Journal of Animal Research: v.15 n.05, p. 165-176. October 2025

DOI: 10.30954/2277-940X.05.2025.1

The Critical Influence of lncRNA *NEAT1* on Porcine Skeletal Muscle Satellite Cells: A Molecular Insight

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Received: 27 July, 2025 **Revised:** 02 Sept., 2025 **Accepted:** 12 Sept., 2025

ABSTRACT

To investigate the biological role of the lncRNA *NEAT1* in porcine skeletal muscle development, this study employed a series of molecular and cellular approaches. First, the full-length cDNA sequence of porcine *NEAT1* was determined using RACE technology, revealing a 3284-base pair transcript. RT-qPCR was then used to analyze the spatiotemporal expression patterns of *NEAT1* in various porcine tissues and PSCs at different developmental stages, while its subcellular localization was characterized. Finally, siRNA-mediated silencing of *NEAT1* was performed to assess its effects on PSCs proliferation and differentiation. RT-qPCR analysis showed that *NEAT1* is highly expressed in porcine heart and skeletal muscle tissues. During PSCs development, *NEAT1* expression is dynamically regulated: it is significantly upregulated during the proliferation phase and markedly downregulated upon entry into the differentiation phase. Subcellular localization studies demonstrated that *NEAT1* is distributed in both the cytoplasm and nucleus of PSCs, with predominant cytoplasmic localization during proliferation; as differentiation proceeds, cytoplasmic *NEAT1* abundance decreases, resulting in distinct subcellular expression patterns between the two stages. Functional validation revealed that silencing *NEAT1* significantly reduces PSC proliferative activity, accompanied by downregulation of key proliferation markers (*PCNA*, *CCNA2*, *CCNB1*, *CCNE2*, *CCND1*, and *CDK4*; $P < 0.05$) and upregulation of the cell cycle inhibitor *CDKN1A* ($P < 0.05$). Concurrently, *NEAT1* silencing enhances PSC differentiation, as evidenced by increased expression of muscle differentiation markers (*MyoD*, *MyoG*, and *MyHC*; $P < 0.05$). In conclusion, *NEAT1* plays a critical role in porcine skeletal muscle development by promoting PSCs proliferation and inhibiting their differentiation. These findings provide a foundation for further exploration of the molecular regulatory mechanisms underlying *NEAT1* function in porcine skeletal muscle development.

HIGHLIGHTS

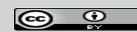
- This study is the first to systematically characterize the full-length cDNA sequence of porcine *NEAT1* (3284 bp) and clarify its dynamic spatiotemporal expression patterns.
- *NEAT1* acts as a key regulator of porcine skeletal muscle satellite cells, promoting proliferation, and inhibiting differentiation, with distinct subcellular localization patterns.

Keywords: lncRNA, *NEAT1*, skeletal muscle satellite cells, proliferation, differentiation

Pork isn't just a staple on Chinese dinner tables—it's a global powerhouse. China produces and consumes over half the world's pork, making it the cornerstone of animal protein for billions. To sustainably meet this massive demand, we must delve deeper into the molecular blueprints that govern how pigs build muscle. After all, skeletal muscle growth directly determines meat yield and

How to cite this article: An, Y., Li, Q., Ren, K., Zhou, K., Xu, H., Cheng, X., Li, C., Zhang, P. and Xu, Y. (2025). The Critical Influence of lncRNA *NEAT1* on Porcine Skeletal Muscle Satellite Cells: A Molecular Insight. *J. Anim. Res.,* **15**(05): 165-176.

Source of Support: None; **Conflict of Interest:** None



quality—yet its regulation remains a complex puzzle. For decades, scientists focused on transcription factors (protein “switches” turning genes on/off) as the master regulators of muscle development (Zhai *et al.* 2022). But recent breakthroughs reveal another layer of control: long non-coding RNAs (lncRNAs) (Zhang *et al.* 2024). These RNA molecules—once considered “genomic noise”—are now recognized as precision engineers of gene activity. Though they don’t produce proteins themselves, lncRNAs excel at fine-tuning biological processes like muscle formation. They act as molecular directors, guiding everything from how DNA is packaged to how genes are silenced or activated (Mercer *et al.* 2009; Wilusz *et al.* 2009; Chen *et al.* 2024). What makes them especially intriguing? Their uncanny specificity: different lncRNAs operate in distinct tissues or developmental stages (Chen 2016), hinting at tailor-made roles in muscle growth.

Among the lncRNAs gaining attention is *NEAT1* (Nuclear Enriched Abundant Transcript 1), a highly conserved molecule with a surprisingly versatile portfolio (Zhang *et al.* 2025). Beyond its role as a structural core of nuclear paraspeckles—dynamic nuclear compartments that regulate RNA processing—*NEAT1* influences everything from cancer progression (prostate, gastric) to autoimmune and neurodegenerative diseases (Chakravarty *et al.* 2014; Fu *et al.* 2016; Zhang *et al.* 2016; Sunwoo *et al.* 2017; Long *et al.* 2024). Critically, *NEAT1* has also carved out a niche in muscle biology: in muscle atrophy models, it is stabilized by FAM129B, driving FoxO1-mediated protein degradation (Wang *et al.* 2025). In myoblasts, it partners with the PRC2 complex to suppress myogenic genes like *MyoG* and *Myh4*, balancing proliferation and differentiation (Wang *et al.* 2019). In addition, *NEAT1*, as the structural core of nuclear plaques, indirectly reflects its extensive regulatory ability in cell fate determination by mediating nuclear retention of specific mRNAs (such as *Smurf1*) and affecting osteoblast function and bone formation (Liu *et al.* 2022). These findings highlight *NEAT1* as a regulatory hub—a molecule capable of rewiring muscle development.

Pigs are more than just livestock. As a biomedical model, their skeletal muscle physiology closely mirrors humans, making them invaluable for studying muscle diseases. Yet, the role of *NEAT1* in pig skeletal muscle development remains a black box. This knowledge gap is significant: understanding how *NEAT1* operates in pigs could bridge agricultural and medical research, offering insights to both improve meat production and inform therapies for muscle disorders. To address this, our study focuses on porcine skeletal muscle satellite cells (PSCs)—the stem cells responsible for muscle growth and regeneration. We aim to: (1) systematically identify the porcine *NEAT1* gene and characterize its full-length cDNA sequence; (2) map its spatiotemporal expression patterns and subcellular localization in skeletal muscle tissues and satellite cells across developmental stages; (3) dissect its functional role in regulating satellite cell proliferation and differentiation. By unraveling *NEAT1*’s mechanisms in pigs, we hope to clarify the complex regulatory networks governing skeletal muscle development, deepen our understanding of muscle formation, and provide a theoretical foundation for two critical goals: enhancing meat production efficiency in animal husbandry and advancing treatments for human muscle-related diseases. In doing so, this research bridges species and disciplines, turning a single lncRNA into a key that unlocks insights across agriculture and biomedicine.

MATERIALS AND METHODS

Cells and experimental materials

Three healthy 7-day-old Large White boars were obtained from the Breeding Farm of Henan Hongzhan Livestock Technology Co., Ltd. for the experiment. Six tissue types—heart, spleen, lung, kidney, dorsal muscle, and fat—were collected, immediately snap-frozen in liquid nitrogen, and subsequently stored at -80 °C. PSCs used in this study were isolated from the leg muscles of these piglets. All animal procedures were reviewed and approved by the Institutional Ethics Committee of Xinyang Normal University (Approval No.: XFEC-20223-023).

Isolation of porcine skeletal muscle satellite cells (PSCs)

PSCs were isolated from the hind leg muscles of 7-day-old Large White piglets using a modified protocol established by our research group (Xu *et al.* 2024), with the following steps. After removing fascia from the muscle tissue, the muscle was minced and digested with 2 mg/mL type II collagenase at 37 °C for 2 h. Then the digested suspension was filtered through a 100-mesh sieve, and the filtrate was centrifuged. The resulting pellet was resuspended in PBS

and sequentially filtered through 200-mesh and 400-mesh sieves. Subsequently, the pellet was centrifuged, washed with RPMI 1640 medium, and resuspended in growth medium (RPMI 1640 supplemented with 20% fetal bovine serum, chicken embryo extract, and 0.25 µg/100 mL bFGF). Finally, the cell suspension was seeded into a polylysine-coated culture dish and incubated at 37 °C in a cell culture incubator. After 2.5 h, cells were transferred to a substrate-coated dish for further culture. When cell confluency reached 60–70%, cells were washed three times with PBS and cultured in differentiation medium (DM) to induce myogenic differentiation.

Primer and siRNA design

Human NEAT1 cDNA (NR_003513.3) and mouse NEAT1 cDNA (NR_028272.1) sequences were retrieved from the GenBank database. Highly homologous segments between these human and mouse sequences were used as queries for BLAST searches against porcine expressed sequence tags (ESTs) to identify all porcine NEAT1-related sequences. These sequences were filtered for high homology and spliced to generate longer porcine *NEAT1* cDNA fragments. Using Primer 5.0, conserved region amplification primers and quantitative analysis primers were designed based on the spliced porcine sequence, with reference to human and mouse *NEAT1* sequences (Table 1). Additionally, 5' rapid amplification of cDNA ends (RACE) primers (5' GSP) and 3' RACE primers (3' GSP) were designed using the conserved region sequences obtained from sequencing (Table 1) to amplify the terminal cDNA sequences of the porcine *NEAT1* gene. Three pairs of specific siRNAs and one pair of negative control siRNAs were designed for the conserved region of the spliced porcine NEAT1 gene, following established siRNA design principles (Samuel-Abraham *et al.* 2010). All primers and siRNAs were synthesized by General Bio (Anhui) Co., Ltd. (Table 1).

RNA extraction, reverse transcription and RACE amplification

Total RNA was extracted from porcine tissues and PSCs using the Trizol method. RNA purity was verified by 2% agarose gel electrophoresis, and concentration was measured using an ultramicro spectrophotometer. The extracted total RNA was reverse-transcribed into cDNA following the manufacturer's instructions for the reverse transcription kit; the resulting cDNA was stored at -20 °C for subsequent use. Additionally, 5' and 3' RACE cDNA libraries of porcine dorsal muscle were constructed using the SMARTer RACE kit (TakaRa) according to the provided protocol.

In order to obtain the transcript information and full-length sequence of porcine *NEAT1*, we used the SMARTer RACE cDNA Amplification Kit (TakaRa) for RACE PCR amplification, and spliced the full-length transcript of pig NEAT1 by amplifying the conserved region sequences of pigs, humans, and mice. Design 5' and 3' RACE specific primers GSP 5-1 and GSP 3-1 based on the published porcine *NEAT1* (GenBank number: MN784916.1) sequence by NCBI (Table 1). Reaction system: 15.5 µL of H_2O, 2 × SeqAmpTM Buffer 25.0 µL, SeqAmp DNA Polymerase 1.0 µL, 5' or 3' RACE-Ready cDNA 2.5 µL, 10 × UPM 5.0 µL, Reaction conditions: 94° for 30 s, 72° for 3 min, 1 cycle; 94° for 30 s, 70° for 30 s, 72° for 3 min, 5 cycles; 94° for 30 s, 68° for 30 s, 72° for 3 min, 25 cycles. After connecting the PCR product to the pMD19-T vector according to the instructions of the kit, positive colonies were selected and sent to Universal Biotech (Anhui) Co., Ltd. for sequencing after transformation screening.

Real time fluorescence quantitative PCR (RT-qPCR)

RT-qPCR was used to analyze gene expression, employing the BioRad C1000 Real-Time PCR System and SYBR Green qPCR Master Mix kit. The 20 µL reaction system contained 50 ng template cDNA, 0.4 µM of each primer, 10 µL SYBR Green I mixture, and deionized water to reach the total volume. Reactions were prepared on ice in a dark environment, with three technical replicates and three biological replicates. The PCR program was set as follows: pre-denaturation at 95 °C for 1 min; 30 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 30 s; final extension at 72 °C for 5 min. The dissolution curve was automatically generated by the CFX-96 (Bio-Rad) detection system. Housekeeping genes 18S and GAPDH were used for normalization. Relative mRNA expression levels were calculated using the $2^{(-\Delta\Delta Ct)}$ method, and statistical analyses were performed using SPSS 18.0 software (Xu *et al.* 2025).

Table 1: The PCR Primers used for full-length cDNA cloning and RT-qPCR analysis of porcine *NEAT1*

Gene or Primer name	Primer	Primer sequence (5'→3')
P-NEAT1	Forward primer	TTCTCCAATGTCCCGTGTCC
	Reverse primer	AACACATCTGCTGACGACCC
B-NEAT1	Forward primer	GTGTGGTGGCAACTGACCTT
	Reverse primer	TGTACCCTCCCAGACCACAG
5' GSP	5' RACE primer	CCGAACAGGAGAGGTGCCTGCTGGC
3' GSP	3' RACE primer	CCAATGTCCCGTGTCCAGGCCTTGGC
UMP	primer	CTCACTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT
siNEAT1-1	Forward primer	CCAGGCCUGGGUGUCGCUGTT
	Reverse primer	CAGCGACACCCAGGCCUGGTT
siNEAT1-2	Forward primer	UGGCCAGUGUGAGUCCUAGTT
	Reverse primer	CUAGGACUCACACUGGCCATT
siNEAT1-3	Forward primer	AUUGUUUUGCUUUGCAAGATT
	Reverse primer	UCUUGCAAAGCAAAACAAUTT
18S RNA	Forward primer	CCCACGGAATCGAGAAAGAG
	Reverse primer	TTGACGGAAGGGCACCA
GAPDH	Forward primer	CGTCCCTGAGACACGATGGT
	Reverse primer	GCCTTGACTGTGCCGTGGAAC
PCNA	Forward primer	TCGTTGTGATTCCACCACCAT
	Reverse primer	GGCCTCGTTGATGAGGTCTT
*CCNA*2	Forward primer	ATGAGACCCTGCATTTGGCT
	Reverse primer	AGTGCCCACAAGCTGAAGTT
*CCN*B1	Forward primer	AGATCGCAGCAGGAGCTTTT
	Reverse primer	CCTCGATTCACCACGACGAT
*CCN*E2	Forward primer	GAAGAGCACAGAGTAAGGGGTC
	Reverse primer	GGCTCCCTCTTCTCATCCCAG
*CCND*1	Forward primer	GGCGTGTGACCTTCACCTTA
	Reverse primer	AGCCTCTTTCAGCATCGGAC
*CD*K4	Forward primer	GGCCCTCAAGAGCGTAAGAG
	Reverse primer	GTCTCTCGATCAGTTCGGGC
*Myo*D	Forward primer	CTACAGCGGTGACTCAGACG
	Reverse primer	AATAGGTGCCGTCGTAGCAG
*Myo*G	Forward primer	GAGCTGTATGAGACATCCCCC
	Reverse primer	GTGGACGGGCAGGTAGTTTT
MyHC	Forward primer	GTTCAGAGAAAGGCATCCCAAA
	Reverse primer	GAGAGTGACCGACACCACAAGTG

Subcellular localization

PSCs at 70–80% confluency during proliferation and differentiation stages were collected. Nuclear and cytoplasmic fractions were isolated using cytoplasmic separation technology, followed by RNA extraction and reverse transcription. Quantitative analysis was performed initially to assess *NEAT1* expression in both fractions,

with *18S* and *GAPDH* as cytoplasmic reference genes. RT-qPCR was then used to quantify *NEAT1* expression in the nucleus and cytoplasm, confirming its subcellular localization.

Cell transfection

Transfections were performed following the instructions for LipoRNAi™ Transfection Reagent (Beyotime, Shanghai Biyun Tian Biotechnology Co., Ltd.). Briefly, cells were seeded in 6-well plates with 2 mL antibiotic-free medium per well. Transfection was initiated when cell density reached 40–60%. siRNA was added to a final concentration of 80 nM, along with 12 μL LipoRNAi™. The mixture was gently vortexed and incubated at room temperature for 15 minutes to form siRNA-transfection reagent complexes. Complexes were added to each well, mixed gently, and incubated for 6–8 h before replacing with fresh medium. Cells were harvested 48 h post-transfection for RNA extraction and reverse transcription.

EdU detection of cell proliferation activity

Cell proliferation efficiency was measured using the BeyoClick™ EdU-488 Cell Proliferation Detection Kit (Beyotime). EdU (10 mM) was diluted in FBS to prepare a 1× working solution, and cells were incubated with this solution for 2 h. Cells were then fixed in 4% paraformaldehyde for 30 min, neutralized with 2 mg/mL glycine, and permeabilized with 0.5% Triton X-100. Click reaction solution was added, and cells were incubated at room temperature for 30 min, followed by nuclear staining with DAPI for another 30 min. An inverted fluorescence microscope was used to capture three randomly selected fields, and the number of EdU-positive cells was counted.

Cell immunofluorescence

Immunofluorescence staining for MyHC was performed on differentiated porcine skeletal muscle satellite cells (PSCs) as follows. Firstly, Cells were fixed with 4% paraformaldehyde for 10 min, then neutralized using 2 mg/mL glycine solution. After washing three times with PBS, cells were permeabilized with 0.5% Triton X-100 at room temperature for 10 min, followed by three additional PBS washes. Then nonspecific binding was blocked by incubating cells with 0.5% goat serum at room temperature for 1 h, and cells were then washed three times with PBS. Cells were incubated with diluted MyHC primary antibody (Santa, sc-32732) overnight at 4 °C, then a fluorescent secondary antibody (homologous to the primary antibody) was added, and cells were incubated at room temperature in the dark for 1 h. Subsequently, the secondary antibody was removed, and cells were washed three times with PBS in the dark. Nuclei were stained with DAPI solution at room temperature in the dark for 10 min, followed by three PBS washes to remove excess DAPI. Finally, PBS was added to the cells, and three randomly selected fields were imaged using an inverted fluorescence microscope for observation and documentation.

RESULTS

Isolation and culture of PSCs

PSCs activity is essential for skeletal muscle development, coordinating processes like proliferation, differentiation, and fusion. To study this in pigs, we isolated PSCs from 7-day-old pig leg muscles. Following 24-48 h of adherent culture, cells were induced to differentiate. PSCs in the logarithmic growth phase exhibited a healthy spindle-shaped morphology with minimal cell death. Differentiation progressed normally: cells elongated by 16 h, small myotubes appeared at 24 h, myotube numbers increased by 28 h, and numerous thick, multinucleated myotubes formed by 36 h (Fig. 1). Although PSC growth was relatively slow, their proliferation and differentiation capacity remained intact. These results confirm that the isolated PSCs were healthy and underwent normal differentiation and fusion into mature myotubes, fulfilling experimental requirements for further study.

Full length cDNA sequence of porcine lncRNA *NEAT1*

Total RNA was extracted from the dorsal muscle of 7-day-old Landrace pigs. Using the TaKaRa RACE kit, we constructed 5′/3′ RACE cDNA libraries and amplified the terminal sequences of the *NEAT1* gene (Fig. 2A–B). Intermediate fragments were amplified by PCR using porcine cDNA and conserved-region primers designed from pig, human, and mouse sequences (Fig. 2C). Sequencing revealed a 1,334 bp 5′-RACE product, 2,154 bp 3′-RACE product, and 1,216 bp conserved intermediate

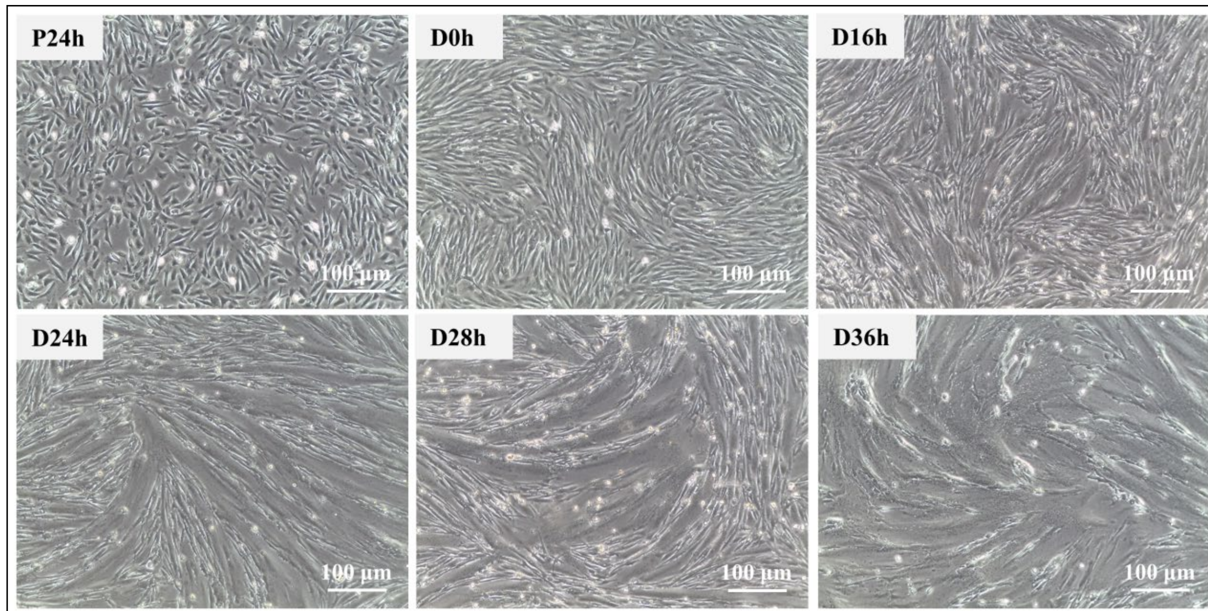


Fig. 1: Isolation and culture of PSCs at different periods. P24h, at 24-hour post-isolation of PSCs underwent proliferation; D0h, at 48-hour post-isolation of PSCs underwent proliferation; D16h, PSCs were in the 16-hour stages of induced differentiation; D24h, PSCs were in the 24-hour stages of induced differentiation; D28h, PSCs were in the 28-hour stages of induced differentiation. D36h, PSCs were in the 28-hour stages of induced differentiation. Scale bar = 100 μm

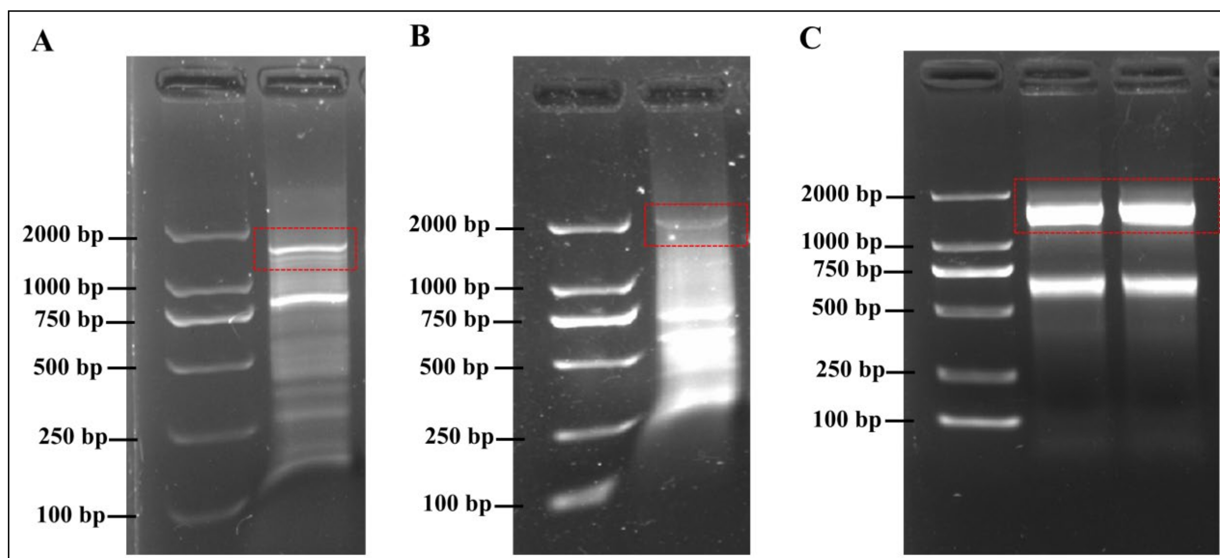


Fig. 2: Agarose gel electrophoresis detection of PCR amplification of porcine *NEAT1*. (A) PCR amplification result of 5' RACE. **(B)** PCR amplification results of 3' RACE; **(C)** PCR amplification results of the intermediate conserved region sequence. The red dashed box is the target sequence

fragment. These segments were assembled into a full-length 3,284 bp *NEAT1* cDNA sequence, localized to pig chromosome 2. Coding potential analysis using Coding Potential Calculator (CPC) (Kong *et al.* 2007) and Coding–Noncoding Index (CNCI) (Sun *et al.* 2013) software yielded negative scores for both algorithms, confirming *NEAT1* as a non-coding gene.

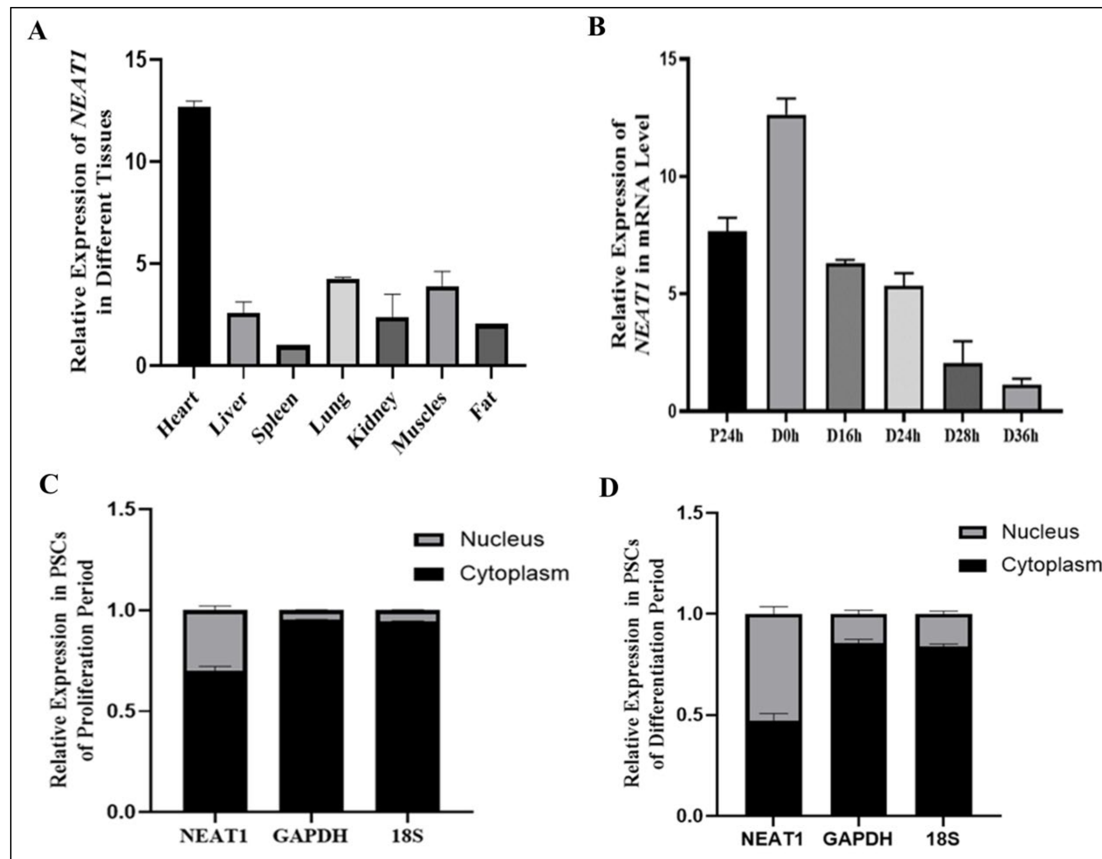


Fig. 3: RT-qPCR detection of spatiotemporal expression and nuclear cytoplasmic localization of *NEAT1* in pigs. (A) Expression of *NEAT1* gene in different tissues of pigs. **(B)** Expression of *NEAT1* gene during proliferation and differentiation of PSCs. **(C)** Expression localization analysis of *NEAT1* gene during the proliferation period of PSCs. **(D)** Localization analysis of *NEAT1* gene expression during differentiation of PSCs

Spatiotemporal expression and subcellular localization analysis of porcine *NEAT1*

We quantified *NEAT1* expression across seven tissues of 7-day-old Landrace pigs using RT-qPCR. *NEAT1* levels varied significantly, with highest expression in heart tissue, intermediate levels in muscle and lung, and lowest levels in spleen (Fig. 3A), indicating tissue-specific functional roles. To assess *NEAT1* dynamics during myogenesis, we analyzed its expression in porcine skeletal muscle satellite cells (PSCs) during proliferation (24 h) and differentiation (0-36 h post-induction). *NEAT1* was highly expressed during proliferation and downregulated progressively during differentiation (Fig. 3B), suggesting involvement in both proliferation maintenance and differentiation regulation.

Nuclear-cytoplasmic fractionation revealed distinct subcellular localization patterns: during proliferation, *NEAT1* was predominantly cytoplasmic (~ 70%), shifting toward nuclear enrichment during differentiation (cytoplasmic proportion decreased to ~ 47%; Figs. 3C-D). This dynamic redistribution implies compartment-specific functions in PSC fate determination.

Effect of *NEAT1* on proliferation and differentiation of PSCs

We designed three *NEAT1*-specific siRNA sequences (siNEAT1-1, 2, 3) and a negative control siRNA (siNC). Transfection into porcine skeletal muscle satellite cells (PSCs) revealed >60% knockdown efficiency for all siNEAT1 constructs at 48h post-transfection, with

siNEAT1-1 achieving >80% suppression (Fig. 4). This optimal construct was selected for subsequent experiments.

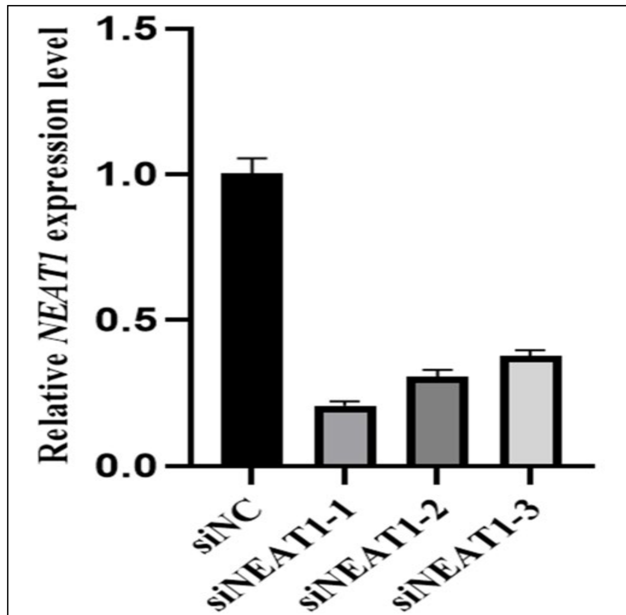


Fig. 4: RT-qPCR detection of interference effect of siNEAT1

To evaluate the role of *NEAT1* in cell proliferation, we transfected adherent PSCs at 40% confluency with either siNEAT1-1 or siNC. Following a 48-hour incubation in growth medium, RT-qPCR analysis confirmed effective knockdown of *NEAT1* expression (Fig. 5B). EdU incorporation assays revealed a significant reduction in the number of EdU-positive cells in the siNEAT1-1 transfected group ($P < 0.05$), indicating diminished proliferative capacity (Figs. 5A and C). Correspondingly, RT-qPCR analysis showed a downregulation of cell cycle regulators (*PCNA*, *CCNA2*, *CCNB1*, *CCNE2*, *CCND1*, *CDK4*; $P < 0.05$) and an upregulation of the cell cycle inhibitor *CDKN1A* ($P < 0.05$) (Fig. 5D), supporting the pro-proliferative function of *NEAT1*.

For differentiation analysis, PSCs at 80% confluency were transfected with siNEAT1-1 or siNC, followed by induction of differentiation. After 72 h, effective knockdown of *NEAT1* was confirmed (Fig. 6B). MyHC immunofluorescence showed enhanced differentiation in siNEAT1-1 groups, with thicker, denser multinucleated myotubes, significantly increased $MyHC^+$ area, and higher fusion indices ($P < 0.05$) (Figs. 6A, C).

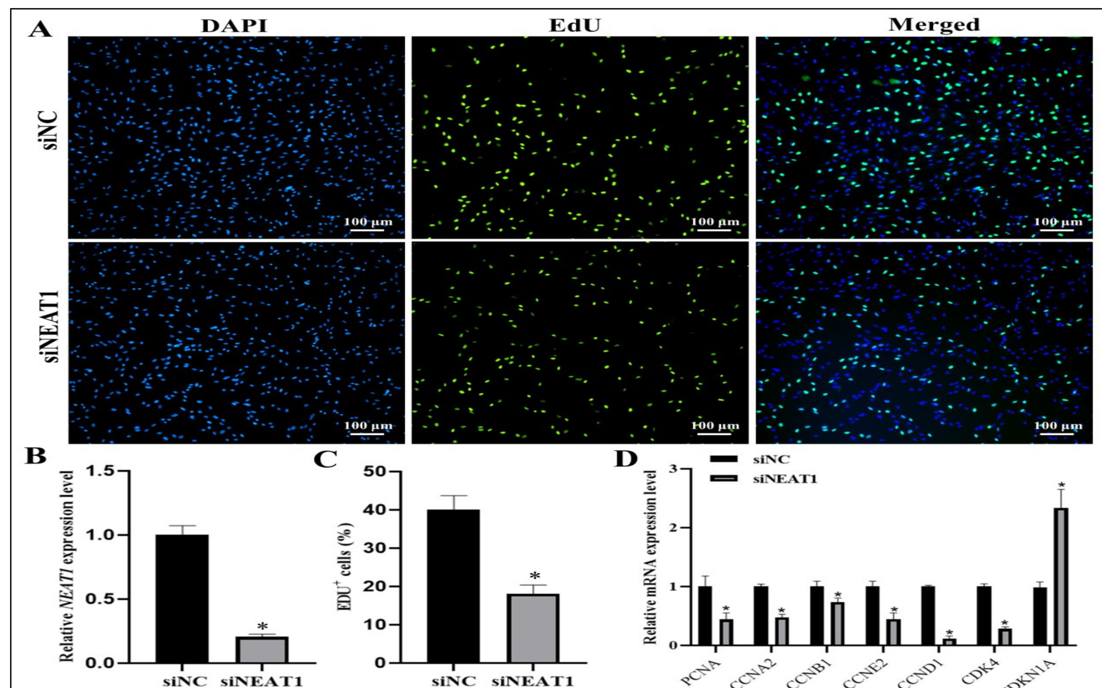


Fig. 5: Silencing *NEAT1* expression inhibits PSCs proliferation. (A) After silencing *NEAT1* expression, EdU detection of PSCs proliferation activity, Scale bar = 100 μm. **(B)** Expression detection of *NEAT1*. **(C)** EDU positive cell ratio statistics, n = 3, * $P < 0.05$. **(D)** RT-qPCR was used to detect the expression changes of cell cycle related genes such as *PCNA*, *CCNA2, CCNB1, CCNE2,CCND1*, and *CDK4* after inhibiting *NEAT1*, n = 3, * $P < 0.05$

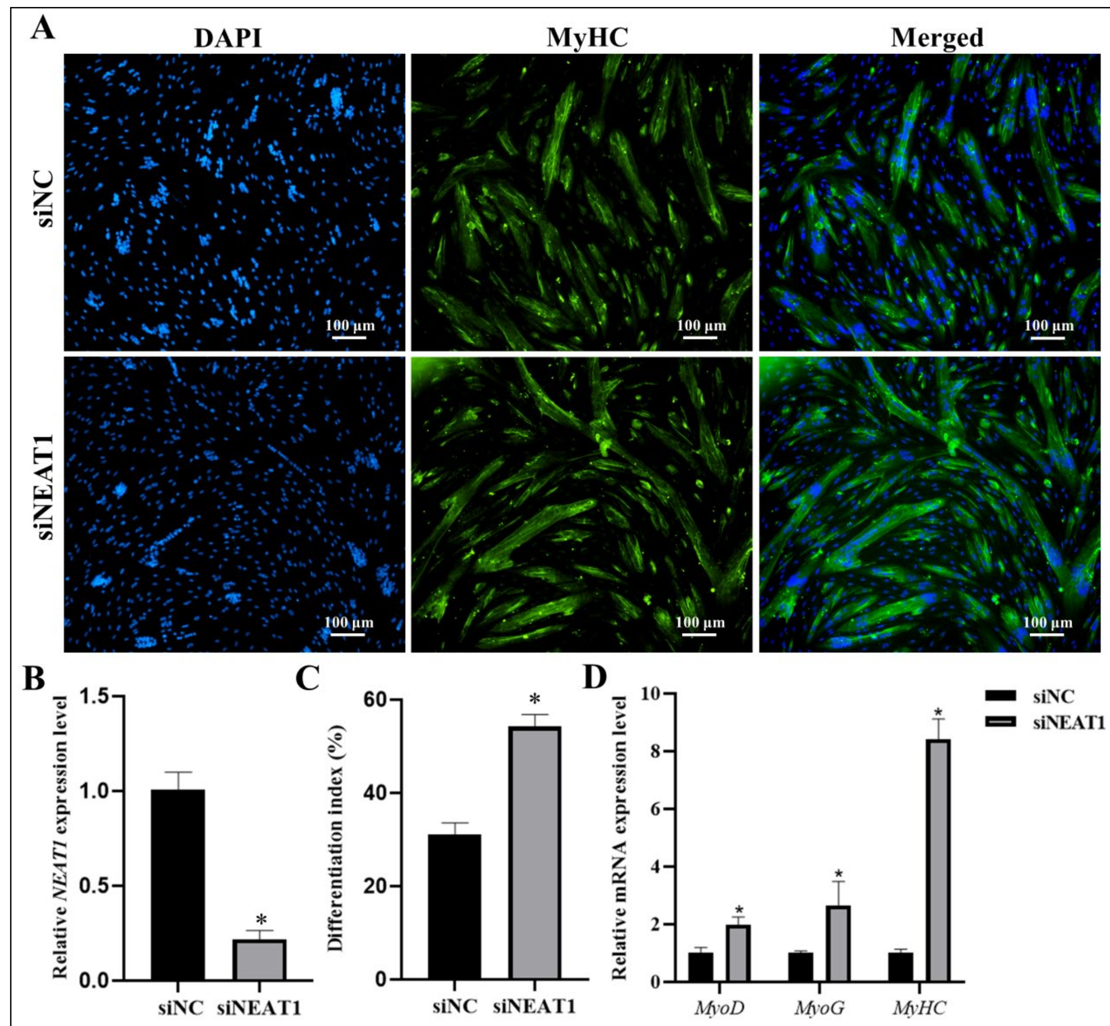


Fig. 6: Silencing *NEAT1* expression promotes PSCs differentiation. (A) MyHC immunofluorescence detection of PSCs differentiation after silencing *NEAT1* expression, Scale bar = 100 μm. **(B)** Expression detection of *NEAT1*. **(C)** MyHC^{+} muscle tube area statistics, n = 3, ** $P < 0.01$. **(D)** RT-qPCR was used to detect the expression changes of myogenic differentiation markers such as *MyoD*, *MyoG*, and *MyHC* after inhibiting *NEAT1*, n = 3, ** $P < 0.05$

Consistently, myogenic factors (*MyoD*, *MyoG*, *MyHC*) were significantly upregulated ($P < 0.05$) (Fig. 6D). These findings demonstrate that *NEAT1* acts as an inhibitor of PSC differentiation.

DISCUSSION

LncRNAs have emerged as pivotal regulators of skeletal muscle development, with implications for both livestock production and human health. However, their functional mechanisms in porcine skeletal muscle—directly linked to pork yield and quality—remain poorly characterized. This study focused on the lncRNA *NEAT1,* uncovering its molecular characteristics, expression patterns, and functional roles in PSCs, thereby addressing critical gaps in our understanding of lncRNA-mediated regulation of porcine myogenesis.

A key finding of this study is the identification of a 942-bp conserved region in *NEAT1* across humans, mice, and pigs, alongside the full-length 3284-bp porcine *NEAT1* cDNA. While lncRNAs are generally less conserved than mRNAs, functional conservation often resides in specific genomic regions or structural motifs (Yao *et al.* 2019; Heydarnezhad Asl *et al.* 2022). It is worth noting that

some lncRNAs contain “ultraconserved” regions and have conserved functions (Ulitsky *et al.* 2011). For instance, the lncRNA THOR contains a “super-conserved” region across vertebrates, enabling conserved interactions with IGF2BP1 to drive carcinogenesis (Hosono *et al.* 2023) . Similarly, the conserved region in porcine *NEAT1*—particularly the 480–1421 bp segment—may underpin conserved functions in myogenesis. This speculation aligns with murine studies, where *Neat1* promotes myoblast proliferation by repressing the cell cycle inhibitor *P21* and inhibits differentiation by downregulating myogenic genes such as *MyoD*, *MyoG*, and *MyHC* (Wang *et al.* 2019). The presence of a conserved sequence in porcine *NEAT1* suggests that its regulatory role in muscle development may be evolutionarily conserved, laying a foundation for cross-species comparative studies and highlighting *NEAT1* as a candidate for conserved myogenic regulatory networks.

The spatiotemporal expression and subcellular localization of *NEAT1* provide critical insights into its role in porcine myogenesis. *NEAT1* was highly expressed in porcine heart and skeletal muscle tissues, consistent with its proposed role in muscle-related processes. In PSCs, NEAT1 expression was dynamically regulated: upregulated during proliferation and downregulated upon differentiation. This pattern mirrors the expression of known myogenic regulators (e.g., *Myf5* in proliferation, *MyoD* in differentiation) (Zammit 2017), strongly suggesting *NEAT1*’s involvement in balancing SC proliferation and differentiation. Notably, *NEAT1* exhibited dual subcellular localization—predominantly cytoplasmic during proliferation and increasingly nuclear during differentiation. This shift aligns with reports that lncRNAs often exert context-specific functions via compartmentalization (Mercer *et al.* 2009). For example, cytoplasmic lncRNAs may regulate mRNA stability or translation, while nuclear lncRNAs often modulate chromatin structure or transcription. In murine myoblasts, *Neat1* interacts with the Polycomb Repressive Complex 2 (PRC2) in the nucleus to repress differentiation genes (Wang *et al.* 2019), suggesting a potential nuclear role in transcriptional silencing. Our observation of nuclear accumulation during PSCs differentiation raises the hypothesis that *NEAT1* may similarly engage epigenetic regulators in pigs, a mechanism warranting further investigation.

Functional assays confirmed that *NEAT1* is a key regulator of porcine SC behavior. Silencing *NEAT1* reduced the expression of proliferation markers (e.g., *PCNA*, *CCNA2*, *CDK4*) and decreased EdU-positive cells, indicating impaired proliferation. Conversely, it upregulated differentiation markers (*MyoD*, *MyoG*, *MyHC*) and enhanced myotube formation, consistent with accelerated differentiation. These findings align with murine studies showing that *Neat1* suppresses myoblast differentiation by repressing myogenic genes (Wang *et al.* 2019), supporting a conserved role for *NEAT1* in balancing muscle stem cell fate across species. Notably, *NEAT1*’s function in porcine SCs distinguishes it from other porcine muscle-related lncRNAs. For example, lnc-MD1 promotes myoblast differentiation by sequestering miR-133 (Zhu *et al.* 2017), whereas *NEAT1* acts as a brake on differentiation. This functional diversity highlights the complexity of lncRNA-mediated regulatory networks in porcine muscle development and underscores the need to characterize individual lncRNAs to unravel their unique roles.

Theoretically, this study fills a critical gap in understanding lncRNA function in porcine skeletal muscle. By identifying *NEAT1*’s conserved sequence, dynamic expression, and regulatory role in SCs, we provide evidence for conserved lncRNA-mediated mechanisms governing muscle development across mammals. This contributes to a broader framework linking lncRNA biology to skeletal muscle stem cell fate. Practically, these findings have implications for livestock production. PSCs are the primary drivers of postnatal muscle growth, and their proliferation/ differentiation balance directly influences muscle mass. Targeting *NEAT1* may offer a strategy to optimize this balance, potentially enhancing pork yield. Furthermore, given that pigs serve as a translational model for human muscle physiology, our findings may inform research on human muscle disorders, such as sarcopenia, where satellite cell dysfunction is a characteristic feature (Boyer *et al.* 2022).

This study is subject to several limitations. Firstly, it predominantly examines *in vitro* SCs, necessitating in vivo validation through porcine models, such as *NEAT1* knockout, to establish physiological relevance. Secondly, the molecular mechanisms through which *NEAT1* exerts its effects remain inadequately understood. For instance, it is unclear whether *NEAT1* interacts with specific microRNAs, such as miR-133—a known regulator of

MyoD—in the cytoplasm, or whether it associates with PRC2 or other epigenetic modifiers within the nucleus. Employing techniques such as RIP-seq or RNA pull-down assays could facilitate the identification of direct binding partners. Thirdly, the functional importance of the 942-base pair conserved region requires further elucidation; mutational analyses could ascertain whether this region is critical for *NEAT1*'s interactions or subcellular localization. Future investigations should also consider the interplay between *NEAT1* and established myogenic pathways, such as mTOR and TGF-β, which regulate SC proliferation and differentiation (Burks *et al.* 2011; Liu *et al.* 2022). Furthermore, exploring *NEAT1*'s role in muscle regeneration or its response to nutritional stimuli may yield valuable insights for optimizing pork quality traits.

CONCLUSION

This study identifies *NEAT1* as a conserved lncRNA that promotes proliferation and inhibits differentiation of porcine skeletal muscle satellite cells, with dynamic expression and subcellular localization supporting context-specific functions. These findings advance our understanding of lncRNA-mediated regulation of porcine myogenesis, offering theoretical basis for improving livestock production and informing translational research on muscle disorders. Further exploration of *NEAT1*'s molecular mechanisms and *in vivo* roles will deepen its significance in both agricultural and biomedical contexts.

ACKNOWLEDGEMENTS

This research was funded by the National Natural Science Foundation of China (31972537 and 32202654), the Department of Science and Technology in Henan Province (242102111015), the Natural Science Foundation of Henan Province (242300421337 and 242300420509), the Postgraduate Education Reform and Quality Improvement Project of Henan Province (YJS2025KC33), and the Nanhu Scholars Program of Xinyang Normal University.

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