






# Serum miR-195-5p Exhibits Clinical Significance in the Diagnosis of Essential Hypertension with Type 2 Diabetes Mellitus by Targeting DRD1

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**OBJECTIVES:** Diagnosis and management of essential hypertension (EH) or type 2 diabetes mellitus (T2DM) by combining comprehensive treatment and classificatory diagnosis have been continuously improved. However, understanding the pathogenesis of EH patients with concomitant T2DM and subsequent treatment remain the major challenges owing to the lack of non-invasive biomarkers and information regarding the underlying mechanisms.

**METHODS:** Herein, we collected 200 serum samples from EH and/or T2DM patients and healthy donors (N). Gene-expression profiling was conducted to identify candidate microRNAs with clinical significance. Then, a larger cohort of the aforementioned patients and 50 N were used to identify the correlation between the tumor suppressor miR-195-5p and EH and/or T2DM. The dual-luciferase reporter assay was used to explore the target genes of miR-195-5p. The suppressive effects of miR-195-5p on the 3'-UTR of the dopamine receptor D1 (DRD1) transcript in EH patients with concomitant T2DM were verified as well.

**RESULTS:** Compared with that in other groups, serum miR-195-5p was highly downregulated in EH patients with concomitant T2DM. miR-195-5p overexpression efficiently suppressed DRD1 expression by binding to the two 3'-UTRs. Additionally, two single nucleotide polymorphisms, including 231T-A and 233C-G, in the miR-195-5p binding sites of the DRD1 3'-UTR were further identified. Collectively, we identified the potential clinical significance of *DRD1* regulation by miR-195-5p in EH patients with concomitant T2DM.

**CONCLUSIONS:** Our data suggested that miR-195-5p circulating in the peripheral blood served as a novel biomarker and therapeutic target for EH and T2DM, which could eventually help address major challenges during the diagnosis and treatment of EH and T2DM.

**KEYWORDS:** Essential Hypertension (EH); Type 2 Diabetes Mellitus (T2DM); miR-195-5p; Single Nucleotide Polymorphism (SNP); DRD1.

## INTRODUCTION

Essential hypertension (EH)—defined as the unexplained rise in blood pressure—is a major cause of cardiovascular and cerebrovascular diseases with a worldwide prevalence ranging from 26.4% in 2000 to 29.2% in 2025 that is mainly

attributable to genetic and environmental factors (1,2). For decades, longitudinal studies have indicated the involvement and clusters of predisposing factors, such as being overweight, the aging process, hyperlipidemia, and insulin resistance-associated type 2 diabetes mellitus (T2DM), yet the precise pathogenesis of EH requires further understanding (2,3). For instance, multifaceted T2DM and/or complication-associated inducements, such as hyperglycemia, insulin resistance, excess fatty acids, and the malfunction of pancreatic beta cells are sufficient for inducing thrombosis, vasoconstriction, vascular inflammation, and atherogenesis, which collectively result in the development of EH-associated cardiovascular diseases (3-5). Moreover, more than 39% of the patients with newly diagnosed T2DM were more hypertensive than normotensive patients, whereas EH occurred in up to 75% of the adult T2DM

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patients (6,7). Long-term EH increases the risk of coronary heart disease, stroke, heart failure, and peripheral vascular disease (7).

MicroRNAs (miRNAs) are small, conserved, endogenously-initiated non-coding RNAs and play a crucial role in multifaceted biological processes, including cell proliferation, apoptosis, development, and tumorigenesis (8,9). Briefly, miRNAs play multidimensional roles in mRNA degradation or post-transcriptional inhibition of expression (8,10). Over 5300 human genes are coordinatively modulated by numerous miRNAs and computational predictions indicate that each microRNA may target thousands of genes (11,12). Recently, emerging evidence has indicated the possibility of using serum/plasma miRNAs—including miR-29b, miR-126, miR-146, miR-130b, and miR-320a—as onset or prognostic biomarkers of T2DM or EH (13,14). Generally, miRNAs function by binding to the recognition element in the 3'-UTR of the target genes, thereby triggering the degradation of the mRNA or inhibiting its translation, negatively regulating the expression of the target mRNA and influencing various physiological and pathophysiological processes (15). Furthermore, increasing numbers of miRNA-based therapies are being successfully employed in murine disease models (16). Circulating miRNAs with differential expression in blood are recognized as novel diagnostic markers and potential therapeutic targets for predicting T2DM or EH. miRNA dysfunction in the pathogenesis of EH combined with T2DM remains to be fully elucidated (9,14).

In this study, we used the miRNA qRT-PCR array to evaluate the expression profile of serum miRNAs among Chinese patients with EH and/or T2DM and healthy donors. Bioinformatic analysis, qRT-PCR validation, and promoter activity detection revealed that miR-195-5p exhibited clinical significance in the aforementioned 50 EH patients with T2DM by directly targeting the 3'-UTR of dopamine receptor D1 (DRD1) and inducing its subsequent downregulation. Furthermore, the variation and susceptibility of two major single nucleotide polymorphisms (SNPs) in the miR-195-5p-binding site in the 3'-UTR of DRD1 were identified in Chinese individuals with EH and T2DM.

## ■ MATERIALS AND METHODS

### Subjects and study design

This study was conducted in accordance with principles and guidelines of Declaration of Helsinki, and approved by the Ethics Committee of First Affiliated Hospital of Kunming Medical University, China (approval number: 2020-L-12). All blood samples were collected from participants after obtaining written informed consent. Generally, the participants were divided into four groups, *i.e.*, EH patients (E), type 2 diabetes mellitus patients (D), E with D (ED), and unrelated healthy individuals (negative controls (N)). Fifty individuals per group were recruited at the First Affiliated Hospital of Kunming Medical University from January 2017 to March 2018. Of the participants, five individuals from each group (age and sex difference <12 months) were randomly selected for serum miRNA expression profile analysis. The differentially expressed miRNAs were screened by bioinformatic analysis and validated by qRT-PCR (for all 50 patients). The general information of representative patients (E, D, ED) and healthy donors (N) is listed in Supplementary Information: Supplementary Table S1.

### Inclusion criteria and exclusion criteria

Diagnosis of diabetes mellitus was made in accordance with the recommendations of the American Diabetes Association (ADA), *i.e.*, fasting plasma glucose (FPG)  $\geq 7.0$  mmol/L; or HbA1c  $\geq 6.5\%$ ; or oral glucose tolerance test (OGTT) for 2h blood glucose  $\geq 11.1$  mmol/L. Fasting was defined as not eating or drinking (except water) for at least 8h (17).

EH was diagnosed according to the 1999 World Health Organization (WHO) criteria, *i.e.*, diastolic blood pressure (DBP)  $\geq 90$  mmHg or systolic blood pressure (SBP)  $\geq 140$  mmHg, and no antihypertensive medication (18).

Exclusion criteria included type 1 diabetes, secondary hypertension, acute myocardial infarction, severe liver and kidney dysfunction, cancer, use of drugs that may affect HRV, development of a non-sinus rhythm (such as atrial flutter or atrial fibrillation) or use of a pacemaker.

### Plasma collection from the peripheral blood

Whole peripheral blood was collected as previously reported (19). Briefly, plasma was isolated from whole peripheral blood using anticoagulant tube, followed by centrifugation at 3000 rpm for 10 min at room temperature (25°C). After phase separation, plasma was collected using a micropipette, divided into two parts, and frozen at -80°C for subsequent analyses.

### Blood sugar status assessment

The fasting blood glucose (FBG), 2h postprandial glucose in OGTT, fasting insulin, and HbA1c were measured. The homeostasis model assessment-insulin resistance index (HOMA-IR) was calculated using the following formula:  $HOMA-IR = FBG \times \text{fasting insulin} / 22.5$ .

### Measurement of blood pressure

Blood pressure was evaluated in subjects who had been seated and rested for 5 min by the nurses in the First Affiliated Hospital of Kunming Medical University. The measurement was performed twice and the mean value was calculated.

### Plasma miRNA enrichment

Total plasma RNAs, including miRNAs were extracted from the plasma samples using TRIzol (Invitrogen, Carlsbad, CA, USA), as previously described, with some modifications (19-21). Briefly, the absorbance ( $A_{260/280} > 1.70$ ) and concentration of RNA samples were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). RNA integrity was evaluated using agarose gel electrophoresis.

### miRNA qRT-PCR array

For miRNA qRT-PCR array, ~20 ng total RNA was reverse transcribed into cDNA using the microRNA Reverse-Transcription kit and the RT Primer Pools (Exiqon A/S, Vedbaek, Denmark), according to the manufacturer's instructions. Then, miRNA qRT-PCR was conducted on an ABI PRISM7900 system and miRCURY LNA™ Universal RT microRNA PCR system (Applied Biosystems; Thermo Fisher Scientific). The detailed procedure is available in the Supplementary Information and the primers used for miRNA amplification are listed in Supplementary Table S2.

### Validation of miRNA expression by qRT-PCR analysis

cDNA was synthesized using the miScript Reverse Transcription Kit (Qiagen, Germany), according to manufacturer's



instructions. The expression levels of candidate miRNAs (miR-197-5p, miR-130a-5p, miR-27a-5p, miR-195-5p, U6) were quantified by qRT-PCR; the miScript SYBR<sup>®</sup>-Green PCR kit (Qiagen GmbH) and ABI PRISM7900 system (Applied Biosystems; Thermo Fisher Scientific) were used for the same. The relative miRNA expression level was determined using the  $2^{-\Delta\Delta CT}$  method, with several modification (3,22-24). The detailed procedure is available in the Supplementary Information, primers used for miRNA amplification are listed in Table S2, and the significantly upregulated, downregulated, and total miRNAs are listed in Supplementary Tables S3–S4 and Table S6.

### Luciferase reporter assay

The 3'UTR of the DRD1 comprising the putative miR-195-5p binding sequence and corresponding mutant segment were constructed into the pmir-RB-REPORT<sup>™</sup> vector (RiboBio Co., Ltd., Guangzhou, China), according to the manufacturer's instructions. Then,  $3 \times 10^5$  HEK-293T cells were seeded into 24-well plates in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS for 24h before transfection. Luciferase constructs, miR-195-5p mimics, or a negative control were co-transfected into HEK-293T cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific). A dual-luciferase reporter assay was performed 48h after transfection (Promega). The activities of Renilla and Firefly luciferase were determined by the dual-luciferase reporter system (Promega), according to manufacturers' instructions (21,25). Three independent experiments were performed. The detailed procedure is available in the Supplementary Information.

### Total protein collection and western-blot assay

RIPA peptide lysis buffer (Beyotime Biotechnology, Jiangsu, China) supplemented with 1% protease inhibitors (Pierce) was used for extracting total protein from HEK-293T cells (48h post-transfection), according to the manufacturer's instructions. The proteins were denatured and then separated by electrophoresis on a 12% SDS-PAGE gel. They were transferred onto on a PVDF membrane (Millipore, Ireland). Thereafter, the membranes were incubated with primary antibodies against DRD1 (ab216644, Abcam) or  $\beta$ -actin (49675, Sigma) for 12h at 4°C, followed by incubation with HRP-labeled mouse IgG secondary antibody (HAF007, R&D Systems) for 1h at 4°C. The ECL Detection Reagent (ThermoFisher) and Super-signal West Pico Chemiluminescent Substrate (Pierce) was utilized to develop the blot (21,25,26). Finally, gray-scale analysis of the detected protein bands was performed using ImageJ (version 1.8.0, Rawak Software, Inc., Germany).

### Genotyping

Genotyping was performed for the miR-195-5p-binding site in the 3'-UTR of DRD1. Peripheral blood samples were collected and subjected to genomic DNA isolation using the standard phenol-chloroform extraction method, with several modification (21). The purity ( $A_{260/280}$ ) and concentration of genomic DNA were determined using a Biophotometer (Eppendorf, Germany). ABI Prism<sup>®</sup> 7900HT (Applied Biosystems) was used for PCR and sequencing, according to the manufacturer's instructions. The following primers were used to amplify miRNAs: 5'-AGACCCCTGGAGAAGCTG TC-3' (upstream) and 5'-GGAAATGCAGGGTTTGGAG-3' (downstream). The SNP locus mutations (231T-A, 233C-G)

in the miR-195-5p-binding site of DRD1 3'-UTR are listed in Supplementary Table S5.

### Statistical analysis

All statistical analyses were conducted, as previously reported (20,23,25-28). SPSS 21.0 (SPSS Inc.) was used for the statistical analyses. The data are presented as the mean  $\pm$  standard deviation (SD). Differences between two groups were compared using the Student's *t*-test. One-way analysis of variance (ANOVA) was performed to compare the differences among three or more groups. Enumeration data are expressed as percentages or rates, and comparisons between groups were performed using the chi-square test.  $p < 0.05$  was considered significant (NS, not significant; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ).

### Ethics

All procedures performed in the current study were approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University (Approval number: 2020-L-12). Hence, the procedures were in accordance with the Helsinki Declaration and the ethical standards established by the committee of the abovementioned institution responsible for human experimentation (institutional). Written informed consent forms were obtained from all participants or their guardians.

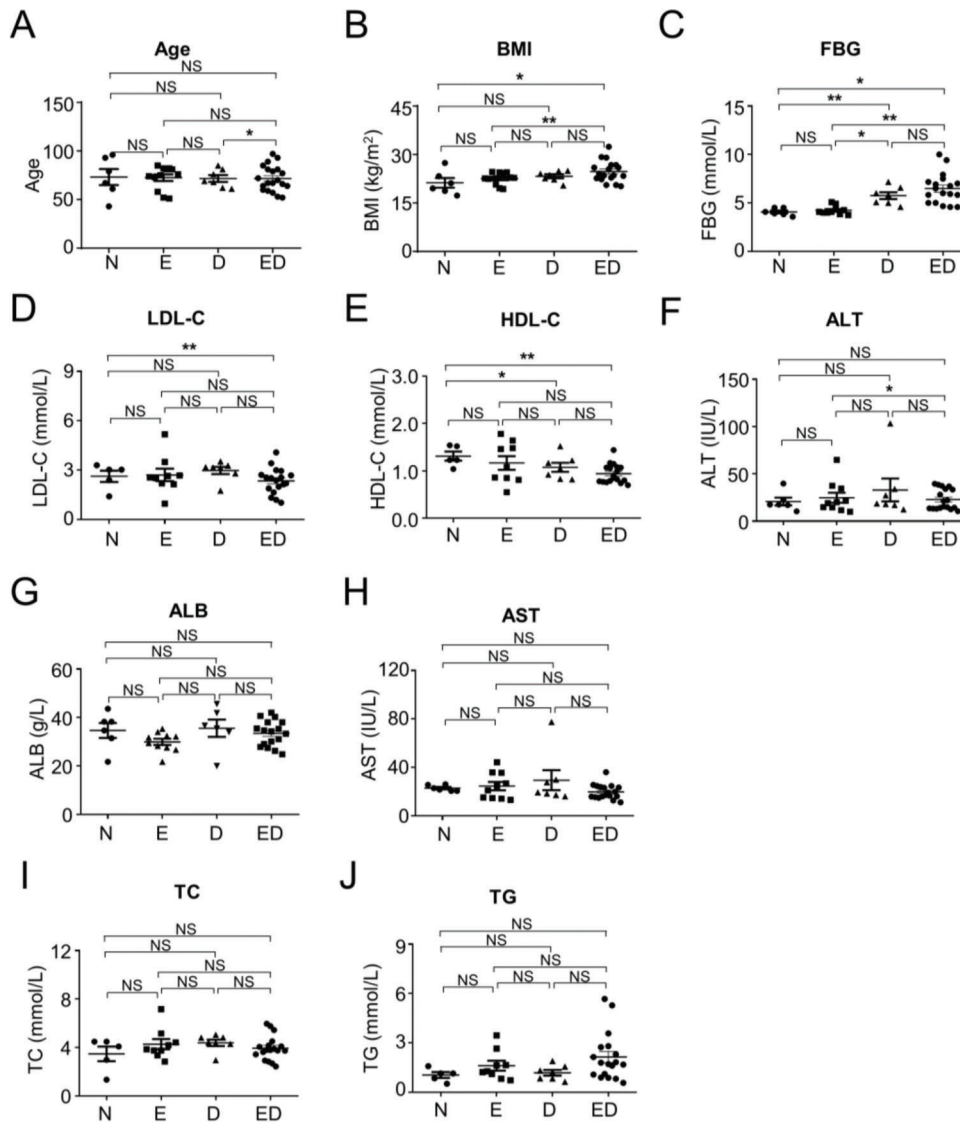
## ■ RESULTS

### Minimal parameters exhibited satisfactory correlation in diagnosis of EH with T2DM

For decades, researchers have been assiduously attempting to optimize the treatment program and evaluating the curative effect for E, D, or ED patients. Consequently, we enrolled 150 patients (50 E, 50 D, 50ED) and 50 unrelated healthy individuals (50 N) in the First Affiliated Hospital of Kunming Medical University during 2017 and 2018. There were no significant differences in age among the groups, except the D and ED patients (Figure 1A). Although multiple parameters, such as body mass index (BMI), fasting blood-glucose (FBG), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) in the N group were different from those in ED patients, differences also occurred among E, D and ED patients (Figures 1B–1F). Furthermore, as shown by the statistical analyses of other clinicopathological parameters involved in clinical trials for comprehensive diagnosis, the current differentiation in the similarities and differences among the aforementioned patients were far from satisfactory (Figures 1G–1J, Figures S1A–S1F, Supplementary Table S1). Hence, there is an urgency for the exploration of more convenient, precise, and non-invasive means for distinguishing ED patients from other groups.

### Characterization of serum miRNA expression profiling

For the purpose of separating the differentially expressed miRNA spectra and identifying potentially novel biomarkers with clinical significance, a miRNA array containing 192 human miRNAs was performed to evaluate the similarities and differences of plasma miRNA expression profiles among the patients with EH (E group), T2DM (D group), EH and T2DM (ED group) together with unrelated healthy control group (N). Compared with the N group, a subset of

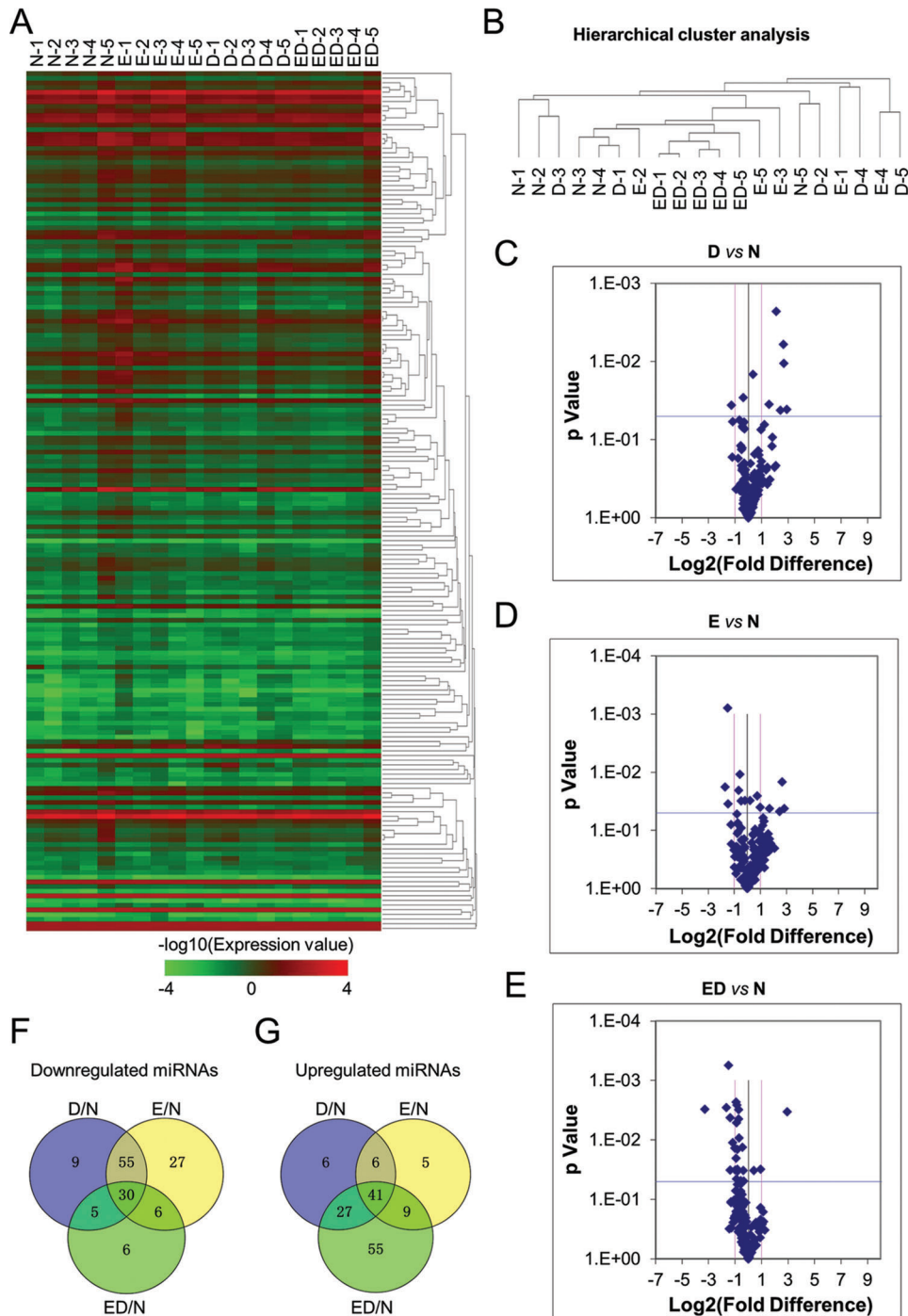


**Figure 1** - Clinicopathological parameters involved in E, D, ED, and N groups. (A) The comparison of age distribution among E (essential hypertension), D (type 2 diabetes mellitus), ED (patients with essential hypertension and concomitant type 2 diabetes mellitus), and N (healthy donors) groups. (B-J) Comparisons of clinicopathological parameters among N, E, D, and ED groups, including BMI (B), FBG (C), LDL-C (D), HDL-C (E), ALT (F), ALB (G), AST (H), TC (I), and TG (J). All data are shown as Mean  $\pm$  SEM. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; NS, not significant.

31 miRNAs showed a significantly different expression profile in the ED group, and in particular, the top 10 upregulated or downregulated ones (Figure 2A, Supplementary Table S3-S4, Table S6). In the hierarchical cluster analysis, we found all the ED samples were clustered together and displayed a more distinguishable miRNA expression pattern, whereas those in the other three groups did not exhibit visible distinctions (Figure 2B). Simultaneously, as shown by the scatter plots, the majority of the 192 miRNAs between E/N, D/N, an ED/N were located in regions with fold change lower than 1 ( $\log_2FC < 1$ ) (Figure 2 C-2E, Figure S2A-S2F, Supplementary Table S6). Furthermore, Venn Map diagrams showed that a cohort of 30 and 41 differentially downregulated and upregulated miRNAs, respectively, were collectively enriched among the four indicated groups (Figure 2F-2G).

### Verification of candidate serum miRNAs involved in patients with EH and T2DM

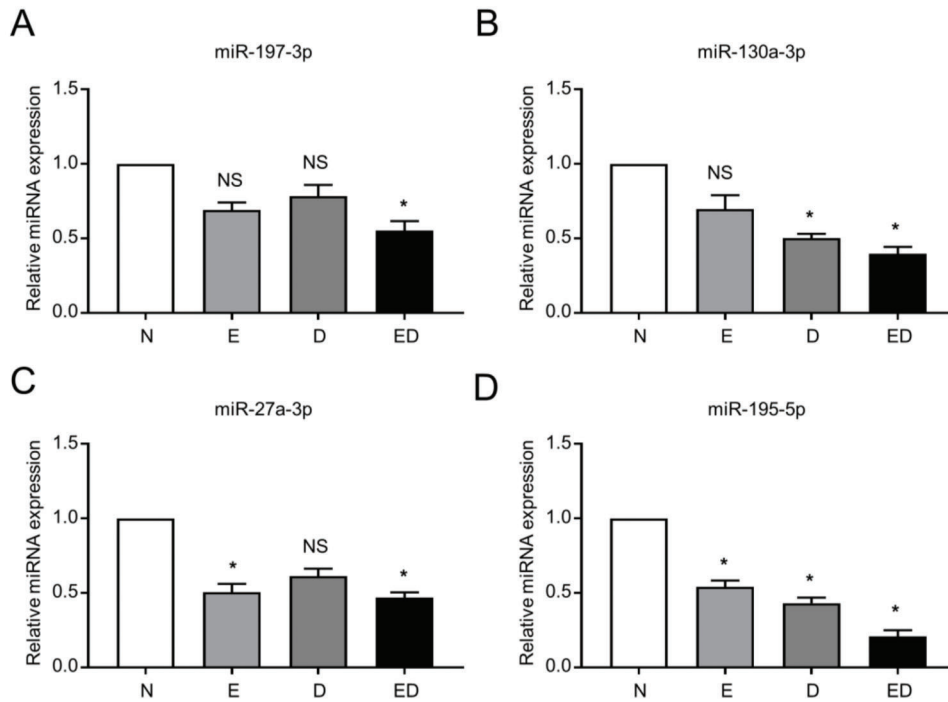
Having preliminarily verified the serum miRNA expression pattern, we further attempted to explore the feasibility of candidate miRNAs as novel diagnostic indicators for patients with EH and T2DM. Of the 71 differentially regulated plasma miRNAs, only the four most downregulated ones, including miR-197-5p, miR-130a-5p, miR-27a-, and miR-195-5p, with rigorous variation screening ( $\log_2FC > 2$ ) were candidates for the significant discriminate of the ED and N groups (Figure 2F-2G, Supplementary Table S3-S4). Along with the miRNA chip, qRT-qPCR analysis was conducted to further identify the expressions of the aforementioned miRNAs among the four groups. Consistent with the trend in the miRNA chip, the expression levels of all candidate miRNAs were downregulated in the ED group



**Figure 2** - Comparison of plasma miRNA expression profile among patients and healthy donors. **A** Heatmap assay of the differentially expressed miRNAs in plasma samples of healthy donors (N-1, N-2, N-3, N-4, N-5), patients with essential hypertension (E-1, E-2, E-3, E-4, E-5), type 2 diabetes (D-1, D-2, D-3, D-4, D-5), patients with essential hypertension and type 2 diabetes mellitus (ED-1, ED-2, ED-3, ED-4, ED-5). **B** Hierarchical cluster analysis of plasma miRNA expression profile among patients (E, D, ED) and healthy donors (N). **C-E** The scatter plots illustration of differentially expressed miRNAs between the D and N groups (**C**), E and N groups (**D**), and ED and N groups (**E**). **F-G** Venn Map analysis of the distributions of the differentially downregulated (**F**) or upregulated (**G**) miRNAs in plasma samples among the indicated groups (N, E, D, and ED).

(Figure 3A-3D, Figure S3A-S3B). For instance, miR-197-3p was only differentially downregulated in patients with ED, whereas miR-130a-3p and miR-27a-3p revealed the opposite trend in E and D groups, respectively (Figure 3A-3C).

However, to distinguish from the other candidate miRNAs (miR-197-3p, miR-130a-3p, and miR-27a-3p), only miR-195-5p was also downregulated in the E and D groups. Above all, miR-195-5p expression in patients with EH and T2DM



**Figure 3** - Plasma miR-195-5p expression was downregulated in the patients. **A-D** Validation of plasma levels of significantly downregulated miRNAs, including miR-197-5p (**A**), miR-130a-5p (**B**), miR-27a-5p (**C**), and miR-195-5p (**D**) by miRNA chip (**A**) and qRT-PCR. U6 was used as an internal control. All data are shown as the mean  $\pm$  SEM (n=5), \* $p$ <0.05; NS, not significant.

(the ED group) displayed a sharper decrease than that in the E and D groups (Figure 3D). Taken together, our data indicated that plasma miR-195-3p could potentially serve as a novel diagnostic biomarker for patients with EH and T2DM.

#### DRD1 as a downstream target gene of miR-195-5p

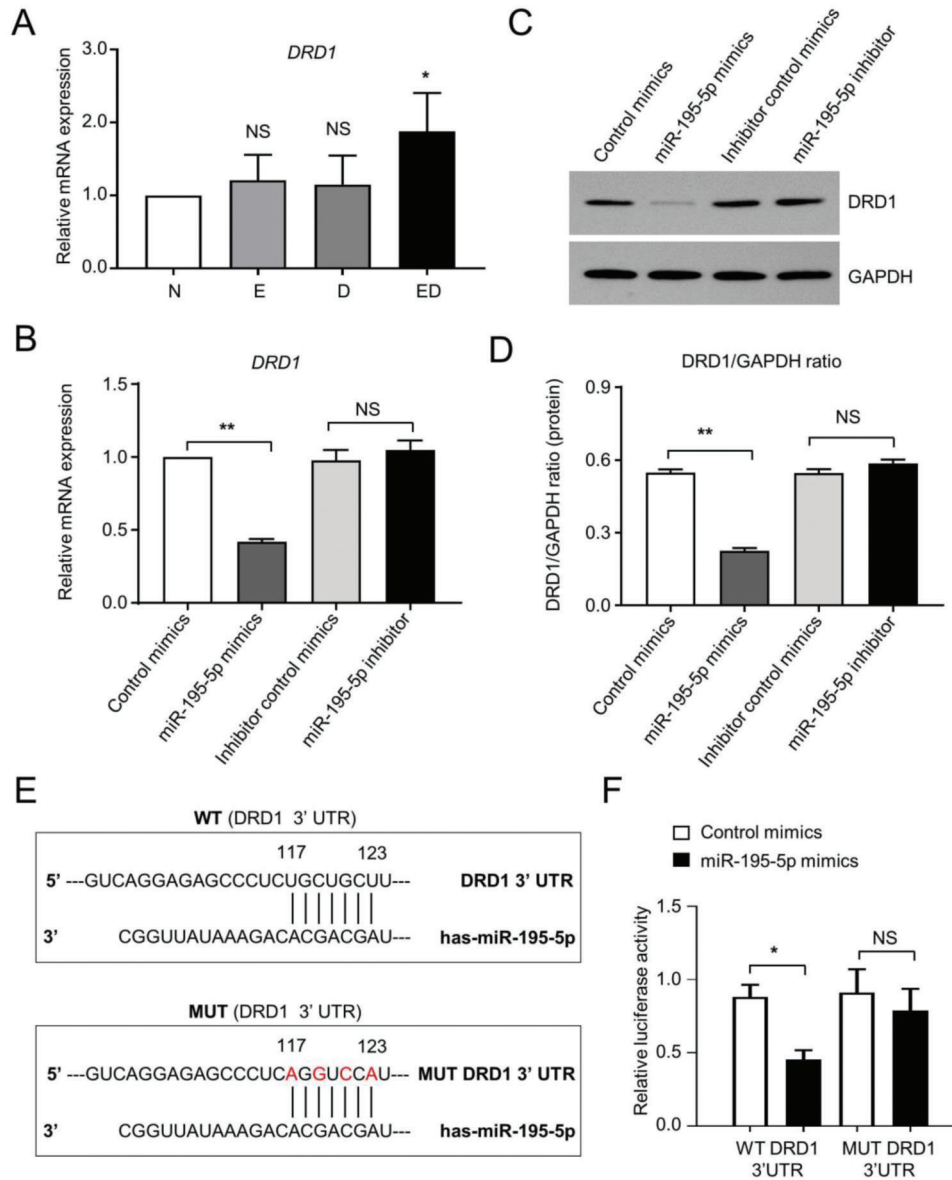
To further explore the molecular mechanism of serum miR-195-5p in patients with EH with T2DM, we took advantage of multiple bioinformatic platforms for target prediction, including the miRDB, TargetScanHuman, and Target-Miner. By overlapping with the Venn Map, we found that DRD1, which was the most abundant dopamine receptor in the central nervous system, showed a preferably negative correlation with miR-195-5p. Hence, we detected the expression of circulating *DRD1* among the indicated groups by utilizing qRT-PCR analysis, and found that the ED group had higher level of *DRD1* mRNA expression did the other groups (N, E, and D), which preliminarily indicated the potential of *DRD1* as the candidate target of miR-195-5p in the ED group (Figure 4A). Thereafter, we delivered the over-expressive and inhibitory mimics of miR-195-5p, and the corresponding control mimics into HEK-293T cells through transfection. After 48h post-transfection, total RNAs and proteins were isolated from HEK-293T cells for *DRD1* expression detection. Quantitative analysis by qRT-PCR and western blotting showed that miR-195-5p overexpression significantly suppressed *DRD1* expression and the suppressive effect was eliminated by miR-195-5p inhibition (Figures 4B–4D).

To assess whether miR-195-5p directly targets *DRD1* to result in dysfunction in patients with EH and T2DM, the putative binding site for miR-195-5p in the 3'-UTR of *DRD1* was predicted using TargetScan (Figure 4E). Then, the

luciferase-based reporter constructs either with the wild type (WT) or mutant (MUT) binding site were respectively co-transfected with miR-195-5p or Control mimics into HEK-293T cells to test their response to ectopic expression of miR-195-5p (Figure 4F, Figure S4A). Strikingly, the relative luciferase activity in the WT + miR-195-5p mimic group was lower than that in the WT DRD1 3' UTR + Control mimic group (WT DRD1 3' UTR + miR-195-5p mimic *vs.* WT DRD1 3' UTR + Control mimic,  $17.96 \pm 2.84$  *vs.*  $9.10 \pm 1.75$ ,  $p=0.0388$ ), whereas there was minimal difference between the MUT + miR-195-5p mimic group and MUT + miR-195-5p mimic group (Figure 4E, Figure S4A). Taken together, *DRD1* functioned as a direct downstream target of miR-195-5p through the abovementioned binding site of miR-195-5p on the 3'-UTR.

#### SNP mutation identification in the DRD1 3'-UTR and diagnostic arability of serum miR-195-5p

To further validate the SNPs in the 3'-UTR of *DRD1* and miR-195-5p-binding regions in patients with EH with T2DM in Chinese individuals, a case control study was performed. For the purpose, we identified two SNPs in the miR-195-5p-binding region of the *DRD1* 3'-UTR in a group of 200 Chinese individuals by sequencing (Figure 5A). We distinguished two common SNPs, 231T-A and 233C-G, in the *DRD1* 3'-UTR (based on allele frequencies) in 100 ED patients and 100 control individuals (Figure 5A, Figure S5A-S5D, Supplementary Table S5). The allele frequency of both the 231A and 233G loci in the ED group was higher than that in the N group (231A: ED *vs.* N, 39% *vs.* 7%; 233G: ED *vs.* N, 54% *vs.* 9%; Figure 5B). Collectively, these data showed that the frequencies of these two polymorphisms were closely related to EH with T2DM ( $p$ <0.05).



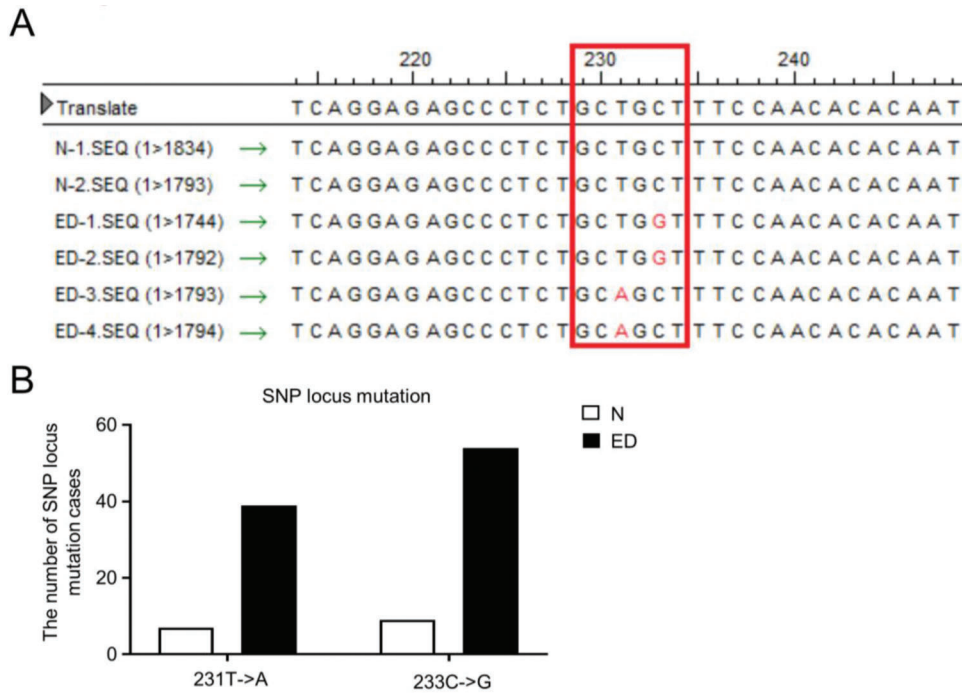
**Figure 4** - DRD1 functions as the direct inhibitory target of miR-195-5p. **A** Validation of plasma levels of DRD1 by qRT-PCR. **B** Fold change of *DRD1* mRNA expression in HEK-293T cells after transfection of miR-195-5p mimic or miR-195-5p inhibitor for 48h. Control mimics and inhibitor control mimics served as negative controls of miR-195-5p mimics and miR-195-5p inhibitors, respectively. **C-D** Expression levels of DRD1 proteins in the aforementioned four groups were quantified by western-blot assay (**C**), and gray-scale scanning with ImageJ software (**D**). GAPDH was used as an internal control. All data are shown as the mean  $\pm$  SEM (n=3), \* $p$ <0.05. **E** Putative wildtype (WT) and mutant type (MUT) sequences of miR-195-5p binding sites in the DRD1 3' UTR. **F** Luciferase reporter assay of the inhibitory activity of miR-195-5p upon transfection with DRD1 3'-UTR and DRD1-mut 3'-UTR and/or miR-195-5p mimic or control mimic in HEK-293T cells for 24h. All data are shown as the mean  $\pm$  SEM (n=3), \* $p$ <0.05; NS, not significant.

Additionally, to further verify the potentially clinical significance and correlation of serum miR-195-5p in diagnosis of patients with EH and T2DM, we conducted a correlation analyses with multiple clinicopathologic indexes. Of them, miR-195-5p showed a positive correlation with Cr and ALP ( $p$ <0.05) whereas weak or no correlation occurred with other parameters (Figures 6A–6H, Figures S6A–6H). Moreover, to further elucidate the clinical significance of miR-195-5p in patients with EH and T2DM, conducted an ROC analysis of miR-195-5p between the N group and patients with EH. We found that the area under the curve (AUC) was 0.772, and the sensitivity and specificity was 0.846 and 0.769 under the

cut-off level 4.020, respectively (Figure 6I). Taken together, our data indicated that circulating miR-195-5p in peripheral blood held the potential of serving as a novel biomarker for EH and T2DM diagnosis.

## DISCUSSION

For decades, researchers have focused on the potential etiology and pathogenesis of EH or T2DM; however, novel non-invasive biomarkers and candidate targets, including miRNAs for the diagnosis and treatment of patients with EH and T2DM are still unclear (1,29). In this study, we identified



**Figure 5** - ED patients exhibited miR-195-5p binding SNP locus mutation in the 3'-UTR of DRD1. **A** Two miR-195-5p binding SNP locus mutations (231T->A, 233C->G) were identified in the 3'-UTR of DRD1 in patients with essential hypertension and type 2 diabetes mellitus (ED group). **B** Number of patients with SNP locus mutation in the ED and N groups.

serum miR-195-5p as a pivotal factor in patients with EH and T2DM. Interestingly, miR-195-5p expression displayed more remarkable downregulation than that in patients with either EH or T2DM, which indicated the potential of miR-195-5p to serve as a novel biomarker and therapeutic target for EH with T2DM. Furthermore, with the aid of the luciferase-based reporter assay and SNP analyses, we identified *DRD1* as a direct target of miR-195-5p in patients with EH and T2DM.

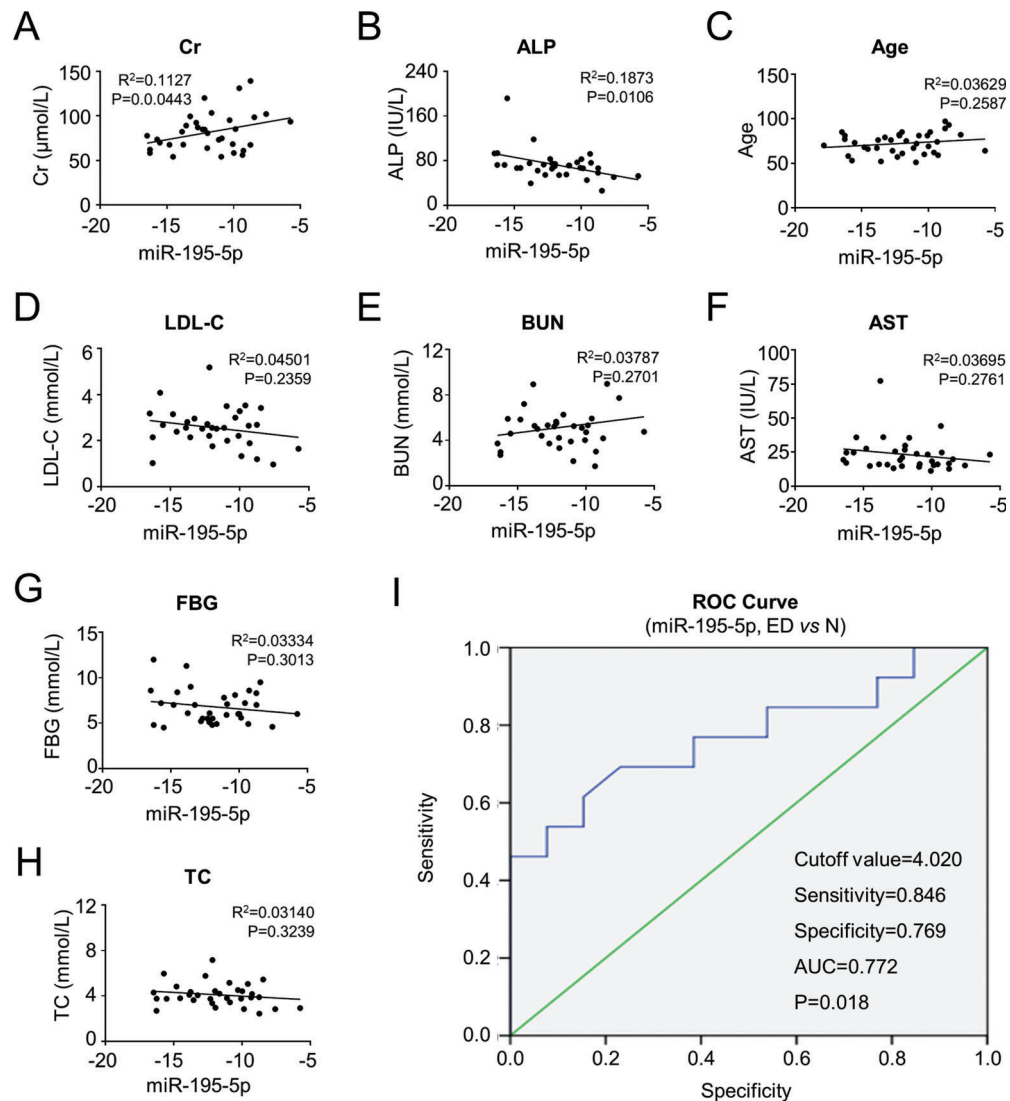
Hypertension is a complicated multifactorial disease attributable to dysregulation of angiogenesis and vascular smooth muscle, myocardial hypertrophy, activation of the renin-angiotensin-aldosterone system and platelet functional impairment. Because of the development and progression of EH and T2DM, morbidity and mortality in cardiovascular diseases collectively deteriorated as well. Furthermore, hypertension is acknowledged as being involved with more than two-thirds of patients with T2DM (3). Despite notable progress for the diagnosis and management of patients with EH or T2DM according to the guidelines, the development of more accurate clinical therapeutics is far from satisfactory. This is mainly caused by the deficiency in novel non-invasive biomarkers and therapeutic target identification.

MicroRNAs are evolutionarily conserved single-stranded transcripts of hairpin structures with 21–23 nucleotides throughout the genome, which are involved in multifaceted physiological and pathological processes by regulating gene-expression post-transcriptionally, and serving as promising candidates for biomarker and targeted therapy development (30). Numerous functional studies have indicated the dysregulation and potential clinical significance of miRNAs in EH (e.g., miR-92a, miR-31a-5p) and T2DM (e.g., miR-15a, miR-223, miR-375, miR-30d) patients (13,14). For instance,

serum miR-92a holds potential to function as a non-invasive marker of atherosclerosis in hypertension, whereas miR-31a-5p and miR-184 are involved in hypertension and glucose metabolism by bidirectional regulation of arterial smooth muscle cell and  $\beta$  cell function, respectively (31,32). Herein, with the aid of an miRNA qRT-PCR array, serum miR-195-5p was identified as a novel non-invasive diagnostic biomarker in patients with EH and T2DM, which was identified as a tumor suppressor and an indicator of poor prognosis in tumor progression (33). Thus, in this study we identified the novel dysregulation and malfunction of miR-195-5p in Chinese individuals with T2DM and EH, which collectively indicated the multidimensional functionality and pathogenesis of miRNAs. Therefore, studies on the characteristics of miRNAs in the etiology and pathogenesis of patient with EH and T2DM should be undertaken systematically and meticulously.

Dopamine is an important neurotransmitter that regulates diverse physiological processes including behavior, hormone synthesis and release, blood pressure, and transmembrane ion transport. Dopamine receptor D1 (DRD1) belongs to the superfamily of G-protein-coupled receptors (GPCRs), and is classified by structure and pharmacology, which is acknowledged as the most important DR subtype for sodium reuptake and renal sodium excretion, while dysfunction of DRD1 commonly results in chronic hypertension in patients with EH (34). Herein, we further illuminated the negative regulation of *DRD1* by miR-195-5p via miRNA-mediated cleavage and translational repression of target genes. Furthermore, we identified two SNPs, 231T-A and 233C-G, in the miR-195-5p-binding sites in the DRD1 3'-UTR. Distinguished from those in the health donors, frequencies of the 231A and 233G alleles were much higher in the patients with EH





**Figure 6** - Relevance of miR-195-5p with multiple clinicopathological parameters. **A-B** The relevance of miR-195-5p with positively related ED-associated clinicopathological parameters, including the Cr (**A**) and ALP (**B**). **C** Relevance of miR-195-5p with age. **D-H** MiR-195-5p showed no significant difference with other ED-associated clinicopathological parameters, including LDL-C (**D**), BUN (**E**), AST (**F**), FBG (**G**), and TC (**H**). **I** ROC analysis of miR-195-5p between N and ED groups. Cut-off value=4.020, Sensitivity=0.846, Specificity=0.769, AUC=0.772,  $p=0.018$ .

and T2DM, which subsequently altered the binding affinity of miR-195-5p toward the 3'-UTR and deregulated the post-transcriptional regulation of *DRD1*. Taken together, our research revealed that serum miR-195-5p exhibits clinical significance with respect to non-invasive diagnosis and interventional therapeutics and provides insights into the pathogenesis of EH with T2DM.

## CONCLUSIONS

Overall, in this study we identified serum miR-195-5p as a novel non-invasive biomarker for clinical diagnosis and interventional therapeutics for EH with concomitant T2DM that functions by directly suppressing *DRD1* expression. Taken together, our findings further elucidated the pathogenesis of EH with T2DM and provided promising candidates for the development of novel targeted agents.

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## AUTHOR CONTRIBUTIONS

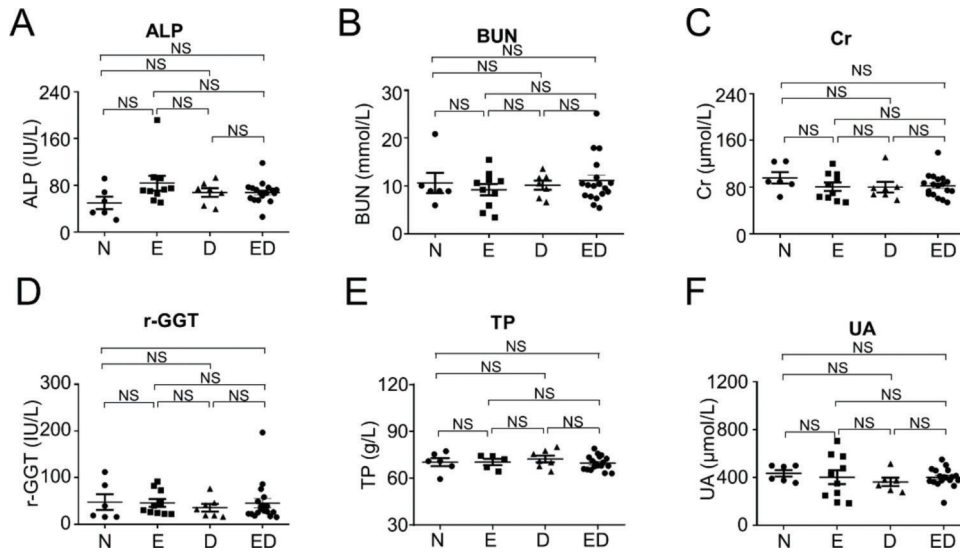
Hu Y, Li Q and Zhang L designed and performed the experiments, were responsible for the data collection and assembly, and manuscript writing. Zhong L, Gu M, He B, Qu Q, Lao Y and Gu K helped with collection and assembly of data. Zhang L, Zheng B and Yang H were responsible for the conception and design, data analysis and interpretation, manuscript writing and revision, approval of the final version of the manuscript. The final version of the manuscript has been read and approved by all the authors.

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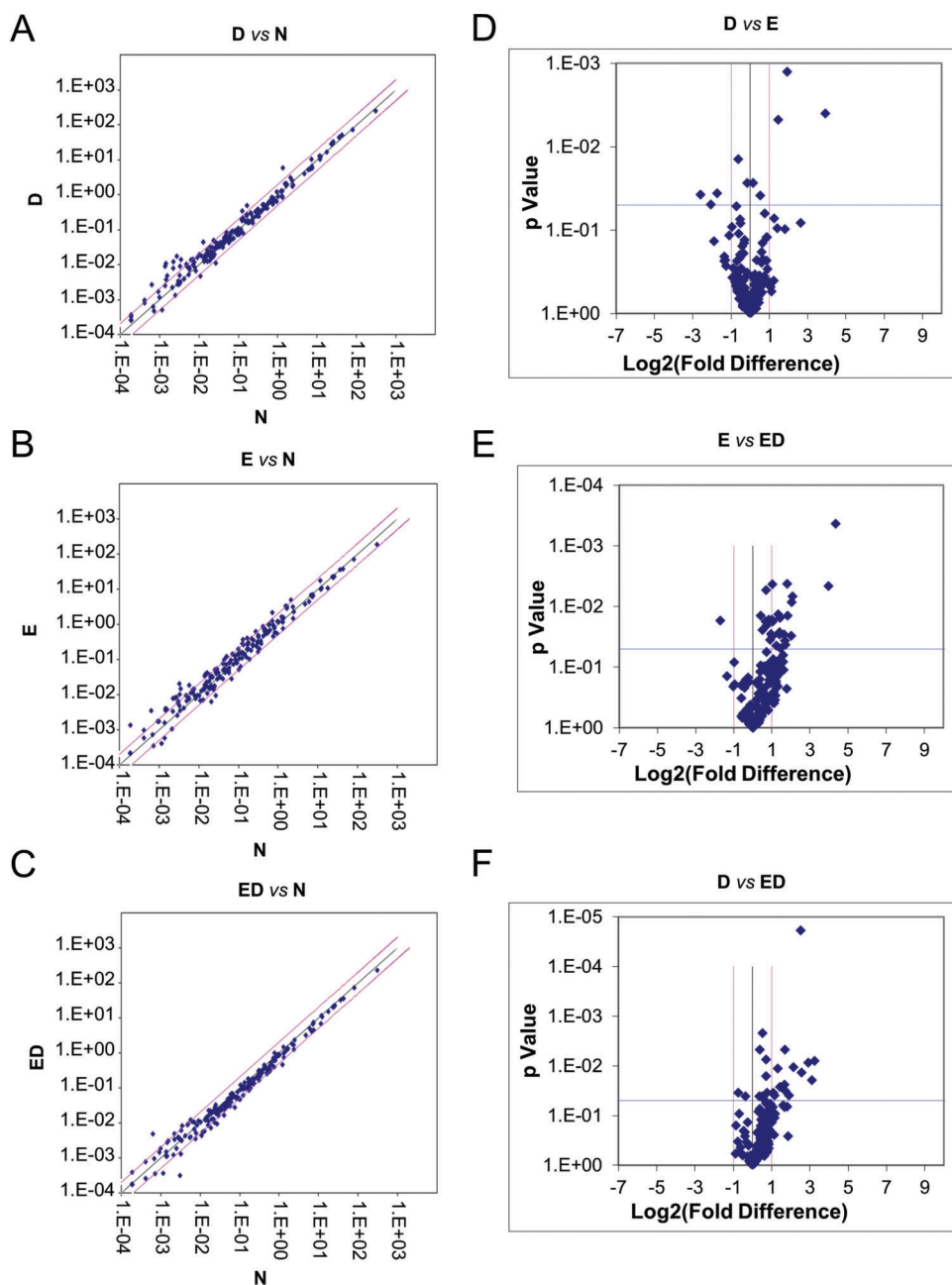
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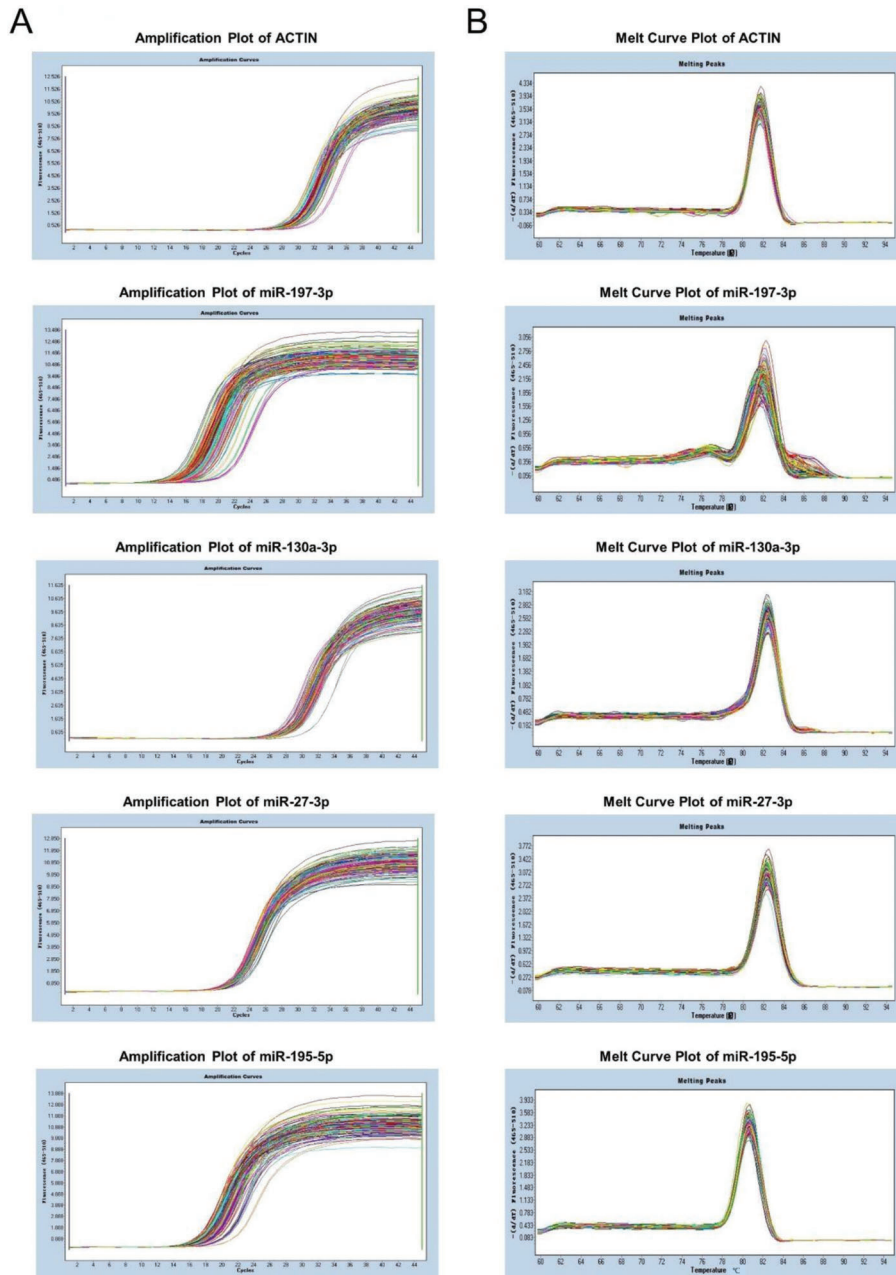
■ APPENDIX



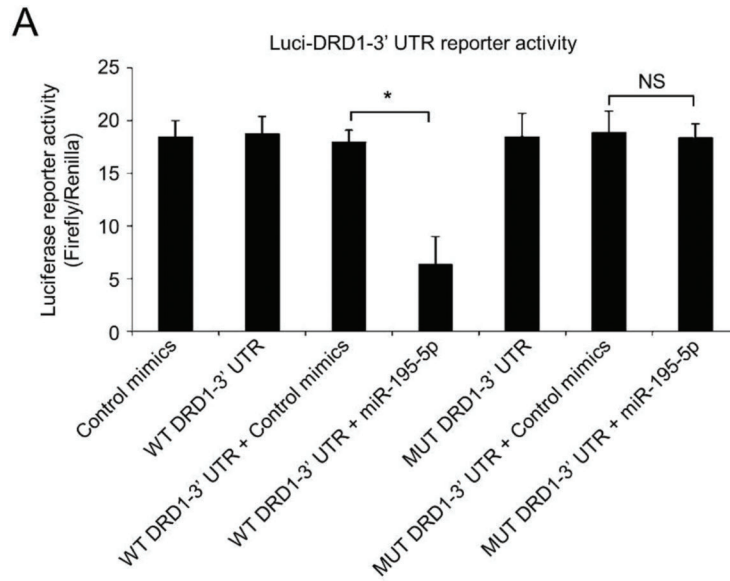
**Figure S1** - Clinicopathological parameters involved in E, D ED, and healthy donor (N) groups. **A-F** ED-associated clinical parameters in E, D, ED, and N groups, including ALP (A), BUN (B), Cr (C), r-GGT (D), TP (E), and UA (F). All data are shown as the mean  $\pm$  SEM. NS, not significant.



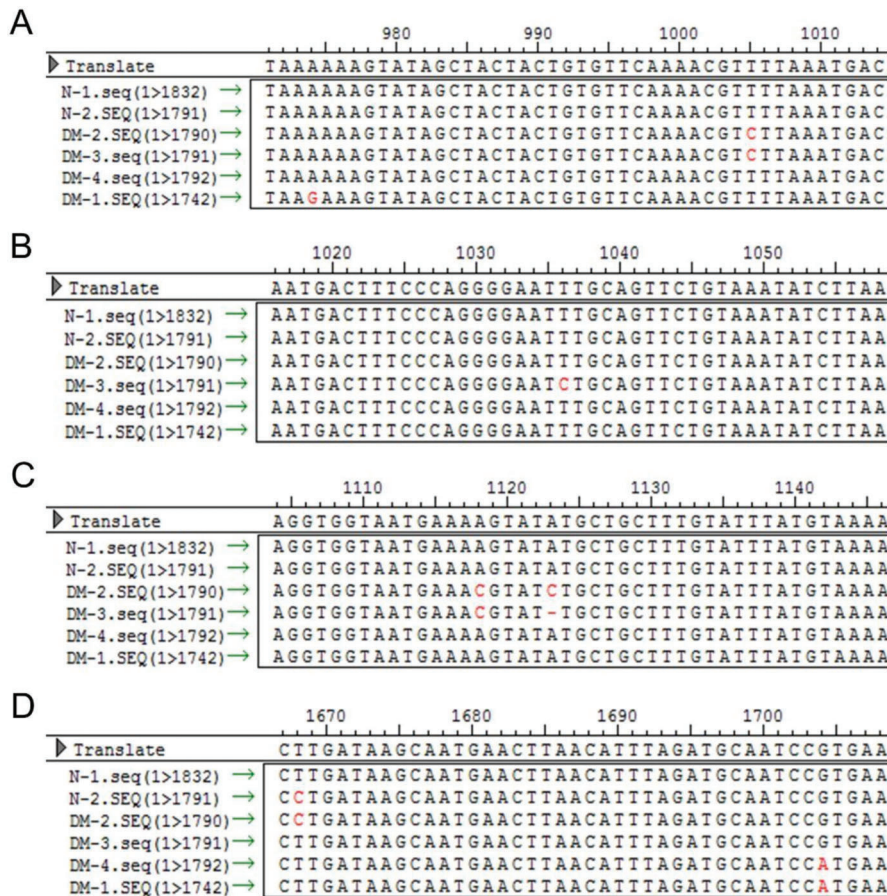
**Figure S2** - Comparison of plasma miRNA expression profiling among patients and healthy donors. **A-C** Scatter plot illustration of serum miRNAs between the D and N groups (**A**), E and N groups (**B**), and ED and N groups (**C**). **D-F** Scatter plot illustration of serum miRNAs between the D and E groups (**D**), E and ED groups (**E**), and D and ED groups (**F**).



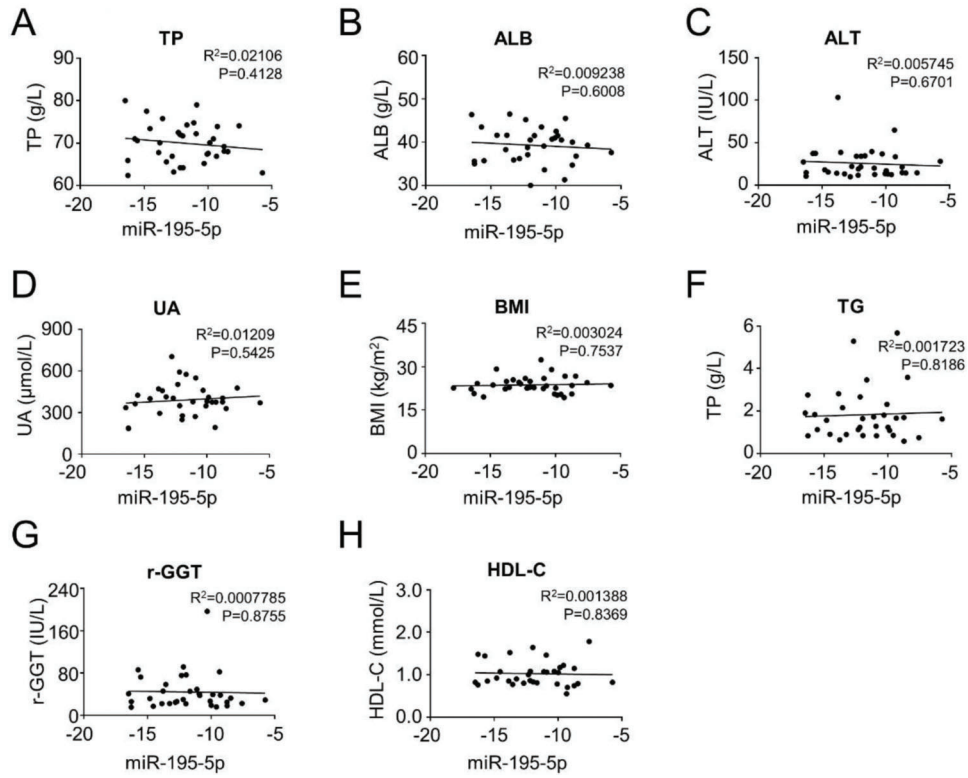
**Figure S3** - Identification of differentially expressed miRNAs among patients and healthy donors. **A-B** Amplification plot (**A**) and melting curve plot (**B**) of ACTIN, miR-197-3p, miR-130a-3p, miR-27-3p, and miR-195-5p.



**Figure S4** - Luciferase reporter assay to investigate the binding of miR-195-5p to the DRD1 3'-UTR. **A** Luciferase reporter activity indicating the inhibitory effect of miR-195-5p on DRD1 in HEK-293T cells transfected with DRD1-3'-UTR and DRD1-mut 3'-UTR (MUT DRD1-3' UTR) and/or miR-195-5p mimic or control mimic for 24h. All values are shown as firefly/renilla ratio. All data are shown as the mean  $\pm$  SEM (n=3), \* $p$ <0.05; NS, not significant.



**Figure S5** - miRNA-binding SNP locus mutation analyses in the 3'-UTR of DRD1 in the ED group. **A** Analyses of miRNA-binding SNPs in other regions of the 3'-UTR of DRD1 in healthy donors (N-1, N-2) and ED groups with essential hypertension and type 2 diabetes mellitus (DM-1, DM-2, DM-3, DM-4), such as 971-1014 (A), 1016-1058 (B), 1104-1146, (C) and 1667-1708 (D).



**Figure S6** - The relevance of miR-195-5p with multiple clinicopathological parameters. **A-H** MiR-195-5p showed no significant difference with other ED-associated clinicopathological parameters, including the TP (**A**), ALB (**B**), ALT (**C**), UA (**D**), BMI (**E**), TG (**F**), r-GGT (**G**), and HDL-C (**H**) values.



**Table S1** - General information of representative groups (E, D, ED) and healthy donors (N).

| Group | NO. | Gender | Age (Year) | Weight (kg) | Height (m) | BMI  | TP (g/L) | ALB (g/L) | Liver Function (IU/L) |      |      |       | Renal Function |              |             | Blood Lipid (mmol/L) |      |      |       |
|-------|-----|--------|------------|-------------|------------|------|----------|-----------|-----------------------|------|------|-------|----------------|--------------|-------------|----------------------|------|------|-------|
|       |     |        |            |             |            |      |          |           | ALT                   | AST  | ALP  | r-GGT | Cr (μmol/L)    | BUN (mmol/L) | UA (μmol/L) | FBG (mmol/L)         | TC   | TG   | HLC-C |
| N     | 1   | F      | 93         | 50          | 1.70       | 17.3 | 70.9     | 43.7      | 17.6                  | 20.5 | 33.6 | 15.9  | 89.4           | 4.47         | 4.48        | 1.15                 | 1.23 | 3.03 |       |
|       | 2   | M      | 43         | 69          | 1.72       | 23.3 | 75.3     | 47.7      | 39.8                  | 22.9 | 68.3 | 39.4  | 85.2           | 2.98         | 1.34        | 1.59                 | 1.04 | 3.3  |       |
|       | 3   | M      | 61         | 63          | 1.72       | 21.1 | 70.9     | 43.7      | 17.6                  | 20.5 | 33.6 | 15.9  | 89.4           | 4.47         | 4.48        | 1.15                 | 1.23 | 3.03 |       |
|       | 4   | F      | 67         | 60          | 1.48       | 27.4 | 77.3     | 41.0      | 22.5                  | 25.5 | 91.7 | 83.7  | 63.5           | 4.45         | 2.98        | 0.53                 | 1.52 | 1.41 |       |
| E     | 5   | M      | 96         | 45          | 1.55       | 18.7 | 59.4     | 31.3      | 17                    | 26   | 21   | 112   | 123.7          | 10.43        | 4.7         | 4.08                 | 0.86 | 1.54 | 2.4   |
|       | 6   | M      | 79         | 50          | 1.60       | 19.5 | 67.7     | 38.6      | 10.4                  | 21.7 | 52.9 | 18.9  | 124.3          | 5.03         | 3.75        | 0.9                  | 1.23 | 0.86 | 2.21  |
|       | 7   | M      | 82         | 60          | 1.63       | 22.6 | 72.0     | 38.8      | 11.7                  | 14.7 | 65.8 | 30.1  | 120.1          | 5.64         | 3.74        | 1.12                 | 0.85 | 2.68 |       |
|       | 8   | F      | 73         | 52          | 1.63       | 19.6 | 70.6     | 35.7      | 37.4                  | 35.9 | 192  | 72.5  | 69.9           | 4.6          | 4.5         | 0.83                 | 1.48 | 2.15 |       |
|       | 9   | F      | 81         | 53          | 1.60       | 20.7 | 62.4     | 35.0      | 14.8                  | 24.6 | 93.7 | 26    | 62.2           | 2.96         | 4.8         | 0.83                 | 1.48 | 2.15 |       |
|       | 10  | M      | 76         | 67          | 1.65       | 24.6 | 66.9     | 36.2      | 9.9                   | 13.1 | 73   | 24.7  | 92.3           | 5.32         | 5.2         | 0.83                 | 1.48 | 2.15 |       |
|       | 11  | F      | 85         | 55          | 1.55       | 22.9 | 71.7     | 40.5      | 21.6                  | 26.7 | 70   | 22.2  | 63.8           | 4.22         | 4.8         | 1.64                 | 1.64 | 2.56 |       |
|       | 12  | F      | 54         | 54          | 1.74       | 22.8 | 74.3     | 41.5      | 30.5                  | 35.5 | 54.3 | 45.5  | 103.3          | 6.26         | 5.9         | 1.28                 | 1.46 | 3.5  |       |
| D     | 1   | M      | 85         | 66          | 1.74       | 24.5 | 74.1     | 39.3      | 14.5                  | 15.2 | 50.8 | 22.6  | 102            | 7.73         | 4.9         | 3.46                 | 0.81 | 2.52 |       |
|       | 2   | M      | 82         | 75          | 1.75       | 24.5 | 74.1     | 39.3      | 14.5                  | 15.2 | 50.8 | 22.6  | 102            | 7.73         | 4.6         | 2.83                 | 0.74 | 1.78 |       |
|       | 3   | M      | 81         | 53          | 1.53       | 22.6 | 64.2     | 37.1      | 19                    | 21   | 75.1 | 91.7  | 84.5           | 5.5          | 6.1         | 1.66                 | 1.08 | 5.18 |       |
|       | 4   | F      | 74         | 47          | 1.56       | 19.3 | 66.9     | 31.3      | 64.8                  | 44.2 | 92.2 | 82.3  | 56             | 5.5          | 7.9         | 1.32                 | 1.17 | 3.16 |       |
|       | 5   | M      | 85         | 60          | 1.64       | 22.3 | 75.5     | 49.2      | 18.7                  | 17.1 | 73.4 | 18.8  | 78.4           | 6.79         | 4.8         | 1.9                  | 0.82 | 3.18 |       |
|       | 6   | M      | 68         | 66          | 1.67       | 23.7 | 80.0     | 46.4      | 27.3                  | 19.4 | 93.1 | 40.9  | 77.7           | 3.72         | 7.9         | 1.32                 | 1.17 | 3.16 |       |
|       | 7   | M      | 76         | 72          | 1.70       | 24.9 | 77.5     | 41.6      | 18.1                  | 27.6 | 66.9 | 31.9  | 67.5           | 5.85         | 8.6         | 1.56                 | 0.92 | 3.15 |       |
|       | 8   | M      | 62         | 51          | 1.58       | 20.4 | 70.1     | 41.6      | 103.3                 | 77.4 | 39.5 | 45.9  | 67.7           | 5.27         | 7           | 4.82                 | 1.56 | 0.92 |       |
| ED    | 1   | M      | 69         | 67          | 1.72       | 22.6 | 71.0     | 40.5      | 12.4                  | 16   | 45.4 | 16    | 131.1          | 5.92         | 6.1         | 4.35                 | 0.84 | 1.52 |       |
|       | 2   | M      | 81         | 72          | 1.73       | 24.1 | 64.2     | 42.5      | 17                    | 18.3 | 82.9 | 19.2  | 58.3           | 4.72         | 6.1         | 5.06                 | 0.85 | 1.22 |       |
|       | 3   | F      | 61         | 60          | 1.55       | 25.0 | 67.6     | 42.5      | 17                    | 18.3 | 82.9 | 19.2  | 58.3           | 4.72         | 6           | 4.41                 | 1.23 | 3.28 |       |
|       | 4   | F      | 81         | 72          | 1.70       | 26.0 | 79.0     | 33.6      | 34.1                  | 29.8 | 74.2 | 76.4  | 80.6           | 3.31         | 6           | 2.95                 | 0.84 | 0.83 |       |
|       | 5   | M      | 72         | 75          | 1.70       | 26.0 | 79.0     | 33.6      | 12.6                  | 16.2 | 71.4 | 37.8  | 74.8           | 5.25         | 5.5         | 2.95                 | 0.84 | 0.83 |       |
|       | 6   | M      | 66         | 66          | 1.67       | 24.5 | 71.0     | 43.5      | 37.3                  | 24.7 | 72.6 | 86.1  | 73.5           | 5.89         | 7.1         | 3.43                 | 0.83 | 1.06 |       |
|       | 7   | M      | 53         | 63          | 1.68       | 22.3 | 67.8     | 38.3      | 14.1                  | 16   | 75.4 | 22.4  | 82.2           | 8.95         | 7.2         | 5.96                 | 1.83 | 1.44 |       |
|       | 8   | F      | 66         | 64          | 1.48       | 29.2 | 73.4     | 41.6      | 15.4                  | 14.8 | 67   | 17.4  | 54.1           | 7.2          | 11.3        | 4.1                  | 2.81 | 0.85 |       |
| ED    | 9   | M      | 52         | 70          | 1.75       | 22.9 | 75.8     | 46.5      | 38.5                  | 36   | 118  | 58.4  | 89             | 5.01         | 8.4         | 3.79                 | 0.9  | 1.07 |       |
|       | 10  | M      | 60         | 90          | 1.76       | 29.1 | 65.2     | 40.7      | 36.8                  | 23.3 | 77   | 196.5 | 95.2           | 5.1          | 9           | 3.62                 | 2.15 | 0.77 |       |
|       | 11  | M      | 57         | 75          | 1.70       | 26.0 | 72.5     | 45.2      | 34                    | 19.1 | 83.1 | 75.6  | 84.8           | 5.1          | 8.1         | 4.52                 | 1.8  | 1.08 |       |
|       | 12  | M      | 59         | 81          | 1.74       | 26.8 | 73.9     | 45.5      | 33.4                  | 24.7 | 76.2 | 38.2  | 60.9           | 3            | 5.5         | 3.73                 | 1.12 | 1    |       |
|       | 13  | M      | 68         | 88          | 1.68       | 24.1 | 63.2     | 38.1      | 22                    | 25.5 | 54.8 | 26.2  | 87.1           | 3            | 8.6         | 4.16                 | 5.67 | 0.7  |       |
|       | 14  | M      | 93         | 81          | 1.74       | 26.8 | 68.0     | 36.8      | 14.3                  | 19.8 | 26.4 | 32.8  | 98.4           | 3.71         | 8.6         | 5.76                 | 5.28 | 0.8  |       |
|       | 15  | M      | 85         | 55          | 1.65       | 20.2 | 70.1     | 41.5      | 13.2                  | 15.4 | 66.4 | 39.2  | 85.2           | 8.98         | 5.5         | 5.44                 | 3.58 | 0.79 |       |
|       | 16  | M      | 67         | 83          | 1.60       | 32.4 | 74.8     | 43.5      | 39.5                  | 23.8 | 55.3 | 49.2  | 73.2           | 5.32         | 9.5         | 5.44                 | 3.58 | 0.79 |       |
|       | 17  | M      | 97         | 68          | 1.70       | 23.5 | 68.1     | 40        | 14.8                  | 12.7 | 66.7 | 18.4  | 139.2          | 3.89         | 7.8         | 2.83                 | 1.08 | 1.17 |       |
|       | 18  | F      | 77         | 64          | 1.62       | 23.1 | 65.9     | 35.6      | 10.5                  | 17.1 | 72.6 | 15.4  | 58.355         | 12.58        | 11.3        | 2.43                 | 0.58 | 1.15 |       |
| ED    | 19  | M      | 79         | 78          | 1.75       | 25.5 | 65.6     | 35.9      | 13.3                  | 15.8 | 62.3 | 22.5  | 99.4           | 2.7          | 12          | 2.68                 | 2.75 | 0.76 |       |
|       | 20  | M      | 81         | 54          | 1.62       | 20.6 | 67.4     | 41        | 13.6                  | 11.2 | 67.3 | 27.1  | 68.3           | 4.4          | 7           | 4.06                 | 0.89 | 0.9  |       |
|       | 21  | F      | 89         | 56          | 1.65       | 20.6 | 69.2     | 34.7      | 16.3                  | 16.6 | 58.2 | 25    | 67.4           | 4.02         | 6           | 3.75                 | 2.31 | 0.78 |       |
|       | 22  | M      | 64         | 61          | 1.61       | 23.5 | 63.0     | 37.6      | 28                    | 23.2 | 52.8 | 29    | 93.6           | 4.76         | 6           | 3.88                 | 1.69 | 0.74 |       |





**Table S2** - Primer sequences for the amplification of candidate miRNAs.

| miR name (human) | microRNA target sequence |
|------------------|--------------------------|
| hsa-miR-652-3p   | AAUGGCGCCACUAGGGUUGUG    |
| hsa-miR-502-3p   | AAUGCACCUGGGCAAGGAUUCA   |
| hsa-miR-181a-5p  | AACAUUCAACGCUGUCGGGUGAGU |
| hsa-miR-339-3p   | UGAGCGCCUCGACGACAGAGCCG  |
| hsa-miR-221-3p   | AGCUACAUUGUCUGCGGGUUC    |
| hsa-miR-409-3p   | GAAUGUUGCUCGGUGAACCCCU   |
| hsa-let-7f-5p    | UGAGGUAGUAGAUUGUAUGUU    |
| hsa-miR-154-5p   | UAGGUUAUCCGUGUUGCCUUCG   |
| hsa-miR-27b-3p   | UUCACAGUGGCUAAGUUCUGC    |
| hsa-miR-155-5p   | UUAUAGCUAAUCGUGAUAGGGGU  |
| hsa-miR-374b-5p  | AUAUAAUACAACUCGUAAGUG    |
| hsa-miR-140-3p   | UACCACAGGGUAGAACCACGG    |
| hsa-miR-93-5p    | CAAAGUGCUGUUCGUGCAGGUAG  |
| hsa-miR-92b-3p   | UAUUGCACUCGUCCCGGCCUCC   |
| hsa-miR-200a-3p  | UAACACUGUCUGGUAACGAUGU   |
| hsa-miR-505-3p   | CGUCAACACUUGCUGGUUCCU    |
| hsa-miR-23b-3p   | UCACACUUGCCAGGGUUAUCC    |
| hsa-miR-484      | UCAGGCUCAGUCCCUCCGAU     |
| hsa-miR-141-3p   | UAACACUGUCUGGUAAGAUGG    |
| hsa-miR-10a-5p   | UACCCUGUAGAUCGAAUUUGUG   |
| hsa-miR-106a-5p  | AAAAGUGCUUACAGUGCAGGUAG  |
| hsa-miR-27a-3p   | UUCACAGUGGCUAAGUUCGCG    |
| hsa-miR-652-3p   | AAUGGCGCCACUAGGGUUGUG    |
| hsa-miR-502-3p   | AAUGCACCUGGGCAAGGAUUCA   |
| hsa-miR-181a-5p  | AACAUUCAACGCUGUCGGGUGAGU |
| hsa-miR-339-3p   | UGAGCGCCUCGACGACAGAGCCG  |
| hsa-miR-221-3p   | AGCUACAUUGUCUGCGGGUUC    |
| hsa-miR-409-3p   | GAAUGUUGCUCGGUGAACCCCU   |
| hsa-let-7f-5p    | UGAGGUAGUAGAUUGUAUGUU    |
| hsa-miR-154-5p   | UAGGUUAUCCGUGUUGCCUUCG   |
| hsa-miR-27b-3p   | UUCACAGUGGCUAAGUUCUGC    |
| hsa-miR-155-5p   | UUAUAGCUAAUCGUGAUAGGGGU  |
| hsa-miR-374b-5p  | AUAUAAUACAACUCGUAAGUG    |
| hsa-miR-140-3p   | UACCACAGGGUAGAACCACGG    |
| hsa-miR-93-5p    | CAAAGUGCUGUUCGUGCAGGUAG  |
| hsa-miR-92b-3p   | UAUUGCACUCGUCCCGGCCUCC   |
| hsa-miR-200a-3p  | UAACACUGUCUGGUAACGAUGU   |
| hsa-miR-505-3p   | CGUCAACACUUGCUGGUUCCU    |
| hsa-miR-23b-3p   | AUCACAUUGCCAGGGUUAUCC    |
| hsa-miR-484      | UCAGGCUCAGUCCCUCCGAU     |
| hsa-miR-141-3p   | UAACACUGUCUGGUAAGAUGG    |
| hsa-miR-10a-5p   | UACCCUGUAGAUCGAAUUUGUG   |
| hsa-miR-106a-5p  | AAAAGUGCUUACAGUGCAGGUAG  |
| hsa-miR-27a-3p   | UUCACAGUGGCUAAGUUCGCG    |
| hsa-miR-145-5p   | GUCCAGUUUCCCGAAUCCCU     |
| hsa-miR-1        | UGGAAUGUAAAGAAGUAUGUAU   |
| hsa-miR-185-5p   | UGGAGAGAAAGGAGUUCUGA     |
| hsa-miR-382-5p   | GAAGUUGUUCGUGGUGGAUUCG   |
| hsa-miR-486-5p   | UCCUGUACUGAGCUGCCCGAG    |
| hsa-miR-32-5p    | UAUUGCACAUUACUAAGUUGCA   |
| hsa-miR-26a-5p   | UUCAAGUAAUCCAGGAUAGGCU   |
| hsa-miR-133b     | UUUGGUCCCUCAACCAGCUG     |
| hsa-miR-143-3p   | UGAGAUGAAGCACUGUAGCUC    |
| hsa-let-7d-5p    | AGAGGUAGUAGGUUGCAUAGUU   |
| hsa-miR-30a-5p   | UGUAAACAUCUCGACUGGAAG    |
| hsa-miR-133a     | UUUGGUCCCUCAACCAGCUG     |
| hsa-miR-222-3p   | AGCUACAUCUGGCUACUGGGU    |
| hsa-miR-20b-5p   | CAAAGUGCUCUAGUGCAGGUAG   |
| hsa-miR-342-3p   | UCUCACACAGAAAUCGCACCCGU  |
| hsa-miR-328      | CUGGCCUCUCUGCCCUUCGCU    |
| hsa-miR-324-5p   | CGCAUCCCUAGGGCAUUGGUGU   |
| hsa-miR-532-3p   | CCUCCACACCAAGGCUUGCA     |
| hsa-miR-15a-5p   | UAGCAGCACAUAAUGGUUUGUG   |
| hsa-let-7e-5p    | UGAGGUAGGAGGUUGUAUGUU    |
| hsa-miR-532-5p   | CAUGCCUUGAGUGUAGGACCGU   |
| hsa-miR-145-5p   | GUCCAGUUUCCCGAAUCCCU     |
| hsa-miR-1        | UGGAAUGUAAAGAAGUAUGUAU   |
| hsa-miR-185-5p   | UGGAGAGAAAGGAGUAGUAGU    |
| hsa-miR-382-5p   | GAAGUUGUUCGUGGUGGAUUCG   |

(Continued)

**Table S2** - Continued.

| miR name (human) | microRNA target sequence |
|------------------|--------------------------|
| hsa-miR-486-5p   | UCCUGUACUGAGCUGCCCCGAG   |
| hsa-miR-32-5p    | UAUUGCACAUUACUAAGUUGCA   |
| hsa-miR-26a-5p   | UUCAAGUAAUCCAGGAUAGGCU   |
| hsa-miR-133b     | UUUGGUCCCCUUAACCAGCUG    |
| hsa-miR-143-3p   | UGAGAUGAAGCACUGUAGCUC    |
| hsa-let-7d-5p    | AGAGGUAGUAGGUUGCAUAGUU   |
| hsa-miR-30a-5p   | UGUAAACAUCUCGACUGGAAG    |
| hsa-miR-133a     | UUUGGUCCCCUUAACCAGCUG    |
| hsa-miR-222-3p   | AGCUACAUCUGGCUACUGGGU    |
| hsa-miR-20b-5p   | CAAAGUGCUCUAGUGCAGGUAG   |
| hsa-miR-342-3p   | UCUCACACAGAAAUCGCACCCGU  |
| hsa-miR-328      | CUGGCCUCUCUGCCCUUCGCU    |
| hsa-miR-324-5p   | CGCAUCCCUAGGGCAUUGGUGU   |
| hsa-miR-532-3p   | CCUCCACACCAAGGCUUGCA     |
| hsa-miR-15a-5p   | UAGCAGCACAUAAUGGUUUGUG   |
| hsa-let-7e-5p    | UGAGGUAGGAGGUUGUAUGUU    |
| hsa-miR-532-5p   | CAUGCCUUGAGUGUAGGACCGU   |
| hsa-miR-139-5p   | UCUACAGUGCACGUGUCUCCAGU  |
| hsa-miR-194-5p   | UGUAAACAGCAACUCCAUGGGA   |
| hsa-miR-375      | UUUGUUCGUUCGGCUCGCGUGA   |
| hsa-miR-660-5p   | UACCAUUGCAUUCGGAGUUG     |
| hsa-miR-451a     | AAACCGUUAACCAUUCAGUUGU   |
| hsa-miR-574-3p   | CACGCUCAUGCACACCCACA     |
| hsa-let-7a-5p    | UGAGGUAGUAGGUUGUAUGUU    |
| hsa-miR-551b-3p  | GCGACCAUACUUGGUUUCAG     |
| hsa-miR-128      | UCACAGUGAACCGGUCUCUUU    |
| hsa-miR-130a-3p  | CAGUGCAAUGUUAAAAGGGCAU   |
| hsa-miR-125a-5p  | UCCUGAGACCCUUUAACCGUGA   |
| hsa-miR-28-5p    | AAGGAGCUCACAGUCUAUUGAG   |
| hsa-miR-485-3p   | GUCAUACACGGCUCUCCUCUCU   |
| hsa-miR-497-5p   | CAGCAGCACACUGUGUUUGU     |
| hsa-let-7b-3p    | CUAUACAACCUACUGCCUCCU    |
| hsa-miR-425-3p   | AUCGGGAAUGUCGUGUCGCCC    |
| hsa-miR-132-3p   | UACAGUCUACAGCCAUGGUCG    |
| hsa-miR-25-3p    | CAUUGCACUUGUCUGGUCUGA    |
| hsa-let-7c       | UGAGGUAGUAGGUUGUAUGGUU   |
| hsa-miR-18a-5p   | UAAGGUAGCAUCUAGUGCAGUAG  |
| hsa-miR-33a-5p   | GUGCAUUGUAGUUGCAUUGCA    |
| hsa-miR-29b-3p   | UAGCACC AUUUGAAAUCAGUGUU |
| hsa-miR-139-5p   | UCUACAGUGCACGUGUCUCCAGU  |
| hsa-miR-194-5p   | UGUAAACAGCAACUCCAUGGGA   |
| hsa-miR-375      | UUUGUUCGUUCGGCUCGCGUGA   |
| hsa-miR-660-5p   | UACCAUUGCAUUCGGAGUUG     |
| hsa-miR-451a     | AAACCGUUAACCAUUCAGUUGU   |
| hsa-miR-574-3p   | CACGCUCAUGCACACCCACA     |
| hsa-let-7a-5p    | UGAGGUAGUAGGUUGUAUGUU    |
| hsa-miR-551b-3p  | GCGACCAUACUUGGUUUCAG     |
| hsa-miR-128      | UCACAGUGAACCGGUCUCUUU    |
| hsa-miR-130a-3p  | CAGUGCAAUGUUAAAAGGGCAU   |
| hsa-miR-125a-5p  | UCCUGAGACCCUUUAACCGUGA   |
| hsa-miR-28-5p    | AAGGAGCUCACAGUCUAUUGAG   |
| hsa-miR-485-3p   | GUCAUACACGGCUCUCCUCUCU   |
| hsa-miR-497-5p   | CAGCAGCACACUGUGUUUGU     |
| hsa-let-7b-3p    | CUAUACAACCUACUGCCUCCU    |
| hsa-miR-425-3p   | AUCGGGAAUGUCGUGUCGCCC    |
| hsa-miR-132-3p   | UACAGUCUACAGCCAUGGUCG    |
| hsa-miR-25-3p    | CAUUGCACUUGUCUGGUCUGA    |
| hsa-let-7c       | UGAGGUAGUAGGUUGUAUGGUU   |
| hsa-miR-18a-5p   | UAAGGUAGCAUCUAGUGCAGUAG  |
| hsa-miR-33a-5p   | GUGCAUUGUAGUUGCAUUGCA    |
| hsa-miR-29b-3p   | UAGCACC AUUUGAAAUCAGUGUU |
| hsa-miR-16-5p    | UAGCAGCACGUAUUUUGGGCG    |
| hsa-miR-136-5p   | ACUCCAUUUGUUUGAUGAUGGA   |
| hsa-let-7b-5p    | UGAGGUAGUAGGUUGUUGGUU    |
| hsa-let-7i-3p    | CUGCGCAAGCUCACUGCCUUGU   |
| hsa-miR-152      | UCAGUGCAUGACAGAACUUGG    |
| hsa-miR-106b-5p  | UAAAGUGCUGACAGUGCAGAU    |
| hsa-miR-30c-5p   | UGUAAACAUCUACACUCUCAGC   |
| hsa-miR-15b-3p   | CGAACCAUUAUUGUCUCUA      |
| hsa-miR-197-3p   | UUCACCACCUUCCACCACGC     |

(Continued)



**Table S2 - Continued.**

| miR name (human) | microRNA target sequence |
|------------------|--------------------------|
| hsa-miR-142-5p   | CAUAAAGUAGAAAGCACUACU    |
| hsa-miR-99b-5p   | CACCCGUAGAACCAGCCUUGCG   |
| hsa-miR-296-5p   | AGGGCCCCCUCAUCCUGU       |
| hsa-miR-30e-5p   | UGUAAACAUCUUGACUGGAAG    |
| hsa-miR-326      | CCUCUGGGCCUUCUCCAG       |
| hsa-miR-146a-5p  | UGAGAACUGAAUCCAUGGGUU    |
| cel-miR-39-3p    | TCACGGGTGTAATCAGCTTG     |
| hsa-miR-421      | AUCAACAGACAUAAUUGGGCGC   |
| hsa-miR-424-5p   | CAGCAGCAAUUAUGUUUGAA     |
| hsa-miR-223-5p   | CGUGUAUUUGACAAGCUGAUU    |
| hsa-miR-146b-5p  | UGAGAACUGAAUCCAUGGGCU    |
| hsa-miR-107      | AGCAGCAUUGUACAGGGCUAUGA  |
| hsa-miR-205-5p   | UCCUUAUUCACCGGAGUCUG     |
| hsa-miR-16-5p    | UGAGCAGCAGUAAUUAUGGGC    |
| hsa-miR-136-5p   | ACUCCAUUUGUUUGAUGAUGGA   |
| hsa-let-7b-5p    | UGAGGUAGUAGGUUGUGUGUU    |
| hsa-let-7i-3p    | CUGCGCAAGCUACUGCCUUGCU   |
| hsa-miR-152      | UCAGUGCAUGACAGAACUUGG    |
| hsa-miR-106b-5p  | UAAAGUGCUGACAGUCAGAU     |
| hsa-miR-30c-5p   | UGUAAACAUCUACACUCUCAGC   |
| hsa-miR-15b-3p   | CGAAUCAUUUUUGCUCUCUA     |
| hsa-miR-197-3p   | UUAACCAUUCUCCACCCAGC     |
| hsa-miR-142-5p   | CAUAAAGUAGAAAGCACUACU    |
| hsa-miR-99b-5p   | CACCCGUAGAACCAGCCUUGCG   |
| hsa-miR-296-5p   | AGGGCCCCCUCAUCCUGU       |
| hsa-miR-30e-5p   | UGUAAACAUCUUGACUGGAAG    |
| hsa-miR-326      | CCUCUGGGCCUUCUCCAG       |
| hsa-miR-146a-5p  | UGAGAACUGAAUCCAUGGGUU    |
| cel-miR-39-3p    | TCACGGGTGTAATCAGCTTG     |
| hsa-miR-421      | AUCAACAGACAUAAUUGGGCGC   |
| hsa-miR-424-5p   | CAGCAGCAAUUAUGUUUGAA     |
| hsa-miR-223-5p   | CGUGUAUUUGACAAGCUGAUU    |
| hsa-miR-146b-5p  | UGAGAACUGAAUCCAUGGGCU    |
| hsa-miR-107      | AGCAGCAUUGUACAGGGCUAUGA  |
| hsa-miR-205-5p   | UCCUUAUUCACCGGAGUCUG     |
| hsa-miR-148b-3p  | UCAGUGCAUCACAGAACUUUGU   |
| hsa-miR-339-5p   | UCCUGUCCUCCAGGAGCAGC     |
| hsa-miR-20a-5p   | UAAAGUGCUAUAGUGCAGGUAG   |
| hsa-miR-17-5p    | CAAAGUGCUAACAGUGCAGGUAG  |
| hsa-miR-500a-5p  | UAAUCCUUGCUACCGGGUGAGA   |
| hsa-miR-30d-5p   | UGUAAACAUCUCCGACUGGAAG   |
| hsa-miR-378a-3p  | ACUGGACUUGGAGUCAGAAGG    |
| hsa-miR-186-5p   | CAAAGAAUUCUCCUUUGGGCU    |
| hsa-miR-425-5p   | AAUGACACGAUCACUCCCGUUGA  |
| hsa-let-7i-5p    | UGAGGUAGUAGUUUGUGUGUU    |
| hsa-miR-346      | UGUCUGCCCGCAUGCCUGCCUCU  |
| hsa-miR-26b-5p   | UUCAAGUAUUUCAGGAUAGGU    |
| hsa-miR-193b-3p  | AACUGGCCUCAAGUCCCGCU     |
| hsa-miR-34a-5p   | UGGAGUGUCUUAUGUCCGUUGU   |
| hsa-miR-320b     | AAAAGCUGGGUUGAGAGGGCAA   |
| hsa-miR-885-5p   | UCCAUAUACACUACCCUGCCUCU  |
| hsa-miR-590-5p   | GAGCUUAUUCAUAAAAGUCAG    |
| hsa-miR-127-3p   | UCGGAUCCGUCUGAGCUGGCU    |
| hsa-miR-191-5p   | CAACGGAAUCCCAAAGCAGCUG   |
| hsa-miR-208a     | AUAAGACGAGCAAAAAGCUUGU   |
| hsa-miR-99a-5p   | AACCCGUAGAUCCGAUCUUGUG   |
| hsa-miR-16-2-3p  | CCAAUAUUACUGUGUCUUUA     |
| hsa-miR-148b-3p  | UCAGUGCAUCACAGAACUUUGU   |
| hsa-miR-339-5p   | UCCUGUCCUCCAGGAGCUCAG    |
| hsa-miR-20a-5p   | UAAAGUGCUAUAGUGCAGGUAG   |
| hsa-miR-17-5p    | CAAAGUGCUAACAGUGCAGGUAG  |
| hsa-miR-500a-5p  | UAAUCCUUGCUACCGGGUGAGA   |
| hsa-miR-30d-5p   | UGUAAACAUCUCCGACUGGAAG   |
| hsa-miR-378a-3p  | ACUGGACUUGGAGUCAGAAGG    |
| hsa-miR-186-5p   | CAAAGAAUUCUCCUUUGGGCU    |
| hsa-miR-425-5p   | AAUGACACGAUCACUCCCGUUGA  |
| hsa-let-7i-5p    | UGAGGUAGUAGUUUGUGUGUU    |
| hsa-miR-346      | UGUCUGCCCGCAUGCCUGCCUCU  |
| hsa-miR-26b-5p   | UUCAAGUAUUUCAGGAUAGGU    |
| hsa-miR-193b-3p  | AACUGGCCUCAAGUCCCGCU     |

(Continued)

**Table S2 - Continued.**

| miR name (human) | microRNA target sequence |
|------------------|--------------------------|
| hsa-miR-34a-5p   | UGGCAGUGUCUJAGCUGGUUGU   |
| hsa-miR-320b     | AAAAGCUGGGUUGAGAGGGCAA   |
| hsa-miR-885-5p   | UCCAUAUACACUACCCUGCCUCU  |
| hsa-miR-590-5p   | GAGCUUAUUCAUAAAAGUCAG    |
| hsa-miR-127-3p   | UCGGAUCCGUCUGAGCUUGGCU   |
| hsa-miR-191-5p   | CAACGGAAUCCCAAAGCAGCUG   |
| hsa-miR-208a     | AUAAGACGAGCAAAAAGCUUGU   |
| hsa-miR-99a-5p   | AACCCGUAGAUCCGAUCUUGUG   |
| hsa-miR-16-2-3p  | CCAAUAUUACUGUGCUCUUUA    |
| hsa-miR-301a-3p  | CAGUGAAUUGCCAAAGCAGCUG   |
| hsa-miR-140-5p   | CAGUGUUUUACCCUAGGUAG     |
| hsa-miR-151a-5p  | UCGAGGAGCUCACAGUCUAGU    |
| hsa-miR-130b-3p  | CAGUGCAAUGAUGAAAGGGCAU   |
| hsa-miR-122-5p   | UGGAGUUGGCAAUUGGUGUUUG   |
| hsa-miR-20a-3p   | ACUGCAUUUAGAGCACUUAAAG   |
| hsa-miR-423-5p   | UGAGGGGCGAGAGCGAGACUUU   |
| hsa-miR-629-5p   | UGGGUUUACGUUGGGAGAACU    |
| hsa-miR-101-3p   | UACAGUACUGUAUACUGAA      |
| hsa-miR-200c-3p  | UAAUACUGCCGGUAAUGAUGGA   |
| hsa-miR-365a-3p  | UAAUGCCCUAAAAUCCUUUAU    |
| hsa-miR-501-3p   | AAUGCACCCGGGCAAGGAUUUCU  |
| hsa-miR-23a-3p   | AUCACAUUGCCAGGGAUUCC     |
| hsa-miR-423-3p   | AGCUCGGUCUGAGGCCCCUCAGU  |
| hsa-miR-215      | AUGACCUAUGAAUUGACAGAC    |
| hsa-miR-376a-3p  | AUCAUAGAGGAAAAUCCAGCU    |
| hsa-miR-320a     | AAAAGCUGGGUUGAGAGGGCGA   |
| hsa-miR-22-5p    | AGUUCUUCAGUGGCAAGCUUUA   |
| hsa-miR-338-3p   | UCCAGCAUCAGUAAUUUGUUUG   |
| hsa-miR-2110     | UUGGGAAACUGCCGUGAGUG     |
| hsa-miR-223-3p   | UGUCAGUUUGUCAAAUACCCCA   |
| hsa-miR-361-3p   | UCCCCAGGUGUGAUUCUGAUUU   |
| hsa-miR-103a-3p  | AGCAGCAUUGUACAGGGCUAUGA  |
| hsa-miR-93-3p    | ACUGCUGAGGAAUCCGUAUCCG   |
| hsa-miR-301a-3p  | CAGUGCAAUAGUAUUGUCAAAAGC |
| hsa-miR-140-5p   | CAGUGUUUUACCCUAGGUAG     |
| hsa-miR-151a-5p  | UCGAGGAGCUCACAGUCUAGU    |
| hsa-miR-130b-3p  | CAGUGCAAUGAUGAAAGGGCAU   |
| hsa-miR-122-5p   | UGGAGUGUGACAUUGGUGUUUG   |
| hsa-miR-20a-3p   | ACUGCAUUUAGAGCACUUAAAG   |
| hsa-miR-423-5p   | UGAGGGGCGAGAGCGAGACUUU   |
| hsa-miR-629-5p   | UGGGUUUACGUUGGGAGAACU    |
| hsa-miR-101-3p   | UACAGUACUGUAUACUGAA      |
| hsa-miR-200c-3p  | UAAUACUGCCGGUAAUGAUGGA   |
| hsa-miR-365a-3p  | UAAUGCCCUAAAAUCCUUUAU    |
| hsa-miR-501-3p   | AAUGCACCCGGGCAAGGAUUUCU  |
| hsa-miR-23a-3p   | AUCACAUUGCCAGGGAUUCC     |
| hsa-miR-423-3p   | AGCUCGGUCUGAGGCCCCUCAGU  |
| hsa-miR-215      | AUGACCUAUGAAUUGACAGAC    |
| hsa-miR-376a-3p  | AUCAUAGAGGAAAAUCCAGCU    |
| hsa-miR-320a     | AAAAGCUGGGUUGAGAGGGCGA   |
| hsa-miR-22-5p    | AGUUCUUCAGUGGCAAGCUUUA   |
| hsa-miR-338-3p   | UCCAGCAUCAGUAAUUUGUUUG   |
| hsa-miR-2110     | UUGGGAAACUGCCGUGAGUG     |
| hsa-miR-223-3p   | UGUCAGUUUGUCAAAUACCCCA   |
| hsa-miR-361-3p   | UCCCCAGGUGUGAUUCUGAUUU   |
| hsa-miR-103a-3p  | AGCAGCAUUGUACAGGGCUAUGA  |
| hsa-miR-93-3p    | ACUGCUGAGGAAUCCGUAUCCG   |
| hsa-miR-331-3p   | GCCCCUGGGCUAUCCUAGAA     |
| hsa-miR-144-5p   | GGUAUAUCAUAUACUGUAAG     |
| hsa-miR-142-3p   | UGUAGUUGUUCCUACUUUAUGGA  |
| hsa-miR-210      | CUGUGCGUUGAGACAGCGGUGA   |
| hsa-let-7d-3p    | CUAUACGACCUGCUGCCUUUCU   |
| hsa-miR-199a-5p  | CCCAGUGUUCAGACUACCCUUCU  |
| hsa-miR-605      | UAAAUCCUAGGGUCCUUCUCCU   |
| hsa-miR-766-3p   | ACUCCAGCCCAAGCUCAGC      |
| hsa-miR-19a-3p   | UGUGCAAUCUUAUGCAAACUGA   |
| hsa-miR-584-5p   | UUUAGUUUGCCUGGGCUGAG     |
| hsa-miR-144-3p   | UACAGUAUAGUAUGUAUGU      |
| hsa-miR-92a-3p   | UAAUAGCUCUUGCCGGCCUCU    |
| hsa-miR-126-3p   | UCGUACCGUGAGUAUAAUGCG    |

(Continued)



**Table S2** - Continued.

| miR name (human) | microRNA target sequence |
|------------------|--------------------------|
| hsa-miR-363-3p   | AAUUGCACGGUAUCCAUCUGUA   |
| hsa-miR-148a-3p  | UCAGUGCACUACAGAACUUUGU   |
| hsa-miR-374a-5p  | UUUAUAUACAACCUGAUAGUG    |
| hsa-miR-10b-5p   | UACCCUGUAGAACCGAAUUUGU   |
| hsa-miR-190a     | UGAUAGUUUGAUUAUUUAGGU    |
| hsa-miR-195-5p   | UAGCAGCACAGAAUAUUGGC     |
| hsa-miR-29a-5p   | ACUGAUUUUUUGGUGUUCAG     |
| hsa-miR-125b-5p  | UCCUGAGACCCUAACUUGUGA    |
| hsa-miR-18a-3p   | ACUGCCUAAGUGCUCCUUCUGG   |
| hsa-miR-192-5p   | CUGACCUAUGAAUUGACAGCC    |
| hsa-miR-151a-3p  | CUAGACUGAAGCUCCUUGAGG    |
| hsa-miR-331-3p   | GCCCCUGGGCCUAUCCUAGAA    |
| hsa-miR-144-5p   | GGUAUCAUCAUAUACUGUAAG    |
| hsa-miR-142-3p   | UGUAGUGUUUCCUACUUUUGGA   |
| hsa-miR-210      | CUGUGCGUGACAGCGGCUGA     |
| hsa-let-7d-3p    | CUAUACGACCCUGCCUUUCU     |
| hsa-miR-199a-5p  | CCCAGUGUUCAGACUACCUUUC   |
| hsa-miR-605      | UAAAUCCCAUGGGUCCUUCUCCU  |
| hsa-miR-766-3p   | ACUCCAGCCCCACAGCCUCAGC   |
| hsa-miR-19a-3p   | UGUGCAAUUCUAUGCAAAACUGA  |
| hsa-miR-584-5p   | UUUUGUUUGCCUGGGACUGAG    |
| hsa-miR-144-3p   | UACAGUAUAGAUAGUAGUACU    |
| hsa-miR-92a-3p   | UAUUGCACUUGUCCCGGCCUGU   |
| hsa-miR-126-3p   | UCGUACCGUGAGUAAUAAUGCG   |
| hsa-miR-363-3p   | AAUUGCACGGUAUCCAUCUGUA   |
| hsa-miR-148a-3p  | UCAGUGCACUACAGAACUUUGU   |
| hsa-miR-374a-5p  | UUUAUAUACAACCUGAUAGUG    |
| hsa-miR-10b-5p   | UACCCUGUAGAACCGAAUUUGU   |
| hsa-miR-190a     | UGAUAGUUUGAUUAUUUAGGU    |
| hsa-miR-195-5p   | UAGCAGCACAGAAUAUUGGC     |
| hsa-miR-29a-5p   | ACUGAUUUUUUGGUGUUCAG     |
| hsa-miR-125b-5p  | UCCUGAGACCCUAACUUGUGA    |
| hsa-miR-18a-3p   | ACUGCCUAAGUGCUCCUUCUGG   |
| hsa-miR-192-5p   | CUGACCUAUGAAUUGACAGCC    |
| hsa-miR-151a-3p  | CUAGACUGAAGCUCCUUGAGG    |
| hsa-miR-18b-5p   | UAAGGUGCAUCUAGUGCAGUUAG  |
| hsa-miR-28-3p    | CACUAGAUUGUGAGCUCCUGGA   |
| hsa-miR-335-5p   | UCAAGAGCAAUAACGAAAAAUGU  |
| hsa-miR-324-3p   | ACUGCCCCAGGUGCUGCUGG     |
| hsa-miR-204-5p   | UUCCUUUGUCAUCCUUAUGCCU   |
| hsa-miR-182-5p   | UUUGGCAAUGGUAGAUCACACU   |
| hsa-let-7g-5p    | UGAGGUAGUAGUUUGUACAGUU   |
| hsa-miR-15b-5p   | UAGCAGCACAUCAUGUUUACA    |
| hsa-miR-22-3p    | AAGCUGCCAGUUGAAGAACUGU   |
| hsa-miR-106b-3p  | CCGCACUGUGGGUACUUGCUGC   |
| hsa-miR-199a-3p  | ACAGUAGUCUGCACAUUGGUUA   |
| hsa-miR-29c-3p   | UAGCACC AUUUGAAUUCGGUUA  |
| hsa-miR-19b-3p   | UGUGCAAUCCAUGCAAAACUGA   |
| hsa-miR-95       | UUCAACGGGUUUUUUUGAGCA    |
| hsa-miR-29a-3p   | UAGCACC AUUCUGAAUUCGGUUA |
| hsa-miR-21-5p    | UAGCUUAUCAGACUGAUUGUA    |
| hsa-miR-150-5p   | UCUCCCAACCCUUGUACCAGUG   |
| hsa-miR-30e-3p   | CUUUCAGUCGGAUGUUUACAGC   |
| hsa-miR-30b-5p   | UGUAAACAUCUACACUCAGCU    |
| hsa-miR-543      | AAACAUCGCGGUGCACUUCU     |
| hsa-miR-24-3p    | UGGCUCAGUUCAGCAGGAACAG   |
| hsa-miR-29b-2-5p | CUGGUUUCACAUGGUGC UUAG   |
| hsa-miR-495-3p   | AAACAACAUGGUGCACUUCU     |
| hsa-miR-18b-5p   | UAAGGUGCAUCUAGUGCAGUUAG  |
| hsa-miR-28-3p    | CACUAGAUUGUGAGCUCCUGGA   |
| hsa-miR-335-5p   | UCAAGAGCAAUAACGAAAAAUGU  |
| hsa-miR-324-3p   | ACUGCCCCAGGUGCUGCUGG     |
| hsa-miR-204-5p   | UUCCUUUGUCAUCCUUAUGCCU   |
| hsa-miR-182-5p   | UUUGGCAAUGGUAGAUCACACU   |
| hsa-let-7g-5p    | UGAGGUAGUAGUUUGUACAGUU   |
| hsa-miR-15b-5p   | UAGCAGCACAUCAUGUUUACA    |
| hsa-miR-22-3p    | AAGCUGCCAGUUGAAGAACUGU   |
| hsa-miR-106b-3p  | CCGCACUGUGGGUACUUGCUGC   |
| hsa-miR-199a-3p  | ACAGUAGUCUGCACAUUGGUUA   |
| hsa-miR-29c-3p   | UAGCACC AUUUGAAUUCGGUUA  |
| hsa-miR-19b-3p   | UGUGCAAUCCAUGCAAAACUGA   |
| hsa-miR-95       | UUCAACGGGUUUUUUUGAGCA    |
| hsa-miR-29a-3p   | UAGCACC AUUCUGAAUUCGGUUA |
| hsa-miR-21-5p    | UAGCUUAUCAGACUGAUUGUA    |
| hsa-miR-150-5p   | UCUCCCAACCCUUGUACCAGUG   |
| hsa-miR-30e-3p   | CUUUCAGUCGGAUGUUUACAGC   |
| hsa-miR-30b-5p   | UGUAAACAUCUACACUCAGCU    |
| hsa-miR-543      | AAACAUCGCGGUGCACUUCU     |
| hsa-miR-24-3p    | UGGCUCAGUUCAGCAGGAACAG   |
| hsa-miR-29b-2-5p | CUGGUUUCACAUGGUGC UUAG   |
| hsa-miR-495-3p   | AAACAACAUGGUGCACUUCU     |
| hsa-miR-18b-5p   | UAAGGUGCAUCUAGUGCAGUUAG  |
| hsa-miR-28-3p    | CACUAGAUUGUGAGCUCCUGGA   |
| hsa-miR-335-5p   | UCAAGAGCAAUAACGAAAAAUGU  |
| hsa-miR-324-3p   | ACUGCCCCAGGUGCUGCUGG     |
| hsa-miR-204-5p   | UUCCUUUGUCAUCCUUAUGCCU   |
| hsa-miR-182-5p   | UUUGGCAAUGGUAGAUCACACU   |
| hsa-let-7g-5p    | UGAGGUAGUAGUUUGUACAGUU   |
| hsa-miR-15b-5p   | UAGCAGCACAUCAUGUUUACA    |
| hsa-miR-22-3p    | AAGCUGCCAGUUGAAGAACUGU   |
| hsa-miR-106b-3p  | CCGCACUGUGGGUACUUGCUGC   |
| hsa-miR-199a-3p  | ACAGUAGUCUGCACAUUGGUUA   |
| hsa-miR-29c-3p   | UAGCACC AUUUGAAUUCGGUUA  |

(Continued)

**Table S2** - Continued.

| miR name (human) | microRNA target sequence |
|------------------|--------------------------|
| hsa-miR-19b-3p   | UGUGCAAUCCAUGCAAAACUGA   |
| hsa-miR-95       | UUCAACGGGUUUUUUUGAGCA    |
| hsa-miR-29a-3p   | UAGCACC AUUCUGAAUUCGGUUA |
| hsa-miR-21-5p    | UAGCUUAUCAGACUGAUUGUA    |
| hsa-miR-150-5p   | UCUCCCAACCCUUGUACCAGUG   |
| hsa-miR-30e-3p   | CUUUCAGUCGGAUGUUUACAGC   |
| hsa-miR-30b-5p   | UGUAAACAUCUACACUCAGCU    |
| hsa-miR-543      | AAACAUCGCGGUGCACUUCU     |
| hsa-miR-24-3p    | UGGCUCAGUUCAGCAGGAACAG   |
| hsa-miR-29b-2-5p | CUGGUUUCACAUGGUGC UUAG   |
| hsa-miR-495-3p   | AAACAACAUGGUGCACUUCU     |

**Table S3** - Ten significant upregulated miRNAs in patients with EH and T2DM.

| miRNA ID         | ED/N  | p-value |
|------------------|-------|---------|
| hsa-miR-409-3p   | 2.391 | 0.327   |
| hsa-miR-200c-3p  | 2.231 | 0.236   |
| hsa-miR-33a-5p   | 2.153 | 0.161   |
| hsa-miR-605      | 2.082 | 0.258   |
| hsa-miR-543      | 1.986 | 0.318   |
| hsa-miR-485-3p   | 1.912 | 0.215   |
| hsa-miR-1        | 1.902 | 0.299   |
| hsa-miR-497-5p   | 1.895 | 0.137   |
| hsa-miR-10a-5p   | 1.884 | 0.031   |
| hsa-miR-29b-2-5p | 1.867 | 0.436   |

miR, microRNA; EH, essential hypertension; T2DM, type 2 diabetes mellitus; ED, essential hypertension with type 2 diabetes mellitus; N, healthy controls.

**Table S4** - Ten significant downregulated miRNAs in patients with EH and T2DM.

| miRNA ID        | ED/N  | p-value |
|-----------------|-------|---------|
| hsa-miR-195-5p  | 0.319 | 0.003   |
| hsa-miR-130a-5p | 0.353 | 0.001   |
| hsa-miR-197-5p  | 0.372 | 0.309   |
| hsa-miR-27a-5p  | 0.383 | 0.004   |
| hsa-miR-374a-5p | 0.389 | 0.031   |
| hsa-miR-204-5p  | 0.404 | 0.108   |
| hsa-miR-551b-3p | 0.405 | 0.112   |
| hsa-miR-99a-5p  | 0.416 | 0.301   |
| hsa-miR-20b-5p  | 0.442 | 0.011   |
| hsa-miR-885-5p  | 0.454 | 0.243   |

miR, microRNA; EH, essential hypertension; T2DM, type 2 diabetes mellitus; ED, essential hypertension with type 2 diabetes mellitus; N, healthy controls.

**Table S5** - SNP in the miR-195-5p-binding region of DRD1 3'-UTR.

| Base change | Position | Allele frequency |            |
|-------------|----------|------------------|------------|
|             |          | Control (n=100)  | ED (n=100) |
| T/A         | 231      | 0.93/0.07        | 0.61/0.39  |
| C/G         | 233      | 0.91/0.09        | 0.46/0.54  |

SNPs, single nucleotide polymorphisms; 3'-UTR, 3' untranslated region; miR, microRNA; ED, essential hypertension with type 2 diabetes mellitus.



**Table S6** - Relative miRNA expression levels.

| Name            | N (healthy donor group) |          |          |          |          | E (essential hypertension group) |          |          |          |          |
|-----------------|-------------------------|----------|----------|----------|----------|----------------------------------|----------|----------|----------|----------|
|                 | N-1                     | N-2      | N-3      | N-4      | N-5      | E-1                              | E-2      | E-3      | E-4      | E-5      |
| hsa-miR-652-3p  | 27.4994                 | 27.5214  | 26.23896 | 27.14213 | 24.93589 | 24.32263                         | 26.86717 | 25.86409 | 26.73846 | 27.80176 |
| hsa-miR-502-3p  | 30.2259                 | 30.10943 | 29.7773  | 30.27932 | 28.40177 | 30.21685                         | 30.85675 | 30.01515 | 29.86859 | 31.08951 |
| hsa-miR-181a-5p | 29.54401                | 29.57566 | 29.74752 | 28.77795 | 27.38079 | 25.18815                         | 28.21215 | 30.17283 | 28.61724 | 29.11461 |
| hsa-miR-339-3p  | 31.36388                | 32.40051 | 30.55619 | 31.90127 | 30.49482 | 28.37407                         | 31.48838 | 30.51381 | 30.85313 | 31.85437 |
| hsa-miR-221-3p  | 26.5359                 | 28.22711 | 25.10158 | 25.71106 | 25.07761 | 21.84271                         | 24.72138 | 24.28112 | 26.37955 | 26.3767  |
| hsa-miR-409-3p  | 31.96837                | 38.06064 | 33.62046 | 32.75995 | 32.62264 | 27.68179                         | 32.03365 | 31.9195  | 32.74971 | 35.14991 |
| hsa-let-7f-5p   | 29.54303                | 29.93954 | 28.70305 | 29.26361 | 27.42158 | 25.53563                         | 27.97526 | 27.91023 | 29.57095 | 29.82001 |
| hsa-miR-154-5p  | 34.32638                | 36.63269 | 35.10727 | 35.29539 | 34.30911 | 37.98412                         | 37.05836 | 36.92326 | 33.8621  | 36.93676 |
| hsa-miR-27b-3p  | 28.00136                | 29.86563 | 26.67595 | 27.87492 | 25.19875 | 23.6541                          | 26.27471 | 26.41524 | 27.72376 | 28.4739  |
| hsa-miR-155-5p  | 33.69112                | 33.86313 | 31.35646 | 32.51689 | 31.94231 | 29.92997                         | 32.84324 | 31.56445 | 31.83759 | 33.48145 |
| hsa-miR-374b-5p | 30.44037                | 30.39161 | 28.17487 | 29.30584 | 27.41831 | 26.5144                          | 28.78996 | 28.74502 | 29.58283 | 30.49701 |
| hsa-miR-140-3p  | 26.59032                | 26.13342 | 25.87901 | 26.76519 | 24.22189 | 26.73265                         | 27.04536 | 25.98797 | 26.03578 | 27.16548 |
| hsa-miR-93-5p   | 24.46973                | 23.68834 | 23.50438 | 24.14214 | 21.15665 | 22.87703                         | 25.23616 | 23.3616  | 22.91347 | 25.20253 |
| hsa-miR-92b-3p  | 33.44775                | 34.48301 | 33.60299 | 33.9209  | 33.747   | 33.03883                         | 33.51449 | 34.24391 | 34.80063 | 38.24825 |
| hsa-miR-200a-3p | 35.81265                | 38.98334 | 33.88347 | 34.43107 | 33.55525 | 33.82429                         | 34.22566 | 35.04956 | 34.51918 | 38.24019 |
| hsa-miR-505-3p  | 31.6744                 | 31.90579 | 30.44626 | 31.41989 | 28.23391 | 29.68319                         | 30.87034 | 30.72041 | 32.1862  | 31.49747 |
| hsa-miR-23b-3p  | 27.61481                | 29.69939 | 26.08749 | 26.75518 | 24.41731 | 23.32742                         | 26.15857 | 25.65728 | 27.48725 | 27.53347 |
| hsa-miR-484     | 26.49639                | 25.92376 | 25.3414  | 26.28128 | 24.53492 | 24.33617                         | 26.44572 | 24.85307 | 25.60827 | 26.54247 |
| hsa-miR-141-3p  | 35.24291                | 34.65108 | 32.7037  | 33.28511 | 31.5345  | 31.35641                         | 32.457   | 33.04189 | 34.0213  | 37.80652 |
| UniSp3 IPC      | 20.83079                | 21.03874 | 20.97121 | 20.86166 | 21.05546 | 21.12578                         | 21.0701  | 21.48895 | 21.81917 | 21.03903 |
| hsa-miR-10a-5p  | 37.82522                | 38.1038  | 35.64824 | 37.69761 | 35.18068 | 33.68993                         | 35.55281 | 35.26032 | 37.86531 | 36.75699 |
| hsa-miR-106a-5p | 23.88025                | 23.17466 | 22.8301  | 23.46748 | 21.1071  | 21.88073                         | 24.02368 | 22.61029 | 22.34813 | 24.51563 |
| hsa-miR-27a-3p  | 31.58713                | 32.98496 | 30.04809 | 30.9465  | 28.51177 | 27.05272                         | 29.31883 | 32.19897 | 32.12651 | 31.7395  |
| hsa-miR-145-5p  | 28.08021                | 30.09938 | 27.49263 | 27.98185 | 25.90704 | 26.24076                         | 27.82468 | 28.6746  | 29.511   | 29.21755 |
| hsa-miR-1       | 33.44626                | 38.73879 | 35.06868 | 36.06837 | 35.488   | 32.42819                         | 34.57653 | 35.34016 | 34.9604  | 37.65683 |
| hsa-miR-185-5p  | 26.74238                | 25.62303 | 26.13874 | 27.10768 | 27.1559  | 25.58529                         | 27.46045 | 27.38226 | 26.56527 | 27.78893 |
| hsa-miR-382-5p  | 33.20625                | 36.29013 | 34.48356 | 32.96202 | 35.24107 | 29.57438                         | 32.53288 | 32.76921 | 33.75302 | 35.46727 |
| hsa-miR-486-5p  | 21.8098                 | 21.41052 | 28.07665 | 22.217   | 20.28876 | 22.90534                         | 23.14312 | 21.79153 | 21.52337 | 22.69885 |
| hsa-miR-32-5p   | 30.03758                | 29.06372 | 28.96416 | 29.30087 | 27.07852 | 28.30837                         | 29.17505 | 28.73644 | 29.27338 | 29.78134 |
| hsa-miR-26a-5p  | 27.37512                | 28.07665 | 25.75058 | 26.57973 | 24.09008 | 23.22924                         | 25.82531 | 25.87128 | 26.90529 | 27.33678 |
| hsa-miR-133b    | 31.78462                | 35.69064 | 32.35449 | 34.1036  | 32.78946 | 30.92826                         | 33.01174 | 31.86402 | 33.24655 | 33.6403  |
| hsa-miR-143-3p  | 30.68927                | 32.58359 | 30.24534 | 31.91434 | 27.04269 | 29.36839                         | 31.41239 | 30.34436 | 31.00669 | 32.02716 |
| hsa-let-7d-5p   | 29.06523                | 28.92637 | 27.73608 | 28.1984  | 26.71953 | 24.7453                          | 27.53663 | 27.4304  | 28.91053 | 29.06821 |
| hsa-miR-30a-5p  | 32.54282                | 33.56037 | 31.08787 | 31.929   | 29.76558 | 30.63907                         | 31.43457 | 30.14122 | 32.97329 | 32.44356 |
| hsa-miR-133a    | 31.7527                 | 36.39534 | 31.89617 | 33.0822  | 31.62113 | 30.89086                         | 32.51809 | 32.61858 | 33.02648 | 32.43336 |
| hsa-miR-222-3p  | 27.68221                | 27.50594 | 26.40857 | 27.68184 | 24.89188 | 25.32533                         | 27.14656 | 25.12012 | 26.07643 | 27.65367 |
| hsa-miR-20b-5p  | 31.66225                | 30.92777 | 31.70095 | 31.58779 | 29.40001 | 32.7573                          | 32.53058 | 31.27701 | 30.40097 | 32.25677 |
| hsa-miR-342-3p  | 29.89237                | 29.63649 | 26.8432  | 28.16519 | 26.61326 | 26.41042                         | 29.11737 | 26.63868 | 27.28963 | 29.24671 |
| UniSp3 IPC      | 20.12914                | 20.28845 | 20.25332 | 20.26003 | 20.20704 | 20.44624                         | 20.62674 | 20.66848 | 20.94368 | 20.34843 |
| hsa-miR-328     | 30.53617                | 31.93652 | 29.36688 | 30.37124 | 29.14912 | 27.0294                          | 29.92333 | 28.56399 | 30.14203 | 32.02518 |
| hsa-miR-324-5p  | 31.54158                | 30.34379 | 29.76351 | 30.50898 | 28.56602 | 27.89933                         | 30.71882 | 29.8955  | 29.66767 | 31.92703 |
| hsa-miR-532-3p  | 30.91136                | 30.6021  | 29.60564 | 30.05227 | 28.0432  | 29.54509                         | 30.75792 | 29.9071  | 30.25387 | 30.56456 |
| UniSp3 IPC      | 21.0251                 | 21.1843  | 21.0683  | 21.02428 | 20.86283 | 21.27783                         | 21.2916  | 21.61786 | 21.82626 | 20.9291  |
| hsa-miR-15a-5p  | 25.1894                 | 24.59376 | 24.23149 | 24.98174 | 22.69894 | 24.10404                         | 24.88989 | 24.65292 | 24.35845 | 25.23549 |
| hsa-let-7e-5p   | 30.35847                | 32.25116 | 29.30433 | 29.61547 | 28.48113 | 25.80256                         | 28.39368 | 29.44182 | 30.68124 | 30.53901 |
| hsa-miR-532-5p  | 30.70271                | 30.20063 | 29.86886 | 30.05227 | 28.0432  | 29.54509                         | 30.75792 | 29.9071  | 30.25387 | 30.56456 |
| hsa-miR-139-5p  | 30.95367                | 32.44723 | 28.65154 | 29.53386 | 29.18265 | 27.16527                         | 29.13087 | 28.40357 | 30.41887 | 30.6824  |
| hsa-miR-194-5p  | 31.02555                | 29.47414 | 29.51723 | 30.14846 | 24.50118 | 30.29932                         | 30.30292 | 29.48233 | 30.43364 | 30.43364 |
| hsa-miR-375     | 32.59305                | 34.16055 | 32.32737 | 32.53798 | 29.59822 | 32.27096                         | 31.01291 | 31.55889 | 35.12284 | 32.78225 |

(Continued)



Table S6 - Continued.

| Name            | N (healthy donor group) |          |          |          |          | E (essential hypertension group) |           |          |          |          |
|-----------------|-------------------------|----------|----------|----------|----------|----------------------------------|-----------|----------|----------|----------|
|                 | N-1                     | N-2      | N-3      | N-4      | N-5      | E-1                              | E-2       | E-3      | E-4      | E-5      |
| hsa-miR-660-5p  | 28.21718                | 27.24476 | 26.78669 | 28.15467 | 25.0884  | 27.9019                          | 27.86754  | 26.45333 | 26.72004 | 28.1124  |
| hsa-miR-451a    | 18.48677                | 17.46424 | 17.48924 | 18.30064 | 15.18831 | 19.38609                         | 18.59371  | 17.71829 | 17.13805 | 18.65624 |
| hsa-miR-574-3p  | 30.30313                | 32.10363 | 29.35911 | 30.5051  | 27.7014  | 27.84048                         | 29.86473  | 28.73419 | 30.71109 | 30.9359  |
| hsa-let-7a-5p   | 25.83652                | 25.76985 | 24.54204 | 24.96715 | 23.49537 | 22.12276                         | 24.56216  | 23.9065  | 25.5391  | 25.50868 |
| hsa-miR-551b-3p | 34.82532                | 35.38679 | 33.09311 | 34.55676 | 31.66249 | 29.36407                         | 32.92147  | 31.11393 | 33.36134 | 30.4085  |
| hsa-miR-128     | 28.83977                | 28.75929 | 28.05337 | 28.3804  | 26.66791 | 26.35832                         | 28.94085  | 27.55607 | 28.27582 | 29.61382 |
| hsa-miR-130a-3p | 29.41045                | 29.12493 | 28.49799 | 29.20877 | 26.51403 | 26.27691                         | 28.55954  | 27.63877 | 28.80502 | 27.51954 |
| hsa-miR-125a-5p | 29.5871                 | 32.28732 | 29.0844  | 28.54903 | 27.80309 | 25.65482                         | 28.55138  | 28.81979 | 30.28619 | 29.83327 |
| hsa-miR-28-5p   | 31.96777                | 33.49418 | 29.86786 | 31.09059 | 29.33175 | 30.81703                         | 30.2412   | 29.72644 | 31.34855 | 31.63658 |
| hsa-miR-485-3p  | 36.83944                | 36.15921 | 34.96649 | 32.98107 | 34.30843 | 29.79036                         | 34.21365  | 33.45903 | 35.47761 | 36.79858 |
| hsa-miR-497-5p  | 33.79824                | 35.26284 | 31.51067 | 34.26224 | 31.63743 | 31.15742                         | 32.94233  | 31.74152 | 35.81569 | 33.64935 |
| hsa-let-7b-3p   | 31.96007                | 33.13511 | 31.27911 | 30.86678 | 30.59552 | 30.5542                          | 31.48372  | 29.57355 | 33.34782 | 33.48306 |
| hsa-miR-425-3p  | 31.23219                | 31.62689 | 30.10234 | 31.40576 | 28.13178 | 27.86525                         | 31.29121  | 31.0194  | 31.09642 | 32.55162 |
| hsa-miR-132-3p  | 31.86506                | 32.28435 | 31.21421 | 31.55189 | 28.77818 | 29.83697                         | 31.49297  | 29.85232 | 31.03606 | 31.87405 |
| hsa-miR-25-3p   | 23.75698                | 22.97704 | 23.20976 | 23.96692 | 21.38269 | 23.6522                          | 24.84752  | 23.01605 | 22.41704 | 24.52008 |
| hsa-let-7c      | 31.46542                | 31.38333 | 30.07255 | 30.23474 | 28.09053 | 27.28307                         | 29.82328  | 30.31844 | 31.88651 | 31.42961 |
| UniSp3 IPC      | 21.06835                | 21.14273 | 21.1418  | 20.90977 | 21.33057 | 21.23945                         | 21.29383  | 21.45312 | 21.79418 | 20.99621 |
| hsa-miR-18a-5p  | 28.97923                | 27.89222 | 27.63545 | 28.0697  | 25.98354 | 25.69059                         | 28.33493  | 26.8864  | 27.09946 | 28.60085 |
| hsa-miR-33a-5p  | 32.97207                | 36.15842 | 31.90488 | 32.62524 | 31.75563 | 28.8476                          | 31.71632  | 30.68616 | 33.47268 | 33.15192 |
| hsa-miR-29b-3p  | 30.20344                | 29.24642 | 29.19008 | 29.84276 | 27.14238 | 28.68591                         | 29.76318  | 29.53538 | 28.57231 | 30.54786 |
| hsa-miR-16-5p   | 20.1034                 | 19.65565 | 19.08557 | 19.97363 | 17.79215 | 20.0053                          | 20.33983  | 18.8456  | 18.72069 | 20.58713 |
| hsa-miR-136-5p  | 36.34523                | 39.34459 | 35.4565  | 35.38076 | 34.4506  | 29.69584                         | 36.30419  | 33.92336 | 34.35291 | 35.63195 |
| hsa-let-7b-5p   | 25.25075                | 24.60535 | 24.59361 | 24.91743 | 22.61386 | 24.04738                         | 25.98504  | 25.20323 | 25.04149 | 26.07109 |
| hsa-let-7i-3p   | 33.99617                | 34.63456 | 33.09503 | 36.62936 | 31.78859 | 31.46923                         | 33.33028  | 32.4646  | 35.60875 | 33.30779 |
| hsa-miR-152     | 30.22637                | 30.69527 | 29.26405 | 30.62641 | 27.58311 | 27.17961                         | 29.78639  | 28.64776 | 30.2661  | 31.12018 |
| hsa-miR-106b-5p | 26.21872                | 25.4469  | 25.4626  | 25.73227 | 23.33224 | 24.99771                         | 26.6251   | 26.2454  | 26.54981 | 26.7477  |
| hsa-miR-30c-5p  | 28.37185                | 28.64766 | 26.76322 | 27.31799 | 25.45133 | 24.66385                         | 26.98539  | 26.10352 | 28.28804 | 28.33102 |
| hsa-miR-15b-3p  | 27.83004                | 26.95826 | 27.13908 | 27.51223 | 24.99949 | 26.54021                         | 27.89947  | 26.49076 | 26.03033 | 27.69174 |
| hsa-miR-197-3p  | 30.33917                | 32.13727 | 28.78687 | 29.41822 | 27.26965 | 27.06521                         | 29.50554  | 28.41073 | 30.59773 | 30.4379  |
| hsa-miR-142-5p  | 29.96804                | 30.20316 | 28.15688 | 29.1513  | 26.17647 | 26.65147                         | 28.54387  | 28.14291 | 29.29087 | 29.89351 |
| hsa-miR-99b-5p  | 30.7004                 | 32.89823 | 30.56781 | 30.1983  | 29.4475  | 26.78618                         | 30.05549  | 29.86919 | 32.12754 | 31.33938 |
| hsa-miR-30e-5p  | 26.61948                | 26.57397 | 31.03687 | 31.72988 | 30.3647  | 33.78673                         | 34.98366  | 30.78675 | 32.39336 | 33.70561 |
| hsa-miR-326     | 32.81226                | 34.08975 | 30.67696 | 26.79168 | 23.89947 | 24.92905                         | 26.77394  | 26.01474 | 26.1682  | 26.9855  |
| hsa-miR-146a-5p | 28.85095                | 30.44415 | 26.69238 | 31.02576 | 29.99435 | 27.49887                         | 31.10153  | 30.67121 | 30.85221 | 32.48916 |
| UniSp3 IPC      | 20.38931                | 20.55089 | 20.50438 | 28.24585 | 26.97342 | 24.42608                         | 27.65545  | 26.59086 | 28.15089 | 28.85262 |
| hsa-miR-421     | 34.28639                | 33.37249 | 32.75791 | 32.75096 | 30.73877 | 30.26873                         | 32.85915  | 31.63228 | 32.05    | 33.19094 |
| hsa-miR-424-5p  | 31.76299                | 32.27545 | 32.62981 | 32.28143 | 27.88008 | 30.86078                         | 31.74688  | 30.94585 | 32.34484 | 32.25546 |
| hsa-miR-223-5p  | 35.32374                | 34.57774 | 32.54866 | 33.78231 | 29.17605 | 31.431                           | 33.64744  | 31.06877 | 32.93565 | 35.26397 |
| hsa-miR-146b-5p | 33.0107                 | 33.87771 | 31.15066 | 33.73035 | 29.9455  | 29.67134                         | 32.23698  | 32.94191 | 32.63085 | 33.10917 |
| hsa-miR-107     | 29.08695                | 28.74306 | 27.24541 | 28.19932 | 25.43937 | 25.09633                         | 27.76247  | 28.23588 | 29.725   | 28.43518 |
| hsa-miR-205-5p  | 34.7469                 | 34.37409 | 34.29313 | 33.98984 | 32.98815 | 34.0923                          | 33.43979  | 32.87469 | 39.40604 | 33.72156 |
| hsa-miR-148b-3p | 27.66843                | 27.3128  | 26.59612 | 27.73573 | 24.60881 | 24.83809                         | 27.61614  | 25.7985  | 25.96808 | 28.16562 |
| hsa-miR-339-5p  | 32.09259                | 33.79491 | 29.99133 | 31.09841 | 30.92345 | 30.523405                        | 30.523405 | 29.97265 | 31.91004 | 32.08718 |
| hsa-miR-20a-5p  | 23.63481                | 22.95393 | 22.79511 | 23.21    | 21.07748 | 22.22794                         | 23.96483  | 22.8081  | 22.38545 | 24.31785 |
| hsa-miR-17-5p   | 30.44374                | 30.12178 | 29.82937 | 30.58703 | 27.80768 | 28.49124                         | 30.77487  | 29.61528 | 29.70941 | 31.20881 |
| hsa-miR-500a-5p | 31.999                  | 30.67757 | 30.88746 | 31.49914 | 29.23199 | 31.6266                          | 33.08257  | 32.71986 | 32.19905 | 33.15011 |
| hsa-miR-30d-5p  | 31.03976                | 31.03976 | 29.43331 | 30.35775 | 28.53174 | 28.02511                         | 30.7582   | 30.16572 | 31.38217 | 31.54103 |
| hsa-miR-378a-3p | 29.07881                | 28.89016 | 28.21669 | 29.24694 | 26.09562 | 28.50094                         | 29.65974  | 27.90733 | 28.29309 | 30.21653 |

(Continued)



Table S6 - Continued.

| Name            | N (healthy donor group) |          |          |          |          | E (essential hypertension group) |          |          |          |          |
|-----------------|-------------------------|----------|----------|----------|----------|----------------------------------|----------|----------|----------|----------|
|                 | N-1                     | N-2      | N-3      | N-4      | N-5      | E-1                              | E-2      | E-3      | E-4      | E-5      |
| hsa-miR-186-5p  | 28.21332                | 27.62693 | 27.5058  | 28.20357 | 25.33418 | 26.22033                         | 28.52195 | 27.08626 | 26.83163 | 28.70276 |
| hsa-miR-425-5p  | 25.98755                | 25.59435 | 25.24202 | 25.83141 | 23.63913 | 25.03722                         | 26.57786 | 25.13631 | 24.97655 | 26.44872 |
| hsa-let-7i-5p   | 27.35768                | 26.86716 | 26.38713 | 26.99316 | 25.01061 | 24.64456                         | 27.10329 | 26.92386 | 26.5123  | 28.05332 |
| hsa-miR-346     | 32.97495                | 35.94007 | 32.91123 | 33.93928 | 33.70149 | 33.73249                         | 34.42959 | 31.81371 | 35.00037 | 32.57941 |
| hsa-miR-26b-5p  | 29.32872                | 28.88938 | 28.11414 | 28.89153 | 25.82461 | 26.31993                         | 28.90173 | 28.05593 | 28.39873 | 26.93873 |
| hsa-miR-193b-3p | 31.56719                | 33.07303 | 31.28595 | 31.72584 | 27.5653  | 30.43094                         | 32.66721 | 31.42588 | 32.07764 | 33.36612 |
| hsa-miR-34a-5p  | 34.06146                | 34.57602 | 31.89062 | 33.01111 | 30.35692 | 30.62814                         | 32.94653 | 30.25604 | 33.65652 | 33.69281 |
| UmiSp3 IPC      | 21.00049                | 21.11922 | 21.10762 | 20.8879  | 21.05193 | 21.21672                         | 21.28537 | 21.6184  | 21.82974 | 21.0888  |
| hsa-miR-320b    | 26.95977                | 26.87001 | 26.15631 | 27.15481 | 24.50027 | 25.43245                         | 26.90346 | 25.60264 | 26.31562 | 27.59269 |
| hsa-miR-885-5p  | 32.69805                | 34.5323  | 32.2734  | 32.13612 | 27.95339 | 32.89874                         | 30.99129 | 30.95694 | 36.17082 | 33.80361 |
| hsa-miR-590-5p  | 29.62653                | 29.52869 | 29.13459 | 29.516   | 26.54636 | 27.76521                         | 29.66522 | 27.94672 | 28.42055 | 29.16378 |
| hsa-miR-127-3p  | 34.42763                | 36.93448 | 37.8028  | 33.25026 | 32.91518 | 33.22889                         | 34.65968 | 33.17507 | 33.67817 | 34.15639 |
| hsa-miR-191-5p  | 27.59792                | 28.21453 | 26.0598  | 26.92878 | 24.94451 | 24.05631                         | 26.86379 | 26.57628 | 27.53786 | 28.23863 |
| hsa-miR-99a-5p  | 31.96146                | 33.06466 | 30.19206 | 31.46272 | 24.67068 | 29.64922                         | 30.6739  | 30.32564 | 32.95655 | 31.29417 |
| hsa-miR-16-2-3p | 29.94031                | 30.1285  | 30.28909 | 30.17707 | 27.17864 | 30.52811                         | 30.67544 | 31.19754 | 31.83175 | 30.63953 |
| hsa-miR-301a-3p | 29.45958                | 30.21708 | 28.82285 | 29.59778 | 27.17686 | 26.68168                         | 29.09125 | 28.04389 | 29.35576 | 30.19157 |
| hsa-miR-140-5p  | 30.0195                 | 30.21419 | 28.93958 | 30.18019 | 26.78533 | 27.18782                         | 30.00977 | 28.51517 | 29.56227 | 30.45989 |
| hsa-miR-151a-5p | 27.17035                | 27.18094 | 25.86517 | 26.77729 | 25.14858 | 23.28316                         | 25.94074 | 25.71939 | 27.03396 | 27.39928 |
| hsa-miR-130b-3p | 32.49076                | 32.67072 | 31.471   | 32.37576 | 30.18452 | 31.91692                         | 31.91692 | 32.12663 | 32.69338 | 33.37513 |
| hsa-miR-122-5p  | 29.75502                | 29.78442 | 27.10293 | 28.70412 | 27.79973 | 26.71362                         | 27.54651 | 27.40428 | 31.30482 | 29.43769 |
| hsa-miR-20a-3p  | 35.50993                | 35.13137 | 32.36831 | 34.00836 | 34.36166 | 30.70102                         | 33.55255 | 33.10722 | 32.23634 | 34.29068 |
| hsa-miR-423-5p  | 26.64392                | 26.57219 | 25.78012 | 26.30782 | 24.83073 | 25.32058                         | 27.01967 | 25.38796 | 25.46094 | 26.92381 |
| hsa-miR-629-5p  | 31.44825                | 31.57955 | 29.95617 | 33.78228 | 29.47496 | 30.71213                         | 31.26098 | 32.63531 | 30.89545 | 32.56001 |
| hsa-miR-101-3p  | 26.97421                | 25.40171 | 25.69379 | 26.37957 | 23.5324  | 24.87663                         | 26.45146 | 25.30316 | 24.63843 | 26.72278 |
| hsa-miR-200c-3p | 34.8623                 | 35.25546 | 33.21792 | 33.02517 | 32.7479  | 29.54656                         | 33.14934 | 35.73673 | 33.75283 | 39.08882 |
| hsa-miR-365a-3p | 32.67135                | 31.8509  | 30.73993 | 32.28486 | 27.3191  | 31.28847                         | 32.62153 | 31.06773 | 31.54612 | 32.00368 |
| hsa-miR-501-3p  | 33.77105                | 32.76588 | 31.79238 | 32.82643 | 31.66015 | 32.8028                          | 33.24609 | 32.67595 | 32.4588  | 32.96821 |
| hsa-miR-23a-3p  | 25.4937                 | 27.17482 | 23.99858 | 25.04817 | 22.82148 | 21.65174                         | 24.54852 | 23.74756 | 25.3092  | 25.56107 |
| hsa-miR-423-3p  | 28.77637                | 28.97673 | 27.11412 | 27.66028 | 26.82952 | 24.90534                         | 27.61923 | 26.91311 | 27.2387  | 28.70288 |
| hsa-miR-215     | 29.44474                | 28.17284 | 28.5431  | 29.54204 | 26.42514 | 29.8439                          | 29.61881 | 28.34815 | 28.29208 | 29.50648 |
| hsa-miR-376a-3p | 36.51092                | 39.01264 | 37.24707 | 35.98175 | 36.59857 | 31.8899                          | 36.9487  | 37.93353 | 38.25774 | 37.89752 |
| hsa-miR-320a    | 27.8443                 | 27.48381 | 26.75114 | 28.01059 | 25.2477  | 26.17948                         | 27.93927 | 27.73173 | 27.9886  | 28.41023 |
| hsa-miR-22-5p   | 32.62365                | 32.34061 | 30.23085 | 32.1987  | 29.67163 | 29.16887                         | 31.50285 | 30.91357 | 31.94666 | 32.54826 |
| hsa-miR-338-3p  | 32.58246                | 32.50407 | 29.90822 | 32.3019  | 27.34408 | 28.10741                         | 31.23338 | 29.76016 | 30.00632 | 31.44597 |
| hsa-miR-2110    | 32.4483                 | 32.1643  | 31.93545 | 32.0053  | 30.66987 | 30.71891                         | 33.22841 | 30.92765 | 31.85031 | 32.51696 |
| hsa-miR-223-3p  | 23.38143                | 24.41653 | 21.55545 | 22.68441 | 18.57904 | 19.74873                         | 22.42085 | 21.36249 | 22.00883 | 23.00233 |
| hsa-miR-361-3p  | 36.01968                | 35.69118 | 34.00154 | 34.39228 | 32.52074 | 32.03904                         | 34.96273 | 35.28903 | 37.48752 | 36.02328 |
| hsa-miR-103a-3p | 26.93517                | 26.89095 | 25.66184 | 26.22617 | 24.55992 | 23.05624                         | 25.88631 | 26.38374 | 26.87309 | 27.19262 |
| hsa-miR-93-3p   | 29.8402                 | 29.72053 | 29.00847 | 29.2478  | 27.57036 | 28.35633                         | 30.50112 | 29.17769 | 28.99668 | 30.3149  |
| hsa-miR-331-3p  | 31.33761                | 31.85614 | 30.39974 | 31.22844 | 29.41814 | 27.70556                         | 30.7745  | 30.38876 | 31.70765 | 31.1916  |
| hsa-miR-144-5p  | 30.5463                 | 28.30142 | 28.60622 | 29.28318 | 27.17848 | 30.53761                         | 30.42263 | 29.09061 | 29.87156 | 29.14756 |
| hsa-miR-142-3p  | 26.91883                | 27.58633 | 25.21892 | 26.30003 | 24.71534 | 23.27428                         | 24.90311 | 25.22482 | 27.20145 | 26.85488 |
| hsa-miR-210     | 30.82277                | 29.96738 | 29.21919 | 30.14465 | 28.89626 | 29.28686                         | 29.89688 | 28.71085 | 28.81251 | 30.61754 |
| hsa-let-7d-3p   | 27.39229                | 28.91246 | 26.92497 | 27.34841 | 25.89912 | 24.91726                         | 27.33879 | 26.2551  | 28.1826  | 28.46649 |
| hsa-miR-199a-5p | 30.74037                | 32.36673 | 28.48457 | 29.20542 | 28.98444 | 25.85809                         | 28.16338 | 29.25032 | 30.84515 | 30.17563 |
| hsa-miR-605     | 38.89225                | 38.70512 | 38.32448 | 37.98623 | 36.87572 | 34.23734                         | 34.17001 | 32.3357  | 38.80757 | 38.11424 |
| hsa-miR-766-3p  | 30.69187                | 32.88527 | 29.49879 | 30.04135 | 30.12567 | 29.89146                         | 29.9058  | 29.03381 | 31.81182 | 31.29208 |
| hsa-miR-19a-3p  | 23.4563                 | 22.99221 | 22.72103 | 23.34391 | 21.25695 | 22.69854                         | 23.66016 | 22.38645 | 22.38587 | 24.07519 |
| hsa-miR-584-5p  | 32.79792                | 34.59336 | 32.19546 | 32.4045  | 33.72847 | 30.19091                         | 32.71464 | 32.9657  | 33.51759 | 34.21453 |

(Continued)



Table S6 - Continued.

| Name             | N (healthy donor group) |          |          |          |          | E (essential hypertension group) |          |          |          |          |
|------------------|-------------------------|----------|----------|----------|----------|----------------------------------|----------|----------|----------|----------|
|                  | N-1                     | N-2      | N-3      | N-4      | N-5      | E-1                              | E-2      | E-3      | E-4      | E-5      |
| hsa-miR-144-3p   | 26.69103                | 25.947   | 25.61288 | 26.22725 | 24.15369 | 27.59957                         | 26.74652 | 26.32333 | 25.81713 | 26.99054 |
| hsa-miR-92a-3p   | 22.52407                | 22.46799 | 22.06299 | 22.27347 | 20.78026 | 22.3568                          | 23.22431 | 21.53899 | 21.73056 | 23.15031 |
| hsa-miR-126-3p   | 25.55144                | 26.61297 | 24.1063  | 25.10461 | 23.72582 | 21.56704                         | 24.56669 | 23.62752 | 24.78778 | 25.95621 |
| hsa-miR-363-3p   | 28.56565                | 27.33586 | 27.26963 | 27.90775 | 25.09547 | 28.43548                         | 28.62302 | 27.64384 | 27.18851 | 28.75017 |
| hsa-miR-148a-3p  | 29.12604                | 29.94817 | 28.70185 | 29.29599 | 25.24286 | 27.40551                         | 28.23301 | 28.72833 | 29.0815  | 29.67859 |
| hsa-miR-374a-5p  | 32.40572                | 32.36877 | 31.07847 | 31.19238 | 29.85797 | 29.19226                         | 33.34733 | 33.34733 | 32.35363 | 32.97143 |
| hsa-miR-10b-5p   | 33.67074                | 33.98075 | 32.04171 | 32.04171 | 30.7248  | 33.58717                         | 32.11369 | 30.90057 | 33.38485 | 32.66679 |
| hsa-miR-190a     | 36.72818                | 35.44731 | 34.60999 | 35.72492 | 34.44106 | 36.20168                         | 38.00856 | 38.32327 | 37.00179 | 36.15786 |
| hsa-miR-195-5p   | 36.76088                | 37.41334 | 35.18873 | 35.27494 | 33.91811 | 33.00945                         | 34.59364 | 34.93774 | 36.36157 | 36.00436 |
| hsa-miR-29a-5p   | 34.49987                | 35.70321 | 32.62996 | 35.66669 | 31.3966  | 33.3523                          | 33.67048 | 31.63377 | 33.19236 | 35.6004  |
| hsa-miR-125b-5p  | 29.24719                | 30.91387 | 28.41159 | 29.92798 | 24.82638 | 28.13419                         | 29.30256 | 27.91069 | 29.80998 | 29.88473 |
| hsa-miR-18a-3p   | 32.28524                | 31.58624 | 31.78024 | 31.89217 | 30.31439 | 31.42086                         | 33.18858 | 31.88136 | 32.14621 | 33.06258 |
| hsa-miR-192-5p   | 29.07099                | 27.54689 | 27.89643 | 28.37286 | 23.74936 | 28.63328                         | 28.94552 | 27.89466 | 27.20621 | 29.42422 |
| hsa-miR-151a-3p  | 31.51415                | 32.30343 | 30.65316 | 31.9832  | 30.03455 | 27.4612                          | 30.95442 | 31.12581 | 32.31164 | 32.39593 |
| hsa-miR-18b-5p   | 28.99933                | 27.94659 | 27.48807 | 28.08904 | 26.35813 | 26.48362                         | 28.52447 | 27.22943 | 27.34803 | 29.13809 |
| hsa-miR-28-3p    | 25.39149                | 32.65604 | 29.45499 | 30.75947 | 28.58719 | 27.13393                         | 29.81975 | 28.47832 | 31.05596 | 31.50272 |
| hsa-miR-335-5p   | 32.17131                | 33.31935 | 30.9563  | 31.99035 | 30.49982 | 28.57853                         | 31.68173 | 29.35351 | 31.32729 | 32.61351 |
| hsa-miR-324-3p   | 28.5075                 | 27.65919 | 27.27883 | 27.91063 | 25.89974 | 26.96228                         | 28.57415 | 27.8928  | 27.26611 | 28.83177 |
| hsa-miR-204-5p   | 32.6358                 | 34.69013 | 32.43379 | 31.41585 | 29.61652 | 32.08724                         | 32.84146 | 30.96939 | 34.26277 | 34.95685 |
| hsa-miR-182-5p   | 37.10955                | 34.85246 | 36.65845 | 37.84937 | 33.86834 | 35.53553                         | 39.14747 | 37.98629 | 37.98236 | 36.9383  |
| hsa-let-7g-5p    | 26.46112                | 25.72285 | 25.48306 | 25.61098 | 23.52012 | 23.27145                         | 25.88853 | 24.8771  | 24.92423 | 26.6523  |
| hsa-miR-15b-5p   | 27.54903                | 26.97654 | 26.12947 | 26.97556 | 24.46608 | 24.49806                         | 27.23634 | 26.48847 | 26.40215 | 27.87269 |
| hsa-miR-22-3p    | 26.27411                | 25.47344 | 24.95457 | 26.14259 | 23.49456 | 24.17081                         | 25.70176 | 24.98878 | 25.41715 | 26.65558 |
| hsa-miR-106b-3p  | 32.81747                | 32.63624 | 31.18234 | 31.35515 | 30.10227 | 28.63553                         | 31.69542 | 31.26469 | 31.61597 | 33.11651 |
| hsa-miR-199a-3p  | 28.62543                | 31.2943  | 27.35934 | 28.50399 | 27.16084 | 24.56451                         | 27.31032 | 27.04197 | 28.37663 | 29.06122 |
| hsa-miR-29c-3p   | 28.07731                | 27.83083 | 27.13386 | 28.44283 | 25.37944 | 26.94735                         | 27.86223 | 26.73084 | 26.99192 | 29.3093  |
| hsa-miR-19b-3p   | 22.80819                | 22.26149 | 21.96556 | 22.62489 | 20.70661 | 22.05826                         | 22.90886 | 22.18024 | 22.0219  | 23.46206 |
| hsa-miR-95       | 35.35854                | 36.1527  | 36.6823  | 38.38045 | 33.2107  | 35.79063                         | 34.90127 | 35.84692 | 36.81309 | 35.6254  |
| hsa-miR-29a-3p   | 29.70453                | 29.75754 | 27.37718 | 28.99457 | 25.45903 | 26.8369                          | 28.18458 | 27.70327 | 28.52023 | 29.59363 |
| hsa-miR-21-5p    | 25.14946                | 25.01872 | 23.84129 | 24.94272 | 23.11337 | 22.66476                         | 23.74803 | 23.78481 | 25.19405 | 25.52586 |
| hsa-miR-150-5p   | 26.68782                | 26.66686 | 24.1158  | 24.84476 | 23.71416 | 25.22556                         | 26.58527 | 24.3579  | 23.57379 | 26.28103 |
| hsa-miR-30e-3p   | 33.98795                | 34.48735 | 31.56219 | 33.11497 | 29.97708 | 29.58775                         | 32.30341 | 32.67778 | 33.89531 | 34.38569 |
| hsa-miR-30b-5p   | 27.96956                | 27.70412 | 26.51385 | 26.71822 | 24.37031 | 24.55574                         | 26.99615 | 26.62218 | 27.21026 | 28.1143  |
| hsa-miR-543      | 33.3403                 | 38.75872 | 35.35503 | 33.76499 | 34.31726 | 29.09432                         | 33.78001 | 32.57128 | 35.80063 | 37.94935 |
| hsa-miR-24-3p    | 25.31418                | 26.14055 | 23.99253 | 25.09914 | 22.29473 | 21.4738                          | 23.97835 | 23.0359  | 23.95369 | 25.2284  |
| hsa-miR-29b-2-5p | 33.70686                | 32.81276 | 32.68963 | 33.68156 | 31.17325 | 31.31618                         | 33.12174 | 31.14022 | 33.00141 | 33.61847 |
| hsa-miR-495-3p   | 34.7331                 | 35.84284 | 34.12085 | 32.59601 | 33.4417  | 28.66639                         | 32.39975 | 32.26211 | 35.36662 | 34.84154 |
| UniSp3 IPC       | 20.74053                | 20.88739 | 20.8411  | 20.73717 | 20.82012 | 20.95567                         | 21.05521 | 21.24502 | 21.54098 | 20.82124 |

(Columns Continued)



Table S6 - Columns Continued.

| Name            | D (type 2 diabetes mellitus group) |          |          |          |          | ED (E with D group) |          |          |          |          |
|-----------------|------------------------------------|----------|----------|----------|----------|---------------------|----------|----------|----------|----------|
|                 | D-1                                | D-2      | D-3      | D-4      | D-5      | ED-1                | ED-2     | ED-3     | ED-4     | ED-5     |
| hsa-miR-652-3p  | 27.12081                           | 27.30996 | 28.1683  | 26.22699 | 27.48216 | 26.81101            | 27.64371 | 28.03684 | 26.98334 | 25.27692 |
| hsa-miR-502-3p  | 30.62679                           | 30.40215 | 29.83363 | 30.81268 | 30.43764 | 29.50065            | 30.59434 | 30.37207 | 29.79478 | 28.5667  |
| hsa-miR-181a-5p | 28.17641                           | 28.47416 | 30.57065 | 27.47061 | 28.01435 | 29.15709            | 29.64834 | 29.88802 | 27.78866 | 27.23969 |
| hsa-miR-339-3p  | 30.27144                           | 31.43263 | 31.95248 | 30.29258 | 31.25646 | 31.55722            | 31.85877 | 31.3485  | 31.84089 | 29.59727 |
| hsa-miR-221-3p  | 25.53376                           | 26.59865 | 27.59387 | 24.44748 | 27.46118 | 25.97825            | 26.8859  | 26.76488 | 25.83128 | 24.66771 |
| hsa-miR-409-3p  | 30.86067                           | 30.08239 | 35.37194 | 29.96329 | 30.93717 | 33.50995            | 33.67864 | 31.52285 | 32.90079 | 30.58131 |
| hsa-let-7f-5p   | 28.51415                           | 29.4214  | 30.92477 | 27.65901 | 29.85581 | 29.6569             | 30.46705 | 29.90785 | 29.76985 | 29.69881 |
| hsa-miR-154-5p  | 33.4407                            | 32.09384 | 35.31864 | 32.00723 | 31.52567 | 35.91077            | 35.04369 | 35.08587 | 35.91708 | 32.61428 |
| hsa-miR-27b-3p  | 27.2078                            | 27.61225 | 28.96364 | 25.70977 | 28.93992 | 27.64356            | 28.91744 | 28.32699 | 28.06383 | 26.05521 |
| hsa-miR-155-5p  | 32.18735                           | 32.78399 | 34.12407 | 31.67273 | 31.68852 | 33.5486             | 35.02567 | 33.00285 | 33.54777 | 33.24107 |
| hsa-miR-374b-5p | 28.70949                           | 30.28804 | 31.29231 | 27.99418 | 24.11983 | 29.90086            | 30.33332 | 30.5164  | 29.81221 | 28.0003  |
| hsa-miR-140-3p  | 26.44001                           | 26.21607 | 26.11365 | 26.98335 | 26.79785 | 25.30769            | 26.18356 | 26.60085 | 26.50047 | 25.08133 |
| hsa-miR-93-5p   | 23.86945                           | 23.80836 | 24.2531  | 24.35304 | 24.11983 | 23.57152            | 24.46046 | 24.82886 | 23.94189 | 22.47515 |
| hsa-miR-92b-3p  | 33.98491                           | 34.25697 | 34.2988  | 33.27515 | 35.95933 | 33.64039            | 35.21371 | 35.0135  | 32.8157  | 32.58928 |
| hsa-miR-200a-3p | 35.71024                           | 33.16651 | 35.51819 | 35.63606 | 33.54199 | 33.23368            | 34.69623 | 35.67212 | 34.91472 | 34.75449 |
| hsa-miR-505-3p  | 30.64051                           | 29.96584 | 31.90407 | 30.08533 | 30.9188  | 31.36671            | 32.1033  | 31.57569 | 32.28345 | 30.08773 |
| hsa-miR-23b-3p  | 26.50705                           | 27.11682 | 28.67356 | 25.53107 | 27.39611 | 27.20536            | 27.6649  | 26.35073 | 26.9346  | 25.24746 |
| hsa-miR-484     | 25.83553                           | 25.49558 | 25.75777 | 25.99555 | 25.99618 | 25.21359            | 25.95281 | 26.35073 | 25.89331 | 24.86213 |
| hsa-miR-141-3p  | 34.66799                           | 35.03629 | 34.25009 | 33.83165 | 33.8719  | 33.08614            | 33.38719 | 32.91007 | 34.46541 | 33.08076 |
| UniSp3 IPC      | 20.92505                           | 20.89359 | 20.90363 | 20.89149 | 21.67327 | 20.9502             | 21.01185 | 21.01761 | 20.91675 | 21.25114 |
| hsa-miR-10a-5p  | 34.3638                            | 36.46663 | 37.19475 | 36.22292 | 36.27803 | 35.83612            | 36.47287 | 36.37754 | 35.891   | 34.71648 |
| hsa-miR-106a-5p | 23.35664                           | 23.31617 | 23.92934 | 23.79013 | 23.79013 | 23.34193            | 24.20332 | 24.36314 | 23.68401 | 21.9606  |
| hsa-miR-27a-3p  | 30.82221                           | 30.66539 | 32.22275 | 28.26848 | 33.39303 | 31.07388            | 32.79878 | 31.52774 | 30.97642 | 29.82726 |
| hsa-miR-145-5p  | 27.45531                           | 27.64322 | 28.39865 | 26.49436 | 29.06153 | 27.93881            | 29.10808 | 29.10705 | 28.70984 | 27.24894 |
| hsa-miR-1       | 36.06906                           | 36.75673 | 38.1973  | 31.90985 | 36.37341 | 34.17597            | 34.81035 | 35.6044  | 34.57869 | 33.7767  |
| hsa-miR-185-5p  | 26.52845                           | 26.33804 | 26.70703 | 26.89922 | 27.79907 | 25.58553            | 26.66526 | 27.28644 | 26.23623 | 25.36239 |
| hsa-miR-382-5p  | 33.21151                           | 33.82644 | 32.32356 | 31.87567 | 31.01063 | 35.51333            | 34.95464 | 33.65596 | 32.94765 | 31.60677 |
| hsa-miR-486-5p  | 21.54243                           | 21.33224 | 21.81741 | 22.88875 | 22.58685 | 20.79542            | 21.97221 | 22.33524 | 21.70398 | 21.22345 |
| hsa-miR-32-5p   | 30.05366                           | 29.15879 | 29.46026 | 29.71922 | 30.83629 | 28.40371            | 28.51788 | 29.66493 | 28.68057 | 27.59264 |
| hsa-miR-26a-5p  | 26.14462                           | 26.82854 | 28.22435 | 25.18349 | 27.47953 | 27.33305            | 28.31028 | 27.85763 | 26.9539  | 25.18696 |
| hsa-miR-133b    | 33.99249                           | 33.56842 | 33.57713 | 29.91051 | 32.3575  | 31.7153             | 32.10364 | 34.17896 | 32.23587 | 31.50515 |
| hsa-miR-143-3p  | 30.76045                           | 31.57299 | 30.97994 | 29.58453 | 30.28759 | 31.27313            | 30.58678 | 31.81768 | 30.40353 | 29.31771 |
| hsa-let-7d-5p   | 27.8516                            | 28.7179  | 29.93562 | 27.04076 | 29.24649 | 28.34693            | 29.86373 | 29.17829 | 28.4986  | 26.76672 |
| hsa-miR-30a-5p  | 31.27983                           | 30.61799 | 31.8724  | 31.7149  | 33.75945 | 31.44139            | 32.69341 | 33.06936 | 31.44052 | 30.96382 |
| hsa-miR-133a    | 33.38371                           | 32.45379 | 33.2342  | 29.35147 | 26.77897 | 26.69998            | 27.5412  | 27.27179 | 32.41451 | 26.14155 |
| hsa-miR-222-3p  | 27.05235                           | 26.88651 | 27.5361  | 26.86411 | 26.77897 | 26.69998            | 27.5412  | 27.27179 | 32.41451 | 26.14155 |
| hsa-miR-20b-5p  | 31.60289                           | 31.78093 | 31.64437 | 32.26431 | 32.50222 | 31.55463            | 32.50263 | 33.67727 | 32.75087 | 30.18692 |
| hsa-miR-342-3p  | 26.34892                           | 27.30435 | 28.63808 | 28.63236 | 26.91957 | 28.65408            | 28.63852 | 29.14133 | 28.36472 | 27.23791 |
| UniSp3 IPC      | 20.3503                            | 20.40245 | 20.26865 | 20.23343 | 20.82561 | 20.32219            | 20.29822 | 20.49263 | 20.19533 | 20.57718 |
| hsa-miR-328     | 30.14473                           | 30.4854  | 31.83453 | 29.59551 | 30.59017 | 30.17805            | 30.57367 | 31.58248 | 30.78035 | 28.99282 |
| hsa-miR-324-5p  | 29.93047                           | 31.16385 | 31.79148 | 29.73488 | 30.60059 | 30.20649            | 31.07916 | 31.87018 | 31.50987 | 28.61568 |
| hsa-miR-532-3p  | 30.18206                           | 29.8714  | 30.09981 | 30.43989 | 30.44165 | 29.77946            | 30.44059 | 30.64341 | 30.70388 | 29.36934 |
| UniSp3 IPC      | 21.15723                           | 21.0395  | 21.05303 | 20.89177 | 21.81205 | 20.95441            | 21.12836 | 21.18615 | 20.96256 | 21.22254 |
| hsa-miR-15a-5p  | 25.27914                           | 24.78283 | 24.33446 | 25.27877 | 26.16628 | 23.69828            | 24.94342 | 24.75539 | 24.79979 | 23.30074 |
| hsa-let-7e-5p   | 29.31629                           | 30.52857 | 30.8044  | 28.96209 | 29.85656 | 29.73802            | 31.08312 | 31.19833 | 30.18432 | 29.34503 |
| hsa-miR-532-5p  | 30.33393                           | 30.31314 | 30.50165 | 30.68427 | 30.90021 | 29.70996            | 30.64305 | 30.41774 | 30.09644 | 28.65405 |
| hsa-miR-139-5p  | 29.50002                           | 30.37273 | 31.34886 | 28.45693 | 31.17327 | 30.64484            | 31.9709  | 30.05839 | 30.3386  | 28.89988 |
| hsa-miR-194-5p  | 29.30925                           | 28.66019 | 30.25448 | 30.45884 | 30.9222  | 29.63083            | 30.49415 | 30.20627 | 30.51679 | 28.80742 |
| hsa-miR-375     | 30.71932                           | 29.54231 | 31.60568 | 32.19861 | 34.78196 | 29.98617            | 30.91087 | 33.37708 | 33.07339 | 33.10255 |

(Continued)





Table S6 - Continued.

| Name            | D (type 2 diabetes mellitus group) |          |          |          |          | ED (E with D group) |          |          |          |          |
|-----------------|------------------------------------|----------|----------|----------|----------|---------------------|----------|----------|----------|----------|
|                 | D-1                                | D-2      | D-3      | D-4      | D-5      | ED-1                | ED-2     | ED-3     | ED-4     | ED-5     |
| hsa-miR-660-5p  | 27.85169                           | 27.53834 | 27.36163 | 28.22911 | 27.51835 | 26.94085            | 27.83518 | 27.5827  | 27.68299 | 26.18647 |
| hsa-miR-451a    | 17.92601                           | 17.51848 | 17.51177 | 18.96806 | 18.70719 | 17.20353            | 18.13001 | 18.42848 | 18.02586 | 16.67711 |
| hsa-miR-574-3p  | 29.85131                           | 29.04526 | 31.34966 | 30.52229 | 30.58157 | 30.15318            | 30.58788 | 31.8007  | 30.62631 | 29.70512 |
| hsa-let-7a-5p   | 24.68851                           | 25.44837 | 26.52234 | 24.11973 | 25.39686 | 25.30265            | 26.06368 | 25.82883 | 25.32109 | 23.35952 |
| hsa-miR-551b-3p | 34.31084                           | 33.78183 | 33.93639 | 32.27083 | 34.45362 | 33.64428            | 33.83847 | 35.76249 | 36.14222 | 36.16309 |
| hsa-miR-128     | 28.58958                           | 28.47557 | 28.81084 | 28.43447 | 29.3721  | 27.84185            | 28.55545 | 29.25512 | 28.09641 | 26.98679 |
| hsa-miR-130a-3p | 29.7015                            | 29.23774 | 29.61988 | 28.65895 | 27.52462 | 28.50284            | 28.64103 | 29.78223 | 27.90754 | 26.8956  |
| hsa-miR-125a-5p | 29.29193                           | 29.48373 | 31.12437 | 28.97035 | 28.83739 | 29.04146            | 30.14285 | 30.51418 | 29.57005 | 28.46987 |
| hsa-miR-28-5p   | 30.87022                           | 31.75866 | 34.60873 | 29.79343 | 30.81774 | 32.03813            | 33.71215 | 32.33774 | 31.80047 | 29.1291  |
| hsa-miR-485-3p  | 33.22994                           | 36.1912  | 35.80698 | 31.94852 | 33.53072 | 34.01696            | 34.85069 | 34.27777 | 34.66191 | 32.28302 |
| hsa-miR-497-5p  | 32.99963                           | 31.92582 | 32.22111 | 32.61117 | 34.64199 | 32.18248            | 32.53742 | 32.81676 | 33.24314 | 30.59141 |
| hsa-let-7b-3p   | 31.9468                            | 30.50366 | 31.9083  | 31.72993 | 31.6154  | 30.76108            | 31.2113  | 31.40703 | 31.21127 | 30.38604 |
| hsa-miR-425-3p  | 30.39668                           | 31.30321 | 30.87244 | 29.71292 | 31.52193 | 30.23074            | 31.73812 | 31.80791 | 30.75182 | 29.72478 |
| hsa-miR-132-3p  | 31.33688                           | 31.22271 | 32.07756 | 31.1991  | 30.75614 | 30.46222            | 31.99404 | 31.51785 | 30.9658  | 30.46886 |
| hsa-miR-25-3p   | 23.70584                           | 23.45254 | 23.71441 | 24.33735 | 23.36906 | 22.60993            | 23.76549 | 23.95233 | 23.68305 | 22.08658 |
| hsa-let-7c      | 30.07534                           | 30.49305 | 32.35527 | 29.02953 | 32.19058 | 30.83719            | 32.37699 | 31.34012 | 30.39614 | 29.62025 |
| UmiSp3 IPC      | 21.19244                           | 21.06163 | 21.10684 | 21.06488 | 21.89592 | 21.19666            | 21.21782 | 21.2475  | 21.01051 | 21.26308 |
| hsa-miR-18a-5p  | 27.78891                           | 28.31283 | 28.84043 | 28.44304 | 28.03572 | 28.04255            | 28.77613 | 29.06246 | 27.78856 | 26.34025 |
| hsa-miR-33a-5p  | 33.268                             | 32.73966 | 33.05554 | 31.2481  | 35.57744 | 32.0154             | 32.57646 | 32.57681 | 31.88531 | 30.34244 |
| hsa-miR-29b-3p  | 29.55242                           | 29.07911 | 29.94887 | 29.13524 | 30.25496 | 28.90917            | 29.73547 | 30.34319 | 29.52467 | 27.66038 |
| hsa-miR-16-5p   | 19.92686                           | 19.68035 | 19.40052 | 20.81589 | 19.73464 | 18.9951             | 19.633   | 19.90282 | 19.83699 | 18.36417 |
| hsa-miR-136-3p  | 32.9208                            | 34.28623 | 39.74125 | 31.41521 | 32.79159 | 33.35181            | 34.01268 | 34.19381 | 34.33761 | 30.15464 |
| hsa-let-7b-5p   | 24.87489                           | 24.6023  | 25.83438 | 25.34926 | 25.61287 | 24.89552            | 25.77196 | 26.39036 | 24.9645  | 23.96882 |
| hsa-let-7i-3p   | 33.41705                           | 34.45986 | 34.53857 | 32.9489  | 36.07883 | 33.14796            | 34.50572 | 34.83747 | 33.59931 | 32.26686 |
| hsa-miR-152     | 29.73712                           | 29.95367 | 30.33964 | 29.21488 | 30.46405 | 29.73417            | 30.98352 | 30.53451 | 30.52707 | 29.08578 |
| hsa-miR-106b-5p | 25.99509                           | 25.53234 | 26.3705  | 26.50316 | 27.26551 | 25.58824            | 26.77437 | 26.62033 | 25.74315 | 25.05995 |
| hsa-miR-30c-5p  | 26.78388                           | 27.60242 | 28.70506 | 26.25275 | 27.88244 | 27.27392            | 27.80614 | 27.83106 | 27.39672 | 25.74457 |
| hsa-miR-15b-3p  | 27.63359                           | 27.46765 | 26.98485 | 27.8718  | 27.43242 | 26.2113             | 26.87783 | 27.5419  | 27.15345 | 25.63427 |
| hsa-miR-197-3p  | 29.29697                           | 29.51136 | 31.8563  | 28.34583 | 29.34933 | 29.21643            | 29.82783 | 30.19058 | 29.60906 | 28.00565 |
| hsa-miR-142-5p  | 29.03771                           | 29.51081 | 30.37655 | 28.00583 | 29.95366 | 28.78869            | 29.22298 | 29.82101 | 28.56376 | 27.30625 |
| hsa-miR-99b-5p  | 30.5717                            | 31.09753 | 31.50773 | 30.33964 | 30.55146 | 30.24098            | 30.97712 | 31.96217 | 30.83422 | 30.1991  |
| hsa-miR-296-5p  | 32.91222                           | 31.30762 | 31.65395 | 32.18618 | 32.62407 | 31.28663            | 31.11049 | 32.37693 | 31.37152 | 29.50946 |
| hsa-miR-30e-5p  | 26.7092                            | 26.396   | 26.53903 | 26.51903 | 27.27281 | 25.74514            | 26.62144 | 26.72083 | 26.75272 | 25.33763 |
| hsa-miR-326     | 30.84852                           | 31.9943  | 34.36186 | 30.20116 | 32.96125 | 27.40442            | 31.69891 | 33.60544 | 31.06181 | 29.38402 |
| hsa-miR-146a-5p | 26.75819                           | 28.46425 | 29.9837  | 26.50301 | 28.96867 | 27.87789            | 28.8551  | 28.99847 | 27.61624 | 26.53414 |
| UmiSp3 IPC      | 20.56365                           | 20.57537 | 20.45766 | 20.55502 | 21.0418  | 20.56197            | 20.44547 | 20.56457 | 20.37643 | 20.61126 |
| hsa-miR-421     | 32.31892                           | 32.78222 | 33.62451 | 31.94183 | 33.04474 | 32.08919            | 33.46824 | 33.80203 | 33.1664  | 31.27593 |
| hsa-miR-424-5p  | 31.8635                            | 31.1219  | 31.4844  | 31.41708 | 32.66533 | 32.18581            | 32.18581 | 31.91502 | 30.87414 | 30.09237 |
| hsa-miR-223-5p  | 33.99865                           | 33.27614 | 34.06653 | 32.02735 | 31.70437 | 32.66264            | 34.20536 | 33.90226 | 33.72893 | 32.94328 |
| hsa-miR-146b-5p | 30.22475                           | 32.92095 | 33.21209 | 33.21209 | 32.67757 | 33.01573            | 34.34956 | 34.03154 | 32.02308 | 31.73014 |
| hsa-miR-107     | 28.02919                           | 28.30266 | 29.45064 | 27.03219 | 31.06113 | 28.16983            | 29.38462 | 29.13248 | 28.10419 | 27.29096 |
| hsa-miR-205-5p  | 34.36265                           | 32.58087 | 33.58212 | 34.79503 | 37.6055  | 34.76176            | 34.69407 | 35.40566 | 32.14544 | 32.68945 |
| hsa-miR-148b-3p | 27.32233                           | 27.4031  | 27.63031 | 26.89429 | 27.40427 | 26.6445             | 27.49121 | 27.81897 | 27.24875 | 25.39712 |
| hsa-miR-339-5p  | 30.61545                           | 31.40527 | 33.11576 | 29.74201 | 31.8802  | 31.8997             | 32.3461  | 31.86757 | 31.74854 | 29.57191 |
| hsa-miR-20a-5p  | 23.47965                           | 22.96101 | 23.63756 | 23.88515 | 23.82668 | 22.81731            | 23.99888 | 24.0398  | 23.96101 | 22.02838 |
| hsa-miR-17-5p   | 30.3924                            | 29.99724 | 30.53359 | 30.37871 | 31.89964 | 29.72308            | 31.61963 | 31.74181 | 30.64851 | 29.13463 |
| hsa-miR-500a-5p | 31.63047                           | 32.10685 | 34.39571 | 32.72407 | 32.00803 | 32.27414            | 32.68087 | 33.42032 | 32.25312 | 30.67178 |
| hsa-miR-30d-5p  | 30.01038                           | 30.52382 | 30.82912 | 29.72977 | 31.51934 | 30.15021            | 30.50127 | 31.09861 | 30.12171 | 29.55901 |
| hsa-miR-378a-3p | 28.9845                            | 27.958   | 28.6598  | 28.66897 | 28.90623 | 28.44772            | 29.20752 | 29.41204 | 28.83443 | 28.09078 |

(Continued)



Table S6 - Continued.

| Name            | D (type 2 diabetes mellitus group) |          |          |          |          | ED (E with D group) |          |          |          |          |
|-----------------|------------------------------------|----------|----------|----------|----------|---------------------|----------|----------|----------|----------|
|                 | D-1                                | D-2      | D-3      | D-4      | D-5      | ED-1                | ED-2     | ED-3     | ED-4     | ED-5     |
| hsa-miR-186-5p  | 27.38388                           | 27.83187 | 28.04749 | 27.34616 | 27.33942 | 26.97155            | 27.9403  | 28.42387 | 28.46801 | 26.25981 |
| hsa-miR-425-5p  | 25.63775                           | 25.41631 | 25.76954 | 26.08943 | 25.77297 | 25.20617            | 25.98444 | 26.03029 | 26.34618 | 24.23706 |
| hsa-let-7i-5p   | 27.00024                           | 27.49013 | 27.70028 | 26.17175 | 28.51793 | 26.46903            | 27.9125  | 27.71668 | 27.7384  | 25.89654 |
| hsa-miR-346     | 33.29454                           | 33.96266 | 34.68408 | 36.64476 | 33.94651 | 34.34383            | 34.75624 | 33.18891 | 32.75082 | 33.88271 |
| hsa-miR-26b-5p  | 27.92162                           | 28.08031 | 29.82573 | 28.01764 | 29.51037 | 30.11016            | 30.11016 | 29.79684 | 27.32803 | 27.32803 |
| hsa-miR-193b-3p | 29.32218                           | 27.17879 | 31.51554 | 31.47475 | 32.66997 | 31.63136            | 32.2887  | 31.69425 | 28.71753 | 29.08636 |
| hsa-miR-34a-5p  | 31.99438                           | 30.33749 | 32.48767 | 32.2882  | 33.98626 | 32.14907            | 32.38044 | 32.59329 | 32.02737 | 31.32315 |
| UniSp3 IPC      | 21.28841                           | 20.9936  | 21.09703 | 21.11533 | 21.70503 | 21.11499            | 21.17326 | 21.20608 | 21.03429 | 21.2736  |
| hsa-miR-320b    | 26.7624                            | 26.41115 | 26.45954 | 26.70227 | 27.56089 | 26.12977            | 26.68162 | 27.00547 | 26.57443 | 25.86712 |
| hsa-miR-885-5p  | 30.79626                           | 27.96267 | 32.48107 | 31.04595 | 30.62359 | 31.75146            | 32.98113 | 33.11964 | 33.17522 | 33.77075 |
| hsa-miR-590-5p  | 29.55649                           | 29.67328 | 29.49773 | 29.82452 | 29.12372 | 28.45526            | 29.07391 | 29.90583 | 29.84341 | 27.71594 |
| hsa-miR-127-3p  | 32.76609                           | 34.73983 | 33.252   | 31.35259 | 31.00222 | 34.45742            | 37.37908 | 34.64361 | 35.42044 | 34.66831 |
| hsa-miR-191-5p  | 26.63509                           | 27.49481 | 28.13501 | 26.08828 | 28.24809 | 27.35439            | 28.44825 | 28.17356 | 27.11774 | 25.80836 |
| hsa-miR-99a-5p  | 30.21591                           | 29.07325 | 31.99554 | 31.12979 | 32.9957  | 30.84759            | 32.09533 | 31.29944 | 32.01439 | 30.9326  |
| hsa-miR-16-2-3p | 29.81005                           | 29.2398  | 29.71399 | 30.30368 | 32.63086 | 29.07293            | 30.6186  | 30.26246 | 29.07335 | 29.70464 |
| hsa-miR-301a-3p | 29.76094                           | 29.47846 | 30.25572 | 29.43878 | 30.45325 | 30.0342             | 30.30558 | 30.86009 | 29.7785  | 27.98571 |
| hsa-miR-140-5p  | 29.48979                           | 29.7663  | 30.28615 | 28.46059 | 29.5461  | 28.85058            | 30.38588 | 30.46991 | 30.38224 | 28.42335 |
| hsa-miR-151a-5p | 26.32515                           | 27.16764 | 27.84965 | 25.42245 | 27.74338 | 26.90835            | 28.02356 | 28.01242 | 27.67413 | 25.71401 |
| hsa-miR-130b-3p | 33.38481                           | 34.10626 | 34.85632 | 31.66996 | 34.01106 | 31.39933            | 32.31535 | 32.40686 | 31.96604 | 30.91024 |
| hsa-miR-423-5p  | 26.43603                           | 26.30937 | 26.48308 | 27.4189  | 32.06776 | 28.45429            | 28.69315 | 29.56806 | 28.51405 | 29.51792 |
| hsa-miR-101-3p  | 32.5333                            | 31.2678  | 31.27802 | 34.68712 | 34.7523  | 35.65752            | 34.55519 | 35.24951 | 35.33783 | 33.13094 |
| hsa-miR-200c-3p | 26.79647                           | 25.98135 | 25.70853 | 26.399   | 26.4995  | 25.31666            | 25.44091 | 26.61329 | 25.5438  | 24.6391  |
| hsa-miR-365a-3p | 31.04312                           | 28.96717 | 30.93914 | 30.82579 | 30.70286 | 30.54227            | 31.21945 | 31.97037 | 29.07414 | 29.44951 |
| hsa-miR-501-3p  | 33.91291                           | 33.53212 | 32.47366 | 33.28887 | 33.35595 | 31.97056            | 32.92544 | 33.35352 | 32.56938 | 31.0937  |
| hsa-miR-23a-3p  | 24.90077                           | 25.20611 | 26.34066 | 23.61326 | 25.11126 | 25.08897            | 25.89022 | 25.91361 | 25.13085 | 23.46818 |
| hsa-miR-423-3p  | 27.8058                            | 27.87058 | 29.48853 | 26.8052  | 28.05927 | 27.82365            | 28.39541 | 28.98639 | 28.47561 | 26.15592 |
| hsa-miR-215     | 28.38792                           | 27.19236 | 29.31909 | 29.54315 | 29.10647 | 28.18778            | 29.15725 | 29.52944 | 29.46916 | 27.96056 |
| hsa-miR-376a-3p | 35.04425                           | 37.42385 | 39.41479 | 34.83206 | 36.52796 | 38.81685            | 37.89062 | 37.81674 | 38.27707 | 35.32907 |
| hsa-miR-320a    | 27.34566                           | 27.52831 | 27.51764 | 27.07989 | 29.14034 | 26.90961            | 27.89774 | 27.84139 | 27.4643  | 26.94567 |
| hsa-miR-22-5p   | 31.44608                           | 31.20769 | 32.90216 | 30.55532 | 33.09369 | 30.7609             | 31.68508 | 32.39146 | 30.12033 | 30.21621 |
| hsa-miR-338-3p  | 31.17906                           | 31.67855 | 33.25192 | 29.1628  | 30.93922 | 30.75279            | 31.6276  | 31.61378 | 31.02343 | 29.27295 |
| hsa-miR-2110    | 32.09001                           | 32.5285  | 32.27613 | 32.54137 | 31.81155 | 31.19553            | 31.90546 | 33.44205 | 31.97486 | 30.94209 |
| hsa-miR-223-3p  | 22.3176                            | 22.77316 | 24.46379 | 21.05343 | 21.47469 | 22.72187            | 23.34807 | 23.55475 | 23.11802 | 21.02463 |
| hsa-miR-361-3p  | 33.71529                           | 35.12146 | 34.67405 | 32.95112 | 34.08602 | 34.33622            | 35.59987 | 35.95909 | 35.17646 | 34.96614 |
| hsa-miR-103a-3p | 26.44444                           | 26.54104 | 28.15291 | 25.29328 | 28.57471 | 26.79884            | 28.23468 | 27.70967 | 26.45439 | 25.14162 |
| hsa-miR-93-3p   | 29.32768                           | 29.26221 | 30.2138  | 29.95811 | 29.63111 | 29.93006            | 29.02666 | 31.10624 | 30.05894 | 28.2526  |
| hsa-miR-331-3p  | 30.7668                            | 30.80864 | 32.14602 | 29.70831 | 31.76879 | 30.70878            | 31.92244 | 32.29941 | 31.28941 | 29.84973 |
| hsa-miR-144-5p  | 28.94647                           | 29.26965 | 29.81558 | 30.7608  | 29.37571 | 29.17263            | 29.94614 | 29.30577 | 28.89575 | 27.33498 |
| hsa-miR-142-3p  | 25.84844                           | 26.71925 | 27.82249 | 24.52527 | 27.36963 | 26.58507            | 27.85983 | 26.99565 | 27.36211 | 25.07437 |
| hsa-miR-210     | 30.52575                           | 29.44265 | 29.8384  | 30.11612 | 30.11612 | 29.27381            | 29.67077 | 30.35948 | 29.69189 | 28.25408 |
| hsa-let-7d-3p   | 27.35308                           | 27.08252 | 28.25494 | 26.57891 | 28.28829 | 26.9092             | 27.81939 | 28.09588 | 27.69543 | 26.4758  |
| hsa-miR-199a-5p | 29.16799                           | 29.77893 | 32.29275 | 26.92736 | 30.09551 | 30.05381            | 30.35315 | 31.47994 | 29.78496 | 37.62966 |
| hsa-miR-605     | 36.61058                           | 36.85348 | 36.08793 | 38.75356 | 37.08973 | 38.82518            | 36.1274  | 35.16719 | 37.29751 | 37.62966 |
| hsa-miR-766-3p  | 30.40158                           | 30.85566 | 33.94442 | 28.95795 | 31.50229 | 30.53629            | 31.54236 | 32.47547 | 31.93192 | 28.87702 |
| hsa-miR-19a-3p  | 23.62305                           | 23.16023 | 22.65393 | 23.99387 | 23.48868 | 22.26454            | 22.78923 | 23.12068 | 23.21873 | 21.77285 |
| hsa-miR-584-5p  | 33.27483                           | 33.9204  | 34.42934 | 32.15987 | 34.59285 | 33.6272             | 35.33808 | 33.69693 | 33.16641 | 31.99856 |

(Continued)



Table S6 - Continued.

| Name             | D (type 2 diabetes mellitus group) |          |          |          |          | ED (E with D group) |          |          |          |          |
|------------------|------------------------------------|----------|----------|----------|----------|---------------------|----------|----------|----------|----------|
|                  | D-1                                | D-2      | D-3      | D-4      | D-5      | ED-1                | ED-2     | ED-3     | ED-4     | ED-5     |
| hsa-miR-144-3p   | 26.81098                           | 25.96434 | 25.93203 | 27.29754 | 27.66445 | 25.47022            | 26.14856 | 26.47072 | 26.19278 | 24.7817  |
| hsa-miR-92a-3p   | 22.5224                            | 21.67895 | 22.20973 | 23.16956 | 22.69553 | 21.71139            | 22.46906 | 22.61225 | 22.38742 | 21.38729 |
| hsa-miR-126-3p   | 24.77132                           | 25.35007 | 26.16188 | 24.13579 | 24.80885 | 25.40429            | 26.07971 | 25.45147 | 25.6893  | 23.6741  |
| hsa-miR-363-3p   | 27.54857                           | 27.94684 | 27.62754 | 28.39699 | 29.18393 | 27.60034            | 28.27741 | 27.83662 | 28.50162 | 26.44453 |
| hsa-miR-148a-3p  | 28.44513                           | 27.77618 | 28.80755 | 27.7924  | 29.88059 | 28.38501            | 29.51613 | 29.35132 | 29.26181 | 27.9475  |
| hsa-miR-374a-5p  | 31.38966                           | 31.58953 | 34.5748  | 31.14826 | 33.0492  | 33.37634            | 33.82884 | 33.61235 | 31.79604 | 30.59642 |
| hsa-miR-10b-5p   | 31.65718                           | 31.30852 | 31.33387 | 32.53956 | 31.4356  | 31.4356             | 32.58463 | 31.08337 | 32.03518 | 32.7045  |
| hsa-miR-190a     | 35.56296                           | 39.9922  | 36.96162 | 36.58842 | 37.8623  | 35.64421            | 37.90305 | 38.90798 | 37.13423 | 36.02359 |
| hsa-miR-195-5p   | 34.81803                           | 36.10756 | 33.75375 | 34.36822 | 37.39947 | 35.40641            | 36.0197  | 34.40466 | 35.86294 | 33.09562 |
| hsa-miR-29a-5p   | 33.62799                           | 34.79367 | 34.28976 | 33.2996  | 33.22906 | 34.80042            | 38.57678 | 37.89062 | 37.7499  | 37.23841 |
| hsa-miR-125b-5p  | 28.15728                           | 27.19051 | 29.69214 | 29.76095 | 27.19072 | 30.33122            | 30.34946 | 30.34946 | 28.97484 | 28.53726 |
| hsa-miR-18a-3p   | 32.00536                           | 32.00099 | 32.72459 | 32.47067 | 32.8706  | 31.53223            | 32.61909 | 33.26028 | 31.60923 | 31.05726 |
| hsa-miR-192-5p   | 27.34306                           | 26.18886 | 28.63762 | 28.85185 | 28.05868 | 27.59172            | 28.44694 | 28.99431 | 28.66389 | 26.8717  |
| hsa-miR-18b-5p   | 28.40923                           | 27.97903 | 32.0797  | 30.09848 | 32.8249  | 31.15724            | 32.20752 | 32.96207 | 31.79728 | 30.82891 |
| hsa-miR-28-3p    | 29.5291                            | 30.47097 | 29.01157 | 28.29912 | 28.93529 | 27.59172            | 29.48258 | 29.40135 | 28.55834 | 26.62752 |
| hsa-miR-335-5p   | 32.37259                           | 32.34742 | 31.39117 | 28.99073 | 30.75623 | 29.88137            | 32.48067 | 31.30003 | 30.68423 | 29.13814 |
| hsa-miR-324-3p   | 27.76575                           | 27.52392 | 28.42872 | 31.03586 | 28.24235 | 31.68464            | 31.80368 | 32.62725 | 32.28526 | 30.70428 |
| hsa-miR-204-5p   | 33.90165                           | 31.81868 | 34.49849 | 33.61979 | 35.21167 | 33.12917            | 33.18265 | 28.6657  | 28.26685 | 26.59406 |
| hsa-miR-182-5p   | 37.04663                           | 36.20702 | 38.41929 | 36.38702 | 36.88975 | 38.07404            | 36.89752 | 34.07457 | 36.41492 | 35.64703 |
| hsa-let-7g-5p    | 25.08185                           | 25.44943 | 27.26788 | 24.88479 | 26.01689 | 25.97483            | 27.30828 | 26.98093 | 26.3918  | 26.56339 |
| hsa-miR-15b-5p   | 26.73571                           | 27.12242 | 27.42981 | 25.85077 | 27.16329 | 26.8371             | 27.41397 | 28.34147 | 27.19159 | 26.35056 |
| hsa-miR-22-3p    | 26.2033                            | 25.44125 | 26.40162 | 25.58852 | 27.39152 | 24.8139             | 27.54486 | 27.3851  | 26.55589 | 21.048   |
| hsa-miR-106b-3p  | 31.51329                           | 32.23705 | 32.53256 | 30.10619 | 32.31112 | 32.73843            | 32.62348 | 25.85736 | 24.90096 | 35.05575 |
| hsa-miR-199a-3p  | 27.75777                           | 28.90052 | 30.13633 | 26.03986 | 28.54507 | 28.54625            | 29.527   | 33.9879  | 31.5128  | 27.39969 |
| hsa-miR-29c-3p   | 27.72294                           | 27.45336 | 27.93699 | 27.47156 | 28.3465  | 26.8371             | 29.527   | 29.5624  | 28.6566  | 27.12385 |
| hsa-miR-19b-3p   | 22.70167                           | 22.34631 | 22.06381 | 23.1515  | 23.3283  | 21.66688            | 22.20088 | 28.34147 | 22.45527 | 26.35056 |
| hsa-miR-95       | 36.1368                            | 35.17346 | 36.75823 | 37.03688 | 37.23497 | 35.45067            | 37.7169  | 22.57058 | 22.45527 | 21.048   |
| hsa-miR-29a-3p   | 27.75886                           | 27.77    | 29.27981 | 26.70421 | 28.96121 | 28.35319            | 29.33983 | 35.09236 | 34.45426 | 35.05575 |
| hsa-miR-21-5p    | 24.7081                            | 24.43927 | 24.95989 | 23.85697 | 25.72037 | 24.31495            | 29.33983 | 29.43939 | 28.56105 | 27.39969 |
| hsa-miR-150-5p   | 22.86076                           | 23.9268  | 23.27749 | 23.41396 | 25.72037 | 25.07351            | 25.75683 | 25.00069 | 25.0331  | 23.75778 |
| hsa-miR-30e-3p   | 32.33401                           | 31.9388  | 35.73007 | 30.9821  | 33.88789 | 33.25337            | 25.86813 | 26.33533 | 32.93573 | 24.73113 |
| hsa-miR-30b-5p   | 26.5017                            | 26.73009 | 28.80591 | 25.81084 | 24.21265 | 27.54214            | 34.48325 | 33.59615 | 28.16653 | 25.52254 |
| hsa-miR-543      | 33.20444                           | 38.4225  | 34.60691 | 31.31915 | 31.77762 | 34.07417            | 28.84336 | 34.38651 | 27.47687 | 31.38476 |
| hsa-miR-24-3p    | 24.25223                           | 24.8208  | 25.77845 | 23.43045 | 24.0378  | 24.548              | 25.13255 | 33.29401 | 33.29401 | 32.80764 |
| hsa-miR-29b-2-5p | 32.35545                           | 32.50351 | 33.15458 | 33.26297 | 33.16234 | 31.89248            | 25.36255 | 25.36255 | 24.97988 | 23.30529 |
| hsa-miR-495-3p   | 31.52793                           | 33.24242 | 31.32449 | 31.03782 | 32.51954 | 33.92587            | 33.61815 | 34.13213 | 33.03684 | 31.70088 |
| UniSp3 IPC       | 20.91285                           | 20.82769 | 20.81447 | 20.79199 | 21.49244 | 20.83724            | 20.87916 | 20.95243 | 20.74931 | 21.03314 |



## ■ SUPPLEMENTARY PROCEDURES

### miRNA qRT-PCR array

A total of 192 mature human miRNAs were detected in the plasma samples. Specifically, the reaction consisted of 4 µl diluted cDNA template, 5 µl SYBR Green master mix, and 1 µl PCR primer mix (Exiqon A/S). The reaction was denatured at 95°C for 8 min, followed by 36 cycles at 95°C for 10s, and at 60°C for 60s. Melt curve analysis was performed at the end of the PCR.

### RNA extraction and assay of real-time PCR

TRIZol reagent (Thermo Fisher Scientific) was used to isolate total RNA from HEK-293T cells, followed by reverse-transcription with a PrimeScript™ RT Reagent Kit, according to the manufacturer's instructions and as we previously described with several modifications (1-5). mRNA expression level of DRD1 and GAPDH was determined by RT-PCR with Power SYBR Green PCR Master Mix (Thermo Fisher Scientific) in an ABI Prism 7900 (Applied Biosystems), following the manufacturer's protocol. Data were normalized against the mean CT value of GAPDH, and the relative mRNA expression level was determined by the  $2^{-\Delta\Delta CT}$  method, as we previously described (5-11).

### Validation of miRNA expression by qRT-PCR analysis

miR-197-5p, miR-130a-5p, miR-27a-5p, and miR-195-5p were chosen for verification with the qPCR array data by RT-qPCR. The above miRNAs were selected based on expression levels and biological significance. The miScript Reverse Transcriptase Kit (Qiagen, Germany) was used to perform cDNA synthesis, following the manufacturer's instructions. In brief, an RT reaction consisting of 1 µg RNA, 2 µl of Nucleic Mix, 2 µl of miScript Reverse Transcriptase Mix, and 4 µl of miScript HiSpec Buffer was created. The reaction was incubated at 37°C for 60 min, at 95°C for 5 min, and then held at 4°C. A miScript SYBR®-Green PCR kit (Qiagen GmbH) was adopted to perform qPCR. The reaction system contained 10 µl RT-qPCR mix, 0.5 µl upstream primer (miRNA-197-FW: 5'-TGCGGCGGGTAGAGAGGGCAGTGG-3'; miRNA-130a-FW: 5'-TGCGGTTACATTGTGCTACTGTCTGC-3'; miRNA-27a-FW: 5'-TGCGGAGGGCTTAGCTGCTTGTGAGC-3'; miRNA-195-FW: 5'-TGCGGTAGCAGCACAGAAATATTGGC-3'), 0.5 µl downstream primer (common primer supplied by the kit), 7 µl ddH<sub>2</sub>O, and 2 µl template of cDNA. The steps of thermal cycle reaction are as follows: initial denaturation for 10 min at 95°C, 38 cycles for 1 min at 95°C, and 30s at 60°C. Calculation by the  $2^{-\Delta\Delta CT}$  method (20) was performed to evaluate the expression level of miR-199a-3p normalized to U6 (forward primer, 5'-GCTCGCTTCGGCAGCACACA-3' and the reverse primer, 5'-AACGCTTCACGAATTTGCGTG-3').

### Cell culture and transfection

HEK-293T cells were cultured in DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS, 100 units/ml Invitrogen penicillin-streptomycin (Thermo Fisher Scientific, Inc.) in a humidified incubator with 5% CO<sub>2</sub> at 37°C as we previously described (12,13). miRNA was transiently transfected with Lipofectamine 2000 reagent,

according to the manufacturer's instructions (Invitrogen). All experiments were done in triplicate.

### Luciferase reporter assay

The miR-195-5p mimic and miR-195-5p inhibitor, and their negative control mimics were purchased from GenePharma (Shanghai, China). Chemically-modified miRNA inhibitors were optimized to specifically inhibit miR-195-5p expression. miRNA mimics and inhibitors were transfected into HEK-293T cells, as described above. At 24h, the transfected HEK-293T cells were used for the luciferase report assay and target mRNA expression detection as we previously reported (12,13).

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