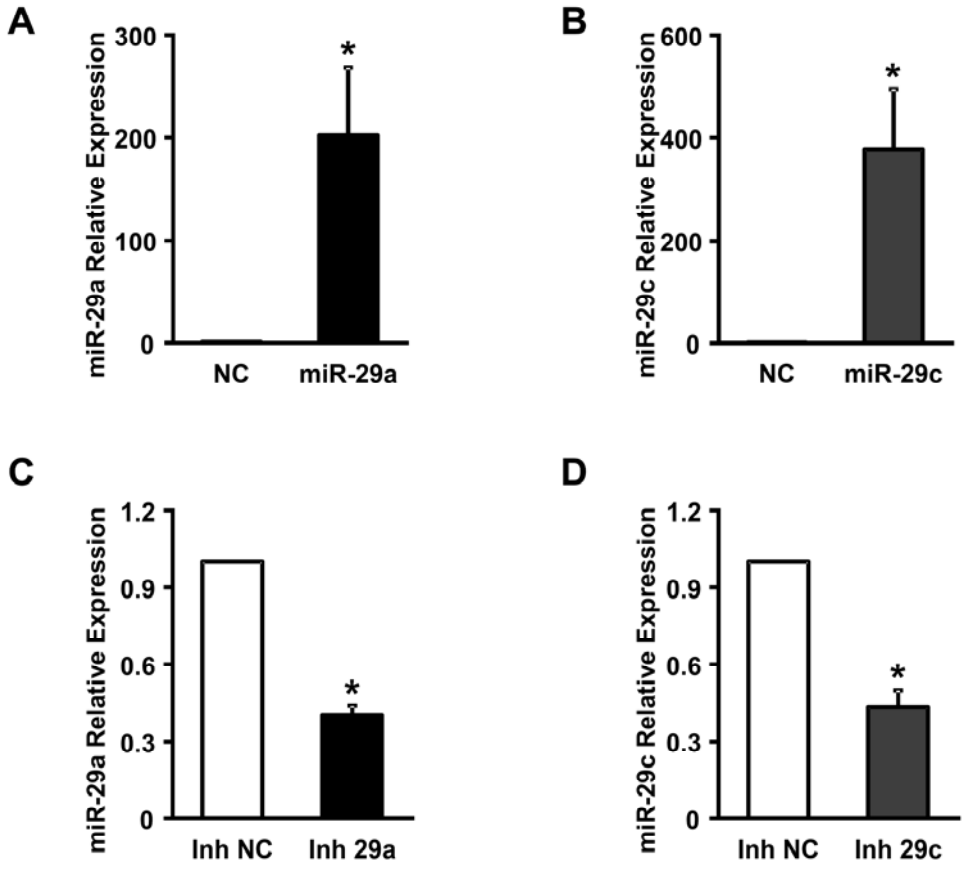
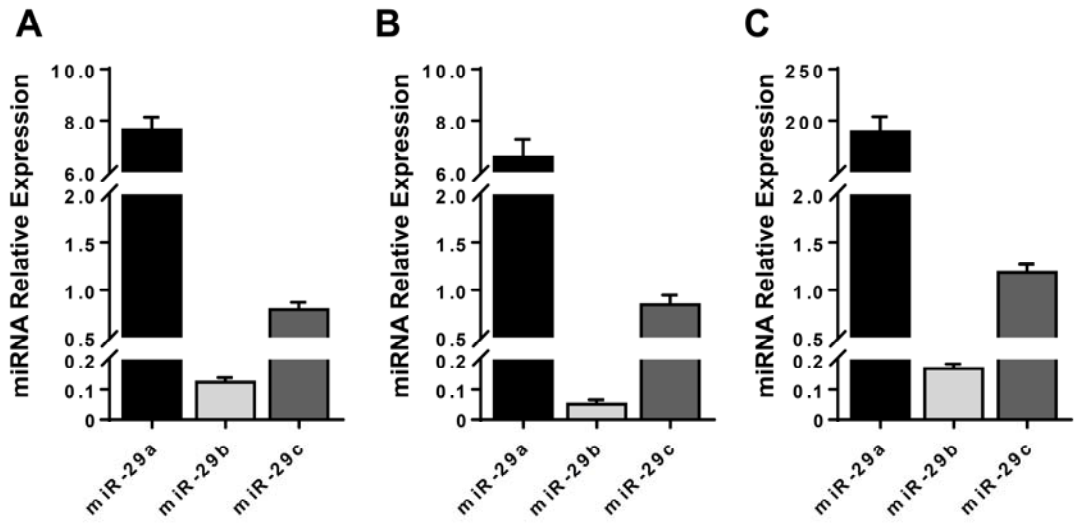
SUPPLEMENTARY DATA

Supplementary Figure 1. Transfection efficiency in human primary skeletal muscle myotubes. Overexpression level of (A) miR-29a and (B) miR-29c was determined by qPCR (n=6). Transfection efficiency using (C) miR-29a inhibitor or (D) miR-29c inhibitor was estimated by qPCR, reflecting the inhibition capacities (n=6). Data is presented as mean ± SEM. *p<0.05.



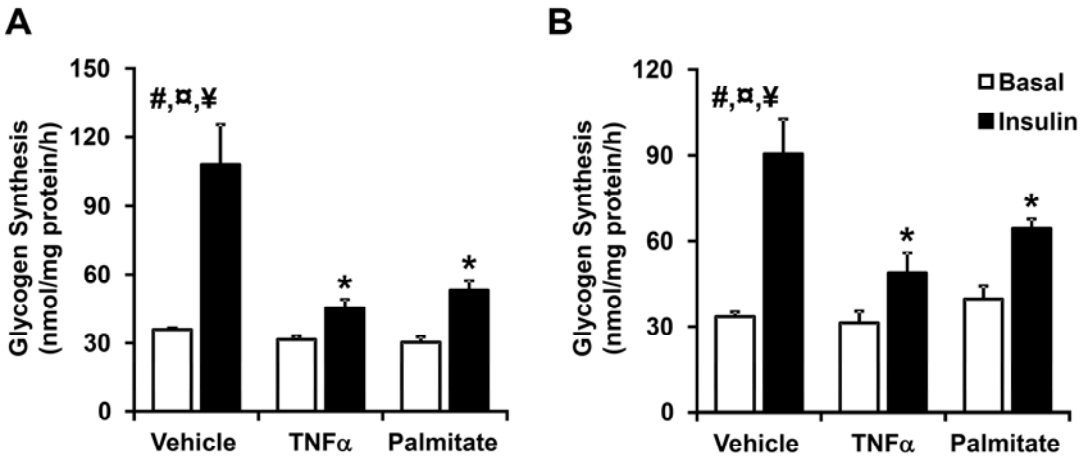
SUPPLEMENTARY DATA

Supplementary Figure 2. Relative expression of miR-29 family members in skeletal muscle. The relative expression of miR-29a, miR-29b and miR-29c was determined in (A) human *vastus lateralis* muscle (n=10), (B) primary human myotubes (n=6) and (C) mouse *tibialis anterior* muscle (n=10). Data is presented as mean ± SEM.



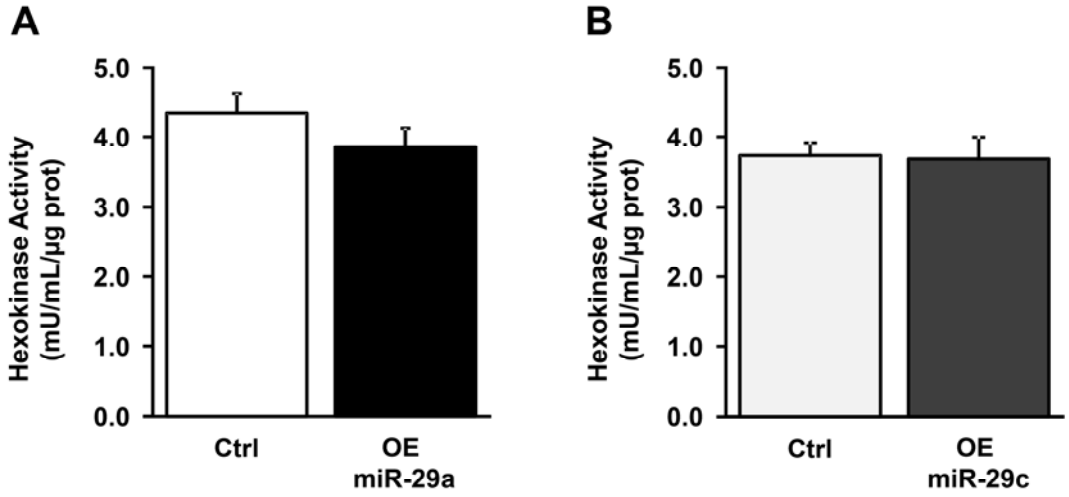
SUPPLEMENTARY DATA

Supplementary Figure 3. Effect of exposure to either TNF or palmitate on insulin-stimulated glucose incorporation into glycogen. Primary human muscle cells were treated for with either TNFα or palmitate (A) 24 h (40 ng/ml TNFα; 0.2 mM palmitate) or (B) 96 h (20 ng/ml TNFα; 0.1 mM palmitate) and ^{14}C-glucose incorporation into glycogen was assessed in the absence or presence of insulin (120 nM) (n=4). #: Transfection effect; ¤: Insulin effect; ¥: Interaction. Data is presented as mean ± SEM. *p<0.05.



SUPPLEMENTARY DATA

Supplementary Figure 4. Hexokinase activity in mouse *tibalis anterior* muscle. Hexokinase activity was assessed following overexpression of either (A) miR-29a or (B) miR-29c (n=10). Data is presented as mean ± SEM.



SUPPLEMENTARY DATA

Supplementary Table 1. Primer Sequences

Mouse primers			Human primers		
mIrs1	**Fw**	GCCAGAGGATCGTCAATAGC	**hIRS1**	**Fw**	TATGCCAGCATCAGTTTCCA
	Rev	GAGGAAGACGTGAGGTCCTG		**Rev**	GGATTTGCTGAGGTCATTTAGG
mPik3r3	**Fw**	GTGATGCCTCAACGAAAATG	**hPIK3R1**	**Fw**	TGGACGGCGAAGTAAAGCATT
	Rev	AAGTAAACGTCAGGGGCTCA		**Rev**	AGTGTGACATTGAGGGAGTCG
mPik3r1	**Fw**	ACGCTATCTCGCCGACTTAC	**hPIK3R3**	**Fw**	AGACTGGAGGGAGGTGATGA
	Rev	CAGCTGGATGCAGTCTTCAG		**Rev**	TTAGGTGGCTTTGGTGGAAG
mAkt2	**Fw**	CGCCTCTTTGAGCTCATTCT	**hAKT2**	**Fw**	AGGCACGGGCTAAAGTGAC
	Rev	ATGACCTCCTTCGCATCACT		**Rev**	CTGTGTGAGCGACTTCATCCT
mSlc2a4	**Fw**	ACACTGGTCCTAGCTGTATTCT	**hGSK3b**	**Fw**	AGACGCTCCCTGTGATTTATGT
	Rev	CCAGCCACGTTGCATTGTA		**Rev**	CCGATGGCAGATTCCAAAGG
mHk2	**Fw**	ATGATCGCCTGCTTATTCACG	**hHK2**	**Fw**	GCACCCAGCTGTTTGACCA
	Rev	CGCCTAGAAATCTCCAGAAGGG		**Rev**	CAACGTCTCTGCCTTCCACT
mSlc2A1	**Fw**	GCAGTTCGGCTATAACACTGG	**hSLC2A1**	**Fw**	GGCCAAGAGTGTGCTAAAGAA
	Rev	GCGGTGGTTCCATGTTTGATTG		**Rev**	ACAGCGTTGATGCCAGACAG
mCd36	**Fw**	ATGGGCTGTGATCGGAACTG	**hFABP3**	**Fw**	TGGAGTTCGATGAGACAACAGC
	Rev	TTTGCCACGTCATCTGGGTTT		**Rev**	CTCTTGCCCGTCCCATTTCTG
mPpargc1a	**Fw**	TATGGAGTGACATAGAGTGTGCT	**hPPARGC1A**	**Fw**	TCTGAGTCTGTATGGAGTGACAT
	Rev	CCACTTCAATCCACCCAGAAAG		**Rev**	CCAAGTCGTTCACATCTAGTTCA
mPdk4	**Fw**	AGGGAGGTCGAGCTGTTCTC	**hPDK4**	**Fw**	GGAAGCATTGATCCTAACTGTGA
	Rev	GGAGTGTTCACTAAGCGGTCA		**Rev**	GGTGAGAAGGAACATACACGATG
mTbp	**Fw**	CCTTGTACCCTTCACCAATGAC	**hTBP**	**Fw**	AACAACAGCCTGCCACCTTA
	Rev	ACAGCCAAGATTCACGGTAGA		**Rev**	GCCATAAGGCATCATTGGAC
mRplp0	**Fw**	AGATTCGGGATATGCTGTTGGC	**hRPLP0**	**Fw**	AGCCCAGAACACTGGTCTC
	Rev	TCGGGTCCTAGACCAGTGTTC		**Rev**	ACTCAGGATTTCAATGGTGCC

SUPPLEMENTARY DATA

Supplementary Table 2. Antibodies Used

Target	Cat. number	Company
Akt	9272	Cell Signaling
Phospho-Akt (Ser473)	9271	Cell Signaling
Phospho-Akt (Thr308)	4056	Cell Signaling
GSK-3α/β	5676	Cell Signaling
Phospho-GSK-3α/β (Ser21/9)	9331	Cell Signaling
IRS1	06-248	Merck Millipore
Phospho-IRS1 (Tyr612)	44816G	Thermo Fisher Scientific

SUPPLEMENTARY DATA

Supplementary Table 3. Quantification of Western blot analysis of primary human myotubes (A.U.)

	Basal			Insulin		
	NC	miR-29a	miR-29c	NC	miR-29a	miR-29c
pAkt(Thr308) ¤	0.02 ± 0.05	0.04 ± 0.06	0.07 ± 0.08	8.61 ± 1.47	9.47 ± 1.89	7.10 ± 1.50
pAkt(Ser473) #, ¤, ¥	0.01 ± 0.03	0.05 ± 0.06	0.09 ± 0.03	8.91 ± 1.63	7.61 ± 1.09*	6.66 ± 0.93*
pIRS1(Tyr612) #, ¤, ¥	0.63 ± 0.19	0.38 ± 0.19	0.43 ± 0.17	2.90 ± 0.64	4.13 ± 1.09*	4.03 ± 0.87*
pGSK3α(Ser21) #, ¤, ¥	0.50 ± 0.08	0.31 ± 0.05	0.36 ± 0.06	4.42 ± 0.60	3.92 ± 0.71	2.99 ± 0.40*
pGSK3β(Ser9) #, ¤, ¥	0.67 ± 0.10	0.35 ± 0.07	0.42 ± 0.07	4.53 ± 0.74	3.52 ± 0.58*	3.01 ± 0.44*

Myotubes were incubated in the absence (Basal) or presence of insulin (120 nM) for 10 min and phosphorylation of components of the canonical insulin signaling cascade were assessed by Western Blot. Myotubes were transfected with 20 nM of miR-29a or miR-29c Pre-miRNA Precursors or with negative control (NC) miRNA. Results are mean ± SEM. Quantification of data reported in Fig 2E for n=8. #: Transfection effect; ¤: Treatment effect; ¥: Interaction. * $p<0.05$ versus NC (Bonferroni *post hoc* test).

SUPPLEMENTARY DATA

Supplementary Table 4. Quantification of Western blot analysis of primary human myotubes (A.U.)

	NC	miR-29a	miR-29c
Akt	4.08 ± 0.13	4.25 ± 0.09	4.16 ± 0.13
IRS1	2.37 ± 0.30	1.96 ± 0.21*	1.92 ± 0.18*
GSK3α	4.07 ± 0.19	4.27 ± 0.20	4.16 ± 0.27
GSK3β	4.60 ± 0.36	3.99 ± 0.33*	3.92 ± 0.35*

Myotubes were transfected with 20 nM of miR-29a or miR-29c Pre-miRNA Precursors or with negative control (NC) miRNA and protein abundance of components of the canonical insulin signaling cascade were assessed by Western Blot. Results are mean ± SEM. Quantification of data reported in Fig 2E for n=8. * $p<0.05$ versus NC (paired Student's t-test).

SUPPLEMENTARY DATA

Supplementary Table 5. Quantification of Western blot analysis of *tibialis anterior* mouse muscle (A.U.)

	Ctrl	OE 29a	Ctrl	OE 29c
pIRS1(Tyr612)	4.81 ± 0.29	4.11 ± 0.09*	4.46 ± 0.25	3.88 ± 0.29*
IRS1	5.18 ± 0.72	4.00 ± 0.48*	4.48 ± 0.42	3.86 ± 0.37*
pAkt(Ser473)	5.39 ± 0.86	2.94 ± 0.47*	4.95 ± 0.85	3.38 ± 0.58*
pAkt(Thr308)	3.97 ± 0.57	4.10 ± 0.60	4.21 ± 0.54	4.12 ± 0.59
Akt	4.26 ± 0.25	4.07 ± 0.18	4.32 ± 0.44	4.02 ± 0.32
pGSK3α(Ser21)	3.41 ± 0.22	3.50 ± 0.28	4.14 ± 0.30	4.19 ± 0.29
pGSK3β(Ser9)	3.50 ± 0.30	3.83 ± 0.38	4.20 ± 0.26	4.14 ± 0.29
GSK3α	4.03 ± 0.18	4.30 ± 0.20	4.34 ± 0.21	3.99 ± 0.20
GSK3β	4.20 ± 0.22	4.13 ± 0.23	2.10 ± 0.12	2.07 ± 0.11

Mouse *tibialis anterior* muscle was electroporated with vectors expressing either pri-miR-29a (OE 29a) or pri-miR-29c (OE 29c) and a control vector in the contralateral leg (Ctrl). Protein phosphorylation and abundance of components of the canonical insulin signaling cascade were assessed by Western Blot. Results are mean ± SEM. Quantification of data reported in Fig. 3F for n=10. * $p<0.05$ versus respective contralateral control leg (paired Student's t-test).