

Supplementary Table 1. The sequences of qPCR primers used in this study.

miRNA	Sequences	Primer
miR-122-5p	UGGAGUGUGACAAUGGUGUUUG	GCAGTGGAGTGTGACAATG
miR-148a-3p	UCAGUGCACUACAGAACUUUGU	GCAGTCAGTGCCTACAGA
miR-99a-5p	AACCCGUAGAUCCGAUCUUGUG	CAGAACCCGTAGATCCGA
miR-423-5p	CUGACCUAUGAAUUGACAGCC	CAGCTGACCTATGAATTGACA
miR-192-5p	UGAGGUAGUAGAUUGUAUAGUU	GCCGTGAGGTAGTAGATTGTATAGTT
miR-151a-3p	CUAGACUGAAGCUCCUUGAGG	GCTAGACTGAAGCTCCTTG
let-7f-5p	UAGCUUAUCAGACUGAUGUUGA	GCGTAGCTTATCAGACTGATGTTGA
miR-21-5p	UGAGGUAGUAGGUUGUAUGGUU	TGAGGTAGTAGGTTGTATGGTT
let-7c-5p	AACCCGUAGAUCCGAACUUGUG	CAGAACCCGTAGATCCGA
miR-100-5p	UUCACAGUGGCUAAGUUCUGC	GCAGTTCACAGTGGCTAAG
miR-27b-3p	UUUGUUCGUUCGGCUCGCGUGA	AGTTTGTTCGTTCCGGCTC
miR-375-3p	UGUAACAGCAACUCCAUGUGGA	TGTAACAGCAACTCCATGTGG
miR-16-2-3p	AAUGGAUUUUUGGAGCAGG	GCAATGGATTTTTTGGAGCAGG
miR-194-5p	UCCCUGAGACCCUAACUUGUGA	TCCCTGAGACCCTAACTTGTGA
miR-186-5p	UUCAAGUAAUUCAGGAUAGGU	GCGCAGTTCAAGTAATTCAG
miR-1246	AGUUCUUCAGUGGCAAGCUUUA	AGTTCTTCAGTGGCAAGCTTTA
miR-125b-5p	UGGAUUUUUGGAUCAGGGA	GTGGATTTTTTGGATCAGGGA
miR-26b-5p	UGAGGGGCAGAGAGCGAGACUUU	CAGTGAGGGGCAGAGAG
miR-22-5p	CCAAUAUUACUGUGCUGCUUUA	CGCAGCCAATATTACTGTG
miR-1-3p	CAAAGAAUUCUCCUUUUGGGCU	CGCAGCAAAGAATTCTCCT
miR-19b-3p	UGGAAUGUAAAGAAGUAUGUAU	CGGTGGAATGTAAAGAAGTATGTA
miR-1290	UGUGCAAAUCCAUGCAAAACUGA	GCTGCAAATCCATGCAAAACTGA

Universal anti-sense was used for reverse primer.

Supplementary Table 2. Spearman correlation analysis investigating the association between exosomal miRNAs and clinical indicators in the NGT and GDM group separately.

	miR-423-5p		miR-122-5p		miR-148a-3p		miR-192-5p		miR-99a-5p	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
NGT group										
Age	0.05	0.63	0.14	0.16	-0.07	0.49	0.03	0.78	0.00	1.00
Prepregnancy-BMI	0.03	0.79	-0.09	0.40	-0.02	0.88	-0.01	0.94	-0.02	0.86
BMI at OGTT	-0.05	0.66	-0.07	0.53	-0.03	0.80	-0.03	0.77	-0.04	0.75
GWG	-0.13	0.21	0.07	0.50	-0.03	0.81	0.01	0.96	-0.14	0.18
TG	0.04	0.72	0.05	0.64	0.01	0.94	-0.01	0.91	-0.04	0.67
CHOL	0.02	0.81	0.17	0.09	0.05	0.60	0.08	0.45	-0.01	0.90
HDL	0.08	0.41	0.04	0.73	0.11	0.29	0.11	0.26	0.19	0.06
LDL	0.05	0.62	0.14	0.16	0.08	0.41	0.07	0.49	-0.02	0.85
FPG	0.09	0.35	0.09	0.36	0.06	0.57	0.06	0.58	-0.01	0.95
1h-PG	-0.15	0.14	0.01	0.95	-0.18	0.07	-0.04	0.69	-0.04	0.71
2h-PG	-0.27	0.01	-0.07	0.47	-0.29	<0.01	-0.22	0.02	-0.06	0.53
HbA1c	-0.02	0.82	-0.08	0.40	-0.08	0.41	-0.05	0.64	-0.07	0.47
Birth weight	-0.06	0.55	-0.06	0.60	-0.07	0.48	0.03	0.76	-0.06	0.60
GDM group										
Age	-0.06	0.58	0.07	0.47	0.01	0.94	-0.06	0.56	0.07	0.46
Prepregnancy-BMI	-0.03	0.75	-0.18	0.08	-0.19	0.08	-0.15	0.15	-0.06	0.58
BMI at OGTT	-0.04	0.73	-0.20	0.09	-0.24	0.04	-0.11	0.37	-0.11	0.37
GWG	0.02	0.87	-0.01	0.94	-0.08	0.46	-0.03	0.77	-0.05	0.62
TG	-0.01	0.89	-0.06	0.59	0.09	0.40	0.03	0.80	-0.02	0.88
CHOL	0.04	0.67	0.04	0.67	0.08	0.40	0.08	0.42	0.19	0.06
HDL	0.08	0.42	-0.04	0.68	0.07	0.51	0.05	0.63	0.08	0.45
LDL	-0.02	0.84	0.02	0.87	0.03	0.75	0.02	0.88	0.12	0.23
FPG	0.16	0.10	0.10	0.31	0.06	0.58	0.01	0.89	0.16	0.11
1h-PG	0.10	0.31	0.10	0.30	0.02	0.86	0.04	0.72	0.01	0.95
2h-PG	0.07	0.50	0.11	0.25	-0.05	0.63	-0.04	0.67	-0.09	0.39
HbA1c	0.09	0.38	-0.10	0.33	0.02	0.84	-0.01	0.89	-0.02	0.81
Birth weight	-0.16	0.13	0.06	0.60	-0.12	0.27	-0.02	0.86	0.05	0.64

Supplementary Table 3. Risk estimates for GDM based on single miRNA levels in prediction set.

miRNAs	Model 1			Model 2			Model 3		
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
miR-423-5p	0.65	0.31-1.38	0.591	0.62	0.28-1.37	0.235	0.63	0.28-1.41	0.259
miR-122-5p	0.58	0.34-0.97	0.041	0.49	0.26-0.92	0.026	0.33	0.14-0.79	0.012
miR-148a-3p	0.12	0.03-0.44	0.001	0.10	0.02-0.39	0.001	0.10	0.02-0.41	0.001
miR-192-5p	0.13	0.04-0.46	0.001	0.10	0.03-0.40	0.001	0.10	0.02-0.40	0.001
miR-99a-5p	0.04	0.006-0.28	0.001	0.02	0.003-0.20	<0.001	0.02	0.002-0.19	<0.001

Logistic regression analysis was used. Model 1 included no adjustment. Model 2 was adjusted for age and BMI at enrollment. Model 3 was adjusted for age, BMI at enrollment, alanine aminotransferase (ALT), and aspartate transaminase (AST). GDM, gestational diabetes mellitus; 95% CI, 95% confidence intervals; OR: odds ratio.

Supplementary Table 4. Net reclassification improvement for GDM prediction with the addition of miRNAs to base model (clinical parameters).

base model + miRNAs	Categorical NRI		Continuous NRI	
	Estimate (95% CI)	<i>P</i>	Estimate (95% CI)	<i>P</i>
Overall	0.86 (0.29-1.33)	<0.01	0.95 (0.70-1.64)	<0.01
GDM	0.41 (0.06-0.70)	0.01	0.52 (0.27-0.85)	<0.01
NGT	0.45 (0.12-0.70)	<0.01	0.43 (0.31-0.85)	<0.01

Reclassification of GDM Risk based on net reclassification improvement (NRI).

Separate analyses were performed in the overall, GDM, and NGT cohorts. Base model:

FPG + age + BMI; miRNAs: miR-423-5p + miR-122-5p + miR-148a-3p + miR-192-5p + miR-99a-5p. 95% CI, 95% confidence intervals; GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; FPG, fasting plasma glucose; BMI, pre-pregnancy body mass index.

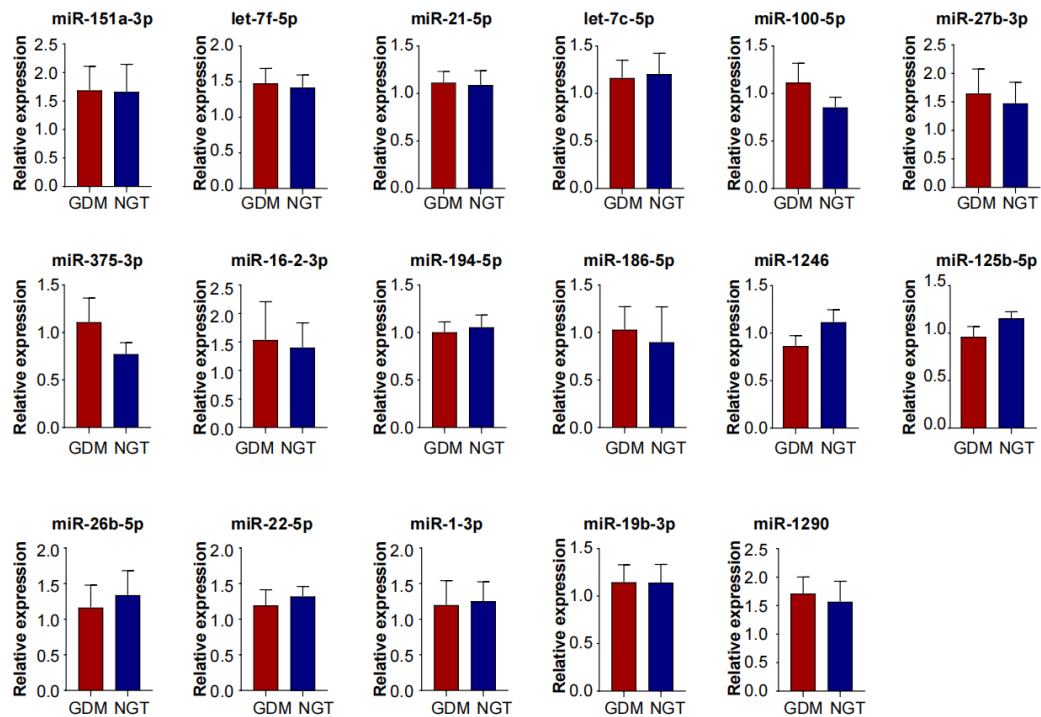
Supplementary Table 5. The miRNA mimic or inhibitor sequences.

Mimic/inhibitor	Sequence (5'-3')
has-miR-122-5p mimic (double strand)	UGGAGUGUGACAAUGGUGUUUG ACCUCACACUGUUACCACAAAC
has-miR-122-5p inhibitor (single strand)	ACCUCACACUGUUACCACAAAC
has-miR-148a-3p mimic (double strand)	UCAGUGCACUACAGAACUUUGU ACAAAGUUCUGUAGUGCACUGA
has-miR-148a-3p inhibitor (single strand)	ACAAAGUUCUGUAGUGCACUGA
has-miR-192-5p mimic (double strand)	CUGACCUAUGAAUUGACAGCC GACUGGAUACUUAACUGUCGG
has-miR-192-5p inhibitor (single strand)	GACUGGAUACUUAACUGUCGG
has-miR-423-5p mimic (double strand)	UGAGGGGCAGAGAGCGAGACUUU ACUCCCCGUCUCUCGCUCAGAAA
has-miR-423-5p inhibitor (single strand)	ACUCCCCGUCUCUCGCUCAGAAA
has-miR-99a-5p mimic (double strand)	AACCCGUAGAUCCGAUCUUGUG UUGGGCAUCUAGGCUAGAACAC
has-miR-99a-5p inhibitor (single strand)	UUGGGCAUCUAGGCUAGAACAC

Supplementary Table 6. The association of differential expression miRNAs with metabolic regulation reported previously.

miRNA	Expression	Function and key targets
miR-423-5p (1-4)	Increase	Overexpression inhibits IGF1R and GYS1, interfering with insulin signal transduction and promotes gluconeogenesis and lipid deposition.
miR-122-5p (5-9)	Decrease	Underexpression elevates G6PC3 and FDFT1, leading to hepatic insulin resistance, glycolysis inhibition and glucose uptake reduction.
miR-148a-3p (10-13)	Decrease	Negatively correlated with glucose and HbA1c levels and BMI. Overexpression alleviated high glucose induced diabetic retinopathy by targeting TGFB2 and FGF2
miR-192-5p (14, 15)	Decrease	Underexpression increases blood glucose. Overexpression decreases adipose deposition and triglyceride.
miR-99a-5p (16)	Decrease	Overexpression inhibits NOX4, ameliorating liver metabolic disorders.

Supplementary Figure 1



Supplementary Figure 1. The qRT-PCR validation results of miRNAs that were inconsistent with the results of small RNA sequencing data.

In dysregulated exosomal miRNAs identified as differential expressed miRNA in the Small RNA next-generation sequencing data, 17 miRNAs of them did not show significant difference between GDM and NGT groups in the validation set.

A

miR-423-5p	ctrl	Gene
-0.8	0.2	MIR3615
-0.7	0.1	IGF2
-0.6	0.0	AL162151.2
-0.5	0.1	GLUL
-0.4	0.2	PPT1
-0.3	0.3	AP002990.1
-0.2	0.4	BANF1
-0.1	0.5	MRPL49
0.0	0.6	RAD51C
0.1	0.7	PAQR4
0.2	0.8	RPS26P19
0.3	0.9	AC011603.2
0.4	1.0	AC004967.2
0.5	1.1	NOMO2
0.6	1.2	DHCR24
0.7	1.3	TMBIM6
0.8	1.4	TSPAN3
0.9	1.5	AL662899.2
1.0	1.6	TEN1
1.1	1.7	CFL2
1.2	1.8	S100A6
1.3	1.9	AC004922.1
1.4	2.0	IFITM2
1.5	2.1	SUOX
1.6	2.2	OR7E38P
1.7	2.3	KIAA1191
1.8	2.4	C1GALT1C1
1.9	2.5	RAB5B
2.0	2.6	AIDA
2.1	2.7	RPL13AP7
2.2	2.8	CCDC69
2.3	2.9	AL157400.5
2.4	3.0	ARPIN-AP3S2
2.5	3.1	PPP2R2C
2.6	3.2	PCCB
2.7	3.3	RNF114
2.8	3.4	TIMP1
2.9	3.5	CNP
3.0	3.6	S100A11
3.1	3.7	SPHK1
3.2	3.8	PGRMC2
3.3	3.9	AC011448.1
3.4	4.0	LGALS1
3.5	4.1	COL1A1
3.6	4.2	COL1A2
3.7	4.3	RPS2
3.8	4.4	GRB2

B

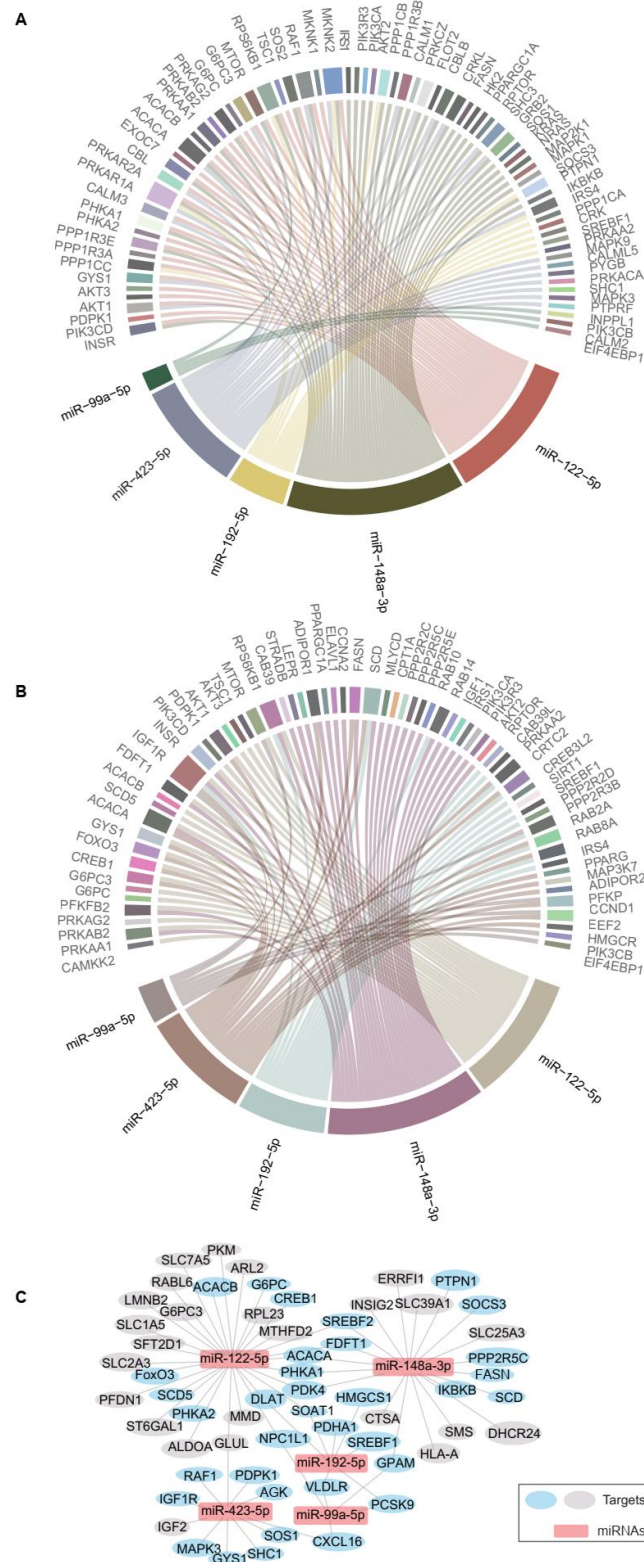
miR-122-5p	ctrl	Gene
0.8	0.2	RPL13AP7
0.7	0.1	LGCN1
0.6	0.0	OR7E38P
0.5	-0.1	AC010732.1
0.4	-0.2	SNORD36A
0.3	-0.3	SNORD2
0.2	-0.4	OSBP1
0.1	-0.5	BP62899.2
0.0	-0.6	LIB1
-0.1	-0.7	TIMP1
-0.2	-0.8	ARIP1
-0.3	-0.9	SLC25A3
-0.4	-1.0	GATL1
-0.5	-1.1	CCDC137
-0.6	-1.2	CDOP1
-0.7	-1.3	SPHK1
-0.8	-1.4	AL157400.5
-0.9	-1.5	SNRPB
-1.0	-1.6	TNFRSF12A
-1.1	-1.7	SLC25A3
-1.2	-1.8	DANCER
-1.3	-1.9	TNMD1
-1.4	-2.0	AL162151.2
-1.5	-2.1	SKIV
-1.6	-2.2	SVOR6
-1.7	-2.3	TRX2
-1.8	-2.4	YTHDF2
-1.9	-2.5	SRB1
-2.0	-2.6	SLC25A3
-2.1	-2.7	RBP1
-2.2	-2.8	CARDSP1
-2.3	-2.9	MA1LC3B
-2.4	-3.0	LMNB2
-2.5	-3.1	ADPF2
-2.6	-3.2	ADNP
-2.7	-3.3	ALDOA
-2.8	-3.4	MMD
-2.9	-3.5	IGFBP1
-3.0	-3.6	SLC25A3
-3.1	-3.7	RPL19
-3.2	-3.8	AP00356.5
-3.3	-3.9	CLDN8
-3.4	-4.0	AC049839.2
-3.5	-4.1	DEC1
-3.6	-4.2	SLC14A5
-3.7	-4.3	SP1
-3.8	-4.4	ABL2
-3.9	-4.5	STKRAL1
-4.0	-4.6	SLC25A3

C

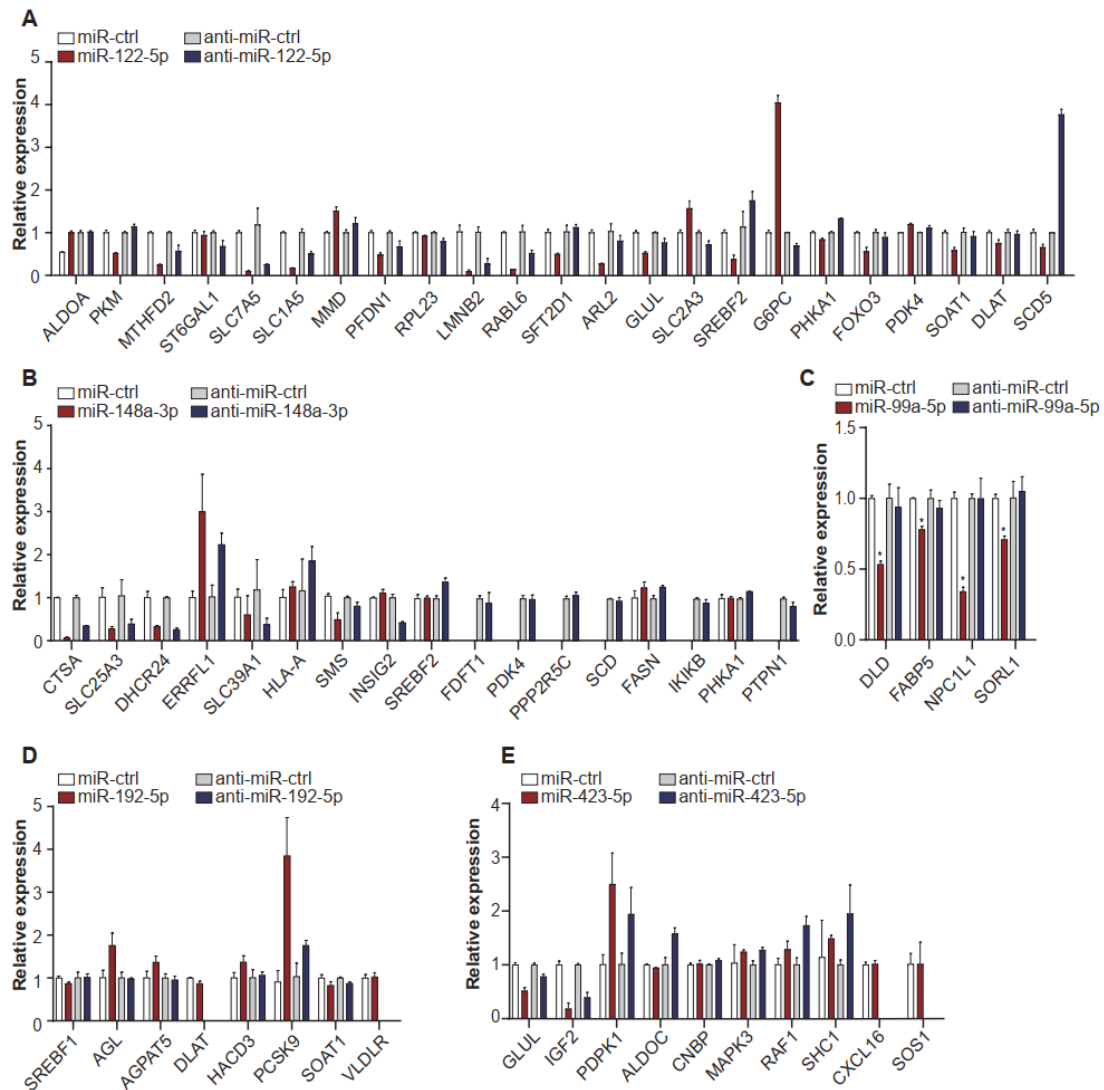
miR-148a-3p	ctrl	Gene
-0.8	0.2	BANF1
-0.7	0.1	CSNK2B
-0.6	0.0	RPS10-NUDT3
-0.5	-0.1	DHCR24
-0.4	-0.2	CUX1
-0.3	-0.3	SLC25A3
-0.2	-0.4	DANCER
-0.1	-0.5	MIR3615
0.0	-0.6	SNORD36A
0.1	-0.7	DEPP1
0.2	-0.8	LCN15
0.3	-0.9	CCN2
0.4	-1.0	SLC25A3
0.5	-1.1	AP002990.1
0.6	-1.2	RPL19
0.7	-1.3	CP
0.8	-1.4	ELI1
0.9	-1.5	RNASSET2
1.0	-1.6	AL162151.2
1.1	-1.7	GSP1
1.2	-1.8	IKBKGP1
1.3	-1.9	PPT1
1.4	-2.0	PAQR4
1.5	-2.1	INSIG2
1.6	-2.2	AC092032.2
1.7	-2.3	YTHDF2
1.8	-2.4	F362260.2

Supplementary Figure 2. The mRNA expression profile in HepG2 cells using next-generation sequencing after miRNAs transfection. The mRNA expression profile of miR-423-5p overexpressed HepG2 cells **(A)**, miR-122-5p overexpressed HepG2 cells **(B)**, miR-148a-3p overexpressed HepG2 cells **(C)** and miR-99a-5p overexpressed HepG2 cells **(D)**.

Supplementary Figure 3. (A) Predicted targets involved in the insulin signaling pathway. **(B)** Predicted targets involved in the AMPK signaling pathway. **(C)** Potential miRNA-mRNA regulatory networks in miRNA overexpressed cells.

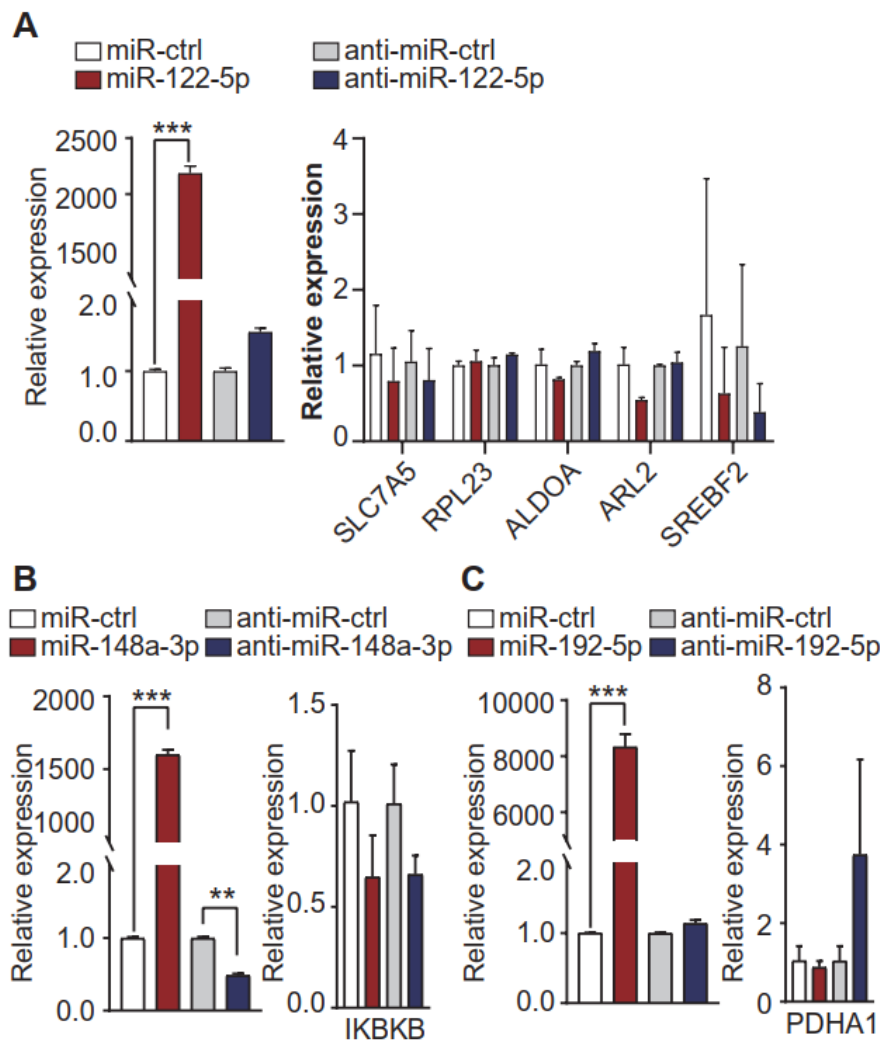


Supplementary Figure 4



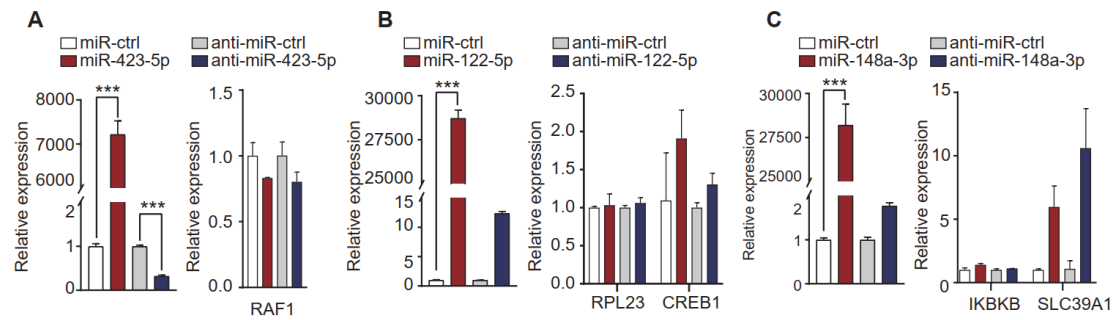
Supplementary Figure 4. The expression of target genes in cells transfected with mimics or inhibitors of miRNAs. The functional targets identified in computational prediction and transcriptome sequencing and insulin signal related genes but not respectively reverse related with the levels of miR122-5p (A), miR-148a-3p (B), miR-99a-5p (C), miR-192-5p (D), and miR-423-5p (E).

Supplementary Figure 5



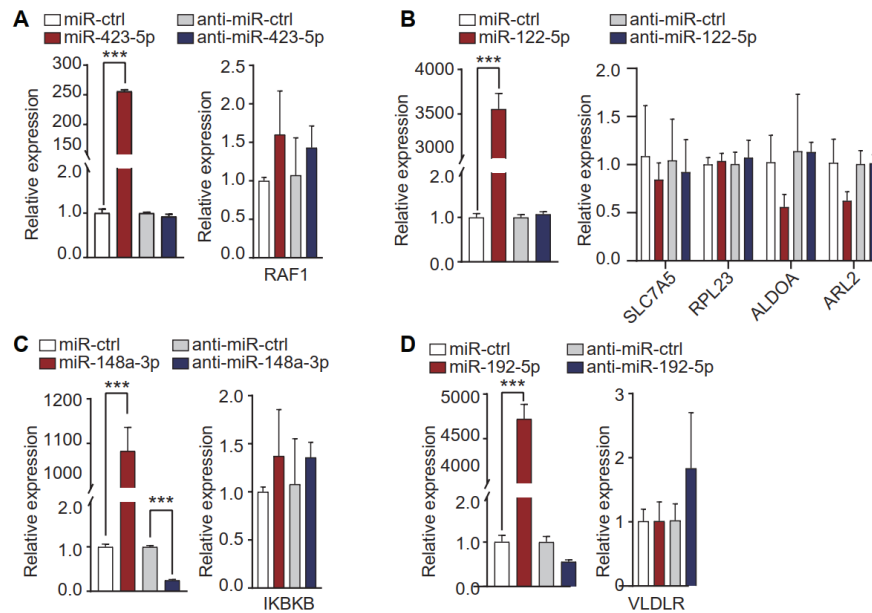
Supplementary Figure 5. The expression of target genes in C2C12 cells transfected with mimics or inhibitors of miRNAs. (A-C) Transfection efficiencies (left) and expression of target genes (right) of miRNAs, miR-122-5p (A), miR-148a-3p (B), and miR-192-5p (C).

Supplementary Figure 6



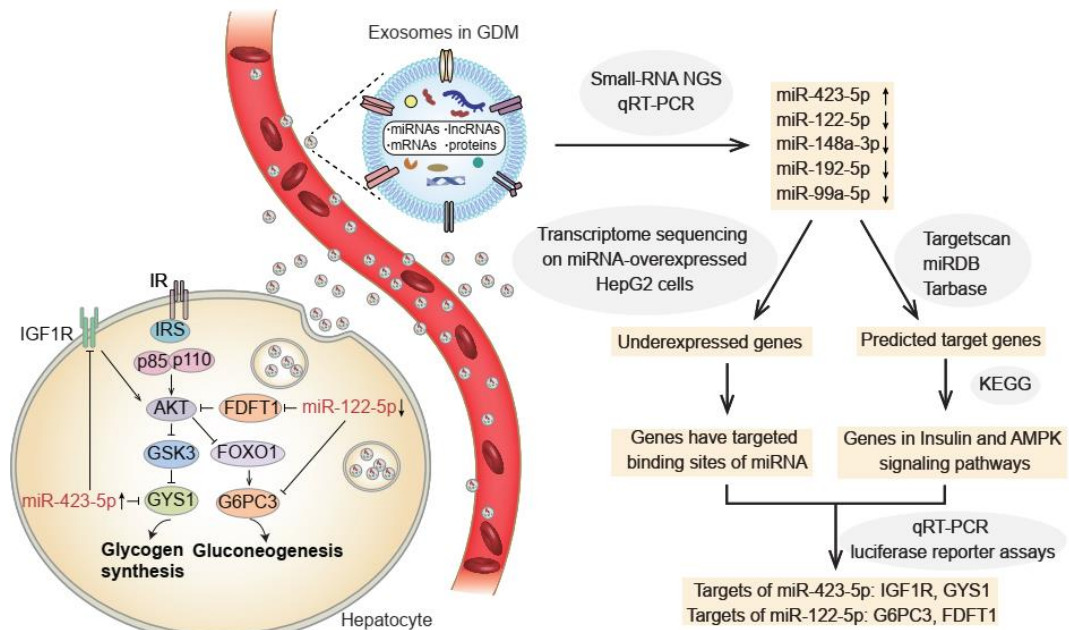
Supplementary Figure 6. The expression of target genes in MIN6 cells transfected with mimics or inhibitors of miRNAs. (A-C) Transfection efficiencies (left) and expression of target genes (right) of miRNAs, miR-423-5p (**A**), miR-122-5p (**B**), and miR-148a-3p (**C**).

Supplementary Figure 7



Supplementary Figure 7. The expression of target genes in 3T3-L1 cells transfected with mimics or inhibitors of miRNAs. (A-D) Transfection efficiencies (left) and expression of target genes (right) of miRNAs, miR-423-5p (A), miR-122-5p (B), miR-148a-3p (C), and miR-192-5p (D).

Supplementary Figure 8



Supplementary Figure 8. Schematic presentation of the research process and the mechanisms of miR-423-5p and miR-122-5p regulated metabolism.

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