

# Ubiquitin-specific peptidase 8 regulates proliferation and early differentiation of sheep skeletal muscle satellite cells

SHAORYU WANG<sup>1</sup>, KUI LI<sup>2</sup>, HUI GAO<sup>2</sup>, ZEPENG LIU<sup>1</sup>, SHUANG SHI<sup>1</sup>, QIANG TAN<sup>1</sup>, ZHENG GUANG WANG<sup>1,3\*</sup>

<sup>1</sup>College of Animal Sciences, Zhejiang University, Hangzhou, P.R. China

<sup>2</sup>Zhejiang Animal Husbandry Techniques Extension Station, Hangzhou, P.R. China

<sup>3</sup>Huzhou Southern Taihu Lake Modern Agricultural Technology Center, Zhejiang University, P.R. China

\*Corresponding author: [wzhguang68@zju.edu.cn](mailto:wzhguang68@zju.edu.cn)

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**Abstract:** Ubiquitin-specific protease 8 (USP8), a member of the ubiquitin-specific protease (USP) family, was originally identified as playing a role in the regulation of growth and cell cycle. However, its functional role in myogenesis is unknown. In this study, we investigated the role of USP8 in proliferation and differentiation of sheep skeletal muscle satellite cells. The results showed that the expression level of *USP8* was significantly increased on days 2 and 3 following the induction of the differentiation process. Furthermore, knocking down *USP8* resulted in a significant increase in myogenin-positive cells, and promoted early differentiation of satellite cells by regulating the expression level of paired box 7 (PAX7). Additionally, knocking down *USP8* suppressed muscle satellite cell proliferation, possibly explaining that the relative mRNA level of *USP8* was linearly related to muscle fibre density of Hu sheep. Overall, our research demonstrates that USP8 plays a role in proliferation and early differentiation of skeletal muscle satellite cells.

**Keywords:** myogenesis; myogenin; desmin; myosin heavy chain 3; PAX7

Myogenesis includes systematically organized and highly regulated sequences of cellular processes that commence with the commitment of muscle satellite cells (Yablonka-Reuveni 2011). As myogenic progenitors, muscle satellite cells are activated and differentiated into myocytes. Then, the cell fusion from mononucleated muscle fibres into multinucleated myofibres occurs, ultimately forming myotubes (Charge and Rudnicki 2004; Bentzinger et al. 2012). These processes involve the paired box

transcription factor PAX7 and myogenic regulatory factors (MRFs), including myogenin (MYOG), myogenic differentiation 1 (MYOD1), myogenic factor 5 (MYF5), myogenic factor 6 (MYF6) and myocyte enhancer factor 2 (MEF2) proteins family, which are critical in regulating the expression of target genes during myogenesis (Seale et al. 2000; Taylor and Hughes 2017; Zammit 2017).

Ubiquitin-specific protease 8 (USP8) is a widely expressed ubiquitin-specific protease (USP) pro-

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tein family, which has previously been implicated in the endocytosis of several receptors by virtue of their deubiquitination (Niendorf et al. 2007; Berlin et al. 2010; Kawaguchi et al. 2018). In addition, as a regulator of cell growth that was originally described, USP8 plays an important role in a series of early cellular processes involved in regulating the cell cycle, maintaining cell stemness and inhibiting neuronal differentiation (Naviglio et al. 1998; Mizuno et al. 2007; Ceriani et al. 2015; Gu et al. 2019). Nevertheless, little is known about the relevance of USP8 for myogenesis.

Since deubiquitinating enzymes remove ubiquitin from substrates, they may also play an important role in controlling ubiquitination in skeletal muscle development (Kitajima et al. 2018). There is some evidence that myogenesis is controlled by USPs. For example, endoplasmic reticulum-localized ubiquitin specific peptidase 19 decreases the myoblast fusion during myocyte differentiation (Wiles et al. 2015), and ubiquitin-specific peptidase 4 (USP4) regulates MRFs in a catalytic-independent manner to participate in the regulation of myogenic differentiation (Yun and Kim 2017). As for USP8, regulation of its stability is dependent on the phosphorylation of USP8 mediated by the protein kinase B signalling, which is closely related to the biological process of myogenesis, suggesting that USP8 may play a role in myogenesis (Cao et al. 2007; Daviet and Colland 2008; Mareco et al. 2015). Additionally, a repertoire of ubiquitin ligases that regulate the myofibre size in *Drosophila* was identified, and the loss of USP8 promoted myofibre hypertrophy (Hunt et al. 2019). Therefore, it can be expected that this ubiquitin-related enzyme regulates the myofibre size.

To date, the biological function of USP8 in skeletal muscle is poorly understood. The aim of this study was to investigate the role of USP8 in myogenesis. Our data suggested that USP8 plays an important role in proliferation and early differentiation of skeletal muscle satellite cells.

## MATERIAL AND METHODS

### Tissue samples

Samples were obtained from the skeletal muscle tissue of the three-month-old Hu sheep (*Ovis aries*) from a farm in Zhejiang Province, China ( $n = 6$ ). The stunned animals were slaughtered by jugu-

lar vein bloodletting, and all efforts were made to minimize the animals' suffering. All experiments were carried out following the guidelines for the care and use of laboratory animals and approved by Zhejiang University. Subsequently, the *biceps femoris* muscle (BFM), *longissimus dorsi* muscle (LDM) and *gluteus medius* muscle (GMM) were either collected for fixation or immediately snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until use. Skeletal muscle satellite cells of Hu sheep were obtained by the primary culture method from leg muscle tissues (Wu et al. 2012).

### Cell culture

Muscle satellite cells were grown in Dulbecco's modified Eagle's medium (DMEM; HyClone Laboratories, Logan, UT, USA) supplemented with 10% heat-inactivated Foetal Bovine Serum (FBS; Invitrogen, Waltham, MA, USA) and 1% penicillin-streptomycin by incubation at  $37^{\circ}\text{C}$  in a humidified atmosphere of 5%  $\text{CO}_2$ . To induce differentiation, cells were grown to 60–80% confluence and then switched to the differentiation medium consisting of DMEM supplemented with 2% horse serum and 1% penicillin-streptomycin. The medium was changed every two days.

### RNA interference

Small interfering RNA (siRNA) was chemically synthesized by GenePharma (Shanghai, China). Their sequences are listed in Table 1. Briefly, cells were allowed to grow in 24-well plates to 30–50% confluence and then transfected with non-target siRNA (siNT) or USP8 siRNA (siUSP8) using siRNA-Mate transfection reagents (GenePharma, Shanghai, China) following the manufacturer's instructions. Cells were harvested for subsequent experimentation after two days of incubation in differentiation medium.

### Reverse transcription – quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from the muscle tissues and cells using RNAiso Plus reagent (Takara Bio Inc., Otsu, Japan) according to the manufacturer's proto-

Table 1. The sequences of primers for qPCR or RNA interference

Target gene	GenBank accession No.	Primer sequence (5'-3')	Annealing temperatures (°C)	Length of the amplicons (bp)	Purpose
<i>USP8</i>	XM_004010617.4	F:CTGCTTCTGTTCCTAATGGATGGTCTC R:TGAGGCACTGTACTGTGGACTTGA	53.8	199	qPCR
<i>MYOG</i>	GU550517.1	F:AGGTGAATGAAGCCTTCGAG R:TCCTGGTTGAGGGAGCTGAG	58.6	138	qPCR
<i>MYOD1</i>	NM_001009390.1	F:CGCAACGCAATCCGCTATATCG R:CCATCATGCCGTCGGAACAGTT	62.1	184	qPCR
<i>MYF5</i>	XM_015094556.2	F:GCAAGAGGAAGTCCACCACCATG R:GCAGGCTCTCAATGTAGCGGATG	59.9	180	qPCR
<i>MYF6</i>	NM_001134782.1	F:GCTCGTGATAACTGCCAAGGAAGG R:GTTACTTCTCCACCACCTCTCCA	57.3	143	qPCR
<i>MEF2A</i>	XM_012098343.3	F:TCGGAGGAAGAGGAATTGGAGTTGA R:TGTTGTAGGCGGTCGGCATTG	57.4	148	qPCR
<i>MEF2B</i>	XM_027969352.1	F:AACCTTCCCTTACCCTTTGCTCCT R:GTGGTAACTGCTGCGTCTTCTCC	62.4	211	qPCR
<i>MEF2C</i>	NM_001159277.1	F:AACCAGACCTCCGAGTCTTATTCCA R:GGTAGCCAATGACTGAGCAGACTG	53.6	101	qPCR
<i>MEF2D</i>	XM_027976382.1	F:CGTTAGCAGTGTAACAGTCCAGGAA R:GGAGCAAGAACAACAGCAGAAGAATC	52.1	153	qPCR
<i>MYH3</i>	XM_027974881.1	F:GCTGGCTGGAGAAGAACAAGGAC R:AGTGGTGAAGGTGGCGTAGAGG	57.8	104	qPCR
<i>PAX7</i>	XM_027965643.1	F:GAAGAAGGAGGAGGAGGACGAAGG R:TGCTTACGCTTCAGAGGGAGGTC	59.2	156	qPCR
<i>18S rRNA</i>	AY753190.1	F:GTCTGCCCTATCAACTTTCGATGGTAG R:TGCTGCCTTCCTTGGATGTGGTA	58.0	126	qPCR
siUSP8	–	F:GCUCGUAUUCACGCAGAAATT R:UUUCUGCGUGAAUACGAGCTT	–	–	RNA interference
siNT	–	F:UUCUCCGAACGUGUCACGUTT R:ACGUGACACGUUCGGAGAATT	–	–	RNA interference

*18S rRNA* = 18S ribosomal RNA; *MEF2A* = myocyte enhancer factor 2A; *MEF2B* = myocyte enhancer factor 2B; *MEF2C* = myocyte enhancer factor 2C; *MEF2D* = myocyte enhancer factor 2D; *MYF5* = myogenic factor 5; *MYF6* = myogenic factor 6; *MYH3* = myosin heavy chain 3; *MYOD1* = myogenic differentiation 1; *MYOG* = myogenin; *PAX7* = paired box 7; siNT = non-target siRNA; siUSP8 = siRNA-USP8; *USP8* = ubiquitin-specific protease 8

col. RNA purity and integrity were analysed by RNA electrophoresis and NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). All RNA samples were treated with genomic DNA Eraser and then reversely transcribed into cDNA using PrimeScript™ RT reagent Kit (Takara Bio Inc., Otsu, Japan). The cDNA was used for expression analysis of the following muscle-related genes, including *USP8*, *MYOG*, *MYOD1*, *MYF5*, *MYF6*, *MEF2A*, *MEF2B*, *MEF2C*, *MEF2D*, *MYH3* and *PAX7* (full names of the genes are presented in Table 1). The *18S rRNA* gene was chosen as the reference for internal standardization. The qPCR primers were designed based on the sequences acquired from

NCBI database (Table 1), and their amplification efficiency was evaluated before quantification. The PCR fragments were sequenced to check for specificity. The qPCR was performed in triplicate using SYBR Premix Ex Taq™ II Kit (Takara Bio Inc., Otsu, Japan) on the LightCycler 480 II system (F. Hoffmann-La Roche Ltd., Basel, Switzerland). The amplification of qPCR was carried out as follows: 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s and 60 °C for 30 s, and a final dissociation cycle of 95 °C for 15 s, 60 °C for 15 s, and 95 °C for 15 s. All qPCR experiments were performed in triplicate. The relative mRNA expression level was calculated using the  $2^{-\Delta\Delta C_T}$  method (Livak and Schmittgen 2001).

## Western blot

Total proteins were extracted from the cultured cells with RIPA Lysis Buffer containing a protease inhibitor cocktail (Beyotime Biotech, Shanghai, China). The protein concentration from the resultant supernatant was estimated by the Bradford method (Bradford 1976). The proteins in the samples were electrophoresed through 10% SDS-PAGE and electro-transferred onto PVDF membranes (Millipore Co., Billerica, MA, USA). The membranes were blocked with 5% skimmed milk in TBST buffer [50 mM Tris-HCl pH 7.4, 150 mM NaCl and 0.2% (vol/vol) Tween20 (Sigma Aldrich, St. Louis, MO, USA)] for 1 h, and then incubated, respectively, with primary antibodies including a rabbit anti-mouse USP8 polyclonal antibody (Proteintech, Rosemont, IL, USA, 27791-1-AP, 1:2 000), mouse anti-human MYOG monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA, sc-12732, 1:1 000), rabbit anti-human MYOD1 monoclonal antibody (Huabio, Hangzhou, China, ET1703-91, 1:1 000), rabbit anti-mouse PAX7 monoclonal antibody (Huabio, Hangzhou, China, ET1612-60, 1:1 000), mouse anti-human MYH3 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA, sc-376157, 1:1 000) and rabbit anti-mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (Abcam, Cambridge, MA, USA, ab181602, 1:10 000) overnight at 4 °C. The membranes were then washed with TBST buffer and incubated with the goat anti-mouse or goat anti-rabbit IgG secondary antibodies (Thermo Pierce, Waltham, MA, USA, 1:5 000) for 1 hour. The bands were visualized with the BeyoECL Plus Detection System (Beyotime Biotech, Shanghai, China) and quantified by densitometry using the Image J software v1.8.0 (National Institutes of Health, Bethesda, MD, USA).

## Immunocytochemistry

Cells were fixed with 4% paraformaldehyde for 30 min and permeabilized with 0.25% Triton X-100 (Sigma Aldrich, St. Louis, MO, USA) for 30 min, followed by blocking with PBS containing 5% goat serum for 1 hour. The cells were then incubated with primary mouse anti-human MYOG monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA, sc-12732, 1:200) or primary rabbit anti-mouse DES polyclonal antibody (Huabio, Hangzhou, China,

ER40113, 1:200) overnight at 4 °C. After washes, cells were incubated with the goat anti-mouse or goat anti-rabbit IgG secondary antibodies (Thermo Life, Waltham, MA, USA, 1:500) for 1 h at room temperature. Samples were then rinsed with PBS and nuclei were counterstained with DAPI (4',6-diamino-2-phenylindole; Sigma Aldrich, St. Louis, MO, USA). The images were taken by an inverse fluorescence microscope (DM IL LED; Leica, Wetzlar, Germany). Each experiment was performed in triplicate, and five non-overlapping random regions at 200× magnification were analyzed in each sample. The level of cell differentiation was determined as percentage of MYOG-positive cells to the muscle satellite cells in the selected region. The fusion index was calculated as percentage of DES-positive myotubes that contained at least two nuclei. The measurement of the numbers of these cells and myotubes was conducted by the Image Pro Plus software (Media Cybernetics, Rockville, MD, USA).

## Cell proliferation assay

After transfection for 0–2 days, muscle satellite cells were seeded in 96-well plates at  $2 \times 10^3$  cells per well in 100 µl of growth medium. To detect cell proliferation, a Cell Counting Kit-8 (CCK-8) (Beyotime Biotech, Shanghai, China) was used according to the manufacturer's instructions. The different siRNA-treated cells were incubated with 10 µl per well CCK-8 reagent in growth medium for 2 hours. The optical density values were detected at 450 nm using a microplate reader (Biotek, Winooski, VT, USA).

## Histological analysis

The muscle samples from sheep (BFM, LDM and GMM) were fixed in 10% neutral buffered formalin for 24 hours. After being dehydrated in graded ethanol concentrations and embedded in paraffin wax, sections were stained with Masson's trichrome, and then prepared for a light microscope (Eclipse E100; Nikon, Tokyo, Japan).

In the stained sections, photographic documentation was obtained of 15 non-overlapping random histological regions at 100× magnification in each sample. The measurement of muscle fibre density was conducted by the Image Pro Plus software. The results are listed in Table 2.



Table 2. The fiber density of different types of muscle in Hu sheep

Muscle category	Density (root/mm <sup>2</sup> )
<i>Biceps femoris</i> muscle	574.84 ± 25.75 <sup>c</sup>
<i>Gluteus medius</i> muscle	656.11 ± 29.85 <sup>b</sup>
<i>Longissimus dorsi</i> muscle	741.98 ± 34.89 <sup>a</sup>

<sup>a–c</sup>Different lowercase letters in the same indicator for the same muscle mean significant differences at  $P < 0.05$   
Data are shown as mean ± SD ( $n = 6$ )

### Statistical analysis

All data are presented as the mean values ± SEM of each group. Statistically significant differences were calculated by one-way ANOVA followed by Tukey's test or two-way ANOVA followed by Bonferroni post-tests using the SPSS software v19.0 (SPSS Inc./IBM

Corp., Armonk, NY, USA). Differences were considered to be significant at  $P < 0.05$  and highly significant at  $P < 0.01$ . Graphs were generated by GraphPad Prism v5.0 (GraphPad, San Diego, CA, USA).

## RESULTS

### Expression profiles of *USP8* in sheep muscle satellite cells at various stages of differentiation

In sheep muscle satellite cells, characteristic morphological changes in the form of myotubes were observed during myogenesis (Figure 1A). Over the course of the five-day myogenic differentiation, the mRNA level of *USP8* increased significantly on days 2 and 3 after the induction of the differen-

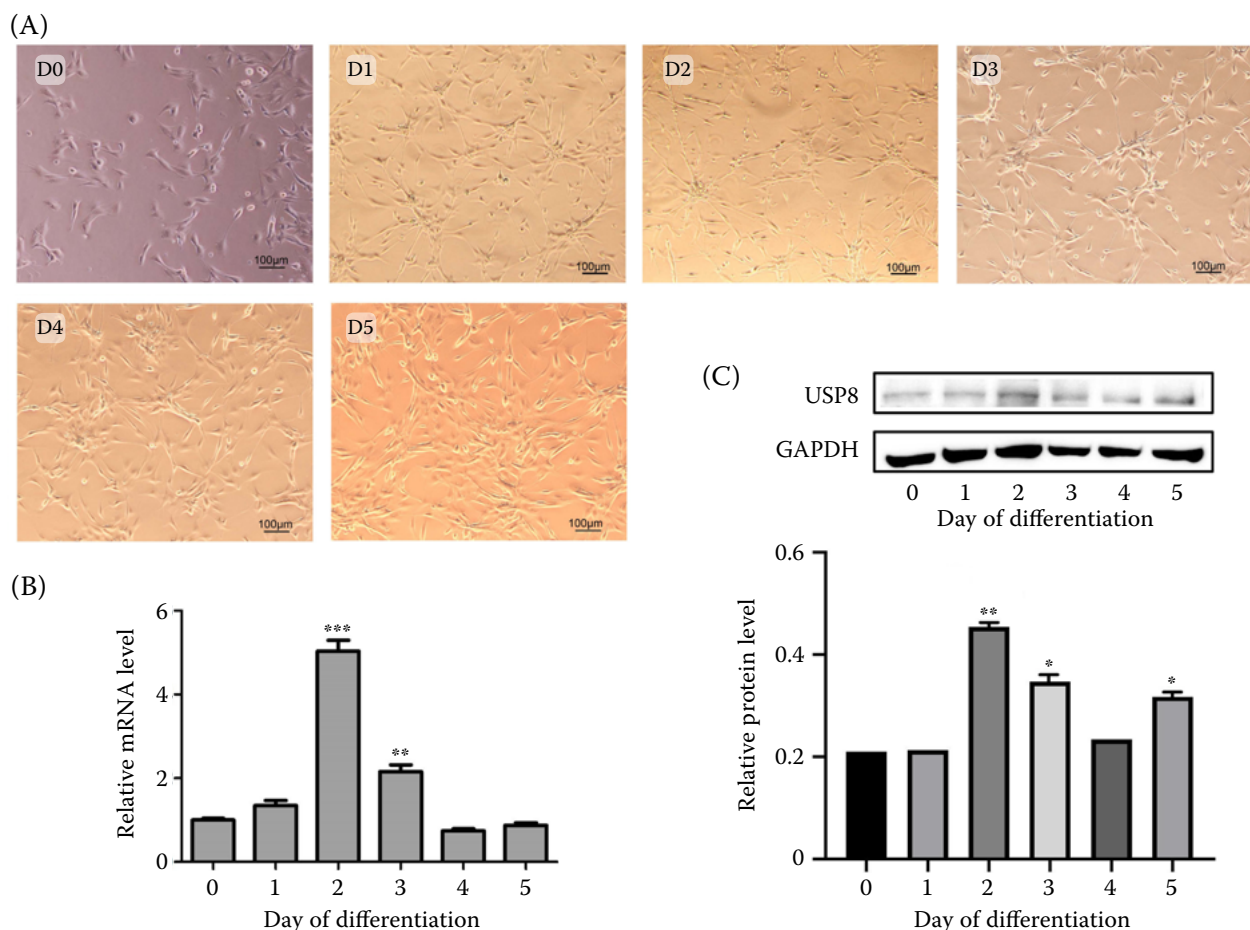


Figure 1. Expression profiles of *USP8* in sheep muscle satellite cells at various stages of differentiation (A) Representative cell morphology during 0–5 days (D0–D5) of differentiation, scale bars = 100 μm; (B) relative mRNA level of *USP8* during cell differentiation; (C) western blot analysis of *USP8* protein expression  
GAPDH = glyceraldehyde-3-phosphate dehydrogenase; *USP8* = ubiquitin-specific protease 8  
Data are shown as mean ± SEM,  $n = 3$ , \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  as compared with day 0

tiation process (Figure 1B). Its protein expression was found to follow a similar pattern to that observed via Western blot (Figure 1C).

### Knocking down *USP8* promotes early differentiation of sheep muscle satellite cells

To further explore the biological function of *USP8* in the myogenic process, sheep muscle satellite

cells were transfected with siRNA against *USP8* to inhibit its expression, which resulted in a significant decrease in both the mRNA and protein level (Figure 2A and 2B). MYOG and DES, the markers of myogenic differentiation, were used to monitor the differentiation process. Immunocytochemistry assay showed that the rate of MYOG-positive cells treated with siUSP8 was remarkably higher than that in the control group (Figure 2C). However, the fusion index represented by DES-positive myo-

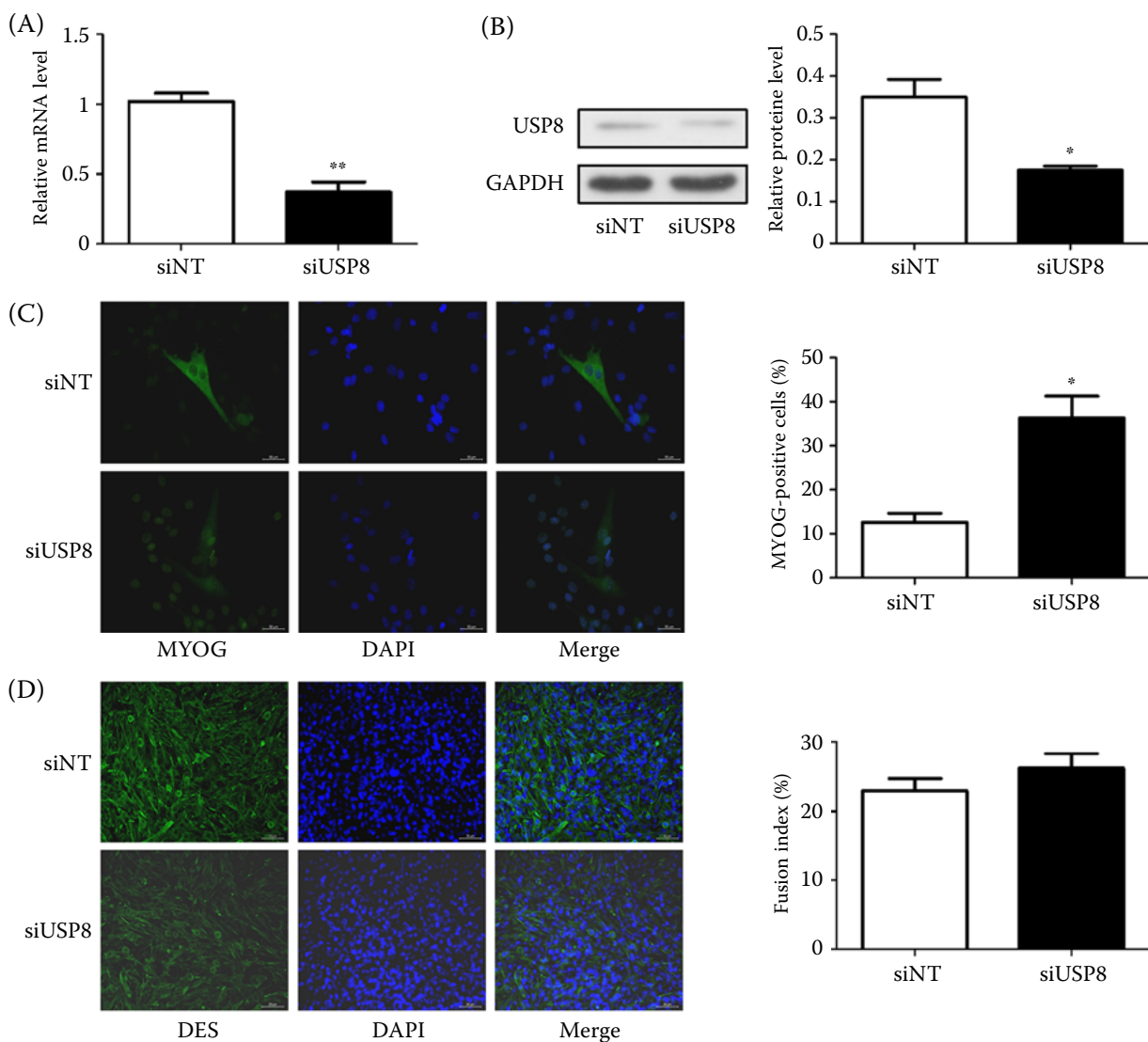


Figure 2. Knocking down *USP8* promotes early differentiation of sheep muscle satellite cells

(A) Relative mRNA level of *USP8* in muscle satellite cells treated with siNT or siUSP8; (B) protein expression level of *USP8* and normalised expression levels of *USP8* by densitometric analysis; (C) immunocytochemistry assay of the MYOG-positive cells (green); (D) immunocytochemistry assay of DES-positive myotubes (green), the fusion index of myotubes is presented DAPI = 4',6-diamino-2-phenylindole; DES = DES polyclonal antibody; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; MYOG = myogenin; siNT = treatment with non-target siRNA as control; siUSP8 = treatment with siRNA targeting for *USP8*; *USP8* = ubiquitin-specific protease 8

Scale bars = 50  $\mu$ m; data are shown as mean  $\pm$  SEM,  $n = 3$ , \* $P < 0.05$  and \*\* $P < 0.01$

tubes showed no significant difference in the treatment group (Figure 2D).

### Effects of knocking down *USP8* on myogenesis marker genes

To gain insight into the effects of *USP8* on myogenic factors during satellite cell differentiation, we checked the expression levels of myogenesis marker genes treated with siNT or siUSP8 (Figure 3A and 3B). The results showed that *MYOG* and *PAX7* were significantly upregulated at both mRNA and protein levels after treatment with siUSP8 and in-

duction of differentiation for two days. The *MYH3* increased significantly only at the mRNA level, and there was no significant difference in the protein level. There was no significant difference of other genes treated with siUSP8 compared to the control group at the mRNA level.

### Knocking down *USP8* inhibits cell proliferation and its correlation with muscle fibre density

To investigate the role played by *USP8* in the proliferation of muscle satellite cells, CCK-8 as-

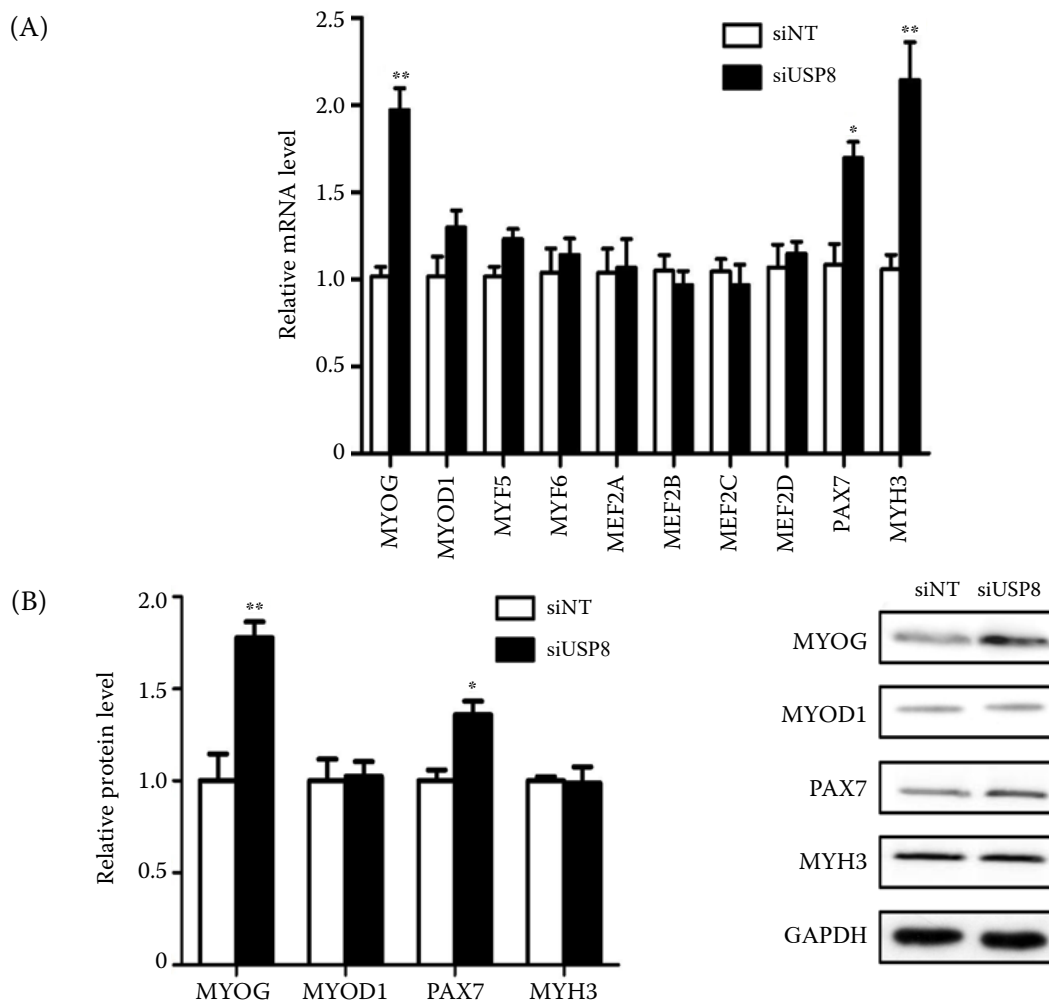


Figure 3. Effects of knocking down *USP8* on myogenesis marker genes

(A) Relative mRNA level of myogenic genes treated with siNT or siUSP8; (B) protein expression level of *MYOG*, *MYOD1*, *PAX7* and *MYH3*

MEF2 = myocyte enhancer factor 2; MYF5 = myogenic factor 5; MYF6 = myogenic factor 6; MYH3 = myosin heavy chain 3; MYOD1 = myogenic differentiation 1; MYOG = myogenin; PAX7 = paired box 7; siNT = treatment with non-target siRNA as control; siUSP8 = treatment with siRNA targeting for *USP8*; *USP8* = ubiquitin-specific protease 8

Data are shown as mean  $\pm$  SEM,  $n = 3$ , \* $P < 0.05$  and \*\* $P < 0.01$

say was employed to assess the cell proliferation in growth medium. The CCK-8 reagents were added into different siRNA-treated media and incubated, and then the optical density values were measured. Compared with the control group, two days of *USP8* knockdown significantly reduced the muscle satellite cell proliferation (Figure 4A). Furthermore, the results showed that the relative level of *USP8* was different in various muscle categories, and was roughly linearly related to the corresponding muscle fibre density (Figures 4B–4D).

## DISCUSSION

Given the potential function of *USP8* in regulating cellular processes, sheep muscle satellite cells were isolated and induced to differentiate for five

days, followed by determination of expression profiles of *USP8* (Figure 1). The mRNA and protein level of *USP8* exhibited significant upregulation during the differentiation stage, which provides evidence that *USP8* likely plays an important role in myogenesis.

To obtain a more accurate idea of its function in myogenesis, a si*USP8* knockdown approach was used to inhibit its expression in muscle satellite cells. After treatment with si*USP8* followed by two days of differentiation, we first confirmed the knockdown efficiency of *USP8* in satellite cells at both mRNA and protein levels (Figure 2A and 2B). Furthermore, when muscle satellite cells began to differentiate into myocytes expressing MYOG, it is regarded as early differentiation stage (Yuan et al. 2017). When the myocytes differentiated and fused into myotubes expressing DES at high levels,

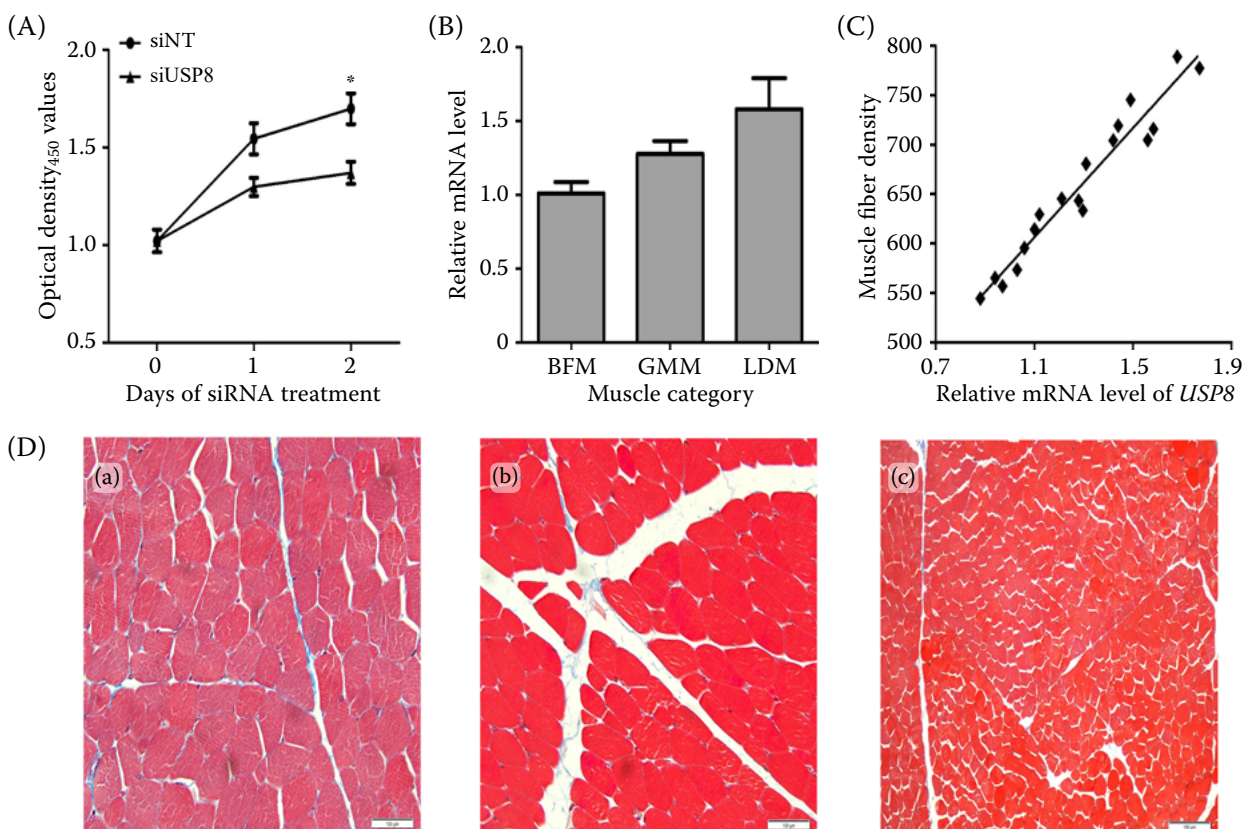


Figure 4. Knocking down ubiquitin-specific protease 8 (*USP8*) inhibits cell proliferation and its correlation with muscle fibre density

(A) Cells in growth medium treated with siNT or siUSP8 for 0–2 days; (B) relative mRNA level of *USP8* in different muscle category; (C) correlation analysis between the relative mRNA level of *USP8* and muscle fibre density; (D) light micrographs of muscle cross-sections in Hu sheep stained with Masson's trichrome. (a) *Biceps femoris* muscle (BFM); (b) *gluteus medius* muscle (GMM); (c) *longissimus dorsi* muscle (LDM)

siNT = treatment with non-target siRNA as control; siUSP8 = treatment with siRNA targeting for *USP8*

Data are shown as mean  $\pm$  SEM,  $n = 6$ , \* $P < 0.05$



it is considered to be the late differentiation stage (Rantanen et al. 1995; Costa et al. 2004; Liu et al. 2017). In this study, the knockdown of *USP8* markedly promoted the differentiation of satellite cells with an increase in MYOG-positive cells, suggesting that *USP8* suppresses myogenic differentiation. Immunostaining data revealed that MYOG, but not DES, was significantly upregulated in the treatment group (Figure 2C and 2D), indicating that *USP8* is involved in regulating early differentiation of satellite cells but it may not significantly influence late differentiation.

Myogenic differentiation is subtly controlled by various signalling paths and MRFs during myogenesis, thus the expression level of marker proteins can fluctuate across differentiation stages (Bentzinger et al. 2012; Zammit 2017). Among the tested myogenesis marker genes (Figure 3), the mRNA and protein levels of *MYOG* increased dramatically, which is consistent with the results of immunostaining. Simultaneously, the expression levels of *PAX7* were elevated after two days differentiation in the treatment group, suggesting that the knockdown of *USP8* promoted early differentiation of satellite cells by regulating *PAX7*, which may be attributed to the maintenance and specialization of *PAX7* in satellite cells (Seale et al. 2000; Kitajima and Ono 2018). Detailed investigation needs to be conducted to determine the specific relationship between *USP8* and *PAX7* in the near future. Furthermore, *MYH3*, an indicator of the terminal differentiation process, showed a dramatic increase in the mRNA level but no significant difference in the protein level, confirming the inability of *USP8* to regulate satellite cell late differentiation, which is consistent with the conclusion of DES immunostaining.

Stable skeletal muscle satellite cells are suitable for describing muscle development as they represent a model close to the *in vivo* situation of farm animals (Will et al. 2015). In this study, knocking down *USP8* inhibited muscle satellite cell proliferation, suggesting that it may positively regulate cell proliferation, which is consistent with the results in other studies about *USP8* (Naviglio et al. 1998; Niendorf et al. 2007). This suggests that the linear correlation between the relative mRNA level of *USP8* and muscle fibre density could be partly attributed to the effect of high levels of *USP8* on the promotion of cell proliferation, which may become a potential objective for future work in animal genetics and breeding.

## CONCLUSION

In conclusion, this study presents evidence showing for the first time that *USP8* is involved in the regulation of proliferation and differentiation of skeletal muscle satellite cells. Our data show that expression levels of *USP8* were upregulated during differentiation. Functionally, the results suggest that *USP8* is important for the early differentiation of satellite cells rather than for the late differentiation, and knockdown of *USP8 in vitro* further promotes differentiation by upregulating the expression level of *PAX7*. Furthermore, *USP8* may positively regulate the cell proliferation and is linearly related to muscle fibre density. Overall, *USP8* has an important impact on myogenesis and these novel data might contribute to a better understanding of the biological function of *USP8*.

## Conflict of interest

The authors declare no conflict of interest.

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