

SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

miRNA expression profiling

RNA extraction and small RNA sequencing

Total RNA was extracted from serum samples using the Qiagen miRNeasy Serum/Plasma Kit (cat. No. 217184, QIAGEN, Germany) according to the manufacturer's protocol. Quantitation of total RNA was carried out using the Nanodrop 2000 (Thermo Fisher Scientific Inc., USA). RNA integrity was assessed by Agilent 2100 Bioanalyzer (Agilent Technology, USA).¹⁶ 1 µg total RNA of each sample was used for the small RNA library construction using TruSeq Small RNA Sample Prep Kits (Cat. No. RS-200-0012, Illumina, USA). The libraries were finally sequenced using the Illumina HiSeq X Ten platform and 150 bp paired-end reads were generated.¹⁷

Confirmation and validation of miRNA by RT-qPCR in PCR-discovery and validation cohorts.

Quantification of miRNAs was performed via two stepwise processes: reverse transcription and polymerase chain reaction (PCR). First, total RNA extracted was reverse transcribed using the miRNA 1st Strand cDNA Synthesis Kit (cat. No. AT351, TransGene Biotech Inc., China). Real-time PCR was performed using LightCycler® 480 II Real-time PCR Instrument (Roche, Swiss) with 10 µL PCR reaction mixture that included 1 µL of cDNA, 5 µL of 2×PerfectStart™ Green qPCR SuperMix (cat. No.

AQ601, TransGene, China), 0.2 μ L of universal primer, 0.2 μ L of miRNA-specific primer and 3.6 μ L of nuclease-free water. Each sample was run in triplicate for analysis.¹⁸ The miRNA-specific primer sequences were designed based on the miRNA sequences (primers were exhibited below). The expression levels of miRNAs were normalized to 5S rRNA and were calculated using the $2^{-\Delta\Delta Ct}$ method.¹⁹

Bioinformatics analysis of RNA-sequence data

The raw data generated by Illumina sequencing were analyzed as previously described.²⁰ The known miRNAs were identified by aligning against miRBase v22 database (<http://www.mirbase.org/>)²¹, and the miRNA expression was calculated by transcript per million (TPM, the number of reads per miRNA matched/all reads $\times 1 \times 10^6$). Differentially expressed miRNAs were calculated and filtered with the threshold of P value < 0.05 and absolute \log_2 (fold change) > 1 using DESeq R package²² (version 1.18.0). The targets of differentially expressed miRNAs were predicted by miRanda software²³ with parameters as follows: $S \geq 150$, $\Delta G \leq -30$ kcal/mol and demand strict 5' seed pairing. GO enrichment and KEGG pathway enrichment analysis of DEM-target-Genes were respectively performed using R based on the hypergeometric distribution.

Table S1. Primer sequences of 40 miRNAs.

ID	Primer sequences (5'-3')
miR-19b-3p	TGTGCAAATCCATGCAAAACTGA
miR-4732-3p	CCTGACCTGTCCTGTTCTG
miR-205-5p	TTCCTTCATTCCACCGGAGTCTG
miR-16-2-3p	CGCGCCAATATTACTGTGCTGCTTTA
miR-15b-5p	CGCTAGCAGCACATCATGGTTTACA
miR-148b-3p	TATCCTGCCCCGAGCTGAGC
miR-3615	CTCGGCTCCTCGCGGCTC
miR-2110	TATTGGGGAAACGGCCGCT
miR-3940-3p	ATATATACAGCCCGGATCCCAGCC
miR-142-5p	GCGCGCATAAAGTAGAAAGCACTACT
miR-143-3p	CGCTGAGATGAAGCACTGTAGCTC
miR-4685-3p	TCTCCCTTCCTGCCCTGG
miR-885-5p	CCTCCATTACACTACCCTGCCTCT
miR-1180-3p	TATATATTTCCGGCTCGCGTGGGT
miR-484	GGCTCAGTCCCCTCCCGAT
miR-424-3p	CCAAAACGTGAGGCGCTGCTAT
miR-1292-5p	TATATATGGGAACGGGTTCCGGCA
let-7b-3p	CGCTATAACAACCTACTGCCTTCCC
miR-4732-5p	GCCCTGACCTGTCCTGTTCT
miR-103a-3p	CAGCAGCATTGTACAGGGCTATGA
miR-204-5p	CGTTCCCTTTGTTCATCCTATGCCT
miR-200c-3p	GCGTAATACTGCCGGGTAATGATGGA
miR-144-3p	GCGCTACAGTATAGATGATGT
miR-100-5p	CCAACCCGTAGATCCGAACCTTGTG
miR-24-3p	TGGCTCAGTTCAGCAGGAACAG
miR-183-5p	TCGCTATGGCACTGGTAGAATTCCT
miR-21-5p	CGCCGTAGCTTATCAGACTGATGTTGA
miR-939-5p	TGGGGAGCTGAGGCTCTG
miR-223-3p	CGCTGTCAGTTTGTCAAATACCCCA
let-7c-5p	CGCCTGAGGTAGTAGGTTGTATGGTT
miR-1908-5p	CGGGGACGGCGATTGGTC
miR-605-3p	CCGCGAGAAGGCACTATGAGATTTAGA
miR-365a/b-3p	GCGCGTAATGCCCTAAAATCCTTAT
let-7e-5p	CGCGTGAGGTAGGAGGTTGTATAGTT
miR-199a/b-3p	CGCACAGTAGTCTGCACATTGGTTA
miR-7706	CGCCTGTGCTCTGCCGAGA
miR-1271-5p	CTTGGCACCTAGCAAGCACTCA
miR-101-3p	CGCTACAGTACTGTGATAACTG
miR-17-5p	GCCAAAGTGCTTACAGTGCAGGTAG
miR-664a-3p	CCGCTATTCATTTATCCCCAGCCTACA

Table S2. Patient characteristics of screening and PCR-discovery cohorts.

	Screening cohort			PCR-discovery cohort		
	control (N=5)	AHF (N=15)	P-value	control (N=26)	AHF (N=50)	P-value
Age (years)	58.2±7.7	65.7±10.2	0.115	62.4±10.6	67.8±11.1	0.948
Male/female (n/n)	1/4	9/6	0.303	10/16	13/37	0.262
Current smoking, n (%)	0 (0)	6 (40)	0.260	4 (15.38)	13 (26.00)	0.292
LVEF (%)	65.4±2.6	28.7±7.9	<0.001*	63.0 (62.0-64.5)	42.0 (30.5-60.0)	0.001*
NYHA functional class	n.a.	2.93±0.26	n.a.	n.a.	2.60±0.64	n.a.
HF history, n (%)	0 (0)	9 (60)	0.038*	4(15.38)	13(26.00)	0.292
NT-proBNP (ng/L)	78 (55-110)	2521 (634-7867)	<0.001*	40.6 (24.3-108.0)	1281.0 (733.0-3380.0)	<0.001*
SBP (mmHg)	127.4±21.6	129.3±26.5	0.874	142.2±25.0	131.9±21.0	0.135
DBP (mmHg)	76.6±10.4	72.8±17.4	0.568	75.8±22.9	79.5±14.1	0.589
DM, n (%)	0 (0)	5 (33.3)	0.266	4 (15.38)	18 (36.00)	0.060
HTN, n (%)	1 (20)	7 (46.7)	0.603	8 (30.77)	22 (44.00)	0.263
HDL (mmol/L)	1.71±0.33	1.02±0.32	0.004*	1.19 (0.98-1.37)	0.87 (0.78-1.24)	0.044*
LDL (mmol/L)	2.73±0.69	2.05±1.17	0.235	2.61±1.15	2.30±0.93	0.104
ALT (IU/L)	29.4±18.1	37.4±25.4	0.461	27.6±19.2	36.8±41.3	0.768
WBC (/nL)	6.6±1.0	7.1±1.8	0.480	6.72 (5.18-7.25)	6.64 (5.43-8.53)	0.517
HB (g/L)	121.0±9.4	135.1±21.3	0.058	130.4±10.4	127.1±23.5	0.062
CRP (mg/L)	3.02 (2.34-3.23)	3.13 (3.02-8.41)	0.343	3.02(2.25-3.75)	8.24(2.43-17.64)	0.058
eGFR (mL/(min*1.73m ²))	107.7±22.5	76.4±24.4	0.021*	99.6±20.8	74.1±29.3	0.009*
BUN (mmol/L)	6.53±1.44	8.69±7.18	0.287	5.35±1.83	8.57±5.36	0.004*

Continuous variables are presented as means±SD if conform normal distribution or median with interquartile range (IQR) if not. Categorical variables are presented as percentage (%).

* Significant P value (<0.05).

AHF, acute heart failure; LVEF, left-ventricular ejection fraction; NYHA, New York Heart Association; HF, heart failure; NT-proBNP, N-terminal brain natriuretic peptide precursor; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; HTN, hypertension; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; WBC, white blood cell count; HB, hemoglobin; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (calculated by MDRD formula); BUN, blood urea nitrogen; n.a., not applicable.

Table S3. Medication at discharge of the validation cohort.

	Overall (N=564)	Q1 (N=141)	Q2 (N=141)	Q3 (N=141)	Q4 (N=141)	P-value
β-blockers, n (%)	319 (56.6)	76 (53.9)	79 (56.1)	80 (56.7)	84 (59.6)	0.815
ACEIs, n (%)	95 (16.8)	26 (18.4)	24 (17.1)	21 (14.9)	24 (17.1)	0.886
ARBs, n (%)	404 (71.6)	109 (77.3)	95 (67.4)	98 (69.3)	102 (72.3)	0.279
ARNI, n (%)	73 (12.9)	16 (11.3)	19 (13.5)	17 (12.1)	21 (14.9)	0.819
MRAs, n (%)	356 (63.1)	87 (61.7)	90 (63.5)	90 (64.1)	89 (63.0)	0.980
Diuretics, n (%)	131 (23.2)	35 (24.8)	29 (20.6)	29 (20.6)	38 (27.0)	0.803
CCBs, n (%)	208 (36.9)	56 (39.7)	45 (31.9)	49 (34.8)	58 (41.1)	0.341
Nitrates, n (%)	97 (17.2)	24 (17.0)	23 (16.4)	24 (16.9)	23 (16.5)	0.997
Digoxin, n (%)	51 (9.0)	9 (6.4)	11 (7.8)	14 (9.9)	17 (12.1)	0.366
Statins, n (%)	368 (65.2)	91 (64.5)	85 (60.3)	97 (68.8)	95 (67.4)	0.453
Anticoagulants and antiplatelets, n (%)	391 (69.3)	102 (72.3)	89 (63.1)	93 (66.0)	107 (75.9)	0.080

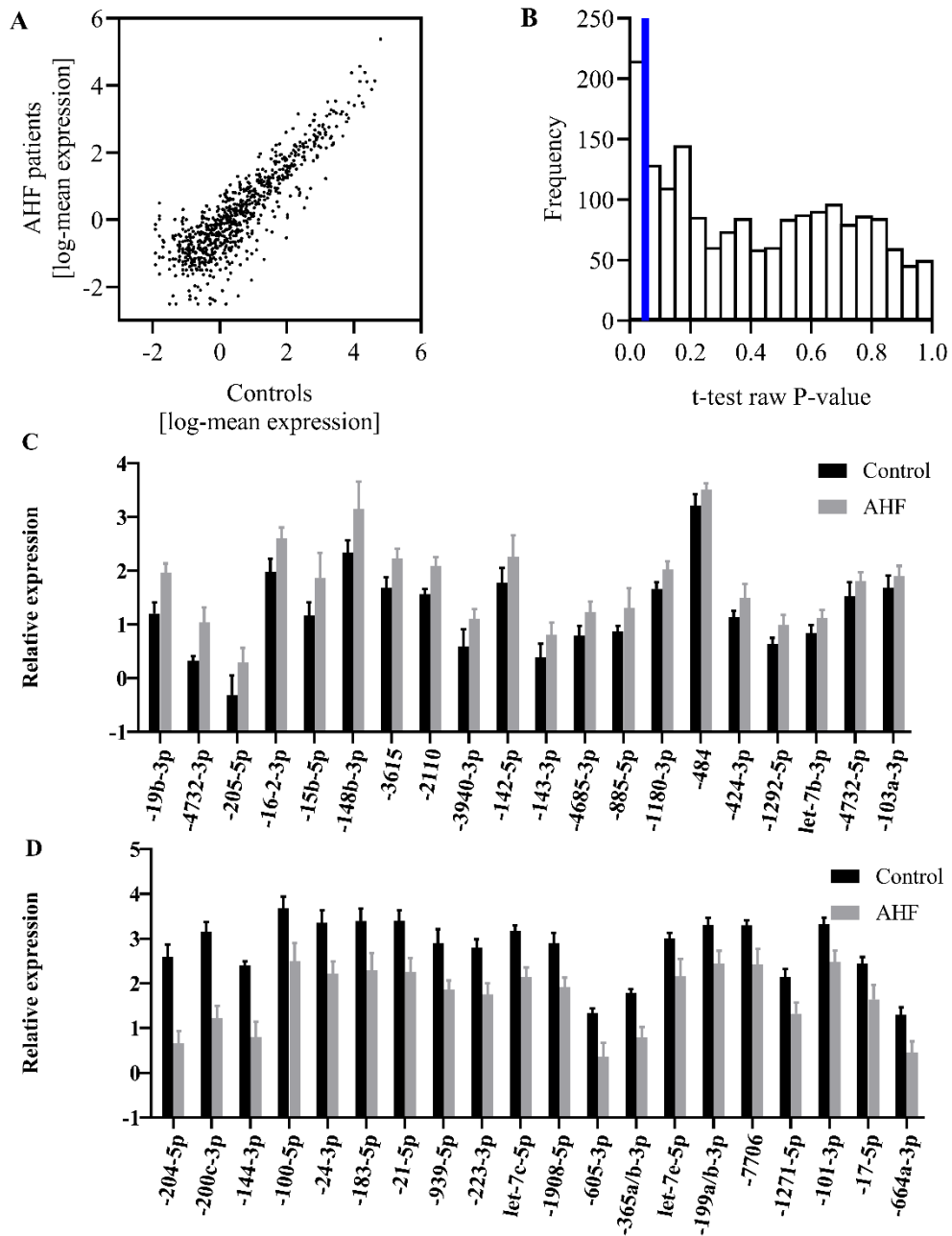
Medication condition is described as categorical variable according to whether specific medicine was taken.

* Significant p value (<0.05).

ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; MRAs, mineralocorticoid receptor antagonists; ARNI, angiotensin receptor enkephalin inhibitor; CCBs, calcium-channel blockers.

Figure S1. miRNA screening by RNA-sequencing and PCR discovery of 40 miRNAs

candidates.



A, Matrix plot showed a positive linear correlation of the mean expression between 15 AHF

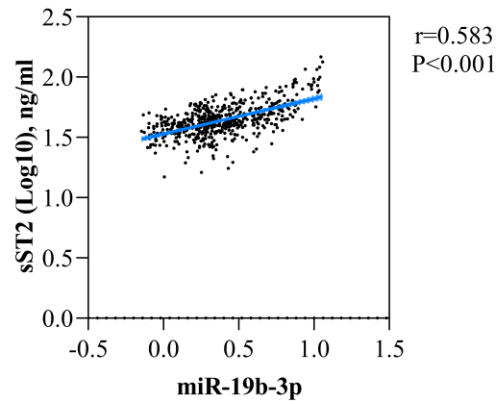
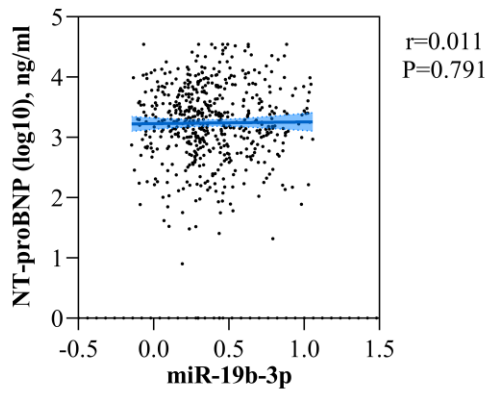
patients and 5 controls. **B**, the histogram of presented the distribution of raw P-values of screened

miRNAs. The blue vertical line draws the boundary of P value=0.05. The bar on the left indicates

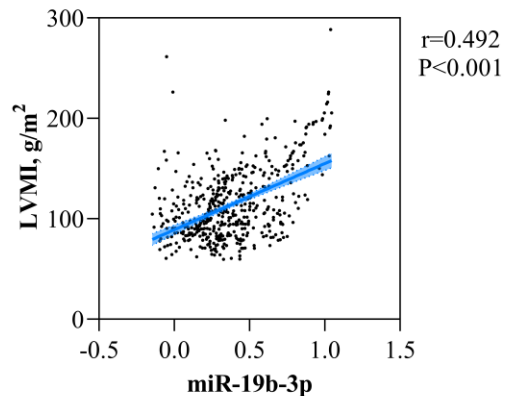
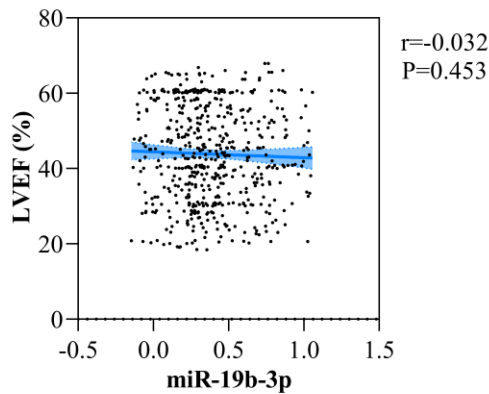
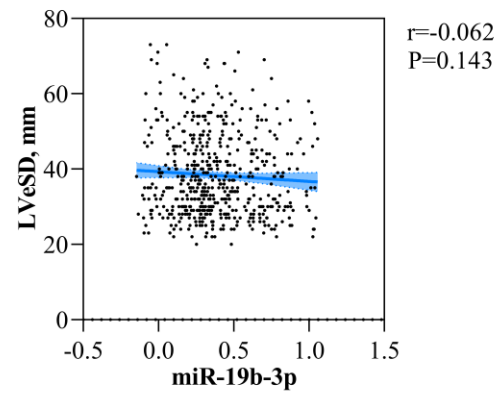
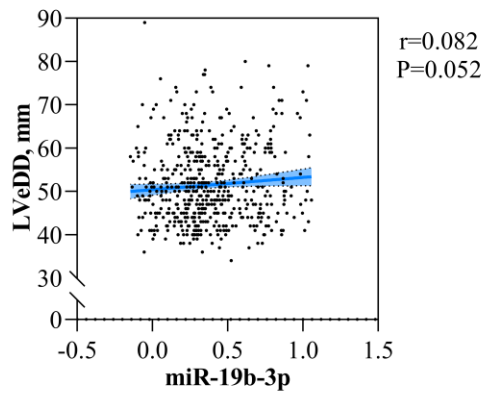
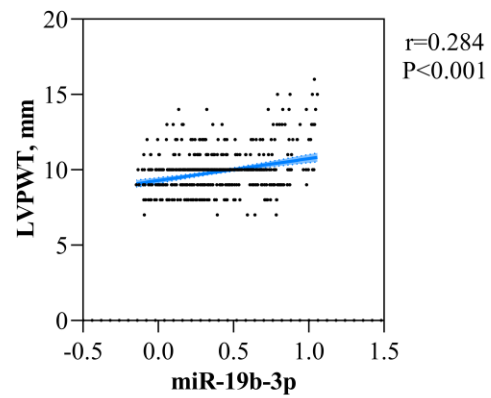
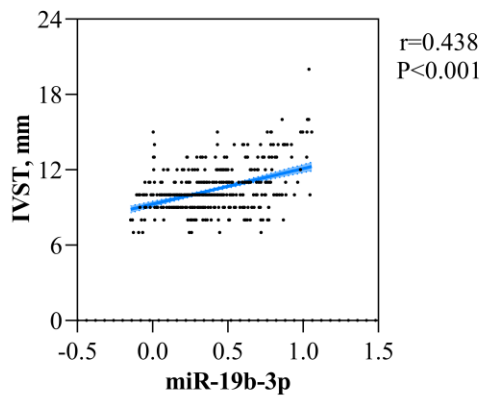
218 differential expressed miRNAs. **1C & 1D** show 20 Up-regulated and 20 down-regulated miRNAs with the highest absolute fold change from the PCR-discovery cohort. Mean relative expression value were normalized to the corresponding values of control subjects and expressed after logarithmic transformation. miRNAs expression levels were calculated by transcript per million. $TPM=N/M*10^6$, N represents reads count for each miRNA, and M represents total reads count of sample. AHF denotes acute heart failure.

Figure S2. Correlations of miR-19b-3p with serological and echocardiographic measurements.

Serological measurements



Echocardiographic measurements



Spearman correlation analysis showed relative expression level of miR-19b-3p significantly positively correlated with sST2, IVST, LVPWT, LVeDD, LVMI, but not NT-proBNP, LVeSD and left ventricular ejection fraction. The levels of NT-proBNP and sST2 were logarithmic transformed. NT-proBNP denotes N-terminal pro brain natriuretic peptide, sST2 soluble suppression of tumorigenicity 2, IVST interventricular septum thickness, LVeDD left ventricular end-diastolic diameter, LVeSD left ventricular end-systolic diameter, LVMI left ventricular mass index, LVPWT left ventricular posterior wall thickness. The light blue area represents 95% confidence interval of correlation coefficient.