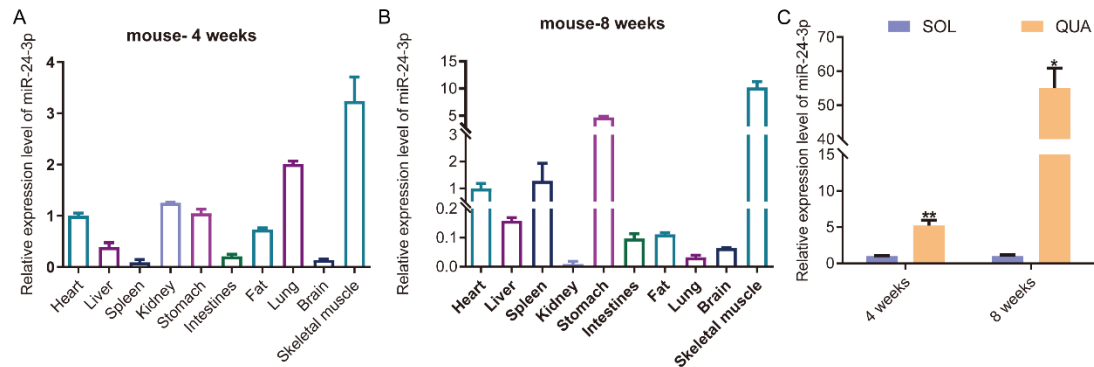


## Supplementary information



**Figure S1 miR-24-3p expression pattern in skeletal muscle**

(A-B) miR-24-3p expression in different tissues at weeks 4 and 8 of mouse development. (C) miR-24-3p expression in fast and slow muscles at weeks 4 and 8 of mouse development. Data are presented as mean  $\pm$  S.E.M and analyzed for statistical differences between groups using unpaired two-tailed t-tests. \* $p < 0.05$ , \*\* $p < 0.01$ .

**Table S1 Primer sequences for reverse transcription and qPCR**

Name	Sequence(5'-3')
miR-24-3p	RT: GTCGTATCCAGTGCAGGGTCCGAGGTATTTCG CACTGGATACGACCTGTTC F: GCGTGGCTCAGTTCAGCAG R: GCCCTGCATCTTGAAATCTGA
Pig-U6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT
Pig- $\beta$ -ACTIN	F: GCGGCATCCACGAAACTAC R: TGATCTCCTTCTGCATCCTGTC
Pig-GAPDH	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA
Pig-NEAT	F: GTCGATGCCCTGAACATG R: GGTTAGCACGGAACCTACA
Pig-KI67	F: AGCCCGTATCTGGTGCAAAA R: CCTGCATCTGTGTAAGGGCA
Pig-PCNA	F: AAGTCAAATCTGGTCTTGTTAGCC R: CACTGTCCTGGGATGCTTGAA
Pig-CDK4	F: TGGTTACAAGTGGTGGGACA R: CTGGAGCACGGTACCAGAGT

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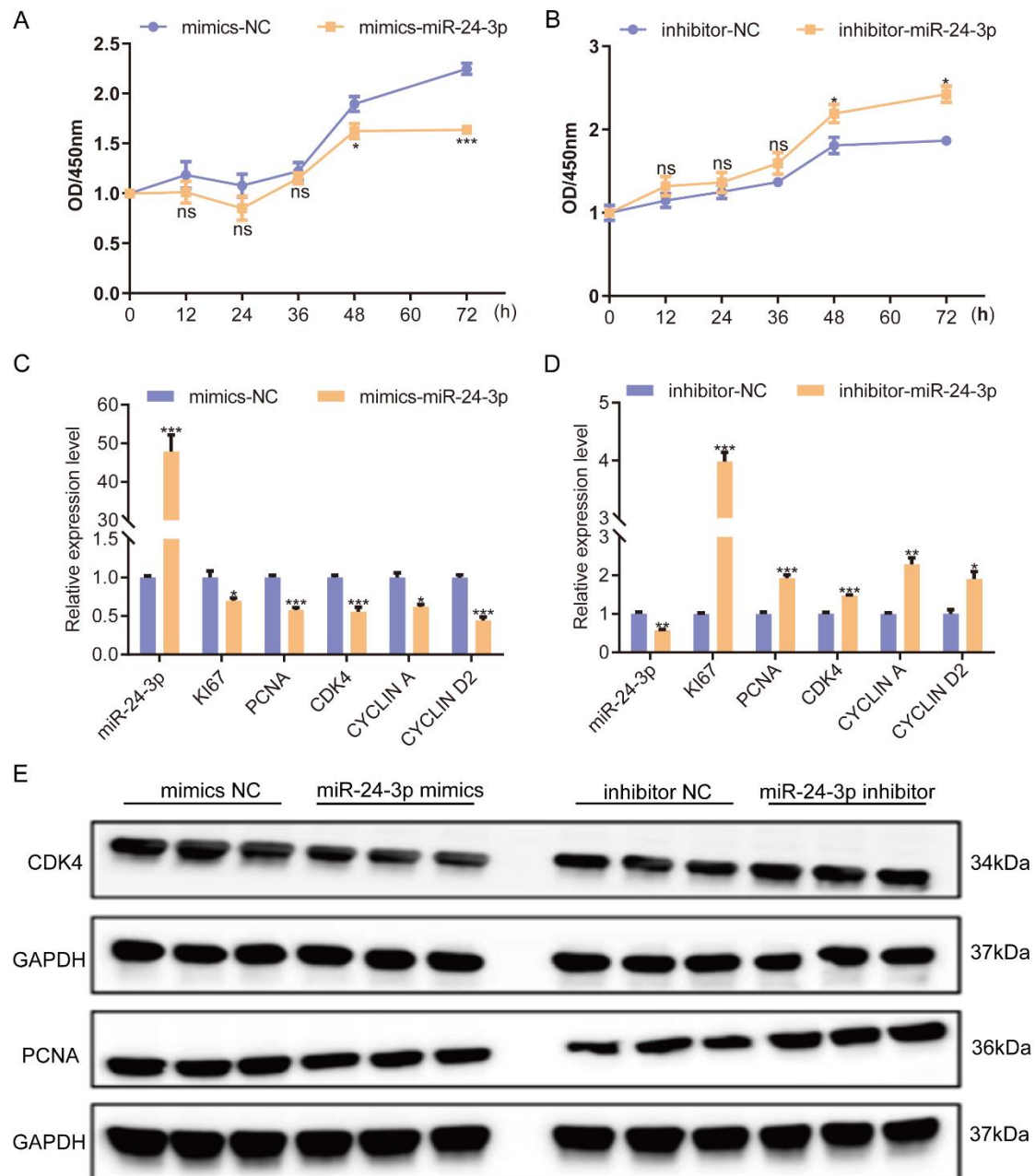
Pig-CYCLIN A	F: GCAGCAGCCTTTCATTTAGC R: GGTGAAGGTCCAGGAGACAA
Pig-CYCLIN D2	F: GTGCTGGGCAAGTTGAAGTG R: GCGAACTTGAAGTCAGTGGC
Pig-MyoD	F: ATGAGACATCCCCCTACTTCTACCA R: GTCCCCAGCCCCTTATCTTCC
Pig-MyoG	F: CCTGCTCAGCTCCCTCAAC R: CACAGCCACATCCTCCACT
Pig-MyHC	F: GTTCAGAGAAAGGCATCCCCAA R: GAGAGTGACCGACACCACAAGTG
Pig-MYH7	F: AAGGGCTTGAACGAGGAGTAGA R: TTATTCTGCTTCCTCCAAAGGG
Pig-MYH4	F: ATGAAGAGGAACCACATTA R: TTATTGCCTCAGTAGCTTG
Pig-MYH1	F: AGAAGATCAACTGAGTGAAC R: AGAGCTGAGAACTAACGTG
Pig-MAPK14	F: CTACAGAGAACTGCGGTTACT R: GTAAGCTTCTGACATTTACAA
Pig-NLK	F: TGGATATTGAGCCGGATAGA R: GCAAGAGACCAGATTCTGGAA
Pig-NEK4	F: CAATTAGATGCCTCTAATGAGCT R: CTCTAGTAATATTGTCAGAGG
Pig-PIM1	F: GCAAGACCTCTTCGACTTTATC R: CGATGAGGATGTTCTCGTCCTT
Pig-PSKH1	F: CATCATCGCCAAGGGTTCTTTCA R: TGCCTGGATGGTAGTAGAGCA
Mouse-U6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT
Mouse-Gapdh	F: CCTGTTGCTGTAGCCGTATT R: CATCAAGAAGGTGGTGAAGC
Mouse-Pax7	F: CCTGGAACAGACAGAGAGGAGCAGGAGAG R: GTGAGTTCCTTCACTCTGCGCTCGTGC
Mouse-Ki67	F: TGCCCGACCCTACAAAATG R: GAGCCTGTATCACTCATCTGC
Mouse-Pcna	F: GGGTGAAGTTTTCTGCAAGTG R: GTACCTCAGAGCAAACGTTAGG
Mouse-Cdk4	F: ACAAGTAATGGGACCGTCAAG R: GGGTGTTGCGTATGTAGACTG

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Mouse-Cyclin D1	F: GCCCTCCGTATCTTACTTCAAG R: GCGGTCCAGGTAGTTCATG
Mouse-Cyclin E1	F: GCGAGGATGAGAGCAGTTC R: AAGTCCTGTGCCAAGTAGAAC
Mouse-MyOD	F: CCAGCACTACATTTGGCGACTAAT R: GCTCCAATATGCTGGACAGGCAGT
Mouse-MyOG	F: GCCCAGTGAATGCAACTCCCACA R: CAGCCGCGAGCAAATGATCTCCT
Mouse-MyHC	F: ACAAGCTGCAGCTGAAGGTG R: TCATTCAGGCCCTTGGCAC
Mouse-Myh7	F: ACAAGCTGCAGCTGAAGGTG R: TCATTCAGGCCCTTGGCAC
Mouse-Myh1	F: TGCAACAGTTCTTCAACCAC R: GCCAGGTCCATCCCAAAGT
Mouse-Myh2	F: CCAGCTGCACCTTCTCGTTTGCCAG R: CATGGGGAAGATCTGGTCTTCTT
Mouse-Myh4	F: CCTGGAACAGACAGAGAGGAGCAGGAGAG R: GTGAGTTCCTTCACTCTGCGCTCGTGC
Mouse-Mapk14	F: GTTTCTCATCTACCAGATCCT R: TAGCCTGTCATCTCATCATCA
Mouse-Nlk	F: GAGTCTTCCGGGAATTGAAGAT R: AGATCACTCTGCATCAATTCT
Mouse-Nek4	F: ATTACATGAGCCCTGAGCTGTT R: GTGCTGTAAACTTTTGGCATT
Mouse-Pim1	F: TTCGGCTCGGTCTACTCTGGCAT R: ACCTTCTTCAACAGGACCA
Mouse-Pskh1	F: TATCAAGATGATAGAGACCA R: AGCTCCATCACCATATATACAC

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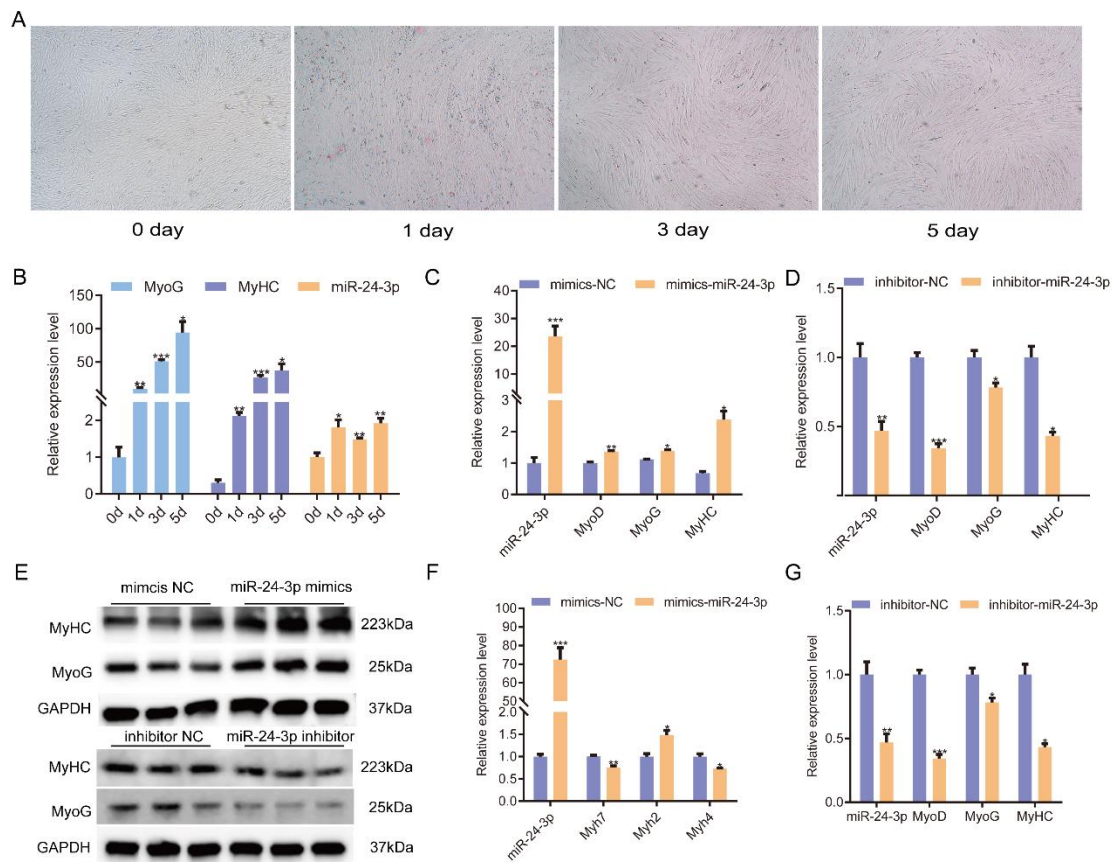


**Figure S2 Effect of miR-24-3p on C2C12 myoblasts differentiation**

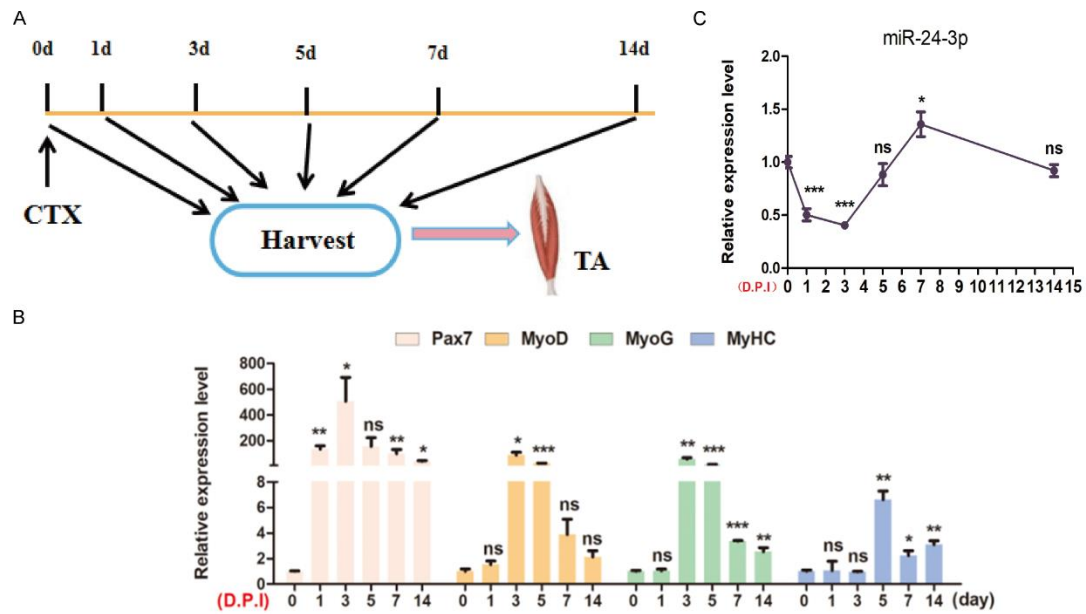
(A-B) CCK-8 analyzed cell proliferation after miR-24-3p overexpression and knockdown in PSMCs. (C-D) qRT-PCR analyzed cell proliferation markers (KI67, PCNA, CDK4, CYCLIN A and CYCLIN D2) mRNA expression after miR-24-3p overexpression and knockdown in PSMCs. (E) Western blot analyzed proliferation markers (CDK4 and PCNA) protein expression after miR-24-3p overexpression and knockdown in PSMCs. Data are presented as mean  $\pm$  S.E.M and analyzed for statistical differences between groups using unpaired two-tailed t-tests.  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ , *ns* (not significant).

		<u>seed sequence</u>
Pig	ssc-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG
Mouse	mmu-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG
Rat	rno-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG
Human	hsa-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG
Rhesus	mml-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG
Cow	bta-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG
Chicken	gga-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG
		*****

**Figure S3 conservation analysis of miR-24-3p sequence in different species**

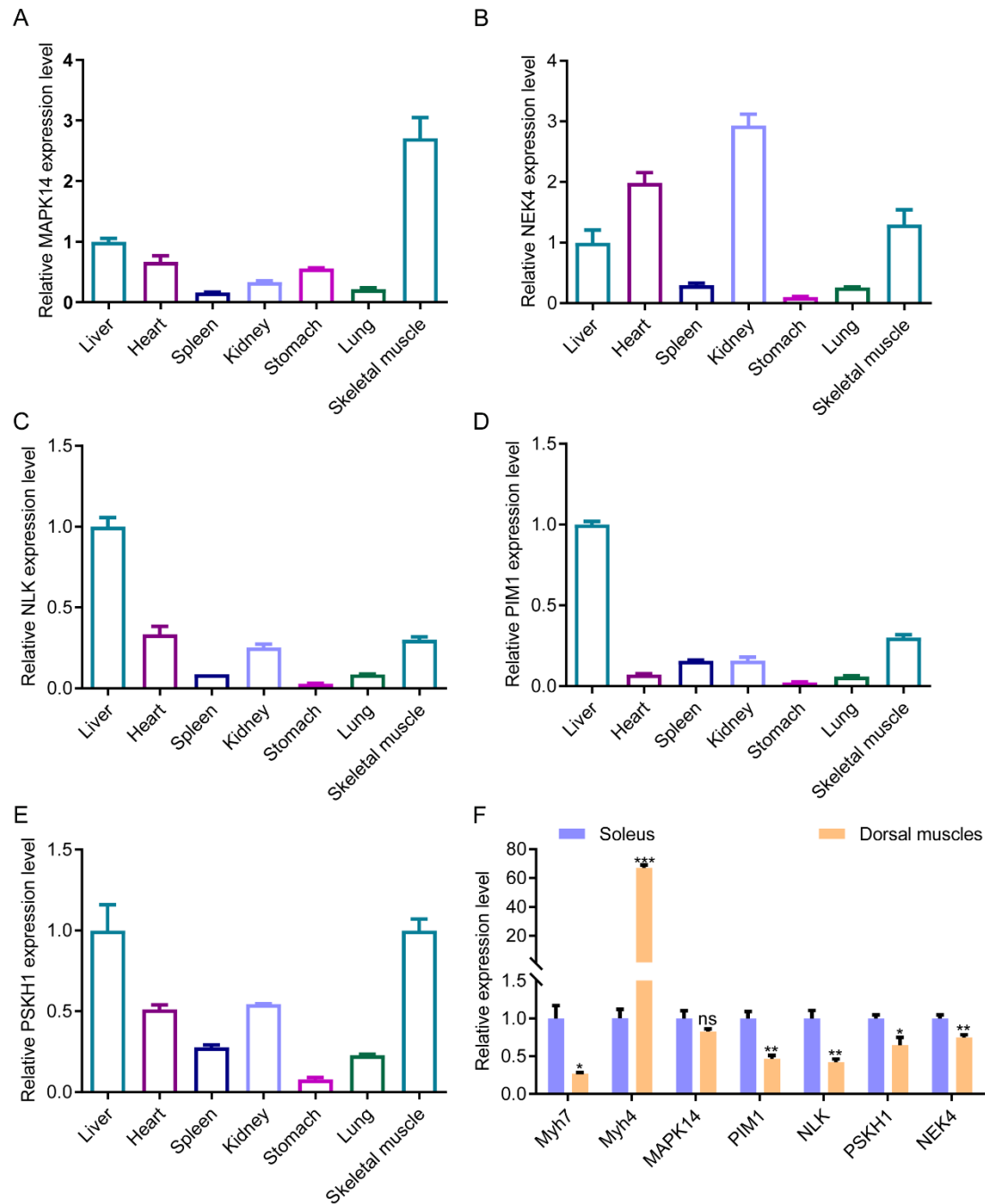


**Figure S4 Effect of miR-24-3p on skeletal muscle fiber type transformation in pig**  
 (A) PSMCs were induced to differentiate for different days (0d, 1d, 3d and 5d). Scale bar: 50  $\mu$ m.  
 (B) qRT-PCR analyzed the expression pattern of MyoG, MyHC and miR-24-3p during PSMCs differentiation. (C-D) qRT-PCR analyzed the expression of miR-24-3p, MyoG, MyHC and MyoD after miR-24-3p overexpression and knockdown. (E) Western blot analyzed the protein expression of MyoG and MyHC after miR-24-3p overexpression and knockdown. (F-G) qRT-PCR analyzed the expression of differentiation and transformation markers expression after miR-24-3p overexpression and knockdown. Data are presented as mean  $\pm$  S.E.M and analyzed for statistical differences between groups using unpaired two-tailed t-tests. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**Figure S5 The miR-24-3p expression during skeletal muscle regeneration**

(A) Schematic representation of the skeletal muscle regeneration. (B-C) qRT-PCR analyzed the expression pattern of Pax7, MyoD, MyoG, MyHC and miR-24-3p during skeletal muscle regeneration. Data are presented as mean  $\pm$  S.E.M and analyzed for statistical differences between groups using unpaired two-tailed t-tests. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , *ns* (not significant)



**Figure S6 The effects of ssc-miR-24-3p on proliferation marker genes of porcine skeletal muscle primary cells**

The expression levels of MAPK14 (A), NEK4 (B), PSKH1 (C), PIM1 (D) and NLK (E) in pig tissues. (F) Expression levels of potential target genes of ssc-miR-24-3p in pig fast and slow muscle. Data are presented as mean  $\pm$  S.E.M and analyzed for statistical differences between groups using unpaired two-tailed t-tests. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ,  $ns$  (not significant).

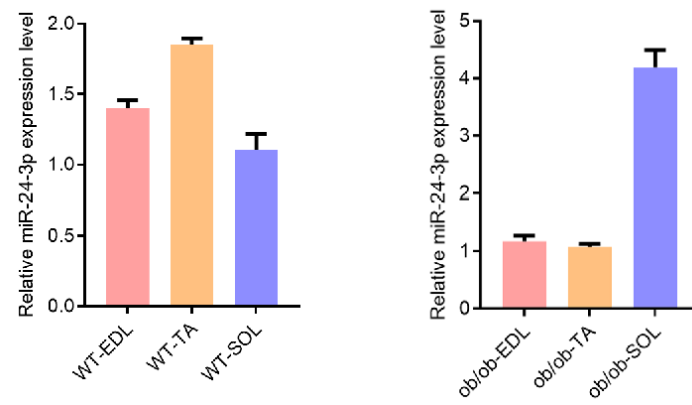


Figure S7: Fast and slow muscle expression in wild mice and ob/ob mice.