

Supplemental Tables, Figures, Methods, and References

Supplemental Tables and Figures

Table S1: Plasma and serum miRNAs reported in the literature as solid tumor biomarkers and blood cell and plasma expression. MiRNAs shown in **BOLD** were selected for further analysis based on blood-cell enriched expression (RBC: miR-451, miR-486-5p, miR-92a, miR-16; myeloid: miR-223, miR-197, miR-574-3p, let-7a; lymphoid: miR-150; non-blood cell expressed negative control: miR-122). Note that miR-150 is not listed because it was selected as a lymphocyte-enriched miRNA and to our knowledge has not yet been proposed as a solid tumor biomarker.

miRNA Biomarker	Cancer Association(s)	Highly Expressed in Blood Cells?*	Percentile Rank in Normal Plasma**
miR-223	↑NSCLC (1, 2), ↑Esophageal Squamous CA (3), ↓Prostate CA (4), ↑HCC (5)	Yes	90 th
miR-16	↑Prostate CA (6), ↓HCC (7)	Yes	90 th
miR-126	↑Ovarian (8), ↑Early Stage NSCLC (9), NSCLC (10)	Yes	90 th
miR-142-3p	NSCLC (10)	Yes	90 th
miR-21	↑Ovarian CA (8, 11), Gastric CA (12), ↑Glioblastoma (13), Pancreatic CA (14), ↑NSCLC short survival group (15), ↑Early Stage NSCLC (9), ↑HCC (5), NSCLC (16)	Yes	90 th
miR-24	↑Oral CA, ↓Prostate CA(4), ↑NSCLC (2)	Yes	90 th
miR-19b	NSCLC (16)	Yes	90 th
miR-103	↑Prostate CA (6)	Yes	90 th
let-7a	↓Breast CA (17), ↓Gastric CA (12), ↑Pancreatic CA (14), ↓NSCLC short survival group (15), ↓NSCLC (18)	Yes	90 th
miR-451	↑Prostate CA (4), NSCLC (16)	Yes	90 th
miR-92a	↑Colon CA (19, 20), ↑Ovarian CA (8), ↑Prostate CA (6), NSCLC (16)	Yes	90 th
miR-106a	↑Gastric CA (12), ↑Prostate CA (4), NSCLC (16, 18)	Yes	90 th
miR-19a	↑Prostate CA (4)	Yes	90 th
miR-30b	NSCLC (16)	Yes	90 th

miR-17	↑Colon CA (miR-17-3p) (19), ↑Gastric CA (miR-17-5p) (12) NSCLC(16, 18)	Yes	90 th
miR-15b	NSCLC (16)	Yes	90 th
miR-107	↑Prostate CA (6)	Yes	90 th
let-7f	↓NSCLC (21)	Yes	90 th
miR-221	↑Melanoma (22), ↑↓NSCLC (2, 16, 18)	Yes	80 th
miR-93	↑Ovarian CA (8), ↑Prostate CA	Yes	80 th
miR-30c	↓Prostate CA (4), NSCLC (16)	Yes	80 th
miR-151-5p	↓Early Breast CA (23)	Yes	80 th
miR-30e	↓NSCLC (21)	Yes	80 th
miR-30d	↑NSCLC short survival group (15)	Yes	80 th
miR-486-5p	↑NSCLC short survival group (15), ↑Early Stage NSCLC (9), NSCLC (16) ↑Prostate CA (6)	Yes	80 th
miR-25	↑NSCLC (1, 2)	Yes	80 th
miR-181a	↓Early breast CA (23)	Yes	70 th
miR-146a	↑HCC (24), ↓Pancreatic CA (14)	Yes	70 th
let-7d	↓Pancreatic CA (14)	Yes	70 th
miR-197	↑Prostate CA (6), NSCLC (16)	Yes	70 th
miR-106b	↑Gastric CA (12)	Yes	70 th
miR-148b	↑Esophageal squamous CA (3)	Yes	70 th
miR-766	↑Prostate CA (6)	Yes	70 th
miR-20b	↓NSCLC (21)	Yes	70 th
miR-328	↑Prostate CA (6)	Yes	60 th
miR-574-3p	↑Prostate CA (6), ↑HCC (24), ↓Early breast CA (23)	Yes	60 th
miR-155	↓Early Breast CA (23), ↑PR+ Breast CA (25), ↓Ovarian CA (8), Altered in pancreatic CA (26), ↓NSCLC (18)	Yes	60 th
miR-140-5p	NSCLC (10)	Yes	60 th
miR-425*	↑Early breast CA (23)	Yes	60 th
miR-148a	↓Early breast CA (23), NSCLC (16)	Yes	60 th
miR-127-3p	↑Esophageal squamous CA (3)	No	50 th
miR-29a	↑Ovarian (6, 8), ↑Colon CA (20)	Yes	50 th
miR-10a	↑Esophageal squamous CA (3)	No	50 th
miR-200c	↑Ovarian CA (11)	Yes	50 th
miR-92b	↑Prostate CA (6)	Yes	40 th
miR-195	↑Breast CA (17, 27)	No	40 th
miR-122	↑Liver disease (28, 29), ↑HCC (5)	No	40 th
miR-1	↓NSCLC short survival group (15)	No	40 th
miR-215	↑HCC (24)	Yes	40 th
miR-133a	↑Esophageal squamous CA (3)	No	40 th
miR-210	↑Pancreatic CA (26, 30), ↑Early Stage NSCLC (9)	No	40 th
miR-141	↑Prostate (31, 32), ↑Ovarian CA (11)	No	40 th

miR-410	↓ Early Breast CA (23)	No	30 th
miR-431	↑ Early breast CA (23)	No	30 th
miR-660	NSCLC (16)	No	30 th
miR-589	↑ Early breast CA (23)	No	30 th
miR-100	↑ Esophageal squamous CA (3)	No	20 th
miR-500	↑ HCC (33)	Yes	20 th
miR-200b	↑ Ovarian CA (11)	No	20 th
miR-31	↑ Oral squamous CA (34)	Yes	20 th
miR-196a	Altered in pancreatic ductal CA (26)	No	20 th
miR-187	↑ Early breast CA (23)	No	10 th
miR-493	↑ Early breast CA (23)	No	10 th
miR-224	↑ HCC (24)	No	10 th
miR-668	↓ Early breast CA (23)	No	10 th
miR-203	↑ Ovarian CA (11)	No	0 th
miR-595	↑ Early breast CA (23)	No	0 th
miR-206	↑ Rhabdomyosarcoma (35), ↓ NSCLC Short Survival Group (15)	No	0 th
miR-34b	↑ Prostate CA (6)	No	0 th
miR-22	↑ Esophageal squamous CA (3)	No	ND
miR-184	↑ Squamous tongue CA (36)	No	ND
miR-200a	↑ Ovarian CA (11)	No	ND
miR-214	↑ Ovarian CA (11)	No	ND
miR-885-5p	↑ HCC (24)	No	ND
miR-205	↑ Ovarian CA (11)	No	ND
miR-377	↓ Early breast CA (23)	No	ND
miR-375	↑ Prostate CA (31)	No	ND
miR-499-5p	↓ NSCLC short survival group (15)	No	ND
miR-518b	↑ Early breast CA (23)	No	ND

CA: cancer; NSCLC: non-small cell lung cancer; HCC: hepatocellular carcinoma; PR: progesterone receptor

*An miRNA was classified as highly expressed in blood cells if consistently expressed above the median in whole blood and in at least one blood cell class (myeloid, RBC, lymphocytes).

**Percentile rank in normal plasma among 257 detected miRNAs profiled using Exiqon v1 miRcury LNA qRT-PCR arrays. ND = not detected.

Table S2: Patient and Specimen Characteristics for Residual Blood Samples R1-R42

Study Code	Inpatient (I)/Outpatient (O)	Age	Gender	Specimen Notes	Primary Diagnosis	Complete Blood Count (CBC) Data														
						White Blood Cells (thou/uI)	Red Blood Cells (million/uI)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets (thou/uI)	Red Cell Dist. Width (%)	Neutrophils (thou/uI)	Lymphocytes (thou/uI)	Monocytes (thou/uI)	Eosinophils (thou/uI)	Basophils (thou/uI)	Immature Gran. (thou/uI)
R1	I	30s	M	Howell-Jolly bodies	Sickle-cell pain crisis	15.2	2.94	8.5	26	87	28.9	33.1	496	17%	10.5	2.89	1.37	0.46	0	0
R2	I	70s	F		Status post kidney transplant	5.93	3.09	10.6	31	99	34.3	34.8	247	17%	4.31	0.88	0.59	0.1	0.02	0.03
R3	O	40s	F		Status post kidney transplant	6.06	4.49	14.1	0.43	95	31.4	32.9	190	12%	4.6	0.45	0.75	0.23	0.01	0.02
R4	I	50s	M		Non-Hodgkin's lymphoma	1.95	2.99	8.6	24	80	28.8	35.8	30	13%	1.37	0.27	0.23	0.08	0	0
R5	I	40s	M		C. difficile colitis	6.59	3.45	9.9	31	90	28.7	32	265	13%	5.4	0.57	0.52	0.05	0.01	0.04
R6	I	50s	M		Squamous cell carcinoma of the tongue	5.54	4.33	13.3	0.39	89	30.7	34.4	210	12%	4.49	0.49	0.45	0.08	0.01	0.02
R7	I	80s	M		Staph. Aureus bacteremia	7.97	3.11	9.8	30	95	31.5	33.2	275	17%	5.97	0.88	0.98	0.08	0.02	0.04
R8	O	60s	M		Heart transplant for ischemic cardiomyopathy	9.96	3.93	11.3	35	90	28.8	31.9	178	15%	4.8	3.01	1.83	0.24	0.04	0.04
R9	I	30s	M	20% Nucleated RBC	Severe congestive heart failure	13.3	2.7	8.8	27	102	32.6	32.1	227	23%	11.2	1.19	0.53	0.13	0	0.13
R10	I	50s	M		Acute myeloid leukemia	0.64	3.16	8.8	25	80	27.8	34.8	23	13%	0.13	0.23	0.27	0.01	0	0
R11	I	20s	F		Acute myeloid leukemia	0.37	3	8.9	26	85	29.7	34.8	20	14%	0	0.37	0	0	0	0
R12	I	50s	M		Metastatic esophageal cancer	7.87	3.29	10.3	31	94	31.3	33.2	241	16%	4.74	1.42	1.65	0.02	0.02	0.02
R13	I	50s	F		Dysfunctional uterine bleeding	8.9	3.39	9.5	32	93	28	30.1	397	20%	6.5	1.51	0.36	0.18	0	0.36
R14	I	60s	F		Relapsed acute myeloid leukemia	0.7	2.69	9.1	26	98	33.8	34.6	24	14%	0.61	0.09	0	0	0	0
R15	I	30s	M	Icteric specimen	Fever, history of chronic myelogenous leukemia	2.38	3.66	10.9	31	80	28.2	35.5	42	15%	1.67	0.48	0.24	0	0	0
R16	I	30s	M		Non-ischemic dilated cardiomyopathy	10.5	3.05	8.9	28	91	29.2	32.2	524	17%	8	2	0.32	0.11	0	0.11
R17	I	60s	M	Hemolyzed	Autoimmune hemolytic anemia	3.03	2.18	7.5	23	106	34.4	32.5	49	20%	2.91	0	0.06	0	0	0
R18	I	50s	M		Multiple myeloma	2.62	3.36	9.8	29	85	29.2	34.1	74	14%	1.41	0.71	0.39	0	0	0.1
R19	I	40s	M		Urosepsis, cardiogenic shock	16	3.81	10.9	32	85	28.6	33.9	137	19%	14.4	0.64	0.32	0.48	0	0.16
R20	I	50s	M		Metastatic renal cell carcinoma	11.2	4.46	12.6	0.38	85	28.3	33.2	134	14%	10.1	0.25	0.35	0.5	0.01	0.04
R21	I	40s	M		Follicular dendritic cell sarcoma	6.18	3.53	10.2	31	89	28.9	32.5	261	16%	5.13	0	0.49	0.06	0.06	0.43
R22	O	60s	M		End stage renal disease, on dialysis	10.2	3.94	11.3	34	87	28.7	32.8	178	19%	ND	ND	ND	ND	ND	ND
R23	O	50s	M		Glioblastoma	3.97	3.72	13.2	0.39	104	35.5	34	51	15%	2.42	0.83	0.36	0	0	0.36
R24	O	70s	F	Hemolyzed	Unknown	19.8	4.05	10.7	33	81	26.4	32.7	265	16%	18.6	0.25	0.5	0.25	0.01	0.16
R25	O	30s	F		Status post kidney transplant for hereditary nephritis	7.39	3.38	11.3	0.36	105	33.4	31.7	305	15%	3.42	3.54	0.24	0.07	0.1	0.02
R26	O	60s	F		Status post liver transplant for hepatitis C	1.23	2.53	7.8	25	99	30.8	31.2	98	15%	0.73	ND	ND	ND	ND	ND
R27	I	20s	F	Icteric specimen	Acute myeloid leukemia in remission	13.3	3.67	10.9	31	86	29.7	34.7	63	17%	10.7	0.53	1.72	0	0.13	0.13
R28	O	80s	M	Hemolyzed	Chronic lymphocytic leukemia	2.74	ND	10.6	30	ND	ND	ND	90	36%	1.85	0.6	0.2	0.06	0	0.03
R29	O	40s	M		Status post kidney transplant	5.45	4.94	13.1	0.4	81	26.5	32.8	194	13%	3.33	1.38	0.54	0.15	0.04	0.01
R30	I	60s	F		Angioimmunoblastic T-cell lymphoma	0.4	3.09	9	27	86	29.1	33.8	11	14%	0.14	0.15	0.11	0	0	0
R31	I	50s	F		Aspiration pneumonia	12.3	3.33	9.8	31	92	29.4	32	166	15%	8.98	1.5	1.54	0.17	0.04	ND
R32	O	20s	F		Fever and rigors following cesarean delivery	9.65	3.49	10.3	30	86	29.5	34.3	207	12%	9.26	0.29	0	0.1	0	0
R33	I	70s	F	Toxic Granulation	Beta-hemolytic Strep. bacteremia	9.69	2.97	8.8	27	91	29.6	32.7	229	17%	5.72	1.97	1.12	0.3	0.04	0.54
R34	O	30s	F		Postpartum, HIV positive	5.3	3.93	10.8	34	87	27.5	31.6	316	14%	3.04	1.58	0.47	0.19	0.01	0.01
R35	I	50s	M		Weakness and anorexia	12.4	4.94	12.4	0.4	80	25.1	31.3	196	17%	11.6	0.25	0.12	0.32	0.01	0.1
R36	O	ND			Unknown	9.15	4.06	12.8	40	99	31.5	32	335	15%	5.34	3.01	0.62	0.09	0.03	0.06
R37	I	60s	F		Hip fracture	3.79	2.85	10	30	106	35.1	33.2	144	14%	2.38	0.94	0.28	0.14	0.01	0.04
R38	O	50s	M		Followup of bilateral lung transplant	4.32	4.83	12.6	0.4	84	26.1	31.2	163	15%	2.91	0.85	0.49	0.01	0	0.06
R39	I	60s	F		Bleeding esophageal varices	1.11	3.47	9.6	29	84	27.7	33	27	15%	0.53	0.49	0.04	0.04	0	0
R40	O	60s	F		Followup of unilateral lung transplant	4.9	2.92	8.9	29	99	30.5	30.7	360	24%	1.86	2.22	0.59	0.14	0.03	0.06
R41	O	50s	F		Followup of lung transplant	4.97	3.93	12.2	0.38	98	31	31.8	132	14%	2.95	1.12	0.77	0.07	0.01	0.05
R42	O	60s	M		Followup of lung transplant	7.36	3.81	11.6	36	93	30.4	32.6	247	14%	5.51	0.9	0.81	0.1	0.02	0.02

ND=No data

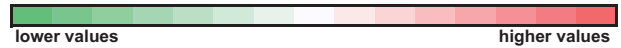


Table S3: Detail On Serial Specimens Collected From a Single Male Patient (Study Code R46) During the Course of Autologous Hematopoietic Cell Transplant

Transplant Day	Transfusions (ml) in prior 24 hours				Complete Blood Count (CBC) data													miRNA Copies/µl Plasma**			
	Apheresis Platelets	Pooled Platelets	Packed RBC	Hematopoietic Stem Cells	White Blood Cells (thou/u)	Red Blood Cells (million/u)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets (thou/u)	Red Cell Dist. Width (%)	Neutrophils (thou/u)	Lymphocytes (thou/u)	Monocytes (thou/u)	Eosinophils (thou/u)	Basophils (thou/u)	Immature Gran. (thou/u)	miR-150	miR-223
Day -5 (BEAM* conditioning)	0	0	0	0	4.9	3.5	11	32	91	31	33	138	14%	4.7	0.1	0.1	0	0	0	10,105	14,810
Day -4 (BEAM conditioning)	0	0	0	0	6	3	9.1	27	91	31	34	127	15%	5.1	0.3	0.6	0	0	0	9,766	8,245
Day -3 (BEAM conditioning)	0	0	0	0	4.1	3.2	10	29	90	31	34	121	15%	3.9	0.1	0.1	0	0	0	9,073	15,259
Day -2 (BEAM conditioning)	0	0	0	0	3.1	3.3	9.8	29	90	30	33	115	14%	2.8	0.1	0.1	0	0	0	7,997	8,278
Day -1 (BEAM conditioning)	0	0	0	0	2.3	3	9	26	89	30	34	108	14%	2.2	0.1	0	0.1	0	0	6,966	6,575
Day 0 (autologous transplant)	0	0	350	198	1.9	3.4	10	30	90	31	34	95	14%	1.8	0.1	0	0	0	0	12,222	25,738
Day 1	0	0	0	194	0.1	3.4	11	30	89	31	35	50	14%	0.1	0	0	0	0	0	25,829	19,819
Day 2	0	0	0	0	0.1	3.4	11	30	89	31	35	50	14%	0.1	0	0	0	0	0	No Data	No Data
Day 3	0	0	0	0	0.1	3.5	11	31	89	31	35	37	14%	0	0	0	0	0	0	No Data	No Data
Day 4	0	0	0	0	0	3	9	26	88	31	35	29	14%	0	0	0	0	0	0	5,905	291
Day 5	0	0	350	0	0	3.3	9.9	28	87	30	35	15	14%	0	0	0	0	0	0	7,747	484
Day 6	250	0	0	0	0	3.1	9.2	27	87	30	34	30	14%	0	0	0	0	0	0	7,198	865
Day 7	0	0	0	0	0	3.2	9.8	28	88	30	35	19	14%	0	0	0	0	0	0	10,225	5,755
Day 8	256	0	0	0	0	3	9.2	27	88	31	35	31	14%	0	0	0	0	0	0	5,429	1,098
Day 9	0	0	0	0	0.2	2.9	8.8	25	87	30	35	19	14%	0	0.2	0	0	0	0	No Data	No Data
Day 10	0	256	720	0	0.3	3.4	10	30	87	30	34	33	14%	0	0.2	0.1	0	0	0	13,672	8,683
Day 11	249	0	0	0	0.7	3.4	10	30	88	30	34	28	14%	0.1	0.3	0.4	0	0	0	18,369	6,706
Day 12	0	0	0	0	1.1	3.3	10	29	88	30	34	66	14%	0.2	0.3	0.6	0	0	0	23,137	11,828
Day 13	0	0	0	0	1.6	3.4	10	30	87	30	35	58	14%	0.5	0.5	0.5	0	0	0.1	21,390	3,694
Day 14	0	0	0	0	2.1	3.4	10	30	87	30	35	47	14%	1	0.6	0.6	0	0	0	26,221	5,998
Day 15	0	0	0	0	2.4	3.6	11	31	87	31	35	46	14%	1.4	0.4	0.6	0	0	0	28,224	5,667
Day 16	0	0	0	0	2.6	3.5	11	31	88	30	34	48	14%	1.6	0.3	0.6	0	0	0.1	23,685	5,540
Day 17	0	0	0	0	2.3	3.6	11	32	88	31	35	61	14%	1.3	0.3	0.7	0	0	0	No Data	No Data
Day 18	0	0	0	0	2.3	3.4	10	30	88	31	35	69	14%	1.2	0.6	0.5	0	0	0	No Data	No Data
Day 19	0	0	0	0	2.8	3.6	11	32	88	31	35	68	15%	1.8	0.5	0.5	0	0	0	No Data	No Data
Day 20	0	0	0	0	3.1	3.5	11	31	88	30	34	81	15%	1.5	0.9	0.5	0.1	0.1	0	22,989	4,079
Day 23	0	0	0	0	3.9	3.7	11	33	88	30	35	98	15%	1.4	1.7	0.5	0.2	0	0	No Data	No Data
Day 27	0	0	0	0	5.7	3.6	11	31	88	31	35	116	15%	1.8	2.2	1.1	0.5	0.1	0	47,234	7,624

*BEAM stands for: BCNU, Etoposide, Ara-C, Melphalan

**Average of 2 independent RNA preparations

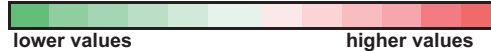


Figure S1

miRNA Biomarker	Plasma Percentile	Whole Blood	Myeloid				Lymphoid			Highly Expressed in Blood Cells?	Highest Expression	
			RBC	Platelets	Neutrophils	Eosinophils	Monocytes	T-cells	B-cells			Nk-cells
miR-223	90th	1	1	1	1	1	ND	1	1	Yes	Neutrophils	
miR-16	90th	1	1	1	1	1	1	1	1	Yes	RBC	
miR-126	90th	1	1	1	2	1	2	2	2	Yes	Platelets	
miR-142-3p	90th	1	1	1	1	1	1	1	1	Yes	Neutrophils	
miR-21	90th	1	1	1	1	1	1	1	1	Yes	Monocytes	
miR-24	90th	1	1	1	1	1	1	2	1	Yes	Neutrophils	
miR-19b	90th	1	1	1	1	1	1	1	1	Yes	Neutrophils	
miR-103	90th	1	1	1	1	1	1	1	1	Yes	Monocytes	
let-7a	90th	1	1	1	1	1	1	1	1	Yes	Neutrophils	
miR-451	90th	1	1	1	1	1	1	1	1	Yes	RBC	
miR-92a	90th	1	1	1	1	1	1	1	1	Yes	RBC	
miR-106a	90th	1	1	1	1	1	1	1	1	Yes	Neutrophils	
miR-19a	90th	1	1	1	1	1	1	1	1	Yes	Neutrophils	
miR-30b	90th	1	1	1	1	1	1	1	1	Yes	Neutrophils	
miR-17	90th	1	1	1	1	1	1	1	1	Yes	Neutrophils	
miR-15b	90th	1	1	1	1	1	1	1	1	Yes	Neutrophils	
miR-107	90th	1	1	1	1	1	2	2	2	Yes	Neutrophils	
let-7f	90th	1	1	1	1	1	1	1	1	Yes	Monocytes	
miR-221	80th	2	ND	1	1	1	1	ND	ND	3	Yes	Platelets
miR-93	80th	1	1	1	1	1	1	2	1	1	Yes	Neutrophils
miR-30c	80th	1	1	1	1	1	1	1	1	1	Yes	Neutrophils
miR-151-5p	80th	1	1	2	3	2	2	2	3	Yes	Platelets	
miR-30e	80th	1	1	2	1	1	1	1	1	1	Yes	Monocytes
miR-30d	80th	1	2	1	1	2	1	2	2	2	Yes	Neutrophils
miR-486-5p	80th	1	1	2	2	2	2	2	2	2	Yes	RBC
miR-25	80th	1	1	2	1	1	1	2	2	2	Yes	Neutrophils
miR-181a	70th	1	2	2	1	2	1	1	1	1	Yes	Neutrophils
miR-146a	70th	2	4	2	3	ND	3	2	2	2	Yes	Lymphocytes
let-7d	70th	1	2	2	2	2	2	3	2	3	Yes	Neutrophils
miR-197	70th	2	3	2	1	1	1	2	2	2	Yes	Neutrophils
miR-106b	70th	1	1	2	1	2	1	2	2	2	Yes	Neutrophils
miR-148b	70th	1	2	2	2	ND	1	ND	ND	ND	Yes	Neutrophils
miR-766	70th	2	4	2	2	2	2	3	3	3	Yes	Neutrophils
miR-20b	70th	1	1	2	2	2	2	2	2	2	Yes	RBC
miR-328	60th	2	3	3	2	2	2	3	4	3	Yes	Neutrophils
miR-574-3p	60th	2	3	2	2	2	1	4	4	4	Yes	Monocytes
miR-155	60th	2	3	3	2	3	1	2	1	2	Yes	Lymphocytes
miR-140-5p	60th	2	2	3	1	2	1	2	3	2	Yes	Neutrophils
miR-425*	60th	2	3	ND	2	2	2	ND	ND	3	Yes	Neutrophils
miR-148a	60th	1	2	ND	1	2	2	ND	ND	ND	Yes	Neutrophils
miR-127-3p	50th	3	ND	3	3	ND	ND	ND	ND	ND	No	NA
miR-29a	50th	2	ND	ND	2	ND	2	2	1	2	Yes	Lymphocytes
miR-10a	50th	2	4	3	3	ND	3	3	3	3	No	NA
miR-200c	50th	2	3	ND	2	3	2	3	3	3	Yes	Neutrophils
miR-92b	40th	2	3	3	3	ND	3	ND	ND	ND	No	NA
miR-195	40th	2	4	4	3	ND	ND	3	2	3	No	NA
miR-122	40th	3	ND	ND	ND	ND	ND	ND	ND	ND	No	NA
miR-1	40th	3	ND	4	ND	ND	ND	4	ND	ND	No	NA
miR-215	40th	1	2	ND	2	3	3	3	3	3	Yes	Neutrophils
miR-133a	40th	3	ND	3	3	ND	4	ND	ND	ND	No	NA
miR-210	40th	2	2	ND	3	3	3	ND	3	3	No	NA
miR-141	40th	2	4	ND	2	3	3	4	3	3	No	NA
miR-410	30th	3	ND	3	4	ND	4	ND	ND	ND	No	NA
miR-431	30th	3	ND	4	4	4	4	ND	ND	ND	No	NA
miR-660	30th	1	2	ND	2	ND	2	ND	4	4	No	NA
miR-589	30th	2	4	ND	3	ND	3	ND	ND	ND	No	NA
miR-100	20th	2	3	3	2	3	3	4	ND	4	No	NA
miR-500	20th	2	2	ND	3	ND	2	ND	ND	ND	Yes	Monocytes
miR-200b	20th	3	ND	ND	3	ND	3	ND	ND	4	No	NA
miR-31	20th	3	ND	ND	3	ND	3	2	ND	ND	No	NA
miR-196a	20th	2	3	ND	3	ND	ND	3	ND	3	No	NA
miR-187	10th	3	ND	ND	4	ND	4	ND	ND	ND	No	NA
miR-493	10th	3	4	4	4	4	4	4	ND	4	No	NA
miR-224	10th	4	ND	4	ND	ND	ND	ND	ND	ND	No	NA
miR-668	10th	4	ND	ND	3	ND	4	ND	ND	ND	No	NA
miR-203	0th	4	4	4	4	4	4	ND	ND	4	No	NA
miR-595	0th	4	4	4	4	4	4	ND	ND	4	No	NA
miR-206	0th	ND	ND	ND	ND	ND	ND	ND	ND	ND	No	NA
miR-34b	0th	4	ND	ND	3	4	4	ND	ND	ND	No	NA
miR-22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	No	NA
miR-184	ND	4	4	4	ND	ND	ND	ND	ND	4	No	NA
miR-200a	ND	3	ND	ND	ND	ND	ND	ND	ND	ND	No	NA
miR-214	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	No	NA
miR-885-5p	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	No	NA
miR-205	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	No	NA
miR-377	ND	4	ND	ND	4	ND	4	ND	ND	ND	No	NA
miR-375	ND	3	ND	ND	ND	ND	ND	ND	ND	ND	No	NA
miR-499-5p	ND	3	ND	ND	4	ND	ND	ND	4	ND	No	NA
miR-518b	ND	4	ND	ND	ND	ND	ND	ND	ND	ND	No	NA

Quartile 1 2 3 4 ND = Not Detected
Higher Median Lower

Figure S1: Blood Cell Expression of Published Circulating microRNA Biomarkers Parallels Plasma Expression. Whole blood, flow-sorted blood cell subsets, and matched plasma were collected from a healthy individual and profiled using Exiqon v1 qRT-PCR arrays. miRNAs are sorted in descending order of expression (i.e., increasing C_t value) in normal plasma. Reliably detected miRNAs are denoted with a 1, 2, 3, or 4 corresponding to the quartile of expression. miRNAs that were expressed in the top 50th percentile (quartile 1 or 2) in whole blood and at least one blood cell class (myeloid, lymphoid, or RBC) were designated as highly expressed in blood cells. Raw and normalized C_t values are available by request.

Figure S2

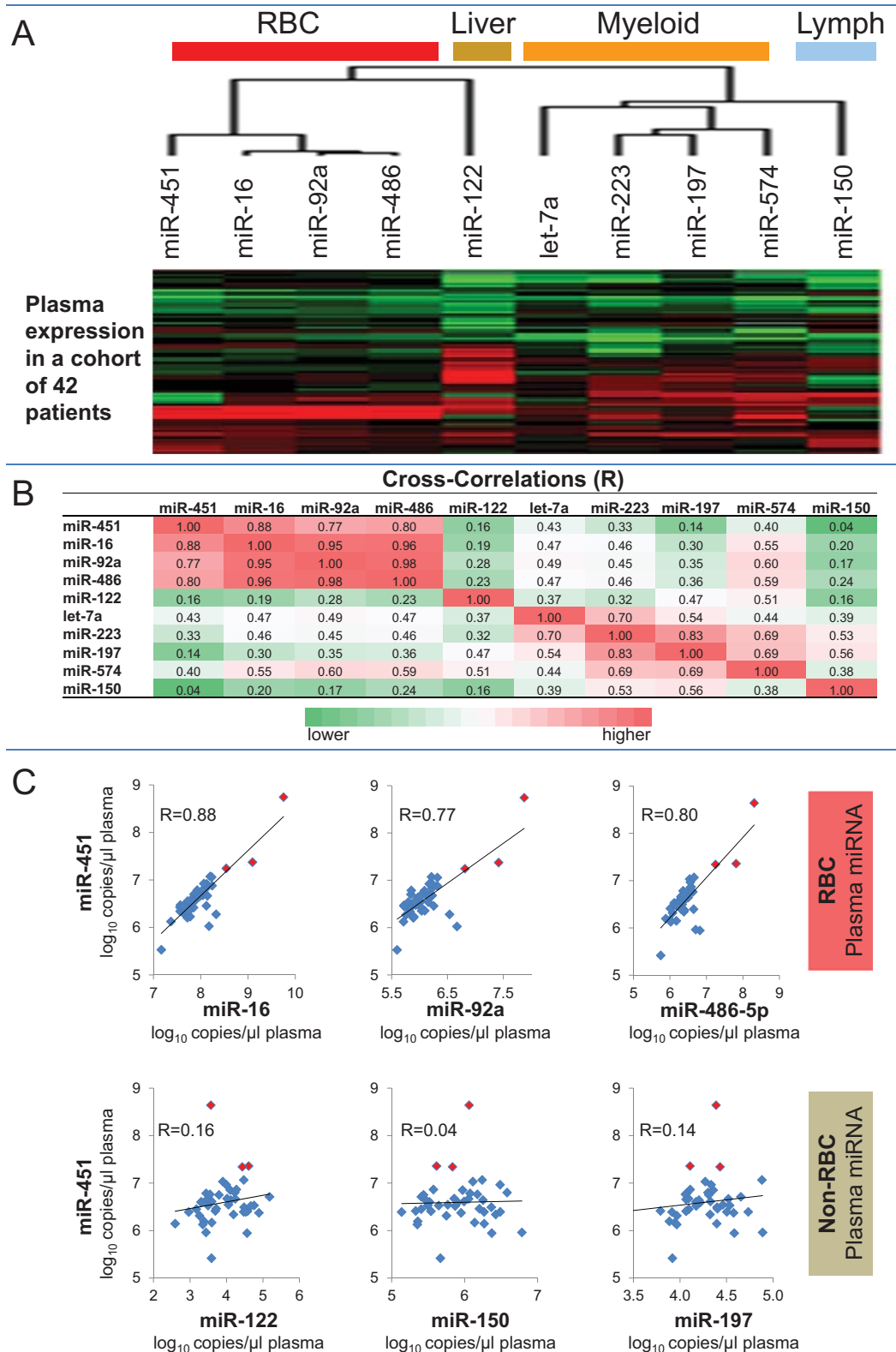


Figure S2: Plasma MicroRNA Biomarker Levels Cluster According to Blood Cells in Which They are Highly Expressed. (A) Unsupervised hierarchical clustering of plasma miRNA biomarkers levels in 42 patient samples demonstrates that plasma miRNAs group according to blood cell expression levels, with an RBC group (miR-451, miR-16, miR-92a, and miR-486-5p), a WBC group (let-7a, miR-223, miR-197, miR-574-3p, miR-150), and a non-blood cell associated miRNA outlier (liver-specific miR-122). (B) Pair-wise Pearson correlations of each of the 10 biomarkers in the cohort of 42 plasma specimens demonstrate that miRNAs expressed most highly in RBC (miR-451, miR-16, miR-92a, and miR-486-5p) have very highly correlated plasma expression patterns (Red indicates higher, and green lower Pearson correlation coefficients). (C) Detail on correlation of RBC-associated biomarkers. miR-16, miR-92a, and miR-486-5p are correlated to plasma levels of erythrocyte-related miR-451, both in **non-hemolyzed** (blue), and **hemolyzed** (red) specimens. miRNA that were not expressed in RBC (such as miR-122, miR-150, and miR-197) did not strongly correlate with miR-451. Together, these data suggest that the published cancer biomarkers miR-16, miR-92a, and miR-486-5p are released into plasma by RBC.

SUPPLEMENTAL METHODS

Fluorescence activated cell sorting (FACS) detailed methods

A total of 4 independent FACS were performed on separate days (designated FS1, FS2, FS4, FS5), using peripheral blood from the same healthy male volunteer donor (H1-1). Blood was drawn in K₂EDTA tubes as described above and run within 1 hour of collection. Automated complete differential blood counts were measured prior to each experiment using a Sysmex XE 2100 instrument. A total of 10 cell types were sorted: red blood cells (RBC; 5 million), platelets (5 million), neutrophils (250,000), eosinophils (100,000), basophils (10,000), B-lymphocytes (100,000), T-lymphocytes (100,000), NK-cells (100,000), plasmacytoid dendritic cells (pDC; 10,000), and monocytes (100,000). Basophils and pDC were not used in additional experiments due to limited sample quantity.

For white blood cell sorts, the red blood cells were removed by ammonium chloride lysis at room temperature for 5 minutes using a 14:1 ratio of lysing buffer to whole blood patient sample. The sample was spun at 530 x g on a Juoun C3i swinging bucket centrifuge with no brake at room temperature for 10 minutes. The cells were washed to remove excess lysis buffer by re-suspending them in 15 mL of PBS and spinning them for 5 minutes and transferring to a 75 mm test tube. The appropriate antibody combination was added to each tube and incubated in the dark for 15 minutes, cells were washed in 3 mL of PBS-BSA to remove unbound antibody, and re-suspended with PBS to an appropriate concentration for cell sorting. Samples were filtered before running on a Becton Dickinson ARIA cell sorter. The instrument was cleaned with 10% bleach between each sort. The following two antibody cocktails were used for white blood cells:

- (1) Lymphocyte/monocytes: CD3 FITC/CD19 PE/CD56 PE-Cy5/CD45 PE-Cy7/CD14 APC, and
- (2) Granulocytes/pDCs: CD66b FITC/CD123 PE/CD16 PE-Cy5/CD45 PE-Cy7/HLA-DR PB.

Aggregates were removed using forward scatter area (FSC-A) vs. forward scatter height (FSC-H). Viable cells were gated using FSC-A vs. side scatter height (SSC-H). Each cell type was gated from the viable cell population by the following criteria: B-cells (CD19/CD45,SSC-H), T-cells (CD3+/CD45/SSC-H), NK-cells (CD56+/CD3-/CD45/SSC-H), monocytes (CD14+/SSC-H) neutrophils (CD16+/CD66b+), eosinophils (CD16-/CD66b+/CD45 bright), basophils (CD123+/HLA-DR-), and pDCs (CD123+/HLA-DR+).

For red blood cell sorts, 2 μ L of whole blood (without red blood cell lysis) was added to each tube of antibody. The following combination of antibodies was added to gate red cells for sorting: CD61 FITC/CD235a PE. A gate was drawn around low forward scatter events to include red blood cells and to exclude viable white cells. Red blood cells were then gated using a CD235a+/CD61- phenotype. Platelet sorts were set up by adding the equivalent of about 10 million cells per tube of antibody of platelet-rich plasma. Platelet-rich plasma was prepared as the top three quarters of the supernatant after centrifugation of whole blood at 800 x g 10 minutes at room temperature in a Juoun C3i swinging bucket centrifuge with no brake. The following combination of antibodies was added to gate platelets for sorting: CD61 FITC/CD235a PE, and platelets were gated using a CD235a-/CD61+ phenotype. The threshold for forward scatter was reduced ~2 logs (100-fold) for platelet sorts compared to WBC sorts. This was done to include the platelets that would normally be excluded by our typical threshold for forward scatter. The remainder of the FACS procedure was the same as described for the white blood cell sorts.

MicroRNA profiling and data analysis detailed methods

Flow-sorted blood cells and corresponding plasma and whole blood samples were profiled for the relative abundance of 369 miRNAs using microRNA Ready-to-Use PCR, Human panel I, V1.M qRT-PCR arrays (Exiqon). A total of 15 experimental arrays were performed on blood cells, plasma, and whole blood (plasma: 2, RBC: 2, platelets: 2, neutrophils: 2, monocytes: 2, B-lymphocytes: 1, T-lymphocytes: 1, NK-cells: 1 whole blood: 1). An additional 12 control arrays were performed on serial 2-fold dilutions of 30 μ l of plasma from a single healthy donor to determine the analytic measurement ranges for each qPCR assay on the Exiqon array as previously described.(37) For both experimental and control arrays a total of 13 μ l of each sample was used for reverse transcription with the miRCURY LNA Universal RT microRNA PCR kit (Exiqon) according to the manufacturer's protocol. The UniSP6 spike-in oligonucleotide was added to each reaction as directed by the manufacturer for use in normalization. In parallel, a reverse transcription reaction was performed with 13 μ l of water as a no template control. Reverse transcription reaction products were diluted, combined with SYBR Green master mix (Exiqon), and loaded into Human panel I, qRT-PCR arrays as directed. Quantitative PCR was performed on a 7900HT (Applied Biosystems). In addition, each RNA sample was assayed for spike-in *C. elegans* oligonucleotide cel-miR-39 by TaqMan qRT-PCR assay as described above and this data was used to normalize across samples as previously described.(38)

Data from all miRNA qRT-PCR arrays was imported into SDS Enterprise Software (V2.2.2, Applied Biosystems), and CT values were calculated using manual constant thresholding at a value of 0.2. miRNA assays were normalized across plates based on the UniSP6 and Inter-Plate Calibrator control assays. For each assay, the control array dilution series CT values were plotted against the log of the dilution. From the dilution series plots and no template control, the CT value corresponding to the assay-specific limit of detection was determined by visual inspection. For each assay, experimental samples with CT values beyond

the limit of detection or greater than the no template control array (NTC) were considered not detected (ND). The remaining reliably detected assays on each array were median normalized. The number of reliably detected miRNAs (out of a possible 369) for each sample type was as follows: Plasma- 293, Whole Blood- 333, RBC- 224, Platelets- 248, Neutrophils- 276, Eosinophils- 238, Monocytes- 255, T-cells- 203, B-cells- 228, NK-cells- 225. An miRNA was categorized as highly expressed in blood cells if its CT value was lower than the median CT (i.e., expressed in the top half of detectable miRNAs) in whole blood, and in at least one class of blood cell type: RBC, granulocytes (neutrophils and eosinophils), lymphocytes (B-cells, T-cells, and NK-cells), platelets, or monocytes.

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