Supplementary Material (ESI) for Molecular BioSystems

Alteration of microRNA expression correlates to fatty acid-mediated insulin resistance in mouse myoblasts

Supplemental Data

Correlation coefficient matrix		
	con	PA
PA	0.9425	1.0000
PA+OA	0.9407	0.9626

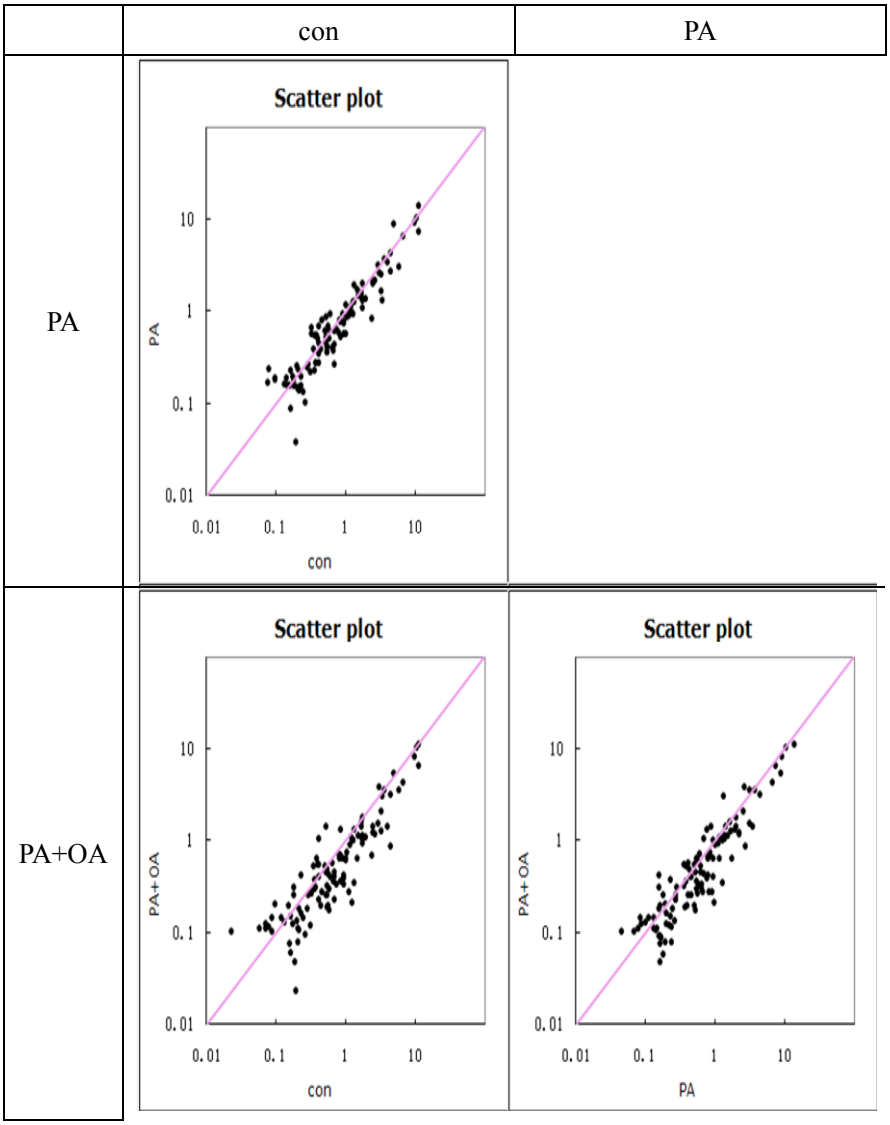

Figure S1 Correlation coefficient matrix

R-values were calculated after array normalization using the Median method. When two samples are the same, their correlation coefficient R reflects the reproducibility of the slides.

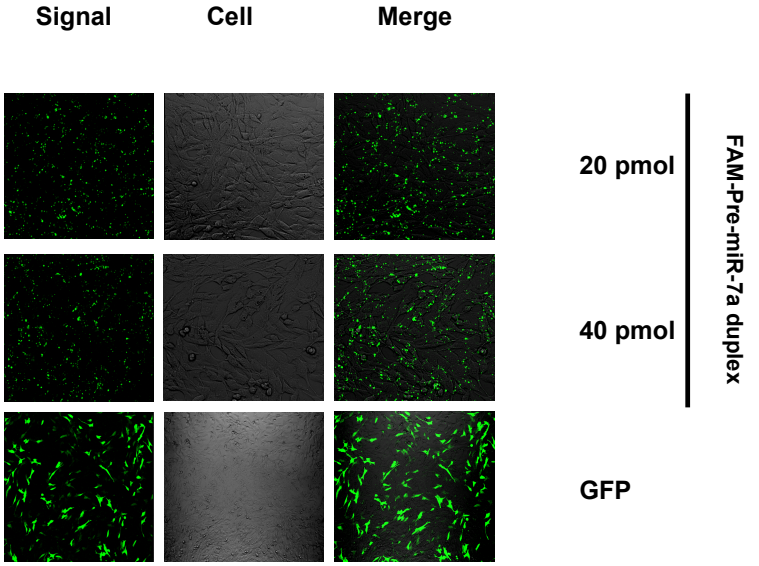

Figure S2 Determination of nucleofection efficiency and optimal conditions
C2C12 cells were transiently nucleofected with FAM-labeled pre-miR-7a siRNA duplex at the indicated amounts, and fluorescence images were obtained by fluorescence microscopy. A GFP vector was used as a positive control to indicate the nucleofection efficiency for optimizing nucleofection conditions.

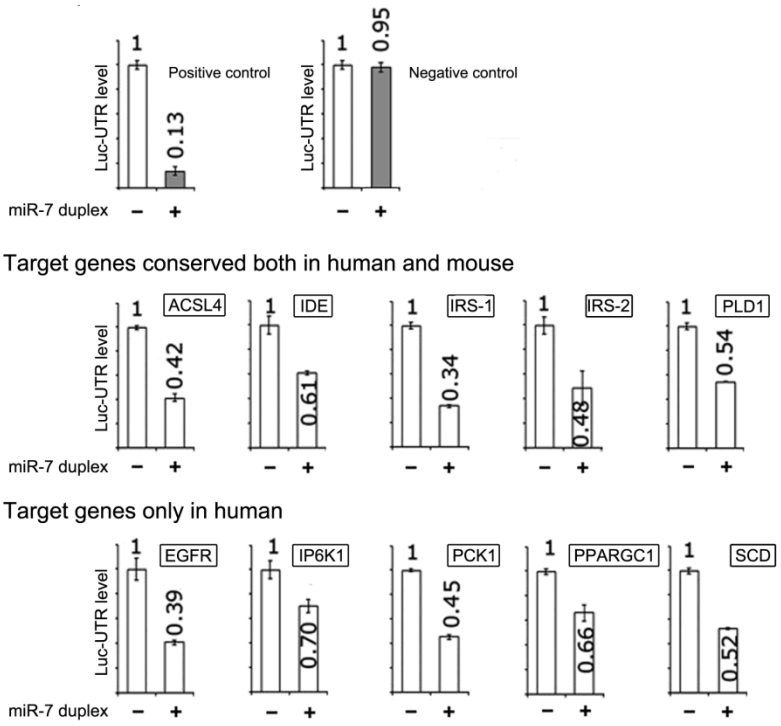

Figure S3 Verification of miR-7 target genes
Luciferase reporter gene constructs containing the 3'UTR of individual target genes were co-transfected with pre-miR-7 duplex or scrambled RNA oligos. Data are presented as the fold-change of the reporter gene ± S.D. in three separate experiments.

Table S1 Primers used in this study

Primer	Sequence	Product
Pos.ctrl-3'UTR-S	5'-CACAACAAAATCACTAGTCTTCCATGCCACAACAAAATCACTAGTCTTCCAC-3'	
Pos.ctrl-3'UTR-A	5'-TCGAGTGGAAGACTAGTGATTTTGTTGTGGCATGGAAGACTAGTGATTTTGTTGTGGTAC-3'	
Neg.ctrl- 3'UTR-S	5'-CGGTTAAGTCGCCCTCGCTCGGCAGGTTAAGTCGCCCTCGCTCC-3'	
Neg.ctrl- 3'UTR-A	5'-TCGAGGAGCGAGGGCGACTTAACCTGCCGAGCGAGGGCGACTTAACCGGTAC-3'	
ACSL4-3'UTR-S(H)	5'-GGGGTACCCCGCCTGGTGGTTTTCAACCTC-3'	**2716 bp**
ACSL4-3'UTR-A(H)	5'-CCGCTCGAGCGGTTGATAACATGGGTGGATTTATTTC-3'	
EGFR-3'UTR-S(H)	5'-GGGGTACCCCCCTCATGCCTTCACGTGTCTG-3'	**522 bp**
EGFR-3'UTR-A(H)	5'-CCGCTCGAGCGGTCTGGATGTTGGATTGGTGGT-3'	
IDE-3'UTR-S(H)	5'-GGGGTACCCCCATGGGAAAGTGCAAGTGGATG-3'	**614 bp**
IDE-3'UTR-A(H)	5'-CCGCTCGAGCGGTAGCATTTTACGAGTTGGCATA-3'	
IP6K1-3'UTR-S(H)	5'-GGGGTACCCCTGCGGGACGAGAACCAGTAG-3'	**2839 bp**
IP6K1-3'UTR-A(H)	5'-CCGCTCGAGCGGACGACCTCAGACACAAGCGG-3'	
IRS1-3'UTR-S(H)	5'-CGACGCGTCGCTCAACTGGACATCACAGCAGAA-3'	**4544 bp**
IRS1-3'UTR-A(H)	5'-CTAGCTAGCTAGGTAGCCAAGAGGTGTAAGGAAAG-3'	
IRS1-3'UTR-S(M)	5'-CGACGCGTCGCGTCAATAGCGTAACTGGACAT-3'	**1030 bp**
IRS1-3'UTR-A(M)	5'-CCGCTCGAGCGGAACACCCACATTCTTCATTCCG-3'	
IRS2-3'UTR-S(H)	5'-CGACGCGTCGGGCTTTATCACCAGGATGTCAC-3'	**2458 bp**
IRS2-3'UTR-A(H)	5'-CCGCTCGAGCGGTGCATATGGCTATTAAGGAGGG-3'	
PLD1-3'UTR-S(H)	5'-GGGGTACCCCAGAGATATTCATTGGCAGCTCA-3'	**2261 bp**
PLD1-3'UTR-A(H)	5'-CCGCTCGAGCGGGCTCTCTCCTTCACCTTGTTTA-3'	
SCD-3'UTR-S(H)	5'-GGGGTACCCCCTTTATTGCTATCGCCCTCCT-3'	**3456 bp**
SCD-3'UTR-A(H)	5'-CCGCTCGAGCGGGGCTAGTTATCCACCGCTTCT-3'	
PCK1-3'UTR-S(H)	5'-GGGGTACCCCTCATTCCCTTTCCCATCCAT-3'	**616 bp**
PCK1-3'UTR-A(H)	5'-CCGCTCGAGCGGTTTCCAAGAAGTAGTAAGGGGG-3'	
PPRGGC1-3'UTR-S(H)	5'-GGGGTACCC ATGGCGAATACCTCATGGGAC-3'	**3775 bp**
PPRGGC1-3'UTR-A(H)	5'-CCGCTCGAGCGGTCCCTTTATTAGCTCAGTGAGGC-3'	
Beta-actin-S(M)	5'-GGCTGTATTCCCCTCCATCG-3'	**154 bp**
Beta-actin-A(M)	5'-CCAGTTGGTAACAATGCCATGT-3'	
IRS1-S(M)	5'-CGATGGCTTCTCAGACGTG-3'	**209 bp**
IRS1-A(M)	5'-CAGCCCGCTTGTTGATGTTG-3'	
Footnotes: H, human. M, mouse		

Table S2 Alteration of miRNAs in C2C12 cells treated with palmitate and oleate

Group 1

ID	Name	Fold Change (PA/con)
29490	mmu-miR-7a	1.612
16528	mmu-miR-706	2.040
46930	mmu-miR-26b	1.503
46639	mmu-miR-467f	1.694
14272	mmu-miR-542-3p	1.727
14301	mmu-miR-361	1.774
10988	mmu-miR-194	1.571
27575	mmu-miR-711	1.802
42790	mmu-miR-337-3p	1.815

Group 2

ID	Name	Fold Change (PA/con)
17825	mmu-miR-338-5p	0.660
11253	mmu-miR-467*/m	0.642
42826	mmu-miR-300*	0.624
46374	mmu-miR-466i	0.390
46807	mmu-miR-466f-3p	0.532
42445	mmu-miR-693-5p	0.574
42878	mmu-miR-882	0.667
42727	mmu-miR-668	0.570
46485	mmu-miR-669f	0.362
27672	mmu-miR-615-3p	0.629
17489	mmu-miR-710	0.619
46436	mmu-let-7e	0.630
42467	mmu-miR-129-5p	0.637
42917	mmu-miR-551b	0.632
46306	mmu-miR-466a-5p	0.527
17495	mmu-miR-697	0.391

Group 3

ID	Name	Fold Change (PA+OA/PA)
11253	mmu-miR-467a*/m	1.624
11007	mmu-miR-206	1.550
46374	mmu-miR-466i	2.371
27565	mmu-miR-423-5p	1.714
46639	mmu-miR-467f	1.539
14272	mmu-miR-542-3p	1.605

Group 4

ID	Name	Fold Change (PA+OA/PA)
17953	mmu-miR-183	0.522
42703	mmu-miR-490	0.504
42626	mmu-miR-30b*	0.559
42659	mmu-miR-290-3p	0.311
29490	mmu-miR-7a	0.610
29650	mmu-miR-714	0.537
46978	mmu-miR-669i	0.620
42605	mmu-miR-503*	0.428
14288	mmu-miR-503	0.474
17851	mmu-miR-200c*	0.394
17859	mmu-miR-200b*	0.339
42706	mmu-miR-325	0.563
42872	mmu-miR-138*	0.643
17669	mmu-miR-690	0.489
16528	mmu-miR-706	0.417
42880	mmu-miR-466d-5p	0.660
27672	mmu-miR-615-3p	0.568
42519	mmu-miR-465c-5p	0.660
28944	mmu-miR-667	0.368
42917	mmu-miR-551b	0.313
11229	mmu-miR-341	0.524
46930	mmu-miR-26b	0.548
14301	mmu-miR-361	0.474

10988	mmu-miR-194	0.429
27575	mmu-miR-711	0.605
42500	mmu-miR-483*	0.570
17749	mmu-let-7b	0.547
42790	mmu-miR-337-3p	0.345