systemic blood circulation and oxygen delivery to peripheral tissues and organs, the heart exhibits the highest sensitivity to hypoxic stimuli. During the short-term hypoxic acclimation of Holstein cattle, differentially expressed genes (DEGs) in the heart were primarily enriched in pathways related to organic substance metabolism and macromolecule metabolism. The greater number of DEGs identified during long-term adaptation indicated that the heart undergoes prominent metabolic reprogramming under sustained hypoxia. Given the heart’s inherent high energy expenditure and constant workload, this continuous transcriptional

reprogramming constitutes a fundamental mechanism for maintaining circulatory efficiency under hypoxic conditions. The liver exhibited a hypoxic response pattern distinct from that of the heart. A larger number of DEGs were detected in hepatocytes during short-term hypoxic acclimation, whereas fewer DEGs were identified during long-term adaptation. This observation suggested that hepatocytes mount a rapid stress response through extensive transcriptional changes in the early stage of hypoxia exposure, and gradually establish a stable regulatory mode during long-term high-altitude adaptation. The pattern observed in the present study, together with closely consistent findings reported in analogous investigations on Escherichia coli, guppies, and chickens[28-30]. Notably, DEGs associated with long-term hepatic adaptation were predominantly enriched in immune and hematopoietic pathways. This finding is consistent with previous studies on plateau-adapted animals such as yaks, which highlighted the involvement of immune regulation in high-altitude adaptation, indicating that immune homeostasis may represent a conserved regulatory module in hypoxic adaptation. Far fewer DEGs were detected in the lung compared with the heart and liver, with only 324 DEGs identified during short-term acclimation and 247 during long-term adaptation, demonstrating a milder transcriptional response of the lung to hypoxia. DEGs linked to long-term pulmonary adaptation were mainly enriched in nucleic acid and RNA metabolic pathways, suggesting that pulmonary adaptation to hypoxic environments relies more on cellular renewal, transcriptional regulation, and modifications to basic metabolic processes, rather than extensive transcriptional remodeling. This adaptive pattern is analogous to the hypoxia-induced pulmonary cellular proliferation and tissue remodeling processes reported in previous studies. In addition, the expression pattern of GNB1, a core candidate gene, exhibited a regulatory mode specific to oxygen-sensitive organs. Its high expression in cardiomyocytes and at the alveolar-capillary interface implied that GNB1 may be involved in angiogenesis and local oxygen exchange under hypoxic conditions. As a gene associated with the PI3K-AKT signaling pathway, GNB1 acts as a key molecular hub in bovine hypoxic adaptation[19.20].

The immune system acts as a key regulatory hub for Tibetan cattle in high-altitude adaptation, maintaining immune surveillance, inflammatory homeostasis, and energy metabolism under hypoxia, low temperature, and intense ultraviolet stress. As an activating receptor widely expressed in NK cells, CD8+ T cells, monocytes, and dendritic cells, SLAMF7 mediates NK cell adhesion, promotes NK and innate immune activation, sustains adaptive and long-term immune responses, and suppresses pro-inflammatory cytokine secretion in activated monocytes through the Toll-like receptor pathway[31.32]. Functioning as a pivotal immune regulator, SLAMF7 modulates NK cell adhesion and activation, coordinates innate and adaptive immunity, and inhibits excessive pro-inflammatory cytokine production[33.34]. Our results revealed significantly upregulated expression of SLAMF7 in the liver of Tibetan cattle native to high-altitude regions, and we further verified the direct antibacterial activity of recombinant SLAMF7 protein against both Gram-positive and Gram-negative bacteria. These observations imply that SLAMF7 may strengthen the immune surveillance and antibacterial capability of Tibetan cattle in harsh highland environments, thereby acting as a crucial adaptive strategy supporting their survival under long-term hypoxic stress. Collectively, our results suggest that bovine hypoxic

adaptation is not mediated by a single organ, but by a tissue-specific regulatory network centered on core oxygen-sensitive organs such as the heart, with coordinated participation of the liver and lung.

In summary, bovine adaptation to high-altitude hypoxic environments is not a unidimensional change, but a dynamic process transitioning from short-term phenotypic plasticity to long-term genetic adaptation. The former involves rapid regulatory responses under stress, while the latter encompasses stable phenotypes and molecular networks shaped by long-term natural selection. Together, these two processes constitute the comprehensive mechanism underlying bovine hypoxic adaptation in high-altitude environments.

# Declarations

### Ethics approval and consent to participate

All animal experiments were approved by the Animal Ethics Committee of Shandong Academy of Agricultural Sciences. Written informed consent was obtained from the owners of the commercial farms where the cattle were raised. All procedures complied with national and institutional guidelines for the care and use of experimental animals.

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### Data Availability Statement

The datasets generated and/or analysed during the current study are available in the Genome Sequence Archive (GSA) repository, with the accession number CRA068915 (https://ngdc.cncb.ac.cn/gsa/browse/CRA068915).

### Ethics declarations

Ethics, Consent to Participate, and Consent to Publish declarations: not applicable.

### Competing interests

The authors declare no competing interests.

### Acknowledgments

Not applicable.

### Animal Ethics and Consent to Participate declarations

All animal experiments and sample collection procedures in this study were reviewed and approved by the Animal Welfare Committee of Shandong Academy of Agricultural Sciences. The care, feeding, management, and slaughter sampling of experimental cattle were conducted in strict accordance with the national and institutional guidelines for the ethical use and welfare of laboratory animals.

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# Figures

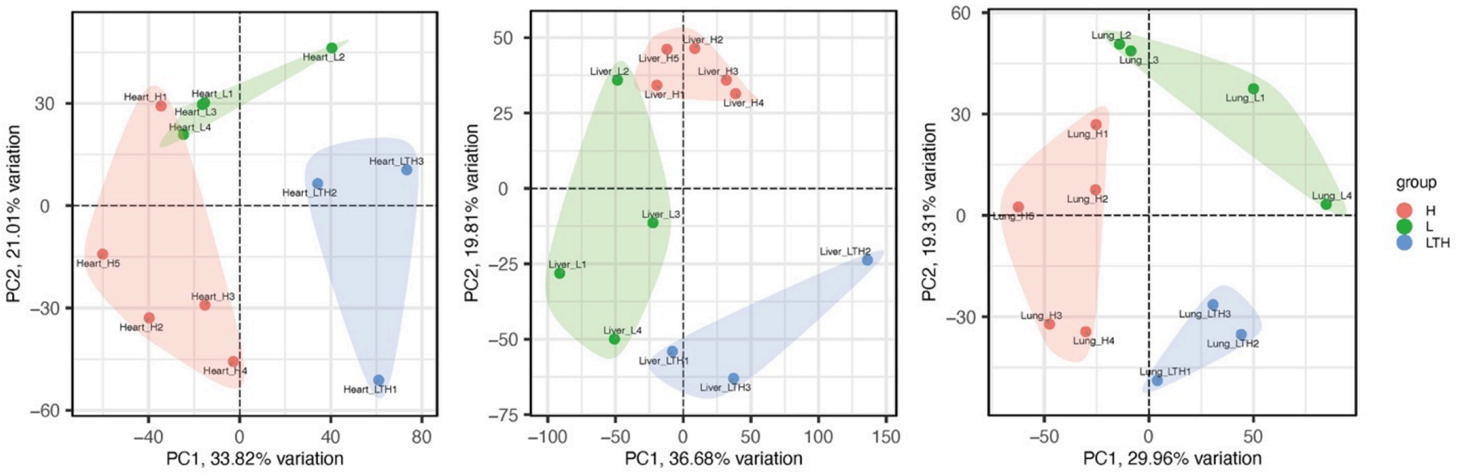



**Figure 1**

PCA score plots illustrating the separation of H (red), L (green), and LTH (blue) sample groups in three tissue types: (A) Heart, (B) Liver, and (C) Lung. Each plot displays the first two principal components (PC1, PC2) with the percentage of total variance explained labeled for each axis. Convex hulls enclose samples from the same group, demonstrating distinct clustering patterns between groups in each tissue —consistent with differential molecular profiles across health/experimental conditions.

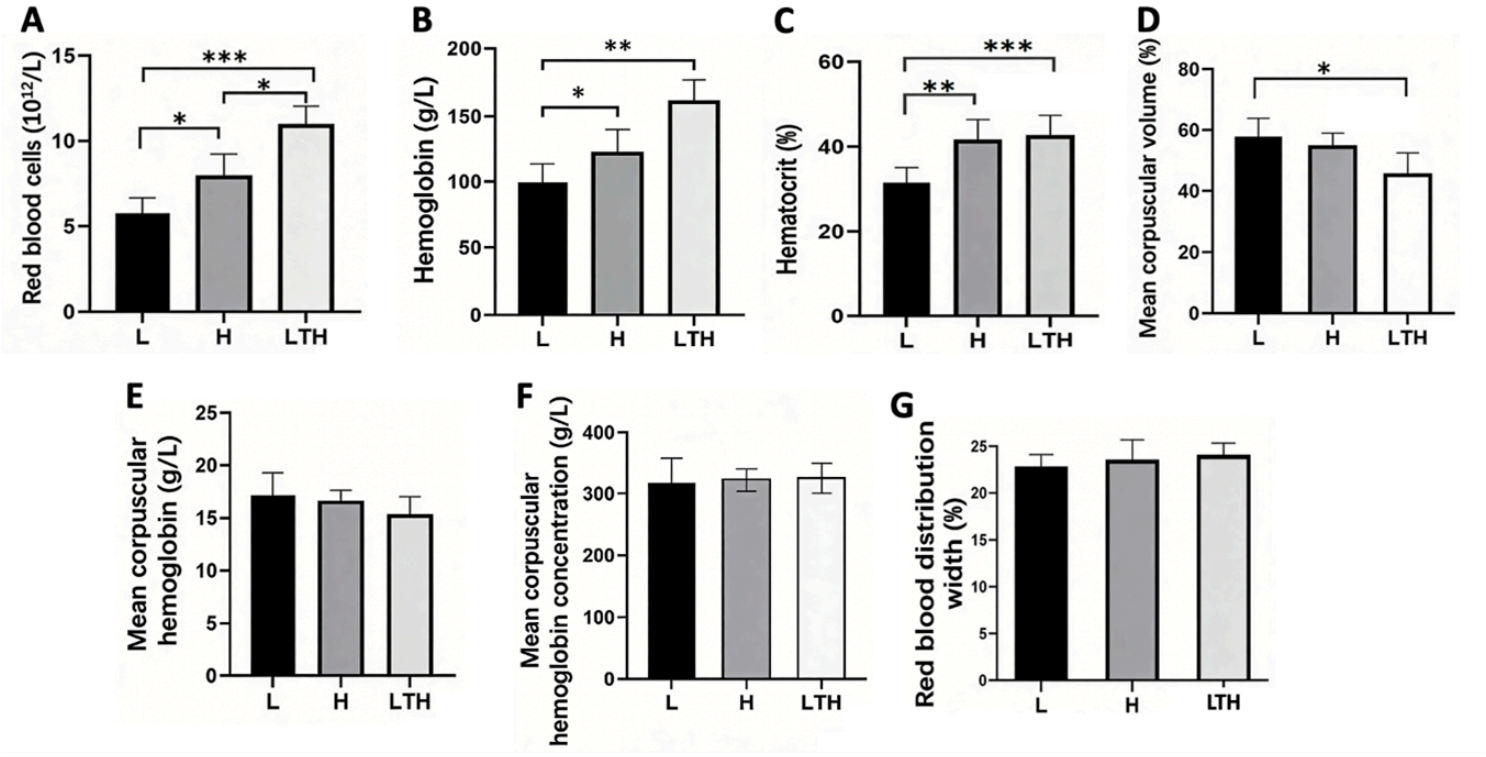



**Figure 2**

The differences in blood physiological indicators among the low-altitude Holstein cattle group（L）, the high-altitude Xizang cattle group（H）, and the high-altitude Holstein cattle group（LTH）.（A）Red blood cell number,（B）hemoglobin,（C）hematocrit,（D）mean hematocrit volume,（E）average erythrocyte hemoglobin content,（F）average erythrocyte hemoglobin content and red blood cell volume distribution width（G）are presented as indicators of blood physiology. Breeds are indicated by different symbols, while environments are indicated by different colors. Error bars show one standard error based on the binomial distribution. P values determined by a T test of independence.

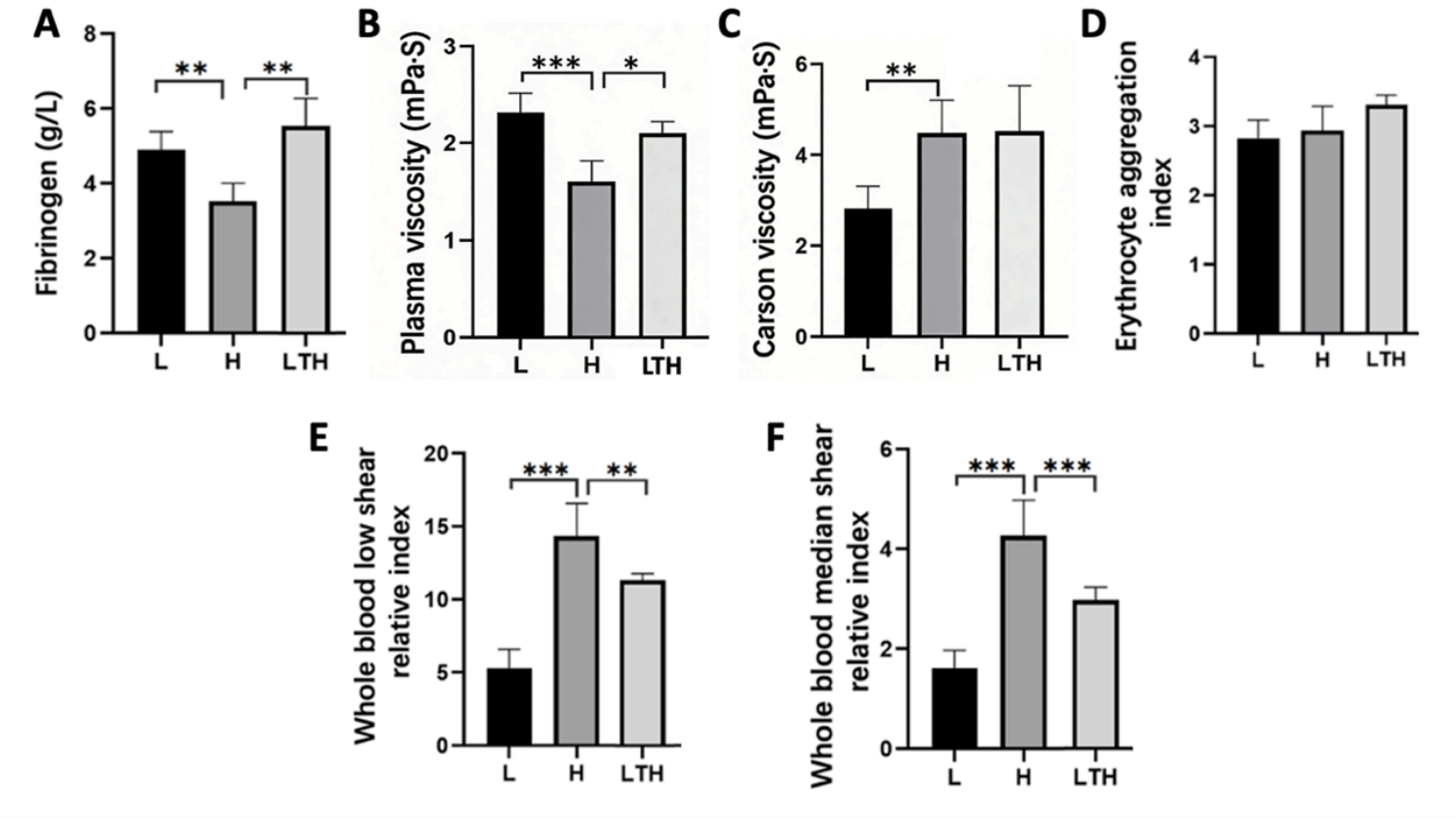



**Figure 3**

The differences in hemorheological indicators among the low-altitude Holstein cattle group（L）, the high-altitude Xizang cattle group（H）, and the high-altitude Holstein cattle group（LTH）.（A）Red blood cell number,（B）hemoglobin,（C）hematocrit,（D）mean hematocrit volume,（E）average erythrocyte hemoglobin content,（F）average erythrocyte hemoglobin content and red blood cell volume distribution width（G）are presented as indicators of blood physiology. Breeds are indicated by different symbols, while environments are indicated by different colors. Error bars show one standard error based on the binomial distribution. P values determined by a T test of independence.（A）fibrinogen,（B）plasma viscosity,（C） casson viscosity,（D）erythrocyte aggregation index,（E）whole blood undercutting relative index,（F）whole blood tangent relative index and whole blood high incision relative index（G）are presented as indicators of hemorheology phenotype. Breeds are indicated by different symbols, while environments are indicated by different colors. Error bars show one standard error based on the binomial distribution. P values determined by a T test of independence.

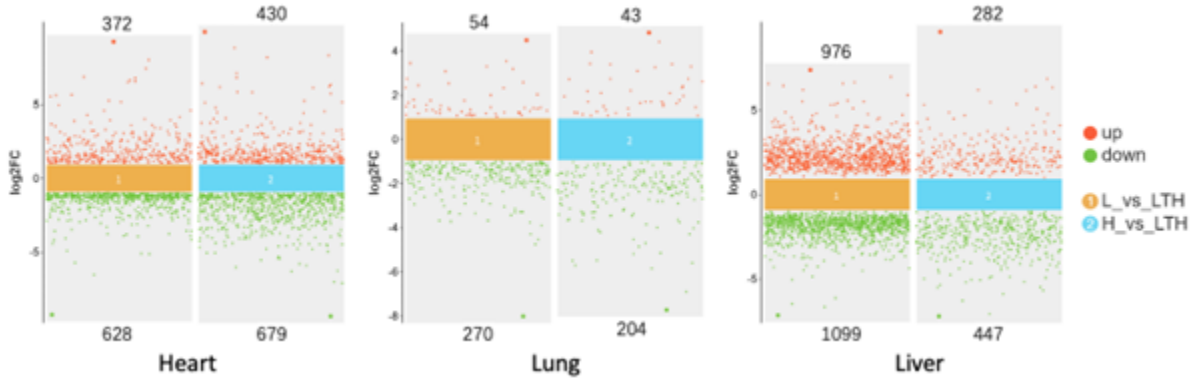



**Figure 4**

The volcano plot of significantly differential expressed genes. The red dots represented up-regulated genes and blue dots represented down-regulated genes, respectively; Orange module represented short-term high-altitude acclimatization (lowland Holstein cattle VS highland Holstein cattle) and blue module represented long-term high-altitude adaptation (highland Holstein cattle VS highland Tibetan cattle).

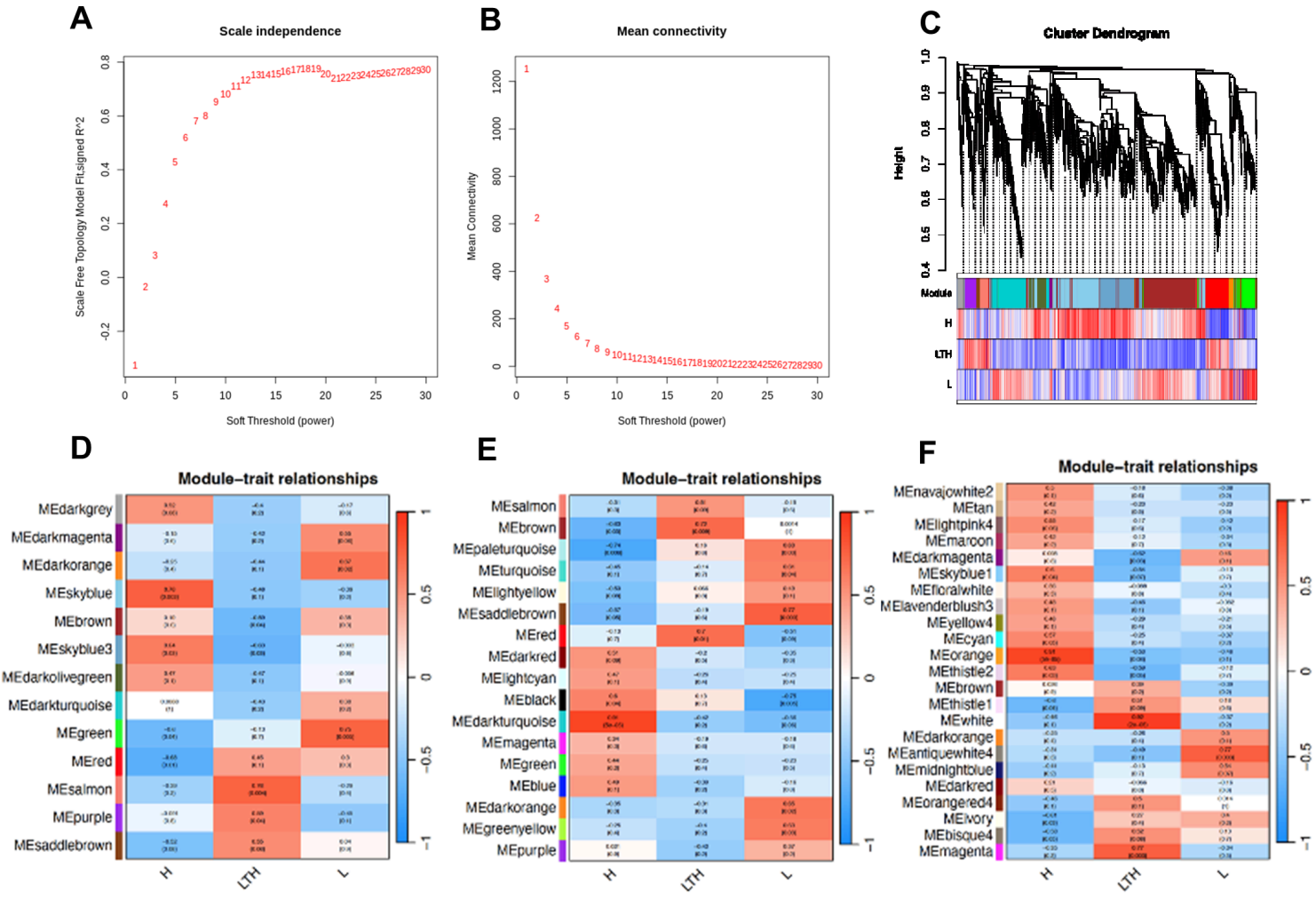



**Figure 5**

Core results of weighted gene co-expression network analysis. (A) Gene cluster dendrogram: The hierarchical clustering tree of genes (top) and the corresponding module color bar (bottom), where different colors represent distinct co-expression modules identified by dynamic tree cutting. (B) Scale independence analysis: The curve shows the scale-free topology fitting index ($R^2$) across different soft-thresholding powers (β), used to select the optimal β that satisfies the scale-free network property. (C) Mean connectivity analysis: The trend of average gene connectivity with increasing soft-thresholding powers, assisting in confirming the appropriate β parameter. (D–F) Module-trait relationship heatmaps: Three heatmaps displaying the correlation between co-expression modules and target traits. The color intensity (red = positive correlation, blue = negative correlation) represents the correlation coefficient, and values in each cell indicate the correlation coefficient (upper) and corresponding P-value (lower, in parentheses). These heatmaps were used to identify modules significantly associated with the traits of interest. (D) heart, (E) lung, (F) liver.

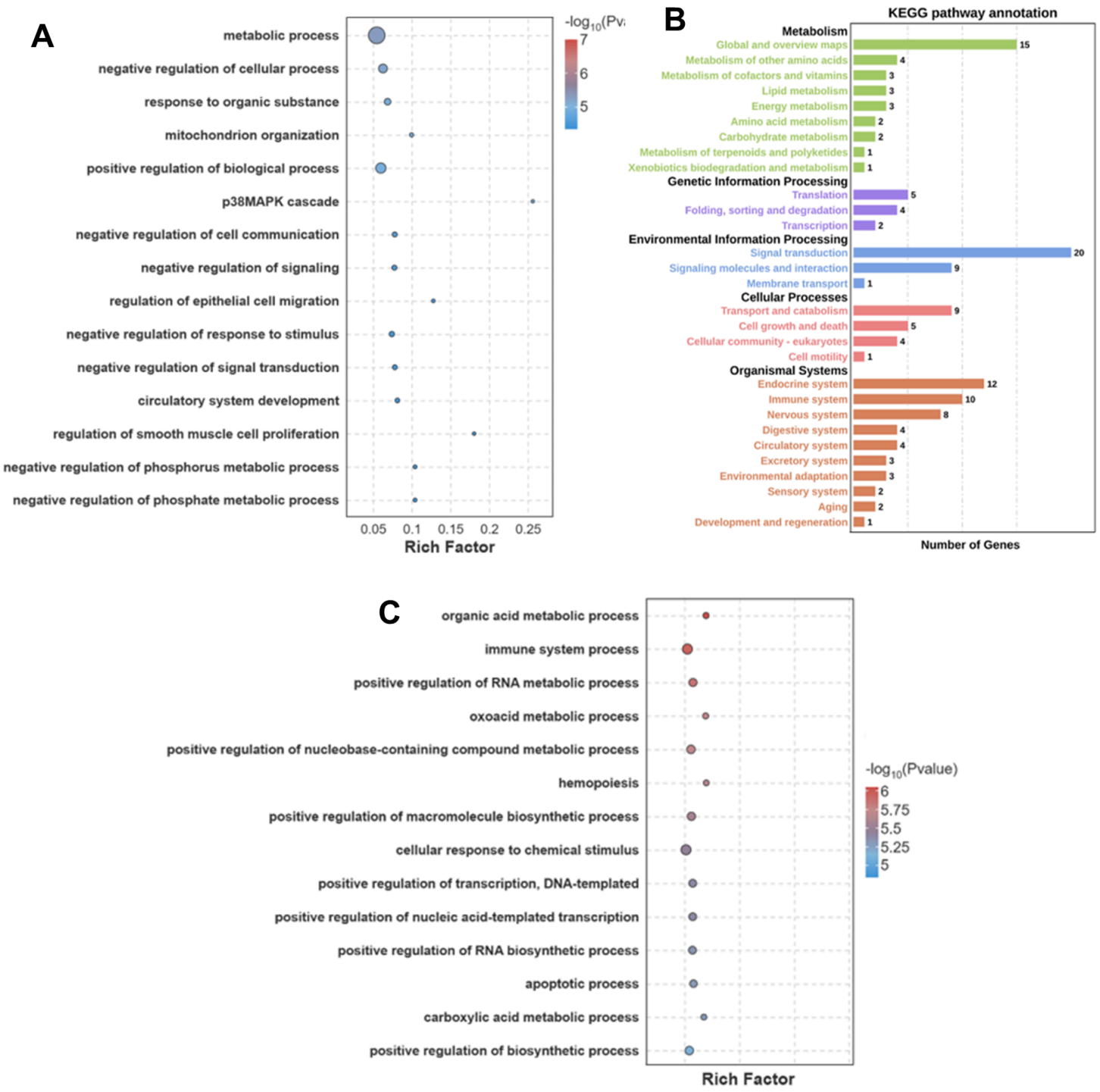



**Figure 6**

Enrichment analysis of differentially expressed genes in oxygen-sensitive organs. (A) Bubble plot of GO functional enrichment (biological process), showing significantly enriched functional entries. Rich Factor indicates the enrichment degree, and the color gradient represents −log10(P-value) (red for higher significance); (B) Bar chart of KEGG pathway enrichment, displaying significantly enriched pathways and the corresponding number of genes across five categories: Metabolism, Genetic Information Processing, Environmental Information Processing, Cellular Processes, and Organismal Systems; (C) Bubble plot of GO functional enrichment (biological process), focusing on core functional entries such as organic acid

metabolic process and immune system process, with Rich Factor and color gradient having the same meanings as in (A).

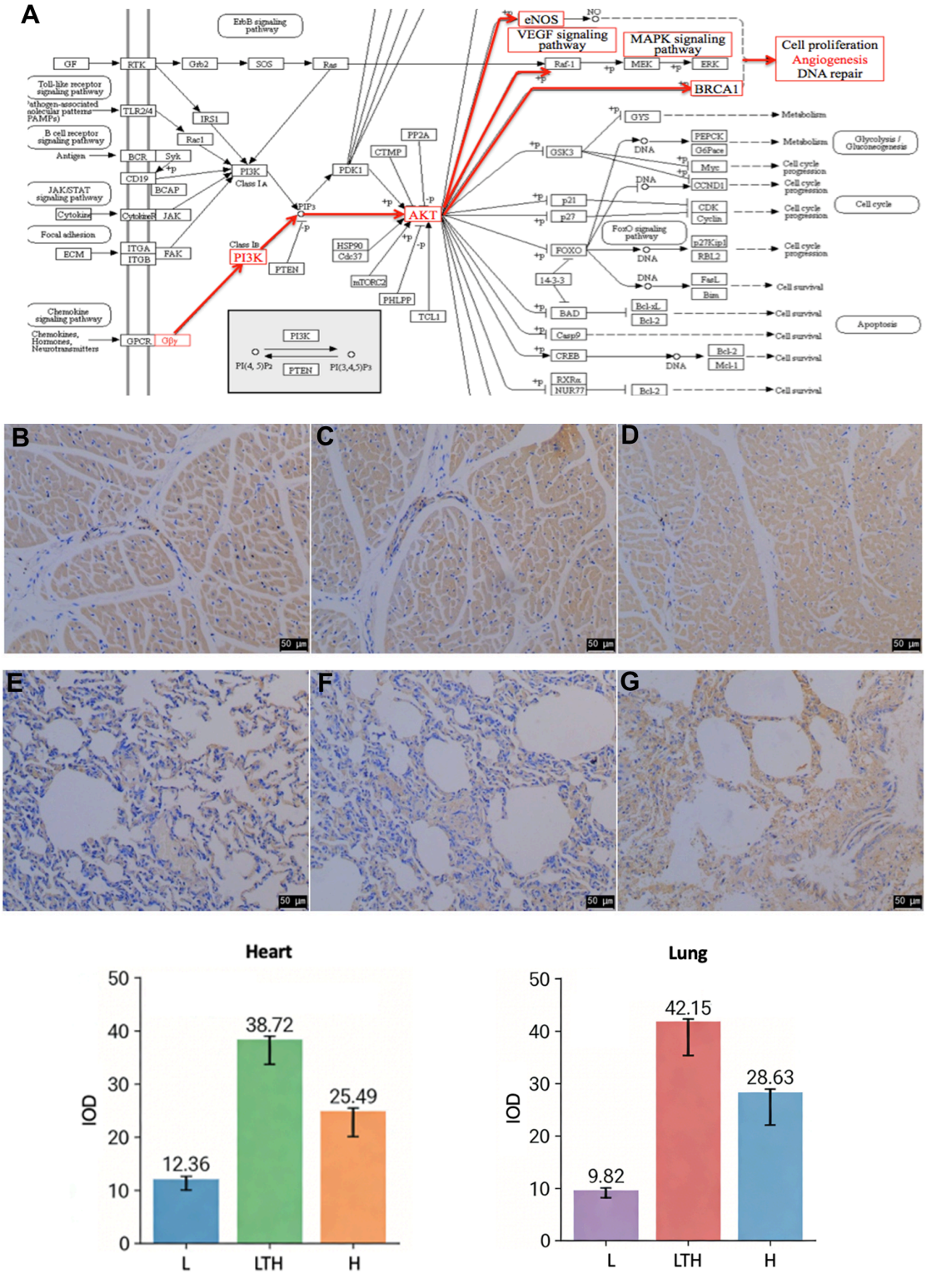



**Figure 7**

Immunohistochemical staining of GNB1 gene expression in heart and lung tissue. (A) The keg pathway of GNB1; (B) Immunohistochemical staining of heart tissue with GNB1 in lowland Holstein cattle; (C)

Immunohistochemical staining of heart tissue with GNB1 in highland Holstein cattle (D) Immunohistochemical staining of heart tissue with GNB1 in highland Tibetan cattle (E) Immunohistochemical staining of lung tissue with GNB1 in lowland Holstein cattle; (F)Immunohistochemical staining of lung tissue with GNB1 in highland Holstein cattle (G) Immunohistochemical staining of lung tissue with GNB1 in highland Tibetan cattle. Brown or tan particles are immune positive cells.

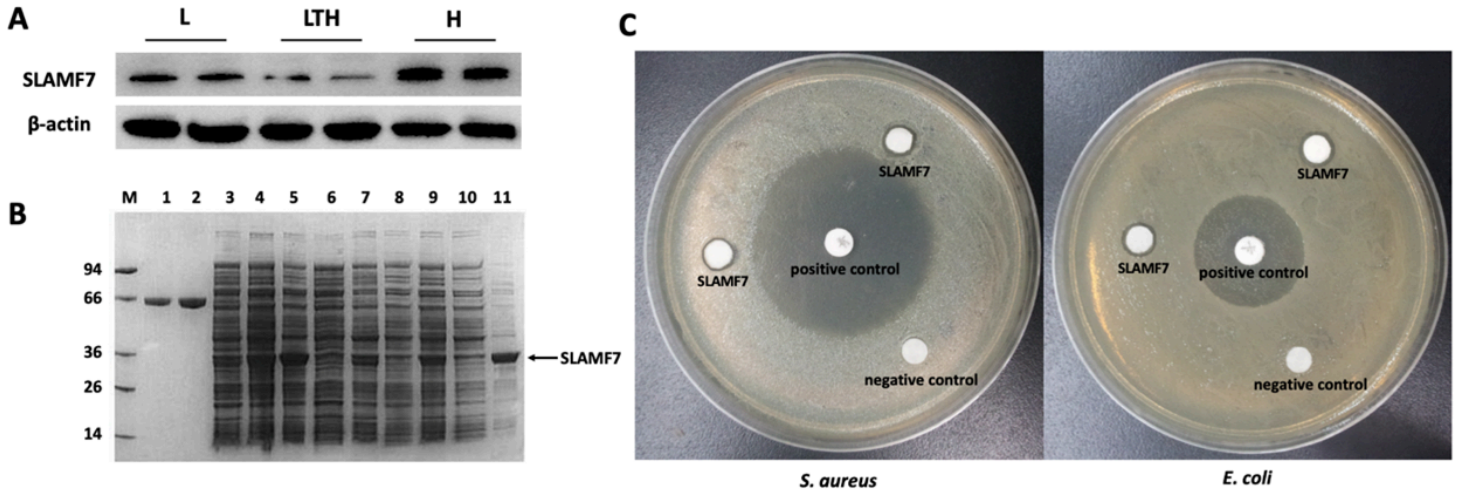



**Figure 8**

Expression validation and functional characterization of SLAMF7. (A) Western blot analysis of SLAMF7 protein expression in liver tissues from three cattle groups. (B) SDS-PAGE analysis of prokaryotic expression and purification of recombinant SLAMF7 protein. Lane M: protein molecular weight marker; Lanes 11: purified recombinant SLAMF7 protein (~35 kDa, consistent with the predicted molecular weight (C) Antimicrobial activity assay of recombinant SLAMF7 protein against Staphylococcus aureus (left) and Escherichia coli (right) using the agar diffusion method. The positive control (ampicillin) and negative control (PBS) were set as references.

## Supplementary Files

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