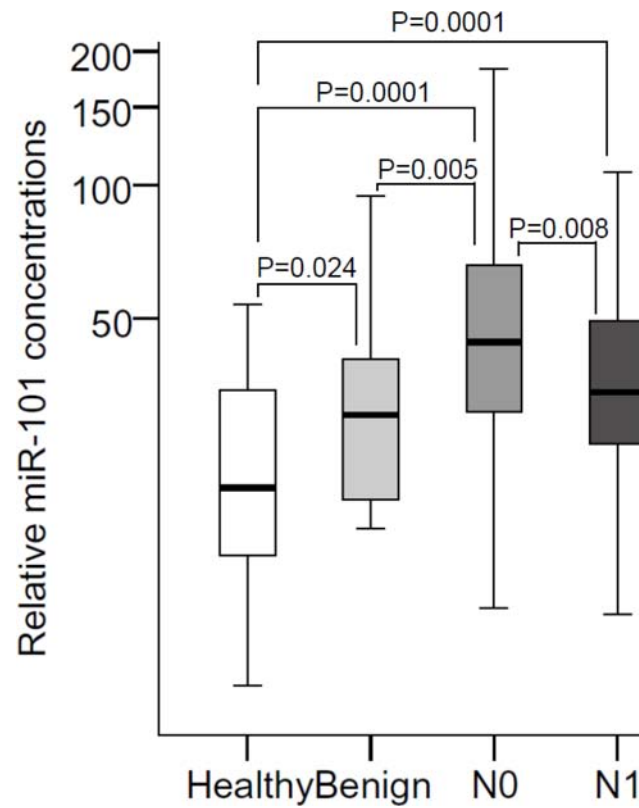
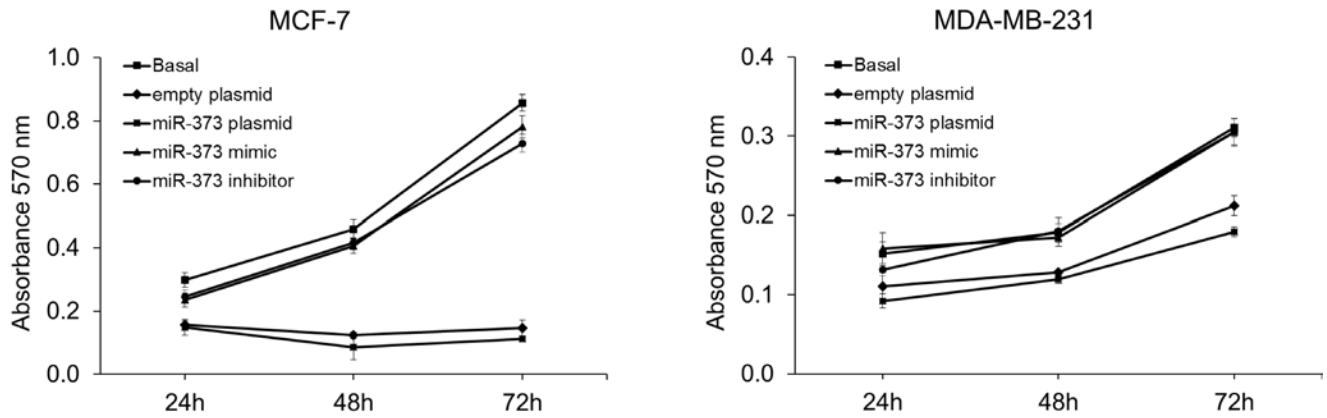


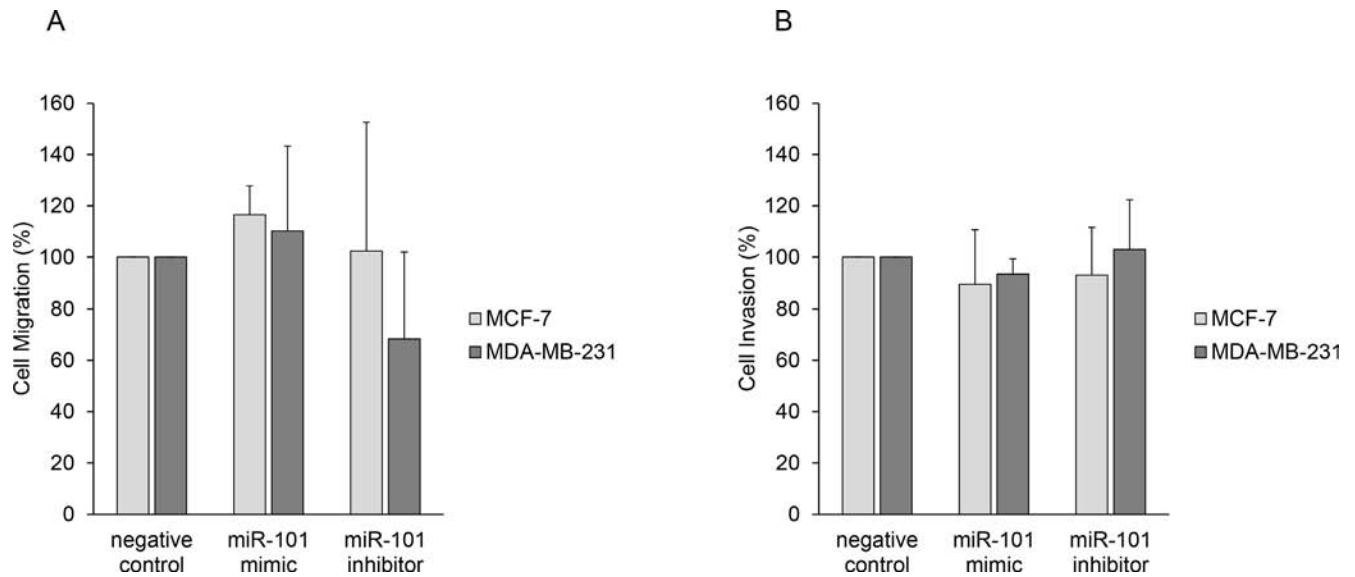
SUPPLEMENTARY FIGURES AND TABLES



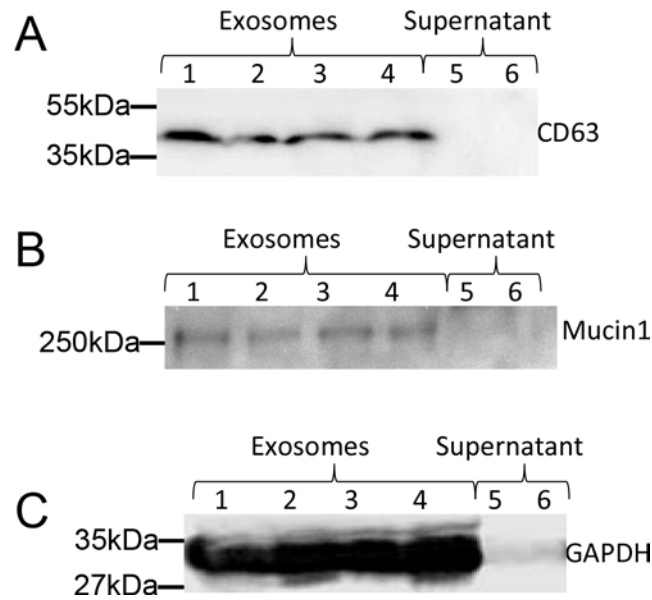
Supplementary Figure S1: Decreased serum levels of cell-free miR-101 are associated with lymph node metastasis. The box plot compares the serum concentrations of miR-101 in lymph node-negative (n=114) with those in lymph node-positive patients (n=53). As determined by the Mann-Whitney-U test, the significant p-values of the statistical evaluations are indicated.



Supplementary Figure S2: miR-373 does not affect cell proliferation. MCF-7 and MDA-MB-231 cells were transiently transfected with empty expression plasmid or plasmid encoding for miR-373, with mimics or inhibitors of miR-373, and treated with MTT. Cell proliferation after overexpression and inhibition of miR-373 for 24, 48 and 72 hours. The standard deviations from triplicate experiments are indicated.



Supplementary Figure S3: miR-101 does not affect cell migration and invasion of breast cancer cells. Cell migration and invasion of MCF-7 and MDA-MB-231 cells were analyzed by transwell assays. Cells were transfected with mimics or inhibitors of miR-101 prior to serum starvation for 24 hours. Cells were added to the upper transwell chamber that was uncoated for the migration assay and BME-coated for the invasion assay, and allowed to migrate or invade for 24 hours. Migrated or invaded cells were stained with Calcein-AM, and fluorescence was measured at excitation of 485nm and emission of 535nm.



Supplementary Figure S4: Extraction of serum exosomes. Exosomes were extracted and pelleted from serum and analyzed by Western blot using an antibody specific for the proteins (A) CD63, (B) Mucin1 and (C) GAPDH. As a negative control served the supernatant of the pellet that displayed no exosomes when the antibodies against CD63, Mucin1 and GAPDH were added. The Western blot shows representative examples of the exosome extraction from pellets of four breast cancer patients (lane 1, 2, 3, 4) and the negative control of two patients (lane 5, 6).

Supplementary Table S1. Patient characteristics at the time of primary diagnosis of breast cancer and correlation to the relative miR concentrations in serum.

Parameters	n (%)	miR-101		miR-372		miR-373				
		relative values		p values	relative values		p values	relative values		
		Median	Mean		Median	Mean		Median	Mean	
		5%	95%		5%	95%		5%	95%	
Total		CI			CI			CI		
	168 (100)	42	49		0.2	0.6		3.8	8.4	
		16	106		0	2.6		0.4	43	
Age		45 - 54			0.4 - 0.8			5.3 - 10.6		
Histology	59 (30-85)									
ductal				0.464			0.613			0.934
	109 (65)	41	51		0.2	0.7		3.8	8.4	
		17	118		0	3.1		0.4	40	
ductu-lobular		45 - 58			0.4 - 0.9			6.0 - 11		
	9 (5)	48	47		0.2	0.3		2.2	8	
		29	-		0	-		0.7	-	
lobular		36 - 58			0.1 - 0.6			0 - 20		
	31 (19)	41	43		0.2	0.4		4.3	10	
		12	93		0	1.8		0.2	73	
others		35 - 50			0.2 - 0.5			3.6 - 17		
	19 (11)	43	48		0	0.4		3.2	5.5	
		15	-		0	-		0.3	-	
Lymph node metastasis		37 - 59			0.0 - 1.0			1.1 - 9.8		
neg.				0.008			0.975			0.418
	114 (68)	44	52		0.2	0.6		4.2	9.5	
		18	106		0	3.1		0.4	49	
pos.		46 - 57			0.4 - 0.9			6.7 - 12		
	53 (32)	35	41		0.2	0.4		3.3	6.1	
		13	87		0	2.4		0.4	23	
Tumor stage		35 - 48			0.2 - 0.7			3.4 - 8.8		
pT1				0.397			0.659			0.106
	93 (56)	43	50		0.2	0.6		2.8	8.0	
		16	106		0	2.6		0.4	49	
pT2- 4		44 - 56			0.3 - 0.8			5.2 - 11		

(Continued)

Parameters	n (%)	miR-101		miR-372		miR-373				
		relative values	p values	relative values	p values	relative values	p values			
		Median	Mean		Median	Mean		Median	Mean	
	74 (44)	39	47		0.2	0.6		4.3	9.1	
		16	110		0	3		0.5	37	
Grading		41 - 54			0.3 - 0.9			5.7 - 12.4		
1.2				0.624			0.525			0.625
	98 (60)	42	47		0.3	0.6		3.6	8.7	
		15	100		0	2.9		0.3	49	
3		42 - 53			0.4 - 0.9			5.8 - 12		
	64 (40)	40	46		0.2	0.6		4.1	8.5	
		16	106		0	3.3		0.7	43	
Estrogen receptor status		40 - 53			0.3 - 0.8			5.2 - 12		
neg.				0.596			0.484			0.735
	38 (23)	42	50		0.2	0.5		4.1	7.4	
		18	123		0	3.3		0.7	31	
pos.		41 - 60			0.2 - 0.9			3.6 - 11		
	127 (77)	41	48		0.2	0.6		3.4	8.9	
		14	102		0	2.9		0.3	48	
Progesterone receptor status		43 - 53			0.4 - 0.8			6.3 - 11		
neg.				0.672			0.479			0.095
	68 (41)	38	50		0.3	0.7		4.2	8.3	
		19	127		0	3.4		0.8	32	
pos.		42 - 59			0.4			5.4 - 11		
	98 (59)	44	48		0.2	0.5		2.7	8.6	
		14	100		0	1.9		0.4	49	
HER2 status		43 - 53			0.3 - 0.7			5.6 - 12		
neg.				0.829			0.644			0.548
	129 (82)	43	49		0.2	0.6		3.6	7.7	
		15	106		0	3.2		0.4	43	
pos.		43 - 55			0.4 - 0.8			5.5 - 10		
	28 (18)	40	40		0.2	0.4		3.8	5.4	
		16	117		0	2.4		0.3	15	
Subtyp		32 - 49			0.1 - 0.7			3.4 - 7.4		
Luminal				0.630			0.362			0.445
	119 (76)	41	47		0.2	0.6		3	7.9	

(Continued)

Parameters	n (%)	miR-101		miR-372		miR-373				
		relative values	p values	relative values	p values	relative values	p values			
		Median	Mean		Median	Mean		Median	Mean	
		14	103		0	3.1		0.3	41	
HER2 pos.		41 - 53			0.4 - 0.8			5.5 - 10		
	5 (3)	42	33		0.1	0.7		4.2	6.7	
		15	-		0	-		1.2	-	
Triple neg.		6.2 - 60			0 - 2.7			0 - 19		
	33 (21)	43	53		0.2	0.5		4.4	8.4	
		21	142		0	3		0.8	40	
Overall survival		39 - 66			0.1 - 0.8			3.6 - 13		
alive				0.691			0.586			0.361
	147 (90)	43	49		0.2	0.6		3.6	8.8	
		16	104		0	3		0.4	48	
dead		44 - 54			0.4 - 0.8			6.4 - 11.1		
	16 (10)	40	46		0.3	0.3		3.8	4.1	
		5.7	-		0	-		0.6	-	
Disease-free survival		30 - 62			0.1 - 0.4			2.5 - 5.7		
Recurrence-free				*			0.318			*
	147 (90)	43	51		0.2	0.6		4.2	8.9	
		17	110		0	3		0.5	48	
Recurrence		46 - 56			0.4 - 0.8			6.5 - 11.3		
	16 (10)	35	39		0.2	0.3		2.9	3.3	
		10	-		0	-		0	-	
		29 - 49			0.1 - 0.6			1.7 - 4.9		
Healthy										
	28 (100)	20	24	0.0001	0.1	0.3	0.509	1.5	2.3	0.001
		7.9	51		0	-		0.1	6.5	
Benign		19 - 28			0 - 0.6			1.5 - 3.1		
	19 (100)	30	35	0.013	0.3	0.4	0.495	7.8	9.6	0.024
		16	-		0	-		0.3	-	
		25 - 45			0.1 - 0.7			6.1 - 13.0		

*no statistical evaluation possible

significant p values in bold

Supplementary Table S2. Patient characteristics at the time of primary diagnosis of breast cancer and correlation to the relative miR concentrations in exosomes.

Parameters	n (%)	miR-101		p values	miR-372		p values	miR-373		p values
		relative values			relative values			relative values		
		Median	Mean		Median	Mean		Median	Mean	
		5%	95%		5%	95%		5%	95%	
		CI			CI			CI		
Total	50 (100)	101	118		1.1	1.6		16	29	
	38	271		0	4.7		1.5	104		
		99 - 137			1.2 - 2.0			20 - 38		
Age	56 (30-83)									
Histology				0.759			0.883			0.217
ductal	38 (76)	102	120		1.3	1.6		13	26	
		32	295		0	4.4		1.2	88	
		97 - 142			1.1 - 2.1			16 - 36		
ductu-lobular	2 (4)	81	81		1.0	1.0		33	33	
		64	-		0	-		13	-	
		0 - 286			0 - 13			0 - 283		
lobular	7 (11)	129	119		0.8	2.0		35	62	
		63	-		0.7	-		14	-	
		66 - 172			0 - 4.9			1.7 - 122		
others	3 (6)	73	71		0.9	0.9		13	56	
		69	-		0.8	-		7.0	-	
		44 - 98			0 - 2.3			0 - 677		
Lymph node metastasis				0.212			1.000			0.276
neg.	35 (71)	118	130		1.2	1.5		14	22	
		29	297		0	5.4		1.2	112	
		100 - 160			0.9 - 2.1			11 - 32		
pos.	14 (29)	90	90		1.2	1.3		29	37	
		63	-		0	-		1.6	-	
		79 - 101			0.8 - 1.9			16 - 59		
Tumor stage				0.435			0.174			0.271
pT1	25 (50)	112	113		1.0	1.2		13	20	
		21	289		0	4.3		1.4	92	
		95 - 132			0.6 - 1.9			10 - 30		
pT2- 4	25 (50)	83	119		1.6	1.6		17	35	
		40	280		0	-		1.1	132	

(Continued)

Parameters	n (%)	miR-101		p values	miR-372		p values	miR-373		p values
		relative values	relative values		relative values	relative values				
		Median	Mean		Median	Mean		Median	Mean	
		78 - 160			1.1 - 2.2			17 - 53		
Grading				0.377			0.636			0.133
1.2	27 (61)	97	112		1.2	1.5		14	22	
		21	240		0	5.8		1.0	122	
		85 - 138			0.8 - 2.2			10 - 34		
3	17 (39)	101	121		1.0	1.3		21	33	
		63	-		0	-		7.0	-	
		86 - 156			0.7 - 1.9			16 - 49		
Estrogen receptor status				0.242			0.852			0.021
neg.	14 (29)	108	127		1.3	1.4		24	45	
		43	-		0	-		9.7	-	
		72 - 181			0.5 - 2.2			20 - 69		
pos.	35 (71)	100	111		1.1	1.5		13	19	
		29	240		0	5.3		1.2	112	
		90 - 133			0.9 - 2.0			11 - 28		
Progesterone receptor status				0.488			0.851			0.013
neg.	22 (44)	97	113		1.2	1.3		23	39	
		35	304		0	-		6.2	103	
		75 - 151			0.7 - 2.0			20 - 57		
pos.	28 (56)	112	118		1.1	1.5		13	18	
		28	243		0	5.6		1.0	124	
		93 - 144			0.9 - 2.1			8.5 - 27		
HER2 status				0.668			0.608			0.492
neg.	36 (80)	113	121		1.0	1.5		16	27	
		50	257		0	5.1		1.2	112	
		97 - 144			1.0 - 2.0			16 - 38		
pos.	9 (20)	100	87		1.3	1.1		23	28	
		45	-		0.8	-		5.6	-	
		66 - 108			0.7 - 1.5			0 - 69		
Subtyp				0.424			0.941			0.074
Luminal	29 (66)	100	111		1.1	1.5		13	19.3	
		29	240		0	5.3		1.2	112	
		90 - 133			0.9 - 2.0			11 - 28		

(Continued)

Parameters	n (%)	miR-101			miR-372			miR-373		
		relative values		p values	relative values		p values	relative values		p values
		Median	Mean		Median	Mean		Median	Mean	
HER2 pos.	3 (7)	120	-		1.3	-		23	-	
		79	-		1.3	-		10	-	
		-		-		-				
Triple neg.	12 (27)	108	132		1.0	1.4		23	43	
		43	-		0	-		10	-	
		71 - 194			0.3 - 2.4			15 - 70		
Overall survival				*			*			*
alive	45 (94)	100	116		1.2	1.5		17	28	
		36	282		0	4.9		1.4	104	
		95 - 137			1.0 - 1.9			18 - 38		
dead	3 (6)	118	-		0.9	-		16	-	
		63	-		0	-		13	-	
			-			-			-	
Disease free survival				*			*			*
Recurrence free	45 (96)	101	117		1.1	1.5		16	27	
		36	282		0	4.2		1.4	96	
		95 - 139			1 - 1.9			16 - 37		
Recurrence	2 (4)	99	99		0.6	0.6		37	37	
		79	-		0	-		13	-	
		0 - 347			0 - 8.8			0 - 342		
Healthy	12 (100)	55	56	0.0001	0.4	0.6	0.021	12	11	0.082
		27	-		0	-		1.0	-	
		43 - 68			0 - 1.1			6.6 - 15		

*no statistical evaluation possible

significant p values in bold