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Research Article

Circulating miR-206 and Wnt-signaling are associated with cardiovascular complications and a history of preeclampsia in women

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Women with a history of preeclampsia (PE) have increased risk of cardiovascular disease (CVD) later in life. However, the molecular determinants underlying this risk remain unclear. We sought to understand how circulating miRNA levels are affected by prior PE, and related to biological pathways underpinning cardiovascular disease. RNA sequencing was used to profile plasma levels of 2578 miRNAs in a retrospective study of women with a history of PE or normotensive pregnancy, in two independent cohorts with either acute coronary syndrome (ACS) ($n = 17$–18/group) or no ACS ($n = 20$/group). Differential miRNA alterations were assessed in relation to a history of PE (within each cohort) or ACS (across cohorts), and compared with miRNAs previously reported to be altered during PE. A history of PE was associated with altered levels of 30 and 20 miRNAs in the ACS and non-ACS cohorts, respectively, whereas ACS exposure was associated with alterations in 259 miRNAs. MiR-206 was identified at the intersection of all comparisons relating to past/current PE and ACS exposure, and has previously been implicated in atherogenic activities related to hepatocytes, vascular smooth muscle cells and macrophages. Integration of all differentially altered miRNAs with their predicted and experimentally validated targets *in silico* revealed a number of highly targeted genes with potential atherogenic functions (including NFAT5, CCND2 and SMAD2), and one significantly enriched KEGG biological pathway (Wnt signaling) that was shared between all exposure groups. The present study provides novel insights into miRNAs, target genes and biological pathways that may underlie the long-term cardiovascular sequelae of PE.

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Introduction

Preeclampsia (PE) is estimated to affect up to 8% of all pregnancies worldwide, and thus represents a substantial burden on the cardiovascular health of millions of women [1]. While the high blood pressure and proteinuria associated with PE usually resolve after placental delivery, women are left with a higher risk of premature cardiovascular complications and mortality [1]. Elevated rates of metabolic syndrome and hypertension have been reported after preeclamptic pregnancies [2]. In addition, vascular abnormalities in arterial stiffness revealed by pulse wave velocity and other metrics have been reported in women with a history of PE [3]. Notwithstanding these broader pathophysiological insights, the underlying molecular determinants that lead to elevated risk of cardiovascular disease (CVD) following PE remain unclear.

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MicroRNAs (miRNAs) are small non-coding RNAs that bind to the messenger RNAs of protein-coding genes in a sequence-dependent fashion to direct their repression. To date, over 2500 mature human miRNAs are known to exist, and each may have the capacity to regulate hundreds of different genes to support diverse biological roles [4,5]. A number of miRNAs have been implicated causally in cardiovascular health and disease [6,7], and altered levels of systemic circulating miRNAs (as identified in blood plasma or serum) have also been investigated as potential biomarkers and/or messengers of disease activity and signaling [8,9]. While a large number of studies have reported acute changes in the levels of circulating and/or placental miRNAs during PE [10], relatively little is known about the long-term impact of PE on cardiovascular health. Thus, we have previously sought to understand how dysregulation of miRNA expression, manifested through alterations in circulating levels, may contribute to CVD activity even after PE is resolved [11]. In a limited screen of 372 predefined miRNAs commonly found in circulation, we identified alterations in several potential atherogenic miRNAs in a cohort of women with premature acute coronary syndrome (ACS) and a history of PE or normotensive (NT) pregnancy [11]. These miRNAs have previously been implicated in endothelial dysfunction, angiogenesis and inflammation, consistent with their potential contribution to cardiovascular abnormalities.

In the present study, we sought to identify miRNAs and biological pathways that may contribute to the long-term cardiovascular sequelae of PE, using a far more comprehensive platform to investigate alterations in circulating miRNAs. Next-generation RNA sequencing was used to probe global changes in plasma levels of all 2578 mature human miRNAs (annotated in miRBase V.20), in two independent cohorts of women with and without a history of PE versus NT control pregnancy. The first cohort consisted of women with premature ACS and many traditional atherosclerotic risk factors. A second cohort of similarly aged women with no acute ischemic event and significantly lower burden of CVD risk factors was used to probe the generalizability of candidate miRNAs associated with prior PE, independent of overt CVD and atherosclerotic risk factors. Differentially altered circulating miRNAs were identified in relation to prior PE and current ACS exposure, combined with their predicted and experimentally validated gene targets and used in a pathway enrichment analysis to identify shared mechanisms linking PE to CVD.

Methods

Cohort 1: ACS subjects with a history of PE versus NT pregnancy

Women with premature acute coronary syndrome (ACS) and a history of PE or NT pregnancy were identified from the GENESIS-PRAXY multicentre study of adults hospitalized with ACS. Detailed methods have been previously described [12]. Participants were previously recruited between 2009 and 2013 from 24 centers across Canada, one in the US and one in Switzerland. All participating sites received ethics approval from their respective ethics review boards, and participants provided written informed consent. Eligible participants were aged 18–55 years diagnosed with ACS, able to provide informed consent and had sufficient plasma specimens drawn at study entry ($n = 30$ with prior preeclampsia and $n = 146$ with prior normotensive pregnancy). A detailed self-reported questionnaire was used to collect pregnancy data from all female participants at study entry, and the classification of prior PE was made if they reported either PE or high blood pressure in addition to proteinuria. Subjects were excluded if they were unsure about the presence or absence of a pregnancy complication or if completion of these questions was incomplete. The time since last pregnancy was estimated using the age of the youngest biological child, to serve as a proxy for the interval between pregnancy and incident ACS. Venipuncture was performed on all consenting participants within 24 h of hospital admission for ACS. Whole blood collected in citrate Vacutainers was spun at 4°C at 3000 rpm for 10 min and the plasma supernatant removed and frozen at −80°C. A total of 40 subjects were selected for miRNA sequencing ($n = 20$ subjects/exposure group) after matching for CVD risk factors including hypertension, diabetes, smoking and age. The final sample size was reduced to $n = 17$–18 subjects/exposure group after several plasma specimens failed RNA- or library- quality control tests prior to sequencing (details provided in the online data supplement).

Cohort 2: Non-ACS subjects with a history of PE versus NT pregnancy

Women without ACS and a history of either PE or NT pregnancy were identified from The Cardiovascular Consequences of Pre-eclampsia (COPS) study at the British Heart Foundation Glasgow Cardiovascular Research Centre (BHF GCRC), which previously recruited 86 women with a history of PE and 80 NT pregnancy controls. Women were recruited from multiple sources including the previous Generation Scotland: Scottish Family Health Study [13], the Proteomics in Pre-eclampsia study [14], patients who attended blood pressure clinics and friends and colleagues of participants who contacted us with interest in participating. The study was approved by the West of Scotland Research Ethics Committee 3 (Reference 12/WS/0306), and participants provided written informed consent. The index pregnancy was defined as the first pregnancy in NT women and the first pre-eclamptic pregnancy in those with PE.

Women were excluded if they were >60 years old, already had established cardiovascular disease or if they were unable to give informed consent. Participants completed a questionnaire asking for obstetric history, past medical history, drug history, smoking history and family history. Blood samples were taken from the antecubital fossa using a standard tourniquet and Vacutainer system, and centrifuged at 4°C at 2500 rpm for 15 min and plasma supernatant removed and frozen at −80°C. A total of 40 subjects ($n = 20$ subjects/exposure group) matched on hypertension, diabetes, and age were selected and used for miRNA sequencing after passing all quality control tests.

Cohort 3: ACS versus non-ACS subjects

The comparison of all subjects in cohort 1 ($n = 35$ total ACS subjects) versus cohort 2 ($n = 40$ total non-ACS subjects) was used to assess potential associations between ACS (including related risk factors)and circulating miRNA levels.

Cohort 4: Women with PE versus NT pregnancy

Information for this cohort was derived from six prior independent studies of preeclamptic women (with no ACS) reported in the systematic review by Sheikh et al. [10]. A total of 104 circulating miRNAs were identified via high-throughput screening methods as differentially altered in plasma, serum or whole blood collected from women during pregnancies complicated by PE versus NT control pregnancies.

RNA isolation and quality control, library construction/quality control and sequencing, sequence trimming, UMI-consolidation and Data Mapping, miRNA-target integration and pathway enrichment analysis, pre- and post-study sample size and power estimations

Details are provided in the online data supplement including quality control assessment of extracted RNA (Supplementary Figure S1) and sequencing libraries (Supplementary Figure S2).

Statistical analysis

All statistical tests comparing cohort characteristics were performed in Graphpad Prism 8.0. Data normality was assessed using the D'Agostino Pearson test. Differences between exposure groups for continuous data were assessed using a Mann–Whitney or unpaired t-test, depending on data normality as appropriate. Differences in categorical variables were assessed via Fisher's exact test. Data are presented as mean ± standard deviation (SD) unless otherwise specified. Differential expression analysis was conducted on the subset of samples related to the specific groups being compared, using UMI-corrected miRNA counts as input into the EdgeR statistical software package (Bioconductor, http://www.bioconductor.org/). Data were preprocessed to exclude poorly detectable miRNAs such that the sum of the counts per million mapped reads (CPM) for each miRNA in all samples pertaining to the comparison subset was >10. The filtered data were normalized using the trimmed mean of M-values (TMM) normalization method in EdgeR to compensate for sample specific effects related to variations in sequencing depth and/or RNA composition. MiRNA levels in some figures are presented simply as counts per million mapped reads (CPM), which only correct for sequencing depth. P-values and Benjamini–Hochberg false discovery rate (FDR)-correct P values for differentially altered miRNAs were calculated with an exact test assuming a negative binomial distribution in EdgeR. Differentially altered miRNAs were defined by $P < 0.05$ (for cohorts 1 and 2 with prior exposure events) and FDR < 0.05 (for cohort 3 with a current exposure event). Principal component analysis and unsupervised hierarchical clustering and heatmap construction were performed with default parameters in Partek Genomics Suite 7.2 using log2 transformed TMM-normalized miRNA counts (with offset 1 to account for 0 values). Study-specific post hoc estimations of miRNA dispersion (a measure of variability in miRNA levels), sample size and statistical power for the completed sequencing experiment were calculated with the RNASeqSampleSize software package in R language and online interface at http://cqs.mc.vanderbilt.edu/shiny/RnaSeqSampleSize/. [15]

Results

Two independent cohorts of women with or without premature ACS, and a history of preeclampsia or normotensive pregnancy

The impact of prior PE on circulating miRNA levels was assessed in two cohorts of women with (cohort 1, C1) or without (cohort 2, C2) premature ACS. Within each cohort, subjects were closely matched and showed no significant differences in baseline characteristics between the prior PE versus NT pregnancy groups including age, body mass index (BMI), menopausal status and several cardiovascular disease (CVD) risk factors (Table 1). At the time of plasma

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Table 1 Cohort characteristics

Characteristics	Cohort 1 (ACS)			Cohort 2 (non-ACS)			ACS versus non-ACS
	PE (n = 18)	NT (n = 17)	P-val	PE (n = 20)	NT (n = 20)	P-val	P-val
Age (years)†	49 ± 6	48 ± 6	0.70	47 ± 11	49 ± 11	0.55	0.81
Sex, % female	100	100	1	100	100	1	1
Caucasian, n (%)	15 (83%)	12 (71%)	0.44	20 (100%)	20 (100%)	>0.99	0.001
Time since index pregnancy (yr)†	16 ± 9 (n = 14)	22 ± 8 (n = 16)	0.10	19 ± 11	22 ± 13	0.34	0.35
Primiparous, n (%)	5 (28%)	2 (12%)	0.40	4 (20%)	5 (25%)	>0.99	>0.99
Menopausal, n (%)	11 (61%)	7 (41%)	0.32	7 (35%)	11 (55%)	0.34	0.65
CV risk factors							
BMI (kg/m^2)†	33 ± 12.4	35 ± 13.4	0.53	27.5 ± 4.0	26.6 ± 5.0	0.54	0.005
Current smoker, n (%)	6 (33%)	5 (29%)	>0.99	1 (5%)	2 (10%)	>0.99	0.02
Ex smoker, n (%)	9 (50%)	8 (47%)	>0.99	9 (45%)	5 (25%)	0.32	0.25
Hypertension, n (%)	14 (78%)	13 (76%)	>0.99	4 (20%)	1 (5%)	0.34	<0.0001
Diabetes, n (%)	2 (11%)	5 (29%)	0.23	0 (0)	0 (0)	>0.99	0.003
Dyslipidemia, n (%)	14 (78%)	8 (47%)	0.09	0 (0)	2 (10%)	0.49	<0.0001
History of CAD, n (%)	6 (33%)	8 (47%)	0.50	0 (0)	0 (0)	>0.99	<0.0001
Systolic BP† (mmHg)	134.1 ± 25.9	134.1 ± 15.2 (n = 16)	0.99	129.8 ± 14.7	124.6 ± 11.1	0.22	0.09
Diastolic BP† (mmHg)	82.2 ± 14.2	81.6 ± 13.6 (n = 16)	0.89	80.8 ± 9.3	77.3 ± 6.3	0.14	0.26
Biomarkers							
Standardized troponin (μg/l)†	8.5 ± 22.5 (n = 13)	30.1 ± 67.8	0.18	nd	nd		n/a
C-reactive protein (mg/l)†	38.4 ± 64.7 (n = 17)	32.1 ± 45.5 (n = 16)	0.97	nd	nd		n/a
ACS type							
STEMI	12 (66%)	5 (29%)	0.04	n/a	n/a		n/a
non-STEMI	6 (33%)	9(53%)	0.31	n/a	n/a		n/a
Unstable angina	0 (0)	3 (18%)	0.10	n/a	n/a		n/a

ACS: acute coronary syndrome
PE: prior preeclampsia
NT: prior normotensive pregnancy
†Mean ± SD
n/a: not applicable
nd: not determined
Additional sample sizes are noted where data were not available for all subjects.

sampling, an average of 19.7 ± 10.7 years had passed since the index pregnancy event, as measured across all subjects in both cohorts (n = 75). Between cohorts, ACS and non-ACS subjects were similarly aged, and showed no significant differences in the time from index pregnancy, or proportion of primiparous and menopausal women. Both cohorts were comprised primarily of Caucasian women, though the proportion of Caucasians in cohort 2 was significantly higher than cohort 1. In addition, the ACS cohort contained a significantly higher proportion of subjects with traditional atherosclerotic risk factors including elevated BMI, current smokers, hypertension, diabetes, dyslipidemia and history of coronary artery disease (Table 1).

Alterations in circulating miRNA levels associated with a history of PE or current ACS exposure

On average, 455 miRNAs (range 271–586) were detected per sample across cohorts 1 and 2 (Supplementary Figure S3). In ACS cohort 1, 30 miRNAs were differentially altered between women with prior PE and NT pregnancy ($P = 1.6 \times 10^{-6}$ to 0.05), including three miRNAs that passed a false discovery rate threshold < 0.05 (Figure 1A and Supplementary Table S1). The absolute magnitude of miRNA changes ranged from 1.5- to 10-fold in both directions (median magnitude of change of 2.8-fold) (Figure 1A and Supplementary Table S1). In non-ACS cohort 2, a total of

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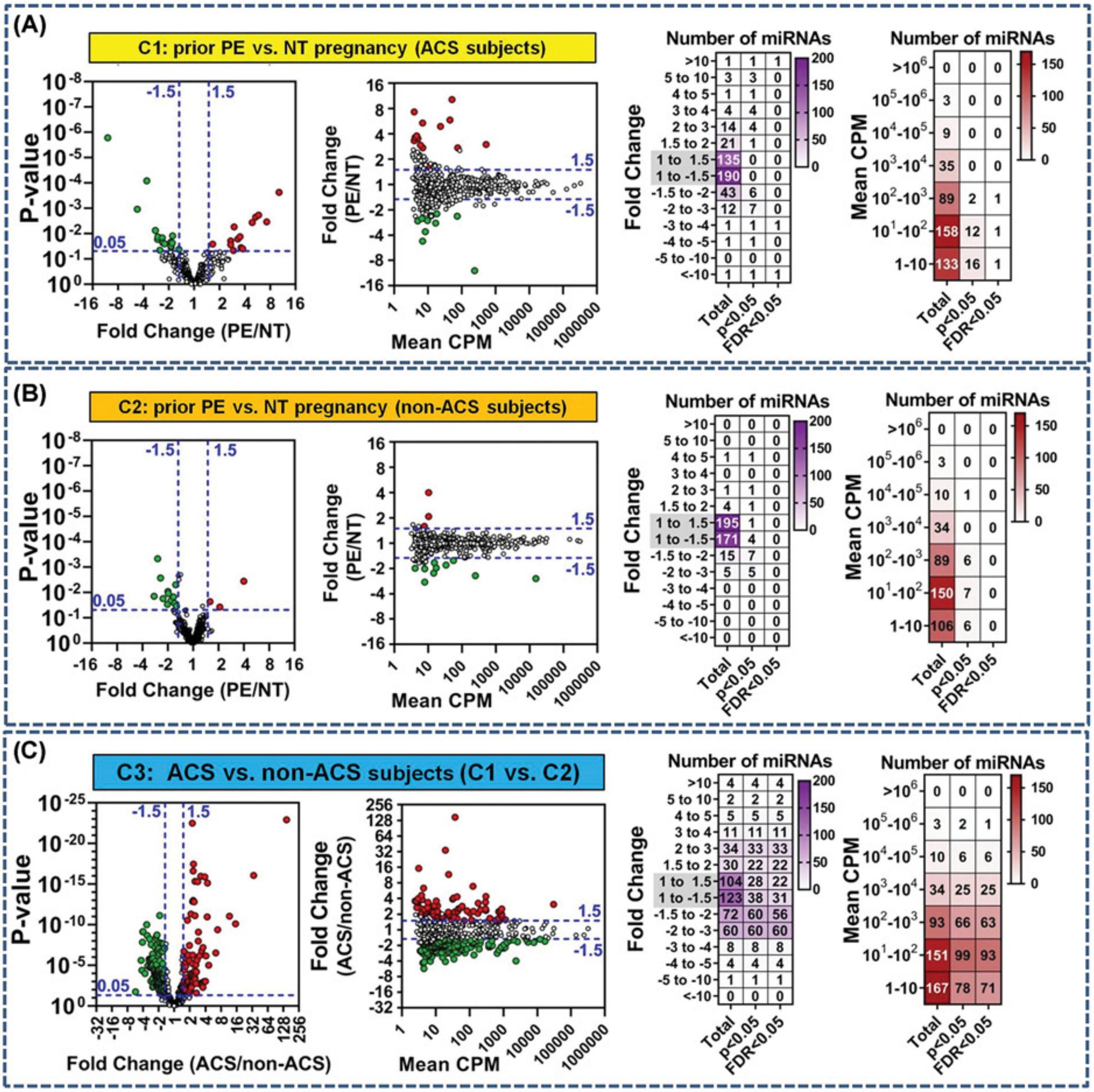

Figure 1. Differentially altered plasma miRNAs in women with and without a history of preeclampsia (PE), and in relation to acute coronary syndrome (ACS)

(**A**) Plasma miRNA levels were assessed by next-generation RNA sequencing and differential analyses performed in (A) women with ACS (cohort 1) and a history of preeclampsia or normotensive (NT) pregnancy ($n = 17$–18/group), (**B**) similarly aged women without ACS (cohort 2) and a history of PE versus NT pregnancy ($n = 20$/group), and (**C**) women with versus without ACS (cohort 1 vs. cohort 2; $n = 35$–40/group). Graphs in panels (A–C) show the distribution of miRNAs (dots) according to unadjusted *P*-values, magnitude of fold change between exposure groups, and plasma level (expressed as the mean counts per million mapped reads, CPM). MiRNAs with $P < 0.05$ are color-coded green or red to denote decreased or increased plasma level, respectively. Specific *P* values for each miRNA are provided in Supplementary Tables S1–S3. Heatmaps in panels (A–C) show details on the number of miRNAs according to specific intervals of fold change or mean CPM for all detected miRNAs (total), and subsets of differentially altered miRNAs based on different statistical thresholds of $P < 0.05$ or FDR < 0.05.

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20 miRNAs were differentially altered in relation to prior PE exposure ($P = 0.0005$–0.05), though none remained significant with FDR < 0.05. The magnitude of observed changes in miRNA levels ranged from 1.2- to 4-fold in cohort 2 (median magnitude of change of 1.8-fold) (Figure 1B and Supplementary Table S2). In both cohorts 1 and 2, the majority of differentially altered miRNAs were detected at less than 100 counts per million mapped reads (Figure 1A,B). To assess the association with ACS, differential alterations in plasma miRNA levels were also evaluated across cohorts 1 and 2 (this comparison is defined as cohort 3, C3, for clarity and labelling purposes). There were marked differences in the number of altered miRNAs, statistical significance and effect sizes related to ACS exposure as compared with prior PE exposure (Figure 1C and Supplementary Table S3). A total of 272 miRNAs were differentially altered ($P < 0.05$) in ACS compared with non-ACS subjects (cohort 1 vs. cohort 2), including 259 miRs with FDR < 0.05. Nominal P values for differentially altered miRNAs in cohort 3 ranged as low as 1.2×10^{-23}, and effect sizes ranged from 1.2- to 148-fold in magnitude (median change of 1.9-fold). The majority of differentially altered miRNAs associated with ACS exposure were detected at less than 100 CPM.

For exploratory purposes and to gain further insight into how the biological features of prior PE (cohorts 1 and 2) and current ACS exposure (cohort 3) relate to the variability in miRNA profiles between subjects, a principal component analysis (PCA) was conducted with the differentially altered miRNAs identified in each cohort. This would allow visual assessment of the relative similarities or differences between subjects on the basis of their plasma miRNA signatures. While cohorts 1 and 2 showed some general clustering according to prior PE or NT pregnancy, there was marked overlap between the exposure groups (Figure 2). By comparison, subjects in cohort 3 appeared more broadly dispersed within clusters according to ACS or non-ACS exposure, but the separation between exposure groups was more prominent (Figure 2A). Unsupervised hierarchical clustering provided similar insights into the heterogeneity of patient miRNA profiles within and between exposure groups (Figure 2B).

We next sought to evaluate the relationship between statistical power, effect size and sample size for the completed sequencing experiment to better inform study outcomes. Since statistical power depends largely on the variability in miRNA levels, which might differ from our original estimates that were based on previously published data [11], we quantified the dispersion in miRNA levels in the present study. Supplementary Figure S4A shows the distribution of dispersion levels as determined by the RNASeqSampleSize program [15]. Although ACS cohort 1 showed a wider range of dispersion levels (from 0.1 to 11.4) as compared with non-ACS cohort 2 (from 0.1 to 4.1), the largest proportion of miRNAs in both cohorts occurred at a dispersion bin center of 0.5 in the frequency histogram (with 39% and 75% of all miRNAs exhibiting a dispersion ≤ 0.5 in cohort 1 and 2, respectively). Therefore, we investigated the relationship between statistical power, sample size and effect size assuming a common dispersion level of 0.5, and corrected for multiple testing of 400 miRNAs with FDR < 0.05. The corresponding power curve (Supplementary Figure S4B) indicates the current study had 85% power (with $n = 20$ subjects per group) to detect a 2.5-fold or greater change in plasma level for the majority of miRNAs, but was relatively underpowered to detect smaller effect sizes that might be associated with a history of PE. However, the power curve suggests a sample size of ∼100 subjects/group would be required to detect a 1.5-fold change in miRNA level (with FDR < 0.05 and 80% power), which exceeds the number of patient samples available in each of our archived cohorts.

miR-206 levels are altered in multiple cohorts related to preeclampsia and acute coronary syndrome

The overlap between differentially altered miRNAs identified in each cohort was investigated to prioritize miRNAs with higher biological relevance. Four miRNAs were altered in relation to a history of PE in both the ACS and non-ACS cohorts (cohorts 1 and 2, respectively), including three miRNAs that showed the same direction of change (miR-206 and miR-376a-3p decreased, and miR-1299 increased; Figure 3A and Supplementary Table S4). To help discern potential molecular mechanisms linking prior PE to CVD, we also examined the overlap with miRNAs that were differentially altered at the time of ACS (cohort 3; Figure 3B and Supplementary Table S5). Eighteen of thirty miRNAs that were differentially altered in ACS subjects with a history of PE (versus prior NT pregnancy; cohort 1) were also altered between ACS and non-ACS subjects (cohort 3 comparison), and the majority of these miRNAs (16 of 18) showed concordant directions of change. Of note, several miRNAs that have previously been associated with acute myocardial infarction including miR-1, miR-133a-3p and miR-499a-5p were also identified in the present study and showed concordant increases in plasma levels in both cohorts 1 and 3. In non-ACS subjects (cohort 2), 12 of 20 miRNAs that were associated with prior PE exposure were also differentially altered in cohort 3 in relation to ACS exposure (Figure 3B and Supplementary Table S5); however, the direction of change differed between cohorts for the majority of these miRNAs (11 of 12). Only one miRNA, miR-206, was differentially altered in all three cohorts, with decreased levels observed in relation to prior PE exposure and increased levels with ACS exposure.

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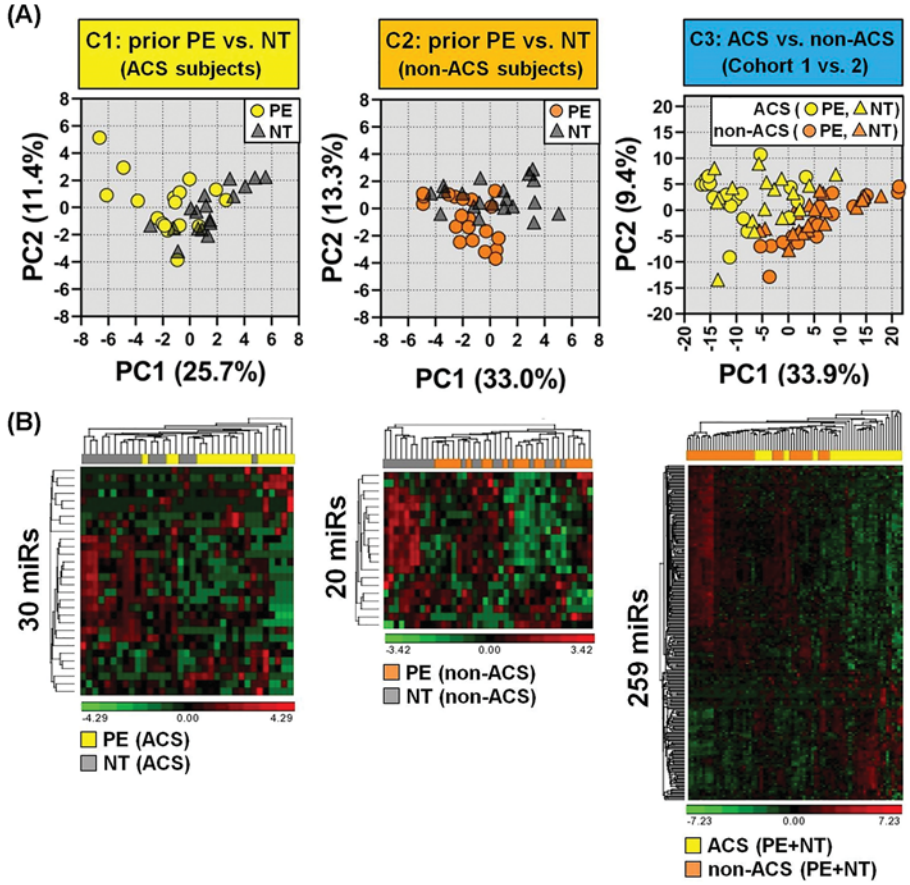

Figure 2. Principal component analysis (PCA) and hierarchical clustering of miRNAs associated with a history of PE or current ACS exposure

(**A**) PCA of 30 differentially altered miRs ($P < 0.05$) in ACS cohort 1 ($n = 17$–18 subjects/group), 20 altered miRs ($P < 0.05$) in non-ACS cohort 2 ($n = 20$ subjects/group), and 259 altered miRs (FDR < 0.05) in cohort 3 comparing ACS versus non-ACS subjects ($n =$ 35–40 /group). (**B**) Unsupervised hierarchical clustering and heatmap of plasma levels of differentially altered miRNAs for each exposure. Rows denote miRNAs and columns denote subjects. High and low relative plasma levels are denoted by red and green, respectively. Log2 transformed TMM-normalized miRNA counts (offset 1) were used for PCA, and further shifted to mean 0 and scaled to a standard deviation of 1 for clustering and heatmap construction.

We next sought to investigate whether the differential miRNA levels were induced at the time of the original PE event, or alternatively reflect progressive changes that occurred after resolution of PE. To address these different possibilities we cross-referenced miRNAs identified in the present study (i.e. assessed ~20 years after the index pregnancy event on average) with miRNAs previously reported to be altered in circulation at or near the time of PE or NT pregnancy [10]. A total of 104 unique circulating miRNAs were identified and curated from six prior screening studies of preeclamptic women [10] (herein defined as cohort 4, C4, for clarity and labeling purposes; Supplementary Table S6). Three of these miRNAs were shared with ACS cohort 1, 8 miRNAs were shared with non-ACS cohort 2, and only 2 miRNAs (miR-206 and miR-376a-3p) were common between all three cohorts, though the direction of regulation differed between prior and current PE exposures (Figure 3C and Supplementary Table S7).

miRNA–gene target integration and pathway enrichment analysis reveal Wnt signaling as a common pathway associated with PE and ACS

Since small changes in multiple miRNAs can potentially produce synergistic biological effects via cooperative and/or redundant mechanisms of gene expression control (i.e. by targeting the same or different genes within the same pathway) [6], we speculated that the largely distinct sets of altered miRNAs associated with prior PE (cohort 1 and 2),

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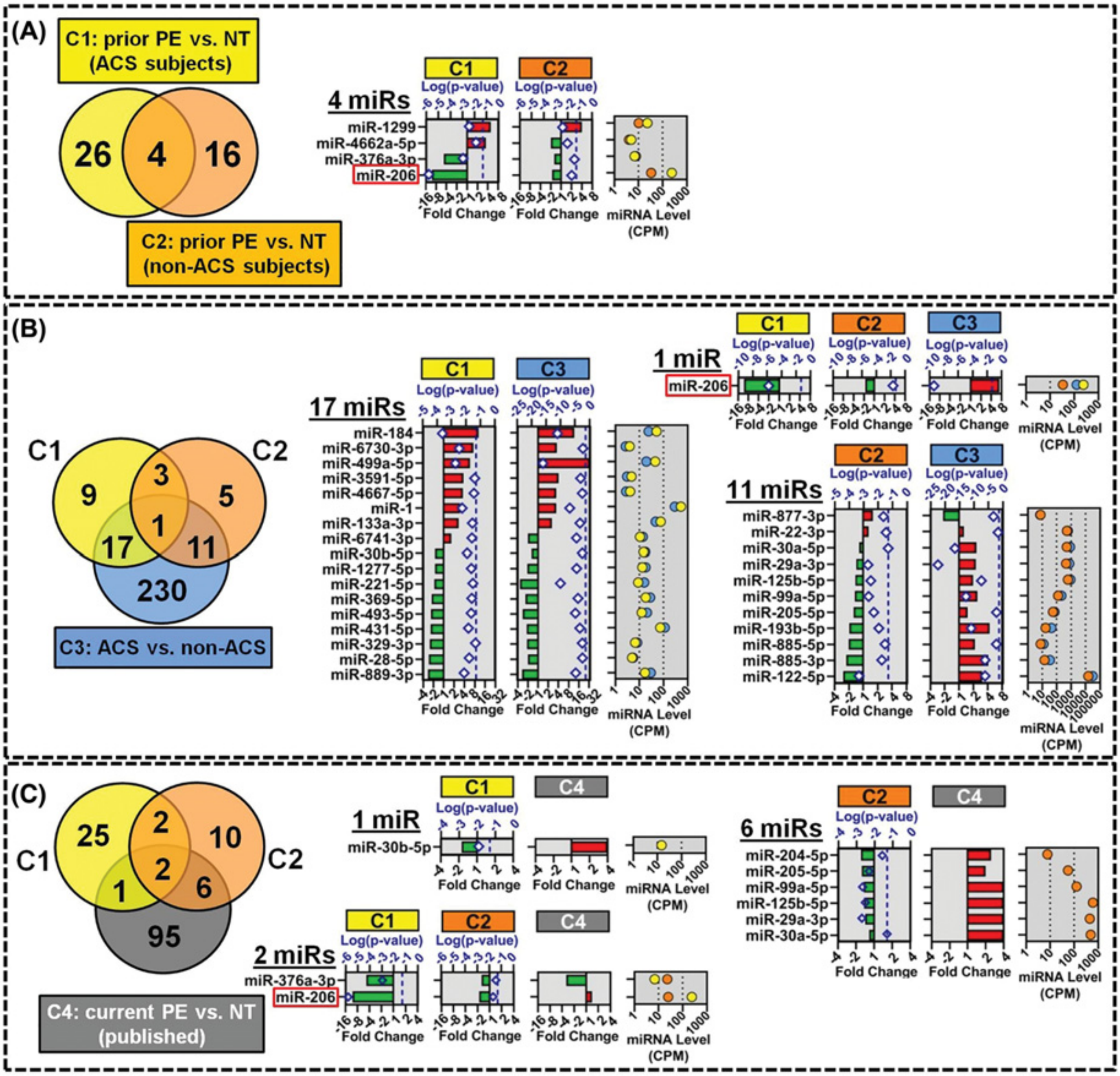

Figure 3. Overlap and identity of circulating miRNAs associated with prior PE or current ACS exposure

(**A**) Venn diagram shows the overlap between 30 and 20 miRNAs that were differentially altered ($P < 0.05$) between women with history of PE versus NT pregnancy in cohorts 1 ($n = 17$–18/group) and 2 ($n = 20$/group), respectively. Right panel shows the identity of the 4 overlapping miRNAs with their direction and magnitude of change (bottom axis scale) and log10 transformed *P*-values (diamonds, top axis scale) separated by exposure cohort (ACS cohort 1, C1; non-ACS cohort 2, C2). Dashed vertical line denotes $P = 0.05$ threshold. Plasma miRNA levels are shown as mean counts per million mapped reads (CPM). Identities of all miRNAs and FDR-adjusted p-values are shown in Supplementary Table S4. (**B**) Venn diagram shows overlap among differentially altered miRNAs associated with prior PE in ACS subjects (C1: 30 miRs $P < 0.05$), prior PE in non-ACS subjects (C2; 20 miRs $P < 0.05$), and current ACS exposure (C3; 259 miRs FDR < 0.05, $n = 35$–40/group). Right panels show the identity, fold-change, *P*-values and CPM plasma level for miRNAs that intersect multiple exposure comparisons. Identities of all miRNAs and FDR-adjusted *P*-values are shown in Supplementary Table S5. (**C**) Venn diagram shows overlap among differentially altered miRNA candidates associated with prior PE in ACS (C1) or non-ACS (C2) subjects, and 104 miRNAs (C4) previously reported to be altered in circulation during PE [10]. Right panels show details on overlapping miRNAs. *P*-values and CPM levels for previously published data are not shown. Identities of all miRNAs and FDR-adjusted *P*-values are shown in Supplementary Table S7.

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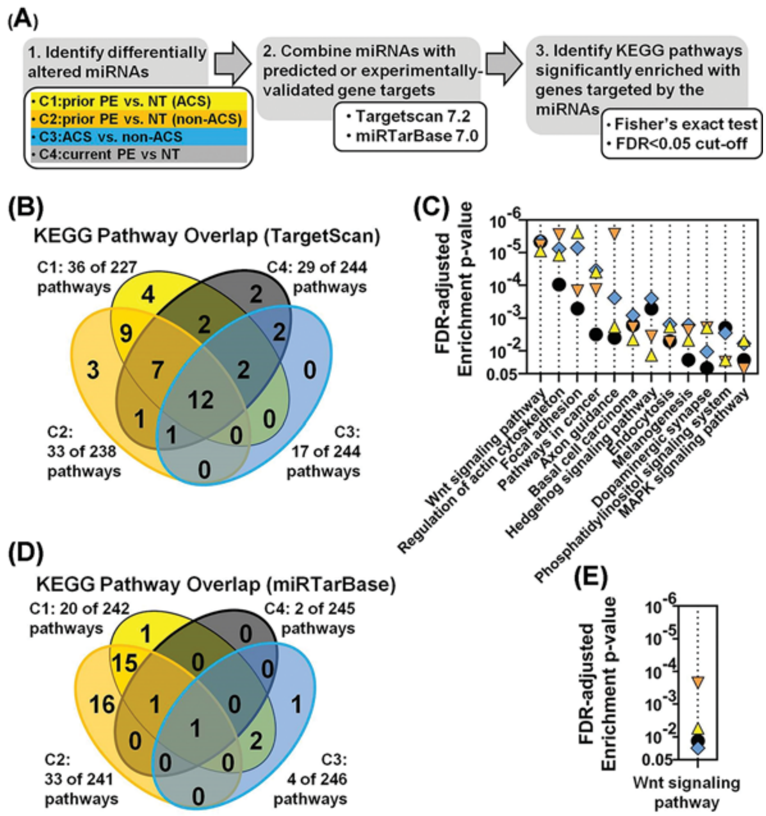

Figure 4. MiRNA target gene integration and pathway enrichment analysis

(**A**) Workflow for identifying KEGG biological pathways significantly enriched with genes targeted by the differentially altered miRNAs. Target integration was conducted with 30 miRs ($P < 0.05$), 20 miRs ($P < 0.05$) and 259 miRs (FDR < 0.05) for cohort 1 (C1: prior PE vs. NT pregnancy in ACS subjects), cohort 2 (C2: prior PE vs. NT pregnancy in non-ACS subjects) and cohort 3 (C3: ACS vs. non-ACS subjects), respectively. A set of 104 miRNAs curated from several previous independent screening studies of miRNA alterations observed in plasma/serum during PE were also assessed (C4: current PE vs. NT pregnancy). Predicted and experimentally validated gene targets were identified using Targetscan7.2 and miRTarBase7.0 databases, respectively. (**B**) Venn diagram showing the number of KEGG pathways significantly enriched (FDR < 0.05) with the predicted gene targets of the altered miRNAs, and the extent of overlap between different exposures. (**C**) Identities of the 12 KEGG pathways at the intersection of all 4 cohorts from panel (B), and associated false discovery rate (FDR)-adjusted enrichment *P*-values for each cohort (C1-triangle; C2-inverted triangle; C3-diamond; C4-circle). (**D**) Venn diagram showing number of pathways significantly enriched (FDR < 0.05) with experimentally-validated gene targets of altered miRNAs. (**E**) Identity of the single pathway at the intersection of all 4 cohorts from panel (D). The identities of all significantly enriched pathways are presented in Supplementary Figures S5 and S6.

current ACS (cohort 3) and current PE (cohort 4) exposure might nevertheless be involved in the regulation of common underlying biological pathways. To address this possibility, differentially altered miRNAs were combined with their predicted gene targets (via TargetScan 7.2 database), and pathway enrichment analysis was performed to identify pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database that were significantly enriched with these gene targets (Figure 4A). From a total of 244 unique pathways, 36 and 33 of these pathways were found to be significantly enriched (FDR < 0.05) with genes targeted by miRNAs associated with prior PE exposure in the ACS and non-ACS cohorts, respectively (Figure 4B). Seventeen pathways were significantly enriched (FDR < 0.05) in relation to current ACS exposure (i.e. cohort 3), and 29 pathways were significantly overrepresented in relation to current PE exposure (cohort 4) (Figure 4B). The majority of pathways associated with a history of PE (i.e. 28 pathways or 78–85% of pathways associated with cohort 1 and 2, respectively) were common between the ACS and non-ACS cohorts, and 12 of these pathways were also implicated in current ACS exposure and current PE exposure, further suggesting the importance of these pathways in the relationship between PE and CVD (Figure 4C). The identities and statistical significance of all biological pathways identified in the pathway enrichment analysis of predicted gene targets are shown in Supplementary Figure S5. Wnt signaling was the most significantly enriched pathway across all four exposure comparisons (based on the mean of the FDR-adjusted *P*-values; Figure 4C). To further prioritize targets, pathway enrichment analysis was also performed after combining the differentially altered miRNAs with a smaller database of experimentally validated gene targets (i.e. miRTarBase7.0 database). In this analysis, a history of preeclampsia in the ACS and non-ACS cohorts was associated with 20 and 33 significantly enriched pathways (FDR < 0.05), respectively, of which the majority (i.e. 17 pathways) were shared between both cohorts (Figure 4D). Four and two KEGG pathways were significantly enriched (FDR < 0.05) with experimentally validated targets of miRNAs related to current ACS and current PE exposure, respectively. Wnt signaling was the only common pathway between all four exposure groups (Figure 4E). Supplementary Figure S6 shows the identities and statistical significance of all pathways identified using the validated gene targets from miRTarBase.

Characterization of miRNA–target gene interactions in Wnt signaling associated with PE and ACS

Since Wnt signaling was the most significantly altered pathway common to both preeclampsia and ACS, we sought to define the network of potential interactions between the altered miRNAs and their predicted/validated gene targets in this pathway. Across all 4 cohorts, there were a total of 2557 and 1676 interactions between the altered miRNAs and their predicted (Targetscan7.2) or experimentally validated (miRTarBase7.0) gene targets, respectively (Figure S7). To further prioritize these miRNA target interactions we focused on overlapping gene targets that were identified in both Targetscan and miRTarBase (Supplementary Table S8) and then identified a further subset of 17 genes that were implicated in all 4 cohorts (Figure 5A). Many of these genes were targeted by multiple miRNAs within the same cohort (Figure 5B) that also showed the same direction of regulation (Figure 5C), suggesting considerable redundancy and/or cooperation in the regulation of Wnt signaling in the cardiovascular sequelae of PE. Among a number of potential effector genes that may be highly regulated in this context were Nuclear Factor of Activated T-cells 5 (NFAT5), Cyclin D2 (CCND2) and Mothers Against Decapentaplegic homolog 2 (SMAD2), which were the most targeted genes showing an average of three different miRNA interactions per gene in cohorts 1 and 2 in relation to a history of PE, 20 miRNA interactions per gene in relation to current PE exposure (cohort 4), and 30 miRNA interactions per gene in relation to ACS exposure (cohort 3) (Figure 5B). The network of experimentally validated (miRTarBase) miRNA interactions with NFAT5, CCND2 and SMAD2 for each cohort is presented in Figure 5C and predicted (Targetscan) miRNA interactions with these genes are presented in Supplementary Figure S8. Of note, each of these genes is a target of miR-206, which was the only miRNA altered in all 4 cohorts.

Discussion

In the present study, we sought to identify miRNAs and biological pathways that may contribute to the long-term cardiovascular sequelae of PE. Our results showed that women with a history of PE exhibit marked perturbations in the circulating levels of miR-206 and other miRNAs, which were also shown to be dysregulated in relation to acute coronary syndrome. These miRNAs are implicated in the regulation of several different KEGG biological pathways, most notably Wnt-signaling, which is known to play important roles in vascular and cardiac function.

Our key finding that circulating levels of miR-206 were differentially altered in women with a history of (or current) PE and in relation to an acute ischemic event suggests that miR-206 may represent an important link between PE and the increased risk of cardiovascular complications that has been reported in many epidemiological studies [1,16]. In women with ACS, a history of PE was associated with ∼10-fold lower plasma levels of miR-206 compared with a history of NT pregnancy, and this was corroborated in a second cohort of women without ACS, though the magnitude of change was more modest at 1.8-fold. If decreased circulating levels of miRNAs reflect underlying changes in cell/tissue expression, a possibility that has been demonstrated elsewhere [17], a number of previous studies suggest how lower miR-206 levels might exert atherogenic effects through distinct cell-type specific mechanisms to elevate the risk of cardiovascular disease. MiR-206 has been shown to be a negative regulator of the Liver X receptor α (LXRα) gene, which serves as a major regulator of lipid homeostasis in the liver by controlling the expression of lipogenic genes [18]. Therefore, the decreased levels of miR-206 could be speculated to facilitate the expression of lipogenic genes that may promote plague formation. Decreased levels of miR-206 have also been associated with increased risk of atherosclerosis via mechanisms involving vascular smooth muscle cells (VSMC). Tao et al. reported decreased VSMC proliferation in cell cultures supplemented with miR-206 mimics, which suggests that a reduction in miR-206 levels might have the opposite effect and contribute to aberrant VSMC proliferation [19]. The authors also reported reduced expression of miR-206 in human atherosclerosis tissue samples [19]. In a separate study, miR-206 was shown to be reduced in the renal artery of rats with hyperlipidemia, which was associated with increased vascular hyperreactivity [20], and the antisense-mediated down-regulation of miR-206 was shown to increase the contractile response of VSMCs in

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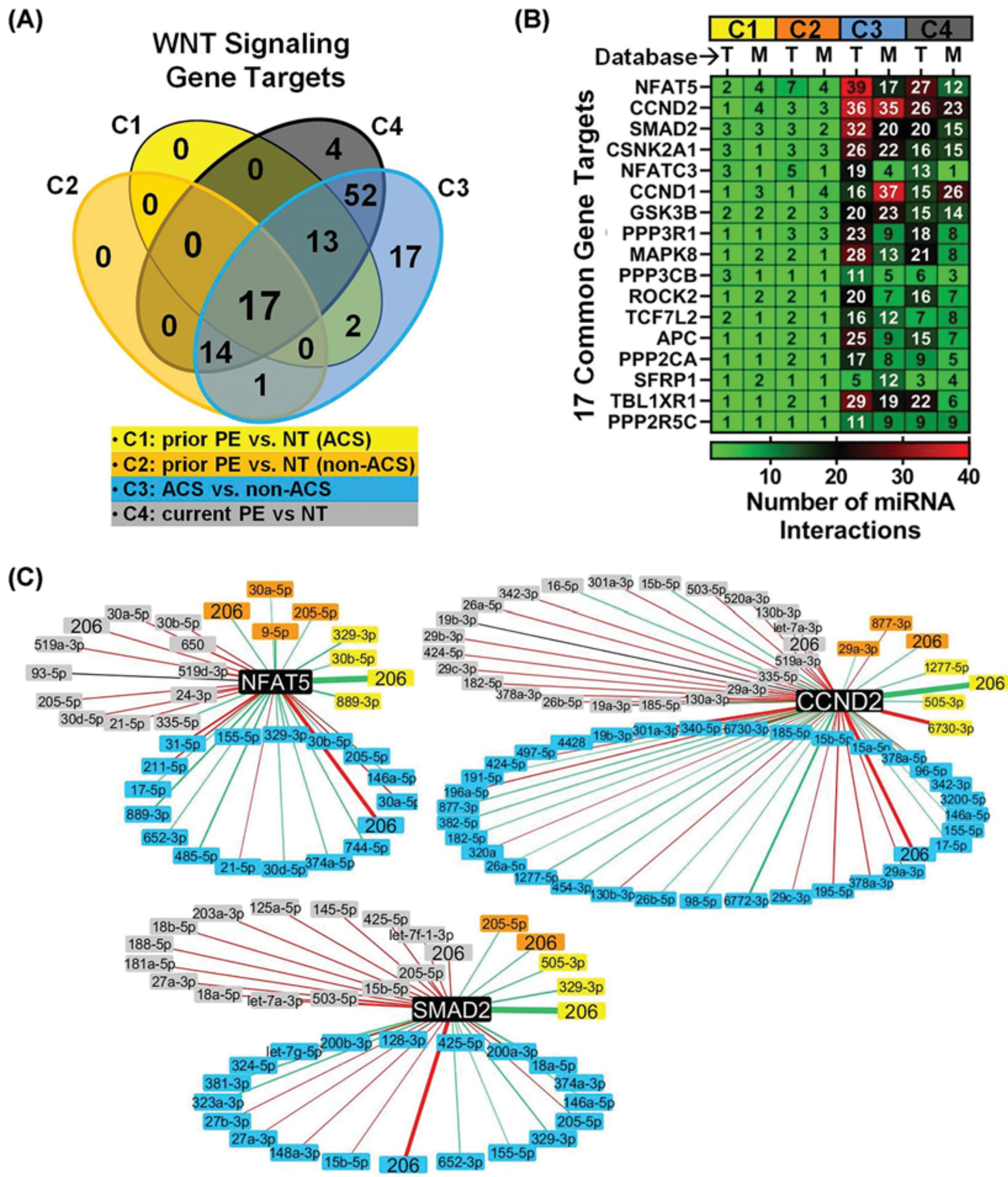

Figure 5. Characterization of miRNA–target gene interactions in Wnt signaling associated with PE and ACS

(**A**) Venn diagram showing the number of miRNA target genes related to Wnt signaling that are shared between different exposure cohorts (C1-C4). Only genes that were identified in both TargetScan (T) and miRTarBase (M) databases were included. (**B**) 17 target genes implicated in Wnt signaling in all 4 exposure cohorts. Heatmap shows the number of miRNAs that target each gene, stratified by database and cohort. (**C**) MiRNA interactions with three highly networked genes (NFAT5, CCND2 and SMAD2) identified in miRTarBase. Nodes represent differentially altered miRNAs color-coded according to cohort and related target genes (black nodes). Red and green lines denote increased and decreased miRNA plasma levels, respectively. Line thickness is scaled to the magnitude of fold change in miRNA level.

cell culture, consistent with a pro-atherogenic effect [20]. Another potential mechanism by which decreased miR-206 levels may contribute to the progression of atherosclerosis is through regulation of macrophage function. Xu et al. reported that overexpression of miR-206 inhibited oxidative stress, while lncRNA-mediated reduction of miR-206 via 'sponging' reversed this effect and exacerbated atherosclerosis events induced by oxidized low-density lipoprotein (oxLDL) in human monocytic/macrophage cells [21]. In yet another study, Vinod et al. showed that the experimental down-regulation of miR-206 decreased cholesterol efflux in mouse peritoneal macrophages [22]. The impairment of

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cholesterol release from macrophages might facilitate the development of foam cells and a pro-thrombotic state that precipitates future ischemic events.

The plasma level of miR-206 has also previously been shown to be altered in women at 28 weeks of gestation who later developed PE (compared with women who experienced a NT pregnancy) [23]. Interestingly, however, these women showed a small 1.4-fold increase in miR-206 plasma levels, which mirrored a 1.4-fold increase in placental tissue that was collected at delivery from another cohort of women [23]. Therefore, we speculate that this previously reported elevation in miR-206 plasma level induced near the time of PE may be a transient effect driven by the increased expression and secretion from the placenta. Of note, we generally observed minimal overlap in the differentially altered miRNAs identified in the present study (in relation to prior PE exposure) as compared with miRNAs previously reported to be altered at the time of PE. This suggests that the majority of miRNA alterations associated with a history of PE in the current study may reflect biological changes that occurred after the resolution of PE.

In the present study, plasma levels of miR-206 were also found to be altered in women with ACS (compared with non-ACS controls); however, levels were elevated with ACS exposure in contrast with the decreased levels observed in women with a history of PE. Since levels of circulating miRNAs may potentially be impacted by changes in release and uptake from many different tissues, we speculate that the observed difference in the direction of change of miR-206 may reflect distinct cell-type specific responses to underlying disease activity. For instance, miR-206 expression has been reported to be up-regulated in the heart tissue of rats with myocardial infarction or diabetic myocardial injury [24,25]. Therefore, marked changes in the myocardium at the time of ACS could potentially mask the vascular and metabolic abnormalities associated with PE described earlier. Of note, several other miRNAs that belong to the myomiR family of miRNAs (i.e. that are abundantly expressed in cardiac and skeletal muscle) were also found to be significantly elevated in plasma in the present study in relation to ACS including miR-499a-5p, miR-1 and miR-133a-3p, which provides support for this theory.

Another key finding of the present study is the identification of Wnt signaling as a potentially important pathway linking PE to increased risk of ACS. This is supported by pathway enrichment analyses conducted with two databases comprising both the predicted and experimentally validated gene targets of the differentially altered miRNA candidates. In addition to providing insights into downstream effector genes that may be regulated by the altered miRNAs, pathway enrichment analysis can offer further insight into shared mechanisms that might not otherwise be discernible from miRNA expression profiles alone. For instance, while only 13–20% of differentially altered miRNAs were common between cohorts 1 and 2 (in relation to prior PE exposure), 78–85% of the biological pathways they are predicted to regulate were shared.

Wnt signaling was the top pathway among 12 pathways that were significantly enriched in the predicted gene targets of miRNAs that were altered in subjects with prior and current PE exposure and ACS, and the only pathway that was also confirmed using the miRTarBase database of experimentally validated gene targets. Of note, a number of prior clinical and preclinical studies have implicated Wnt signaling in both vascular (e.g. atherosclerosis, endothelial dysfunction and vascular calcification) and cardiac disease (e.g. myocardial infarction and heart failure) [26], supporting the relevance of this pathway to pathophysiological activity in both PE and CVD. We also identified potential gene targets that may be regulated by the differentially altered miRNAs, and are also known to have atherogenic activities. In particular, NFAT5, CCND2 and SMAD2 were among the most highly targeted genes with connections to multiple miRNAs (including miR-206), which suggests an increased likelihood for regulation. The transcription factor, NFAT5, has been shown to be up-regulated in relation to atherosclerosis and neointimal hyperplasia [27], consistent with a negative regulatory relationship with miR-206 (which was decreased in women with a history of PE in the present study). Another study reported that up-regulation of NFAT5 may increase the migratory and proliferative activity of VSMCs and promote maladaptive arterial stiffening [28]. CCND2, a cell cycle regulator, has also been implicated in the pathobiology of cardiovascular complications related to diabetes [29,30], with increased levels related to increased endothelial cell proliferation [29]. SMAD2 expression has been reported to be up-regulated in human macrophages from atherosclerotic lesions [31], and to negatively regulate inducible nitric oxide synthase (iNOS) expression in macrophages [32].

Because PE and CVD share several common risk factors and pathophysiological features, a critical question is whether the occurrence of PE merely unmasks this existing risk or contributes directly to future CVD. We attempted to provide insight into this question by investigating prior PE exposure in the context of two cohorts with either a high (cohort 1) or significantly lower (cohort 2) burden of cardiovascular risk factors. While not conclusive, the fact that miR-206 and Wnt signaling were identified in both cohorts suggests these biological features may be affected by PE independent of existing CVD risk factors, and is consistent with the possibility of a causal role in future CVD. Evidence of a possible causal relationship between PE and future CVD has previously been demonstrated in a mouse model of experimentally induced PE with no pre-existing CVD risk factors [33]. Pruthi et al. reported that the blood vessels of exposed mice exhibited an enhanced vascular remodelling response to future injury, which persisted after pregnancy and resolution of PE [33]. While the authors showed the enhanced vascular response in exposed mice could be attributed to an increase in smooth muscle cell (SMC) proliferation and vessel fibrosis, they did not explore specific molecular mechanisms in further detail. However, our finding that miR-206 and Wnt signaling (known to contribute to the regulation of vascular SMC proliferation and/or fibrosis [26]) may be central features linking women with prior PE and future CVD is consistent with and provides a new translational link for these prior experimental findings.

The present study has several strengths that distinguish it from previous studies. To the best of our knowledge, this is the first application of next-generation sequencing to assess circulating miRNAs in women with a history of PE, which in contrast with our previous report that screened only 372 miRNAs by PCR array [11], now provides a comprehensive assessment of all 2578 mature human miRNAs annotated in miRBase version 20. The evaluation of two independent cohorts of women with different cardiovascular backgrounds is another strength of the present study and improvement over our prior study [11], which provides evidence of the generalizability of key findings. Another potential strength of the present study is the relatively large sample sizes used for high-throughput miRNA screening (i.e. 17–20/group). By comparison, the majority of previous high-throughput screening studies that assessed circulating miRNA levels in women during pregnancies complicated by PE used sample sizes of less than 10 subjects per group [10], and two prior RNA-sequencing-based studies employed markedly smaller sample sizes (i.e. $\leq$4 subjects/group) [34,35]. Nevertheless, we also wish to note that while the present study was robustly powered ($\sim$80%) to detect >2.5-fold changes in levels of the majority of miRNAs, the detection of smaller effect sizes would be relatively underpowered and susceptible to false negative errors. This may explain why small alterations (i.e. <2-fold) in several atherogenic miRNAs previously linked to a history of PE (i.e. miR-126-3p, miR-122-5p and miR-146a-5p) [11] were not reproduced in the present study, in addition to other potential factors including differences in methodology and patient heterogeneity.

One key limitation of the present study is the retrospective cross-sectional observational design that limits conclusions about causality, and whether altered miRNAs represent potential mediators or just markers of underlying cardiovascular abnormalities. Although prospective and other clinical study designs with higher evidentiary value would clearly be beneficial, the long latency period of $\sim$20 years between exposure and outcome in this context would be highly impractical to implement. In addition, the identification of common miRNAs and signaling pathways at the intersection of several independent cohorts of women comprising past and current PE and ACS exposures is suggestive, consistent with the known mechanisms by which different sets of miRNAs can regulate overlapping gene networks, and supports the overall biological plausibility of our findings. While it is beyond the scope of the current observational study, our results should help to inform the design of *in vitro* and/or *in vivo* preclinical experiments to further interrogate potential causal mechanisms related to specific miRNA and gene targets.

The present study provides the most comprehensive assessment of circulating miRNAs in women with a history of PE. The biological implications of altered miRNA levels related to a history of PE were interpreted through gene target integration and pathway enrichment analysis in two distinct cohorts of women with and without ACS, and provided novel evidence that miR-206 and Wnt signaling may play important roles in the relationship between PE and future CVD. This helps to address a marked knowledge gap on potential mechanisms underlying the long-term cardiovascular effects of PE, and should help to inform the design of further experimental studies to delineate whether PE is a marker or mediator of CVD. This may lead to improved identification of high risk women who may benefit from closer monitoring and aggressive management of CVD risk factors.

Clinical perspectives

- Epidemiological studies have shown that women with a history of preeclampsia have a higher risk of cardiovascular disease in later life, yet the molecular determinants that contribute to this risk remain poorly defined.
- RNA-sequencing and pathway enrichment analysis showed that alterations in circulating miR-206 and Wnt signaling were associated with acute cardiovascular complications and a history of preeclampsia.
- The present study provides novel insights into miRNAs, target genes and biological pathways that may underlie the long term cardiovascular sequelae of preeclampsia.

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contribution

Provision of clinical specimens: L.P. and C.D.; Study conception and design: K.S., A.K., N.D., L.P. and C.D.; Data analysis and interpretation: K.S., A.K., N.D., D.J.S., L.P. and C.D.; Manuscript preparation and revision: K.S., A.K., N.D., D.J.S., L.P. and C.D.

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Abbreviations

ACS, acute coronary syndrome; CVD, cardiovascular disease; oxLDL, oxidized low-density lipoprotein; PE, preeclampsia; SMC, smooth muscle cell; VSMC, vascular smooth muscle cell.

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Online Data Supplement for

Circulating miR-206 and Wnt-signaling are associated with cardiovascular complications and a history of preeclampsia in women

Running Title: Circulating miRNAs in prior preeclampsia

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Supplementary Methods

Cohort 1: ACS subjects with a history of PE or NT pregnancy

Women with acute coronary syndrome (ACS) and a history of preeclampsia (PE) or normotensive (NT) pregnancy were identified from the GENESIS-PRAXY multicentre cohort study of adults (aged ≤ 55 years) hospitalized with ACS. Detailed methods have been previously described [1]. Participants were recruited between January 2009 and April 2013 from 24 centers across Canada, one in the US and one in Switzerland. All participating sites received ethics approval from their respective ethics review boards, and participants provided written informed consent. Eligible participants were adults aged 18-55 years diagnosed with ACS, and able to provide informed consent. Pregnancy data was collected by detailed self-reported questionnaires of all female participants at study entry. Women were classified as prior PE if they reported either PE or high blood pressure in addition to proteinuria. Women who were unsure about the presence or absence of a pregnancy complication or for whom completion of these questions was incomplete were excluded. The time since last pregnancy was estimated using the age of the youngest biological child, to serve as a proxy for the interval between pregnancy and incident ACS. Venipuncture was performed on all consenting participants within 24 hours of hospital admission for ACS. Whole blood collected in citrate Vacutainers was spun at 4 ˚C at 3000 rpm for 10 minutes and the plasma supernatant removed and frozen at -80 ˚C. A total of 40 subjects were initially selected for miRNA sequencing (n=20 subjects/exposure group) after matching for cardiovascular disease risk factors including hypertension, diabetes, smoking and age. The final sample size was reduced to n=17-18 subjects/exposure group after excluding plasma specimens that failed RNA- and/or library-quality control tests prior to sequencing (further details provided under quality control assays).

Cohort 2: non-ACS subjects with a history of PE or NT pregnancy

Women without ACS and a history of either preeclampsia or normotensive pregnancy were identified from The Cardiovascular Consequences of Pre-eclampsia (COPS) study at the British Heart Foundation Glasgow Cardiovascular Research Centre (BHF GCRC). Women were recruited from multiple sources including the previous Generation Scotland: Scottish Family Health Study [2], the Proteomics in Pre-eclampsia study [3], patients who attended blood pressure clinics and friends and colleagues of participants who contacted us with interest in participating. The study was approved by the West of Scotland Research Ethics Committee 3 (Reference 12/WS/0306), and participants provided written informed consent. The index pregnancy was defined as the first pregnancy in normotensive women and the first pre-eclamptic pregnancy in those with pre-eclampsia. Women were excluded if they were >60 years old, already had established cardiovascular disease or if they were unable to give informed consent. Participants completed a questionnaire asking for obstetric history, past medical history, drug history, smoking history and family history. Blood samples were taken from the antecubital fossa using a standard tourniquet and Vacutainer system, and centrifuged at 4 °C at 2500 rpm for 15 minutes and plasma supernatant removed and frozen -80 °C. A total of 40 subjects (n=20 subjects/exposure group) matched on hypertension, diabetes, and age were selected for miRNA sequencing. All specimens passed pre-sequencing quality control tests.

Cohort 3: ACS and non-ACS subjects

The comparison of all women in cohort 1 (n=35 total ACS subjects) versus cohort 2 (n=40 total non-ACS subjects) was used to assess the impact of ACS on circulating miRNA levels.

Cohort 4: Women with PE or NT pregnancy
Information for this cohort was derived from six prior independent studies of preeclamptic women (with no ACS) reported in the systematic review by Sheikh et al. [4]. A total of 104 circulating miRNAs were identified via high-throughput screening methods as differentially altered in plasma, serum or whole blood between women with preeclampsia versus normotensive pregnancy.

RNA isolation
Total RNA including small RNAs <200 nt in size were purified from a fixed volume of 200 µL of citrate-plasma using the miRNeasy Serum/Plasma kit (i.e., phenol/guanidine-based sample lysis and silica-column-based purification) according to manufacturer instructions (Qiagen Genomic Services; cat #217184). Cel-miR-39 was added as a spike-in control during RNA purification for downstream quality control assessment. RNA was eluted with 14 µL of RNase-free water.

RNA Quality Control
Because the concentration of eluted total RNA purified from plasma is typically too low to assess RNA purity and integrity by standard methods involving UV absorbance ratios and Agilent Bioanalyzer, the quality of the extracted total RNA was assessed using a miScript miRNA QC PCR array (Qiagen Genomic Services; cat #MIHS-989ZE). The array contains primer assays for several types of external spike-in controls to assess variations during RNA extraction (i.e. cel-miR-39), an indicator to monitor relative efficiency and potential inhibition of the reverse transcription reaction (i.e., miRTC) and an indicator to monitor relative efficiency and potential inhibition of the downstream PCR reaction (i.e., PPC). In addition, primer assays for several endogenous miRNAs (i.e., miR-16, miR-21 and miR-191) are included as positive controls since these miRNAs are ubiquitously expressed across many different biologic specimens including body fluids, and several endogenous small nuclear/nucleolar RNAs (i.e., SNORD61, SNORD95, and SNORD96A) are included as negative controls (or markers of cellular contamination) since these are typically expressed abundantly in cells, but poorly in body fluids. During this quality assessment, specimens from 4 subjects in cohort 1 (including 1 prior PE subject and 3 prior normotensive pregnancy subjects) showed evidence of poor RNA quality (Figure S1), and therefore were not sequenced after the corresponding sequencing libraries showed poor yields (noted below; Figure S2). One additional sample from cohort 1 (a subject with prior PE) was excluded prior to sequencing because the qPCR assay showed evidence of cellular contamination (Figure S1). Overall, the results of the quality control assays suggested that the quality and quantity of extracted RNA was generally comparable between samples, with the above noted exceptions.

Library Construction, Quality Control and Sequencing
A fixed volume of 5 µL of the extracted total RNA was used for NGS library construction using the QIAseq miRNA library kit according to manufacturer instructions (Qiagen Genomic Services; cat #331505). In brief, specifically designed 3' and 5' adapters were sequentially ligated to mature miRNAs. The ligated miRNAs were then reverse-transcribed to generate cDNA using a reverse-transcription (RT) primer with a unique molecular index (UMI) tag. Of note, the kit is designed to minimize adapter dimers and hy4 Y RNA contamination during cDNA cleanup. The

cDNA library was then amplified via PCR (21 cycles) and subsequently purified prior to quality control assessment. Library qualification was conducted with High Sensitivity DNA chips on an Agilent Bioanalyzer 2100 instrument (or Agilent TapeStation 4200) to confirm proper library size and yield (Figure S2). In addition, library concentrations were quantified on a Qubit device using the Quant-iT dsDNA High Sensitivity Assay Kit. Libraries were pooled in equimolar ratios and quantified using qPCR. Library pools were then sequenced on an Illumina NextSeq 500 sequencer using NextSeq 500 Mid Output Reagent Cartridge v2 (75 cycles). Raw data was de-multiplexed and FASTQ data files for each sample were generated using the bcl2fastq software (Illumina Inc.), and checked for quality using the FastQC tool.

Sequence Trimming, UMI consolidation and read mapping
Trimming of library and sequencing adaptors was performed after sequencing with Cutadapt (1.11), and reads were analyzed for the presence of the unique molecular index (UMI) tags that were added during library construction. On average, 22.2 million raw reads were obtained per sample, and 10.2 million reads per sample remained after excluding reads that were missing adaptors, too short (insert sequence <16 nt) or did not contain the minimal length UMI tag (i.e., 10 nt). All reads containing identical insert sequence and UMI sequence (insert-UMI pair) were collapsed into a single read (to correct for potential library amplification bias and improve miRNA quantification) and passed into the analysis pipeline. On average, 2.3 million UMI-corrected reads were obtained per sample. Bowtie2 (2.2.2) was used for mapping reads based on the criterion of having a perfect sequence match to the reference sequence. Reads were aligned to miRBase 20 and/or human GRCh37 reference genome. Conversion of raw reads to UMI-corrected mapped counts was conducted by Qiagen Genomic Services.

miRNA-gene target integration and Pathway Enrichment Analysis
miRNA-gene target integration and pathway enrichment analysis were performed in Partek Genomics Suite using default settings in the miRNA integration and biological interpretation pathway analysis features. Differentially-altered miRNA candidates were combined with predicted gene targets using Targetscan 7.2 [5] (conserved miRNA sites database; 1,468,778 records) or experimentally-validated gene targets using miRTarBase 7.0 (422 517 curated miRNA-target interactions) [6], and pathway enrichment determined with a Fisher's exact test.

Pre and post study sample size and power estimations
Study sample sizes of n=20/group were estimated *a priori* to achieve 80% power to detect a >2-fold change in miRNA level with a bonferonni-adjusted alpha value of 0.0001 assuming 500 detectable miRNAs for differential analysis and a coefficient of variation (CV) of 0.47. This variation in miRNA levels was estimated using the median CV from 235 miRs previously measured by (RT)-qPCR array in a similar set of human plasma samples [7], and extrapolated into a common standard deviation assuming mean miR levels between 10-20 counts. These initial sample size calculations were performed with the online calculator at https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html. Post hoc estimations of the dispersion in miRNA count levels from the completed sequencing experiment, and sample size-power relationships were calculated using the RNASeqSampleSize software package in R language and online interface at http://cqs.mc.vanderbilt.edu/shiny/RnaSeqSampleSize/.[8]

Statistical Analysis

All statistical tests comparing cohort characteristics were performed in Graphpad Prism 8.0. Data normality was assessed using the D'Agostino Pearson test. Differences between exposure groups for continuous data was assessed using a Mann-Whitney or unpaired t-test, depending on data normality as appropriate. Differences in categorical variables were assessed via Fisher's exact test. Data are presented as mean ± standard deviation (SD) unless otherwise specified. Differential expression analysis was conducted on the subset of samples related to the specific groups being compared, using UMI-corrected miRNA counts as input into the EdgeR statistical software package (Bioconductor, http://www.bioconductor.org/). Data was preprocessed to exclude poorly detectable miRNAs such that the sum of the counts per million mapped reads (CPM) for each miRNA in all samples pertaining to the comparison subset were > 10. The filtered data was normalized using the trimmed mean of M-values (TMM) normalization method in EdgeR to compensate for sample specific effects related to variations in sequencing depth and RNA composition. MiRNA levels in some figures are presented simply as counts per million mapped reads (CPM), which only corrects for differences in sequencing depth between samples. P-values and Benjamini-Hochberg false discovery rate (FDR)-correct p values for differentially altered miRNAs were calculated with an exact test assuming a negative binomial distribution in EdgeR. Principal component analysis and unsupervised hierarchical clustering and heatmap construction was performed with default parameters in Partek Genomics Suite 7.2 using log2 transformed TMM-normalized miRNA counts (with offset 1 to account for 0 values).

Table S1. Differential expression analysis of 427 miRNAs detected in plasma from women with acute coronary syndrome (cohort 1) and a history of preeclampsia (PE, n=18) versus normotensive (NT, n=17) pregnancy. MiRNA levels are expressed as mean counts per million mapped reads (CPM). MiRNAs are listed in descending order of statistical significance.

miRNA	Fold Change (PE/NT)	p value	FDR-adjusted p value	miR level (CPM)
miR-206	-10.6	1.64E-06	6.98E-04	242
miR-1292-5p	-3.6	8.28E-05	1.77E-02	8
miR-184	10.3	2.35E-04	3.35E-02	52
miR-376a-3p	-4.7	1.10E-03	1.17E-01	7
miR-499a-5p	5.8	1.85E-03	1.58E-01	45
miR-218-5p	5.4	2.24E-03	1.60E-01	7
miR-6730-3p	7.3	3.46E-03	1.96E-01	4
miR-1299	4.9	3.67E-03	1.96E-01	23
miR-1	3.0	5.46E-03	2.59E-01	531
miR-889-3p	-2.9	7.52E-03	3.21E-01	17
miR-30b-5p	-1.8	1.23E-02	4.45E-01	15
miR-4662a-5p	3.5	1.33E-02	4.45E-01	5
miR-136-3p	-2.7	1.35E-02	4.45E-01	10
miR-431-5p	-2.4	1.47E-02	4.49E-01	73
miR-874-5p	3.3	1.64E-02	4.54E-01	4
miR-28-5p	-2.7	1.70E-02	4.54E-01	5
miR-6767-5p	2.7	1.95E-02	4.91E-01	7
miR-505-3p	-1.9	2.22E-02	5.01E-01	9
miR-493-5p	-2.3	2.29E-02	5.01E-01	13
miR-1277-5p	-1.8	2.35E-02	5.01E-01	13
miR-369-5p	-2.2	2.54E-02	5.07E-01	18
miR-6741-3p	1.7	2.61E-02	5.07E-01	11
miR-133a-3p	2.7	2.78E-02	5.16E-01	76
miR-221-5p	-1.9	3.03E-02	5.39E-01	9
miR-2355-3p	-1.9	3.54E-02	5.91E-01	7
miR-4667-5p	3.6	3.60E-02	5.91E-01	4
miR-3591-5p	3.8	4.01E-02	6.34E-01	5
miR-769-5p	-1.5	4.20E-02	6.40E-01	27
miR-329-3p	-2.5	4.56E-02	6.54E-01	7
miR-202-3p	2.9	4.68E-02	6.54E-01	6
miR-130b-3p	1.5	5.16E-02	6.54E-01	47
miR-27a-5p	2.0	5.32E-02	6.54E-01	5
miR-335-3p	-1.9	5.48E-02	6.54E-01	15

miR-195-5p	1.5	5.51E-02	6.54E-01	99
miR-378c	1.6	5.79E-02	6.54E-01	15
miR-5189-3p	2.3	5.89E-02	6.54E-01	6
miR-378a-3p	1.4	6.01E-02	6.54E-01	465
miR-150-3p	1.6	6.19E-02	6.54E-01	21
miR-493-3p	-2.1	6.62E-02	6.54E-01	9
miR-128-3p	-1.3	6.67E-02	6.54E-01	324
miR-29c-3p	1.3	6.82E-02	6.54E-01	1241
miR-339-5p	-1.4	6.95E-02	6.54E-01	117
miR-10b-3p	2.0	7.25E-02	6.54E-01	9
miR-432-5p	-1.8	7.28E-02	6.54E-01	434
miR-628-3p	-1.4	7.42E-02	6.54E-01	61
miR-223-3p	-1.5	7.50E-02	6.54E-01	5871
miR-369-3p	-1.8	7.55E-02	6.54E-01	19
miR-6859-5p	2.0	7.63E-02	6.54E-01	5
miR-423-3p	-1.4	7.72E-02	6.54E-01	553
miR-4775	-2.4	7.99E-02	6.54E-01	4
miR-381-3p	-2.0	8.08E-02	6.54E-01	34
miR-548a-3p	-2.0	8.14E-02	6.54E-01	6
miR-335-5p	1.5	8.34E-02	6.54E-01	397
miR-3127-5p	1.9	8.73E-02	6.54E-01	8
miR-382-5p	-1.8	9.17E-02	6.54E-01	262
miR-5187-5p	-1.8	9.32E-02	6.54E-01	8
miR-3688-3p	-1.5	9.64E-02	6.54E-01	14
miR-181c-3p	-2.0	9.78E-02	6.54E-01	7
miR-4714-3p	2.4	9.79E-02	6.54E-01	5
miR-9-5p	-2.4	9.89E-02	6.54E-01	12
miR-26b-3p	1.7	9.90E-02	6.54E-01	9
miR-4433b-5p	-1.7	1.00E-01	6.54E-01	955
miR-205-5p	-1.5	1.03E-01	6.54E-01	90
miR-1250-5p	2.6	1.03E-01	6.54E-01	4
miR-491-5p	-1.7	1.05E-01	6.54E-01	12
miR-18a-3p	-1.4	1.07E-01	6.54E-01	39
miR-143-5p	1.7	1.09E-01	6.54E-01	16
miR-664b-5p	1.5	1.09E-01	6.54E-01	12
miR-654-3p	-1.8	1.11E-01	6.54E-01	42
miR-208b-3p	2.5	1.12E-01	6.54E-01	88
miR-30d-5p	-1.2	1.12E-01	6.54E-01	12944
miR-376c-3p	-2.2	1.14E-01	6.54E-01	7
miR-4669	-2.3	1.15E-01	6.54E-01	9
miR-1304-3p	-1.5	1.15E-01	6.54E-01	14

miR-652-3p	-1.4	1.16E-01	6.54E-01	36
miR-125b-1-3p	2.1	1.17E-01	6.54E-01	8
miR-574-3p	1.5	1.19E-01	6.54E-01	74
miR-3679-5p	1.9	1.20E-01	6.54E-01	8
miR-3613-3p	-1.8	1.21E-01	6.54E-01	6
miR-323a-3p	-1.7	1.26E-01	6.56E-01	21
miR-379-5p	-1.7	1.26E-01	6.56E-01	53
miR-885-5p	-1.8	1.28E-01	6.56E-01	18
miR-361-3p	-1.3	1.28E-01	6.56E-01	351
miR-145-3p	2.2	1.31E-01	6.56E-01	6
miR-127-3p	-1.9	1.34E-01	6.56E-01	10
miR-4738-3p	1.9	1.36E-01	6.56E-01	6
miR-6721-5p	-1.7	1.36E-01	6.56E-01	7
miR-3168	1.6	1.38E-01	6.56E-01	36
miR-1237-3p	-1.7	1.38E-01	6.56E-01	7
miR-296-5p	1.4	1.38E-01	6.56E-01	22
miR-452-5p	1.8	1.44E-01	6.69E-01	12
miR-338-3p	-1.5	1.45E-01	6.69E-01	17
miR-576-5p	-1.3	1.46E-01	6.69E-01	171
miR-1301-3p	-1.5	1.47E-01	6.69E-01	26
miR-339-3p	-1.3	1.57E-01	6.97E-01	38
miR-1307-3p	-1.3	1.62E-01	6.97E-01	734
miR-17-5p	-1.3	1.64E-01	6.97E-01	117
let-7d-5p	1.2	1.64E-01	6.97E-01	1828
miR-425-5p	-1.2	1.64E-01	6.97E-01	4293
miR-23a-5p	1.9	1.65E-01	6.97E-01	4
miR-27a-3p	1.2	1.66E-01	6.97E-01	201
miR-1273h-3p	-1.6	1.67E-01	6.97E-01	10
miR-196a-5p	-1.7	1.75E-01	7.14E-01	16
miR-151a-3p	-1.3	1.76E-01	7.14E-01	2003
miR-548n	-1.7	1.76E-01	7.14E-01	6
miR-106b-5p	-1.4	1.77E-01	7.14E-01	25
miR-4732-3p	-1.3	1.84E-01	7.21E-01	208
miR-29b-3p	1.2	1.85E-01	7.21E-01	152
miR-126-3p	-1.2	1.88E-01	7.21E-01	9787
miR-93-3p	-1.3	1.88E-01	7.21E-01	32
miR-30a-3p	1.3	1.92E-01	7.21E-01	58
miR-323b-3p	-1.6	1.93E-01	7.21E-01	31
miR-382-3p	-1.7	1.94E-01	7.21E-01	13
miR-1255b-5p	-1.3	1.94E-01	7.21E-01	45
miR-95-3p	1.5	1.96E-01	7.21E-01	18

miR-425-3p	-1.2	1.97E-01	7.21E-01	75
miR-409-3p	-1.6	1.98E-01	7.21E-01	346
miR-208a-3p	2.1	1.99E-01	7.21E-01	5
miR-22-5p	-1.5	2.01E-01	7.23E-01	10
miR-199a-5p	-1.5	2.07E-01	7.31E-01	17
miR-30a-5p	1.3	2.07E-01	7.31E-01	1276
miR-485-5p	-1.5	2.13E-01	7.41E-01	41
miR-144-5p	-1.3	2.13E-01	7.41E-01	197
miR-30d-3p	1.7	2.16E-01	7.41E-01	6
miR-215-5p	1.6	2.19E-01	7.41E-01	82
miR-411-5p	-1.6	2.20E-01	7.41E-01	13
miR-222-3p	1.2	2.20E-01	7.41E-01	113
miR-129-5p	-1.5	2.26E-01	7.52E-01	7
miR-132-3p	1.3	2.27E-01	7.52E-01	46
miR-671-5p	-1.3	2.32E-01	7.59E-01	57
miR-10b-5p	1.3	2.33E-01	7.59E-01	1401
miR-29a-3p	1.2	2.36E-01	7.59E-01	1092
miR-1226-3p	-1.3	2.38E-01	7.59E-01	10
miR-548l	-1.3	2.40E-01	7.59E-01	9
miR-485-3p	-1.6	2.41E-01	7.59E-01	70
miR-7976	-1.4	2.42E-01	7.59E-01	9
miR-4710	2.1	2.47E-01	7.69E-01	4
miR-15a-5p	1.2	2.58E-01	7.98E-01	599
miR-21-3p	-1.3	2.61E-01	8.03E-01	10
miR-23b-3p	1.2	2.64E-01	8.06E-01	154
miR-148a-3p	-1.2	2.67E-01	8.08E-01	3832
miR-200b-3p	-1.6	2.71E-01	8.15E-01	34
miR-340-5p	-1.2	2.82E-01	8.40E-01	173
miR-625-5p	-1.4	2.84E-01	8.40E-01	8
miR-625-3p	-1.3	2.85E-01	8.40E-01	321
miR-584-5p	-1.2	2.94E-01	8.54E-01	723
miR-494-3p	1.5	2.95E-01	8.54E-01	12
miR-23b-5p	1.5	2.97E-01	8.54E-01	9
miR-377-3p	-1.4	3.02E-01	8.54E-01	6
miR-1468-5p	-1.3	3.07E-01	8.54E-01	9
miR-15b-5p	1.2	3.08E-01	8.54E-01	672
miR-3942-5p	-1.5	3.09E-01	8.54E-01	4
miR-421	1.2	3.09E-01	8.54E-01	47
miR-191-3p	-1.3	3.09E-01	8.54E-01	13
miR-107	1.2	3.12E-01	8.54E-01	414
miR-3187-3p	1.3	3.12E-01	8.54E-01	25

miR-628-5p	-1.5	3.15E-01	8.57E-01	13
miR-33a-5p	1.3	3.19E-01	8.61E-01	9
miR-548d-5p	1.3	3.25E-01	8.68E-01	16
miR-5583-3p	-1.7	3.25E-01	8.68E-01	5
miR-106b-3p	-1.2	3.32E-01	8.77E-01	575
miR-548j-5p	-1.3	3.33E-01	8.77E-01	31
miR-4433b-3p	1.7	3.40E-01	8.90E-01	16
miR-24-3p	-1.1	3.42E-01	8.90E-01	583
let-7e-5p	1.1	3.44E-01	8.91E-01	214
miR-214-3p	1.6	3.49E-01	8.93E-01	7
miR-151a-5p	-1.2	3.52E-01	8.93E-01	20
miR-636	-1.2	3.56E-01	8.93E-01	16
miR-181a-5p	-1.2	3.56E-01	8.93E-01	1338
miR-3605-5p	1.3	3.63E-01	8.93E-01	8
miR-3065-5p	1.4	3.63E-01	8.93E-01	9
miR-1908-5p	-1.2	3.66E-01	8.93E-01	49
miR-197-3p	-1.2	3.67E-01	8.93E-01	243
miR-374a-5p	-1.2	3.67E-01	8.93E-01	86
miR-671-3p	-1.3	3.68E-01	8.93E-01	16
miR-1976	-1.2	3.69E-01	8.93E-01	42
miR-3615	-1.2	3.70E-01	8.93E-01	744
miR-542-3p	-1.2	3.73E-01	8.94E-01	20
miR-337-5p	-1.4	3.78E-01	9.01E-01	5
miR-409-5p	-1.6	3.80E-01	9.01E-01	5
miR-199a-3p	-1.2	3.88E-01	9.13E-01	1591
miR-190a-5p	1.2	3.94E-01	9.13E-01	95
miR-34a-5p	1.3	3.96E-01	9.13E-01	56
miR-134-5p	-1.3	3.97E-01	9.13E-01	152
miR-3158-3p	-1.3	3.98E-01	9.13E-01	15
miR-664a-3p	-1.3	3.99E-01	9.13E-01	9
miR-146b-5p	-1.2	4.04E-01	9.13E-01	335
miR-509-3p	1.6	4.05E-01	9.13E-01	5
miR-6514-5p	-1.4	4.07E-01	9.13E-01	6
miR-370-3p	-1.4	4.08E-01	9.13E-01	32
miR-5010-5p	-1.2	4.10E-01	9.13E-01	8
miR-19a-3p	1.1	4.12E-01	9.13E-01	140
miR-181d-5p	-1.3	4.13E-01	9.13E-01	14
miR-454-3p	1.2	4.17E-01	9.16E-01	90
miR-342-3p	-1.1	4.19E-01	9.16E-01	3341
miR-140-3p	1.2	4.21E-01	9.16E-01	963
miR-6515-3p	-1.3	4.23E-01	9.16E-01	5

miR-487b-3p	-1.4	4.26E-01	9.16E-01	8
miR-589-5p	-1.2	4.27E-01	9.16E-01	21
miR-92a-3p	-1.1	4.30E-01	9.19E-01	127379
miR-328-3p	-1.2	4.34E-01	9.21E-01	648
miR-199b-3p	-1.2	4.39E-01	9.26E-01	1256
miR-100-5p	-1.2	4.40E-01	9.26E-01	201
miR-484	-1.2	4.48E-01	9.37E-01	2247
miR-143-3p	1.2	4.50E-01	9.38E-01	1510
miR-125b-2-3p	1.3	4.54E-01	9.38E-01	18
miR-3940-3p	-1.3	4.58E-01	9.38E-01	7
miR-6852-5p	-1.3	4.58E-01	9.38E-01	22
miR-345-5p	-1.2	4.66E-01	9.38E-01	28
miR-25-5p	1.2	4.66E-01	9.38E-01	40
miR-125a-3p	1.5	4.67E-01	9.38E-01	6
miR-6793-5p	-1.2	4.67E-01	9.38E-01	5
miR-497-5p	1.4	4.68E-01	9.38E-01	8
miR-1224-5p	1.2	4.73E-01	9.40E-01	10
miR-3177-3p	-1.3	4.74E-01	9.40E-01	8
miR-4435	1.5	4.75E-01	9.40E-01	5
miR-326	-1.3	4.78E-01	9.40E-01	24
miR-22-3p	1.1	4.91E-01	9.49E-01	685
miR-3613-5p	1.1	4.92E-01	9.49E-01	381
miR-744-5p	-1.2	4.97E-01	9.49E-01	254
miR-99b-5p	-1.1	4.98E-01	9.49E-01	297
miR-223-5p	-1.1	5.01E-01	9.49E-01	349
miR-122-3p	-1.3	5.03E-01	9.49E-01	6
miR-4732-5p	1.1	5.05E-01	9.49E-01	260
miR-1247-5p	1.3	5.05E-01	9.49E-01	10
miR-532-5p	-1.1	5.09E-01	9.49E-01	289
miR-122-5p	-1.2	5.24E-01	9.49E-01	50853
miR-9-3p	1.4	5.27E-01	9.49E-01	5
miR-5001-3p	-1.2	5.30E-01	9.49E-01	9
miR-181c-5p	-1.2	5.32E-01	9.49E-01	9
miR-18b-3p	-1.2	5.33E-01	9.49E-01	6
miR-769-3p	1.2	5.33E-01	9.49E-01	7
miR-660-5p	-1.1	5.34E-01	9.49E-01	470
miR-181b-5p	-1.1	5.39E-01	9.49E-01	197
miR-203a	-1.3	5.41E-01	9.49E-01	272
miR-148b-5p	-1.2	5.45E-01	9.49E-01	13
miR-30e-3p	-1.1	5.46E-01	9.49E-01	112
miR-155-5p	1.1	5.47E-01	9.49E-01	367

miR-210-3p	1.2	5.47E-01	9.49E-01	22
miR-424-5p	-1.2	5.51E-01	9.49E-01	13
miR-502-3p	1.1	5.54E-01	9.49E-01	46
miR-192-5p	-1.1	5.61E-01	9.49E-01	972
miR-324-3p	-1.1	5.61E-01	9.49E-01	32
miR-103a-3p	-1.1	5.64E-01	9.49E-01	3992
miR-6786-3p	-1.2	5.64E-01	9.49E-01	7
miR-651-5p	1.3	5.65E-01	9.49E-01	15
let-7f-5p	1.1	5.65E-01	9.49E-01	15727
miR-181a-3p	1.1	5.65E-01	9.49E-01	26
miR-31-5p	1.4	5.69E-01	9.49E-01	10
miR-96-5p	1.1	5.70E-01	9.49E-01	144
miR-598-3p	-1.1	5.72E-01	9.49E-01	37
miR-320d	-1.1	5.75E-01	9.49E-01	68
miR-3120-3p	-1.2	5.77E-01	9.49E-01	4
miR-18a-5p	-1.1	5.86E-01	9.49E-01	26
miR-378i	1.2	5.88E-01	9.49E-01	10
miR-3138	1.3	5.90E-01	9.49E-01	8
miR-1260b	-1.2	5.91E-01	9.49E-01	10
miR-26b-5p	-1.1	5.94E-01	9.49E-01	3842
miR-627-5p	-1.2	5.95E-01	9.49E-01	8
miR-181a-2-3p	1.1	5.96E-01	9.49E-01	40
miR-200c-3p	-1.2	5.96E-01	9.49E-01	91
miR-4742-3p	-1.1	5.99E-01	9.49E-01	12
miR-483-5p	1.2	6.01E-01	9.49E-01	550
miR-10a-5p	1.1	6.01E-01	9.49E-01	978
miR-1246	-1.1	6.03E-01	9.49E-01	35
miR-193b-5p	1.2	6.03E-01	9.49E-01	78
miR-186-5p	-1.1	6.08E-01	9.49E-01	277
miR-4428	2.3	6.08E-01	9.49E-01	3
miR-125a-5p	1.1	6.08E-01	9.49E-01	2160
miR-6807-5p	1.3	6.11E-01	9.49E-01	4
miR-505-5p	-1.2	6.13E-01	9.49E-01	14
miR-126-5p	1.1	6.14E-01	9.49E-01	3411
miR-199b-5p	-1.2	6.14E-01	9.49E-01	8
miR-320a	1.1	6.15E-01	9.49E-01	2154
miR-1260a	-1.2	6.18E-01	9.49E-01	8
miR-374b-5p	-1.1	6.20E-01	9.49E-01	23
miR-140-5p	-1.1	6.22E-01	9.49E-01	46
miR-4533	-2.0	6.23E-01	9.49E-01	4
miR-7706	1.2	6.25E-01	9.49E-01	16

miR-664a-5p	1.1	6.27E-01	9.49E-01	128
miR-629-5p	-1.1	6.29E-01	9.49E-01	219
miR-3605-3p	-1.1	6.30E-01	9.49E-01	40
let-7a-3p	1.2	6.30E-01	9.49E-01	9
miR-1306-5p	-1.1	6.31E-01	9.49E-01	260
miR-365a-3p	-1.2	6.39E-01	9.49E-01	14
miR-99b-3p	-1.2	6.39E-01	9.49E-01	9
miR-26a-5p	-1.1	6.39E-01	9.49E-01	4679
miR-676-3p	1.5	6.42E-01	9.49E-01	5
miR-193a-5p	1.1	6.46E-01	9.49E-01	305
miR-1270	1.1	6.47E-01	9.49E-01	18
let-7c-5p	1.1	6.52E-01	9.49E-01	765
miR-363-3p	-1.1	6.56E-01	9.49E-01	510
miR-196b-5p	-1.1	6.56E-01	9.49E-01	224
miR-194-5p	-1.1	6.61E-01	9.49E-01	461
miR-7-5p	1.1	6.61E-01	9.49E-01	659
miR-146b-3p	-1.1	6.62E-01	9.49E-01	14
miR-500a-3p	1.1	6.62E-01	9.49E-01	39
miR-941	-1.1	6.62E-01	9.49E-01	139
miR-27b-3p	1.1	6.67E-01	9.51E-01	771
miR-32-5p	1.1	6.68E-01	9.51E-01	535
let-7a-5p	1.1	6.72E-01	9.52E-01	27324
miR-127-5p	-1.5	6.73E-01	9.52E-01	5
miR-125b-5p	-1.1	6.82E-01	9.55E-01	1302
miR-1294	1.1	6.83E-01	9.55E-01	70
miR-766-3p	1.2	6.84E-01	9.55E-01	7
miR-99a-5p	-1.1	6.89E-01	9.55E-01	334
miR-6780a-5p	1.1	6.96E-01	9.55E-01	6
miR-548at-5p	-1.1	6.98E-01	9.55E-01	7
miR-885-3p	1.2	7.02E-01	9.55E-01	61
miR-6862-5p	1.4	7.03E-01	9.55E-01	5
miR-454-5p	1.1	7.04E-01	9.55E-01	21
miR-1343-3p	-1.1	7.05E-01	9.55E-01	8
miR-15b-3p	-1.1	7.06E-01	9.55E-01	38
miR-30c-5p	1.1	7.07E-01	9.55E-01	62
miR-365b-3p	-1.1	7.08E-01	9.55E-01	12
miR-101-3p	1.1	7.08E-01	9.55E-01	4921
miR-28-3p	-1.1	7.10E-01	9.55E-01	359
miR-139-5p	-1.1	7.17E-01	9.55E-01	13
miR-130b-5p	1.1	7.18E-01	9.55E-01	11
miR-224-5p	-1.1	7.20E-01	9.55E-01	23

miR-301a-3p	-1.1	7.22E-01	9.55E-01	17
miR-3200-3p	1.2	7.25E-01	9.55E-01	9
miR-21-5p	1.0	7.27E-01	9.55E-01	23355
miR-486-3p	-1.1	7.31E-01	9.55E-01	87
miR-19b-3p	1.1	7.32E-01	9.55E-01	570
miR-132-5p	1.2	7.34E-01	9.55E-01	7
miR-148b-3p	-1.0	7.35E-01	9.55E-01	785
miR-6803-3p	-1.1	7.35E-01	9.55E-01	15
miR-145-5p	-1.1	7.36E-01	9.55E-01	22
miR-6511a-3p	1.1	7.39E-01	9.56E-01	8
miR-4685-3p	-1.1	7.41E-01	9.56E-01	8
miR-874-3p	1.1	7.44E-01	9.57E-01	32
miR-106a-5p	1.1	7.57E-01	9.70E-01	10
miR-211-5p	1.2	7.61E-01	9.73E-01	7
miR-30e-5p	-1.0	7.75E-01	9.80E-01	4562
miR-141-3p	1.1	7.80E-01	9.80E-01	53
miR-501-3p	1.0	7.83E-01	9.80E-01	204
miR-4429	-1.1	7.85E-01	9.80E-01	7
miR-760	-1.1	7.87E-01	9.80E-01	30
miR-320c	-1.1	7.89E-01	9.80E-01	129
miR-25-3p	-1.0	7.89E-01	9.80E-01	14922
miR-330-3p	-1.1	7.91E-01	9.80E-01	7
miR-191-5p	-1.0	7.91E-01	9.80E-01	3448
miR-3173-5p	1.1	7.95E-01	9.80E-01	22
miR-185-5p	1.0	7.96E-01	9.80E-01	3116
miR-6747-3p	-1.1	7.96E-01	9.80E-01	6
miR-3934-5p	-1.2	7.97E-01	9.80E-01	4
miR-92b-3p	-1.0	8.05E-01	9.84E-01	493
miR-2110	1.1	8.05E-01	9.84E-01	56
miR-16-2-3p	1.0	8.09E-01	9.84E-01	34
miR-1307-5p	1.1	8.09E-01	9.84E-01	21
miR-3198	-1.0	8.15E-01	9.87E-01	5
miR-17-3p	-1.1	8.16E-01	9.87E-01	11
miR-10a-3p	1.2	8.18E-01	9.87E-01	9
miR-130a-3p	1.1	8.21E-01	9.87E-01	74
miR-6764-5p	1.2	8.25E-01	9.89E-01	6
miR-483-3p	-1.1	8.27E-01	9.89E-01	260
miR-503-5p	1.0	8.30E-01	9.90E-01	38
miR-423-5p	-1.0	8.32E-01	9.90E-01	7674
miR-324-5p	-1.0	8.40E-01	9.93E-01	86
miR-1249	-1.0	8.48E-01	9.93E-01	12

miR-152-3p	-1.0	8.54E-01	9.93E-01	156
miR-16-5p	1.0	8.59E-01	9.93E-01	334345
miR-142-5p	1.0	8.61E-01	9.93E-01	2532
miR-532-3p	-1.0	8.61E-01	9.93E-01	56
miR-2116-3p	1.1	8.61E-01	9.93E-01	7
miR-183-5p	-1.0	8.66E-01	9.93E-01	532
miR-486-5p	-1.0	8.70E-01	9.93E-01	217490
miR-1229-3p	-1.0	8.70E-01	9.93E-01	7
miR-320b	1.0	8.71E-01	9.93E-01	151
miR-23a-3p	1.0	8.71E-01	9.93E-01	812
miR-451a	-1.0	8.71E-01	9.93E-01	18628
miR-331-3p	1.0	8.73E-01	9.93E-01	9
miR-361-5p	-1.0	8.75E-01	9.93E-01	257
miR-429	-1.0	8.79E-01	9.93E-01	7
miR-342-5p	-1.1	8.79E-01	9.93E-01	5
miR-185-3p	-1.0	8.81E-01	9.93E-01	66
miR-190b	-1.0	8.81E-01	9.93E-01	32
miR-200a-3p	1.1	8.81E-01	9.93E-01	34
miR-148a-5p	-1.0	8.91E-01	9.96E-01	8
miR-146a-5p	-1.0	8.91E-01	9.96E-01	4532
let-7d-3p	-1.0	8.92E-01	9.96E-01	287
miR-144-3p	-1.0	8.97E-01	9.96E-01	209
miR-942-5p	-1.0	9.02E-01	9.96E-01	71
miR-6511b-3p	1.1	9.03E-01	9.96E-01	9
miR-221-3p	-1.0	9.03E-01	9.96E-01	1293
miR-20a-5p	-1.0	9.06E-01	9.96E-01	416
miR-937-3p	-1.1	9.07E-01	9.96E-01	5
miR-450b-5p	1.0	9.11E-01	9.96E-01	11
miR-374a-3p	-1.0	9.12E-01	9.96E-01	11
miR-1180-3p	1.0	9.12E-01	9.96E-01	164
miR-182-5p	-1.0	9.15E-01	9.97E-01	959
let-7b-5p	1.0	9.17E-01	9.97E-01	33604
miR-548ay-5p	1.0	9.23E-01	1.00E+00	15
miR-6805-5p	1.0	9.26E-01	1.00E+00	7
let-7i-5p	1.0	9.31E-01	1.00E+00	17929
miR-7151-3p	1.1	9.33E-01	1.00E+00	5
miR-7-1-3p	-1.0	9.41E-01	1.00E+00	7
miR-150-5p	-1.0	9.41E-01	1.00E+00	2592
miR-20b-5p	-1.0	9.49E-01	1.00E+00	223
miR-378a-5p	1.0	9.53E-01	1.00E+00	14
let-7g-5p	-1.0	9.53E-01	1.00E+00	1408

miR-3682-3p	1.0	9.57E-01	1.00E+00	6
miR-365a-5p	1.1	9.58E-01	1.00E+00	6
miR-29c-5p	1.0	9.60E-01	1.00E+00	12
miR-375	1.0	9.61E-01	1.00E+00	465
miR-330-5p	1.1	9.66E-01	1.00E+00	4
miR-98-5p	1.0	9.68E-01	1.00E+00	137
miR-142-3p	-1.0	9.72E-01	1.00E+00	1713
miR-4446-3p	1.0	9.72E-01	1.00E+00	13
miR-92b-5p	-1.0	9.82E-01	1.00E+00	33
miR-204-5p	-1.0	9.88E-01	1.00E+00	12
miR-590-3p	-1.0	9.88E-01	1.00E+00	14
miR-93-5p	1.0	9.89E-01	1.00E+00	7457
miR-139-3p	-1.0	9.89E-01	1.00E+00	173
miR-3913-5p	-1.1	9.97E-01	1.00E+00	12
miR-6796-5p	1.2	1.00E+00	1.00E+00	4
miR-3150a-5p	1.2	1.00E+00	1.00E+00	4
miR-877-3p	1.1	1.00E+00	1.00E+00	6
miR-550a-3p	1.0	1.00E+00	1.00E+00	13
miR-3928-3p	-1.0	1.00E+00	1.00E+00	7
let-7b-3p	1.0	1.00E+00	1.00E+00	19
miR-1296-5p	-1.0	1.00E+00	1.00E+00	6
miR-6734-5p	1.0	1.00E+00	1.00E+00	7
miR-6842-3p	1.0	1.00E+00	1.00E+00	7
miR-26a-1-3p	-1.0	1.00E+00	1.00E+00	6
miR-1287-5p	-1.0	1.00E+00	1.00E+00	17

Table S2. Differential expression analysis of 392 miRNAs detected in plasma from women without ACS (cohort 2) and a history of preeclampsia (PE, n=20) versus normotensive (NT, n=20) pregnancy. MiRNA levels are expressed as mean counts per million mapped reads (CPM). MiRNAs are listed in descending order of statistical significance.

miRNA	Fold Change (PE/NT)	p value	FDR-adjusted p value	miR level (CPM)
miR-122-5p	-2.6	4.65E-04	1.82E-01	15405
miR-29a-3p	-1.4	1.98E-03	2.37E-01	449
miR-99a-5p	-1.5	2.17E-03	2.37E-01	126
miR-203a	-2.4	2.67E-03	2.37E-01	251
miR-125b-5p	-1.5	3.07E-03	2.37E-01	603
miR-1299	4.0	3.63E-03	2.37E-01	10
miR-205-5p	-1.6	4.95E-03	2.77E-01	56
miR-4662a-5p	-2.0	9.21E-03	4.51E-01	4
miR-193b-5p	-2.0	1.11E-02	4.84E-01	17
miR-9-5p	-2.9	1.40E-02	5.32E-01	8
miR-382-3p	-1.7	1.49E-02	5.32E-01	15
miR-885-3p	-2.3	1.74E-02	5.70E-01	15
miR-206	-1.8	2.06E-02	6.21E-01	34
miR-877-3p	1.6	2.32E-02	6.50E-01	8
miR-204-5p	-1.7	2.68E-02	7.02E-01	8
miR-885-5p	-2.0	2.95E-02	7.23E-01	8
miR-22-3p	1.3	3.47E-02	7.63E-01	506
miR-376a-3p	-1.6	3.50E-02	7.63E-01	8
miR-1224-5p	2.1	3.78E-02	7.79E-01	10
miR-30a-5p	-1.2	4.93E-02	9.18E-01	491
miR-5189-3p	-1.7	5.07E-02	9.18E-01	5
miR-598-3p	-1.3	5.15E-02	9.18E-01	34
miR-483-5p	-1.5	6.03E-02	1.00E+00	118
miR-329-3p	-1.7	6.26E-02	1.00E+00	9
miR-543	-1.5	7.29E-02	1.00E+00	7
miR-27b-3p	-1.2	7.61E-02	1.00E+00	439
miR-25-5p	1.3	7.65E-02	1.00E+00	37
miR-33a-5p	1.4	7.67E-02	1.00E+00	6
miR-1294	1.4	8.99E-02	1.00E+00	56
miR-652-3p	1.2	9.10E-02	1.00E+00	52
miR-625-5p	1.4	9.20E-02	1.00E+00	8
miR-378a-5p	-1.5	9.48E-02	1.00E+00	8
miR-1287-5p	1.3	9.80E-02	1.00E+00	14
miR-28-5p	-1.5	1.10E-01	1.00E+00	6

miR-483-3p	-1.5	1.14E-01	1.00E+00	117
miR-625-3p	1.3	1.16E-01	1.00E+00	528
miR-143-3p	1.2	1.18E-01	1.00E+00	873
miR-96-5p	-1.4	1.21E-01	1.00E+00	93
miR-181c-5p	-1.4	1.30E-01	1.00E+00	12
miR-196b-5p	1.3	1.31E-01	1.00E+00	202
miR-485-3p	-1.4	1.35E-01	1.00E+00	180
miR-629-5p	1.2	1.35E-01	1.00E+00	198
miR-503-5p	1.3	1.36E-01	1.00E+00	30
miR-423-5p	1.2	1.37E-01	1.00E+00	6692
miR-4646-3p	-1.3	1.45E-01	1.00E+00	7
miR-556-3p	-1.6	1.53E-01	1.00E+00	4
miR-324-3p	1.2	1.60E-01	1.00E+00	31
miR-548a-3p	-1.6	1.60E-01	1.00E+00	4
miR-487b-5p	-1.5	1.66E-01	1.00E+00	5
miR-548l	1.3	1.71E-01	1.00E+00	8
miR-377-3p	-1.5	1.79E-01	1.00E+00	5
miR-140-3p	-1.2	1.80E-01	1.00E+00	650
miR-2110	1.2	1.85E-01	1.00E+00	55
let-7d-3p	1.1	1.91E-01	1.00E+00	317
miR-140-5p	1.2	1.94E-01	1.00E+00	49
miR-484	1.2	1.95E-01	1.00E+00	2471
miR-454-3p	1.3	1.96E-01	1.00E+00	157
miR-196a-5p	1.5	1.99E-01	1.00E+00	7
miR-3613-5p	1.3	2.04E-01	1.00E+00	415
miR-150-5p	-1.3	2.05E-01	1.00E+00	1679
miR-142-3p	1.2	2.10E-01	1.00E+00	1340
miR-99b-5p	-1.3	2.10E-01	1.00E+00	511
miR-130b-3p	1.2	2.13E-01	1.00E+00	24
miR-664b-5p	1.2	2.24E-01	1.00E+00	12
miR-1	-1.3	2.27E-01	1.00E+00	132
miR-17-3p	-1.3	2.29E-01	1.00E+00	7
miR-125b-2-3p	-1.5	2.34E-01	1.00E+00	5
let-7f-5p	1.2	2.39E-01	1.00E+00	19987
miR-7151-3p	-1.5	2.43E-01	1.00E+00	4
miR-95-3p	-1.4	2.43E-01	1.00E+00	6
miR-494-3p	-1.3	2.47E-01	1.00E+00	12
miR-942-5p	1.2	2.50E-01	1.00E+00	77
miR-98-5p	1.1	2.52E-01	1.00E+00	201
miR-1260a	1.3	2.55E-01	1.00E+00	10
miR-338-3p	-1.2	2.69E-01	1.00E+00	18

miR-4738-3p	1.5	2.73E-01	1.00E+00	4
miR-100-5p	-1.3	2.74E-01	1.00E+00	85
miR-3168	-1.4	2.78E-01	1.00E+00	31
miR-1538	1.3	2.78E-01	1.00E+00	5
miR-548d-5p	1.3	2.79E-01	1.00E+00	11
miR-185-3p	1.1	2.80E-01	1.00E+00	85
miR-34a-5p	-1.2	2.80E-01	1.00E+00	14
miR-382-5p	-1.3	2.83E-01	1.00E+00	489
miR-26b-3p	1.2	2.87E-01	1.00E+00	7
miR-550a-3p	1.3	2.87E-01	1.00E+00	11
miR-6805-5p	1.3	2.89E-01	1.00E+00	7
miR-6515-3p	1.3	2.92E-01	1.00E+00	5
miR-27a-3p	-1.1	2.93E-01	1.00E+00	125
miR-210-3p	1.3	2.96E-01	1.00E+00	11
miR-345-5p	-1.2	3.04E-01	1.00E+00	22
miR-26b-5p	1.1	3.04E-01	1.00E+00	5431
miR-1292-5p	-1.2	3.05E-01	1.00E+00	9
miR-3605-3p	1.2	3.08E-01	1.00E+00	37
miR-664a-5p	1.1	3.09E-01	1.00E+00	132
miR-1908-5p	1.2	3.15E-01	1.00E+00	75
miR-16-2-3p	1.2	3.17E-01	1.00E+00	28
miR-337-5p	-1.4	3.17E-01	1.00E+00	5
miR-192-5p	-1.2	3.18E-01	1.00E+00	550
miR-10b-5p	-1.2	3.20E-01	1.00E+00	525
miR-148a-3p	-1.1	3.27E-01	1.00E+00	2413
miR-181a-2-3p	-1.2	3.29E-01	1.00E+00	46
miR-31-5p	1.7	3.31E-01	1.00E+00	4
miR-1247-5p	-1.3	3.32E-01	1.00E+00	7
miR-3173-5p	1.1	3.32E-01	1.00E+00	23
miR-431-5p	-1.2	3.33E-01	1.00E+00	134
miR-6741-5p	1.3	3.34E-01	1.00E+00	6
miR-186-5p	1.1	3.34E-01	1.00E+00	279
miR-532-3p	1.2	3.37E-01	1.00E+00	42
let-7b-3p	-1.2	3.37E-01	1.00E+00	12
miR-1249	-1.2	3.40E-01	1.00E+00	19
miR-374a-3p	1.2	3.45E-01	1.00E+00	13
miR-191-5p	-1.1	3.52E-01	1.00E+00	4263
miR-342-3p	-1.2	3.57E-01	1.00E+00	3886
miR-4742-3p	1.2	3.60E-01	1.00E+00	16
miR-4669	1.6	3.62E-01	1.00E+00	5
let-7i-5p	1.1	3.66E-01	1.00E+00	24167

miR-1296-5p	1.2	3.66E-01	1.00E+00	8
miR-4467	-1.6	3.67E-01	1.00E+00	4
miR-4732-5p	1.2	3.67E-01	1.00E+00	172
miR-3198	1.2	3.68E-01	1.00E+00	5
miR-107	1.2	3.69E-01	1.00E+00	265
miR-223-5p	1.1	3.72E-01	1.00E+00	415
miR-505-5p	1.2	3.73E-01	1.00E+00	12
miR-15b-3p	1.2	3.74E-01	1.00E+00	31
miR-1246	-1.2	3.76E-01	1.00E+00	17
miR-92b-3p	1.1	3.77E-01	1.00E+00	630
miR-222-3p	1.1	3.82E-01	1.00E+00	84
miR-6786-3p	1.3	3.86E-01	1.00E+00	6
miR-136-3p	-1.3	3.86E-01	1.00E+00	9
miR-148a-5p	-1.2	3.87E-01	1.00E+00	8
miR-1270	-1.2	3.88E-01	1.00E+00	16
miR-3613-3p	-1.3	3.91E-01	1.00E+00	5
miR-627-5p	1.2	3.91E-01	1.00E+00	5
miR-1226-3p	1.2	3.91E-01	1.00E+00	13
let-7c-5p	-1.1	3.92E-01	1.00E+00	579
miR-221-3p	-1.1	3.94E-01	1.00E+00	1580
miR-628-3p	1.1	3.96E-01	1.00E+00	83
miR-15b-5p	1.2	3.96E-01	1.00E+00	474
miR-487b-3p	-1.3	4.03E-01	1.00E+00	9
miR-3187-3p	-1.1	4.11E-01	1.00E+00	18
miR-4732-3p	1.2	4.12E-01	1.00E+00	230
miR-4435	1.2	4.14E-01	1.00E+00	4
miR-320a	1.1	4.16E-01	1.00E+00	1427
miR-6764-5p	1.2	4.17E-01	1.00E+00	4
miR-491-5p	1.2	4.18E-01	1.00E+00	17
miR-365b-3p	-1.3	4.20E-01	1.00E+00	5
miR-1976	1.2	4.20E-01	1.00E+00	48
miR-139-5p	-1.2	4.23E-01	1.00E+00	11
miR-654-3p	-1.2	4.24E-01	1.00E+00	82
miR-3928-3p	-1.2	4.26E-01	1.00E+00	6
miR-92a-3p	1.1	4.28E-01	1.00E+00	153484
let-7d-5p	1.1	4.30E-01	1.00E+00	1729
miR-7706	1.2	4.33E-01	1.00E+00	16
miR-103a-3p	1.1	4.38E-01	1.00E+00	6256
miR-485-5p	-1.2	4.45E-01	1.00E+00	93
let-7a-5p	1.1	4.49E-01	1.00E+00	31676
miR-376c-3p	-1.3	4.50E-01	1.00E+00	8

miR-301a-3p	1.1	4.57E-01	1.00E+00	21
miR-125a-5p	-1.1	4.66E-01	1.00E+00	2273
miR-486-3p	1.1	4.66E-01	1.00E+00	100
miR-30e-5p	1.1	4.70E-01	1.00E+00	4051
miR-191-3p	-1.2	4.78E-01	1.00E+00	17
miR-29c-5p	1.1	4.80E-01	1.00E+00	12
miR-1179	1.2	4.82E-01	1.00E+00	6
miR-134-5p	-1.2	4.82E-01	1.00E+00	241
miR-411-5p	-1.2	4.91E-01	1.00E+00	23
miR-136-5p	1.2	4.93E-01	1.00E+00	4
miR-425-5p	1.1	4.99E-01	1.00E+00	5463
miR-320d	-1.1	5.07E-01	1.00E+00	28
miR-5187-5p	1.2	5.10E-01	1.00E+00	10
miR-190a-5p	1.2	5.11E-01	1.00E+00	159
miR-7110-3p	1.3	5.13E-01	1.00E+00	3
miR-193a-5p	-1.1	5.24E-01	1.00E+00	88
miR-493-3p	-1.2	5.24E-01	1.00E+00	14
miR-532-5p	1.1	5.24E-01	1.00E+00	213
miR-106a-5p	1.1	5.26E-01	1.00E+00	10
miR-3127-5p	1.2	5.27E-01	1.00E+00	6
miR-197-3p	1.1	5.35E-01	1.00E+00	436
miR-18b-3p	-1.2	5.36E-01	1.00E+00	6
let-7a-3p	-1.1	5.36E-01	1.00E+00	6
miR-323b-3p	-1.2	5.45E-01	1.00E+00	51
miR-25-3p	1.1	5.50E-01	1.00E+00	13596
miR-1343-3p	-1.1	5.50E-01	1.00E+00	9
miR-194-5p	-1.1	5.50E-01	1.00E+00	175
miR-20b-5p	-1.1	5.52E-01	1.00E+00	174
miR-381-3p	-1.1	5.52E-01	1.00E+00	66
miR-7976	1.2	5.57E-01	1.00E+00	10
miR-1180-3p	1.1	5.61E-01	1.00E+00	191
miR-127-5p	1.1	5.62E-01	1.00E+00	4
miR-221-5p	-1.1	5.72E-01	1.00E+00	20
miR-23b-3p	-1.0	5.76E-01	1.00E+00	86
let-7g-5p	1.1	5.79E-01	1.00E+00	1962
miR-92b-5p	1.1	5.80E-01	1.00E+00	47
miR-3615	1.1	5.81E-01	1.00E+00	635
miR-29c-3p	-1.1	5.81E-01	1.00E+00	648
miR-32-5p	1.1	5.82E-01	1.00E+00	308
miR-5010-3p	-1.1	5.83E-01	1.00E+00	9
miR-143-5p	-1.1	5.94E-01	1.00E+00	8

miR-324-5p	1.1	5.96E-01	1.00E+00	128
miR-375	-1.1	5.99E-01	1.00E+00	404
miR-3200-5p	1.2	6.00E-01	1.00E+00	6
miR-766-3p	1.1	6.01E-01	1.00E+00	11
miR-18a-3p	-1.1	6.01E-01	1.00E+00	51
miR-584-5p	1.1	6.04E-01	1.00E+00	1358
miR-1260b	1.1	6.10E-01	1.00E+00	14
miR-342-5p	-1.2	6.12E-01	1.00E+00	5
miR-409-3p	-1.1	6.12E-01	1.00E+00	846
miR-10a-5p	-1.0	6.17E-01	1.00E+00	572
miR-3200-3p	1.1	6.17E-01	1.00E+00	7
miR-7-5p	1.1	6.17E-01	1.00E+00	545
miR-378c	-1.2	6.18E-01	1.00E+00	5
miR-323a-3p	-1.1	6.22E-01	1.00E+00	36
miR-3177-3p	-1.1	6.23E-01	1.00E+00	8
miR-3913-5p	1.1	6.31E-01	1.00E+00	9
miR-101-3p	1.1	6.33E-01	1.00E+00	4474
miR-548ay-5p	1.1	6.34E-01	1.00E+00	12
miR-224-5p	-1.1	6.38E-01	1.00E+00	18
miR-548j-5p	1.1	6.38E-01	1.00E+00	54
miR-146b-5p	-1.1	6.39E-01	1.00E+00	374
miR-148b-5p	-1.1	6.41E-01	1.00E+00	16
miR-370-3p	1.1	6.41E-01	1.00E+00	55
miR-502-3p	1.1	6.41E-01	1.00E+00	31
miR-3620-3p	-1.1	6.48E-01	1.00E+00	4
miR-320c	-1.1	6.53E-01	1.00E+00	51
miR-21-3p	-1.1	6.57E-01	1.00E+00	9
miR-4750-5p	1.2	6.59E-01	1.00E+00	4
miR-30d-5p	1.0	6.60E-01	1.00E+00	18694
miR-152-3p	1.1	6.64E-01	1.00E+00	142
miR-941	1.1	6.66E-01	1.00E+00	172
miR-21-5p	1.0	6.67E-01	1.00E+00	15203
miR-4433-5p	1.1	6.67E-01	1.00E+00	6
miR-15a-5p	1.1	6.68E-01	1.00E+00	409
miR-3158-3p	-1.1	6.69E-01	1.00E+00	15
miR-542-3p	1.1	6.72E-01	1.00E+00	13
miR-744-5p	-1.1	6.75E-01	1.00E+00	455
miR-589-5p	-1.1	6.76E-01	1.00E+00	20
let-7e-5p	-1.1	6.77E-01	1.00E+00	182
miR-671-3p	1.1	6.89E-01	1.00E+00	27
miR-5698	1.2	6.90E-01	1.00E+00	5

miR-423-3p	1.1	6.92E-01	1.00E+00	1036
miR-636	1.1	6.93E-01	1.00E+00	18
miR-1277-5p	1.1	6.93E-01	1.00E+00	20
miR-486-5p	1.1	6.95E-01	1.00E+00	267381
miR-505-3p	-1.1	7.00E-01	1.00E+00	8
miR-432-5p	-1.1	7.05E-01	1.00E+00	915
miR-361-3p	1.1	7.08E-01	1.00E+00	366
miR-127-3p	-1.1	7.10E-01	1.00E+00	24
miR-3138	1.1	7.14E-01	1.00E+00	11
miR-320b	1.0	7.15E-01	1.00E+00	67
miR-1273h-3p	1.1	7.16E-01	1.00E+00	18
miR-1468-5p	1.1	7.20E-01	1.00E+00	8
miR-142-5p	1.0	7.23E-01	1.00E+00	2015
miR-6780a-5p	-1.2	7.23E-01	1.00E+00	5
miR-18a-5p	1.1	7.25E-01	1.00E+00	32
miR-501-5p	-1.1	7.29E-01	1.00E+00	5
miR-200a-3p	1.1	7.30E-01	1.00E+00	17
miR-130a-3p	1.0	7.33E-01	1.00E+00	56
miR-218-5p	1.3	7.34E-01	1.00E+00	5
miR-4433b-3p	1.1	7.36E-01	1.00E+00	44
miR-452-5p	-1.1	7.39E-01	1.00E+00	6
miR-23b-5p	1.1	7.40E-01	1.00E+00	7
miR-874-3p	-1.0	7.41E-01	1.00E+00	15
miR-760	-1.1	7.42E-01	1.00E+00	28
miR-146b-3p	-1.1	7.43E-01	1.00E+00	12
miR-664a-3p	1.1	7.48E-01	1.00E+00	13
miR-628-5p	-1.1	7.50E-01	1.00E+00	18
miR-30e-3p	-1.0	7.50E-01	1.00E+00	185
miR-4685-3p	1.1	7.54E-01	1.00E+00	10
miR-6881-3p	-1.1	7.56E-01	1.00E+00	6
miR-6842-3p	-1.1	7.57E-01	1.00E+00	10
miR-590-3p	-1.0	7.57E-01	1.00E+00	18
miR-3688-3p	1.1	7.59E-01	1.00E+00	11
miR-24-3p	1.0	7.60E-01	1.00E+00	592
miR-5193	1.1	7.63E-01	1.00E+00	5
miR-145-5p	1.1	7.63E-01	1.00E+00	15
miR-6747-3p	-1.0	7.65E-01	1.00E+00	9
miR-1237-3p	-1.1	7.66E-01	1.00E+00	7
miR-1307-5p	1.1	7.68E-01	1.00E+00	18
miR-369-5p	-1.1	7.69E-01	1.00E+00	34
miR-181d-5p	-1.1	7.72E-01	1.00E+00	21

miR-4446-3p	-1.1	7.73E-01	1.00E+00	25
miR-6511a-3p	1.1	7.74E-01	1.00E+00	9
miR-5189-5p	1.1	7.75E-01	1.00E+00	4
miR-199a-3p	1.0	7.75E-01	1.00E+00	2127
miR-769-5p	-1.0	7.77E-01	1.00E+00	28
miR-1255b-5p	1.1	7.78E-01	1.00E+00	44
miR-4433b-5p	1.1	7.79E-01	1.00E+00	3099
miR-16-5p	1.1	7.79E-01	1.00E+00	310612
miR-30c-5p	1.0	7.80E-01	1.00E+00	51
miR-26a-5p	1.0	7.81E-01	1.00E+00	5795
miR-182-5p	1.1	7.82E-01	1.00E+00	1635
miR-215-5p	-1.1	7.82E-01	1.00E+00	31
miR-495-3p	-1.1	7.88E-01	1.00E+00	4
miR-199b-5p	-1.1	7.93E-01	1.00E+00	6
miR-190b	1.0	7.95E-01	1.00E+00	22
miR-200b-3p	-1.1	7.97E-01	1.00E+00	15
miR-379-5p	-1.1	8.01E-01	1.00E+00	104
miR-19b-3p	-1.0	8.04E-01	1.00E+00	388
miR-126-5p	1.0	8.09E-01	1.00E+00	3886
miR-1304-3p	1.1	8.09E-01	1.00E+00	21
miR-421	1.0	8.11E-01	1.00E+00	35
miR-6803-3p	-1.0	8.13E-01	1.00E+00	24
miR-28-3p	-1.0	8.13E-01	1.00E+00	493
miR-151a-3p	-1.0	8.17E-01	1.00E+00	2949
miR-133a-3p	1.1	8.19E-01	1.00E+00	26
miR-146a-3p	-1.2	8.20E-01	1.00E+00	3
miR-361-5p	-1.0	8.22E-01	1.00E+00	225
miR-1306-5p	-1.0	8.24E-01	1.00E+00	437
miR-144-5p	-1.1	8.33E-01	1.00E+00	147
miR-126-3p	-1.0	8.37E-01	1.00E+00	13164
miR-330-3p	-1.0	8.38E-01	1.00E+00	9
miR-199b-3p	1.0	8.39E-01	1.00E+00	1710
miR-331-3p	1.0	8.41E-01	1.00E+00	13
miR-339-5p	-1.0	8.44E-01	1.00E+00	231
miR-424-5p	1.1	8.44E-01	1.00E+00	7
miR-378a-3p	1.0	8.45E-01	1.00E+00	185
miR-23a-3p	-1.0	8.45E-01	1.00E+00	785
miR-26a-1-3p	-1.1	8.47E-01	1.00E+00	7
miR-30b-5p	-1.0	8.47E-01	1.00E+00	19
miR-183-5p	-1.0	8.48E-01	1.00E+00	703
miR-150-3p	-1.1	8.49E-01	1.00E+00	12

miR-6741-3p	-1.0	8.53E-01	1.00E+00	15
miR-335-5p	-1.0	8.53E-01	1.00E+00	286
miR-671-5p	-1.0	8.53E-01	1.00E+00	65
let-7b-5p	1.0	8.56E-01	1.00E+00	30143
miR-30d-3p	1.1	8.59E-01	1.00E+00	6
miR-106b-3p	-1.0	8.59E-01	1.00E+00	616
miR-6511b-3p	-1.0	8.63E-01	1.00E+00	9
miR-6772-3p	-1.1	8.64E-01	1.00E+00	8
miR-1229-3p	-1.0	8.65E-01	1.00E+00	11
miR-106b-5p	-1.0	8.65E-01	1.00E+00	27
miR-181b-5p	-1.0	8.66E-01	1.00E+00	183
miR-20a-5p	1.0	8.67E-01	1.00E+00	410
miR-363-3p	1.0	8.67E-01	1.00E+00	468
miR-181c-3p	1.1	8.68E-01	1.00E+00	9
miR-660-5p	1.0	8.70E-01	1.00E+00	356
miR-6721-5p	1.0	8.74E-01	1.00E+00	13
miR-181a-5p	1.0	8.75E-01	1.00E+00	1516
miR-148b-3p	1.0	8.76E-01	1.00E+00	902
miR-151a-5p	-1.0	8.82E-01	1.00E+00	26
miR-99b-3p	-1.0	8.85E-01	1.00E+00	9
miR-146a-5p	1.0	8.85E-01	1.00E+00	5516
miR-296-5p	-1.0	8.86E-01	1.00E+00	30
miR-2355-3p	1.1	8.87E-01	1.00E+00	7
miR-185-5p	-1.0	8.96E-01	1.00E+00	2212
miR-10a-3p	1.0	8.96E-01	1.00E+00	4
miR-130b-5p	1.0	8.98E-01	1.00E+00	15
miR-30a-3p	1.0	8.99E-01	1.00E+00	31
miR-141-3p	-1.0	9.02E-01	1.00E+00	23
miR-19a-3p	-1.0	9.03E-01	1.00E+00	108
miR-493-5p	-1.0	9.03E-01	1.00E+00	25
miR-651-5p	-1.0	9.06E-01	1.00E+00	13
miR-29b-3p	-1.0	9.13E-01	1.00E+00	118
miR-1307-3p	1.0	9.13E-01	1.00E+00	1217
miR-369-3p	-1.0	9.16E-01	1.00E+00	27
miR-155-5p	1.0	9.18E-01	1.00E+00	435
miR-17-5p	1.0	9.21E-01	1.00E+00	162
miR-181a-3p	-1.0	9.27E-01	1.00E+00	31
miR-335-3p	-1.0	9.29E-01	1.00E+00	29
miR-326	1.0	9.31E-01	1.00E+00	47
miR-374b-5p	1.0	9.31E-01	1.00E+00	31
miR-501-3p	1.0	9.33E-01	1.00E+00	144

miR-4665-5p	-1.0	9.38E-01	1.00E+00	4
miR-1301-3p	-1.0	9.38E-01	1.00E+00	57
miR-889-3p	1.0	9.43E-01	1.00E+00	36
miR-454-5p	1.0	9.44E-01	1.00E+00	21
miR-139-3p	1.0	9.46E-01	1.00E+00	226
miR-132-3p	1.0	9.47E-01	1.00E+00	32
miR-451a	1.0	9.54E-01	1.00E+00	14636
miR-22-5p	-1.0	9.57E-01	1.00E+00	7
miR-340-5p	-1.0	9.58E-01	1.00E+00	211
miR-500a-3p	-1.0	9.61E-01	1.00E+00	23
miR-128-3p	1.0	9.61E-01	1.00E+00	380
miR-450b-5p	1.0	9.65E-01	1.00E+00	7
miR-223-3p	-1.0	9.70E-01	1.00E+00	9090
miR-576-5p	1.0	9.72E-01	1.00E+00	183
miR-574-3p	1.0	9.72E-01	1.00E+00	49
miR-200c-3p	-1.0	9.77E-01	1.00E+00	58
miR-339-3p	1.0	9.78E-01	1.00E+00	44
miR-6852-5p	1.0	9.82E-01	1.00E+00	38
miR-93-3p	1.0	9.83E-01	1.00E+00	35
miR-93-5p	-1.0	9.85E-01	1.00E+00	7863
miR-144-3p	-1.0	9.89E-01	1.00E+00	223
miR-374a-5p	1.0	9.92E-01	1.00E+00	141
miR-328-3p	-1.0	9.95E-01	1.00E+00	1229
miR-425-3p	-1.0	9.99E-01	1.00E+00	92
miR-4533	-1.1	1.00E+00	1.00E+00	4
miR-3940-3p	1.0	1.00E+00	1.00E+00	9
miR-5010-5p	1.0	1.00E+00	1.00E+00	7
miR-195-5p	-1.0	1.00E+00	1.00E+00	33
miR-409-5p	-1.0	1.00E+00	1.00E+00	6
miR-199a-5p	-1.0	1.00E+00	1.00E+00	29

Table S3. Differential expression analysis of 458 miRNAs detected in plasma between women with (n=35) and without (n=40) acute coronary syndrome. MiRNA levels are expressed as mean counts per million mapped reads (CPM). MiRNAs are listed in descending order of statistical significance.

miRNA	Fold Change (ACS/non-ACS)	p value	FDR-adjusted p value	miR level (CPM)
miR-208b-3p	148.0	1.18E-23	5.39E-21	37
miR-29a-3p	2.2	3.12E-23	7.14E-21	726
miR-378a-3p	2.4	3.39E-18	5.18E-16	303
miR-30a-5p	2.3	2.48E-17	2.84E-15	828
miR-499a-5p	34.2	8.30E-17	7.60E-15	19
miR-193a-5p	3.3	1.08E-16	8.24E-15	181
miR-34a-5p	3.9	1.32E-16	8.64E-15	32
miR-10b-5p	2.5	4.51E-16	2.53E-14	897
miR-195-5p	2.8	4.98E-16	2.53E-14	61
miR-483-5p	4.4	7.31E-16	3.35E-14	305
miR-320c	2.3	1.01E-13	4.20E-12	84
miR-320d	2.3	1.39E-13	5.30E-12	45
miR-99a-5p	2.4	1.53E-13	5.38E-12	214
miR-320b	2.1	4.78E-12	1.56E-10	103
miR-1306-5p	-1.9	7.19E-12	2.19E-10	369
miR-194-5p	2.5	7.64E-12	2.19E-10	298
miR-184	11.7	9.00E-12	2.43E-10	24
miR-193b-5p	4.3	1.07E-11	2.73E-10	43
miR-29c-3p	1.8	2.87E-11	6.91E-10	896
miR-208a-3p	15.2	7.87E-11	1.80E-09	3
miR-221-5p	-3.4	9.56E-11	2.08E-09	16
miR-206	6.9	4.15E-10	8.65E-09	127
miR-4433b-5p	-4.0	7.04E-10	1.38E-08	2369
miR-6772-3p	-4.1	7.21E-10	1.38E-08	7
miR-378c	2.9	8.02E-10	1.47E-08	9
miR-423-3p	-2.1	1.84E-09	3.24E-08	858
miR-5010-3p	-3.3	2.07E-09	3.52E-08	8
miR-584-5p	-2.2	2.23E-09	3.64E-08	1129
miR-328-3p	-2.3	4.61E-09	7.28E-08	1034
miR-4646-3p	-3.0	5.76E-09	8.79E-08	6
miR-95-3p	2.9	1.03E-08	1.52E-07	11
miR-127-3p	-4.1	1.45E-08	2.07E-07	19
miR-30e-3p	-1.9	1.67E-08	2.32E-07	158

miR-103a-3p	-1.7	1.95E-08	2.63E-07	5337
miR-98-5p	-1.6	2.16E-08	2.82E-07	174
let-7i-5p	-1.4	2.29E-08	2.92E-07	21241
miR-197-3p	-2.1	2.60E-08	3.21E-07	375
miR-1301-3p	-2.8	2.86E-08	3.45E-07	46
miR-125b-5p	2.0	3.51E-08	4.12E-07	899
miR-339-5p	-2.4	5.74E-08	6.58E-07	191
miR-125b-2-3p	3.6	6.46E-08	7.21E-07	10
miR-26b-5p	-1.5	7.87E-08	8.58E-07	4744
miR-6803-3p	-2.0	8.60E-08	9.16E-07	21
miR-744-5p	-2.1	1.43E-07	1.49E-06	384
miR-1	3.4	1.55E-07	1.58E-06	296
miR-23b-3p	1.5	1.78E-07	1.76E-06	116
miR-374a-5p	-1.8	1.81E-07	1.76E-06	118
miR-30d-5p	-1.7	1.93E-07	1.84E-06	16794
miR-125b-1-3p	6.3	2.98E-07	2.78E-06	4
miR-1307-3p	-1.9	3.30E-07	3.03E-06	1047
miR-335-3p	-2.6	4.06E-07	3.65E-06	24
miR-874-3p	1.9	5.28E-07	4.65E-06	23
miR-100-5p	2.3	5.54E-07	4.79E-06	132
miR-148a-3p	1.5	5.72E-07	4.85E-06	3030
miR-122-5p	3.1	6.56E-07	5.42E-06	30458
miR-99b-5p	-2.0	6.63E-07	5.42E-06	437
miR-885-3p	4.1	6.85E-07	5.51E-06	34
miR-27a-3p	1.4	7.43E-07	5.87E-06	160
miR-1229-3p	-2.6	1.43E-06	1.11E-05	10
miR-130b-3p	1.8	1.51E-06	1.16E-05	34
miR-1273h-3p	-2.7	1.64E-06	1.23E-05	15
miR-485-3p	-3.1	1.72E-06	1.27E-05	136
miR-365a-3p	2.8	2.21E-06	1.58E-05	9
miR-409-3p	-2.8	2.21E-06	1.58E-05	654
miR-21-5p	1.4	2.26E-06	1.59E-05	18833
miR-3620-3p	-4.4	2.45E-06	1.70E-05	4
miR-485-5p	-2.8	2.56E-06	1.75E-05	73
miR-543	-3.1	2.65E-06	1.79E-05	6
miR-1246	2.0	2.84E-06	1.88E-05	25
miR-1908-5p	-1.9	2.87E-06	1.88E-05	67
miR-6842-3p	-2.3	3.32E-06	2.14E-05	9
miR-192-5p	1.7	3.75E-06	2.38E-05	730
miR-652-3p	-1.7	6.26E-06	3.90E-05	45
miR-378i	2.2	6.30E-06	3.90E-05	7

miR-4446-3p	-2.8	6.57E-06	4.01E-05	21
miR-548j-5p	-2.2	8.20E-06	4.94E-05	46
miR-6747-3p	-2.2	8.31E-06	4.94E-05	8
miR-215-5p	2.6	8.41E-06	4.94E-05	54
miR-10a-5p	1.5	9.16E-06	5.31E-05	752
miR-326	-2.6	9.37E-06	5.36E-05	39
miR-122-3p	4.6	9.81E-06	5.55E-05	4
miR-4433-5p	-2.8	1.20E-05	6.69E-05	6
miR-5193	-3.3	1.21E-05	6.70E-05	5
miR-30a-3p	1.7	1.28E-05	6.98E-05	43
miR-493-5p	-2.8	1.41E-05	7.61E-05	21
miR-6721-5p	-2.8	1.69E-05	8.99E-05	11
miR-889-3p	-2.9	1.88E-05	9.92E-05	30
miR-151a-3p	-1.7	2.18E-05	1.14E-04	2627
miR-432-5p	-2.5	2.60E-05	1.34E-04	736
miR-454-3p	-1.8	2.69E-05	1.37E-04	126
miR-27b-3p	1.5	3.20E-05	1.61E-04	588
miR-6741-3p	-2.0	3.24E-05	1.61E-04	14
miR-126-3p	-1.5	3.63E-05	1.79E-04	12027
miR-379-5p	-2.4	3.82E-05	1.86E-04	85
miR-1277-5p	-2.1	3.92E-05	1.87E-04	18
miR-500a-3p	1.6	3.95E-05	1.87E-04	30
miR-671-3p	-2.3	3.97E-05	1.87E-04	24
miR-5698	-4.2	4.21E-05	1.97E-04	5
miR-331-3p	-2.1	4.28E-05	1.98E-04	12
miR-625-3p	-2.0	4.48E-05	2.05E-04	471
miR-10b-3p	2.5	4.63E-05	2.10E-04	6
miR-1249	-2.2	5.69E-05	2.56E-04	17
miR-3065-5p	2.7	6.32E-05	2.79E-04	6
miR-26a-5p	-1.3	6.34E-05	2.79E-04	5364
miR-1304-3p	-2.0	6.68E-05	2.91E-04	19
miR-374b-5p	-1.6	6.74E-05	2.91E-04	28
miR-654-3p	-2.4	7.06E-05	3.02E-04	68
miR-320a	1.4	7.83E-05	3.32E-04	1742
miR-4429	2.5	8.75E-05	3.65E-04	5
miR-92b-5p	-1.6	8.77E-05	3.65E-04	41
miR-1296-5p	-2.4	9.09E-05	3.75E-04	8
miR-182-5p	-1.7	1.02E-04	4.12E-04	1298
miR-664a-3p	-2.1	1.02E-04	4.12E-04	12
miR-4433b-3p	-3.7	1.02E-04	4.12E-04	34
miR-483-3p	2.1	1.04E-04	4.16E-04	177

miR-590-3p	-1.7	1.09E-04	4.32E-04	17
miR-369-5p	-2.4	1.20E-04	4.68E-04	28
miR-141-3p	2.2	1.27E-04	4.95E-04	37
miR-323a-3p	-2.2	1.36E-04	5.24E-04	31
miR-223-3p	-1.8	1.37E-04	5.25E-04	8005
miR-6852-5p	-2.2	1.46E-04	5.53E-04	33
miR-1179	-2.1	1.54E-04	5.78E-04	6
miR-411-5p	-2.4	1.59E-04	5.93E-04	20
miR-3138	-2.1	1.70E-04	6.30E-04	10
miR-1260b	-2.2	1.85E-04	6.78E-04	13
miR-381-3p	-2.3	1.90E-04	6.91E-04	54
miR-628-3p	-1.6	1.95E-04	7.03E-04	76
miR-185-3p	-1.5	2.13E-04	7.63E-04	79
miR-4685-3p	-1.8	2.37E-04	8.42E-04	9
miR-365b-3p	2.3	2.55E-04	8.97E-04	8
miR-17-5p	-1.5	2.76E-04	9.66E-04	142
miR-382-5p	-2.1	2.81E-04	9.74E-04	402
miR-211-5p	2.5	2.94E-04	1.01E-03	5
miR-199a-5p	-2.1	2.98E-04	1.02E-03	25
miR-181d-5p	-1.9	3.05E-04	1.03E-03	19
miR-32-5p	1.7	3.35E-04	1.13E-03	402
miR-324-5p	-1.6	3.40E-04	1.13E-03	108
let-7g-5p	-1.4	3.42E-04	1.13E-03	1689
miR-10a-3p	2.4	3.63E-04	1.20E-03	6
miR-5187-5p	-2.0	3.83E-04	1.25E-03	9
miR-28-3p	-1.6	4.20E-04	1.36E-03	454
miR-151a-5p	-1.6	4.47E-04	1.43E-03	24
miR-766-3p	-2.6	4.47E-04	1.43E-03	10
miR-130b-5p	-1.9	4.49E-04	1.43E-03	14
miR-431-5p	-2.2	4.74E-04	1.50E-03	112
miR-133a-3p	2.5	5.46E-04	1.71E-03	47
miR-877-3p	-2.1	5.74E-04	1.79E-03	7
miR-200b-3p	2.2	5.79E-04	1.79E-03	23
miR-181c-5p	-1.8	6.18E-04	1.89E-03	11
miR-491-5p	-2.0	6.21E-04	1.89E-03	15
miR-181c-3p	-2.2	6.24E-04	1.89E-03	9
miR-107	1.5	6.52E-04	1.97E-03	328
miR-196a-5p	2.3	6.81E-04	2.04E-03	11
miR-4742-3p	-1.6	6.98E-04	2.07E-03	14
miR-128-3p	-1.3	7.01E-04	2.07E-03	364
miR-1226-3p	-1.8	7.12E-04	2.09E-03	12

miR-191-5p	-1.4	7.54E-04	2.20E-03	3984
miR-425-3p	-1.4	8.07E-04	2.34E-03	87
let-7f-5p	-1.3	8.27E-04	2.38E-03	17925
miR-556-3p	-2.8	8.84E-04	2.53E-03	4
miR-424-5p	1.8	9.05E-04	2.57E-03	9
miR-3591-5p	4.0	9.51E-04	2.68E-03	3
miR-365a-5p	3.8	9.55E-04	2.68E-03	4
miR-143-5p	1.9	9.84E-04	2.75E-03	11
miR-143-3p	1.6	1.07E-03	2.97E-03	1159
miR-210-3p	1.8	1.09E-03	2.99E-03	16
miR-497-5p	2.2	1.17E-03	3.22E-03	5
miR-487b-5p	-2.4	1.24E-03	3.39E-03	5
miR-199b-3p	-1.6	1.34E-03	3.63E-03	1572
miR-425-5p	-1.4	1.40E-03	3.77E-03	4999
miR-31-5p	3.4	1.41E-03	3.78E-03	6
miR-26a-1-3p	-2.1	1.45E-03	3.83E-03	7
miR-155-5p	-1.3	1.45E-03	3.83E-03	414
miR-190a-5p	-1.7	1.66E-03	4.36E-03	128
miR-4428	3.6	1.71E-03	4.48E-03	2
miR-140-3p	1.4	1.73E-03	4.48E-03	781
miR-221-3p	-1.4	1.73E-03	4.48E-03	1500
miR-4750-5p	-2.5	1.75E-03	4.50E-03	4
miR-676-3p	2.7	1.79E-03	4.58E-03	3
miR-370-3p	-2.1	1.83E-03	4.66E-03	47
miR-199a-3p	-1.6	1.90E-03	4.81E-03	1964
miR-628-5p	-1.9	1.95E-03	4.90E-03	17
miR-6807-5p	3.4	1.97E-03	4.92E-03	3
miR-150-3p	1.7	1.98E-03	4.92E-03	16
miR-7110-3p	-2.6	2.13E-03	5.27E-03	4
miR-19b-3p	1.4	2.19E-03	5.39E-03	467
miR-493-3p	-2.1	2.27E-03	5.56E-03	12
miR-301a-3p	-1.6	2.29E-03	5.58E-03	20
miR-323b-3p	-2.0	2.42E-03	5.86E-03	44
miR-92b-3p	-1.4	2.66E-03	6.42E-03	564
miR-139-3p	-1.6	2.71E-03	6.50E-03	214
miR-200a-3p	1.9	2.77E-03	6.60E-03	25
miR-330-3p	-2.2	2.97E-03	7.05E-03	8
miR-3940-3p	-1.8	3.14E-03	7.41E-03	8
miR-329-3p	-2.1	3.32E-03	7.79E-03	8
miR-30b-5p	-1.5	3.43E-03	8.02E-03	17
miR-1260a	-1.8	3.46E-03	8.05E-03	10

miR-4667-5p	3.5	3.53E-03	8.16E-03	3
miR-6515-3p	-1.9	3.60E-03	8.29E-03	5
miR-452-5p	2.0	3.66E-03	8.38E-03	8
miR-28-5p	-2.0	3.76E-03	8.56E-03	5
miR-409-5p	-2.2	3.95E-03	8.95E-03	6
miR-501-3p	1.3	4.04E-03	9.09E-03	168
miR-134-5p	-1.8	4.06E-03	9.09E-03	206
miR-4732-5p	1.4	4.07E-03	9.09E-03	207
miR-433-3p	-2.5	4.28E-03	9.51E-03	4
miR-214-3p	2.1	4.34E-03	9.59E-03	5
miR-126-5p	-1.3	4.35E-03	9.59E-03	3778
miR-191-3p	-1.7	4.47E-03	9.80E-03	16
miR-296-5p	-1.5	4.57E-03	9.97E-03	27
miR-4533	-2.6	4.74E-03	1.03E-02	4
miR-532-5p	1.3	5.03E-03	1.09E-02	245
miR-148b-3p	-1.3	5.08E-03	1.09E-02	871
miR-340-5p	-1.4	5.23E-03	1.12E-02	201
miR-6881-3p	-1.8	5.31E-03	1.13E-02	6
miR-885-5p	2.1	5.33E-03	1.13E-02	12
miR-6730-3p	3.6	5.34E-03	1.13E-02	3
miR-185-5p	1.4	5.54E-03	1.16E-02	2589
miR-18a-3p	-1.4	5.54E-03	1.16E-02	45
miR-342-5p	-1.7	6.13E-03	1.27E-02	5
miR-15b-5p	1.4	6.13E-03	1.27E-02	556
miR-205-5p	1.5	6.15E-03	1.27E-02	70
miR-369-3p	-1.8	6.20E-03	1.27E-02	25
miR-223-5p	-1.4	6.22E-03	1.27E-02	400
miR-502-3p	1.4	6.93E-03	1.41E-02	37
miR-146a-5p	-1.4	7.60E-03	1.54E-02	5306
miR-148b-5p	-1.5	7.69E-03	1.55E-02	15
miR-15a-5p	1.4	7.80E-03	1.57E-02	489
let-7d-3p	-1.3	9.53E-03	1.91E-02	314
miR-378a-5p	1.6	9.94E-03	1.98E-02	10
miR-18a-5p	-1.4	9.97E-03	1.98E-02	30
miR-339-3p	-1.3	1.01E-02	1.99E-02	42
miR-27a-5p	2.0	1.04E-02	2.04E-02	4
miR-5189-5p	-2.0	1.06E-02	2.07E-02	4
miR-941	-1.4	1.16E-02	2.26E-02	159
miR-542-3p	1.5	1.25E-02	2.42E-02	16
miR-181a-2-3p	-1.4	1.25E-02	2.42E-02	44
miR-4665-5p	-1.8	1.40E-02	2.69E-02	5

miR-150-5p	1.5	1.40E-02	2.69E-02	2090
miR-1343-3p	-1.5	1.45E-02	2.77E-02	9
miR-96-5p	1.5	1.49E-02	2.83E-02	113
miR-92a-3p	-1.3	1.50E-02	2.83E-02	140962
miR-660-5p	1.2	1.64E-02	3.08E-02	407
miR-374a-3p	-1.5	1.64E-02	3.08E-02	13
miR-1538	-1.8	1.67E-02	3.13E-02	6
miR-4513	-5.6	1.85E-02	3.44E-02	4
miR-6805-5p	-1.6	1.88E-02	3.47E-02	7
miR-181a-3p	-1.5	1.88E-02	3.47E-02	30
miR-671-5p	-1.4	1.91E-02	3.52E-02	64
miR-142-5p	1.2	2.26E-02	4.15E-02	2247
miR-22-3p	1.3	2.28E-02	4.15E-02	584
miR-342-3p	-1.3	2.39E-02	4.34E-02	3780
miR-3200-5p	-1.6	2.44E-02	4.41E-02	6
let-7c-5p	1.2	2.50E-02	4.51E-02	654
miR-6511a-3p	-1.5	2.54E-02	4.55E-02	9
miR-3934-5p	2.7	2.54E-02	4.55E-02	3
miR-4714-3p	1.9	2.61E-02	4.66E-02	4
miR-200c-3p	1.4	2.67E-02	4.73E-02	73
miR-127-5p	-1.9	2.68E-02	4.74E-02	5
miR-636	-1.3	2.90E-02	5.11E-02	17
miR-190b	1.3	3.04E-02	5.34E-02	26
miR-2355-3p	-1.7	3.11E-02	5.44E-02	7
miR-376a-3p	-1.7	3.22E-02	5.62E-02	8
miR-146b-5p	-1.3	3.27E-02	5.68E-02	369
let-7b-3p	1.4	3.41E-02	5.89E-02	15
miR-183-5p	-1.3	3.45E-02	5.94E-02	610
miR-532-3p	1.3	4.00E-02	6.85E-02	48
miR-6741-5p	-1.5	4.01E-02	6.85E-02	6
miR-7976	-1.4	4.12E-02	7.01E-02	10
miR-1237-3p	-1.6	4.19E-02	7.10E-02	7
miR-132-3p	1.3	4.29E-02	7.26E-02	39
miR-154-5p	-1.7	4.41E-02	7.43E-02	4
miR-222-3p	1.2	4.58E-02	7.68E-02	98
miR-29b-3p	1.2	4.63E-02	7.74E-02	133
miR-3177-3p	-1.5	4.86E-02	8.10E-02	8
miR-486-5p	-1.3	4.95E-02	8.21E-02	242014
miR-574-3p	1.3	5.01E-02	8.28E-02	61
miR-3605-5p	1.5	5.02E-02	8.28E-02	6
miR-29c-5p	-1.3	5.26E-02	8.63E-02	12

miR-129-5p	1.7	5.33E-02	8.71E-02	5
miR-6767-5p	1.6	5.34E-02	8.71E-02	5
miR-6734-5p	1.7	5.70E-02	9.26E-02	5
miR-19a-3p	1.2	5.77E-02	9.33E-02	122
miR-18b-3p	-1.5	5.85E-02	9.43E-02	6
miR-204-5p	1.4	5.95E-02	9.56E-02	10
miR-337-5p	-1.7	6.18E-02	9.90E-02	5
miR-3064-5p	-1.5	6.38E-02	1.02E-01	4
miR-125a-5p	-1.2	6.40E-02	1.02E-01	2302
miR-30d-3p	-1.4	6.55E-02	1.04E-01	6
miR-3198	-1.6	6.60E-02	1.04E-01	5
miR-1299	2.0	6.63E-02	1.04E-01	16
miR-2116-3p	1.5	6.80E-02	1.07E-01	5
miR-9-3p	1.8	6.94E-02	1.09E-01	4
miR-1292-5p	-1.3	6.99E-02	1.09E-01	9
miR-6514-5p	-1.4	7.56E-02	1.17E-01	5
miR-376c-3p	-1.7	7.69E-02	1.19E-01	8
miR-548d-5p	1.4	7.71E-02	1.19E-01	13
miR-93-3p	-1.2	7.85E-02	1.21E-01	34
miR-421	1.2	8.11E-02	1.24E-01	40
miR-145-5p	1.3	8.51E-02	1.30E-01	18
miR-148a-5p	-1.5	8.63E-02	1.31E-01	8
miR-664a-5p	-1.2	8.71E-02	1.32E-01	133
miR-130a-3p	1.2	8.81E-02	1.33E-01	65
miR-99b-3p	-1.5	9.39E-02	1.42E-01	9
miR-625-5p	-1.4	9.44E-02	1.42E-01	8
miR-181a-5p	-1.2	9.50E-02	1.42E-01	1450
miR-450b-5p	1.4	9.65E-02	1.44E-01	9
miR-24-3p	-1.2	9.74E-02	1.45E-01	604
let-7a-5p	-1.2	9.84E-02	1.46E-01	29100
miR-20b-5p	1.2	9.85E-02	1.46E-01	194
miR-3682-3p	1.6	9.93E-02	1.46E-01	4
miR-451a	1.2	9.98E-02	1.46E-01	16308
miR-495-3p	-1.6	1.00E-01	1.46E-01	5
miR-4435	-1.7	1.00E-01	1.46E-01	4
miR-3187-3p	1.2	1.01E-01	1.47E-01	21
miR-769-5p	-1.2	1.04E-01	1.51E-01	28
miR-144-5p	1.3	1.05E-01	1.52E-01	166
miR-382-3p	-1.4	1.06E-01	1.53E-01	15
miR-6796-5p	2.0	1.07E-01	1.54E-01	3
miR-431-3p	-1.6	1.08E-01	1.55E-01	4

miR-4467	-1.7	1.09E-01	1.55E-01	4
miR-1976	-1.2	1.10E-01	1.57E-01	45
miR-627-5p	1.5	1.11E-01	1.57E-01	6
miR-136-5p	-1.5	1.13E-01	1.59E-01	5
miR-3173-5p	-1.2	1.13E-01	1.59E-01	23
miR-664b-5p	-1.4	1.14E-01	1.60E-01	12
miR-942-5p	-1.2	1.14E-01	1.60E-01	74
miR-487b-3p	-1.5	1.17E-01	1.63E-01	9
miR-125a-3p	1.5	1.36E-01	1.90E-01	4
miR-6511b-3p	-1.3	1.37E-01	1.90E-01	9
miR-1180-3p	-1.2	1.39E-01	1.92E-01	175
miR-145-3p	1.6	1.40E-01	1.93E-01	4
miR-338-3p	-1.3	1.47E-01	2.03E-01	18
miR-106a-5p	-1.2	1.49E-01	2.05E-01	10
miR-361-3p	-1.2	1.51E-01	2.06E-01	369
miR-140-5p	-1.1	1.56E-01	2.12E-01	48
miR-3679-5p	1.4	1.58E-01	2.15E-01	6
miR-486-3p	-1.2	1.68E-01	2.28E-01	93
miR-17-3p	1.3	1.74E-01	2.34E-01	9
miR-1250-5p	-1.7	1.77E-01	2.39E-01	3
miR-377-3p	-1.4	1.81E-01	2.43E-01	5
miR-1294	1.2	1.88E-01	2.51E-01	61
miR-642a-5p	1.5	1.93E-01	2.57E-01	4
miR-146a-3p	-1.7	1.93E-01	2.57E-01	4
miR-494-3p	-1.4	1.95E-01	2.59E-01	12
miR-5189-3p	-1.4	2.08E-01	2.75E-01	6
miR-7-5p	1.2	2.16E-01	2.85E-01	588
miR-22-5p	1.2	2.27E-01	2.99E-01	8
miR-7706	-1.2	2.33E-01	3.06E-01	16
miR-93-5p	-1.1	2.35E-01	3.08E-01	7639
miR-7151-3p	-1.5	2.38E-01	3.11E-01	5
miR-454-5p	-1.2	2.42E-01	3.14E-01	21
miR-23a-3p	-1.1	2.43E-01	3.15E-01	820
miR-6786-3p	-1.2	2.43E-01	3.15E-01	7
miR-30c-5p	1.1	2.47E-01	3.19E-01	57
miR-335-5p	1.2	2.48E-01	3.20E-01	345
miR-576-5p	-1.1	2.49E-01	3.20E-01	177
miR-6793-5p	1.4	2.51E-01	3.21E-01	4
miR-484	-1.2	2.53E-01	3.23E-01	2367
let-7a-3p	1.3	2.62E-01	3.33E-01	7
miR-106b-3p	-1.1	2.76E-01	3.50E-01	593

miR-106b-5p	-1.2	2.77E-01	3.50E-01	26
miR-9-5p	1.4	2.92E-01	3.69E-01	9
miR-15b-3p	1.2	2.93E-01	3.69E-01	34
miR-503-5p	1.2	3.12E-01	3.92E-01	34
miR-4732-3p	-1.1	3.13E-01	3.92E-01	217
miR-7-1-3p	1.2	3.22E-01	4.01E-01	5
miR-1224-5p	-1.3	3.22E-01	4.01E-01	10
miR-3928-3p	-1.2	3.35E-01	4.15E-01	6
miR-3615	1.1	3.42E-01	4.23E-01	682
miR-142-3p	1.1	3.48E-01	4.30E-01	1526
miR-16-2-3p	1.1	3.51E-01	4.32E-01	30
miR-548l	-1.2	3.53E-01	4.33E-01	9
miR-202-3p	1.4	3.57E-01	4.38E-01	5
miR-4775	-1.1	3.59E-01	4.39E-01	4
miR-3613-5p	-1.1	3.62E-01	4.41E-01	391
miR-4662a-5p	-1.3	3.65E-01	4.43E-01	5
miR-3158-3p	-1.2	3.73E-01	4.52E-01	15
miR-33a-5p	1.2	3.78E-01	4.57E-01	7
miR-136-3p	-1.2	3.90E-01	4.70E-01	10
miR-139-5p	-1.2	4.00E-01	4.81E-01	12
miR-4669	1.4	4.10E-01	4.91E-01	7
miR-330-5p	1.3	4.12E-01	4.93E-01	3
miR-1247-5p	1.2	4.14E-01	4.94E-01	9
miR-3913-5p	1.1	4.19E-01	4.99E-01	10
miR-5001-3p	1.2	4.43E-01	5.26E-01	7
miR-345-5p	1.1	4.48E-01	5.31E-01	25
miR-760	-1.1	4.60E-01	5.42E-01	30
miR-505-5p	-1.1	4.64E-01	5.47E-01	13
miR-548n	-1.2	4.66E-01	5.47E-01	5
miR-4738-3p	1.2	4.71E-01	5.51E-01	5
miR-324-3p	-1.1	4.77E-01	5.57E-01	32
let-7b-5p	1.1	5.01E-01	5.84E-01	31199
miR-6859-5p	1.2	5.06E-01	5.88E-01	4
miR-423-5p	1.1	5.10E-01	5.91E-01	7124
miR-186-5p	-1.1	5.11E-01	5.91E-01	280
miR-874-5p	1.3	5.14E-01	5.93E-01	3
miR-589-5p	-1.1	5.25E-01	6.04E-01	21
miR-1468-5p	-1.1	5.28E-01	6.06E-01	9
miR-101-3p	1.1	5.37E-01	6.15E-01	4635
miR-144-3p	-1.1	5.43E-01	6.18E-01	213
miR-598-3p	-1.1	5.43E-01	6.18E-01	36

miR-3942-5p	1.3	5.59E-01	6.35E-01	3
miR-25-3p	1.1	5.63E-01	6.38E-01	14035
miR-5583-3p	-1.1	5.71E-01	6.46E-01	5
miR-429	1.2	5.79E-01	6.53E-01	5
miR-152-3p	-1.1	5.80E-01	6.53E-01	153
miR-23a-5p	-1.3	5.88E-01	6.60E-01	4
miR-548at-5p	1.2	5.91E-01	6.62E-01	6
miR-3127-5p	-1.1	5.95E-01	6.65E-01	7
miR-21-3p	-1.1	5.97E-01	6.66E-01	10
miR-30e-5p	1.0	6.00E-01	6.67E-01	4305
miR-5010-5p	-1.0	6.44E-01	7.14E-01	8
miR-548ay-5p	1.1	6.46E-01	7.14E-01	14
miR-2110	-1.0	6.62E-01	7.29E-01	55
miR-224-5p	1.1	6.64E-01	7.29E-01	20
miR-26b-3p	-1.1	6.64E-01	7.29E-01	8
miR-375	1.1	6.65E-01	7.29E-01	419
miR-363-3p	1.1	6.67E-01	7.29E-01	480
miR-196b-5p	1.1	6.70E-01	7.31E-01	209
miR-20a-5p	-1.0	6.72E-01	7.31E-01	414
miR-16-5p	1.1	6.88E-01	7.47E-01	317310
miR-23b-5p	-1.1	7.14E-01	7.73E-01	8
miR-3688-3p	1.1	7.38E-01	7.97E-01	12
miR-505-3p	-1.1	7.54E-01	8.12E-01	9
miR-4710	-1.1	7.59E-01	8.14E-01	3
miR-6764-5p	1.1	7.59E-01	8.14E-01	5
miR-629-5p	1.0	7.66E-01	8.20E-01	207
miR-1255b-5p	-1.0	7.69E-01	8.21E-01	44
miR-5196-3p	-1.2	7.74E-01	8.24E-01	5
let-7d-5p	1.0	7.84E-01	8.34E-01	1747
miR-6780a-5p	1.1	7.92E-01	8.36E-01	5
miR-132-5p	-1.0	7.92E-01	8.36E-01	6
miR-550a-3p	-1.0	7.94E-01	8.36E-01	12
miR-218-5p	1.1	7.94E-01	8.36E-01	6
miR-199b-5p	1.1	8.12E-01	8.53E-01	7
miR-1270	1.1	8.16E-01	8.55E-01	17
let-7e-5p	1.0	8.27E-01	8.65E-01	200
miR-769-3p	-1.0	8.38E-01	8.74E-01	6
miR-25-5p	1.0	8.43E-01	8.77E-01	38
miR-3168	1.0	8.44E-01	8.77E-01	32
miR-3200-3p	1.0	8.48E-01	8.77E-01	8
miR-3150a-5p	-1.2	8.49E-01	8.77E-01	3

miR-937-3p	1.1	8.65E-01	8.92E-01	4
miR-6862-5p	-1.0	8.77E-01	9.01E-01	4
miR-146b-3p	-1.0	8.78E-01	9.01E-01	13
miR-181b-5p	-1.0	8.80E-01	9.01E-01	190
miR-509-3p	-1.1	8.89E-01	9.09E-01	4
miR-3613-3p	-1.0	8.95E-01	9.13E-01	5
miR-1287-5p	-1.0	8.98E-01	9.14E-01	16
miR-1307-5p	-1.0	9.15E-01	9.30E-01	20
miR-501-5p	1.0	9.34E-01	9.47E-01	6
miR-548a-3p	1.0	9.39E-01	9.50E-01	5
miR-361-5p	1.0	9.45E-01	9.53E-01	243
miR-203a	1.0	9.57E-01	9.63E-01	249
miR-3605-3p	1.0	9.78E-01	9.82E-01	38
miR-651-5p	1.0	9.86E-01	9.89E-01	13
miR-3120-3p	-1.0	1.00E+00	1.00E+00	4

Table S4. Overlap in differentially altered miRs (p<0.05) between cohorts 1 and 2 in relation to a history of preeclampsia versus normotensive pregnancy. MiRNA levels are expressed as mean counts per million mapped reads (CPM).

4 common miRNAs	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)			
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-1299	4.9	3.67E-03	1.96E-01	23	4.0	3.63E-03	2.37E-01	10
miR-4662a-5p	3.5	1.33E-02	4.45E-01	5	-2.0	9.21E-03	4.51E-01	4
miR-376a-3p	-4.7	1.10E-03	1.17E-01	7	-1.6	3.50E-02	7.63E-01	8
miR-206	-10.6	1.64E-06	6.98E-04	242	-1.8	2.06E-02	6.21E-01	34

26 miRs unique to cohort 1	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)			
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-184	10.3	2.35E-04	3.35E-02	52	na	>0.05	na	na
miR-6730-3p	7.3	3.46E-03	1.96E-01	4	na	>0.05	na	na
miR-499a-5p	5.8	1.85E-03	1.58E-01	45	na	>0.05	na	na
miR-218-5p	5.4	2.24E-03	1.60E-01	7	na	>0.05	na	na
miR-3591-5p	3.8	4.01E-02	6.34E-01	5	na	>0.05	na	na
miR-4667-5p	3.6	3.60E-02	5.91E-01	4	na	>0.05	na	na
miR-874-5p	3.3	1.64E-02	4.54E-01	4	na	>0.05	na	na
miR-1	3.0	5.46E-03	2.59E-01	531	na	>0.05	na	na
miR-202-3p	2.9	4.68E-02	6.54E-01	6	na	>0.05	na	na
miR-133a-3p	2.7	2.78E-02	5.16E-01	76	na	>0.05	na	na
miR-6767-5p	2.7	1.95E-02	4.91E-01	7	na	>0.05	na	na
miR-6741-3p	1.7	2.61E-02	5.07E-01	11	na	>0.05	na	na
miR-769-5p	-1.5	4.20E-02	6.40E-01	27	na	>0.05	na	na
miR-30b-5p	-1.8	1.23E-02	4.45E-01	15	na	>0.05	na	na
miR-1277-5p	-1.8	2.35E-02	5.01E-01	13	na	>0.05	na	na
miR-221-5p	-1.9	3.03E-02	5.39E-01	9	na	>0.05	na	na
miR-2355-3p	-1.9	3.54E-02	5.91E-01	7	na	>0.05	na	na
miR-505-3p	-1.9	2.22E-02	5.01E-01	9	na	>0.05	na	na
miR-369-5p	-2.2	2.54E-02	5.07E-01	18	na	>0.05	na	na
miR-493-5p	-2.3	2.29E-02	5.01E-01	13	na	>0.05	na	na
miR-431-5p	-2.4	1.47E-02	4.49E-01	73	na	>0.05	na	na
miR-329-3p	-2.5	4.56E-02	6.54E-01	7	na	>0.05	na	na
miR-136-3p	-2.7	1.35E-02	4.45E-01	10	na	>0.05	na	na
miR-28-5p	-2.7	1.70E-02	4.54E-01	5	na	>0.05	na	na
miR-889-3p	-2.9	7.52E-03	3.21E-01	17	na	>0.05	na	na
miR-1292-5p	-3.6	8.28E-05	1.77E-02	8	na	>0.05	na	na

16 miRs unique to cohort 2	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)			
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-1224-5p	na	>0.05	na	na	2.1	3.8E-02	7.8E-01	10
miR-877-3p	na	>0.05	na	na	1.6	2.3E-02	6.5E-01	8
miR-22-3p	na	>0.05	na	na	1.3	3.5E-02	7.6E-01	506
miR-30a-5p	na	>0.05	na	na	-1.2	4.9E-02	9.2E-01	491
miR-29a-3p	na	>0.05	na	na	-1.4	2.0E-03	2.4E-01	449
miR-125b-5p	na	>0.05	na	na	-1.5	3.1E-03	2.4E-01	603
miR-99a-5p	na	>0.05	na	na	-1.5	2.2E-03	2.4E-01	126
miR-205-5p	na	>0.05	na	na	-1.6	5.0E-03	2.8E-01	56
miR-204-5p	na	>0.05	na	na	-1.7	2.7E-02	7.0E-01	8
miR-382-3p	na	>0.05	na	na	-1.7	1.5E-02	5.3E-01	15
miR-193b-5p	na	>0.05	na	na	-2.0	1.1E-02	4.8E-01	17
miR-885-5p	na	>0.05	na	na	-2.0	3.0E-02	7.2E-01	8
miR-885-3p	na	>0.05	na	na	-2.3	1.7E-02	5.7E-01	15
miR-203a	na	>0.05	na	na	-2.4	2.7E-03	2.4E-01	251
miR-122-5p	na	>0.05	na	na	-2.6	4.6E-04	1.8E-01	15405
miR-9-5p	na	>0.05	na	na	-2.9	1.4E-02	5.3E-01	8

Table S5. Overlap in differentially altered miRs ($p<0.05$) identified in cohorts 1, 2 and 3 in relation to a history of PE or NT pregnancy (cohorts 1 and 2) or acute coronary syndrome (cohort 3). MiRNA levels are expressed as mean counts per million mapped reads (CPM).

	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				ACS vs non-ACS (cohort 1 vs cohort 2)			
1 common miR	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-206	-10.6	1.64E-06	6.98E-04	242	-1.8	2.06E-02	6.21E-01	34	6.9	4.15E-10	8.65E-09	127
	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				ACS vs non-ACS (cohort 1 vs cohort 2)			
3 miR overlap	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-1299	4.9	3.67E-03	1.96E-01	23.5	4.0	3.63E-03	2.37E-01	10	na	>0.05	na	na
miR-4662a-5p	3.5	1.33E-02	4.45E-01	5.0	-2.0	9.21E-03	4.51E-01	4	na	>0.05	na	na
miR-376a-3p	-4.7	1.10E-03	1.17E-01	7.0	-1.6	3.50E-02	7.63E-01	8	na	>0.05	na	na
	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				ACS vs non-ACS (cohort 1 vs cohort 2)			
11 miR overlap	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-877-3p	na	>0.05	na	na	1.6	2.32E-02	6.50E-01	8	-2.1	5.74E-04	1.79E-03	7
miR-22-3p	na	>0.05	na	na	1.3	3.47E-02	7.63E-01	506	1.3	2.28E-02	4.15E-02	584
miR-30a-5p	na	>0.05	na	na	-1.2	4.93E-02	9.18E-01	491	2.3	2.48E-17	2.84E-15	828
miR-29a-3p	na	>0.05	na	na	-1.4	1.98E-03	2.37E-01	449	2.2	3.12E-23	7.14E-21	726
miR-125b-5p	na	>0.05	na	na	-1.5	3.07E-03	2.37E-01	603	2.0	3.51E-08	4.12E-07	899
miR-99a-5p	na	>0.05	na	na	-1.5	2.17E-03	2.37E-01	126	2.4	1.53E-13	5.38E-12	214
miR-205-5p	na	>0.05	na	na	-1.6	4.95E-03	2.77E-01	56	1.5	6.15E-03	1.27E-02	70
miR-193b-5p	na	>0.05	na	na	-2.0	1.11E-02	4.84E-01	17	4.3	1.07E-11	2.73E-10	43
miR-885-5p	na	>0.05	na	na	-2.0	2.95E-02	7.23E-01	8	2.1	5.33E-03	1.13E-02	12
miR-885-3p	na	>0.05	na	na	-2.3	1.74E-02	5.70E-01	15	4.1	6.85E-07	5.51E-06	34
miR-122-5p	na	>0.05	na	na	-2.6	4.65E-04	1.82E-01	15405	3.1	6.56E-07	5.42E-06	30457
	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				ACS vs non-ACS (cohort 1 vs cohort 2)			
17 miR overlap	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-184	10.3	2.35E-04	3.35E-02	52	na	>0.05	na	na	11.7	9.00E-12	2.43E-10	24
miR-6730-3p	7.3	3.46E-03	1.96E-01	4	na	>0.05	na	na	3.6	5.34E-03	1.13E-02	3
miR-499a-5p	5.8	1.85E-03	1.58E-01	45	na	>0.05	na	na	34.2	8.30E-17	7.60E-15	19
miR-3591-5p	3.8	4.01E-02	6.34E-01	5	na	>0.05	na	na	4.0	9.51E-04	2.68E-03	3
miR-4667-5p	3.6	3.60E-02	5.91E-01	4	na	>0.05	na	na	3.5	3.53E-03	8.16E-03	3
miR-1	3.0	5.46E-03	2.59E-01	531	na	>0.05	na	na	3.4	1.55E-07	1.58E-06	296
miR-133a-3p	2.7	2.78E-02	5.16E-01	76	na	>0.05	na	na	2.5	5.46E-04	1.71E-03	47
miR-6741-3p	1.7	2.61E-02	5.07E-01	11	na	>0.05	na	na	-2.0	3.24E-05	1.61E-04	14
miR-30b-5p	-1.8	1.23E-02	4.45E-01	15	na	>0.05	na	na	-1.5	3.43E-03	8.02E-03	17
miR-1277-5p	-1.8	2.35E-02	5.01E-01	13	na	>0.05	na	na	-2.1	3.92E-05	1.87E-04	18
miR-221-5p	-1.9	3.03E-02	5.39E-01	9	na	>0.05	na	na	-3.4	9.56E-11	2.08E-09	16
miR-369-5p	-2.2	2.54E-02	5.07E-01	18	na	>0.05	na	na	-2.4	1.20E-04	4.68E-04	28
miR-493-5p	-2.3	2.29E-02	5.01E-01	13	na	>0.05	na	na	-2.8	1.41E-05	7.61E-05	21
miR-431-5p	-2.4	1.47E-02	4.49E-01	73	na	>0.05	na	na	-2.2	4.74E-04	1.50E-03	112
miR-329-3p	-2.5	4.56E-02	6.54E-01	7	na	>0.05	na	na	-2.1	3.32E-03	7.79E-03	8
miR-28-5p	-2.7	1.70E-02	4.54E-01	5	na	>0.05	na	na	-2.0	3.76E-03	8.56E-03	5
miR-889-3p	-2.9	7.52E-03	3.21E-01	17	na	>0.05	na	na	-2.9	1.88E-05	9.92E-05	30
	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				ACS vs non-ACS (cohort 1 vs cohort 2)			
5 miRs unique to cohort 2	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-1224-5p	na	>0.05	na	na	2.1	3.78E-02	7.79E-01	10	na	>0.05	na	na
miR-204-5p	na	>0.05	na	na	-1.7	2.68E-02	7.02E-01	8	na	>0.05	na	na
miR-382-3p	na	>0.05	na	na	-1.7	1.49E-02	5.32E-01	15	na	>0.05	na	na
miR-203a	na	>0.05	na	na	-2.4	2.67E-03	2.37E-01	251	na	>0.05	na	na
miR-9-5p	na	>0.05	na	na	-2.9	1.40E-02	5.32E-01	8	na	>0.05	na	na

Table S5. Continued.

9 miRs unique to cohort 1	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				ACS vs non-ACS (cohort 1 vs cohort 2)			
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-218-5p	5.4	2.24E-03	1.60E-01	7	na	>0.05	na	na	na	>0.05	na	na
miR-874-5p	3.3	1.64E-02	4.54E-01	4	na	>0.05	na	na	na	>0.05	na	na
miR-202-3p	2.9	4.68E-02	6.54E-01	6	na	>0.05	na	na	na	>0.05	na	na
miR-6767-5p	2.7	1.95E-02	4.91E-01	7	na	>0.05	na	na	na	>0.05	na	na
miR-769-5p	-1.5	4.20E-02	6.40E-01	27	na	>0.05	na	na	na	>0.05	na	na
miR-2355-3p	-1.9	3.54E-02	5.91E-01	7	na	>0.05	na	na	na	>0.05	na	na
miR-505-3p	-1.9	2.22E-02	5.01E-01	9	na	>0.05	na	na	na	>0.05	na	na
miR-136-3p	-2.7	1.35E-02	4.45E-01	10	na	>0.05	na	na	na	>0.05	na	na
miR-1292-5p	-3.6	8.28E-05	1.77E-02	8	na	>0.05	na	na	na	>0.05	na	na

230 miRs unique to cohort 3	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				ACS vs non-ACS (cohort 1 vs cohort 2)			
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)
miR-208b-3p	na	>0.05	na	na	na	>0.05	na	na	148.0	1.18E-23	5.39E-21	37
miR-208a-3p	na	>0.05	na	na	na	>0.05	na	na	15.2	7.87E-11	1.80E-09	3
miR-125b-1-3p	na	>0.05	na	na	na	>0.05	na	na	6.3	2.98E-07	2.78E-06	4
miR-122-3p	na	>0.05	na	na	na	>0.05	na	na	4.6	9.81E-06	5.55E-05	4
miR-483-5p	na	>0.05	na	na	na	>0.05	na	na	4.4	7.31E-16	3.35E-14	305
miR-34a-5p	na	>0.05	na	na	na	>0.05	na	na	3.9	1.32E-16	8.64E-15	32
miR-365a-5p	na	>0.05	na	na	na	>0.05	na	na	3.8	9.55E-04	2.68E-03	4
miR-125b-2-3p	na	>0.05	na	na	na	>0.05	na	na	3.6	6.46E-08	7.21E-07	10
miR-4428	na	>0.05	na	na	na	>0.05	na	na	3.6	1.71E-03	4.48E-03	2
miR-6807-5p	na	>0.05	na	na	na	>0.05	na	na	3.4	1.97E-03	4.92E-03	3
miR-31-5p	na	>0.05	na	na	na	>0.05	na	na	3.4	1.41E-03	3.78E-03	6
miR-193a-5p	na	>0.05	na	na	na	>0.05	na	na	3.3	1.08E-16	8.24E-15	181
miR-378c	na	>0.05	na	na	na	>0.05	na	na	2.9	8.02E-10	1.47E-08	9
miR-95-3p	na	>0.05	na	na	na	>0.05	na	na	2.9	1.03E-08	1.52E-07	11
miR-195-5p	na	>0.05	na	na	na	>0.05	na	na	2.8	4.98E-16	2.53E-14	61
miR-365a-3p	na	>0.05	na	na	na	>0.05	na	na	2.8	2.21E-06	1.58E-05	9
miR-3065-5p	na	>0.05	na	na	na	>0.05	na	na	2.7	6.32E-05	2.79E-04	6
miR-3934-5p	na	>0.05	na	na	na	>0.05	na	na	2.7	2.54E-02	4.55E-02	3
miR-676-3p	na	>0.05	na	na	na	>0.05	na	na	2.7	1.79E-03	4.58E-03	3
miR-215-5p	na	>0.05	na	na	na	>0.05	na	na	2.6	8.41E-06	4.94E-05	54
miR-194-5p	na	>0.05	na	na	na	>0.05	na	na	2.5	7.64E-12	2.19E-10	298
miR-10b-5p	na	>0.05	na	na	na	>0.05	na	na	2.5	4.51E-16	2.53E-14	897
miR-211-5p	na	>0.05	na	na	na	>0.05	na	na	2.5	2.94E-04	1.01E-03	5
miR-10b-3p	na	>0.05	na	na	na	>0.05	na	na	2.5	4.63E-05	2.10E-04	6
miR-4429	na	>0.05	na	na	na	>0.05	na	na	2.5	8.75E-05	3.65E-04	5
miR-10a-3p	na	>0.05	na	na	na	>0.05	na	na	2.4	3.63E-04	1.20E-03	6
miR-378a-3p	na	>0.05	na	na	na	>0.05	na	na	2.4	3.39E-18	5.18E-16	303
miR-320c	na	>0.05	na	na	na	>0.05	na	na	2.3	1.01E-13	4.20E-12	84
miR-365b-3p	na	>0.05	na	na	na	>0.05	na	na	2.3	2.55E-04	8.97E-04	8
miR-100-5p	na	>0.05	na	na	na	>0.05	na	na	2.3	5.54E-07	4.79E-06	132
miR-320d	na	>0.05	na	na	na	>0.05	na	na	2.3	1.39E-13	5.30E-12	45
miR-196a-5p	na	>0.05	na	na	na	>0.05	na	na	2.3	6.81E-04	2.04E-03	11
miR-497-5p	na	>0.05	na	na	na	>0.05	na	na	2.2	1.17E-03	3.22E-03	5
miR-378i	na	>0.05	na	na	na	>0.05	na	na	2.2	6.30E-06	3.90E-05	7
miR-141-3p	na	>0.05	na	na	na	>0.05	na	na	2.2	1.27E-04	4.95E-04	37
miR-200b-3p	na	>0.05	na	na	na	>0.05	na	na	2.2	5.79E-04	1.79E-03	23
miR-320b	na	>0.05	na	na	na	>0.05	na	na	2.1	4.78E-12	1.56E-10	103
miR-214-3p	na	>0.05	na	na	na	>0.05	na	na	2.1	4.34E-03	9.59E-03	5
miR-483-3p	na	>0.05	na	na	na	>0.05	na	na	2.1	1.04E-04	4.16E-04	177
miR-27a-5p	na	>0.05	na	na	na	>0.05	na	na	2.0	1.04E-02	2.04E-02	4
miR-452-5p	na	>0.05	na	na	na	>0.05	na	na	2.0	3.66E-03	8.38E-03	8
miR-1246	na	>0.05	na	na	na	>0.05	na	na	2.0	2.84E-06	1.88E-05	25
miR-4714-3p	na	>0.05	na	na	na	>0.05	na	na	1.9	2.61E-02	4.66E-02	4
miR-143-5p	na	>0.05	na	na	na	>0.05	na	na	1.9	9.84E-04	2.75E-03	11
miR-874-3p	na	>0.05	na	na	na	>0.05	na	na	1.9	5.28E-07	4.65E-06	23

Table S5. Continued

miR-200a-3p	na	>0.05	na	na	na	>0.05	na	na	1.9	2.77E-03	6.60E-03	25
miR-424-5p	na	>0.05	na	na	na	>0.05	na	na	1.8	9.05E-04	2.57E-03	9
miR-210-3p	na	>0.05	na	na	na	>0.05	na	na	1.8	1.09E-03	2.99E-03	16
miR-29c-3p	na	>0.05	na	na	na	>0.05	na	na	1.8	2.87E-11	6.91E-10	896
miR-130b-3p	na	>0.05	na	na	na	>0.05	na	na	1.8	1.51E-06	1.16E-05	34
miR-150-3p	na	>0.05	na	na	na	>0.05	na	na	1.7	1.98E-03	4.92E-03	16
miR-32-5p	na	>0.05	na	na	na	>0.05	na	na	1.7	3.35E-04	1.13E-03	402
miR-192-5p	na	>0.05	na	na	na	>0.05	na	na	1.7	3.75E-06	2.38E-05	730
miR-30a-3p	na	>0.05	na	na	na	>0.05	na	na	1.7	1.28E-05	6.98E-05	43
miR-378a-5p	na	>0.05	na	na	na	>0.05	na	na	1.6	9.94E-03	1.98E-02	10
miR-500a-3p	na	>0.05	na	na	na	>0.05	na	na	1.6	3.95E-05	1.87E-04	30
miR-143-3p	na	>0.05	na	na	na	>0.05	na	na	1.6	1.07E-03	2.97E-03	1159
miR-23b-3p	na	>0.05	na	na	na	>0.05	na	na	1.5	1.78E-07	1.76E-06	116
miR-27b-3p	na	>0.05	na	na	na	>0.05	na	na	1.5	3.20E-05	1.61E-04	588
miR-10a-5p	na	>0.05	na	na	na	>0.05	na	na	1.5	9.16E-06	5.31E-05	752
miR-150-5p	na	>0.05	na	na	na	>0.05	na	na	1.5	1.40E-02	2.69E-02	2090
miR-107	na	>0.05	na	na	na	>0.05	na	na	1.5	6.52E-04	1.97E-03	328
miR-96-5p	na	>0.05	na	na	na	>0.05	na	na	1.5	1.49E-02	2.83E-02	113
miR-148a-3p	na	>0.05	na	na	na	>0.05	na	na	1.5	5.72E-07	4.85E-06	3030
miR-542-3p	na	>0.05	na	na	na	>0.05	na	na	1.5	1.25E-02	2.42E-02	16
miR-4732-5p	na	>0.05	na	na	na	>0.05	na	na	1.4	4.07E-03	9.09E-03	207
miR-27a-3p	na	>0.05	na	na	na	>0.05	na	na	1.4	7.43E-07	5.87E-06	160
miR-200c-3p	na	>0.05	na	na	na	>0.05	na	na	1.4	2.67E-02	4.73E-02	73
miR-140-3p	na	>0.05	na	na	na	>0.05	na	na	1.4	1.73E-03	4.48E-03	781
miR-21-5p	na	>0.05	na	na	na	>0.05	na	na	1.4	2.26E-06	1.59E-05	18833
miR-15a-5p	na	>0.05	na	na	na	>0.05	na	na	1.4	7.80E-03	1.57E-02	489
miR-320a	na	>0.05	na	na	na	>0.05	na	na	1.4	7.83E-05	3.32E-04	1741
miR-19b-3p	na	>0.05	na	na	na	>0.05	na	na	1.4	2.19E-03	5.39E-03	467
miR-502-3p	na	>0.05	na	na	na	>0.05	na	na	1.4	6.93E-03	1.41E-02	37
miR-15b-5p	na	>0.05	na	na	na	>0.05	na	na	1.4	6.13E-03	1.27E-02	556
miR-185-5p	na	>0.05	na	na	na	>0.05	na	na	1.4	5.54E-03	1.16E-02	2589
miR-501-3p	na	>0.05	na	na	na	>0.05	na	na	1.3	4.04E-03	9.09E-03	168
miR-532-5p	na	>0.05	na	na	na	>0.05	na	na	1.3	5.03E-03	1.09E-02	245
let-7c-5p	na	>0.05	na	na	na	>0.05	na	na	1.2	2.50E-02	4.51E-02	654
miR-660-5p	na	>0.05	na	na	na	>0.05	na	na	1.2	1.64E-02	3.08E-02	407
miR-142-5p	na	>0.05	na	na	na	>0.05	na	na	1.2	2.26E-02	4.15E-02	2247
miR-92a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.3	1.50E-02	2.83E-02	140957
miR-148b-3p	na	>0.05	na	na	na	>0.05	na	na	-1.3	5.08E-03	1.09E-02	871
let-7d-3p	na	>0.05	na	na	na	>0.05	na	na	-1.3	9.53E-03	1.91E-02	314
miR-126-5p	na	>0.05	na	na	na	>0.05	na	na	-1.3	4.35E-03	9.59E-03	3778
miR-128-3p	na	>0.05	na	na	na	>0.05	na	na	-1.3	7.01E-04	2.07E-03	364
miR-342-3p	na	>0.05	na	na	na	>0.05	na	na	-1.3	2.39E-02	4.34E-02	3780
miR-155-5p	na	>0.05	na	na	na	>0.05	na	na	-1.3	1.45E-03	3.83E-03	414
miR-339-3p	na	>0.05	na	na	na	>0.05	na	na	-1.3	1.01E-02	1.99E-02	42
miR-26a-5p	na	>0.05	na	na	na	>0.05	na	na	-1.3	6.34E-05	2.79E-04	5364
let-7f-5p	na	>0.05	na	na	na	>0.05	na	na	-1.3	8.27E-04	2.38E-03	17925
miR-92b-3p	na	>0.05	na	na	na	>0.05	na	na	-1.4	2.66E-03	6.42E-03	564
miR-941	na	>0.05	na	na	na	>0.05	na	na	-1.4	1.16E-02	2.26E-02	159
miR-425-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	1.40E-03	3.77E-03	4999
miR-181a-2-3p	na	>0.05	na	na	na	>0.05	na	na	-1.4	1.25E-02	2.42E-02	44
miR-671-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	1.91E-02	3.52E-02	64

Table S5. Continued

miR-223-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	6.22E-03	1.27E-02	400
miR-191-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	7.54E-04	2.20E-03	3983
miR-18a-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	9.97E-03	1.98E-02	30
miR-425-3p	na	>0.05	na	na	na	>0.05	na	na	-1.4	8.07E-04	2.34E-03	87
miR-340-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	5.23E-03	1.12E-02	201
miR-221-3p	na	>0.05	na	na	na	>0.05	na	na	-1.4	1.73E-03	4.48E-03	1500
let-7i-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	2.29E-08	2.92E-07	21242
let-7g-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	3.42E-04	1.13E-03	1689
miR-146a-5p	na	>0.05	na	na	na	>0.05	na	na	-1.4	7.60E-03	1.54E-02	5306
miR-18a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.4	5.54E-03	1.16E-02	45
miR-6511a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.5	2.54E-02	4.55E-02	9
miR-374a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.5	1.64E-02	3.08E-02	13
miR-185-3p	na	>0.05	na	na	na	>0.05	na	na	-1.5	2.13E-04	7.63E-04	79
miR-181a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.5	1.88E-02	3.47E-02	30
miR-17-5p	na	>0.05	na	na	na	>0.05	na	na	-1.5	2.76E-04	9.66E-04	142
miR-148b-5p	na	>0.05	na	na	na	>0.05	na	na	-1.5	7.69E-03	1.55E-02	15
miR-126-3p	na	>0.05	na	na	na	>0.05	na	na	-1.5	3.63E-05	1.79E-04	12027
miR-26b-5p	na	>0.05	na	na	na	>0.05	na	na	-1.5	7.87E-08	8.58E-07	4744
miR-296-5p	na	>0.05	na	na	na	>0.05	na	na	-1.5	4.57E-03	9.97E-03	27
miR-1343-3p	na	>0.05	na	na	na	>0.05	na	na	-1.5	1.45E-02	2.77E-02	9
miR-301a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.6	2.29E-03	5.58E-03	20
miR-92b-5p	na	>0.05	na	na	na	>0.05	na	na	-1.6	8.77E-05	3.65E-04	41
miR-199a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.6	1.90E-03	4.81E-03	1964
miR-139-3p	na	>0.05	na	na	na	>0.05	na	na	-1.6	2.71E-03	6.50E-03	214
miR-6805-5p	na	>0.05	na	na	na	>0.05	na	na	-1.6	1.88E-02	3.47E-02	7
miR-324-5p	na	>0.05	na	na	na	>0.05	na	na	-1.6	3.40E-04	1.13E-03	108
miR-199b-3p	na	>0.05	na	na	na	>0.05	na	na	-1.6	1.34E-03	3.63E-03	1572
miR-628-3p	na	>0.05	na	na	na	>0.05	na	na	-1.6	1.95E-04	7.03E-04	76
miR-98-5p	na	>0.05	na	na	na	>0.05	na	na	-1.6	2.16E-08	2.82E-07	174
miR-28-3p	na	>0.05	na	na	na	>0.05	na	na	-1.6	4.20E-04	1.36E-03	454
miR-151a-5p	na	>0.05	na	na	na	>0.05	na	na	-1.6	4.47E-04	1.43E-03	24
miR-374b-5p	na	>0.05	na	na	na	>0.05	na	na	-1.6	6.74E-05	2.91E-04	28
miR-3200-5p	na	>0.05	na	na	na	>0.05	na	na	-1.6	2.44E-02	4.41E-02	6
miR-4742-3p	na	>0.05	na	na	na	>0.05	na	na	-1.6	6.98E-04	2.07E-03	14
miR-191-3p	na	>0.05	na	na	na	>0.05	na	na	-1.7	4.47E-03	9.80E-03	16
miR-652-3p	na	>0.05	na	na	na	>0.05	na	na	-1.7	6.26E-06	3.90E-05	45
miR-30d-5p	na	>0.05	na	na	na	>0.05	na	na	-1.7	1.93E-07	1.84E-06	16794
miR-151a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.7	2.18E-05	1.14E-04	2627
miR-590-3p	na	>0.05	na	na	na	>0.05	na	na	-1.7	1.09E-04	4.32E-04	17
miR-190a-5p	na	>0.05	na	na	na	>0.05	na	na	-1.7	1.66E-03	4.36E-03	128
miR-103a-3p	na	>0.05	na	na	na	>0.05	na	na	-1.7	1.95E-08	2.63E-07	5337
miR-182-5p	na	>0.05	na	na	na	>0.05	na	na	-1.7	1.02E-04	4.12E-04	1298
miR-342-5p	na	>0.05	na	na	na	>0.05	na	na	-1.7	6.13E-03	1.27E-02	5
miR-223-3p	na	>0.05	na	na	na	>0.05	na	na	-1.8	1.37E-04	5.25E-04	8005
miR-1538	na	>0.05	na	na	na	>0.05	na	na	-1.8	1.67E-02	3.13E-02	6
miR-1226-3p	na	>0.05	na	na	na	>0.05	na	na	-1.8	7.12E-04	2.09E-03	12
miR-3940-3p	na	>0.05	na	na	na	>0.05	na	na	-1.8	3.14E-03	7.41E-03	8
miR-374a-5p	na	>0.05	na	na	na	>0.05	na	na	-1.8	1.81E-07	1.76E-06	118
miR-181c-5p	na	>0.05	na	na	na	>0.05	na	na	-1.8	6.18E-04	1.89E-03	11
miR-6881-3p	na	>0.05	na	na	na	>0.05	na	na	-1.8	5.31E-03	1.13E-02	6
miR-369-3p	na	>0.05	na	na	na	>0.05	na	na	-1.8	6.20E-03	1.27E-02	25

Table S5. Continued

miR-134-5p	na	>0.05	na	na	na	>0.05	na	na	-1.8	4.06E-03	9.09E-03	206
miR-1260a	na	>0.05	na	na	na	>0.05	na	na	-1.8	3.46E-03	8.05E-03	10
miR-4665-5p	na	>0.05	na	na	na	>0.05	na	na	-1.8	1.40E-02	2.69E-02	5
miR-454-3p	na	>0.05	na	na	na	>0.05	na	na	-1.8	2.69E-05	1.37E-04	126
miR-4685-3p	na	>0.05	na	na	na	>0.05	na	na	-1.8	2.37E-04	8.42E-04	9
miR-6515-3p	na	>0.05	na	na	na	>0.05	na	na	-1.9	3.60E-03	8.29E-03	5
miR-130b-5p	na	>0.05	na	na	na	>0.05	na	na	-1.9	4.49E-04	1.43E-03	14
miR-1908-5p	na	>0.05	na	na	na	>0.05	na	na	-1.9	2.87E-06	1.88E-05	67
miR-127-5p	na	>0.05	na	na	na	>0.05	na	na	-1.9	2.68E-02	4.74E-02	5
miR-30e-3p	na	>0.05	na	na	na	>0.05	na	na	-1.9	1.67E-08	2.32E-07	158
miR-1306-5p	na	>0.05	na	na	na	>0.05	na	na	-1.9	7.19E-12	2.19E-10	369
miR-181d-5p	na	>0.05	na	na	na	>0.05	na	na	-1.9	3.05E-04	1.03E-03	19
miR-628-5p	na	>0.05	na	na	na	>0.05	na	na	-1.9	1.95E-03	4.90E-03	17
miR-1307-3p	na	>0.05	na	na	na	>0.05	na	na	-1.9	3.30E-07	3.03E-06	1047
miR-491-5p	na	>0.05	na	na	na	>0.05	na	na	-2.0	6.21E-04	1.89E-03	15
miR-6803-3p	na	>0.05	na	na	na	>0.05	na	na	-2.0	8.60E-08	9.16E-07	21
miR-5189-5p	na	>0.05	na	na	na	>0.05	na	na	-2.0	1.06E-02	2.07E-02	4
miR-323b-3p	na	>0.05	na	na	na	>0.05	na	na	-2.0	2.42E-03	5.86E-03	44
miR-625-3p	na	>0.05	na	na	na	>0.05	na	na	-2.0	4.48E-05	2.05E-04	471
miR-5187-5p	na	>0.05	na	na	na	>0.05	na	na	-2.0	3.83E-04	1.25E-03	9
miR-99b-5p	na	>0.05	na	na	na	>0.05	na	na	-2.0	6.63E-07	5.42E-06	437
miR-1304-3p	na	>0.05	na	na	na	>0.05	na	na	-2.0	6.68E-05	2.91E-04	19
miR-26a-1-3p	na	>0.05	na	na	na	>0.05	na	na	-2.1	1.45E-03	3.83E-03	7
miR-370-3p	na	>0.05	na	na	na	>0.05	na	na	-2.1	1.83E-03	4.66E-03	47
miR-664a-3p	na	>0.05	na	na	na	>0.05	na	na	-2.1	1.02E-04	4.12E-04	12
miR-744-5p	na	>0.05	na	na	na	>0.05	na	na	-2.1	1.43E-07	1.49E-06	384
miR-1179	na	>0.05	na	na	na	>0.05	na	na	-2.1	1.54E-04	5.78E-04	6
miR-331-3p	na	>0.05	na	na	na	>0.05	na	na	-2.1	4.28E-05	1.98E-04	12
miR-382-5p	na	>0.05	na	na	na	>0.05	na	na	-2.1	2.81E-04	9.74E-04	402
miR-197-3p	na	>0.05	na	na	na	>0.05	na	na	-2.1	2.60E-08	3.21E-07	375
miR-493-3p	na	>0.05	na	na	na	>0.05	na	na	-2.1	2.27E-03	5.56E-03	12
miR-199a-5p	na	>0.05	na	na	na	>0.05	na	na	-2.1	2.98E-04	1.02E-03	25
miR-423-3p	na	>0.05	na	na	na	>0.05	na	na	-2.1	1.84E-09	3.24E-08	858
miR-3138	na	>0.05	na	na	na	>0.05	na	na	-2.1	1.70E-04	6.30E-04	10
miR-6852-5p	na	>0.05	na	na	na	>0.05	na	na	-2.2	1.46E-04	5.53E-04	33
miR-1249	na	>0.05	na	na	na	>0.05	na	na	-2.2	5.69E-05	2.56E-04	17
miR-409-5p	na	>0.05	na	na	na	>0.05	na	na	-2.2	3.95E-03	8.95E-03	6
miR-584-5p	na	>0.05	na	na	na	>0.05	na	na	-2.2	2.23E-09	3.64E-08	1129
miR-548j-5p	na	>0.05	na	na	na	>0.05	na	na	-2.2	8.20E-06	4.94E-05	46
miR-323a-3p	na	>0.05	na	na	na	>0.05	na	na	-2.2	1.36E-04	5.24E-04	31
miR-1260b	na	>0.05	na	na	na	>0.05	na	na	-2.2	1.85E-04	6.78E-04	13
miR-6747-3p	na	>0.05	na	na	na	>0.05	na	na	-2.2	8.31E-06	4.94E-05	8
miR-181c-3p	na	>0.05	na	na	na	>0.05	na	na	-2.2	6.24E-04	1.89E-03	9
miR-330-3p	na	>0.05	na	na	na	>0.05	na	na	-2.2	2.97E-03	7.05E-03	8
miR-328-3p	na	>0.05	na	na	na	>0.05	na	na	-2.3	4.61E-09	7.28E-08	1034
miR-6842-3p	na	>0.05	na	na	na	>0.05	na	na	-2.3	3.32E-06	2.14E-05	9
miR-381-3p	na	>0.05	na	na	na	>0.05	na	na	-2.3	1.90E-04	6.91E-04	54
miR-671-3p	na	>0.05	na	na	na	>0.05	na	na	-2.3	3.97E-05	1.87E-04	24
miR-379-5p	na	>0.05	na	na	na	>0.05	na	na	-2.4	3.82E-05	1.86E-04	85
miR-339-5p	na	>0.05	na	na	na	>0.05	na	na	-2.4	5.74E-08	6.58E-07	191
miR-487b-5p	na	>0.05	na	na	na	>0.05	na	na	-2.4	1.24E-03	3.39E-03	5

Table S5. Continued

miR-1296-5p	na	>0.05	na	na	na	>0.05	na	na	-2.4	9.09E-05	3.75E-04	8
miR-411-5p	na	>0.05	na	na	na	>0.05	na	na	-2.4	1.59E-04	5.93E-04	20
miR-654-3p	na	>0.05	na	na	na	>0.05	na	na	-2.4	7.06E-05	3.02E-04	68
miR-432-5p	na	>0.05	na	na	na	>0.05	na	na	-2.5	2.60E-05	1.34E-04	736
miR-433-3p	na	>0.05	na	na	na	>0.05	na	na	-2.5	4.28E-03	9.51E-03	4
miR-4750-5p	na	>0.05	na	na	na	>0.05	na	na	-2.5	1.75E-03	4.50E-03	4
miR-7110-3p	na	>0.05	na	na	na	>0.05	na	na	-2.6	2.13E-03	5.27E-03	4
miR-1229-3p	na	>0.05	na	na	na	>0.05	na	na	-2.6	1.43E-06	1.11E-05	10
miR-326	na	>0.05	na	na	na	>0.05	na	na	-2.6	9.37E-06	5.36E-05	39
miR-4533	na	>0.05	na	na	na	>0.05	na	na	-2.6	4.74E-03	1.03E-02	4
miR-335-3p	na	>0.05	na	na	na	>0.05	na	na	-2.6	4.06E-07	3.65E-06	24
miR-766-3p	na	>0.05	na	na	na	>0.05	na	na	-2.6	4.47E-04	1.43E-03	10
miR-1273h-3p	na	>0.05	na	na	na	>0.05	na	na	-2.7	1.64E-06	1.23E-05	15
miR-4433-5p	na	>0.05	na	na	na	>0.05	na	na	-2.8	1.20E-05	6.69E-05	6
miR-485-5p	na	>0.05	na	na	na	>0.05	na	na	-2.8	2.56E-06	1.75E-05	73
miR-1301-3p	na	>0.05	na	na	na	>0.05	na	na	-2.8	2.86E-08	3.45E-07	46
miR-556-3p	na	>0.05	na	na	na	>0.05	na	na	-2.8	8.84E-04	2.53E-03	4
miR-6721-5p	na	>0.05	na	na	na	>0.05	na	na	-2.8	1.69E-05	8.99E-05	11
miR-409-3p	na	>0.05	na	na	na	>0.05	na	na	-2.8	2.21E-06	1.58E-05	654
miR-4446-3p	na	>0.05	na	na	na	>0.05	na	na	-2.8	6.57E-06	4.01E-05	21
miR-4646-3p	na	>0.05	na	na	na	>0.05	na	na	-3.0	5.76E-09	8.79E-08	6
miR-543	na	>0.05	na	na	na	>0.05	na	na	-3.1	2.65E-06	1.79E-05	6
miR-485-3p	na	>0.05	na	na	na	>0.05	na	na	-3.1	1.72E-06	1.27E-05	136
miR-5193	na	>0.05	na	na	na	>0.05	na	na	-3.3	1.21E-05	6.70E-05	5
miR-5010-3p	na	>0.05	na	na	na	>0.05	na	na	-3.3	2.07E-09	3.52E-08	8
miR-4433b-3p	na	>0.05	na	na	na	>0.05	na	na	-3.7	1.02E-04	4.12E-04	34
miR-4433b-5p	na	>0.05	na	na	na	>0.05	na	na	-4.0	7.04E-10	1.38E-08	2369
miR-6772-3p	na	>0.05	na	na	na	>0.05	na	na	-4.1	7.21E-10	1.38E-08	7
miR-127-3p	na	>0.05	na	na	na	>0.05	na	na	-4.1	1.45E-08	2.07E-07	19
miR-5698	na	>0.05	na	na	na	>0.05	na	na	-4.2	4.21E-05	1.97E-04	5
miR-3620-3p	na	>0.05	na	na	na	>0.05	na	na	-4.4	2.45E-06	1.70E-05	4
miR-4513	na	>0.05	na	na	na	>0.05	na	na	-5.6	1.85E-02	3.44E-02	4

Table S6. 104 circulating miRNAs previously identified via high-throughput screening to be differentially altered in plasma, serum or whole blood of women with preeclampsia versus normotensive pregnancy. Data is adapted from a systematic review by Sheikh et al. [4].

MicroRNA ID		Number of Citations	References
Source Nomenclature	Updated Nomenclature		
519d	miR-519d-3p	3	Li et al., 2013[9], Yang et al., 2011[10], Yang et al., 2015[11]
517c	miR-517c-3p	3	Li et al., 2013, Yang et al., 2011, Yang et al., 2015
29a	miR-29a-3p	3	Li et al., 2013, Yang et al., 2011, Yang et al., 2015
144	miR-144-3p	3	Li et al., 2013, Ura et al., 2014[12], Wu et al., 2012[13]
130a	miR-130a-3p	3	Li et al., 2013, Wu et al., 2012, Yang et al., 2015
18a	miR-18a-5p	2	Li et al., 2013, Yang et al., 2015
125b	miR-125b-5p	2	Li et al., 2013, Yang et al., 2011
27a	miR-27a-3p	2	Li et al., 2013, Yang et al., 2015
24	miR-24-3p	2	Li et al., 2013, Wu et al., 2012
518b	miR-518b	2	Li et al., 2013, Ura et al., 2014
25	miR-25-3p	2	Li et al., 2013, Ura et al., 2014
223	miR-223-3p	2	Li et al., 2013, Yang et al., 2011
185	miR-185-5p	2	Li et al., 2013, Yang et al., 2011
126	miR-126-3p	2	Ura et al., 2014, Yang et al., 2015
518e	miR-518e-3p	2	Yang et al., 2011, Yang et al., 2015
19a	miR-19a-3p	1	Li et al., 2013
101	miR-101-3p	1	Li et al., 2013
26b	miR-26b-5p	1	Li et al., 2013
378	miR-378a-3p	1	Li et al., 2013
144*	miR-144-5p	1	Li et al., 2013
182	miR-182-5p	1	Li et al., 2013
29c	miR-29c-3p	1	Li et al., 2013
518c	miR-518c-3p	1	Li et al., 2013
515-3p	miR-515-3p	1	Li et al., 2013
424	miR-424-5p	1	Li et al., 2013
29b	miR-29b-3p	1	Li et al., 2013
21	miR-21-5p	1	Li et al., 2013
19b	miR-19b-3p	1	Li et al., 2013
451	miR-451a	1	Li et al., 2013
210	miR-210-3p	1	Ura et al., 2014
32	miR-32-5p	1	Ura et al., 2014
204	miR-204-5p	1	Ura et al., 2014
296-5p	miR-296-5p	1	Ura et al., 2014

152	miR-152-3p	1	Ura et al., 2014
335	miR-335-5p	1	Ura et al., 2014
26a	miR-26a-5p	1	Wu et al., 2012
151-3p	miR-151-3p	1	Wu et al., 2012
181a	miR-181a-5p	1	Wu et al., 2012
30d	miR-30d-5p	1	Wu et al., 2012
342-3p	miR-342-3p	1	Wu et al., 2012
16	miR-16-5p	1	Wu et al., 2012
520g	miR-520g-3p	1	Yang et al., 2011
542-3p	miR-542-3p	1	Yang et al., 2011
135b	miR-135b-5p	1	Yang et al., 2015
149	miR-149-5p	1	Yang et al., 2015
188-5p	miR-188-5p	1	Yang et al., 2015
18b	miR-18b-5p	1	Yang et al., 2015
203	miR-203-3p	1	Yang et al., 2015
205	miR-205-5p	1	Yang et al., 2015
224	miR-224-5p	1	Yang et al., 2015
301a	miR-301a-3p	1	Yang et al., 2015
518a-3p	miR-518a-3p	1	Yang et al., 2015
126*	miR-126-5p	1	Yang et al., 2015
142-3p	miR-142-3p	1	Yang et al., 2015
93	miR-93-5p	1	Yang et al., 2015
34a	miR-34a-5p	1	Li et al., 2013
517b	miR-517b-3p	2	Li et al., 2013, Yang et al., 2011
221	miR-221-3p	2	Li et al., 2013, Wu et al., 2012
521	miR-521	2	Li et al., 2013, Yang et al., 2011
519a	miR-519a-3p	2	Li et al., 2013, Yang et al., 2011
520h	miR-520h	2	Li et al., 2013, Yang et al., 2011
125a-5p	miR-125a-5p	2	Li et al., 2013, Yang et al., 2011
145	miR-145-5p	2	Li et al., 2013, Wu et al., 2012
15b	miR-15b-5p	2	Li et al., 2013, Ura et al., 2014
320c	miR-320c	2	Li et al., 2013, Yang et al., 2011
let-7f	let-7f-5p	2	Li et al., 2013, Yang et al., 2011
23a*	miR-23a-5p	1	Akehurst et al., 2015 [14]
196b-5p	miR-196b-5p	1	Akehurst et al., 2015
206-5p	miR-206-5p	1	Akehurst et al., 2015
502-5p	miR-502-5p	1	Akehurst et al., 2015
503-5p	miR-503-5p	1	Akehurst et al., 2015
758-3p	miR-758-3p	1	Akehurst et al., 2015
10a	miR-10a-5p	1	Li et al., 2013
114	miR-114	1	Li et al., 2013

15b*	miR-15b-3p	1	Li et al., 2013
30a	miR-30a-5p	1	Li et al., 2013
519e	miR-519e-3p	1	Li et al., 2013
299a-5p	miR-299a-5p	1	Li et al., 2013
23a	miR-23a-3p	1	Li et al., 2013
23b	miR-23b-3p	1	Li et al., 2013
525-3p	miR-525-3p	1	Li et al., 2013
199a-5p	miR-199a-5p	1	Li et al., 2013
100	miR-100-5p	1	Li et al., 2013
99a	miR-99a-5p	1	Li et al., 2013
512-5p	miR-512-5p	1	Li et al., 2013
30b	miR-30b-5p	1	Li et al., 2013
107	miR-107	1	Li et al., 2013
1233	miR-1233-3p	1	Ura et al., 2014
650	miR-650	1	Ura et al., 2014
520a	miR-520a-3p	1	Ura et al., 2014
215	miR-215-5p	1	Ura et al., 2014
193a-3p	miR-193a-3p	1	Ura et al., 2014
668	miR-668-3p	1	Ura et al., 2014
376a	miR-376a-3p	1	Ura et al., 2014
574-5p	miR-574-5p	1	Wu et al., 2012
130b	miR-130b-3p	1	Wu et al., 2012
103	miR-103a-3p	1	Wu et al., 2012
425	miR-425-5p	1	Wu et al., 2012
136	miR-136-5p	1	Yang et al., 2011
let-7f-1-star	let-7f-1-3p	1	Yang et al., 2011
let-7a-star	let-7a-3p	1	Yang et al., 2011
1260	miR-1260a	1	Yang et al., 2011
let-7d	let-7d-5p	1	Yang et al., 2011
1272	miR-1272	1	Yang et al., 2011

Table S7. Overlap in differentially altered miRs ($p<0.05$) identified in cohorts 1, 2 and 4 in relation to a history of PE or NT pregnancy (cohort 1 and 2) or current PE versus NT. Only the direction of change in miRNA level is shown for previously published data. Up/Down denotes conflicting information on direction.

2 common miRs	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				current PE vs NT pregnancy (cohort 4; published literature)					
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Li et al 2013	Yang et al 2011	Wu et al. 2012	Yang et al 2015	Ura et al. 2014	Akehurst et al., 2015
miR-206	-10.6	1.64E-06	6.98E-04	242	-1.8	2.06E-02	6.21E-01	25						Up
miR-376a-3p	-4.7	1.10E-03	1.17E-01	7	-1.6	3.50E-02	7.63E-01	23					Down	
6 miRs common to cohort 2 and 4	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				current PE vs NT pregnancy (cohort 4; published literature)					
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Li et al 2013	Yang et al 2011	Wu et al. 2012	Yang et al 2015	Ura et al. 2014	Akehurst et al., 2015
miR-30a-5p	na	>0.05	na	na	-1.2	4.93E-02	9.18E-01	491	Up					
miR-29a-3p	na	>0.05	na	na	-1.4	1.98E-03	2.37E-01	449	Up	Up		Up		
miR-125b-5p	na	>0.05	na	na	-1.5	3.07E-03	2.37E-01	603	Up	Up				
miR-99a-5p	na	>0.05	na	na	-1.5	2.17E-03	2.37E-01	126	Up					
miR-205-5p	na	>0.05	na	na	-1.6	4.95E-03	2.77E-01	56				Up		
miR-204-5p	na	>0.05	na	na	-1.7	2.68E-02	7.02E-01	8					Up	
1 miR common to cohort 1 and 4	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				current PE vs NT pregnancy (cohort 4; published literature)					
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Li et al 2013	Yang et al 2011	Wu et al. 2012	Yang et al 2015	Ura et al. 2014	Akehurst et al., 2015
miR-30b-5p	-1.8	1.23E-02	4.45E-01	14.88265	na	>0.05	na	na	Up					
2 common miRs	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				current PE vs NT pregnancy (cohort 4; published literature)					
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Li et al 2013	Yang et al 2011	Wu et al. 2012	Yang et al 2015	Ura et al. 2014	Akehurst et al., 2015
miR-1299	4.9	3.67E-03	1.96E-01	23	4.0	3.63E-03	2.37E-01	10	na	na	na	na	na	na
miR-4662a-5p	3.5	1.33E-02	4.45E-01	5	-2.0	9.21E-03	4.51E-01	4	na	na	na	na	na	na
10 miRs unique to cohort 2	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				current PE vs NT pregnancy (cohort 4; published literature)					
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Li et al 2013	Yang et al 2011	Wu et al. 2012	Yang et al 2015	Ura et al. 2014	Akehurst et al., 2015
miR-1224-5p	na	>0.05	na	na	2.1	3.78E-02	7.79E-01	10	na	na	na	na	na	na
miR-877-3p	na	>0.05	na	na	1.6	2.32E-02	6.50E-01	8	na	na	na	na	na	na
miR-22-3p	na	>0.05	na	na	1.3	3.47E-02	7.63E-01	506	na	na	na	na	na	na
miR-382-3p	na	>0.05	na	na	-1.7	1.49E-02	5.32E-01	15	na	na	na	na	na	na
miR-193b-5p	na	>0.05	na	na	-2.0	1.11E-02	4.84E-01	17	na	na	na	na	na	na
miR-885-5p	na	>0.05	na	na	-2.0	2.95E-02	7.23E-01	8	na	na	na	na	na	na
miR-885-3p	na	>0.05	na	na	-2.3	1.74E-02	5.70E-01	15	na	na	na	na	na	na
miR-203a	na	>0.05	na	na	-2.4	2.67E-03	2.37E-01	251	na	na	na	na	na	na
miR-122-5p	na	>0.05	na	na	-2.6	4.65E-04	1.82E-01	15405	na	na	na	na	na	na
miR-9-5p	na	>0.05	na	na	-2.9	1.40E-02	5.32E-01	8	na	na	na	na	na	na
25 miRs unique to cohort 1	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				current PE vs NT pregnancy (cohort 4; published literature)					
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Li et al 2013	Yang et al 2011	Wu et al. 2012	Yang et al 2015	Ura et al. 2014	Akehurst et al., 2015
miR-184	10.3	2.35E-04	3.35E-02	52	na	>0.05	na	na	na	na	na	na	na	na
miR-6730-3p	7.3	3.46E-03	1.96E-01	4	na	>0.05	na	na	na	na	na	na	na	na
miR-499a-5p	5.8	1.85E-03	1.58E-01	45	na	>0.05	na	na	na	na	na	na	na	na
miR-218-5p	5.4	2.24E-03	1.60E-01	7	na	>0.05	na	na	na	na	na	na	na	na
miR-3591-5p	3.8	4.01E-02	6.34E-01	5	na	>0.05	na	na	na	na	na	na	na	na
miR-4667-5p	3.6	3.60E-02	5.91E-01	4	na	>0.05	na	na	na	na	na	na	na	na
miR-874-5p	3.3	1.64E-02	4.54E-01	4	na	>0.05	na	na	na	na	na	na	na	na
miR-1	3.0	5.46E-03	2.59E-01	531	na	>0.05	na	na	na	na	na	na	na	na
miR-202-3p	2.9	4.68E-02	6.54E-01	6	na	>0.05	na	na	na	na	na	na	na	na
miR-133a-3p	2.7	2.78E-02	5.16E-01	76	na	>0.05	na	na	na	na	na	na	na	na
miR-6767-5p	2.7	1.95E-02	4.91E-01	7	na	>0.05	na	na	na	na	na	na	na	na
miR-6741-3p	1.7	2.61E-02	5.07E-01	11	na	>0.05	na	na	na	na	na	na	na	na
miR-769-5p	-1.5	4.20E-02	6.40E-01	27	na	>0.05	na	na	na	na	na	na	na	na
miR-1277-5p	-1.8	2.35E-02	5.01E-01	13	na	>0.05	na	na	na	na	na	na	na	na
miR-221-5p	-1.9	3.03E-02	5.39E-01	9	na	>0.05	na	na	na	na	na	na	na	na
miR-2355-3p	-1.9	3.54E-02	5.91E-01	7	na	>0.05	na	na	na	na	na	na	na	na
miR-505-3p	-1.9	2.22E-02	5.01E-01	9	na	>0.05	na	na	na	na	na	na	na	na
miR-369-5p	-2.2	2.54E-02	5.07E-01	18	na	>0.05	na	na	na	na	na	na	na	na
miR-493-5p	-2.3	2.29E-02	5.01E-01	13	na	>0.05	na	na	na	na	na	na	na	na
miR-431-5p	-2.4	1.47E-02	4.49E-01	73	na	>0.05	na	na	na	na	na	na	na	na
miR-329-3p	-2.5	4.56E-02	6.54E-01	7	na	>0.05	na	na	na	na	na	na	na	na
miR-136-3p	-2.7	1.35E-02	4.45E-01	10	na	>0.05	na	na	na	na	na	na	na	na
miR-28-5p	-2.7	1.70E-02	4.54E-01	5	na	>0.05	na	na	na	na	na	na	na	na
miR-889-3p	-2.9	7.52E-03	3.21E-01	17	na	>0.05	na	na	na	na	na	na	na	na
miR-1292-5p	-3.6	8.28E-05	1.77E-02	8	na	>0.05	na	na	na	na	na	na	na	na

Table S7. Continued.

95 miRs unique to cohort 4	prior PE vs. NT pregnancy (ACS cohort 1)				prior PE vs. NT preg. (non-ACS cohort 2)				current PE vs NT pregnancy (cohort 4; published literature)					
	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Fold Change	p value	FDR-adjusted p value	miR level (CPM)	Li et al 2013	Yang et al 2011	Wu et al. 2012	Yang et al 2015	Ura et al. 2014	Akehurst et al., 2015
let-7a-3p	na	>0.05	na	na	na	>0.05	na	na		Up				
let-7d-5p	na	>0.05	na	na	na	>0.05	na	na		Down				
let-7f-1-3p	na	>0.05	na	na	na	>0.05	na	na		Up				
let-7f-5p	na	>0.05	na	na	na	>0.05	na	na	Down	Down				
miR-100-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-101-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-103a-3p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-107	na	>0.05	na	na	na	>0.05	na	na	Down					
miR-10a-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-114	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-1233-3p	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-125a-5p	na	>0.05	na	na	na	>0.05	na	na	Up	Up				
miR-126-3p	na	>0.05	na	na	na	>0.05	na	na				Up	Down	
miR-126-5p	na	>0.05	na	na	na	>0.05	na	na				Up/Down		
miR-1260a	na	>0.05	na	na	na	>0.05	na	na		Down				
miR-1272	na	>0.05	na	na	na	>0.05	na	na		Down				
miR-130a-3p	na	>0.05	na	na	na	>0.05	na	na	Up			Up		
miR-130b-3p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-135b-5p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-136-5p	na	>0.05	na	na	na	>0.05	na	na		Up				
miR-142-3p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-144-3p	na	>0.05	na	na	na	>0.05	na	na	Up/down		Down			
miR-144-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-145-5p	na	>0.05	na	na	na	>0.05	na	na	Up		Up			
miR-149-5p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-151a-3p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-152-3p	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-15b-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-15b-5p	na	>0.05	na	na	na	>0.05	na	na	Down				Down	
miR-16-5p	na	>0.05	na	na	na	>0.05	na	na			Down			
miR-181a-5p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-182-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-185-5p	na	>0.05	na	na	na	>0.05	na	na	Down	Down				
miR-188-5p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-18a-5p	na	>0.05	na	na	na	>0.05	na	na	Up			Up		
miR-18b-5p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-193a-3p	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-196b-5p	na	>0.05	na	na	na	>0.05	na	na						Up
miR-199a-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-19a-3p	na	>0.05	na	na	na	>0.05	na	na	Up/down					
miR-19b-3p	na	>0.05	na	na	na	>0.05	na	na	Down/Up					
miR-203a-3p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-21-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-210-3p	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-215-5p	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-221-3p	na	>0.05	na	na	na	>0.05	na	na	Up		Up			
miR-223-3p	na	>0.05	na	na	na	>0.05	na	na	Down	Down				

Table S7. Continued.

miR-224-5p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-23a-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-23a-5p	na	>0.05	na	na	na	>0.05	na	na						Down
miR-23b-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-24-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-25-3p	na	>0.05	na	na	na	>0.05	na	na	Down/Up					
miR-26a-5p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-26b-5p	na	>0.05	na	na	na	>0.05	na	na	UP					
miR-27a-3p	na	>0.05	na	na	na	>0.05	na	na	UP			Up		
miR-296-5p	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-29b-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-29c-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-301a-3p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-30d-5p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-32-5p	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-320c	na	>0.05	na	na	na	>0.05	na	na	Down	Down				
miR-335-5p	na	>0.05	na	na	na	>0.05	na	na					Down	
miR-342-3p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-34a-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-378a-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-424-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-425-5p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-451a	na	>0.05	na	na	na	>0.05	na	na	Down					
miR-502-5p	na	>0.05	na	na	na	>0.05	na	na						Up
miR-503-5p	na	>0.05	na	na	na	>0.05	na	na						Up
miR-512-5p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-515-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-517b-3p	na	>0.05	na	na	na	>0.05	na	na	Up	Up				
miR-517c-3p	na	>0.05	na	na	na	>0.05	na	na	Up	Up		Up		
miR-518a-3p	na	>0.05	na	na	na	>0.05	na	na				Up		
miR-518b	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-518c-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-518e-3p	na	>0.05	na	na	na	>0.05	na	na		Up		Up		
miR-519a-3p	na	>0.05	na	na	na	>0.05	na	na	Up	Up				
miR-519d-3p	na	>0.05	na	na	na	>0.05	na	na	Up	Up		Up		
miR-519e-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-520a-3p	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-520g-3p	na	>0.05	na	na	na	>0.05	na	na		Up				
miR-520h	na	>0.05	na	na	na	>0.05	na	na	Up	Up				
miR-521	na	>0.05	na	na	na	>0.05	na	na	Up	Up				
miR-525-3p	na	>0.05	na	na	na	>0.05	na	na	Up					
miR-542-3p	na	>0.05	na	na	na	>0.05	na	na		Up				
miR-574-5p	na	>0.05	na	na	na	>0.05	na	na			Up			
miR-650	na	>0.05	na	na	na	>0.05	na	na					Up	
miR-668-3p	na	>0.05	na	na	na	>0.05	na	na					Down	
miR-758-3p	na	>0.05	na	na	na	>0.05	na	na						Up
miR-93-5p	na	>0.05	na	na	na	>0.05	na	na				Up/Down		
miR-299-5p	na	>0.05	na	na	na	>0.05	na	na	Up					

Table S8. Number of differentially altered miRNAs that target each gene implicated in the Wnt signaling pathway. Data is stratified by cohort and the database that was used for miRNA-target integration. T: Targetscan7.2 (predicted). M: miRTarBase7.0 (experimentally-validated).

Gene Targets	prior PE vs. NT pregnancy (Cohort 1)		prior PE vs. NT pregnancy (Cohort 2)		ACS vs non-ACS (Cohort 3)		current PE vs NT pregnancy (cohort 4; literature)	
	T	M	T	M	T	M	T	M
NFAT5	2	4	7	4	39	17	27	12
CCND2	1	4	3	3	36	35	26	23
SMAD2	3	3	3	2	32	20	20	15
CSNK2A1	3	1	3	3	26	22	16	15
NFATC3	3	1	5	1	19	4	13	1
CCND1	1	3	1	4	16	37	15	26
GSK3B	2	2	2	3	20	23	15	14
PPP3R1	1	1	3	3	23	9	18	8
MAPK8	1	2	2	1	28	13	21	8
PPP3CB	3	1	1	1	11	5	6	3
ROCK2	1	2	2	1	20	7	16	7
TCF7L2	2	1	2	1	16	12	7	8
APC	1	1	2	1	25	9	15	7
PPP2CA	1	1	2	1	17	8	9	5
SFRP1	1	2	1	1	5	12	3	4
TBL1XR1	1	1	2	1	29	19	22	6
PPP2R5C	1	1	1	1	11	9	9	9
FZD4	0	0	3	1	34	8	22	7
TP53	0	0	2	2	12	30	4	17
SMAD4	0	0	1	3	5	21	3	24
FRAT2	0	0	2	2	7	12	8	4
FZD8	0	0	3	1	17	3	6	3
CTNNBIP1	0	0	2	1	19	6	17	6
DVL3	0	0	2	1	12	12	7	8
PPP3CA	0	0	2	1	20	1	13	3
WNT1	0	0	1	2	14	6	9	2
CAMK2G	0	0	2	1	15	3	8	2
PPP2R1B	0	0	1	2	8	7	5	7
TCF7	0	0	2	1	6	4	4	2
EP300	0	0	1	1	10	15	4	7
FOSL1	0	0	1	1	11	7	11	3
RHOA	0	0	1	1	5	16	0	0
NLK	3	2	0	0	33	11	20	6
LRP6	3	2	0	0	35	9	17	7
PRKCA	2	2	0	0	12	5	2	3
CSNK1A1	2	1	0	0	22	11	15	9
FZD5	1	2	0	0	23	10	12	11
PPP2R5E	1	2	0	0	23	8	15	8
ROCK1	2	1	0	0	25	6	16	4
VANGL2	2	1	0	0	17	5	8	3
WNT5A	1	2	0	0	10	8	2	5
CREBBP	1	2	0	0	9	6	4	2
FZD2	1	2	0	0	6	7	4	2
DKK2	2	1	0	0	8	3	3	3
LEF1	1	1	0	0	5	2	5	1
NFATC2	4	1	0	0	27	3	0	0
AXIN1	1	1	0	0	3	3	0	0

Table S8. Continued

Gene Targets	prior PE vs. NT pregnancy (Cohort 1)		prior PE vs. NT pregnancy (Cohort 2)		ACS vs non-ACS (Cohort 3)		current PE vs NT pregnancy (cohort 4; literature)	
	T	M	T	M	T	M	T	M
FZD6	0	0	0	0	10	26	10	19
VANGL1	0	0	0	0	20	8	17	3
PRICKLE2	0	0	0	0	16	7	12	10
PRKACB	0	0	0	0	17	3	16	7
WNT2B	0	0	0	0	22	5	14	2
RAC1	0	0	0	0	11	19	2	7
SMAD3	0	0	0	0	8	16	6	8
DAAM1	0	0	0	0	18	2	15	2
AXIN2	0	0	0	0	9	13	8	6
NKD1	0	0	0	0	17	7	8	4
BTRC	0	0	0	0	12	9	9	4
CCND3	0	0	0	0	12	8	8	6
WNT9B	0	0	0	0	17	5	8	1
MAP3K7	0	0	0	0	7	11	4	8
TBL1X	0	0	0	0	15	3	10	2
MAPK10	0	0	0	0	10	5	11	2
CTNNB1	0	0	0	0	2	15	1	9
WNT4	0	0	0	0	10	4	8	4
FBXW11	0	0	0	0	13	2	9	1
PRICKLE1	0	0	0	0	10	6	6	3
WNT3A	0	0	0	0	11	4	7	3
SENP2	0	0	0	0	11	6	6	1
CAMK2D	0	0	0	0	10	1	10	1
SIAH1	0	0	0	0	11	2	7	2
PRKX	0	0	0	0	6	8	4	3
WNT7B	0	0	0	0	5	6	6	4
DAAM2	0	0	0	0	6	3	8	3
WNT10B	0	0	0	0	10	2	7	1
PPARD	0	0	0	0	5	6	6	2
WNT9A	0	0	0	0	10	1	7	1
CTBP2	0	0	0	0	10	3	4	1
PPP2R1A	0	0	0	0	6	6	4	1
WNT10A	0	0	0	0	4	4	5	4
WNT7A	0	0	0	0	7	4	4	2
FZD7	0	0	0	0	2	8	1	5
SOX17	0	0	0	0	7	4	3	2
WIF1	0	0	0	0	8	1	4	3
WNT3	0	0	0	0	7	3	4	2
PRKCB	0	0	0	0	3	8	1	3
PRKACA	0	0	0	0	4	4	4	1
CHD8	0	0	0	0	6	4	1	1
WNT16	0	0	0	0	4	5	2	1
DKK1	0	0	0	0	2	3	3	3
PLCB4	0	0	0	0	8	1	1	1
PPP2R5A	0	0	0	0	4	1	5	1
SFRP4	0	0	0	0	3	2	4	2
FZD1	0	0	0	0	1	3	1	3
DVL2	0	0	0	0	4	1	1	1
FZD10	0	0	0	0	3	1	2	1
PPP2CB	0	0	0	0	2	3	1	1
RUVBL1	0	0	0	0	2	2	1	1
WNT2	0	0	0	0	1	1	2	2

Table S8. Continued

Gene Targets	prior PE vs. NT pregnancy (Cohort 1)		prior PE vs. NT pregnancy (Cohort 2)		ACS vs non-ACS (Cohort 3)		current PE vs NT pregnancy (cohort 4; literature)	
	T	M	T	M	T	M	T	M
FZD3	0	0	0	0	32	2	0	0
SKP1	0	0	0	0	11	6	0	0
CAMK2A	0	0	0	0	12	3	0	0
PLCB1	0	0	0	0	12	3	0	0
PSEN1	0	0	0	0	10	1	0	0
JUN	0	0	0	0	2	8	0	0
APC2	0	0	0	0	7	1	0	0
TCF7L1	0	0	0	0	4	4	0	0
CTBP1	0	0	0	0	1	5	0	0
PPP2R5D	0	0	0	0	3	3	0	0
CACYBP	0	0	0	0	4	1	0	0
RBX1	0	0	0	0	2	3	0	0
FRAT1	0	0	0	0	1	3	0	0
NFATC1	0	0	0	0	1	3	0	0
PLCB3	0	0	0	0	1	1	0	0
PORCN	0	0	0	0	1	1	0	0
SFRP2	0	0	0	0	1	1	0	0
CXXC4	0	0	0	0	0	0	4	1
DVL1	0	0	0	0	0	0	1	3
LRP5	0	0	0	0	0	0	2	2
PPP3R2	0	0	0	0	0	0	1	1

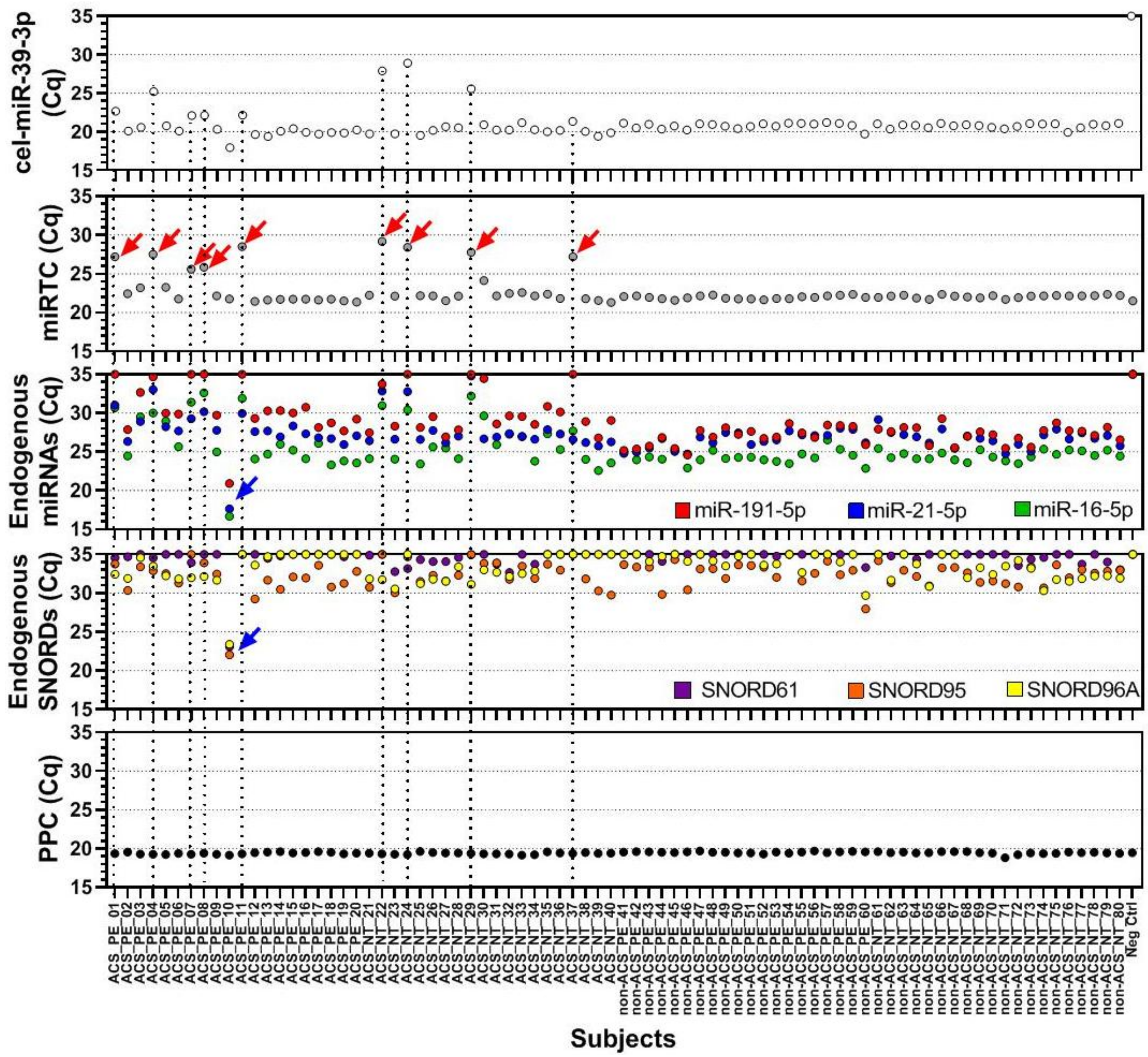

Figure S1. Quality control assessment of total RNA extracted from plasma using the miScript miRNA QC PCR array. The PCR array contains primer assays for several external spike-in controls introduced in fixed quantities at different stages in the RNA extraction and downstream RT-qPCR reactions. Cel-miR-39-3p (a miRNA mimic with no mammalian homolog) is added at the beginning of RNA extraction just after chemical denaturation of nucleases to assess variations in RNA extraction efficiency. miRTC (a synthetic RNA molecule) is incorporated into the reverse transcription reaction to monitor relative reaction efficiencies. PPC is a positive PCR control (synthetic DNA molecule) used to monitor the relative efficiency of downstream PCR reactions. In addition, primer assays for several endogenous miRNAs (i.e., miR-16, miR-21 and miR-191) are included as positive controls since these miRNAs are ubiquitously expressed across many different biologic specimens including body fluids, and several endogenous small nuclear/nucleolar RNAs (i.e., SNORD61, SNORD95, and SNORD96A) are included as negative controls (or markers of cellular contamination) since these are typically expressed abundantly in cells, but relatively poorly in body fluids. Each panel shows the PCR quantification cycles (Cq) assessed for different analytes across all 80 subjects. Each subject is denoted by the cohort (i.e., ACS or non-ACS), prior preeclampsia (PE) or normotensive pregnancy (NT) exposure, and a unique numerical identifier. Red arrows denote subject samples that were flagged for inhibition of the reverse transcription control (miRTC). In most cases these flagged subjects also showed a concomitant decrease in the levels (i.e., higher Cq values) of the external spike-in cel-miR-39-3p and several endogenous miRNAs. Blue arrow denotes a subject sample that showed a marked increase (i.e., lower Cq values) in the levels of both endogenous miRNAs and SNORDs, indicative of potential cellular contamination. Neg Ctrl denotes a mock RNA extraction performed with water instead of plasma. Overall, the majority of samples showed relatively consistent levels in both external and endogenous controls suggesting that the quality and quantity of extracted RNA was generally comparable between samples.

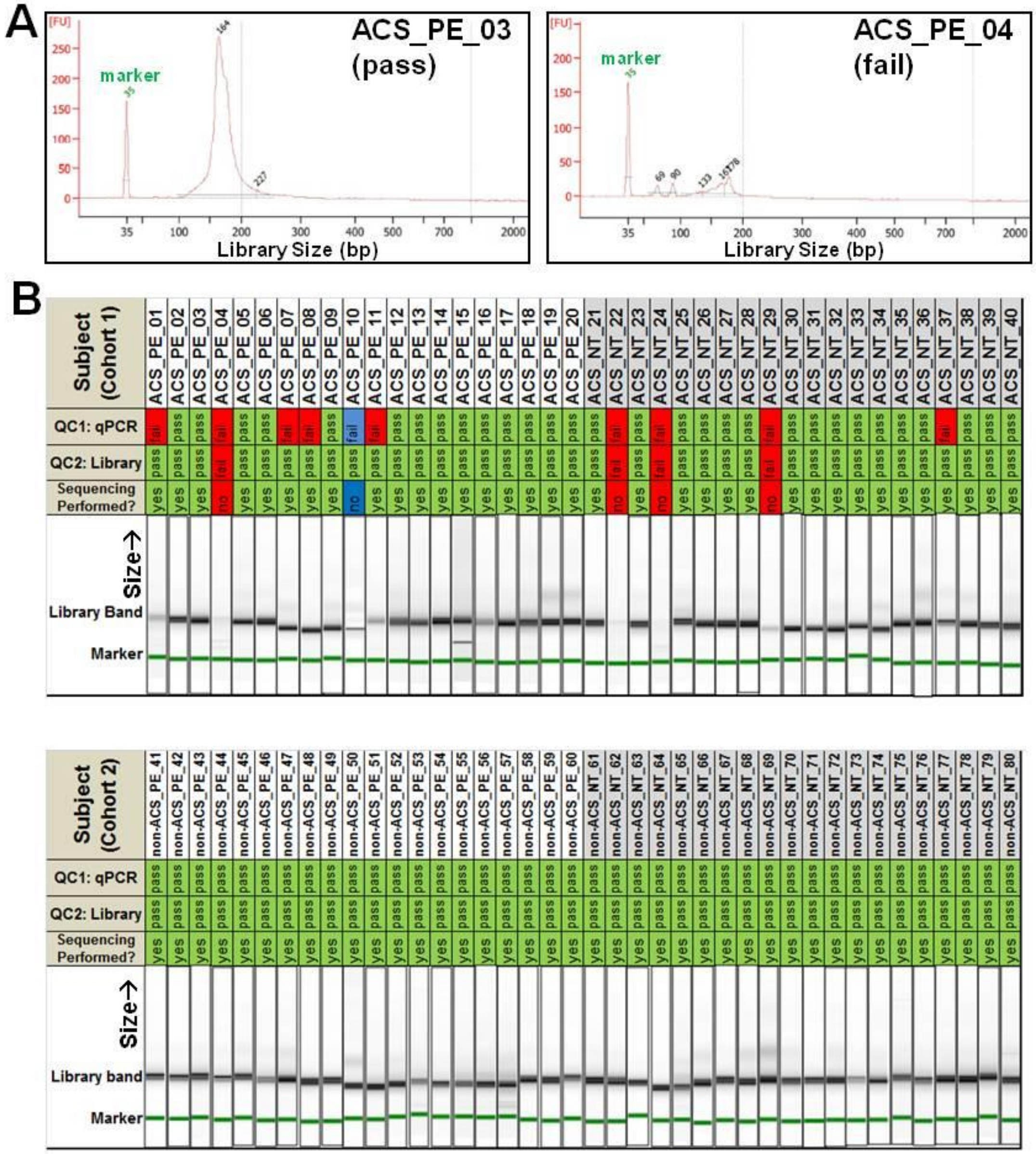

Figure S2. Quality control assessment of sequencing libraries. Library qualification was performed on an Agilent Bioanalyzer using High Sensitivity DNA Chips (A) Electropherogram showing DNA library levels (in arbitrary fluorescence units, FU) versus size in base-pairs (bp). Left panel is an example of a library (generated for subject ACS_PE_03) that showed good yield and was considered to pass this quality control step. Right panel is an example of a library (generated for subject ACS_PE_04) that showed poor yield and quality (consistent with prior PCR QC assays), and therefore was not sequenced. (B) Summary of quality control assays for all 80 subjects. Each subject is denoted by the cohort (i.e., ACS or non-ACS), prior preeclampsia (PE) or normotensive pregnancy (NT) exposure, and a unique numerical identifier. Pass or fail outcomes are indicated for quality control assays conducted with either the plasma-extracted total RNA (i.e., for QC1 using RT-qPCR assays) or subsequently derived next-generation DNA sequencing libraries (i.e., QC2). Four subjects were not sequenced after failing both the PCR and library QC steps. An additional subject (ACS_PE_10; denoted in blue) was not sequenced because it showed evidence of cellular contamination via qPCR assay (i.e., marked elevation in levels of endogenous miRNAs and SNORDs). Virtual gel-like images are presented for each subject to show the quality of the sequencing libraries. Each gel-like image was generated from the corresponding Bioanalyzer electropherogram and manually compiled for presentation. Of note, the extracted RNA from subjects 01,07,08,11 and 37 was flagged for quality issues in the qPCR QC assays, but still generated libraries with sufficient yield/quality to conduct sequencing.

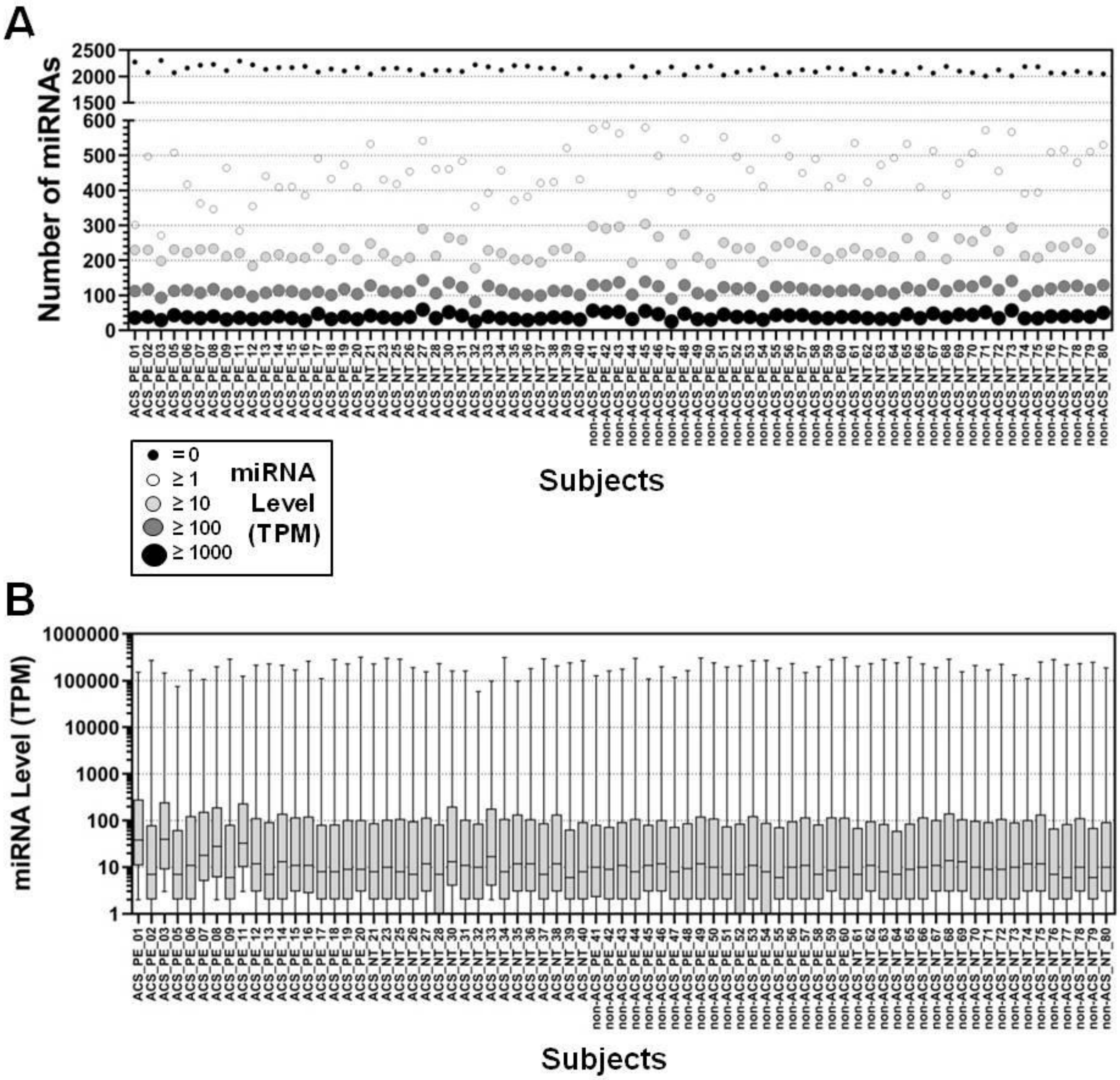

Figure S3. Distribution of plasma miRNA levels per subject identified by RNA-sequencing. **(A)** Number of miRNAs identified per subject per miRNA level. MiRNA levels are expressed as Tags Per Million mapped reads (TPM), which normalizes for differences in miRNA length and sequencing depth. **(B)** Distribution of miRNA levels per subject. Boxplots show median and interquartile range, and whiskers denote min-max range. MiRNAs with 0 TPM were excluded from the graph. Each subject is denoted by the cohort (i.e., ACS or non-ACS), prior preeclampsia (PE) or normotensive pregnancy (NT) exposure, and a unique numerical identifier.

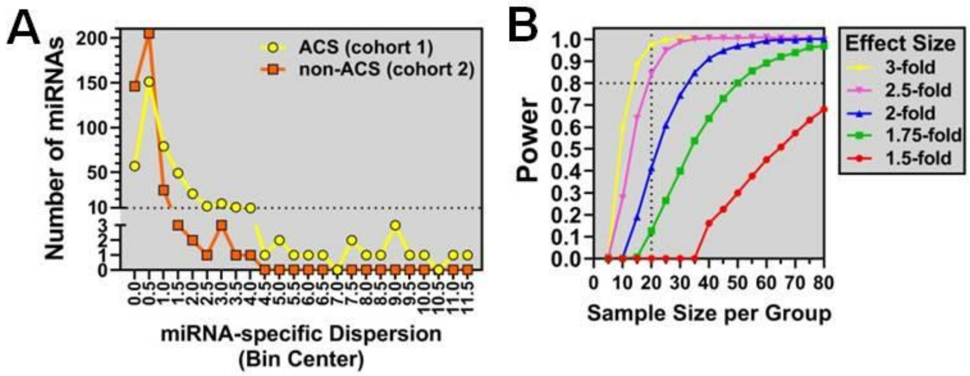

Figure S4. Dispersion of miRNA levels and related power curves. (A) Histogram showing number of miRNAs as a function of their level of dispersion across subjects (bin width = 0.5). miRNA-specific dispersion values were calculated from TMM-normalized counts for 427 miRs in ACS cohort 1 (n=17-18 subjects/exposure group) and 392 miRs in non-ACS cohort 2 (n=20 subjects/exposure group) using the R program RNASeqSampleSize. **(B)** Power curves showing the relationship between statistical power and sample size per group for varying effect sizes (i.e., magnitude of fold-change in miRNA level). Statistical power was estimated by RNASeqSampleSize for a hypothetical scenario assuming 400 assessed miRs, 25 differentially altered miRNAs at FDR<0.05, an average read count of 30 and dispersion level of 0.5.

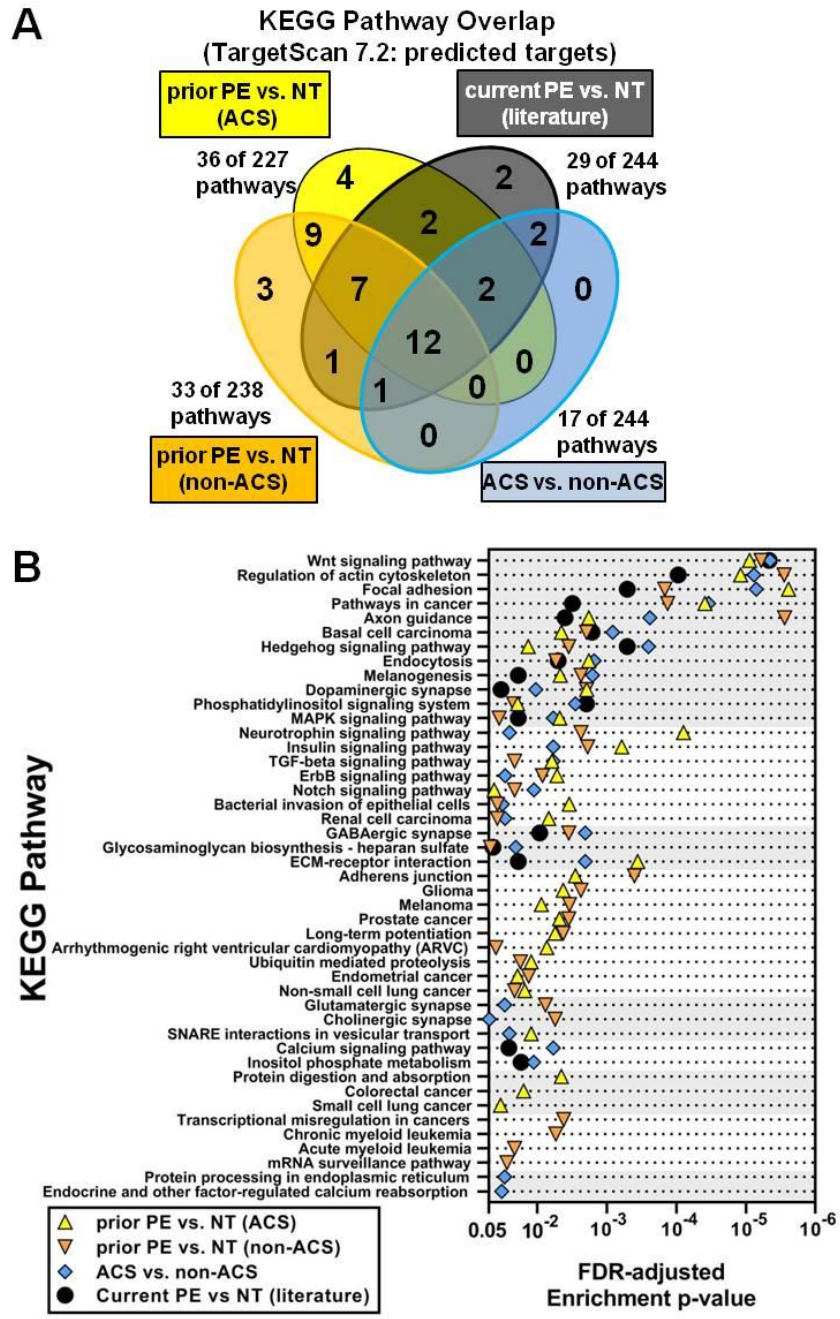

Figure S5. Integration of predicted gene targets and pathway enrichment analysis. Gene target integration was performed with 30 miRs ($p<0.05$), 20 miRs ($p<0.05$), 259 miRs (FDR<0.05) and 104 miRs ($p<0.05$) for cohort 1 (prior PE vs. NT exposure; ACS subjects), cohort 2 (prior PE vs. NT exposure; non-ACS subjects), cohort 3 (ACS vs. non-ACS exposure) and cohort 4 (current PE vs. NT exposure, curated from prior literature). **(A)** Venn diagram shows the number of KEGG pathways significantly enriched (FDR<0.05) with the predicted gene targets of the altered miRNA candidates (via Targetscan 7.2), and the level of overlap between different exposure groups. (**B)** Identity of specific KEGG pathways from panel A and associated false discovery rate-adjusted enrichment p-values for each exposure group.

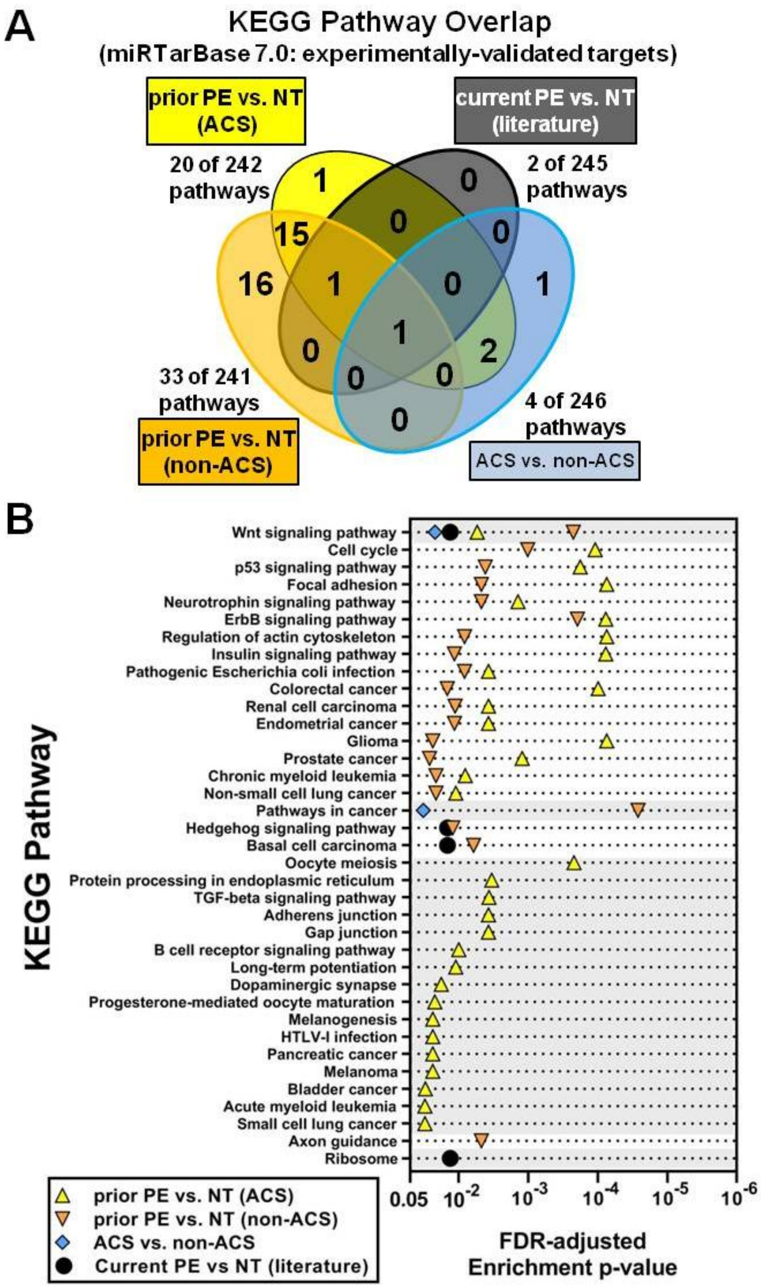

Figure S6. Integration of experimentally-validated gene targets and pathway enrichment analysis. Gene target integration was performed with 30 miRs ($p<0.05$), 20 miRs ($p<0.05$), 259 miRs (FDR<0.05) and 104 miRs ($p<0.05$) for cohort 1 (prior PE vs. NT exposure; ACS subjects), cohort 2 (prior PE vs. NT exposure; non-ACS subjects), cohort 3 (ACS vs. non-ACS exposure) and cohort 4 (current PE vs. NT exposure, curated from prior literature). (**A**) Venn diagram shows the number of KEGG biological pathways significantly enriched (FDR<0.05) with experimentally-validated targets of the altered miRNA candidates (via miRTarBase 7.0 database), and the overlap between different exposure groups. **(B)** Identity of specific KEGG pathways from panel A, and associated false discovery rate-adjusted enrichment p-values for each exposure group.

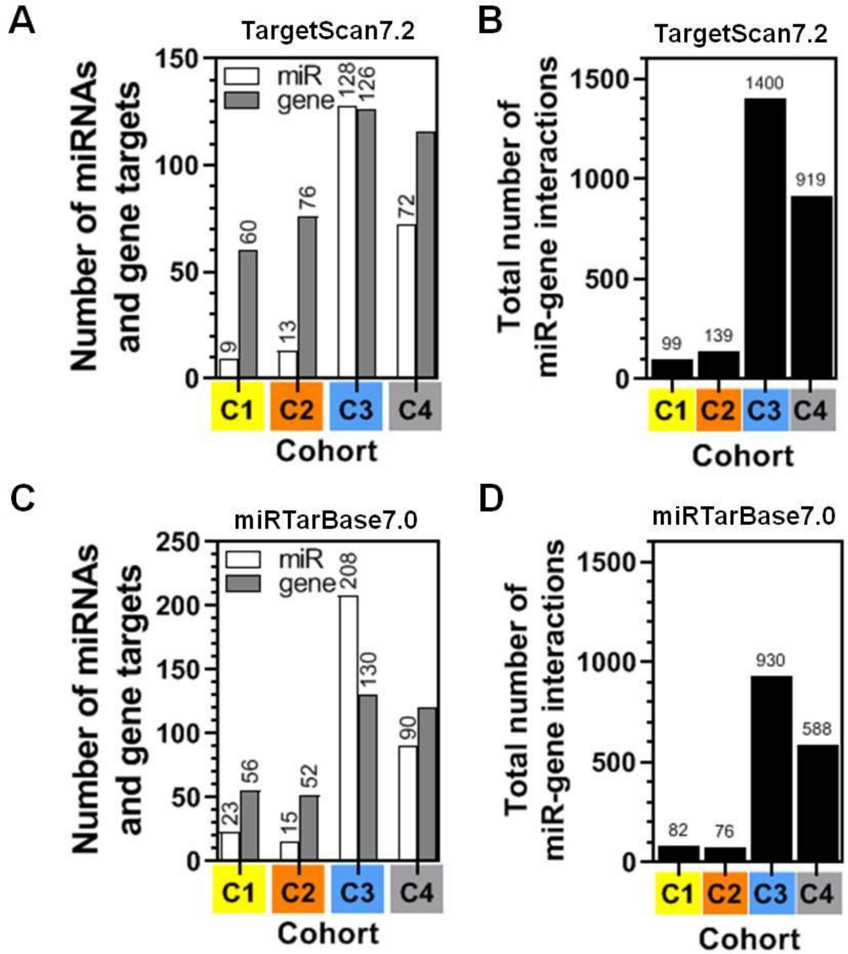

Figure S7. Characterization of miRNA-gene target interactions related to Wnt signaling in subjects with prior PE, current ACS or current PE. (A) Number of miRNAs and predicted gene targets identified in each cohort by Targetscan 7.2. (B) Total number of predicted miRNA-gene target interactions identified in each cohort by Targetscan7.2. (C) Number of miRNAs and experimentally-validated gene targets identified in each cohort by miRTarBase7.0. (D) Total number of experimentally-validated miRNA-gene target interactions identified in each cohort by miRTarBase. Cohort 1 (C1: prior PE vs. NT exposure; ACS subjects), cohort 2 (C2: prior PE vs. NT exposure; non-ACS subjects), cohort 3 (C3: ACS vs. non-ACS exposure) and cohort 4 (C4: current PE vs. NT exposure, curated from prior literature).

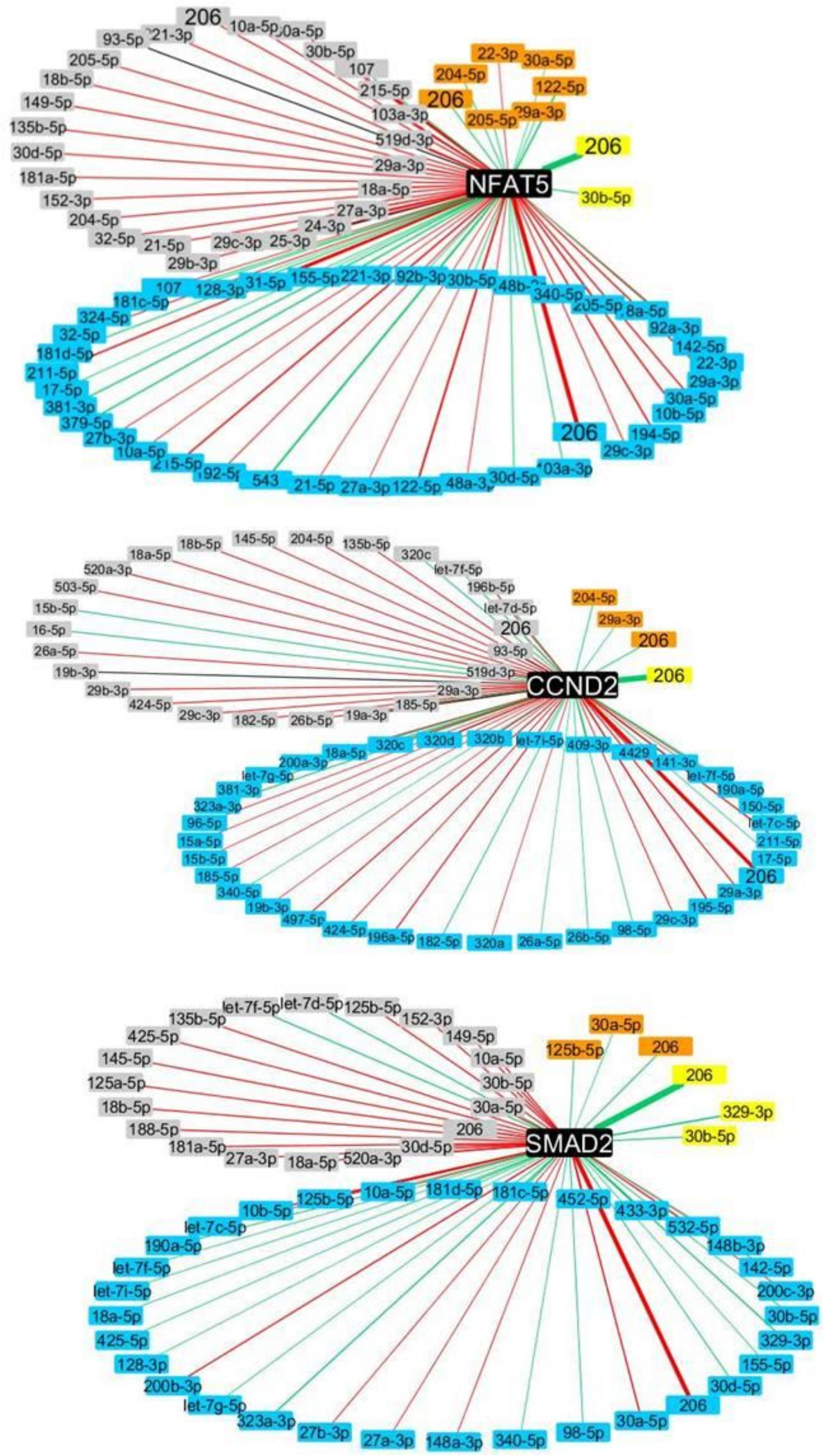

Figure S8. Predicted miRNA-target gene interactions in Wnt signaling associated with PE and ACS. MiRNA interactions with three of the most highly networked genes (NFAT5, CCND2 and SMAD2) identified in Targetscan 7.2. Nodes represent differentially altered miRNAs color-coded according to cohort (cohort 1, yellow; cohort 2, orange; cohort 3, blue; cohort 4, grey) and their common predicted target genes (black nodes). Red and green lines denote increased and decreased miRNA plasma levels, respectively. Line thickness is scale to the magnitude of fold change in miRNA level.

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