SUPPLEMENTARY DATA

Exosomal miR-20b-5p in type 2 diabetes regulates insulin action in human skeletal muscle

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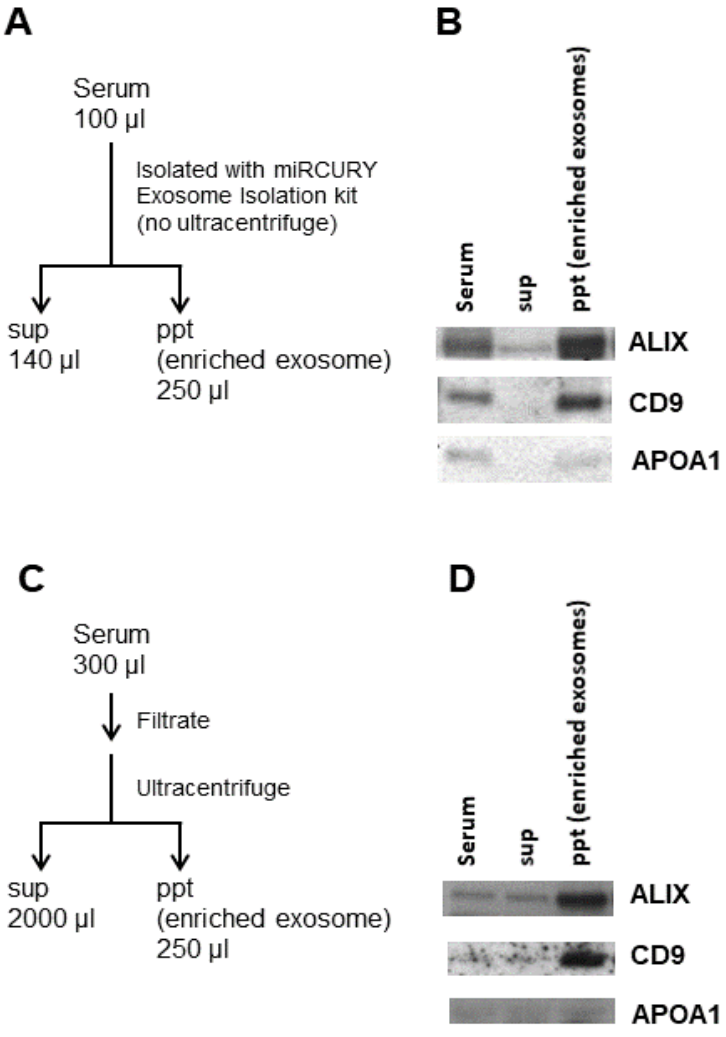
Supplementary Figure 1. Exosomes Were Concentrated in Our Isolation Process. (A) Schematic representation of resultant isolated fractions from serum in the kit-based exosome enrichment method. Human serum obtained from healthy volunteers was used for isolation of exosome-enriched fraction as described in the method section. (B) Immunoblotting of each fraction in the kit-based isolation method was performed with exosome or HDL marker protein antibodies. The same protein amount of each fraction was loaded to each lane. ALIX, CD9 and APOA1 antibodies were purchased from commercial sources (ALIX: #2171 of Cell Signaling, CD9: ab92726 of Abcam, APOA1: ab7613 of Abcam). (C) Schematic representation of ultracentrifugation method for isolation of exosome enriched fraction. Human serum was centrifuged at 500xg and 2000xg twice for 10 min each time to remove cell debris. The supernatant was transferred into a fresh tube, filtered with a 0.22-μm filter (Merck), and pelleted by ultracentrifugation (Beckman Optima TLX, Beckman Coulter) at 100,000xg for 90 min. The precipitate was washed with ice-cold PBS and followed by ultracentrifugation at 100,000xg for 90 min to pellet exosomes. The exosome pellets were dissolved in PBS. (D) Immunoblotting of each fraction was performed with the same exosome or HDL marker proteins as described in (B).



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Supplementary Figure 2. Sequence Alignment of miR-20b-5p and Its Putative Target Sites in the 3'-UTR of (A) STAT3 and (B) AKTIP mRNA. Mutation was generated in the complementary sites of the seed region of miR-20b-5p.

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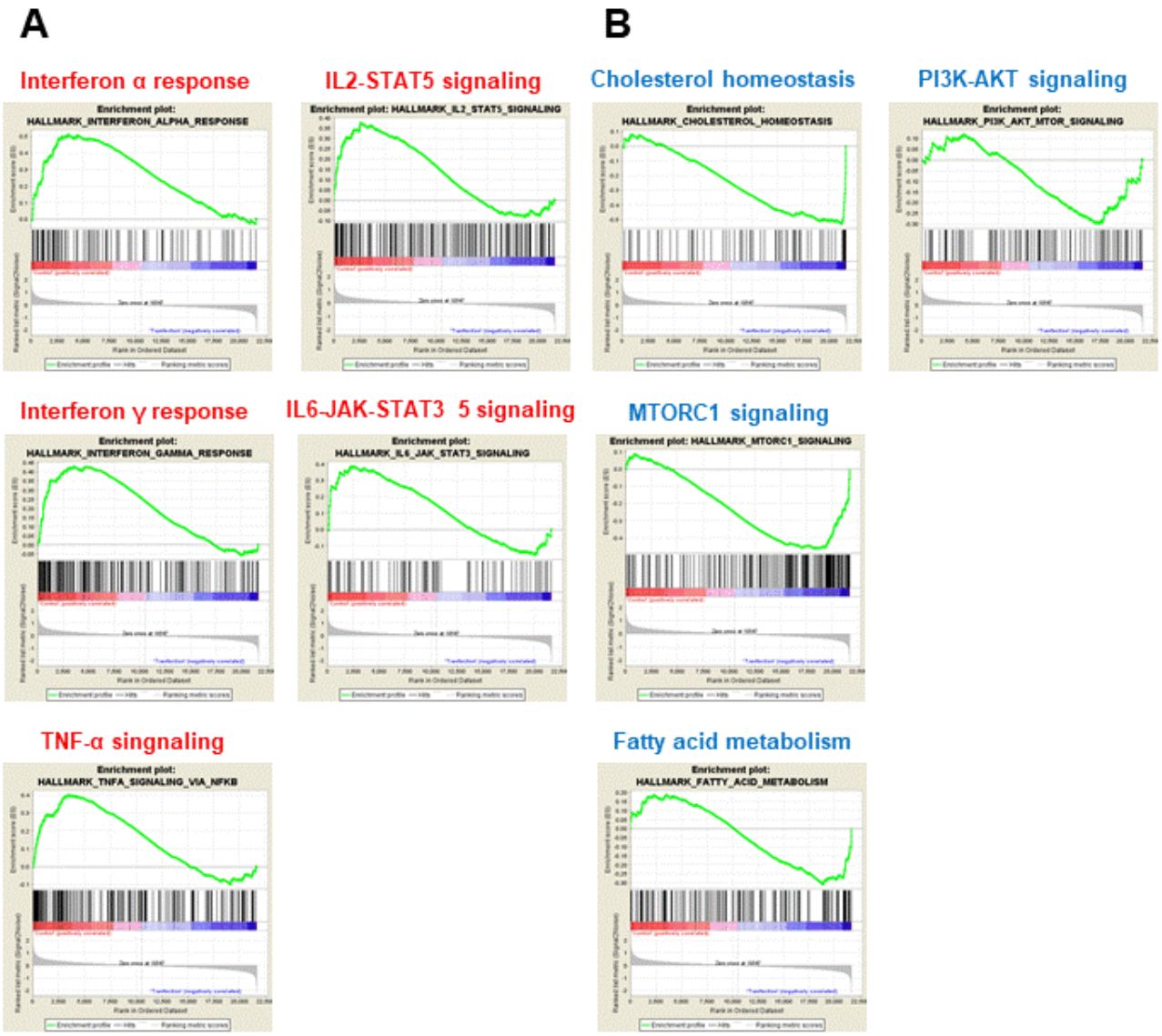
Supplementary Figure 3. Gene Set Enrichment Analysis (GSEA) of mRNA Profiling of miR-20b-5p Transfected Human Skeletal Muscle Cells. Histograms show the distribution of selected GSEA molecular signatures. The leading edge (most significant genes) are shown as vertical bars accumulated either left and below the peak of green enrichment score plot (A) or right of the valley of the green plot (B), indicating the respective up- or down-regulated genes of each shown GSEA characterized by the highest enrichment score.



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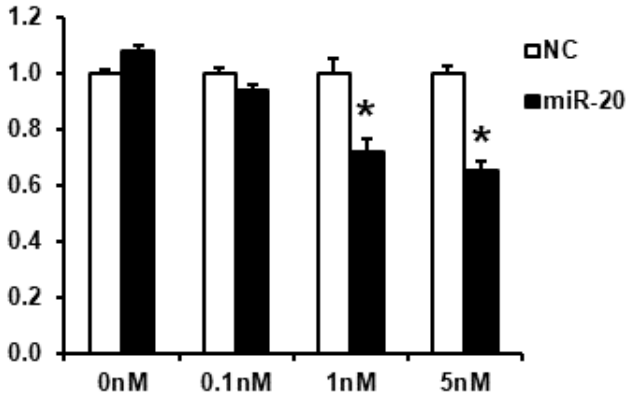
Supplementary Figure 4. Validation of miR-20b-5p Binding to 3'UTR of AKTIP Genes. Luciferase reporter assay of HEK293 cells transfected with AKTIP 3'UTR plasmids containing wild type miRNA seed sequence from AKTIP gene, and either miR-20b-5p or NC (scrambled miRNA control). Samples were analyzed 24 hours after transfection and data normalized to secreted alkaline phosphatase signals. Columns represent the luciferase activity of 3'UTR of AKTIP gene plasmid transfected either NC or miR-20b-5p. Data represents the mean ± SEM, n = 3. Student's t-test comparing NC or miR-20b-5p transfection indicated as $*p < 0.05$



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Supplementary Table 1. Clinical and Anthropometric Characteristics of the Study Participants

	Control	Pre-diabetic	Type 2 Diabetic
n	20	16	21
Age (years)	60 ± 1	63 ± 0.9	61 ± 1
Height (cm)	179.1 ± 1.0	179.1 ± 1.8	180.3 ± 1.3
Weight (kg)	91.7 ± 1.1	91.7 ± 2.0	95.9 ± 1.8
Waist (cm)	103.7 ± 1.1	102.5 ± 1.0	104.8 ± 1.2
BMI (kg/m2)	28.8 ± 0.4	28.8 ± 0.6	29.5 ± 0.6
SBP (mmHg)	132.8 ± 3.1	139.1 ± 3.2	141.9 ± 2.6
DBP (mmHg)	82.0 ± 2.1	87.2 ± 1.4*	83.1 ± 1.52*
Fasting glucose (mmol/L)	5.4 ± 0.1	5.7 ± 0.2	9.1 ± 0.5**
2 hr glucose (mmol/L)	7.1 ± 0.2	10.0 ± 0.2**	16.4 ± 0.8**
Insulin (pmol/L)	61.2 ± 7.3	61.7 ± 8.9	65.4 ± 11.9
HbA_{1c} (%)	4.6 ± 0.1	4.8 ± 0.1	6.34 ± 0.3**
HbA_{1c} (mmol/mol)	27.1 ± 0.8	29.1 ± 0.8	46.3 ± 2.8**
HOMA-IR	2.4 ± 0.3	2.8 ± 0.5	3.91 ± 0.6*
Cholesterol (mmol/L)	5.8 ± 0.2	5.7 ± 0.3	4.3 ± 0.2**
HDL (mmol/L)	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
LDL (mmol/L)	3.6 ± 0.2	3.6 ± 0.3	2.4 ± 0.1**
TG (mmol/L)	1.8 ± 0.3	1.8 ± 0.3	1.4 ± 0.1

Data are mean ± SEM. Statistical comparison between the normal glucose tolerant (Control) versus pre-diabetic or type 2 diabetic men were determined using Student's *t*-test. $p < 0.05$*; $p < 0.0001$**

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA_{1c} (%), NGSP standard; HbA1c (mmol/mol); IFCC standard; HOMA-IR, homeostasis model assessment estimated insulin resistance. Study participants were prescribed various medications, but control and pre-diabetic group was not prescribed any anti-diabetic medications while many of diabetic group were prescribed anti-diabetic medications (N=15 (15/21)). All group were prescribed anti-hypertensive or anti-hypercholesterolemia medication (anti-hypertensive: control N = 4 (4/20), pre-diabetic N = 8 (8/16), diabetic N = 11 (11/21) and anti-hypercholesterolemia: N = 1 (1/20), pre-diabetic N = 3 (3/16), diabetic N = 11 (11/21)).

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Supplementary Table 2. Primer List of Specific Gene qRT-PCR Validation of miR-20b Target Genes

Gene symbol	Forward primer sequence	Reverse primer sequence
CYBRD1	5'-CTCGTCTGGGTCCTCCACTAC-3'	5'-TTCCAGGTCCACGGCAGTCT-3'
TBC1D2	5'-ATCCTCCTTCGGGTCTGGGA-3'	5'-TGGCCAAGGCATAGCGAAAC-3'
AKTIP	5'-GCATTGAAGGCACTGGGGTAAA-3'	5'-TGCAGTTCGTGGAGGACTGG-3'
GINM1	5'-CTCAGAGCAGAGCCGCCATC-3'	5'-ACGTTGCTCCAGAACCTACAC-3'
CFL2	5'-TCCCTTTCGCTTCCACGTCC-3'	5'-CCAGAAGCCATGTAAGTCGTCC-3'