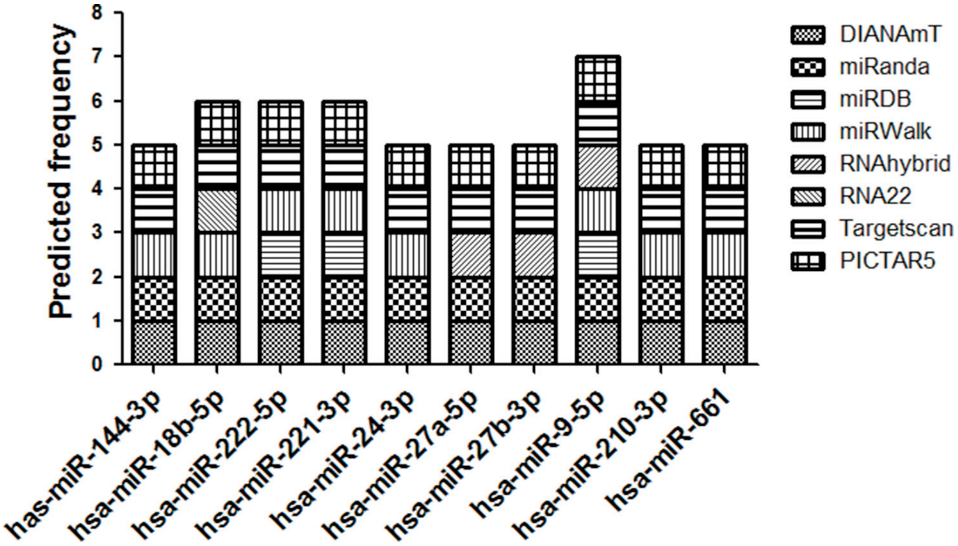
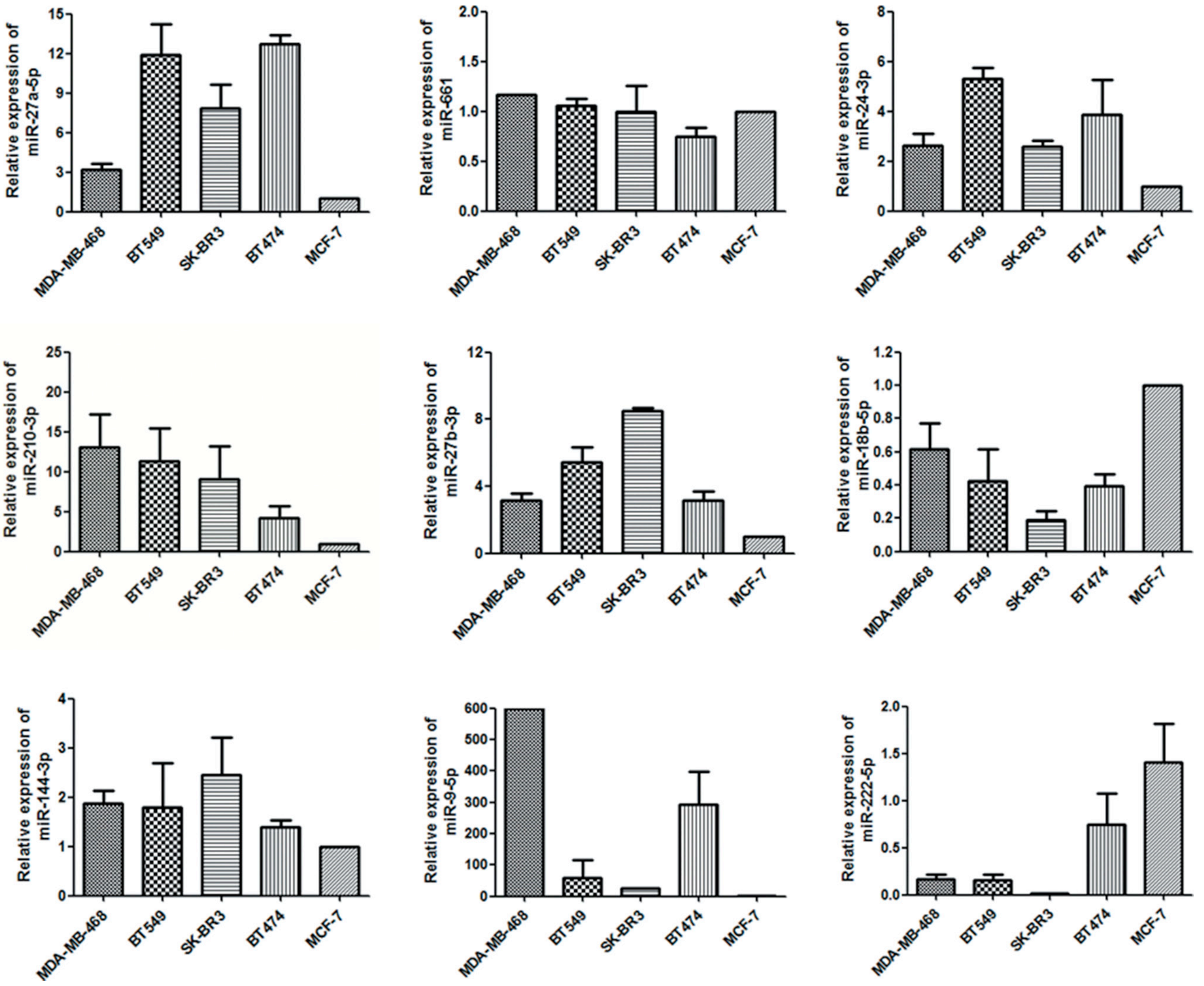
ADAMTS6 suppresses tumor progression via the ERK signaling pathway and serves as a prognostic marker in human breast cancer

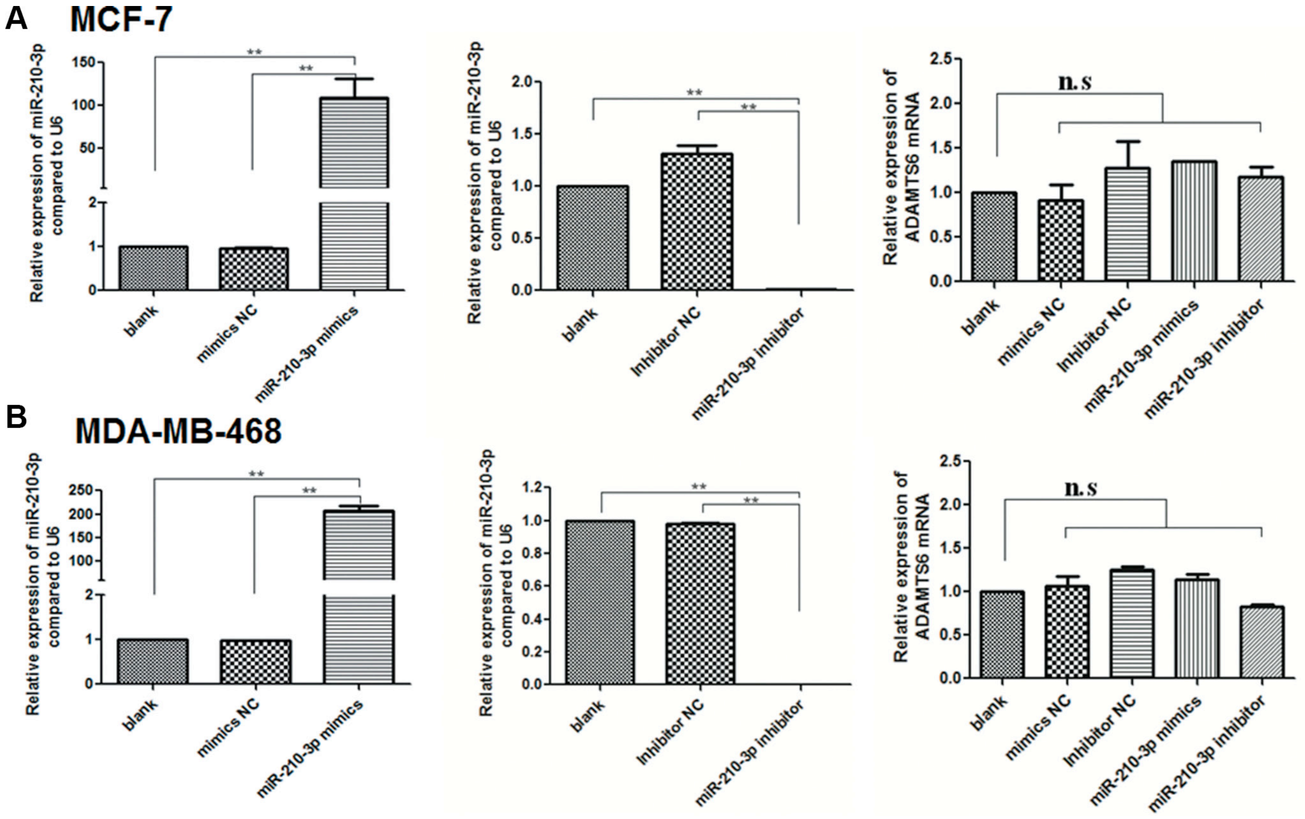
Supplementary Materials



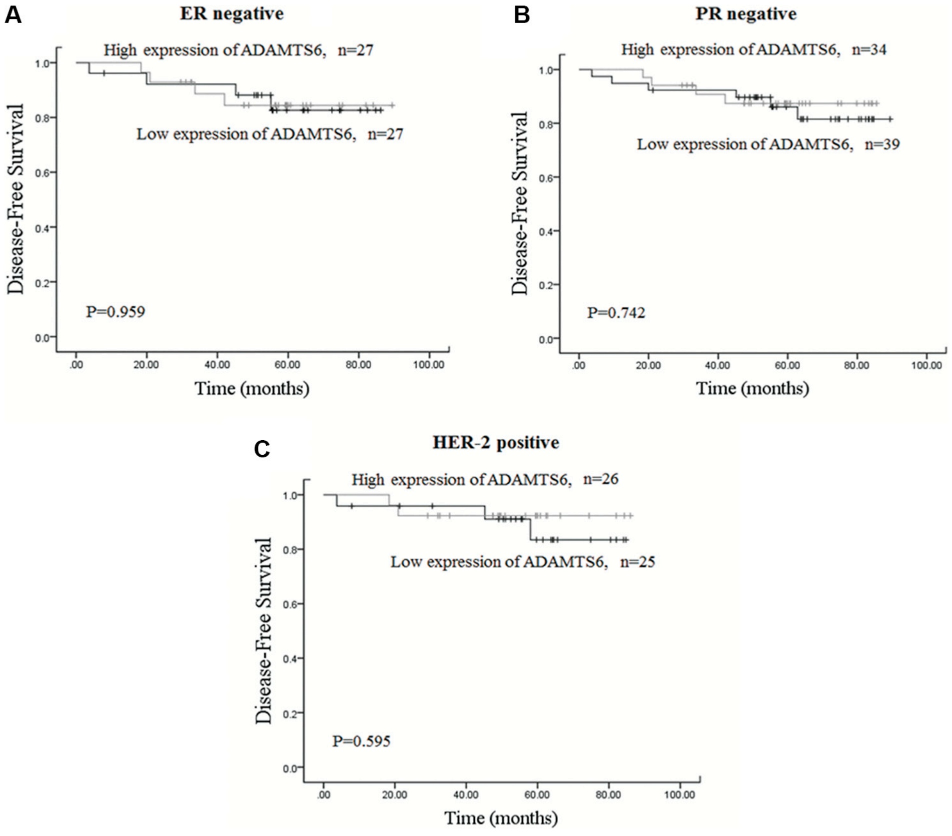
Supplementary Figure S1: Predicted frequencies of candidate miRNAs by online bioinformatics databases (DIANAmT, miRanda, miRDB, miRWalk, RNAhybrid, RNA22, Targetscan, PICTAR5) in BC.



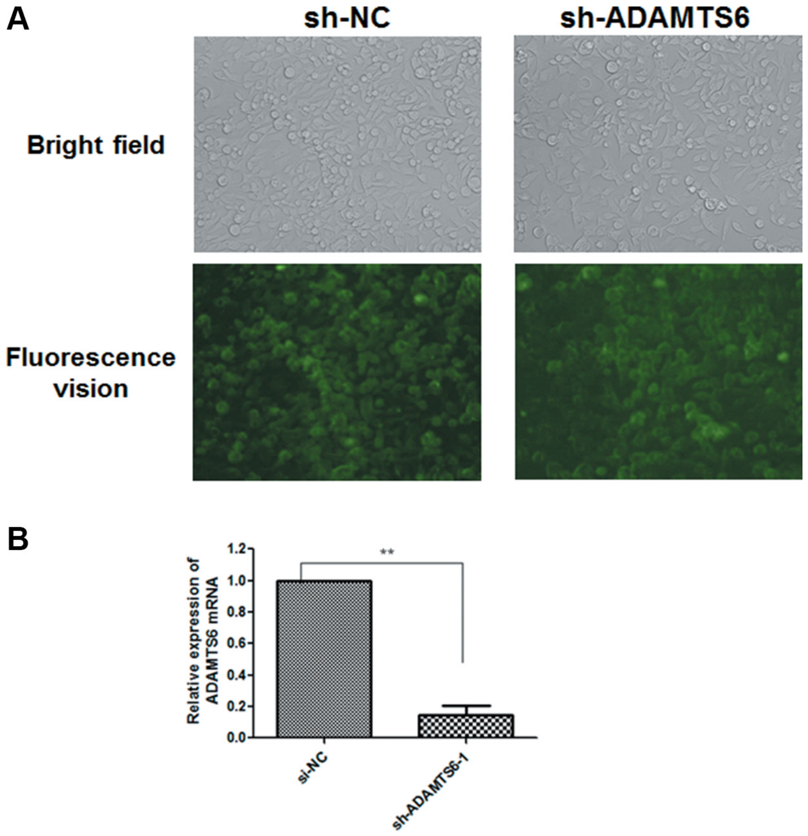
Supplementary Figure S2: The expression of candidate miRNAs was examined using qPCR in five BC cell lines.



Supplementary Figure S3: Cells were transfected with miR-210-3p mimics or miR-210-3p inhibitor and their respective negative controls. After 48 h incubation, cells were treated as depicted in §Methods. MiR-210-3p expression (left and middle), corresponding ADAMTS6 mRNA expression (right) was detected in (**A**) MCF-7 and (**B**) MDA-MB-468 cells.



Supplementary Figure S4: Kaplan-Meier curves for 5-year DFS according to subgroup survival analysis of (A) ER-negative, (B) PR-negative and (C) HER2- positive status based on ADAMTS6 expression.



Supplementary Figure S5: (A) The transduction efficiency of the GV112 lentivirus sh-NC or sh-ADAMTS6 infected MCF-7 cells were > 90% (200× magnification). (**B**) ADAMTS6 mRNA expression was detected by qPCR.

Supplementary Table S1. Basic clinicoathological parameters of the patients and their correlation with ADAMTS6 expression in BC

Parameters	All		ADAMTS6-high		ADAMTS6-low		*P* value
	n = 182		*n* = 90 (49.45%)		*n* = 92 (50.55%)		
	No.	(%)	No.	(%)	No.	(%)	
Age							0.185
Median (range)	48	(27–80)	50	(31–77)	47	(27–80)	
≤ 50	102	(56.0)	46	(45.1)	56	(54.9)	
> 50	80	(40.0)	44	(55.0)	36	(45.0)	
Tumor size (cm)							0.367
≤ 2	77	(42.3)	35	(45.5)	42	(54.5)	
> 2–5	92	(50.5)	50	(54.3)	42	(45.7)	
> 5	13	(7.2)	5	(38.5)	8	(61.5)	
Lymph node metastasis							0.303
No	98	(53.8)	45	(45.9)	53	(54.1)	
Yes	84	(46.2)	45	(53.6)	39	(46.4)	
TNM stage							0.462
I	47	(25.8)	20	(42.6)	27	(57.4)	
II	95	(52.2)	50	(52.6)	45	(47.4)	
III	37	(20.3)	20	(54.1)	17	(45.9)	
Unknown	3	(1.6)	0	(0.0)	3	(100.0)	
Histologic grade							0.156
I	6	(3.3)	5	(83.3)	1	(16.7)	
II	46	(25.3)	26	(56.5)	20	(43.5)	
III	96	(52.7)	45	(46.9)	51	(53.1)	
Unknown	34	(18.7)	14	(41.2)	20	(58.8)	
Molecular subtype							0.561
Luminal A	19	(10.5)	12	(63.2)	7	(36.8)	
Luminal B	98	(53.8)	47	(48.0)	51	(52.0)	
Triple-negative	15	(8.2)	6	(40.0)	9	(60.0)	
HER2-type	26	(14.3)	13	(50.0)	13	(50.0)	
Unknown	24	(13.2)	12	(50.0)	12	(50.0)	
subtyles							1.000
Negative	54	(29.7)	27	(50.0)	27	(50.0)	
Positive	126	(69.3)	63	(50.0)	63	(50.0)	
Unknown	2	(0.1)	0	(0.0)	2	(100.0)	
PR							0.448
Negative	73	(40.1)	34	(37.8)	39	(43.3)	
Positive	107	(58.8)	56	(62.2)	51	(56.7)	
Unknown	2	(1.1)	1	(50.0)	1	(50.0)	
HER-2							0.702
Negative	111	(61.0)	53	(67.1)	58	(69.9)	
Positive	51	(28.0)	26	(32.9)	25	(30.1)	
Uncertainty	20	(11.0)	11	(55.0)	9	(45.0)	
Ki67							0.817
< 14%	31	(17.0)	15	(16.7)	16	(18.0)	
≥ 14%	148	(81.3)	75	(83.3)	73	(82.0)	
Unknown	3	(1.7)	0	(0.0)	3	(100.0)	

Supplementary Table S2: Sequences of RNA and DNA oigonucleotides

Name	Sense Strand/Sense Primer(5′-3′)	Antisense Strand/Antisense Primer (5′-3′)
Primers for RT-qPCR		
ADAMTS6	ATC ACT CGA ACT GGC AGT GG	GTC TTT GGA CAC CTC CAG CA
GAPDH	ATCATCAGCAATGCCTCC	AGTCCTTCCACGATACCAA
microRNA mimics		
anti-miR-221-3p	AGC UAC AUU GUC UGC UGG GUU UC	AAC CCA GCA GAC AAU GUA GCU UU
anti-miR-210-3p	CUG UGC GUG UGA CAG CGG CUGA	AGC CGC UGU CAC ACG CAC AGU U
microRNA inhibitor		
anti-miR-221-3p	GAA ACC CAG CAG ACA AUG UAG CU	
anti-miR-210-3p	UCA GCC GCU GUC ACA CGC ACA G	
siRNA Duplexes		
si-ADAMTS6	GGA CUA CAG UGG UUG UUU A dTdT	UAA ACA ACC ACU GUA GUC C dTdT
shRNA Duplexes		
sh-ADAMTS6	Sense: GGACTACAGTGGTTGTTTA TTCAAGAGA TAAACAACCACTGTAGTCC TTTTT	Antisense: AAAAA GGACTACAGTGGTTGTTTA TCTCTTGAA TAAACAACCACTGTAGTCC