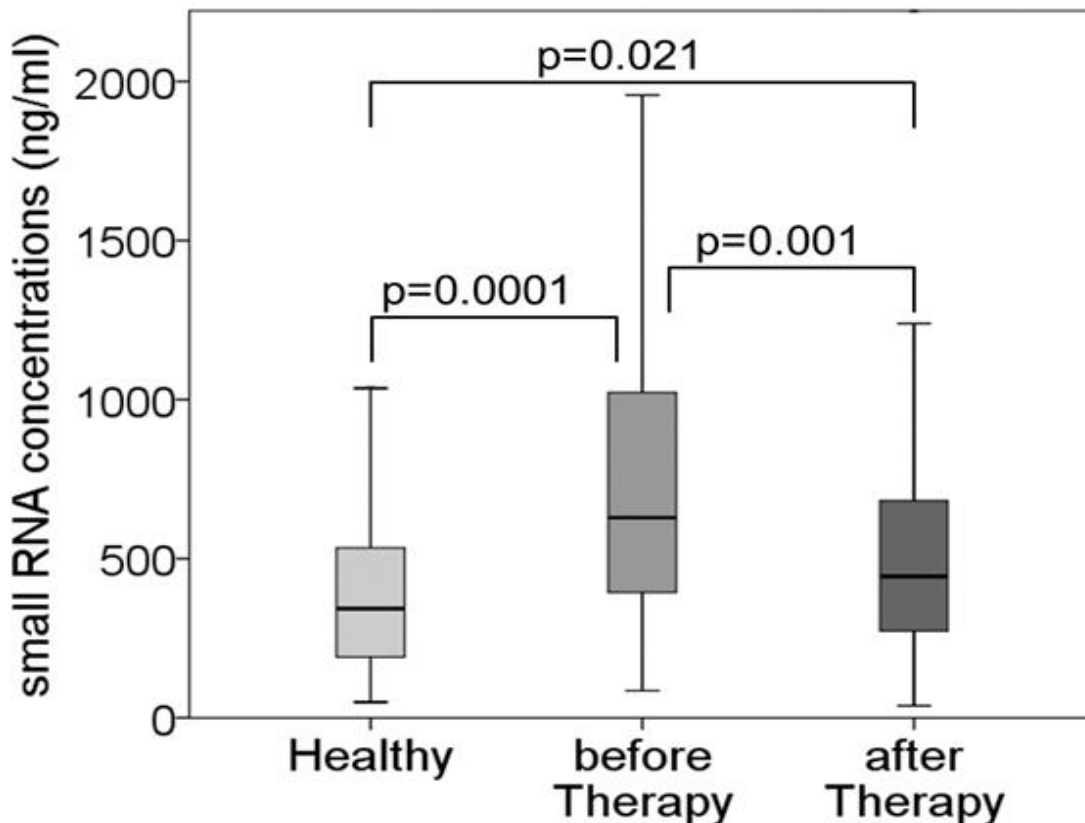


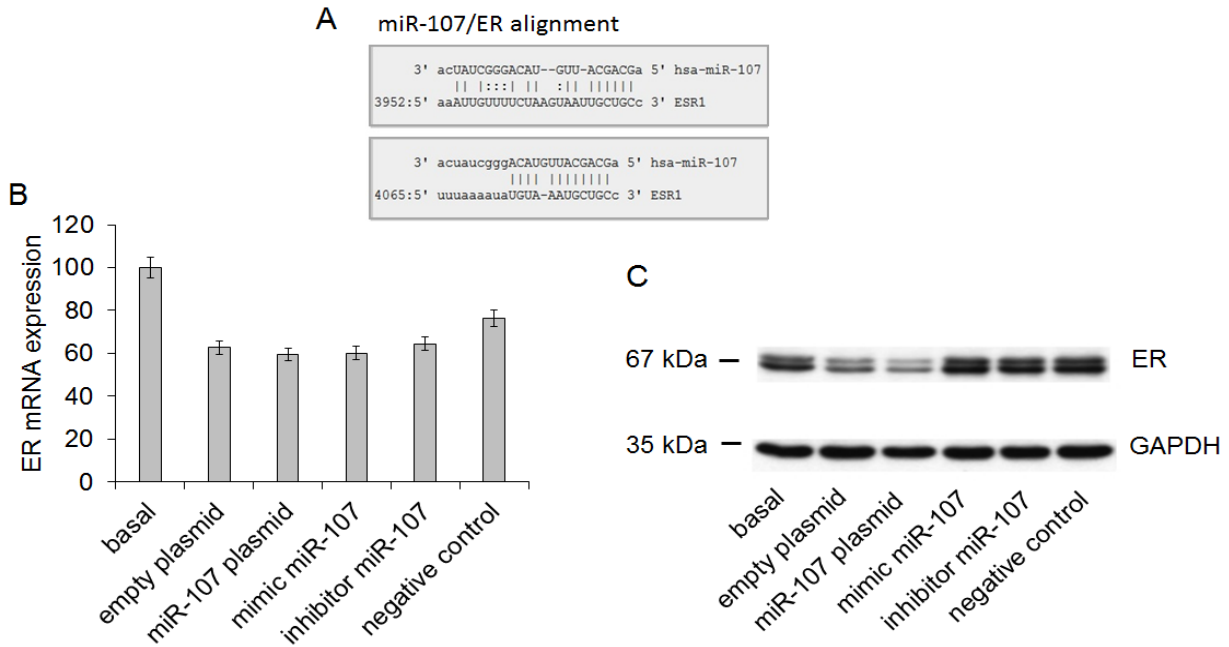
Aberrant plasma levels of circulating *miR-16*, *miR-107*, *miR-130a* and *miR-146a* are associated with lymph node metastasis and receptor status of breast cancer patients

Supplementary Material



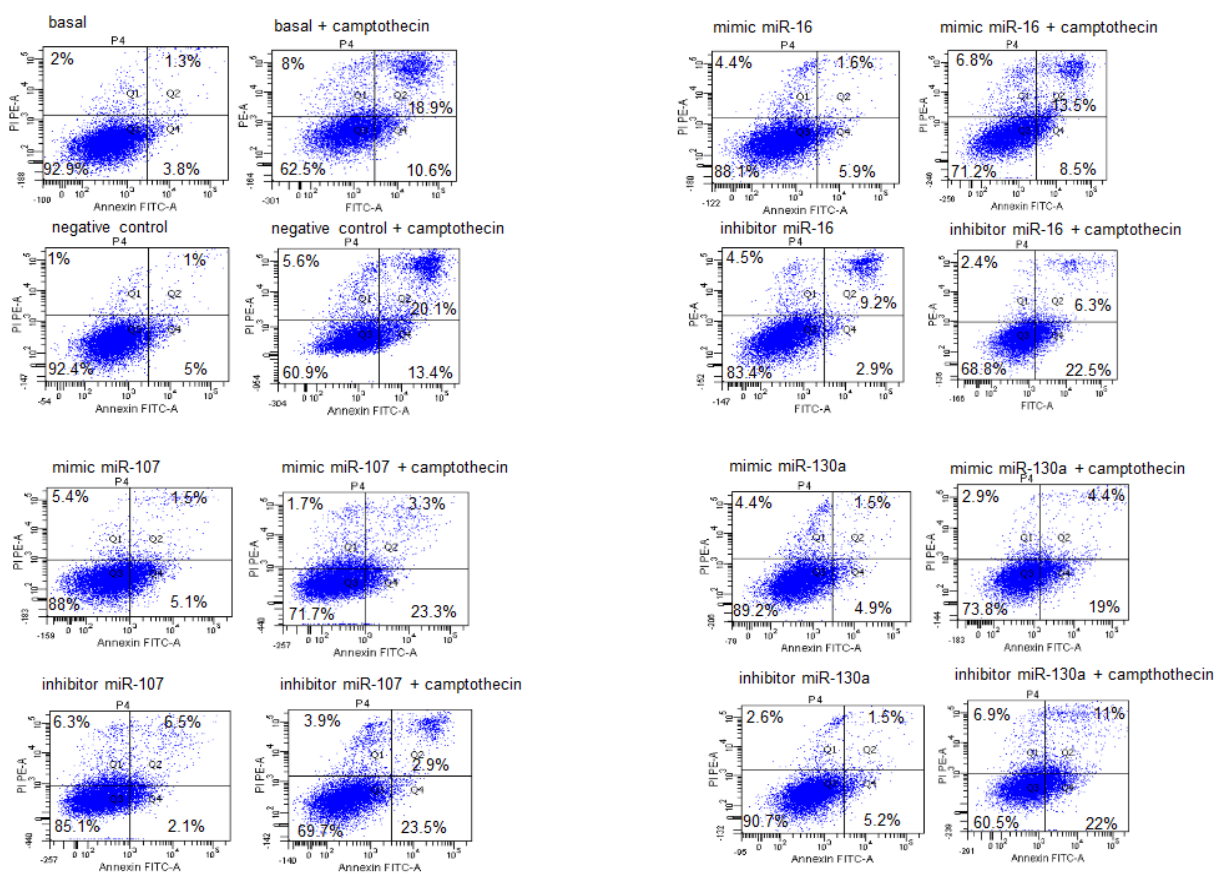
Supplementary Figure S1: Plasma levels of circulating total small RNA in breast cancer patients before and after chemotherapy.

The box plot compares the total small RNA concentrations in the plasma of healthy women (n=46) with breast cancer patients before (n=111) and after chemotherapy (n=111). As determined by the Mann-Whitney-U and Wilcoxon test, the significant p-values of the statistical evaluations are indicated.



Supplementary Figure S2: *MiR-107* does not affect the protein expression of ER.

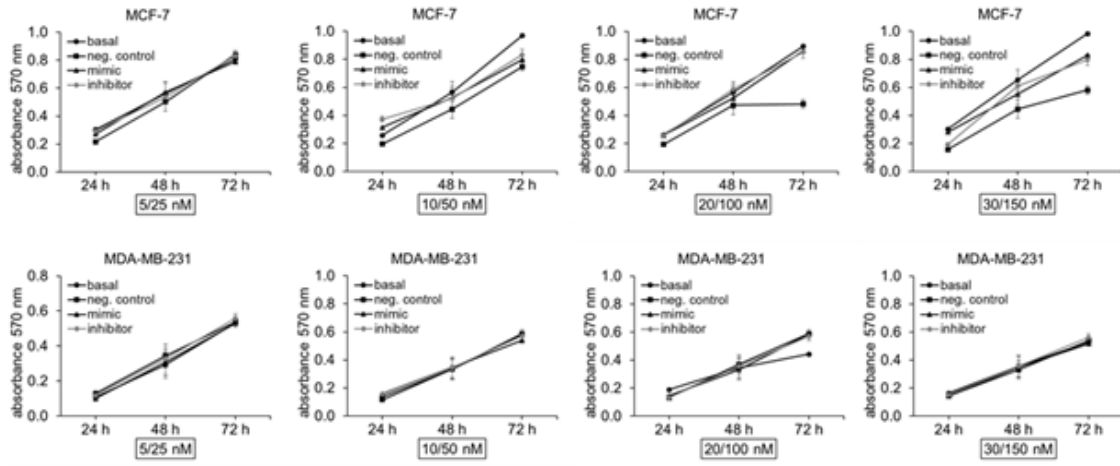
The alignment of *miR-107*/ER indicates the two putative binding sites for *miR-107* in the 3'UTR of ER (**A**). ER mRNA (**B**) and protein (**C**) levels in MCF-7 cells were analysed by real-time PCR and Western blot, respectively. GAPDH signals of the Western blot served as a loading control. ER mRNA and protein levels in MCF-7 cells untreated (basal) or transfected with an empty expression plasmid, a plasmid encoding for *miR-107*, mimic or inhibitor of *miR-107*, or scramble miR (negative control). A representative gel of three independent experiments is shown, all experiments were performed in duplicate.



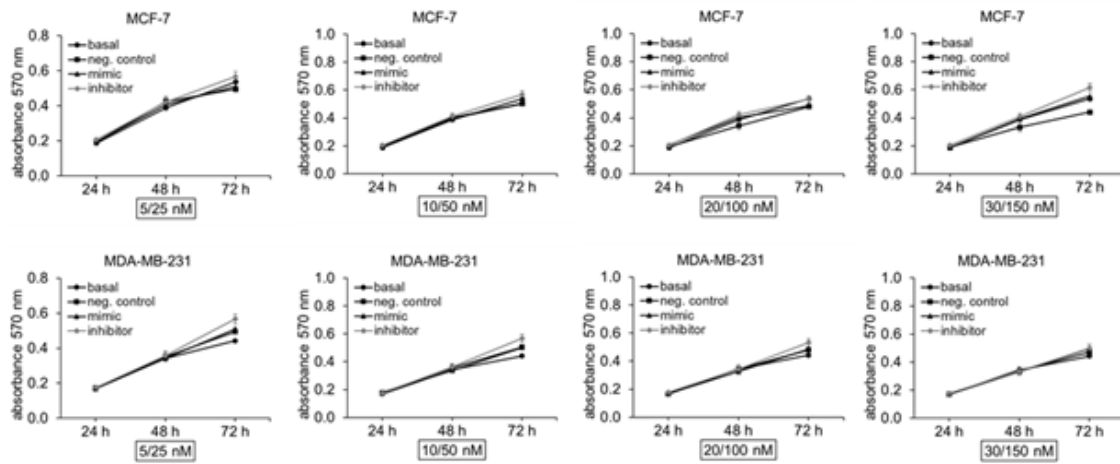
Supplementary Figure S3: *MiR-16*, *miR-107* and *miR-130a* cannot protect cells against induced apoptosis.

MCF-7 cells were transfected with mimics and inhibitors of *miR-16*, *miR-107* and *miR-130a* or scramble miR (negative control). Apoptosis in non-transfected and transfected cells was induced by the topoisomerase I inhibitor camptothecin. For FACS analyses on a FACS Cantoll device, cells were labeled with annexin-V-FITC and propidium iodide. Cell fragments, positive for only propidium iodide, can be found in the upper left corner (Q1). Late apoptotic as well as necrotic cells can be found in the upper right corner (Q2), since they are positive for annexin and propidium iodide. Living cells, negative for annexin and propidium iodide, can be found in the lower left corner (Q3). Early apoptotic cells, positive for annexin, are located in the lower right corner (Q4). The size for each population (%) is given in the corresponding area.

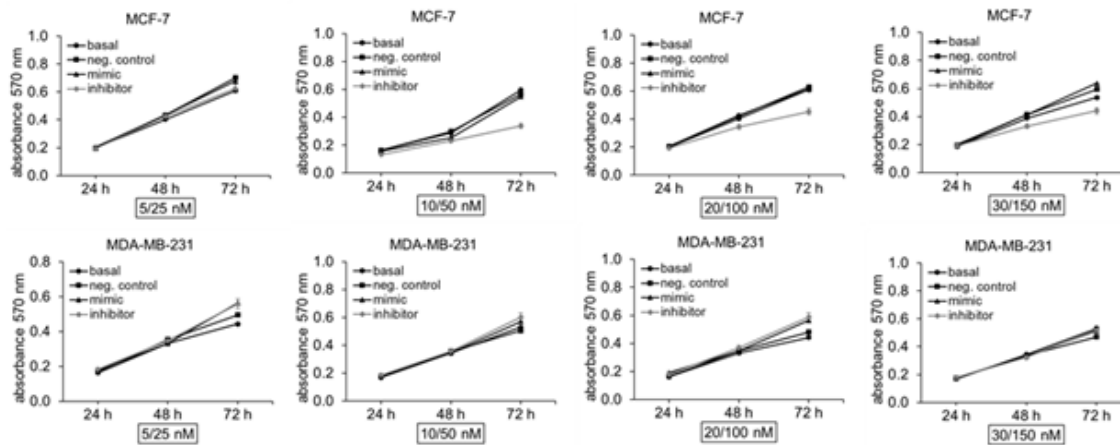
A) miR-16



B) miR-107



C) miR-130a



Supplementary Figure S4: Proliferation of MCF-7 and MDA-MB-231 cells transfected with different concentrations of mimics and inhibitors of *miR-16*, *miR-107* and *miR-130a*.

MCF-7 and MDA-MB-231 cells were transfected with mimic or inhibitor of *miR-16* (**A**), *miR-107* (**B**) and *miR-130a* (**C**) using four different concentrations 5/25 nM, 10/50 nM, 20/100 nM and 30/150 nM for mimics and inhibitors, respectively. Transfection with scramble miR served as a negative control. Cell proliferation was measured after 24 h, 48 h and 72 h incubation with MTT. Reduced cell proliferation in MCF-7 cells due to the Inhibition of endogenous *miR-130a* was observed for the MCF-7 cells transfected with 50 nM, 100 nM or 150 nM. No significant changes on cell proliferation were observed for *miR-16* and *miR-107* in both cell lines.

Table S1: Associations of miR levels with clinicopathological risk factors of patients before chemotherapy.

Parameters	n (%)	small RNA	miR-16	miR-27a	miR-107	miR-130a	miR-132	miR-146a
Breast cancer patients								
Total	111 (100)	647, 834	904, 3179	64, 405	Median, Mean, (95% CI) pvalue	15, 97	2, 9	79, 490
Age (range 33-76 years)	56 years	(703-975)	(1983-4374)	(145-665)	0.4, 1.5 (0.6-2.4)	(19-176)	(3-15)	(73-908)
Tumor stage								
pT1	59 (53)	600, 819	904, 3696	60, 369	0.887	0.817	0.575	0.607
		(616-1021)	(1758-5635)	(95-644)	0.4, 1.3 (0.6-2.1)	10.3, 61.2 (19.6-102)	2.1, 11 (0.7-21)	78, 361 (74-649)
pT2-4	52 (47)	711, 863	762, 2568	69, 447	0.093	0.036	0.027	0.089
		(679-1046)	(1259-3877)	(-31-924)	0.4, 1.8 (0.1-3.6)	15.5, 140 (-27-307)	2.2, 6.5 (1.4-11.5)	78, 642 (-218-1502)
Lymph node metastasis								
N0	71 (64)	737, 889	1633, 3657	94, 493	0.093	0.036	0.027	0.089
		(719-1059)	(2108-5206)	(99-887)	0.4, 1.9 (0.6-3.2)	21, 109 (-5.8-224)	3, 12 (2.6-21)	104, 622 (-17-1261)
N1-3	40 (36)	481, 745	490, 2286	43, 240	0.275	0.138	0.383	0.084
		(510-980)	(385-4186)	(93-387)	0.2, 0.8 (0.3- 1.4)	8, 75 (-2.2-153)	1.4, 3.8 (1.9-5.9)	43, 245 (87-402)
Grading								
II	40 (36)	558, 732	555, 2152	52, 171	0.275	0.138	0.383	0.084
		(537-926)	(968-3336)	(84-258)	0.3, 0.6 (0.3-1)	15, 24 (14-34)	1.4, 3.7 (1.5-5.8)	54, 155 (83-226)
III	71 (64)	652, 902	1071, 3708	225, 523	0.252	0.035	0.542	0.082
		(716-1089)	(1951-5466)	(121-926)	0.4, 2 (0.6-3.4)	13, 136 (13-259)	2.5, 12 (2.5-21)	96, 663 (13-1314)
ER status								
positive	50 (45)	697, 887	717, 2455	59, 430	0.252	0.035	0.542	0.082
		(661-1113)	(894-4016)	(-58-919)	0.1, 1.5 (-0.2-3.3)	27, 125 (-42-292)	1.8, 6.2 (1-11)	40, 620 (-258-1498)
negative	61 (55)	623, 799	2569, 3770	69, 984	0.749	0.748	0.306	0.340
		(630-969)	(1984-5555)	(115-653)	0.4, 1.5 (0.8-2.3)	15, 75 (24-126)	2.5, 11 (1.1-22)	99, 385 (100-670)
PR status								
positive	35 (32)	771, 976	859, 4049	63, 774	0.749	0.748	0.306	0.340
		(696-1256)	(1213-6886)	(-54-1602)	0.3, 2.4 (-0.3-5.1)	10, 164 (-80-408)	3.2, 19 (0.03-39)	90, 1018 (-333-2370)
negative	76 (68)	566, 777	904, 2784	65, 237	0.058	0.058	0.193	0.025
		(622-931)	(1569-4000)	(152-323)	0.4, 1.1 (0.6-1.6)	38, 67 (27-108)	1.5, 4.3 (3-5.7)	76, 251 (155-347)
HER2 status								
positive	15 (14)	584, 1018	1370, 2854	71, 934	0.934	0.058	0.193	0.025
		(469-1567)	(88-5620)	(-746-2615)	0.95, 4.6 (-1.5-11)	43, 331 (-238-899)	3.6, 13 (-4-30)	176, 1703 (-1331-4737)
negative	96 (86)	649, 810	838, 3230	63, 320	0.720	0.331	0.683	0.916
		(674-946)	(1899-4562)	(144-497)	0.3, 1 (0.6-1.5)	12, 60 (24-97)	1.8, 8.4 (1.8-15)	67, 297 (112-482)
Triple neg.								
Triple neg	47 (42)	572, 747	1117, 3032	66, 2	0.720	0.331	0.683	0.916
		(555-939)	(1606-4458)	(124-322)	0.4, 1.1 (0.6-1.5)	15, 64 (9-119)	1.9, 4.3 (2.7-6)	93, 260 (134-387)
other receptor statuses	64 (58)	720, 906	767, 33	61, 538	0.039	0.814	0.065	0.177
		(714-1098)	(1465-5108)	(91-985)	0.3, 1.9 (0.4-3.4)	13, 1 (-10-254)	2, 12 (2-23)	64, 658 (-63-1380)
Healthy women								
Total	46 (100)	343, 417	300, 483	52, 60	0.039	0.814	0.065	0.177
Age (range 50-85 years)	63 years	(326-508)	(294-672)	(41-79)	0.3, 0.6 (0.2-1)	9, 14 (9-18)	1.4, 1.7 (1.1-2.3)	60, 85 (58-112)

Significant p values in bold; p values as determined by Mann-Whitney-U test. *Confidence Interval, CI. Median, Mean of relative values.