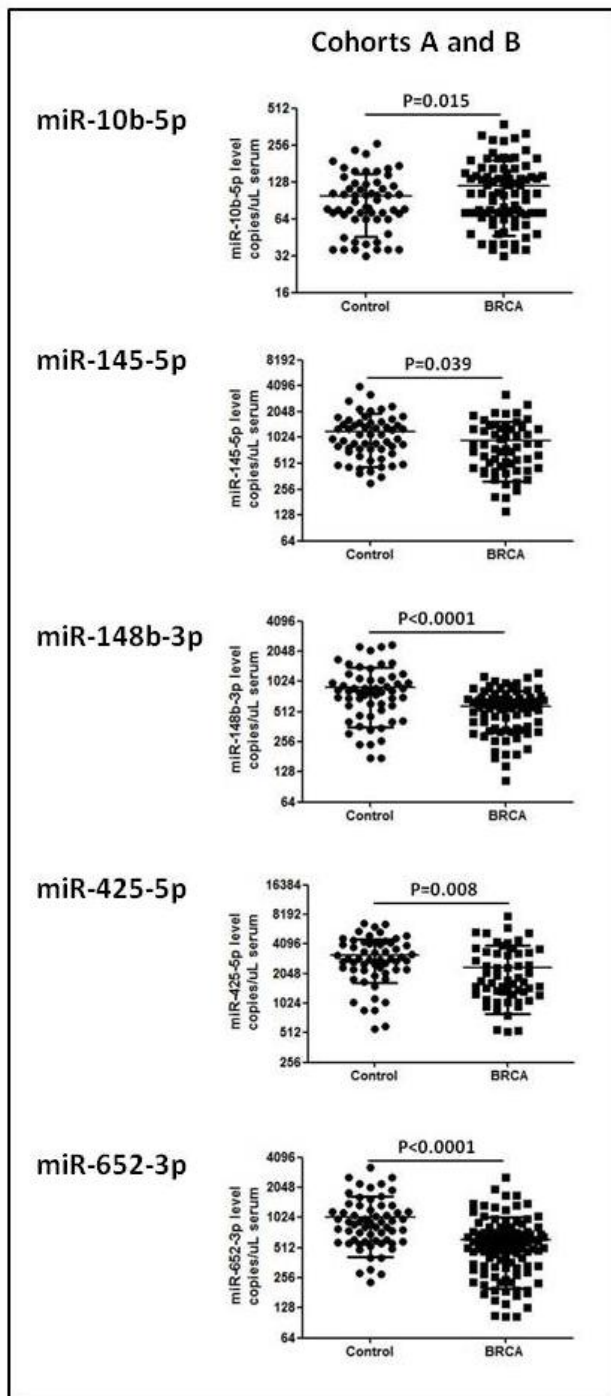## Additional files

Figure S1. Distribution of miRNA levels in sera of the two combined cohorts of breast cancer and disease-free patients. Results of the two cohorts shown in Figure 1 were combined. The unpaired t-test with Welch's correction was performed to assess significance of differences between breast cancer patients and control groups. P-values of less than 0.05 were deemed to be significant. Significant discrimination between breast cancer patients and disease-free controls as well as trends of dysregulation were all confirmed. BRCA = breast cancer patients
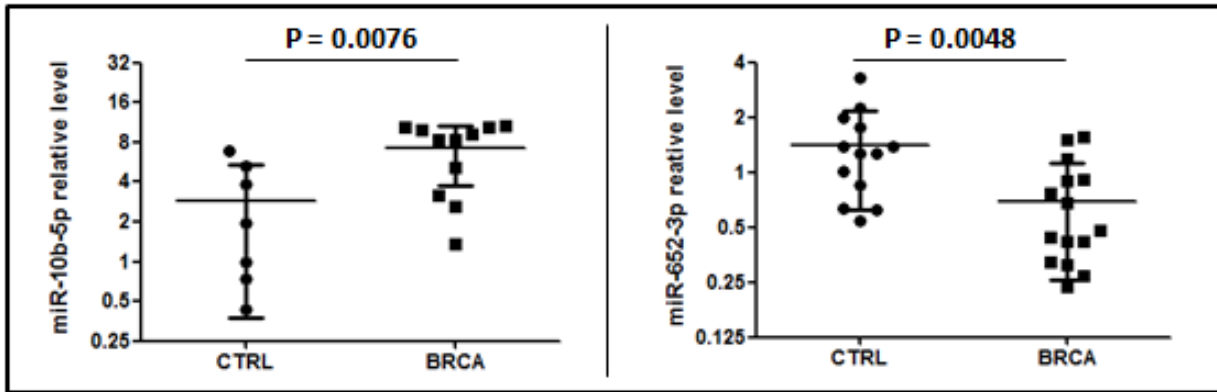
Figure S2. Validation of ddPCR results using Real-Time PCR. The serum levels of miR_10b-5p (left) and miR-652-3p (right) were investigated in a subset of samples from cohort B using Real-Time PCR instead of ddPCR method. The same trend of dysregulation found with ddPCR was also confirmed with this method. Cel-miR-39 was used as standard reference to normalize results as described in manuscript text.
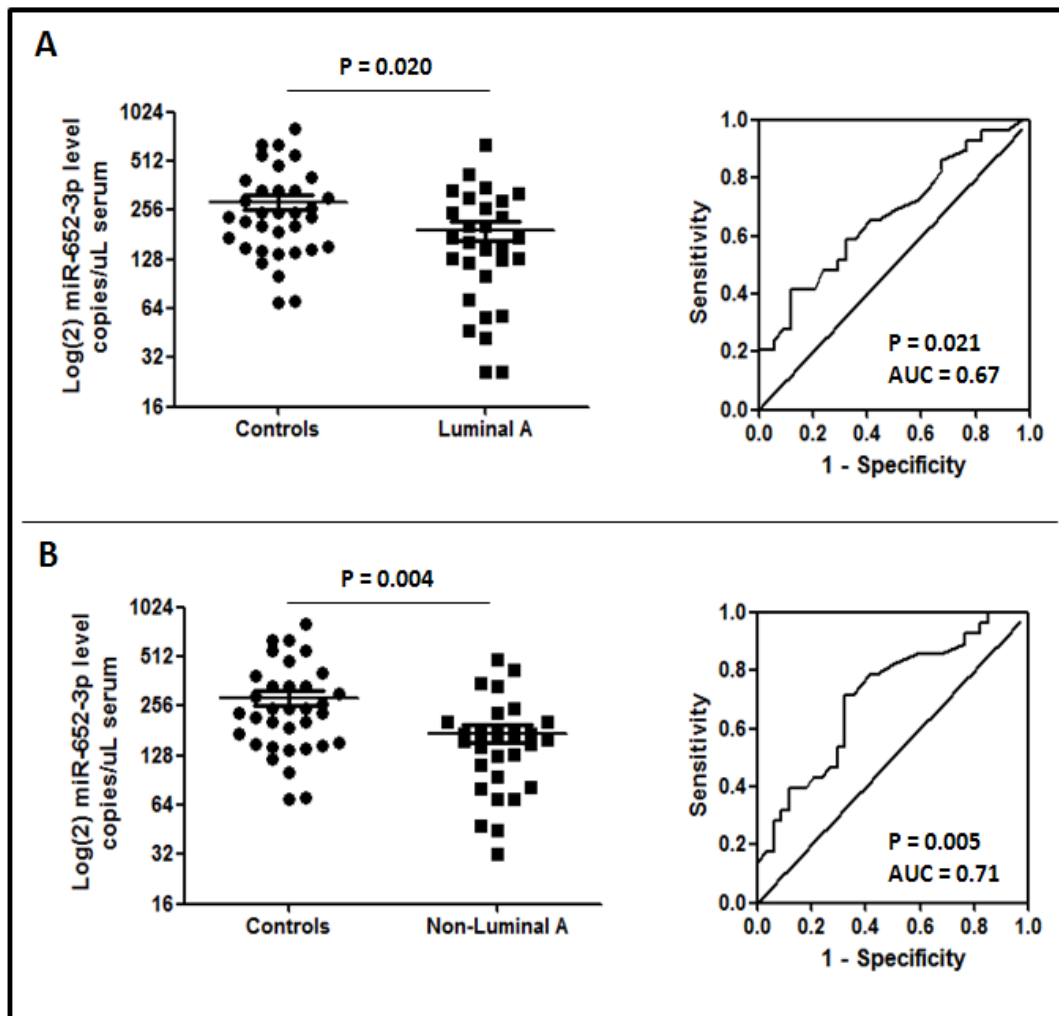
Figure S3. Serum miR-652-3p is significantly reduced in patients affected by either Luminal A or non-Luminal A breast cancers. Analysis of cohort B indicated that the levels of serum miR-652-3p are significantly reduced in Luminal A (ER+/PR+/HER2-) (panel A) and other non-Luminal A cases (panel B) in comparison with disease-free controls. The same results were obtained by analysis of cohort A. AUC = area under the curve.

Table S1. Published data on circulating microRNAs in human breast cancer patients

miRNA	Sample type	Experimental design	Results	References
miR-10b-5p	Serum	SyberGreen qRT-PCR Normalizer: miR-16	Higher in BRCA vs healthy controls	(1)
	Serum	TaqMan qRT-PCR Normalizer: 18S RNA	Higher in BRCA vs healthy controls	(2)
	Serum	TaqMan qRT-PCR Normalizer: miR-16	Higher in pM1 BRCA vs pM0 BRCA	(3)
	Serum/plasma	TaqMan qRT-PCR Normalizer: miR-16	No significant difference between BRCA and healthy controls	(4)
	Serum	LNA RT-PCR human miRNA panel (Exiqon) Normalizer: miR-103, miR-191	Higher in BRCA vs healthy controls	(5)
	Serum	LNA-based qRT-PCR QX200 digital PCR system	Higher in BRCA vs healthy controls Higher in BRCA stage-IV vs I Higher in pN + BRCA vs pN0 BRCA Higher in grade III BRCA vs grade I-II BRCA	This paper
miR-145-5p	Serum	TaqMan qRT-PCR Normalizer: 18S RNA	Higher in BRCA vs healthy controls	(2)
	Plasma	SyberGreen qRT-PCR Normalizer: RNU6B	Lower in BRCA vs healthy controls	(6)
	Serum	LNA-based qRT-PCR Normalizer: based on the mean of the assays detected in all samples	Lower in BRCA vs healthy controls	(7)
	Serum	LNA-based qRT-PCR QX200 digital PCR system	Lower in BRCA vs healthy controls	This paper
miR-148b-3p	Plasma	TaqMan qRT-PCR Normalizer: Spike-in cel-miR-39	Higher in early BRCA vs healthy controls	(8)
	Plasma	TaqMan qRT-PCR Normalizer: Spike-in cel-miR-39	Higher in BRCA vs healthy controls Higher in benign BRCA vs healthy controls	(9)
	Plasma	miRCURY LNA Universal RT microRNA PCR/TaqMan qRT-PCR Normalizer: miR-93	Higher in early BRCA vs healthy controls	(10)
	Serum	LNA-based qRT-PCR QX200 digital PCR system	Lower in BRCA vs healthy controls	This paper
miR-425-5p	Serum	LNA-based qRT-PCR	Higher in ER+ BRCA vs healthy controls	(7)

	Sample	Method	Result	Reference
		Normalizer: based on the mean of the assays detected in all samples		
	Serum	LNA-based qRT-PCR QX200 digital PCR system	Lower in BRCA vs healthy controls	This paper
miR-652-3p	Blood	TaqMan qRT-PCR Normalizer: miR-16	Lower in Luminal A-like BRCA vs healthy controls	(11)
	Plasma	TaqMan qRT-PCR Normalizer: Spike-in cel-miR-39	Higher in BRCA vs healthy controls Higher in benign BRCA vs healthy controls	(9)
	Serum	LNA-based qRT-PCR QX200 digital PCR system	Lower in BRCA vs healthy controls Lower in Luminal A-like BRCA vs healthy controls	This paper

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10.	Shen J, Hu Q, Schrauder M, et al. Circulating miR-148b and miR-133a as biomarkers for breast cancer detection. Oncotarget 2014; 5:5284-94.
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Table S2. Demographic characteristics of study populations

	Cohort A (Italy)		Cohort B (USA)	
	Cancer patients	Controls	Cancer patients	Controls
Total	28	27	59	35
Mean age, years (SD)	65.3 (±14.4)	54.2 (±14.8)	56.73 (±10.4)	53.2 (±11.5)
Range	33-91	28-78	34-81	27-94
< 50	4 (14.3%)	8 (29.6%)	15 (25.4%)	12 (34.3%)
50-60	8 (28.6%)	8 (29.6%)	22 (37.3%)	14 (40.0%)
60-70	6 (21.4%)	3 (11.1%)	15 (25.4%)	8 (22.9%)
> 70	10 (35.7%)	5 (18.5%)	7 (11.9%)	1 (2.9%)
not known	0	3 (11%)	0	0