

Supplemental tables

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Supplementary Table 1. Sequences of the primers used for RT-qPCR.

Systematic Name	Sequence (5'-3')	Accession number
<i>hsa-miR-140-5p</i>	CAGCAGTGGTTTTACCCTATG	MIMAT0000431
<i>hsa-miR-148a-3p</i>	GCAGTCAGTGCACTACAGA	MIMAT0000243
<i>hsa-miR-17-3p</i>	CTGCAGTGAAGGCACTT	MIMAT0000071
<i>hsa-miR-185-5p</i>	CAGTGGAGAGAAAGGCAGT	MIMAT0000455
<i>hsa-miR-194-5p</i>	CAGTGTAACAGCAACTCCA	MIMAT0000460
<i>hsa-miR-19b-3p</i>	AGTGTGCAAATCCATGCAA	MIMAT0000074
<i>hsa-miR-29b-3p</i>	CAGTAGCACCATTTGAAATCAG	MIMAT0000100
<i>hsa-miR-29c-3p</i>	CAGTAGCACCATTTGAAATCG	MIMAT0000681
<i>hsa-miR-362-5p</i>	CGCAGAATCCTTGGAACCT	MIMAT0000705
<i>hsa-miR-3653-3p</i>	CGCAGCTAAGAAGTTGAC	MIMAT0018073
<i>hsa-miR-4685-5p</i>	CCCAGGGCTTGGAGTG	MIMAT0019771
<i>hsa-miR-7114-5p</i>	GTCTGTGGAGTGGGGT	MIMAT0028125

Supplemental Table 2. Validation of the 8 differentially expressed miRNAs in healthy donors and PA-HSOS patients by RT-qPCR.

Systematic Name	HSOS patients in cohort 1 (n=9)			HSOS patients in cohort 2 (n=20)		
	Fold change	<i>p</i> value	<i>p</i> value summary	Fold change	<i>p</i> value	<i>p</i> value summary
<i>hsa-miR-148a-3p</i>	4.7	0.0080	**	2.9	0.0089	**
<i>hsa-miR-194-5p</i>	4.2	0.0168	*	2.5	0.0082	**
<i>hsa-miR-29b-3p</i>	3.6	0.0003	***	1.7	0.0131	*
<i>hsa-miR-185-5p</i>	3.2	0.0014	**	1.9	0.0067	**
<i>hsa-miR-424-5p</i>	3.0	0.0057	**	1.9	0.0293	*
<i>hsa-miR-140-5p</i>	2.7	0.0244	*	1.9	0.0299	*
<i>hsa-miR-29c-3p</i>	2.6	0.0199	*	1.9	0.0165	*
<i>hsa-miR-362-5p</i>	2.5	0.0141	*	2.5	0.0020	**

Data were analyzed by Student's t test and expressed as mean±SD. **p*<0.05, ***p*<0.01, ****p*<0.001 versus healthy donors.

Supplemental Table 3. ROC curve analysis for the discrimination between healthy donors and PA-HSOS patients by the three miRNA biomarkers.

Index	HSOS patients in cohort 1 (n=9)			HSOS patients in cohort 2 (n=20)		
	<i>hsa-miR-148a-3p</i>	<i>hsa-miR-362-5p</i>	<i>hsa-miR-194-5p</i>	<i>hsa-miR-148a-3p</i>	<i>hsa-miR-362-5p</i>	<i>hsa-miR-194-5p</i>
AUC	0.926	0.877	0.938	0.900	0.900	0.933
(95 % CI)	(0.80; 1.00)	(0.70; 1.00)	(0.83; 1.00)	(0.80; 1.00)	(0.75; 1.00)	(0.82;1.00)
Sensitivity %	100.00	100.00	100.00	70.00	100.00	75.00
Specificity %	77.78	77.78	77.78	100.00	66.67	100.00
Standard error	0.07	0.09	0.05	0.06	0.07	0.05
Youden Index	0.778	0.778	0.778	0.70	0.67	0.75

AUC, area under curve; Youden's index=sensitivity+specificity-1.

Supplemental Table 4. Top 30 GO annotations for genes targeted by 3 potential miRNAs biomarkers.

GO_ID	Description	Type	P value	q value	Enrich factor
<i>hsa-miR-148a-3p</i>					
GO:0002040	sprouting angiogenesis	biological_process	2.49E-06	1.69E-04	6.67
GO:0016441	posttranscriptional gene silencing	biological_process	2.96E-06	1.89E-04	7.44
GO:0035194	posttranscriptional gene silencing by RNA	biological_process	2.96E-06	1.89E-04	7.44
GO:0008038	neuron recognition	biological_process	1.59E-06	1.25E-04	9.51
GO:0035195	gene silencing by miRNA	biological_process	1.09E-05	5.06E-04	7.24
GO:0021795	cerebral cortex cell migration	biological_process	1.79E-05	7.54E-04	6.76
GO:0002042	cell migration involved in sprouting angiogenesis	biological_process	1.37E-05	6.15E-04	8.32
GO:0040036	regulation of fibroblast growth factor receptor signaling pathway	biological_process	4.25E-05	1.51E-03	8.45
GO:0060612	adipose tissue development	biological_process	9.11E-05	2.84E-03	7.36
GO:0090207	regulation of triglyceride metabolic process	biological_process	1.09E-04	3.24E-03	7.13
GO:0016442	RISC complex	cellular_component	5.50E-06	3.07E-04	17.28
GO:0070884	regulation of calcineurin-NFAT signaling cascade	biological_process	6.59E-05	2.16E-03	10.01
GO:0032878	regulation of establishment or maintenance of cell polarity	biological_process	1.05E-04	3.18E-03	9.05
GO:0033173	calcineurin-NFAT signaling cascade	biological_process	3.46E-04	7.73E-03	7.04
GO:0021799	cerebral cortex radially oriented cell migration	biological_process	5.70E-04	1.14E-02	6.34
GO:0035068	micro-ribonucleoprotein complex	cellular_component	1.51E-05	6.63E-04	21.73
GO:0035278	negative regulation of translation involved in gene silencing by miRNA	biological_process	3.65E-05	1.34E-03	16.9
GO:0048268	clathrin coat assembly	biological_process	1.06E-04	3.17E-03	12.67
GO:0031061	negative regulation of histone methylation	biological_process	2.48E-04	6.32E-03	10.14
GO:0021542	dentate gyrus development	biological_process	3.19E-04	7.48E-03	9.51
GO:0007413	axonal fasciculation	biological_process	6.25E-04	1.24E-02	8
GO:0005942	phosphatidylinositol 3-kinase complex	cellular_component	6.25E-04	1.24E-02	8
GO:0005545	1-phosphatidylinositol binding	molecular_function	7.65E-04	1.42E-02	7.6
GO:0031290	retinal ganglion cell axon guidance	biological_process	7.65E-04	1.42E-02	7.6
GO:2000209	regulation of anoikis	biological_process	7.65E-04	1.42E-02	7.6
GO:0048103	somatic stem cell division	biological_process	1.11E-03	1.73E-02	6.91
GO:0035196	production of miRNAs involved in gene silencing by miRNA	biological_process	1.11E-03	1.73E-02	6.91
GO:0043567	regulation of insulin-like growth factor	biological_process	1.57E-03	2.13E-02	6.34

	receptor signaling pathway				
GO:0043276	anoikis	biological_process	1.57E-03	2.13E-02	6.34
GO:0060216	definitive hemopoiesis	biological_process	1.57E-03	2.13E-02	6.34
	<i>hsa-miR-362-5p</i>				
GO:0007155	cell adhesion	biological_process	9.38E-04	9.56E-03	3.16
GO:0048514	blood vessel morphogenesis	biological_process	2.31E-04	6.20E-03	4.98
GO:0001568	blood vessel development	biological_process	5.31E-04	7.28E-03	4.39
GO:0001944	vasculature development	biological_process	6.85E-04	8.21E-03	4.22
GO:0016337	single organismal cell-cell adhesion	biological_process	1.46E-04	5.36E-03	6.19
GO:0030155	regulation of cell adhesion	biological_process	2.26E-04	6.16E-03	5.76
GO:0004674	protein serine/threonine kinase activity	molecular_function	4.10E-03	2.03E-02	3.94
GO:0010256	endomembrane system organization	biological_process	4.73E-03	2.24E-02	3.82
GO:0043547	positive regulation of GTPase activity	biological_process	6.33E-03	2.58E-02	3.6
GO:0045664	regulation of neuron differentiation	biological_process	7.05E-03	2.75E-02	3.52
GO:0043087	regulation of GTPase activity	biological_process	1.06E-02	3.52E-02	3.23
GO:0030036	actin cytoskeleton organization	biological_process	1.10E-02	3.61E-02	3.21
GO:0009143	nucleoside triphosphate catabolic process	biological_process	1.16E-02	3.75E-02	3.17
GO:0046130	purine ribonucleoside catabolic process	biological_process	1.19E-02	3.83E-02	3.15
GO:0021510	spinal cord development	biological_process	2.90E-05	1.10E-02	13.97
GO:0021953	central nervous system neuron differentiation	biological_process	3.43E-04	6.11E-03	8.13
GO:0060537	muscle tissue development	biological_process	1.05E-02	3.51E-02	3.69
GO:0048589	developmental growth	biological_process	1.09E-02	3.61E-02	3.66
GO:0043025	neuronal cell body	cellular_component	1.14E-02	3.73E-02	3.62
GO:0032869	cellular response to insulin stimulus	biological_process	1.25E-02	3.92E-02	3.54
GO:0030900	forebrain development	biological_process	1.36E-02	4.15E-02	3.47
GO:0048011	neurotrophin TRK receptor signaling pathway	biological_process	1.38E-02	4.21E-02	3.45
GO:0051493	regulation of cytoskeleton organization	biological_process	1.41E-02	4.27E-02	3.43
GO:0038179	neurotrophin signaling pathway	biological_process	1.43E-02	4.29E-02	3.42
GO:0030425	dendrite	cellular_component	1.47E-02	4.39E-02	3.4
GO:0001525	angiogenesis	biological_process	1.48E-02	4.42E-02	3.39
GO:0071900	regulation of protein serine/threonine kinase activity	biological_process	1.81E-02	5.02E-02	3.22
GO:0032868	response to insulin	biological_process	1.92E-02	5.24E-02	3.18
GO:0044297	cell body	cellular_component	1.92E-02	5.24E-02	3.18
GO:0072657	protein localization to membrane	biological_process	1.93E-02	5.26E-02	3.17
	<i>hsa-miR-194-5p</i>				
GO:0007030	Golgi organization	biological_process	4.93E-07	3.19E-04	8.7
GO:0001838	embryonic epithelial tube formation	biological_process	8.57E-06	1.75E-03	6.19
GO:0072175	epithelial tube formation	biological_process	9.16E-06	1.78E-03	6.14
GO:0035148	tube formation	biological_process	2.18E-05	2.22E-03	5.53
GO:0001843	neural tube closure	biological_process	3.68E-06	1.02E-03	7.91
GO:0001841	neural tube formation	biological_process	1.42E-05	2.12E-03	6.62

GO:0033116	endoplasmic reticulum-Golgi intermediate compartment membrane	cellular_component	1.85E-05	2.18E-03	9.06
GO:0017053	transcriptional repressor complex	cellular_component	6.30E-05	3.94E-03	7.37
GO:0006476	protein deacetylation	biological_process	1.02E-04	5.52E-03	6.79
GO:0001948	glycoprotein binding	molecular_function	4.25E-04	1.17E-02	5.32
GO:0005793	endoplasmic reticulum-Golgi intermediate compartment	cellular_component	4.51E-04	1.22E-02	5.27
GO:0003158	endothelium development	biological_process	4.78E-04	1.21E-02	5.21
GO:0048066	developmental pigmentation	biological_process	6.65E-05	4.10E-03	9.15
GO:0016575	histone deacetylation	biological_process	3.48E-04	1.06E-02	6.62
GO:0051149	positive regulation of muscle cell differentiation	biological_process	3.76E-04	1.09E-02	6.52
GO:0070603	SWI/SNF superfamily-type complex	cellular_component	6.25E-04	1.44E-02	5.89
GO:1901800	positive regulation of proteasomal protein catabolic process	biological_process	9.27E-04	1.44E-02	5.44
GO:0046915	transition metal ion transmembrane transporter activity	molecular_function	2.13E-04	8.09E-03	9.56
GO:0060563	neuroepithelial cell differentiation	biological_process	3.00E-04	9.93E-03	8.82
GO:0046928	regulation of neurotransmitter secretion	biological_process	6.06E-04	1.43E-02	7.48
GO:0060425	lung morphogenesis	biological_process	9.40E-04	1.45E-02	6.75
GO:0021879	forebrain neuron differentiation	biological_process	1.29E-03	1.73E-02	6.26
GO:0051219	phosphoprotein binding	molecular_function	1.62E-03	1.71E-02	5.93
GO:0000118	histone deacetylase complex	cellular_component	1.74E-03	1.81E-02	5.83
GO:1901888	regulation of cell junction assembly	biological_process	1.87E-03	1.90E-02	5.74
GO:0032922	circadian regulation of gene expression	biological_process	1.87E-03	1.90E-02	5.74
GO:0021872	forebrain generation of neurons	biological_process	2.29E-03	2.21E-02	5.46
GO:0032436	positive regulation of proteasomal ubiquitin-dependent protein catabolic process	biological_process	2.61E-03	2.14E-02	5.29
GO:0048041	focal adhesion assembly	biological_process	2.78E-03	2.26E-02	5.21
GO:0019935	cyclic-nucleotide-mediated signaling	biological_process	2.78E-03	2.26E-02	5.21

Supplemental Table 5. Top 30 KEGG pathways for genes targeted by 3 potential miRNAs biomarkers.

Pathway ID	Description	<i>P</i> value	q value	Count	Enrich factor
<i>hsa-miR-148a-3p</i>					
hsa04510	Focal adhesion	1.08E-05	2.32E-04	18	3.42
hsa04810	Regulation of actin cytoskeleton	8.53E-05	1.00E-03	17	3.02
hsa05215	Prostate cancer	6.15E-07	7.94E-05	13	5.55
hsa04722	Neurotrophin signaling pathway	4.98E-04	4.29E-03	11	3.29
hsa05221	Acute myeloid leukemia	1.78E-06	1.15E-04	10	6.56
hsa05214	Glioma	5.21E-06	2.24E-04	10	5.85
hsa05211	Renal cell carcinoma	1.04E-05	2.68E-04	10	5.43
hsa05410	Hypertrophic cardiomyopathy (HCM)	4.86E-05	6.97E-04	10	4.58
hsa05220	Chronic myeloid leukemia	8.40E-05	1.08E-03	9	4.69
hsa04012	ErbB signaling pathway	3.43E-04	3.69E-03	9	3.93
hsa05414	Dilated cardiomyopathy	4.47E-04	4.12E-03	9	3.8
hsa05213	Endometrial cancer	3.35E-05	6.18E-04	8	5.85
hsa05223	Non-small cell lung cancer	4.48E-05	7.23E-04	8	5.63
hsa05218	Melanoma	3.47E-04	3.44E-03	8	4.28
hsa04350	TGF-beta signaling pathway	1.25E-03	1.01E-02	8	3.58
hsa04512	ECM-receptor interaction	1.25E-03	1.01E-02	8	3.58
hsa04916	Melanogenesis	4.00E-03	1.99E-02	8	3.01
hsa04115	p53 signaling pathway	1.37E-03	9.29E-03	7	3.86
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	2.12E-03	1.25E-02	7	3.6
hsa04662	B cell receptor signaling pathway	2.31E-03	1.30E-02	7	3.55
hsa04070	Phosphatidylinositol signaling system	2.94E-03	1.52E-02	7	3.41
hsa05222	Small cell lung cancer	4.96E-03	2.37E-02	7	3.13
hsa00562	Inositol phosphate metabolism	2.08E-03	1.28E-02	6	4
hsa05212	Pancreatic cancer	6.46E-03	2.87E-02	6	3.26
hsa04150	mTOR signaling pathway	6.23E-03	2.87E-02	5	3.66
hsa05210	Colorectal cancer	1.43E-02	4.98E-02	5	3.07
hsa04320	Dorso-ventral axis formation	1.28E-03	9.20E-03	4	6.08
hsa05216	Thyroid cancer	2.46E-03	1.32E-02	4	5.24
hsa05219	Bladder cancer	1.18E-02	4.34E-02	4	3.62
hsa04710	Circadian rhythm	7.26E-03	2.93E-02	3	4.96
<i>hsa-miR-362-5p</i>					
hsa04510	Focal adhesion	7.37E-03	4.08E-02	3	5.53
hsa04810	Regulation of actin cytoskeleton	9.31E-03	4.47E-02	3	5.16
hsa04960	Aldosterone-regulated sodium reabsorption	6.31E-04	4.54E-02	2	17.54
hsa05223	Non-small cell lung cancer	1.30E-03	4.68E-02	2	13.64
hsa00562	Inositol phosphate metabolism	1.52E-03	3.64E-02	2	12.93
hsa05214	Glioma	2.21E-03	3.97E-02	2	11.33
hsa04971	Gastric acid secretion	3.18E-03	4.59E-02	2	9.96
hsa04370	VEGF signaling pathway	3.43E-03	4.12E-02	2	9.69

hsa04070	Phosphatidylinositol signaling system	3.69E-03	3.80E-02	2	9.45
hsa04664	Fc epsilon RI signaling pathway	3.83E-03	3.44E-02	2	9.33
hsa03008	Ribosome biogenesis in eukaryotes	4.10E-03	3.28E-02	2	9.1
hsa03015	mRNA surveillance pathway	4.39E-03	3.16E-02	2	8.88
hsa04012	ErbB signaling pathway	5.01E-03	3.28E-02	2	8.47
hsa04666	Fc gamma R-mediated phagocytosis	6.40E-03	3.84E-02	2	7.76
hsa05146	Amoebiasis	8.65E-03	4.45E-02	2	6.95
hsa04270	Vascular smooth muscle contraction	1.11E-02	4.97E-02	2	6.35
hsa04670	Leukocyte transendothelial migration	1.13E-02	4.79E-02	2	6.3
<i>hsa-miR-194-5p</i>					
hsa04510	Focal adhesion	1.65E-03	6.71E-02	9	3.27
hsa04010	MAPK signaling pathway	1.25E-02	2.18E-01	9	2.44
hsa04120	Ubiquitin mediated proteolysis	4.81E-04	5.87E-02	8	4.19
hsa04360	Axon guidance	1.41E-03	8.58E-02	7	3.92
hsa04350	TGF-beta signaling pathway	3.12E-03	9.52E-02	5	4.28
hsa04114	Oocyte meiosis	1.27E-02	1.94E-01	5	3.19
hsa04722	Neurotrophin signaling pathway	2.08E-02	2.31E-01	5	2.86
hsa04530	Tight junction	2.55E-02	2.23E-01	5	2.74
hsa04514	Cell adhesion molecules (CAMs)	2.82E-02	2.15E-01	5	2.68
hsa04910	Insulin signaling pathway	3.00E-02	2.03E-01	5	2.64
hsa04720	Long-term potentiation	7.07E-03	1.72E-01	4	4.16
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	8.90E-03	1.81E-01	4	3.93
hsa04512	ECM-receptor interaction	1.56E-02	2.12E-01	4	3.42
hsa04012	ErbB signaling pathway	1.71E-02	2.09E-01	4	3.35
hsa04666	Fc gamma R-mediated phagocytosis	2.42E-02	2.46E-01	4	3.06
hsa04916	Melanogenesis	3.07E-02	1.97E-01	4	2.88
hsa05131	Shigellosis	2.44E-02	2.29E-01	3	3.52
hsa05214	Glioma	2.85E-02	2.05E-01	3	3.36
hsa05211	Renal cell carcinoma	3.63E-02	2.21E-01	3	3.12
hsa05218	Melanoma	3.79E-02	2.20E-01	3	3.07
hsa04520	Adherens junction	4.15E-02	2.30E-01	3	2.99
hsa04662	B cell receptor signaling pathway	4.52E-02	2.21E-01	3	2.91
hsa04370	VEGF signaling pathway	4.71E-02	2.21E-01	3	2.87
hsa05410	Hypertrophic cardiomyopathy (HCM)	6.20E-02	2.61E-01	3	2.63
hsa05219	Bladder cancer	4.42E-02	2.24E-01	2	3.47
hsa04930	Type II diabetes mellitus	6.19E-02	2.70E-01	2	3.03
hsa05144	Malaria	7.20E-02	2.93E-01	2	2.85
hsa04150	mTOR signaling pathway	7.55E-02	2.97E-01	2	2.8
hsa04340	Hedgehog signaling pathway	9.05E-02	3.35E-01	2	2.6
hsa04621	NOD-like receptor signaling pathway	1.03E-01	3.69E-01	2	2.47

Supplementary Table 6. The intersection between 3 potential miRNAs biomarkers and angiogenesis pathway genes.

Systematic Name	The intersected angiogenesis pathway genes	Number
<i>hsa-miR-148a-3p</i>	MEOX2, ROBO1, COL4A1, NRP1, PNPLA6, ACVR1, STARD13, C1GALT1, E2F7, DICER1, F3, S1PR1, TEK, TGFB2, ITGA5, NRARP, KLF5, EPAS1, ROCK1, KLF4, EFNB2, SERPINE1, PTEN, PIK3CA, DDAH1, SULF1, VASH2, NR2E1, PTPRM, NAA15, MMP19, EGR3	32
<i>hsa-miR-362-5p</i>	CYP1B1, DLL1, PRKCA, PIK3CB	4
<i>hsa-miR-194-5p</i>	SLC12A6, PTGIS, ARHGAP24, NRP1, EFNB2, THBS1	6

Supplemental Materials

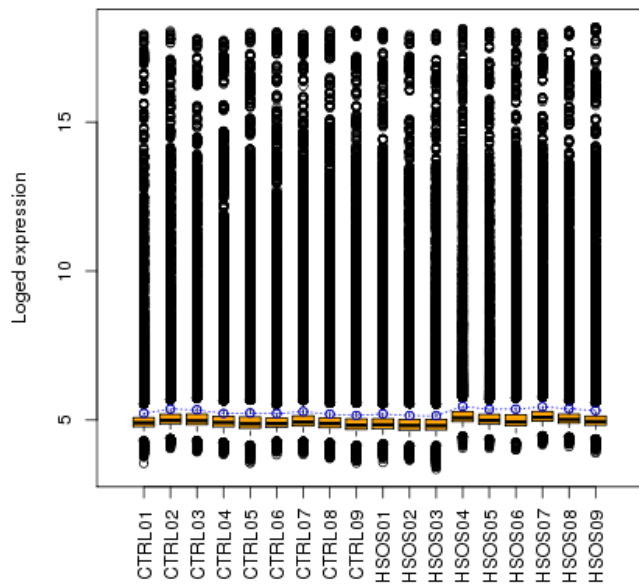
Supplemental Figure 1 Signal distribution of miRNA microarray of blood samples. The middle line of the figure represents the median of the overall data. The yellow box contains 50% of the overall data, and this area is the Inter Quantile Range.

Supplemental Figure 2 Heat map analysis of 12 differentially expressed miRNAs in PA-HSOS patients in cohort 1 by microarray.

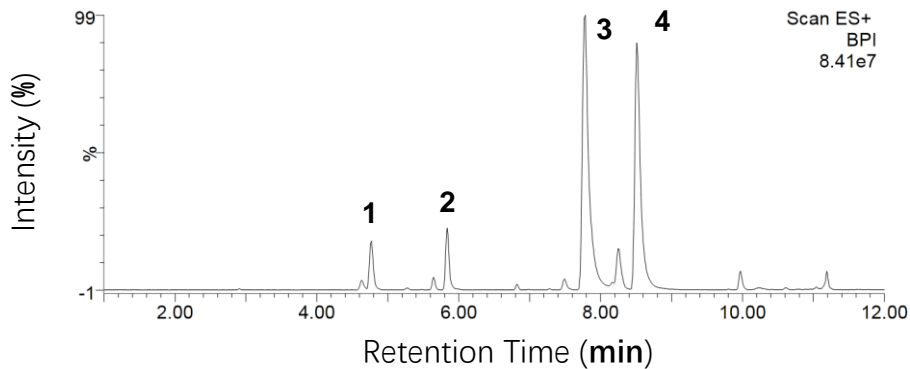
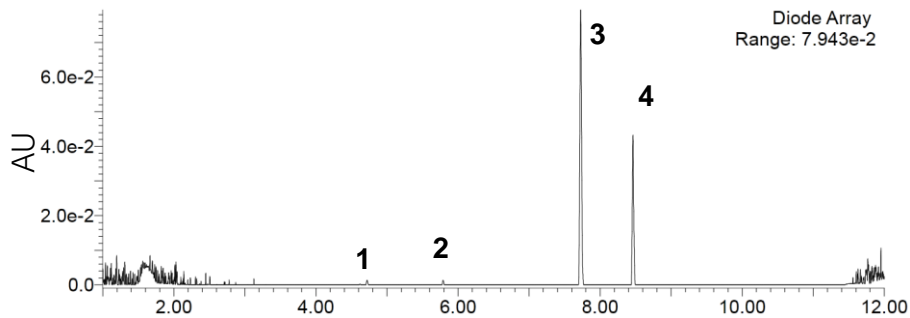
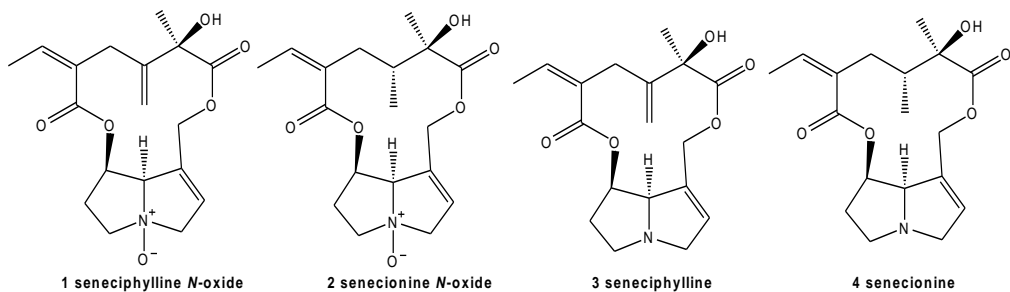
Supplemental Figure 3 Representative UPLC-DAD-MS chromatograms of *G. japonica* total alkaloid extracts. DAD data were acquired from 200-400 nm while MS data were acquired from m/z 50-650.

Supplemental Figure 4 Blood levels of reported miRNAs candidates.

Supplemental Figure 1



Supplemental Figure 3



Supplemental Figure 4

