1	WNT5B regulates myogenesis and fiber type conversion by affecting mRNA stability
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- 32 (A) RNA-seq analysis of the expression level of *WNT11* changes at 27 different developmental time points.
- 33 (B) qRT-PCR analysis of *WNT5B* expression in the cytoplasm and nucleus of Tongcheng pig myoblasts.

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А	<u>m</u>		V	Ĭ	100	V		Ŷ	WW	_γ_		>	seed sequence
	130		50 170	180	190	ZUU	210	220	230	240		hsa-miR-29a	UAGCACCAUCUGAAAUCGGUUA
		MOUSE_PROTEIN.tx PIG_PROTEIN.txt	MPSLLL MPSppLLL	vvvAALLS: aaaAALLa: aall	SWAQLLID SWAQLLID SWApLpsel	ANSWWSLA ANSWWSLA Asswwsla	LNPVQRPE mNPVQRPE	38 40				mmu-miR-29a	UAGCACCAUCUGAAAUCGGUUA
		HUMAN_PROTEIN.t.	t MFIIGAQP	VCSQLPGL:	SPGQRKLC	OLYQEHMA OLYQEHMS	YIGEGAKT	78 78				ssc-miR-29a	UAGCACCAUCUGAAAUCGGUUA
		PIG_PROTEIN.txt Consensus	MFIIGAQP mfiigaqp	VCSQLPGL vcsqlpgl	SPGQRKLCO	QLYQEHMA qlyqehm	YIGEGArT yigega t	80				rno-miR-29a	UAGCACCAUCUGAAAUCGGUUA
		HUMAN_PROTEIN.tx MOUSE_PROTEIN.tx PIG_PROTEIN.txt Consensus	t GIKECOHO t GIRECOHO GIKECOHO gi ecqhq	FRORRWNC: FRORRWNC: FRORRWNC: frqrrwnc:	STADNASVI STVDNtSVI STVgNASVI st n svi	FGRVMQIG FGRVMQIG FGRVvQIG fgrv qig	SRETAFTH SRETAFTY SRETAFTY sretaft	118 118 120				cfa-miR-29a	UA <mark>GCACCA</mark> UCUGAAAUCGGUUA
		HUMAN_PROTEIN.t; MOUSE_PROTEIN.t; PIG_PROTEIN.txt Consensus	t AVSAAGVVI t AVSAAGVVI xVSAAGVVI	NAISRACRI NAISRACRI NAISRACRI	EGELSTCGC EGELSTCGC EGELSTCGC	CSRTARPK CSRaARPK CSRxARPK	DLPRDWLW DLPRDWLW DLPRDWLW dlprdwlw	158 158 160				hsa-miR-29b	UAGCACCAUUUGAAAUCAGUGU
		HUMAN_PROTEIN.to MOUSE_PROTEIN.to	t GGCGDNVE	YGYRFAKE YGYRFAKE	FVDAREREN	KNFAKGSE KNFAKGSE	EQGRVLMN EQGRaLMN	198 198				mmu-miR-29b	UA <mark>GCACCA</mark> UUUGAAAUCAGUGU
		PIG_PROTEIN.txt Consensus	GGCGDNVE ggcgdnve	YGYRFAKE ygyrfake	FVDARERE fvdarere)	KNFAKGSE knfakgse	EQGRVLMN eqgr 1mn	200				ssc-miR-29b	UAGCACCAUUUGAAAUCAGUGU
		HUMAN_PROTEIN.t> MOUSE_PROTEIN.t> PIG_PROTEIN.txt	LQNNEAGRI LQNNEAGRI	RAVYKMAD' RAVYKMAD' RAVYKMAD'	VACKCHGVS VACKCHGVS VACKCHGVS	SGSCSLKT SGSCSLKT SGSCSLKT	CWLQLAEF CWLQLAEF CWLQLAEF	238 238 240				rno-miR-29b	UAGCACCAUUUGAAAUCAGUGU
		HUMAN_PROTEIN.t>	lqnneagr: t RKVGDRLK	EKYDSAAA	WACKChgvs	LELVNSRF	CW1q1aef TQPTPEDL	278				cfa-miR-29b	UAGCACCAUUUGAAAUCAGUGU
		PIG_PROTEIN.txt Consensus	RKVGDRLKI RKVGDqLKI rkvgd lk	EKYDSAAA EKYDSAAA ekydsaaa	MRITRIGKI mr tr g l	LELVNSRF	nQPTPEDL qptpedl	280				haa-miD-90a	
		HUMAN_PROTEIN.t>	t VYVDPSPD	YCLRNEST YCLRNETT	GSLGTQGRI GSLGTQGRI	LCNKTSEG	MDGCELMC MDGCELMC	318 318				nsa-mik-29c	
		Consensus	vyvdpspd	yclrne to	gslgtqgrl	lonktseg	mdgc 1mc	320				mmu-m1R-29c	UAGCACCAUUUGAAAUCGGUUA
		HUMAN_PROTEIN.to MOUSE_PROTEIN.to PIG_PROTEIN.txt	t CGRGYNQFI t CGRGYdrFi CGRGYdQFi	KSVQVERCI KSVQVERCI KSVQVERCI	HCKFHWCCH HCrFHWCCH HCKFHWCCH	FVRCKKCT FVRCKKCT FVRCKKCT	EIVDQYIC EvVDQYvC EvVDQfvC	358 358 360				ssc-miR-29c	UAGCACCAUUUGAAAUCGGUUA
		Consensus HUMAN_PROTEIN.t:	cgrgy fi	ksvqverc	hc fhwcci	fvrckkct	e vdq c	359				rno-miR-29c	UAGCACCAUUUGAAAUCGGUUA
		PIG_PROTEIN.txt Consensus	K K k					359 361				cfa-miR-29c	UAGCACCAUUUGAAAUCGGUUA

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- 45 Figure S2. Analysis of *WNT5B* 3'UTR and miR-29a/b/c sequences in different species.
- 46 (A) Conservation analysis of the *WNT5B* across different species.
- 47 (B) Conservation analysis of the miR-29a/b/c across different species.



- 50 (A-C) The results of CCK-8 (A), cell cycle (B) and cell proliferation status (C) of C2C12 myoblasts after
- 51 transfection with pcDNA3.1 and pcDNA3.1-*WNT5B* vectors. Scale bar, 50 μm.
- 52 (D-E) mRNA (D) and protein (E) expression levels of proliferation marker genes in C2C12 myoblasts after
- 53 *WNT5B* overexpression.
- 54 (F-H) The results of CCK-8 (F), cell cycle (G) and cell proliferation status (H) of in C2C12 myoblasts after
- 55 transfection with siRNA-NC and siRNA-*WNT5B*. Scale bar, 50 μm.
- 56 (I-J) mRNA (I) and protein (J) expression levels of proliferation marker genes in C2C12 myoblasts after
- 57 *WNT5B* knockdown.
- 58 Data are presented as mean ± SEM and analyzed for statistical differences between groups using unpaired
- two-tailed t-tests. *p < 0.05, **p < 0.01, ***p < 0.001, ns means no significant differences.

⁴⁹ Figure S3. The effects of *WNT5B* on the cell proliferation and cell cycle of C2C12 myoblasts *in vitro*



61 Figure S4. In vitro experiments on the effects of WNT5B on cell apoptosis in C2C12 myoblasts

- 62 (A) The results of cell apoptosis of C2C12 myoblasts after *WNT5B* overexpression.
- 63 (B-C) mRNA (B) and protein (C) expression levels of cell apoptosis markers genes in C2C12 myoblasts
- 64 after overexpression of *WNT5B*.
- 65 (D-E) The results of cell apoptosis of C2C12 myoblasts after *WNT5B* knockdown.
- 66 (F-G) mRNA (F) and protein (G) expression levels of cell apoptosis markers genes in C2C12 myoblasts after
- 67 knockdown of *WNT5B*.
- 68 Data are presented as mean ± SEM and analyzed for statistical differences between groups using unpaired
- 69 two-tailed t-tests. *p < 0.05, **p < 0.01, ***p < 0.001, ns means no significant differences.



71 Figure S5. RNAfold tool to analyze the effects of ΔARE1 and ΔARE2 on *WNT5B* mRNA structure

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Position 482-488 of WNT5B 3' UTR		GUUCCGUAAGAGGCC <mark>UGGUG</mark> CUC	
hsa-miR-29a-3p	3'	AUUGGCUAAAGUCUACCACGAU	
Position 482-488 of WNT5B 3' UTR	5'	GUUCCGUAAGAGGCCU <mark>GGUG</mark> CUC	
hsa-miR-29b-3p	3'	UUGUGACUAAAGUUUACCACGAU	

Binding sites

В CAGTGTTCCTGCTGTGGTGCAGTGGGTTAAG PIG MOUSE GAAUGGAUGGAUGGTGGTGCAAUAAUCCAAG

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78	Figure S6. Analysis of WNT5B 3'UTR sequences
79	(A) Prediction of binding sites between the miR-29a/b/c and WNT5B using Target Scan.
80	(B) Amplification of miR-29a/b/c binding sites in the WNT5B 3'UTR.
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91 Figure S7. The efficiency of overexpression and knockdown of miR-29/b/c.

- 92 (A-B) The efficiency of overexpression (A) and knockdown (B) of miR-29/b/c in porcine myoblasts.
- 93 (C-D) The efficiency of overexpression (C) and knockdown (D) of miR-29/b/c in C2C12 myoblasts.



96 Figure S8. The effects of miR-29a/b/c overexpression on cell proliferation, cell cycle and cell apoptosis

97 in porcine myoblasts

- 98 (A-C) The results of cell proliferation (A), cell cycle (B), and cell apoptosis (C) after transfection with miR-
- 99 29a/b/c mimics in porcine skeletal muscle cells. Scale bar, 50 μm.
- 100 (D-F) Quantitative results of cell proliferation (D), cell cycle (E), and cell apoptosis (F).
- 101 (G-H) The mRNA expression of cell cycle (G) and cell apoptosis (H) markers expression after miR-29a/b/c
- 102 overexpression in porcine skeletal muscle cells.
- 103 (I) The protein expression of cell cycle and cell apoptosis markers after miR-29a/b/c overexpression in
- 104 porcine skeletal muscle cells.
- 105 Data are presented as mean ± SEM and analyzed for statistical differences between groups using unpaired
- 106 two-tailed t-tests. *p < 0.05, **p < 0.01, ***p < 0.001, ns means no significant differences.



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Figure S9. The effects of miR-29a/b/c on cell proliferation, cell cycle and cell apoptosis in C2C12
myoblasts

- 110 (A-B) The results of EdU-staining in cell proliferation after miR-29a/b/c overexpression (A) and knockdown
- 111 (B) in C2C12 myoblasts. Scale bar, 50 μ m.

112	(C-D) Cell cycle results (C) and the mRNA (D) expression of cell proliferation marker gene after miR-
113	29a/b/c overexpression in C2C12 myoblasts.
114	(E-F) Cell cycle results (E) and the mRNA (F) expression of cell proliferation marker gene after miR-29a/b/c
115	knockdown in C2C12 myoblasts.
116	(G-H) Cell apoptosis results (G) and the mRNA (H) expression of cell apoptosis marker gene after miR-
117	29a/b/c overexpression in C2C12 myoblasts.
118	(I-J) Cell apoptosis results (E) and the mRNA (F) expression of cell apoptosis marker gene after miR-29a/b/c
119	knockdown in C2C12 myoblasts.
120	(K-L) The protein expression of Cyclin A2, BAX, and BCL2 after miR-29a/b/c overexpression (K) and
121	knockdown (L) in C2C12 myoblasts.
122	Data are presented as mean \pm SEM and analyzed for statistical differences between groups using unpaired
123	two-tailed t-tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns means no significant differences.
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Figure S10. miR29 family targeting *WNT5B* regulates C2C12 myoblast proliferation, cell cycle, and
cell apoptosis

139 (A-C) The results of cell proliferation (A), cell cycle (B), and cell apoptosis (C) after co-transfection with

140 miR-29a/b/c inhibitor and WNT5B siRNA in C2C12 myoblast. Scale bar, 50 μm.

141 (D-F) Quantitative results of cell proliferation (D), cell cycle (E), and cell apoptosis (F).

142 (G-H) The expression of cell cycle (G) and cell apoptosis markers (H) in mRNA level after co-transfection

143 with miR-29a/b/c inhibitor and *WNT5B* siRNA in C2C12 myoblast.

144 Data are presented as mean \pm SEM and analyzed for statistical differences between groups using unpaired

145 two-tailed t-tests. *p < 0.05, **p < 0.01, ***p < 0.001, ns means no significant differences.

147 Supplementary Tables

148 Table S1. Small RNA was used in this manuscript

Name	Target sequence (5'-3')
miR-29c-mimics	UAGCACCAUUUGAAAUCGGUUA
miR-29c-inhibitor	UAACCGAUUUCAAAUGGUGCUA
miR-29b-mimics	UAGCACCAUUUGAAAUCAGUGUU
miR-29b-inhibitor	AACACUGAUUUCAAAUGGUGCUA
miR-29a-mimics	CUAGCACCAUCUGAAAUCGGUUA
miR-29a-inhibitor	UAACCGAUUUCAGAUGGUGCUAG
mimics NC	UUGUACUACAAAAAGUACUG
Inhibitor NC	CAGUACUUUUGUGUAGUACAA
mmu-siRNA-NC	GCGACGAUCUGCCUAAGAU
mmu-siRNA-WNT5B	GGGUGAGUUGCACAGUGAAUC UUCACUGUGCAACUCACCCUG
ssc-siRNA-NC	UGCUCAGACUCGUAACUG
ssc-siRNA-WNT5B	GGUGAGUUGCACAGUGAAUCG AUUCACUGUGCAACUCACCCU

Forward primer (5'-3') Name **Reverse primer (5'-3')** TCTAGATACACTGCAGCTCATGGCA GCTAGCACCAAGGGATATCCACCA WNT5B-ARE TA ACAT CCTTGGCTTTAGTTGCTAGCATGTA GCTAGCAACTAAAGCCAAGGA WNT5B-ARE1 ACCAATAAACCAGCCAG CTGGGGAACCCAACATGTACTTAT TCTAGATACACTGCAGCTCATGGCA WNT5B-ARE2 ATTAGGTGCTCAAAGTGCA ACAT

172 Table S2. Primers for *WNT5B* 3'UTR dual luciferase reporter vector construction in this manuscript

Name	Forward primer (5'-3')	Reverse primer (5'-3')
mmu-WNT5B	CTGCTGACTGACGCCAACT	CCTGATACAACTGACACAGCTTT
mmu-CDK4	GAAGCCAGAGAACATTCTAGTGAC	TCGAGGCCAGTCGTCTTCT
mmu-Cyclin A2	TTACCCGGAGCAAGAAAAC	TCTGGCTGCCTCTTCATG
mmu-Cyclin D	AATGCCAGAGGCGGATGA	AAAATGCCAGAGGCGGATGA
mmu-BAX	GTGATGGCATGGGACATAGCTC	TGGCGTAGACCTTGCGGATAA
mmu-BCL2	GCAGGCAGCTTGAAAGAAAC	GCTGGCCTTTCATGACTCTC
mmu-CASP3	CTGCGGCGGGGGGGGCT	GGTTGGCTGCGTCCACAT
mmu-GAPDH	GGTTGTCTCCTGCGACTTCA	TGGTCCAGGGTTTCTTACTCC
ssc-WNT5B	GGTGGTCCTTGGCCATGA	AGGCTACGTCTGCCATCTTATAC
ssc-CDK4	GCGTAAGAGTCCCCAATGGA	AGACATCCATCAGCCGGACA
ssc-Cyclin A2	TCTATGGCGGAAGTTCTTGCT	CACTGCCCATGCTGGTAGAA
ssc-BAX	GCCCTTTTGCTTCAGGGTTTC	GCCCTTTTGCTTCAGGGTTTC
ssc-BCL2	GGATAACGGAGGCTGGGATG	TTATGGCCCAGATAGGCACC
ssc-CASP3	CTGGCGAAATTCAAAGGAC	AACCATTTCCTCATTTCACATAC
ssc-MYHC	GTTCAGAGAAAGGCATCCCAAA	GAGAGTGACCGACACCACAAGTG
ssc-MYH7	AAGGGCTTGAACGAGGAGTAGA	TTATTCTGCTTCCTCCAAAGGG
ssc-MYH4	ATGAAGAGGAACCACATTA	TTATTGCCTCAGTAGCTTG
ssc-TNNI1	CCCACAGTCTGCAGTCCAC	CCAGCATCAGGCCCTTCAG
ssc-TNNI2	TCCAGGAGCTCTGCAAACAG	GGTTCATGTCCTCCAGCTCC
ssc-TNNT1	CCAAGCCAAGCCGTCCC	CAATACGCTCTTTCAGCGCC
ssc-TNNT3	CATCATCGCCAAGGGTTCTTTCA	TGCCTGGATGGTAGTAGAGCA
ssc-NEAT1	GTCGATGCCCTGAACATG	GTCGATGCCCTGAACATG
ssc-GAPDH	TTATGGCCCAGATAGGCACC	TTATGGCCCAGATAGGCACC
miR-29a	GCGCTAGCACCATCTGAAAT	AGTGCAGGGTCCGAGGTATT
miR-29b	CGCGTAGCACCATTTGAAATC	AGTGCAGGGTCCGAGGTATT
miR-29c	CGCGTAGCACCATTTGAAAT	AGTGCAGGGTCCGAGGTATT

203 Table S3. qRT-PCR Primers used in this manuscript