SUPPLEMENTAL MATERIAL

Data S1. Supplementary Methods

Study samples of nested case-control study

Overall, in 2013, a total of 38 295 participants had complete questionnaires and physical examinations information in the Dongfeng-Tongji (DFTJ) cohort. Each participant’s medical insurance documents, hospital records, and death certificates could be tracked by Dongfeng Motor Corporation’s health-care service system up to 31 December 2016. In further analyses, we excluded participants with cardiovascular disease (CVD), cancer, or severely abnormal electrocardiograms before the date of blood drawing in 2013 (n=10 254), participants without medical insurance record or blood samples (n=3626), as well as participants who were newly diagnosed with coronary heart disease (CHD) <6 months after collection of blood samples (n=490). After exclusion, a total of 23 925 participants were eligible for our analyses. Participants were diagnosed with acute coronary syndrome (ACS) by an expert panel of physicians based on symptoms, clinical examinations according to the guideline of ST-segment elevation or non-ST-segment elevation acute myocardial infarction (AMI) or unstable angina (UA).[40,41] Moreover, we tracked the International Classification of Diseases (ICD) codes to further distinguish AMI (ICD-10 codes I21) and UA (ICD-10 codes I20.0). Finally, we identified 595 incident ACS without stroke or cancer before ACS first-episode, and randomly selected matched controls from the 23 925 eligible participants who were free of ACS at the time of ACS diagnosis.

Data and sample collection

Participants were collected information used semi-structured questionnaires by trained investigators, including sociodemographic factors (e.g., age, sex, and education), health status (e.g., personal and family history of CHD, stroke, and cancer), medical history, and lifestyle, such as smoking status (current, former, never), drinking status (current, former, never) and physical activity. Participants were taken anthropometric examinations (e.g., weight, height, and blood pressure) and clinical examinations (e.g., blood lipid and fasting glucose) after an overnight fast. Fasting blood was drawn with EDTA-anticoagulant tubes and centrifuged immediately, separated into plasma and whole blood, and stored at -80°C until analysis.

Definition of covariates was in line with previously study.[42-44] Current smokers were those who smoked more than one cigarette a day over six months, former smokers were those who quitted smoking for over six months, and never smokers were those who had never smoked in their lifetime. Current drinkers were those who drunk more than once a week over six months, former drinkers were those who quitted drinking for over six months, and never drinkers were those who had never drunk in their lifetime. Physical activity was represented as metabolic equivalent (MET) hours a week multiplied by the coefficient, duration (hours per time) and frequency (times per week) of physical activity. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared, individuals with BMI≥24 were defined as obesity. Hyperlipidemia was defined as individual having self-reported physician-diagnosed hyperlipidemia, or currently taking lipid-lowering medication, or total cholesterol (TC) ≥6.22 mmol/L, or triglyceride (TG) ≥2.26 mmol/L, or high-density lipoprotein cholesterol (HDL-C) <1.04 mmol/L, or low-density lipoprotein cholesterol (LDL-C) ≥4.14 mmol/L. Hypertension was defined as individual having self-reported physician-diagnosed hypertension, or currently taking antihypertensive medication, or blood pressure

≥140/90 mmHg. Diabetes was defined as individual having self-reported physician-diagnosed diabetes, or currently taking antidiabetic medication (oral hypoglycemic medication or insulin), or fasting glucose (FG) ≥7.0 mmol/L. Impaired fasting glucose (IFG) was defined as non-diabetic participant whose FG≥5.6 mmol/L and <7.0 mmol/L.

RNA isolation

In the discovery stage, 400 µl plasma was used to extract RNA using the miRNeasy Serum/Plasma kit (Qiagen, Germany) according to the instruction handbook. In the validation stage, the method and reagents of RNA isolation were similar to that in the discovery stage, except that we added 2 µl synthetic Caenorhabditis elegans miR-39-3p (cel-miR-39-3p, 50 pmol/L, RiboBio, China) as spiked-in control before chloroform extraction in each plasma sample.[45]

MiRNA microarray and data preprocessing

Total RNA from each plasma sample was labeled with the Cyanine 3-pCp and hybridized to the Agilent Human miRNA Microarray, Release 21.0, 8x60K (Agilent Technologies Inc, USA) with 2549 human miRNAs probes according to miRBase database (Release 21.0). The hybridized arrays were scanned on an Agilent Scanner G2565CA after washing. We extracted the array data from the scanned image using the Agilent Feature Extraction software v10.7. After adjusting for background noise by negative control probes, all raw signal values were normalized using the 90th percentile shift and log2-transformed in all statistical analyses. Only the probes with a present or marginal flag passing the 60% detection rate in either ACS or control group were kept for further analysis. Data summarization, quantile-normalization, and quality control were performed using the Agilent GeneSpring GX software v11.5. Of 23 pairs of plasma samples analyzed by microarrays in the discovery stage, 408 out of 2549 miRNAs on the array were left after data filtering. Only the miRNAs at a significant level ($P<0.05$, absolute fold change [FC] >1.3) were included for further analyses by the paired *t*-tests. To reduce potential false positivity of microarray results, we further removed extremely low abundance miRNAs (an average expression value of reads per million mapped reads <1) in plasma with miRmine database (http://guanlab.ccmb.med.umich.edu/mirmine/help.html), which is an integrated database from 304 high-quality miRNA sequencing experiments of 16 different types of human biospecimen from NCBI-SRA datasets. Finally, candidate miRNAs that could be detected (Ct<37) by Taqman Advanced miRNA assay were selected for the validation stage. Unsupervised hierarchical clustering was used to generate a tree cluster showing distinguishable expression patterns of miRNAs.

Quality control and analysis of quantitative real-time polymerase chain reaction assays

The efficiency of amplification in each TaqMan assay was certified to range from 90% ~ 110% by the supplier. Spike-in cel-miR-39-3p was used as an exogenous control to assess RNA extraction and reverse transcription (RT) efficiency. To estimate the inter-assay variations of polymerase chain reaction (PCR) plates, we pooled 20 plasma samples randomly as mix sample and together with validation samples to perform RNA isolation and reverse transcription. We also evaluated hemolysis by calculating the difference in expression level between miR-23a-3p and miR-451a.[46] Plasma samples with a $\Delta Ct_{miR\text{-}23a\text{-}3p\text{-}miR\text{-}451a} > 7$ were defined as hemolysis samples and were removed for further analyses. Assays were carried out in triplicates for each sample. Three miRNAs, miR-16-5p,[47] miR-26b-5p,[48,49] and miR-423-5p[50] were selected as candidate endogenous controls to normalize the Ct values in each target assay. Not only were these miRNAs reported as endogenous controls and recommended in the manual of TaqMan Advanced miRNA assay, but they were most commonly found in plasma[46] and highly expressed in the discovery stage. To enhance the reliability of the normalization strategy, we also performed the global mean normalization.[51] We used the Normfinder program that combined the intragroup and intergroup variation into a relative stability value to evaluate the expression variability of endogenous controls, of which, the stability value is inversely correlated with the stability of endogenous controls.[52] For PCR data cleaning, each baseline was automatically assigned by the PCR system, and each target set the same threshold. The Ct values above 37 were considered missing. Only the miRNAs whose missing rates were less than 20% in all samples were considered stably expressed and included for differential expression analyses.

Overall, the miRNA isolation and reverse transcription of the samples were successfully completed, as endogenous controls (miR-16-5p, miR-26b-5p, and miR-423-5p) and spike-in (cel-miR-39-3p) indicated good technical performance. In each PCR plate, the same volume of mix sample was tested for cel-miR-39-3p, coefficients of variations for all PCR plates were 2.52%, suggesting good technical performance. All of the samples were free of hemolysis as evaluated by the Ct values difference of miR-23a-3p and miR-451a. However, four miRNAs (miR-142-5p, miR-17-3p, miR-381-3p, and miR-744-3p) were excluded for further analysis as over 20% of participants had an undetected signal in the validation stage. For the endogenous control selection, the most stable endogenous control identified by NormFinder was miR-26b-5p, of which the stability was 0.008, while miR-16-5p, miR-423-

5p, and global mean were 0.011, 0.011 and 0.010, respectively. Thus, we used miR-26-5p as the endogenous control to normalize each candidate miRNA.

Target genes screening and functional pathway analysis

Target genes of validated miRNAs were predicted by Targetscan (http://www.targetscan.org/), miRanda (http://www.microrna.org/), miRDB (http://www.mirdb.org/), Pictar (http://pictar.mdc-berlin.de/) and RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/) databases. Target genes successfully imputed by at least three of the above algorithms were considered as predicted genes. We also screened out validated target genes by miRTarBase database that offered the experimental data (http://mirtarbase.mbc.nctu.edu.tw/php/index.php). Furthermore, we extracted the gene expression profiles of coronary artery and heart from the GTEx project (https://gtexportal.org/home/) to filter out low abundance target genes (an average expression value of transcripts per million <5). After target genes screening and filtering, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses (http://www.genome.jp/kegg/) were performed by the clusterProfiler (R Bioconductor).[53] Statistical significance was defined as adjusted *P* value<0.05 based on the Benjamini-Hochberg method.

Study samples of genome-wide-association analysis of target miRNA

We conducted a genome-wide association study (GWAS) using the Affymetrix Genome-Wide Human SNP Array 6.0 chips and the Illumina Infinium OminZhongHua-8 chips in 1452 and 7417 Chinese subjects, respectively. Of these subjects, we included 7027 participants who were enrolled in DFTJ cohort had complete questionnaire information and blood samples in 2013. We further excluded participants with a history of CVD, cancer, or abnormal electrocardiograms (n=2542), as well as participants who were newly diagnosed with CHD<6 months after blood drawing (n=262). To perform bi-directional two-sample Mendelian randomization (MR), we separated two independent populations from the remaining 4223 eligible subjects. Among which, 340 samples were also included in the nested case-control study, thus a GWAS analysis of miR-4286 was performed in these subjects. For the remaining 3883 subjects, after excluding participants using lipid-lowering medication (n=447) or missing TG or HDL-C measurements, we included 3240 subjects who had complete TG measurements and 3238 subjects who had complete HDL-C measurements to estimate the effect sizes of lipid-related SNPs.

Genome-wide-association analyses of target miRNA

Genotyping was carried out with Affymetrix Genome-Wide Human SNP Array 6.0 chips and the Illumina Infinium OminZhongHua-8 chips with standard quality control procedures. We performed SNP imputation to merge the two arrays by Minimac3 (v2.0.1) as reported previously.[54] Briefly, we used 1000 Genomes Project ALL Phase 3 Integrated Release Version 5 Haplotypes (February 5, 2013) as reference panel, after filtering missing call rate >5%, minor allele frequency (MAF) <1%, and Hardy-Weinberg equilibrium P<1E-05, a total of 549 196 and 703 302 eligible SNPs were detected in the Affymetrix and Illumina dataset, respectively. We performed GWAS analyses of miR-4286 in 340 samples with natural log-transformed data after adjusting for age, sex, and 3 principal components (PCs) to account for population structure by SNPTEST (v2.5.4) using additive models.[55] After a meta-analysis of the two array dataset by METAL software,[56] we screened 24 independent loci associated with miR-4286 (*P*<5E-06) with weak linkage disequilibrium (r^2 <0.2).

Selection of lipid-related instrumental variables for the two-sample Mendelian randomization analyses

The genetic instrumental variables (IVs) of TG and HDL-C were selected based on the two larger scale reports in Asian. One is the largest publish GWAS study from the Biobank Japan Project (BBJ) in 162 255 Japanese individuals, the other one is an exome chip study in 47 532 East Asian individuals.[57,58] We included 4280 TG SNPs and 5606 HDL-C SNPs from BBJ, together with 96 TG SNPs and 135 HDL-C SNPs from exome chip study (all *P*<5E-08). Finally, a total of 39 SNPs of TG and 58 SNPs of HDL-C with weakly linkage disequilibrium (r^2<0.2) were selected as IVs in MR analyses, after removing low-quality or MAF<1% loci. Of note, we chose a larger effect size of SNP if it was significant in both two studies. Overall, 10 TG SNPs and 10 HDL-C SNPs were selected from the exome chip results, the remaining were derived from the BBJ study.

Table S1. Baseline characteristics of study participants in the validation stage.

Variables	Acute myocardial infarction			Unstable angina		
	Cases (*n*=137)	Controls (*n*=137)	*P* Value	Cases (*n*=435)	Controls (*n*=435)	*P* Value
Age, y	69.9±7.7	69.9±7.6	0.96	66.4±7.5	66.3±7.5	0.92
Male, No. (%)	101 (73.7)	101 (73.7)	1.00	226 (52.0)	226 (52.0)	1.00
BMI, kg/m2	24.08±3.64	23.97±3.17	0.79	24.91±3.29	24.29±3.00	0.004
SBP, mmHg	150.80±27.95	142.01±21.93	0.004	147.41±23.21	143.02±22.53	0.005
DBP, mmHg	84.22±13.24	80.10±12.60	0.009	82.99±13.28	80.85±12.58	0.02
Smoking status, No. (%)			0.07			0.24
Current smoker	42 (30.7)	24 (17.5)		94 (21.6)	81 (18.6)	
Former smoker	33 (24.1)	37 (27.0)		86 (19.8)	73 (16.8)	
Never smoker	60 (43.8)	75 (54.7)		255 (58.6)	280 (64.4)	
Drinking status, No. (%)			0.43			0.31
Current drinker	44 (32.1)	51 (37.2)		114 (26.2)	134 (30.8)	
Former drinker	23 (16.8)	16 (11.7)		52 (12.0)	40 (9.2)	
Never drinker	69 (50.4)	70 (51.1)		267 (61.4)	260 (59.8)	
Education level, No. (%)			0.002			0.31
Primary school or below	41 (29.9)	41 (29.9)		134 (30.8)	113 (26.0)	
Middle school	68 (49.6)	43 (31.4)		150 (34.5)	169 (38.9)	

High school or beyond	28 (20.5)	51 (37.2)		146 (33.6)	145 (33.3)	
Physical activity, MET-h/week	21.00 (0.00, 40.25)	30.00 (14.50, 42.00)	0.004	21.00 (10.50, 42.00)	21.00 (13.75, 42.00)	0.52
Family history of CHD, No. (%)	7 (5.1)	8 (5.8)	1.00	32 (7.4)	33 (7.6)	1.00
Lipid-lowering medication, No. (%)	14 (10.2)	19 (13.9)	0.46	71 (16.3)	63 (14.5)	0.51
Antihypertensive medication, No. (%)	54 (39.4)	42 (30.7)	0.16	177 (40.7)	151 (34.7)	0.08
Antidiabetic medication, No. (%)	20 (14.6)	15 (10.9)	0.47	56 (12.9)	42 (9.7)	0.16
Hyperlipidemia, No. (%)	86 (62.8)	85 (62.0)	1.00	294 (67.6)	289 (66.4)	0.77
Hypertension, No. (%)	112 (81.8)	100 (73.0)	0.10	337 (77.5)	309 (71.0)	0.04
Diabetes, No. (%)	43 (31.4)	35 (25.5)	0.35	117 (26.9)	99 (22.8)	0.18
FG, mmol/L	5.90 (5.30, 6.70)	5.60 (5.10, 6.30)	0.04	5.80 (5.30, 6.50)	5.69 (5.25, 6.26)	0.02
HDL-C, mmol/L	1.25 (1.12, 1.51)	1.36 (1.17, 1.61)	0.14	1.31 (1.16, 1.54)	1.42 (1.20, 1.67)	<0.001
LDL-C, mmol/L	2.91±0.85	2.71±0.89	0.06	2.89±0.80	2.81±0.85	0.15
TC, mmol/L	4.76 (4.21, 5.39)	4.76 (4.19, 5.26)	0.46	4.96 (4.33, 5.46)	4.88 (4.16, 5.50)	0.28
TG, mmol/L	1.36 (0.92, 2.09)	1.23 (0.86, 1.71)	0.04	1.40 (1.07, 1.96)	1.23 (0.90, 1.67)	<0.001

Continuous variables are presented as mean±SD or median (25th, 75th). Categorical variables are presented as numbers (percentage).

P Values were estimated using Student t-tests or Mann-Whitney U tests for continuous variables, and Chi-square tests for categorical variables.

Abbreviations: BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride.

Table S2. Candidate miRNAs associated with incident ACS in the discovery stage.

miRNAs	Fold change	Regulation	*P* Value	Chromosome location	miRBase ID	Taqman Advanced assay ID
let-7b-3p	2.39	Up	0.04	Chr22: 46113686-46113768[+]	MIMAT0004482	478221_mir
miR-1268b	1.91	Down	0.03	Chr17: 80098828-80098877[+]	MIMAT0018925	480790_mir
miR-1307-3p	1.41	Up	0.01	Chr10: 103394253-103394401[-]	MIMAT0005951	483036_mir
miR-133b	2.42	Down	0.03	Chr6: 52148923-52149041[+]	MIMAT0000770	480871_mir
miR-142-5p	1.61	Down	0.04	Chr17: 58331232-58331318[-]	MIMAT0000433	477911_mir
miR-17-3p	1.56	Down	0.04	Chr13: 91350605-91350688[+]	MIMAT0000071	477932_mir
miR-181c-3p	1.83	Down	0.02	Chr19: 13874699-13874808[+]	MIMAT0004559	477933_mir
miR-21-3p	1.41	Down	0.02	Chr17: 59841266-59841337[+]	MIMAT0004494	477973_mir
miR-28-3p	1.33	Up	0.048	Chr3: 188688781-188688866[+]	MIMAT0004502	477999_mir
miR-320a	1.33	Up	0.02	Chr8: 22244966-22245037[-]	MIMAT0000510	478594_mir
miR-381-3p	2.60	Down	0.048	Chr14: 101045920-101045994[+]	MIMAT0000736	477816_mir
miR-4286	1.56	Up	0.02	Chr8: 10666978-10667070[+]	MIMAT0016916	478096_mir
miR-4485-3p	2.85	Up	0.04	Chr11: 10508270-10508326[-]	MIMAT0019019	479430_mir
miR-500a-3p	1.85	Down	0.04	ChrX: 50008431-50008514[+]	MIMAT0002871	478951_mir
miR-574-5p	1.37	Up	0.04	Chr4: 38868032-38868127[+]	MIMAT0004795	479357_mir
miR-744-3p	2.07	Up	0.046	Chr17: 12081899-12081996[+]	MIMAT0004946	479165_mir
miR-940	1.45	Up	0.02	Chr16: 2271747-2271840[+]	MIMAT0004983	479216_mir

miR-99b-5p	1.59	Up	0.01	Chr19: 51692612-51692681[+]	MIMAT0000689	478343_mir

P Values were estimated by paired *t*-tests using microarray normalized data.

Plasma miR-142-5p, miR-17-3p, miR-381-3p, and miR-744-3p were excluded from analysis because of over 20% participants had undetected signal in the validation stage.

Abbreviations: ACS, acute coronary syndrome.

Table S3. Association of plasma miR-4286 normalized by global mean with incident ACS in the validation stage.

miRNA	Tertiles of plasma miR-4286*			*P*-trend†	OR (95% CI) per one IQR
	T1	T2	T3		
miR-4286	<5.62	5.62-8.75	>8.75		
n (case/control)	149/191	205/190	218/191		
OR (95% CI)	1 [Ref]	1.44 (1.03, 2.02)	1.57 (1.10, 2.24)	0.02	1.17 (1.02, 1.34)

*Plasma miRNA levels were normalized to the average of all miRNAs levels and were expressed as $2^{-\Delta Ct}$.

†*P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in logistic regression model.

OR (95% CI) was obtained using multivariable conditional logistic regression model with adjustment for age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication.

Abbreviations: ACS, acute coronary syndrome; CI, confidence interval; IQR, interquartile range; OR, odds ratio.

Table S4. Association of plasma miR-4286 with incident ACS in the validation stage, excluding ACS cases within the first year of follow-up.

miRNA	Tertiles of plasma miR-4286 (×10^{-2})*			*P*-trend†	OR (95% CI) per one IQR
	T1	T2	T3		
miR-4286	<7.06	7.06-12.85	>12.85		
n (case/control)	110/151	153/151	191/152		
OR (95% CI)	1 [Ref]	1.49 (1.01, 2.20)	2.00 (1.34, 2.98)	<0.001	1.28 (1.06, 1.55)

*Plasma miRNA levels were normalized to miR-26b-5p and were expressed as $2^{-\Delta Ct}$.

†*P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in logistic regression model.

OR (95% CI) was obtained using multivariable conditional logistic regression model with adjustment for age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication.

Abbreviations: ACS, acute coronary syndrome; CI, confidence interval; IQR, interquartile range; OR, odds ratio.

Table S5. Associations of plasma miR-4286 with incident ACS in subgroups stratified by risk factors in the validation stage.

Variables	*n* (case/control)	Tertiles of plasma miR-4286			*P*-trend*	*P*-interaction†
		T1	T2	T3		
Age						0.42
<65 years	214/213	1 [Ref]	0.99 (0.60, 1.64)	1.27 (0.78, 2.08)	0.97	
≥65 years	358/359	1 [Ref]	1.55 (1.06, 2.29)	1.76 (1.20, 2.58)	0.02	
Sex						0.97
Female	245/245	1 [Ref]	1.58 (0.97, 2.56)	1.61 (1.01, 2.57)	0.08	
Male	327/327	1 [Ref]	1.16 (0.78, 1.73)	1.55 (1.04, 2.31)	0.03	
BMI						0.10
<24 kg/m^2	241/277	1 [Ref]	1.34 (0.85, 2.13)	1.30 (0.84, 2.02)	0.31	
≥24 kg/m^2	331/295	1 [Ref]	1.41 (0.92, 2.14)	2.04 (1.34, 3.13)	0.001	
Current smokers						0.22
No	434/465	1 [Ref]	1.38 (0.98, 1.94)	1.78 (1.27, 2.49)	0.001	
Yes	136/105	1 [Ref]	1.23 (0.62, 2.45)	1.07 (0.55, 2.10)	0.90	
Current drinkers						0.19
No	411/386	1 [Ref]	1.39 (0.97, 2.00)	1.78 (1.24, 2.55)	0.002	
Yes	158/185	1 [Ref]	1.10 (0.62, 1.96)	1.19 (0.69, 2.07)	0.53	
Hypertension						0.46
No	123/163	1 [Ref]	1.06 (0.57, 2.00)	1.27 (0.67, 2.41)	0.44	
Yes	449/409	1 [Ref]	1.40 (0.99, 2.00)	1.67 (1.19, 2.35)	0.004	
Diabetes						0.88
No	412/438	1 [Ref]	1.45 (1.02, 2.07)	1.65 (1.16, 2.34)	0.008	
Yes	160/134	1 [Ref]	0.99 (0.53, 1.86)	1.50 (0.81, 2.76)	0.15	
FG level						0.19
Normal	198/233	1 [Ref]	1.61 (0.98, 2.68)	1.35 (0.83, 2.22)	0.34	

IFG	214/205	1 [Ref]	1.32 (0.78, 2.23)	2.00 (1.20, 3.37)	0.007	
Hyperlipidemia						0.51
No	192/198	1 [Ref]	0.96 (0.57, 1.63)	1.43 (0.86, 2.39)	0.11	
Yes	380/374	1 [Ref]	1.62 (1.11, 2.36)	1.75 (1.21, 2.53)	0.007	

Logistic regression models were used in the subgroup analysis with adjustment for age, sex, body mass index, smoking status, drinking status, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, low-density lipoprotein cholesterol, triglyceride, high-density lipoprotein cholesterol, and use of lipid-lowering medication.

[*] *P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in logistic regression model.

[†] *P*-interaction was obtained by continuous indicator*categorical stratifying variable.

Abbreviations: ACS, acute coronary syndrome; BMI, body mass index; FG, fasting glucose; IFG, impaired fasting glucose.

Table S6. Comparison of the associations between plasma miR-4286 and different subtypes of ACS in the validation stage.

ACS subtypes	Tertiles of plasma miR-4286 ($\times 10^{-2}$)*			*P*-trend†	OR (95% CI) per one IQR	$P_{heterogeneity}$‡
	T1 (<7.10)	T2 (7.10-12.23)	T3 (>13.23)			
AMI						
n (case/control)	36/109	38/190	63/191			
OR (95% CI)	1 [Ref]	1.00 (0.59, 1.69)	1.74 (1.07, 2.81)	0.01	1.29 (1.02, 1.63)	0.49
UA						
n (case/control)	109/191	153/190	173/191			
OR (95% CI)	1 [Ref]	1.38 (1.00, 1.91)	1.51 (1.10, 2.08)	0.02	1.18 (1.01, 1.38)	

OR (95% CI) was obtained using multinomial logistic regression model with the outcomes (AMI, UA, or non-ACS) as the dependent variable and miRNA level as independent variable, and adjusted for age, sex, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication.

*Plasma miRNA levels were normalized to miR-26b-5p and were expressed as $2^{-\Delta Ct}$.

†*P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in multinomial logistic regression model.

‡$P_{heterogeneity}$ was obtained from multinomial logistic regression model in the comparison between AMI and UA.

Abbreviations: ACS, acute coronary syndrome; AMI, acute myocardial infarction; CI, confidence interval; IQR, interquartile range; OR, odds ratio; UA, unstable angina.

Table S7. Adjusted PD (95% CI) for plasma miR-4286 according to categorical cardiovascular traits in the validation stage.

Variables	All participants	ACS cases	Controls
Age			
<65 years	0 [Ref]	0 [Ref]	0 [Ref]
≥65 years	2.72 (-8.46, 15.26)	3.47 (-12.06, 21.74)	1.93 (-13.52, 20.14)
Sex			
Female	0 [Ref]	0 [Ref]	0 [Ref]
Male	-13.37 (-23.62, -1.75)	-12.66 (-27.02, 4.52)	-12.53 (-26.91, 4.68)
BMI			
<24 kg/m^2	0 [Ref]	0 [Ref]	0 [Ref]
≥24 kg/m^2	2.05 (-8.27, 13.53)	11.11 (-4.39, 29.13)	-7.93 (-20.96, 7.24)
Current smokers			
No	0 [Ref]	0 [Ref]	0 [Ref]
Yes	-0.53 (-13.55, 14.45)	-4.46 (-21.41, 16.14)	0.88 (-17.96, 24.06)
Current drinkers			
No	0 [Ref]	0 [Ref]	0 [Ref]
Yes	2.73 (-9.09, 16.08)	-0.54 (-16.47, 18.42)	9.18 (-8.17, 29.81)
Education level			
Primary school or below	0 [Ref]	0 [Ref]	0 [Ref]
Middle school	-3.98 (-15.74, 9.43)	-7.17 (-22.64, 11.38)	-0.19 (-17.48, 20.72)
High school or beyond	-7.46 (-19.21, 6.01)	-5.20 (-21.66, 14.72)	-8.00 (-24.26, 11.74)
MET			
<21 h/week	0 [Ref]	0 [Ref]	0 [Ref]
≥21 h/week	-1.56 (-11.69, 9.72)	1.30 (-12.77, 17.63)	-2.40 (-16.70, 14.35)
Hypertension			
No	0 [Ref]	0 [Ref]	0 [Ref]
Yes	4.93 (-5.74, 16.80)	7.60 (-6.32, 23.58)	-0.15 (-15.75, 18.35)

Diabetes			
No	0 [Ref]	0 [Ref]	0 [Ref]
Yes	3.16 (-8.49, 16.31)	2.57 (-13.04, 20.99)	1.25 (-15.17, 20.84)
Hyperlipidemia			
No	0 [Ref]	0 [Ref]	0 [Ref]
Yes	-3.37 (-13.63, 8.09)	-3.02 (-17.33, 13.78)	-3.42 (-17.66, 13.28)

Adjusted PD (95% CI) were estimated using linear regression models, including age (<65 years, ⩾65 years), sex (female, male), BMI (<24 kg/m^2, ⩾24 kg/m^2), current smokers (no, yes), current drinkers (no, yes), education level (primary school or below, middle school, high school or beyond), MET (<21 h/week, ⩾21 h/week), hypertension (no, yes), diabetes (no, yes), and hyperlipidemia (no, yes).

Abbreviations: BMI, body mass index; CI, confidence interval; MET, metabolic equivalent; PD, percent difference.

Table S8. Adjusted PD (95% CI) for plasma miR-4286 according to linear and tertiles of cardiovascular traits in the validation stage.*

Variables	Tertiles of cardiovascular traits			*P*-trend†	PD (95% CI) per one IQR
	T1	T2	T3		
All participants					
SBP	0 [Ref]	3.12 (-9.58, 17.60)	2.46 (-10.52, 17.33)	0.74	-0.73 (-7.50, 6.54)
DBP	0 [Ref]	-0.18 (-12.53, 13.90)	1.30 (-11.04, 15.35)	0.83	-1.36 (-8.06, 5.83)
FG	0 [Ref]	1.25 (-11.05, 15.24)	7.86 (-5.57, 23.21)	0.25	1.94 (-2.68, 6.76)
HDL-C	0 [Ref]	-9.28 (-20.24, 3.18)	-18.08 (-28.21, -6.51)	0.003	-11.05 (-16.65, -5.08)
LDL-C	0 [Ref]	12.42 (-1.01, 27.68)	5.71 (-6.98, 20.13)	0.43	3.18 (-3.69, 10.54)
TC	0 [Ref]	7.01 (-5.82, 21.59)	-3.46 (-15.32, 10.07)	0.63	1.76 (-4.03, 7.91)
TG	0 [Ref]	7.11 (-5.74, 21.70)	23.89 (8.67, 41.24)	0.001	11.04 (3.77, 18.83)
TG/HDL-C	0 [Ref]	4.84 (-7.75, 19.15)	23.63 (8.37, 41.04)	0.001	15.01 (7.16, 23.42)
ACS cases					
SBP	0 [Ref]	-15.53 (-29.75, 1.56)	-1.22 (-18.10, 19.14)	0.96	2.16 (-7.40, 12.71)
DBP	0 [Ref]	-9.50 (-24.48, 8.45)	5.74 (-12.08, 27.17)	0.49	-0.75 (-9.85, 9.27)
FG	0 [Ref]	14.36 (-4.39, 36.80)	4.99 (-13.17, 26.96)	0.67	-1.34 (-7.77, 5.54)
HDL-C	0 [Ref]	0.58 (-15.97, 20.37)	-16.28 (-30.44, 0.76)	0.046	-11.00 (-18.25, -3.11)
LDL-C	0 [Ref]	3.36 (-13.44, 23.42)	4.22 (-12.86, 24.64)	0.65	3.33 (-6.67, 14.41)
TC	0 [Ref]	-0.01 (-16.33, 19.49)	-5.26 (-21.01, 13.64)	0.56	-2.22 (-10.80, 7.19)
TG	0 [Ref]	8.18 (-9.58, 29.43)	20.44 (-0.16, 45.30)	0.05	7.46 (-2.44, 18.36)
TG/HDL-C	0 [Ref]	10.45 (-7.83, 32.34)	24.30 (3.13, 49.81)	0.02	12.13 (1.50, 23.88)
Controls					
SBP	0 [Ref]	-1.23 (-18.10, 19.12)	-4.87 (-21.66, 15.53)	0.61	-5.04 (-14.43, 5.37)
DBP	0 [Ref]	9.83 (-8.63, 32.02)	-2.56 (-19.58, 18.06)	0.78	-3.11 (-12.74, 7.58)
FG	0 [Ref]	-4.52 (-20.51, 14.70)	8.19 (-10.35, 30.58)	0.39	4.37 (-2.60, 11.85)
HDL-C	0 [Ref]	-9.80 (-25.13, 8.65)	-16.47 (-30.56, 0.49)	0.06	-8.72 (-16.85, 0.21)
LDL-C	0 [Ref]	14.78 (-4.35, 37.75)	2.23 (-15.04, 23.02)	0.85	1.61 (-7.41, 11.51)

TC	0 [Ref]	9.51 (-8.99, 31.78)	-1.53 (-18.62, 19.15)	0.92	2.61 (-5.15, 11.01)
TG	0 [Ref]	-9.26 (-24.44, 8.97)	17.09 (-3.01, 41.37)	0.10	11.52 (1.41, 22.64)
TG/HDL-C	0 [Ref]	-2.00 (-18.35, 17.62)	19.14 (-1.24, 43.73)	0.06	13.46 (3.14, 24.80)

*Adjusted PD (95% CI) corresponds to tertiles of trait or an IQR increase in trait with adjustment for age, sex, body mass index, smoking status, drinking status, education levels, and metabolic equivalent. SBP and DBP additionally adjusted for antihypertensive medication use. FG additionally adjusted for antidiabetic medication use. HDL-C, LDL-C, TC, TG, and TG/HDL-C ratio additionally adjusted for lipid-lowering medication use.

†*P*-trend was estimated by assigning the median value of trait to each tertile and using this as a continuous variable in linear regression model.

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; PD, percent difference; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Table S9. Associations of plasma lipid traits with incident ACS in the validation stage.*

Lipids	Tertiles of plasma lipid traits			*P*-trend†	*FDR*	OR (95% CI) per one IQR
	T1	T2	T3			
HDL-C (mmol/L)	<1.25	1.25-1.55	>1.55			
n (case/control)	235/189	200/186	137/197			
OR (95% CI)	1 [Ref]	0.96 (0.71, 1.30)	0.66 (0.48, 0.92)	0.01	0.02	0.86 (0.73, 1.00)
LDL-C (mmol/L)	<2.42	2.42-3.12	>3.12			
n (case/control)	175/188	175/192	222/192			
OR (95% CI)	1 [Ref]	1.00 (0.74, 1.36)	1.35 (0.99, 1.83)	0.05	0.06	1.26 (1.06, 1.48)
TC (mmol/L)	<4.38	4.38-5.26	>5.26			
n (case/control)	161/188	227/192	184/192			
OR (95% CI)	1 [Ref]	1.53 (1.12, 2.09)	1.23 (0.88, 1.71)	0.18	0.18	1.31 (1.13, 1.52)
TG (mmol/L)	<1.00	1.00-1.51	>1.51			
n (case/control)	125/190	194/190	253/192			
OR (95% CI)	1 [Ref]	1.68 (1.21, 2.34)	2.06 (1.47, 2.89)	<0.001	<0.001	1.45 (1.21, 1.74)
TG/HDL-C (ratio)	<0.68	0.68-1.12	>1.12			
n (case/control)	123/191	193/190	256/191			
OR (95% CI)	1 [Ref]	1.53 (1.11, 2.12)	1.99 (1.43, 2.77)	<0.001	<0.001	1.47 (1.22, 1.77)

*Lipid traits were included in the conditional logistic regression models with adjustment for age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, family history of coronary heart disease, diabetes, hypertension, and use of lipid-lowering medication.

†*P*-trend was estimated by assigning the median value of lipid to each tertile and using this as a continuous variable in conditional logistic regression models.

Abbreviations: ACS, acute coronary syndrome; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TC, total cholesterol; TG, triglyceride.

Table S10. SNPs of miR-4286 selected for the Mendelian randomization analyses based on the genome-wide association analysis (*P*<5E-06).

SNPs	Position	Nearest Genes	Allele	EAF	Effect size (SEM)	*P* Value
rs5775955	1:88217744	*RP5-1027O11.1*	CATA/C	0.88	-0.65 (0.12)	4.71E-08
rs185758585	2:83971924	*RNU6-1312P*	T/C	0.01	1.82 (0.37)	8.97E-07
rs75615431	2:157524497	*RPLP0P7*	C/T	0.15	0.60 (0.12)	3.55E-07
rs6434150	2:186825204	*RPL21P32*	A/T	0.90	0.64 (0.13)	6.49E-07
rs17626938	3:20080132	*PP2D1KAT2B*	G/A	0.08	-0.68 (0.15)	4.48E-06
rs6797897	3:139620442	*RP11-166D18.1*	C/G	0.59	-0.47 (0.10)	2.03E-06
rs145356549	4:4689685	*STX18-AS1*	T/C	0.02	1.95 (0.40)	1.09E-06
rs544792245	4:27478020	*IGBP1P5*	CT/C	0.01	1.96 (0.40)	9.70E-07
rs372448036	4:88553224	*RP11-742B18.1*	A/ACC	0.01	2.22 (0.43)	2.09E-07
rs199980924	4:113245645	*ALPK1*	T/G	0.02	1.72 (0.34)	3.31E-07
rs192431740	5:12347309	*RNU6-679P*	C/T	0.01	1.89 (0.41)	4.10E-06
rs143142540	5:26159829	*RNU4-43P*	T/C	0.01	1.71 (0.37)	4.02E-06
rs1057412	6:31321752	*HLA-B*	G/T	0.12	0.57 (0.12)	3.33E-06
rs606578	6:139845597	*RP11-12A2.3*	A/G	0.39	0.46 (0.10)	4.51E-06
rs1721018	7:17004886	*AC098592.6*	T/C	0.29	-0.42 (0.09)	1.11E-06
rs28375190	7:36431478	*ANLN*	G/T	0.18	-0.50 (0.10)	1.17E-06
rs7030875	9:89180003	*RP11-359J6.1*	T/A	0.01	2.49 (0.53)	2.36E-06
rs28374621	9:139219818	*GPSM1, WI2-1959D15.1*	A/G	0.01	1.99 (0.44)	4.80E-06
rs140233303	10:5646881	*RP13-463N16.6*	AG/A	0.08	-0.73 (0.15)	1.25E-06
rs71399823	15:86879954	*AGBL1*	A/G	0.05	0.88 (0.18)	1.99E-06
rs61744697	17:11672607	*DNAH9*	T/G	0.05	0.79 (0.17)	1.66E-06
rs28397896	18:10191496	*RP11-419J16.1*	A/G	0.32	0.43 (0.09)	5.78E-07
rs34047128	19:54266441	*MIR519A2*	T/C	0.24	0.57 (0.13)	4.53E-06
rs116437901	21:29860677	*AF131217.1*	T/G	0.01	1.97 (0.36)	5.14E-08

Positions are reported in human genome build hg19. Alleles are listed as effect/reference alleles.

Abbreviations: EAF, effect allele frequency; SEM, standard error of the mean.

Table S11. SNPs of TG selected for Mendelian randomization analyses based on the previous reports in Asian (*P*<5E-08).

SNPs	Position	Nearest Genes	Allele	EAF	Effect size (SEM)	*P* Value
rs35529421	1:62965621	*DOCK7*	A/T	0.14	-0.07 (0.006)	3.57E-32
rs2114273	1:93854517	*RF00019, FNBP1L*	C/T	0.61	0.03 (0.005)	5.95E-10
rs2144300	1:230294916	*GALNT2*	T/C	0.19	-0.03 (0.005)	4.23E-08
rs12992267	2:21215645	*AC012361.1, AC115619.1*	T/C	0.09	0.05 (0.008)	1.46E-11
rs13306194	2:21252534	*APOB**	A/G	0.13	-0.07 (0.01)	1.38E-12
rs1260326	2:27730940	*GCKR**	C/T	0.52	-0.11 (0.007)	1.26E-62
rs3749147	2:27851918	*GPN1, ZNF512*	A/G	0.38	0.05 (0.005)	8.25E-27
rs3752442	4:3446883	*HGFAC*	G/A	0.43	-0.03 (0.004)	1.27E-14
rs1037814	4:88049850	*AFF1*	C/T	0.56	0.03 (0.004)	9.46E-11
rs154254	5:55820584	*C5orf67*	C/G	0.39	0.03 (0.004)	7.59E-09
rs6882076	5:156390297	*TIMD4, HAVCR1**	C/T	0.73	0.05 (0.008)	4.15E-09
rs6905288	6:43758873	*VEGFA, AL157371.2*	A/G	0.79	0.03 (0.006)	7.90E-09
rs12531645	7:73023881	*MLXIPL, AC005089.1*	A/G	0.10	-0.11 (0.007)	3.23E-49
rs4921914	8:18272438	*NAT2, PSD3**	T/C	0.49	-0.05 (0.007)	4.73E-15
rs1059611	8:19824563	*LPL*	C/T	0.13	-0.17 (0.006)	1.49E-154
rs2954021	8:126482077	*RP11-136O12.2, TRIB1*	G/A	0.55	-0.06 (0.004)	7.43E-42
rs7916868	10:64988931	*JMJD1C*	T/A	0.73	-0.03 (0.005)	1.41E-10
rs4411227	10:94831513	*AL358613.3*	G/C	0.67	-0.04 (0.005)	3.57E-16
rs174551	11:61573684	*FADS2, FADS1*	C/T	0.37	0.04 (0.005)	8.15E-15
rs7350481	11:116586283	*AP000770.1, BUD13**	C/T	0.75	-0.22 (0.008)	1.90E-160
rs75198898	11:116649806	*ZPR1*	A/G	0.08	0.29 (0.008)	1.94E-288
rs603446	11:116654435	*ZPR1*	T/C	0.22	-0.15 (0.005)	3.38E-180
rs662799	11:116663707	*APOA5, AP006216.2**	A/G	0.71	-0.28 (0.009)	2.47E-196

rs9804646	11:116665079	*APOA5, AP006216.2*	T/C	0.11	-0.15 (0.007)	4.99E-106
rs633389	11:116667337	*APOA5, AP006216.2*	T/C	0.40	0.03 (0.004)	1.30E-12
rs6589567	11:116670676	*ZPR1, APOA5**	C/A	0.71	-0.15 (0.01)	1.36E-51
rs7123454	11:116704178	*APOC3, APOA1*	A/C	0.61	-0.08 (0.004)	2.77E-75
rs7112513	11:117037361	*PAFAH1B2*	G/A	0.54	-0.06 (0.004)	6.23E-49
rs508487	11:117075566	*PCSK7**	T/C	0.16	0.09 (0.01)	1.03E-13
rs11542139	11:117100257	*PCSK7**	T/C	0.04	0.11 (0.02)	9.85E-11
rs2075260	12:109696838	*ACACB**	A/G	0.73	0.04 (0.008)	3.95E-08
rs75766425	14:52511911	*NID2*	C/G	0.13	-0.04 (0.007)	1.27E-08
rs261291	15:58680178	*ALDH1A2*	C/T	0.51	0.05 (0.004)	2.57E-26
rs1800588	15:58723675	*LIPC, ALDH1A2*	T/C	0.51	0.06 (0.004)	6.22E-51
rs2679617	15:70207077	*LINC00593, TLE3*	G/A	0.26	-0.03 (0.005)	2.38E-08
rs56156922	16:56987369	*HERPUD1,CETP*	C/T	0.22	-0.04 (0.005)	1.41E-12
rs2278426	19:11350488	*ANGPTL8, DOCK6*	T/C	0.27	-0.04 (0.005)	1.62E-13
rs58542926	19:19379549	*TM6SF2, CILP2*	T/C	0.08	-0.06 (0.009)	9.10E-14
rs75627662	19:45413576	*AC011481.3*	T/C	0.12	0.09 (0.007)	5.65E-42

Positions are reported in human genome build hg19. Alleles are listed as effect/reference alleles.

SNPs of gene marked with asterisk were derived from the East Asian exome meta-analysis study, others were selected from the BioBank Japan Project in Japanese.

Abbreviations: EAF, effect allele frequency; SEM, standard error of the mean; TG, triglyceride.

Table S12. SNPs of HDL-C selected for the Mendelian randomization analyses based on the previous reports in Asian (*P*<5E-08).

SNPs	Position	Nearest Genes	Allele	EAF	Effect size (SEM)	*P* Value
rs6685271	1:93634590	*TMED5*	A/C	0.60	-0.04 (0.006)	1.37E-10
rs2144300	1:230294916	*GALNT2*	T/C	0.19	0.04 (0.007)	4.25E-08
rs117350179	3:12374332	*PPARG*	G/C	0.23	-0.03 (0.006)	2.96E-08
rs28366301	6:32560883	*HLA-DRB1, HLA-DQA1*	A/G	0.39	0.04 (0.006)	7.58E-11
rs1358980	6:43764551	*VEGFA, LINC01512*	T/C	0.54	-0.03 (0.005)	2.69E-09
rs1652507	6:161082461	*LPA*[*]	C/T	0.47	-0.04 (0.007)	2.10E-09
rs1026422	7:50319807	*AC020743.3, IKZF1*	A/G	0.45	0.03 (0.005)	4.84E-10
rs7778167	7:127851628	*MIR129-1, LEP*	A/G	0.11	0.06 (0.008)	1.99E-11
rs301	8:19816934	*LPL*[*]	C/T	0.49	0.11 (0.008)	1.72E-40
rs325	8:19819328	*LPL*	C/T	0.13	0.15 (0.008)	2.09E-79
rs3808447	8:116575459	*TRPS1*	A/G	0.69	0.05 (0.006)	3.64E-15
rs4743758	9:107515814	*NIPSNAP3A*	T/C	0.25	-0.04 (0.006)	1.94E-10
rs2230808	9:107562804	*ABCA1*	C/T	0.61	0.05 (0.005)	9.31E-21
rs4149310	9:107589134	*ABCA1*	T/A	0.69	0.06 (0.006)	2.39E-28
rs1883025	9:107664301	*ABCA1*	T/C	0.27	-0.11 (0.006)	3.03E-76
rs7847628	9:123631225	*PHF19*	G/A	0.37	-0.03 (0.006)	1.62E-08
rs7895716	10:94783777	*EXOC6*	G/C	0.65	0.04 (0.005)	1.24E-10
rs2297991	10:113913222	*GPAM*[*]	C/T	0.76	-0.05 (0.007)	6.52E-12
rs4917630	10:114019830	*GPAM, TECTB*	A/G	0.37	-0.04 (0.005)	7.56E-13
rs2257129	10:122898697	*RPL19P16, LINC01153*	C/T	0.70	-0.04 (0.006)	1.85E-10
rs174570	11:61597212	*FADS2*[*]	T/C	0.56	-0.05 (0.008)	1.49E-08
rs1263056	11:116576415	*AP000770.1*	G/A	0.73	-0.07 (0.006)	1.40E-29
rs1893460	11:116603677	*AP000770.1*	A/G	0.19	0.11 (0.007)	1.42E-62
rs10488698	11:116633947	*BUD13*[*]	A/G	0.07	0.09 (0.01)	2.14E-15

rs3741297	11:116657667	*ZPR1*	T/C	0.08	-0.26 (0.01)	2.52E-157
rs651821	11:116662579	*APOA5*	T/C	0.65	0.14 (0.005)	1.07E-153
rs6589567	11:116670676	*ZPR1, APOA5*[*]	C/A	0.71	0.10 (0.01)	8.86E-24
rs12718465	11:116707736	*APOA1-AS, APOA1*	T/C	0.07	-0.09 (0.01)	7.97E-13
rs4883263	12:7649484	*CD163*[*]	C/T	0.69	-0.05 (0.007)	5.24E-11
rs11067592	12:110069190	*MVK, RN7SKP250*	T/G	0.08	-0.09 (0.01)	5.12E-18
rs11067829	12:110116872	*RN7SKP250, FAM222A*	G/A	0.46	0.04 (0.006)	5.03E-13
rs28577594	12:123895906	*SETD8, RILPL2*	C/G	0.74	-0.04 (0.006)	1.67E-09
rs61005347	12:125325778	*SCARB1*	T/C	0.07	-0.07 (0.01)	1.22E-11
rs67053123	12:125353810	*SCARB1*	A/T	0.29	0.07 (0.006)	1.38E-32
rs76213020	14:52436005	*GNG2, AL358333.3*	C/A	0.14	0.05 (0.008)	1.19E-08
rs118146059	15:58544773	*ALDH1A2*	C/T	0.03	-0.16 (0.02)	2.43E-21
rs12903590	15:58577777	*ALDH1A2*	T/A	0.54	0.07 (0.005)	8.81E-38
rs261291	15:58680178	*ALDH1A2*	C/T	0.51	0.12 (0.005)	7.30E-109
rs13329672	15:58699937	*ALDH1A2*	T/C	0.14	0.10 (0.008)	7.15E-37
rs2070895	15:58723939	*ALDH1A2, LIPC*	A/G	0.51	0.13 (0.005)	1.13E-140
rs77780456	16:56849114	*NUP93*	G/A	0.05	0.17 (0.01)	5.93E-39
rs56156922	16:56987369	*HERPUD1, CETP*	C/T	0.22	0.27 (0.006)	0.00E+00
rs1800775	16:56995236	*HERPUD1, CETP*	A/C	0.55	0.09 (0.005)	9.95E-71
rs7499892	16:57006590	*CETP*	T/C	0.16	-0.16 (0.008)	3.88E-101
rs116893196	16:57010955	*CETP*	C/G	0.07	-0.19 (0.01)	2.96E-59
rs2303790	16:57017292	*CETP*	G/A	0.04	0.42 (0.01)	5.00E-198
rs56303487	16:68029739	*DUS2, DPEP2*	T/C	0.14	0.07 (0.008)	3.09E-19
rs2925979	16:81534790	*CMIP*	C/T	0.67	0.04 (0.006)	7.40E-13
rs12970066	18:47107152	*LIPG*	G/C	0.26	0.05 (0.006)	2.44E-14
rs11082764	18:47119579	*LIPG*	G/A	0.46	0.08 (0.005)	5.63E-51
rs73959590	18:47156188	*LIPG, SMUG1P1*	A/G	0.09	-0.09 (0.01)	9.77E-22
rs4939883	18:47167214	*LIPG*[*]	C/T	0.80	0.06 (0.009)	1.23E-13

rs737337	19:11347493	*DOCK6**	C/T	0.24	-0.07 (0.008)	1.50E-18
rs429358	19:45411941	*APOE*	C/T	0.10	-0.09 (0.009)	2.43E-24
rs7412	19:45412079	*APOE**	T/C	0.10	0.10 (0.02)	3.95E-11
rs5167	19:45448465	*APOC4*, *APOC2*	G/T	0.48	0.04 (0.005)	9.06E-13
rs235314	21:46271452	*PTTG1IP*	T/C	0.41	-0.03 (0.005)	3.30E-10
rs7445	22:21977047	*UBE2L3*	T/C	0.46	-0.04 (0.005)	1.25E-14

Positions are reported in human genome build hg19. Alleles are listed as effect/reference alleles.

SNPs of gene marked with asterisk were derived from the East Asian exome meta-analysis study, others were selected from the BioBank Japan Project in Japanese.

Abbreviations: EAF, effect allele frequency; HDL-C, high-density lipoprotein cholesterol; SEM, standard error of the mean.

Table S13. The proportions of genetic explanation of TG, HDL-C, and miR-4286 related SNPs.

Traits	**#SNP**	**Beta**	**SEM**	***P* Value**	**R^2**	***F* statistics**
TG	39	0.23	0.02	<0.001	0.07	76.50
HDL-C	58	0.14	0.01	<0.001	0.07	83.65
miR-4286	24	0.25	0.01	<0.001	0.48	105.41

Linear regression models adjusted for age and sex were used to examine the effects and F statistics.

Abbreviations: HDL-C, high-density lipoprotein cholesterol; SEM, standard error of the mean; TG, triglyceride.

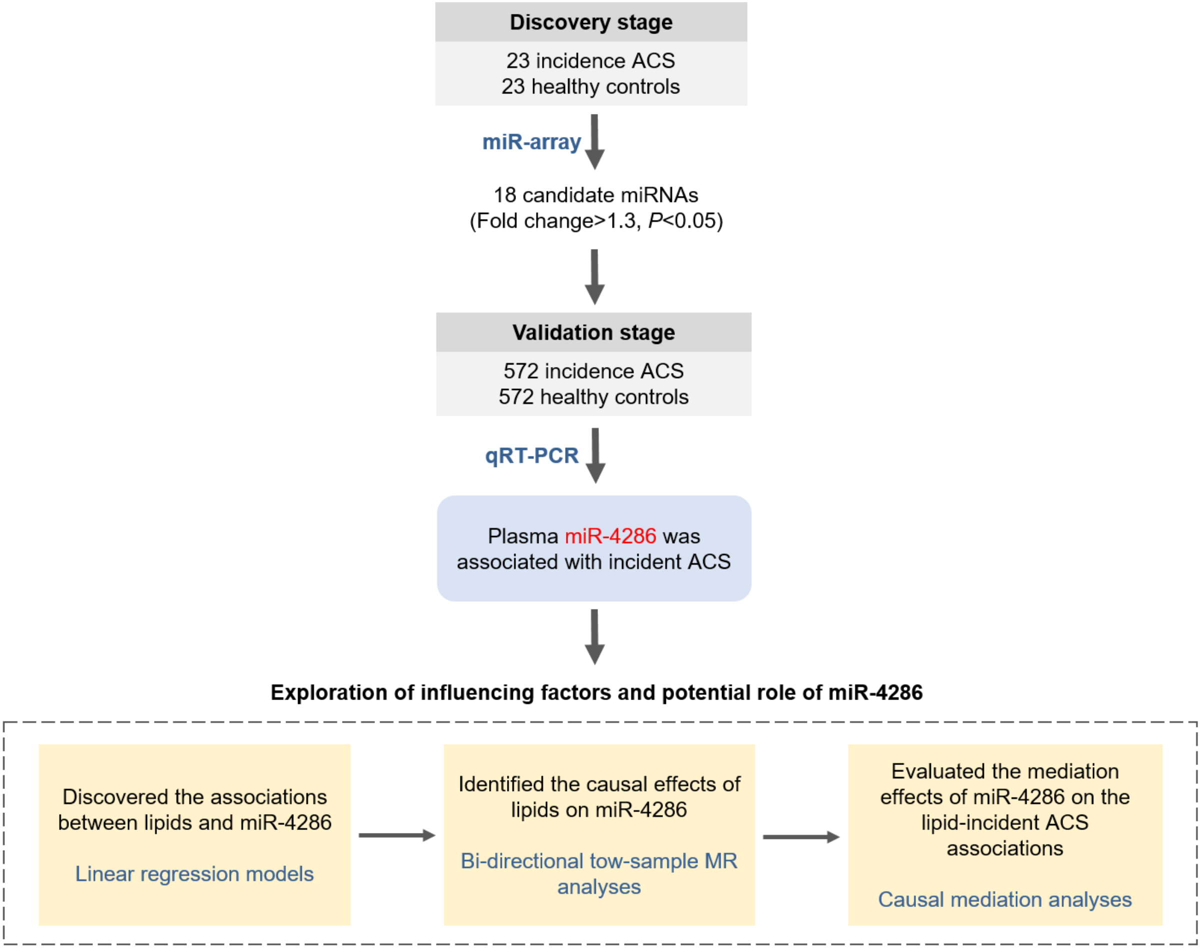

Figure S1. Overview of the study design.

ACS, acute coronary syndrome, MR, Mendelian randomization; qRT-PCR, quantitative real-time polymerase chain reaction.

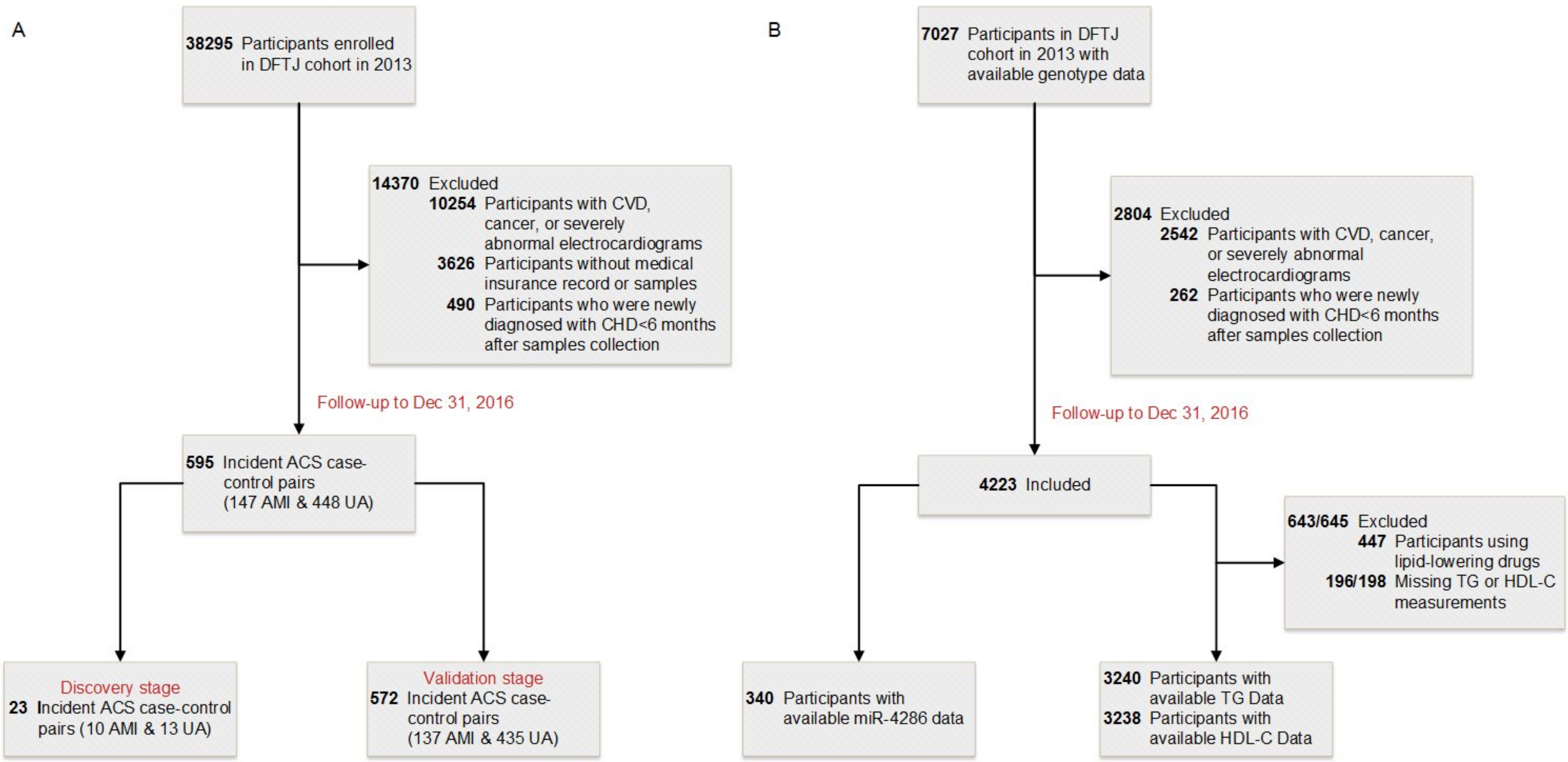

Figure S2. Flowchart of the study participants.

A, Participants of the nested case-control study; **B**, Participants of the genome-wide association study and Mendelian randomization study.

ACS, acute coronary syndrome; AMI, acute myocardial infarction; CHD, coronary heart disease; CVD, cardiovascular disease; DFTJ, Dongfeng-Tongji cohort; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; UA, unstable angina.

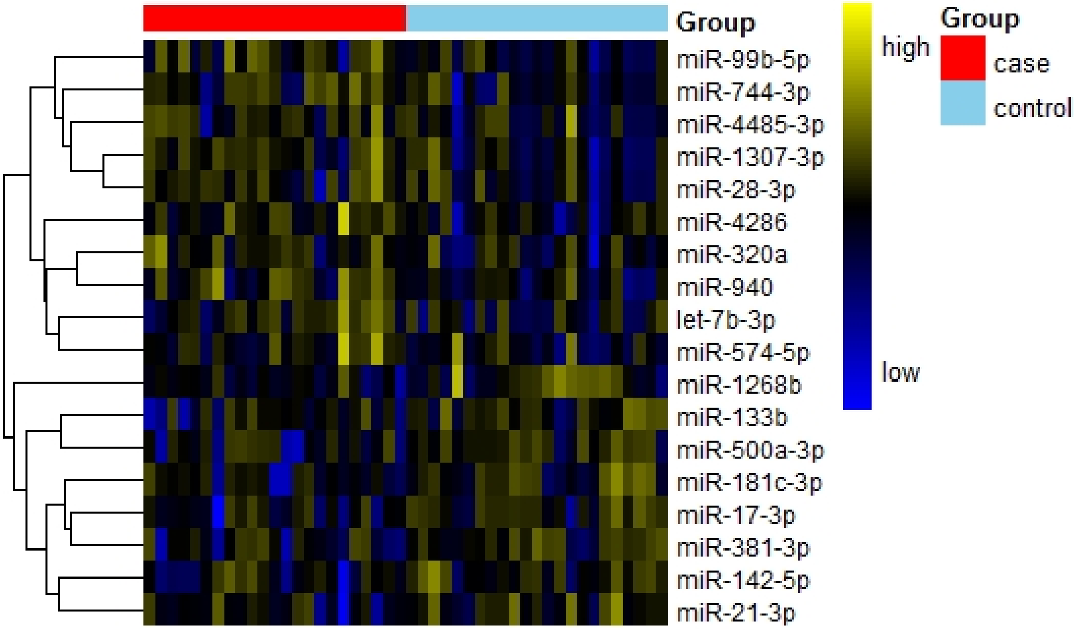

Figure S3. Hierarchical clustering of 18 differentially expressed candidate miRNAs in the discovery stage.

The columns of the heatmap represent incident acute coronary syndrome cases (red) and controls (sky blue), and the rows represent miRNAs. The color gradient range from highest (yellow) to lowest (blue) based on the miRNA levels of each sample.

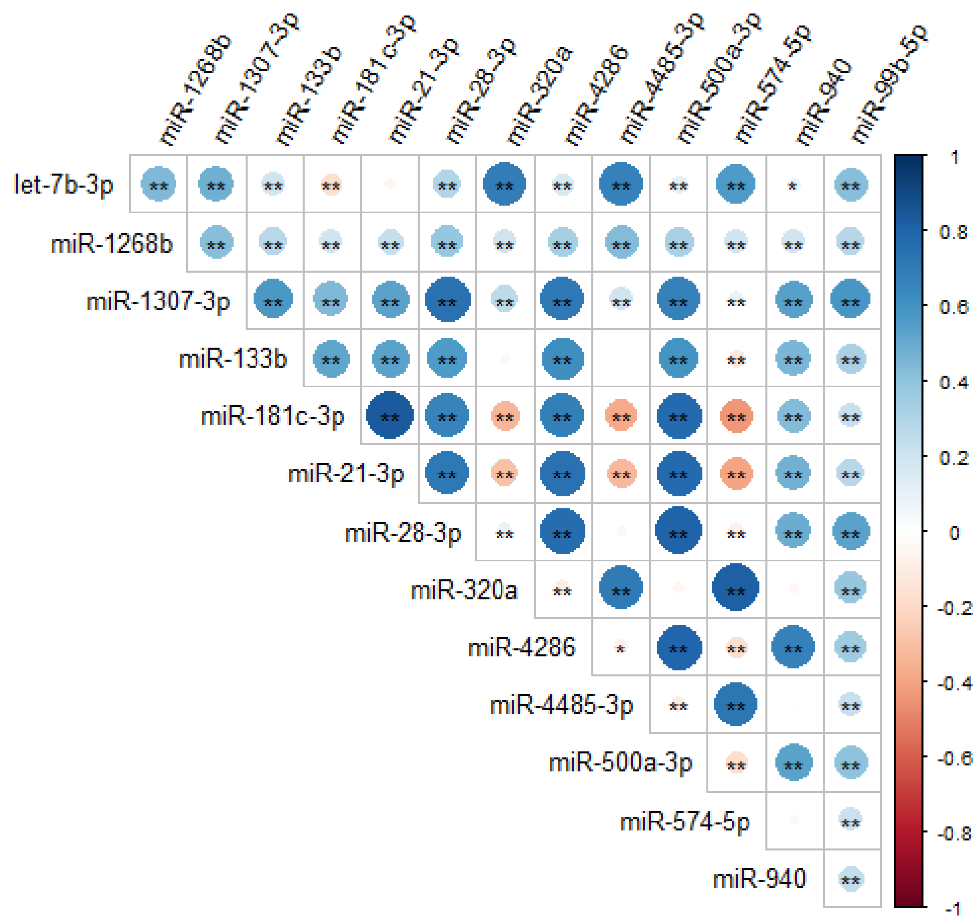

Figure S4. Correlation patterns of plasma miRNAs in the validation stage.

Dot size and shade depth represent the sizes of Spearman's rank correlation coefficients. Significant *P* values are marked with asterisk (**P*<0.05; ***P*<0.01).

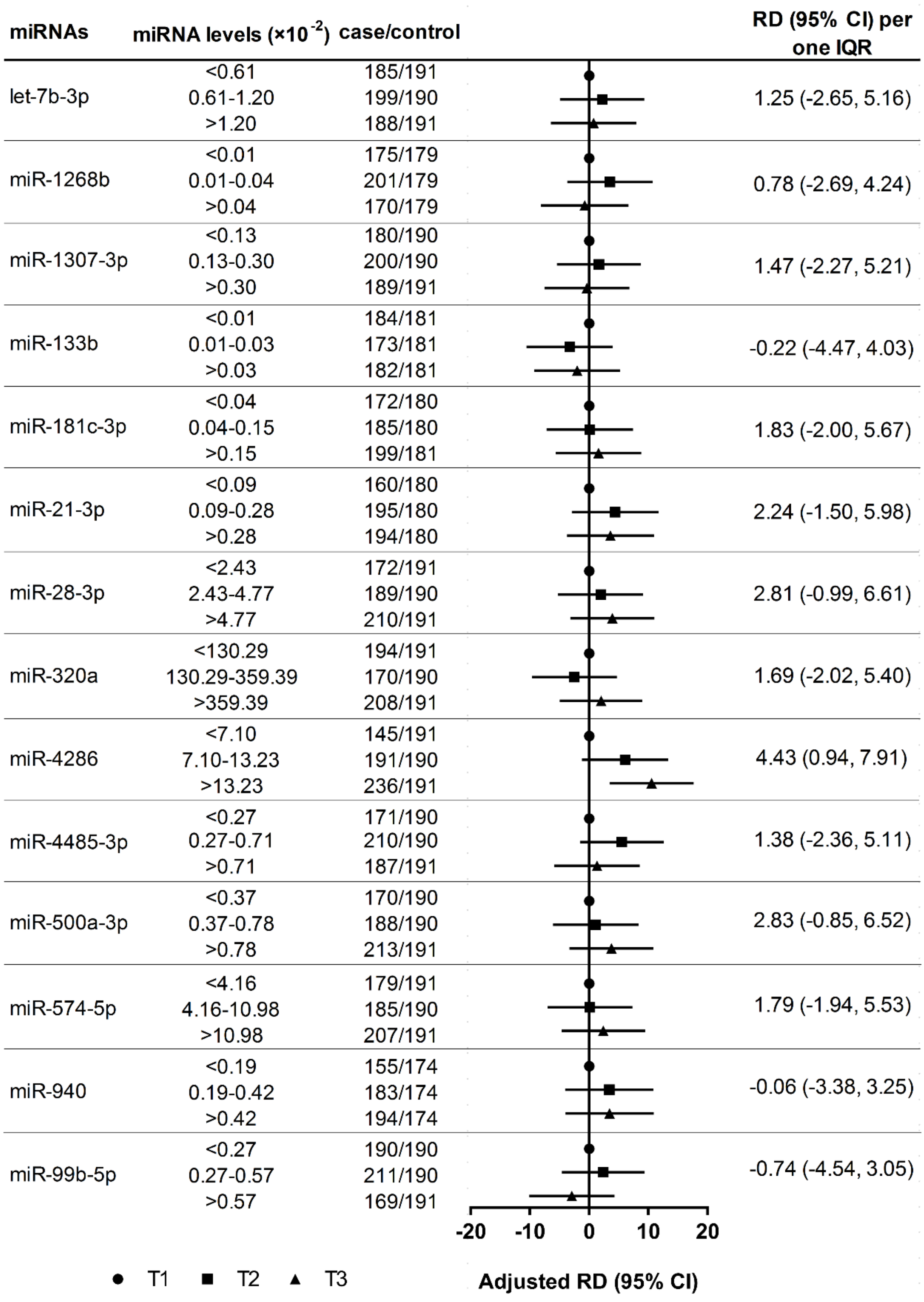

Figure S5. Adjusted RD (95% CI) for incident ACS according to plasma miRNAs in the validation stage.

Plasma miRNA levels were normalized to miR-26b-5p and were expressed as $2^{-\Delta Ct}$. Adjusted RD

(95% CI) for incident ACS was obtained using generalized linear regression model with adjustment for age, sex, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication. ACS, acute coronary syndrome; CI, confidence interval; IQR, interquartile range; RD, risk difference.

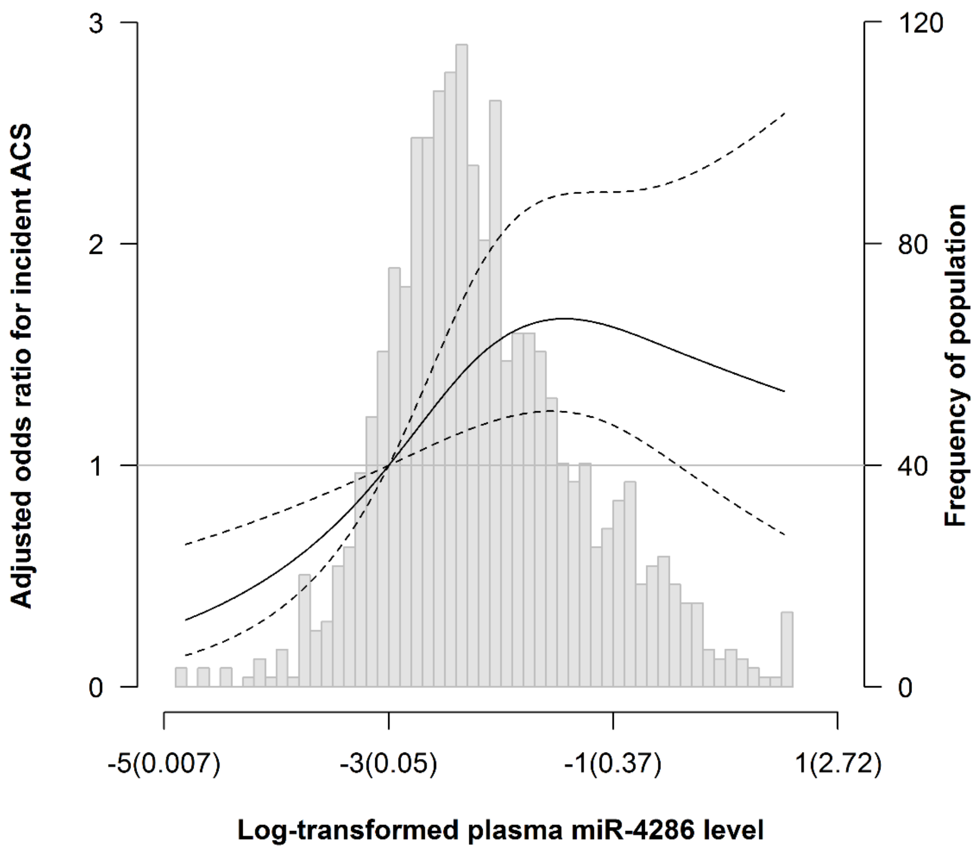

Figure S6. The restricted cubic spline for the associations between plasma miR-4286 and incident ACS.

The lines represent adjusted odds ratios based on restricted cubic splines for the log-transformed level of miR-4286 in the conditional logistic model. Knots were placed at the 5th, 50th and 95th percentiles of the distribution, and the reference value was set at the 10th percentile. Adjustment factors were age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, low-density lipoprotein cholesterol, triglyceride, high-density lipoprotein cholesterol, and use of lipid-lowering medication. ACS, acute coronary syndrome.

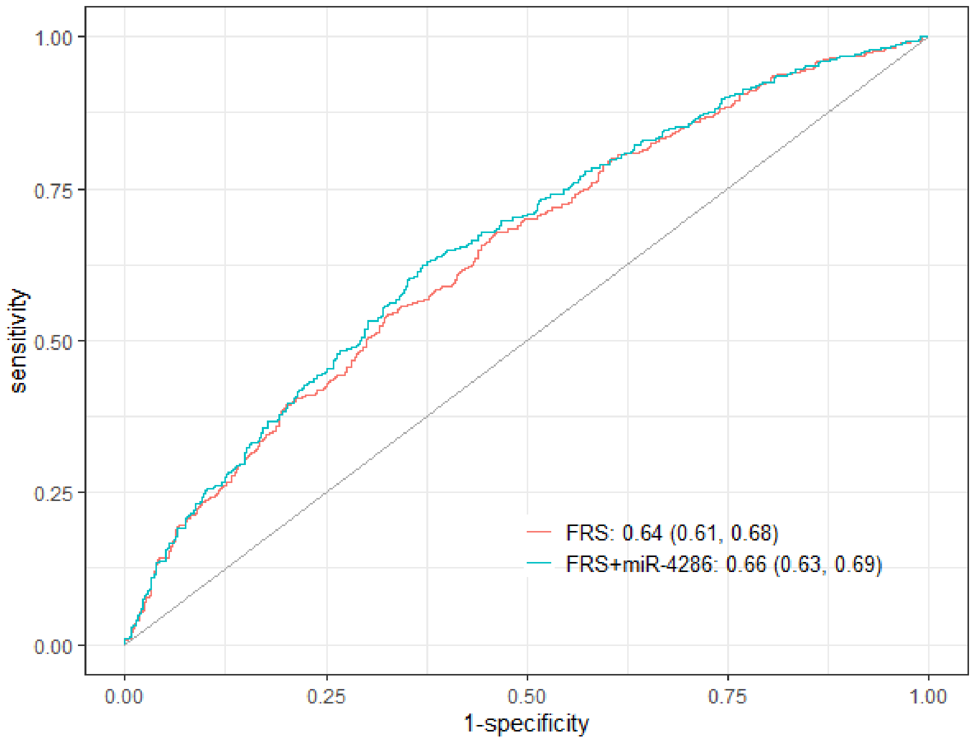

Figure S7. Receiver-operating characteristic curves for prediction of incident ACS.

Red curve represents the model of FRS and blue curve represents FRS model plus plasma miR-4286. Annotation shows the area and 95% confidence intervals under the receiver-operating characteristic curves. ACS, acute coronary syndrome; FRS, Framingham Risk Score.

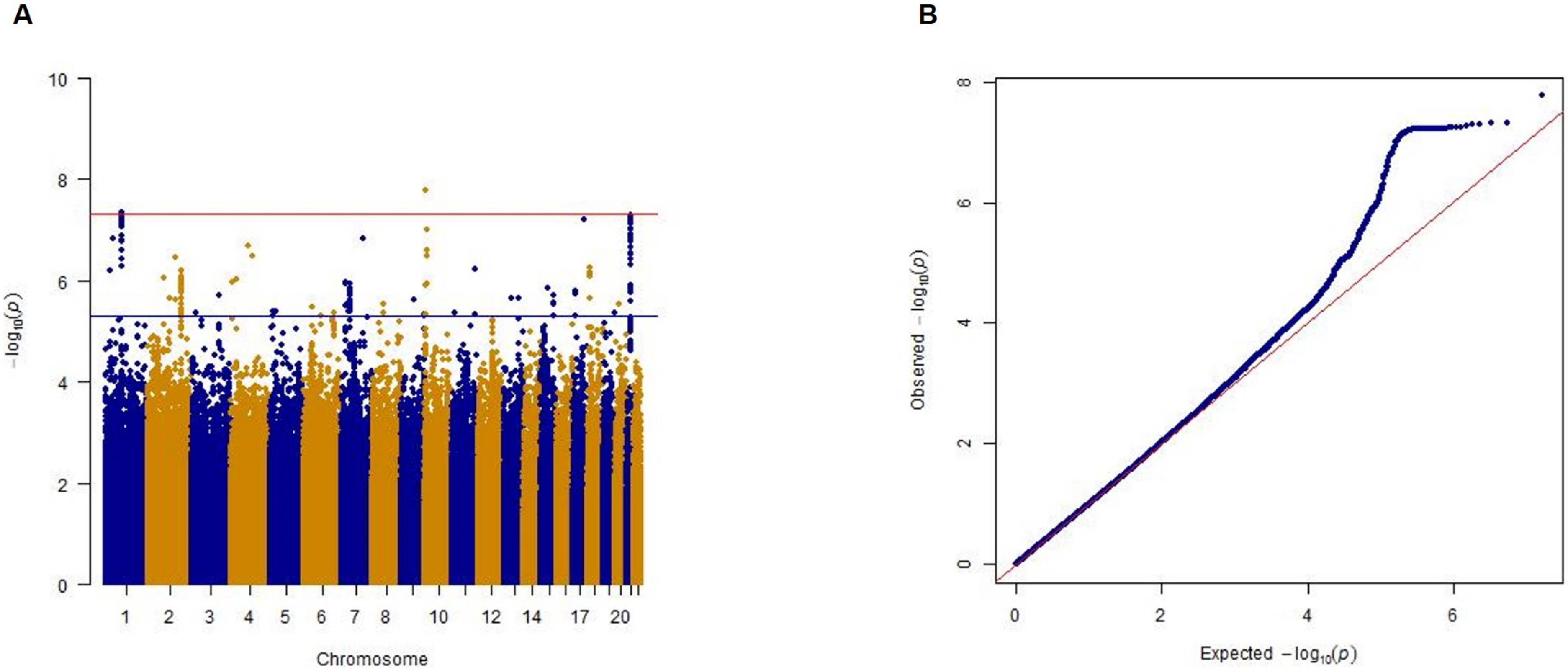

Figure S8. Manhattan Plot and Q-Q plot.

The Manhattan Plot and Q-Q plot of the genome-wide association study of miR-4286 are showed separately in **A** and **B**. The inflation factor lambda is 0.99.

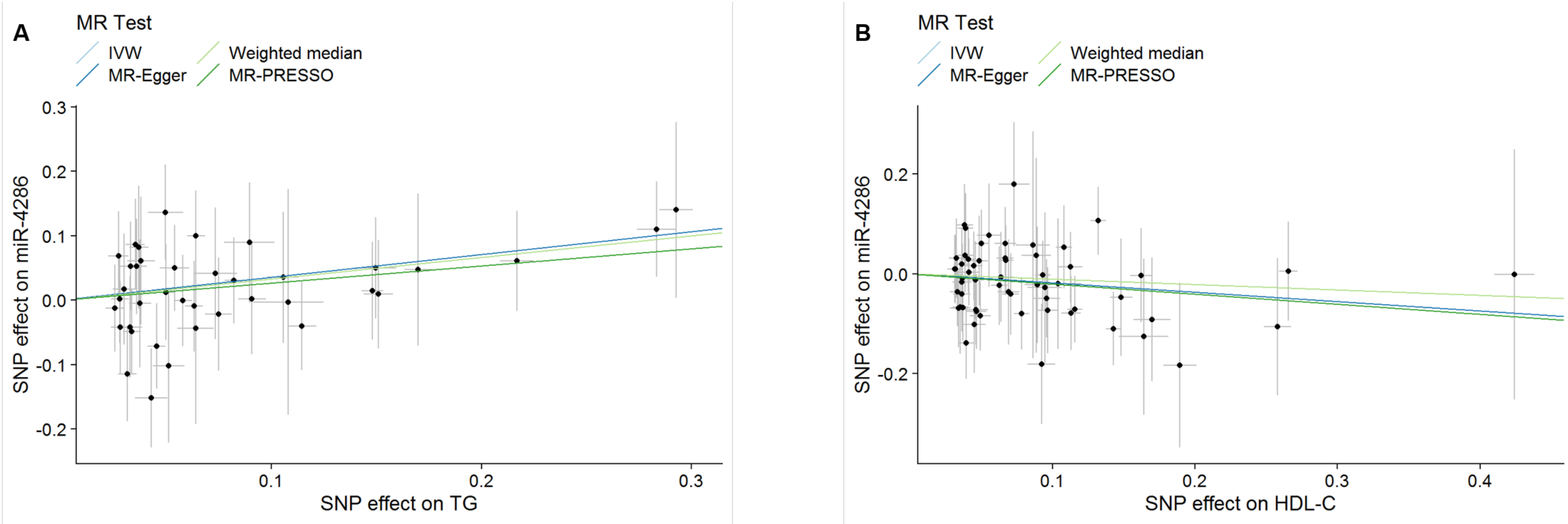

Figure S9. Scatter plots showing the causal effects of TG and HDL-C on plasma miR-4286 by the Mendelian randomization analyses.

A, Causal effects of TG on miR-4286 based on 39 TG SNPs. **B**, Causal effects of HDL-C on miR-4286 based on 58 HDL-C SNPs. Each point represents a SNP, The x-axis plot shows their effect sizes on TG or HDL-C derived from summary data, and the y-axis shows the effect on miR-4286 as estimated in 340 samples using linear regression model adjusted for age and sex. Error bars represent the 95% confidence intervals of each SNP. The slope of different colored lines represent the causal effects estimated by the IVW test, MR-Egger test, weighted median test, or MR-PRESSO test. IVW, inverse variance-weighted; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

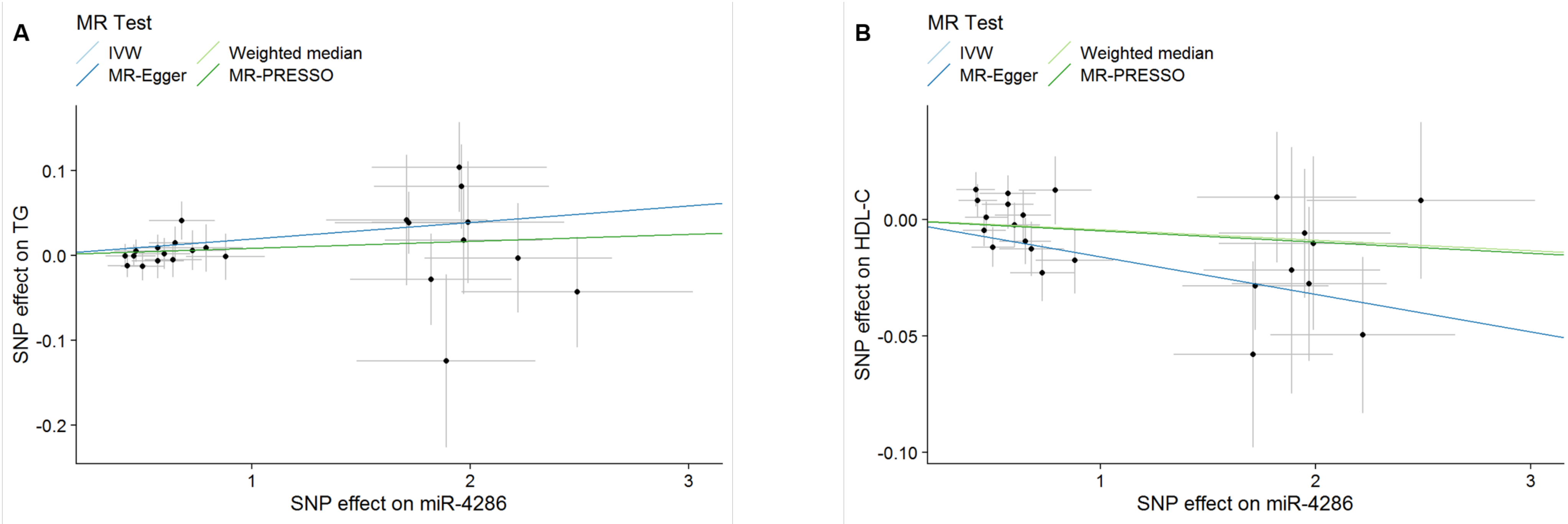

Figure S10. Scatter plots showing the causal effects of miR-4286 on TG and HDL-C by the Mendelian randomization analyses.

A, Causal effects of miR-4286 on TG based on 24 SNPs of miR-4286. **B,** Causal effects of miR-4286 on HDL-C based on 23 SNPs of miR-4286 after removing 1 pleiotropic SNP (rs544792245) that associated with HDL-C. Each point represents a SNP, The x-axis plot shows their effect sizes on miR-4286 obtained from the GWAS results in the present study, and the y-axis shows the effect on TG or HDL-C as estimated in 3240 samples and 3238 samples using linear regression model adjusted for age and sex, respectively. Error bars represent the 95% confidence intervals of each SNP. The slope of different colored lines represent the causal effects estimated by the IVW test, MR-Egger test, weighted median test, or MR-PRESSO test. IVW, inverse variance-weighted; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

MR method	β (95% CI)	Pleiotropy
TG		
IVW	0.01 (0.00, 0.02)	
MR-Egger	0.02 (-0.01, 0.04)	0.32
Weighted Median	0.01 (-0.01, 0.02)	
MR-PRESSO	0.01 (0.00, 0.02)	0.90
HDL-C		
IVW	0.00 (-0.01, 0.00)	
MR-Egger	-0.02 (-0.03, 0.00)	0.08
Weighted Median	0.00 (-0.01, 0.01)	
MR-PRESSO	0.00 (-0.01, 0.00)	0.40

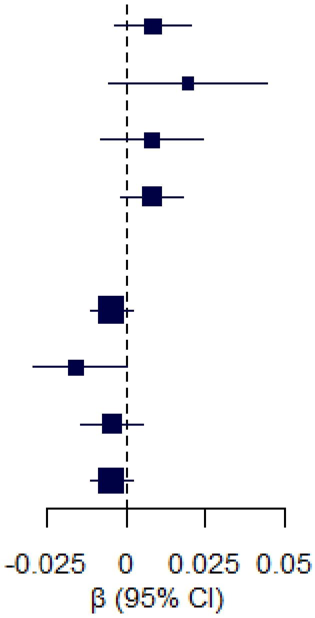

Figure S11. Causal effects of plasma miR-4286 on TG and HDL-C.

The causal effect (95% CI) of miR-4286 on TG used 24 SNPs of miR-4286, and the causal effect (95% CI) of miR-4286 on HDL-C used 23 SNPs of miR-4286, after removing 1 pleiotropic SNP (rs544792245) that associated with HDL-C. Pleiotropy *P* value derived from the intercept of MR-Egger test or MR-PRESSO Global test, a small *P* value indicates an unbalanced pleiotropy. CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; IVW, inverse variance-weighted; TG, triglyceride.

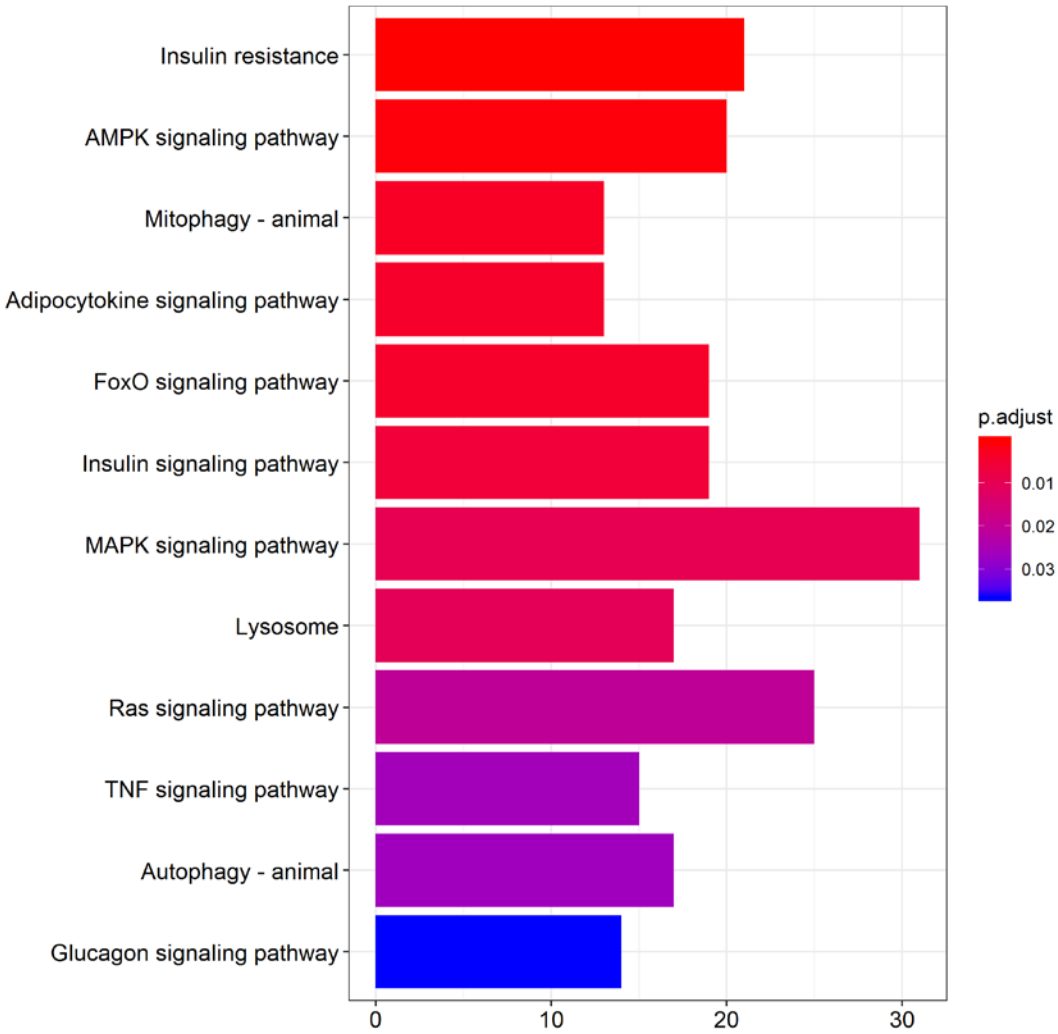

Figure S12. Enriched KEGG pathways for target genes of miR-4286.

Bar chart shows the significant KEGG pathways (Benjamini-Hochberg adjusted $P<0.05$) for miR-4286, using highly expressed target genes in the cardiovascular system. The x-axis represents the counts of target genes enriched in each pathway, and the y-axis represents the pathways in order by increased adjusted *P* value. KEGG, Kyoto Encyclopedia of Genes and Genomes.