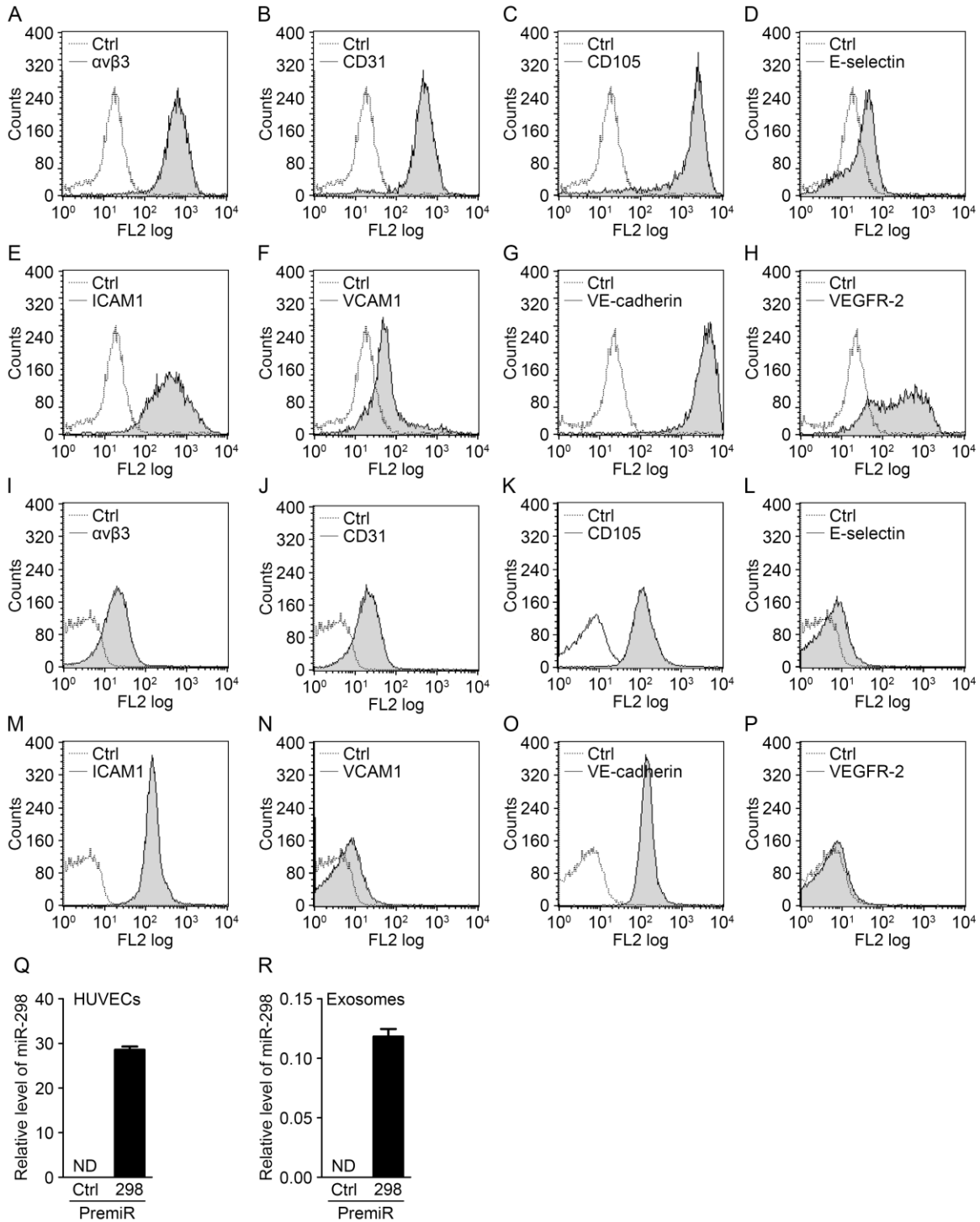


# Endothelial exosomes contribute to the antitumor response during breast cancer neoadjuvant chemotherapy via microRNA transfer

## Supplementary Material



## Figure S1

(A-H) Flow cytometry analysis of HUVECs for the levels of (A)  $\alpha v\beta 3$  integrins, (B) CD31, (C) CD105, (D) E-selectin, (E) ICAM-1, (F) VCAM-1, (G) VE-cadherin and (H) VEGFR2. (I-P) Flow cytometry analysis of HUVEC exosomes for the levels of (I)  $\alpha v\beta 3$  integrins, (J) CD31, (K) CD105, (L) E-selectin, (M) ICAM-1, (N) VCAM-1, (O) VE-cadherin and (P) VEGFR2. (Q) MiR-298 levels evaluated by qRT-PCR in HUVECs transfected with pre-miR-298 or pre-miR-control. (R) MiR-298 levels evaluated by qRT-PCR in the exosomes of HUVECs transfected with pre-miR-298 or pre-miR-control.

A

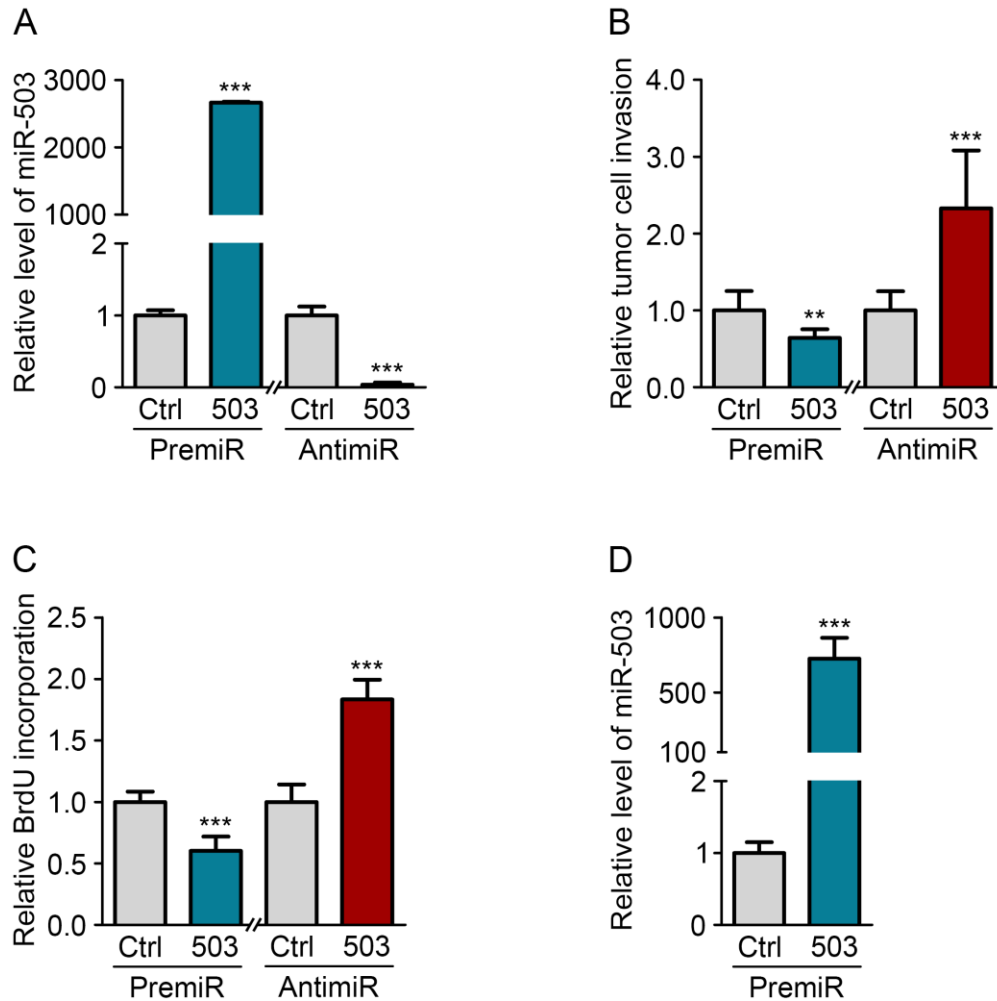
miRNAs only detected in exosomes	Common miRNAs between HUVECs and exosomes	miRNAs only detected in HUVECs
miR-122, miR-133a, miR-133b, miR-142-3p, miR-142-5p, miR-143, miR-144, miR-150, miR-202, miR-223, miR-451, miR-486-5p, miR-506, miR-509-3p, miR-596, miR-631	let-7b, let-7d, let-7d*, let-7g, let-7i, miR-101, miR-106a, miR-107, miR-10b, miR-125a-5p, miR-125b, miR-126, miR-126*, miR-128, miR-130a, miR-130b, miR-132, miR-134, miR-136, miR-137, miR-138, miR-139-5p, miR-140-3p, miR-140-5p, miR-146a, miR-148a, miR-148b, miR-149, miR-151-3p, miR-151-5p, miR-152, miR-154, miR-15a, miR-15b, miR-16, miR-17, miR-181a, miR-181b, miR-181c, miR-185, miR-186, miR-188-5p, miR-18a, miR-18a*, miR-18b, miR-191, miR-192, miR-193a-3p, miR-193a-5p, miR-193b, miR-194, miR-195, miR-196b, miR-197, miR-197b, miR-199a-3p, miR-199b-5p, miR-19a, miR-19b, miR-20a, miR-20b*, miR-21, miR-21*, miR-210, miR-215, miR-217, miR-218, miR-219-5p, miR-22, miR-22*, miR-221, miR-222, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-27a, miR-27b, miR-28-5p, miR-296-5p, miR-29a, miR-29b, miR-29c, miR-30b, miR-30c, miR-30c-2*, miR-30d, miR-30e*, miR-32, miR-320a, miR-323-3p, miR-324-3p, miR-324-5p, miR-326, miR-328, miR-337-3p, miR-337-5p, miR-338-3p, miR-339-5p, miR-33b, miR-342-3p, miR-345, miR-346, miR-34a, miR-34c-3p, miR-365, miR-374b, miR-377, miR-378, miR-381, miR-382, miR-409-3p, miR-411, miR-421, miR-423-3p, miR-423-5p, miR-424, miR-425, miR-425*, miR-431, miR-432, miR-433, miR-455-5p, miR-483-3p, miR-484, miR-490-3p, miR-483, miR-497, miR-501-5p, miR-502-5p, miR-503, miR-505, miR-532-5p, miR-574-3p, miR-582-5p, miR-584, miR-589, miR-590-5p, miR-595, miR-597, miR-598, miR-622, miR-629, miR-651, miR-652, miR-680, miR-683, miR-665, miR-671-5p, miR-720, miR-744, miR-877, miR-886-3p, miR-887, miR-888, miR-92a, miR-92b, miR-93, miR-934, miR-940, miR-99a, miR-99b	miR-141, miR-181a*, miR-26a-2*, miR-362-5p, miR-454, miR-514, miR-548b-3p, miR-576-3p, miR-576-5p, miR-654-5p

B

miRNA	Fold	P value	miRNA	Fold	P value	miRNA	Fold	P value	miRNA	Fold	P value
hsa-miR-502-5p	29,50	0,006	hsa-miR-339-5p	2,38	0,216	hsa-miR-23a	1,49	0,083	hsa-miR-589	0,67	0,234
hsa-miR-744	23,35	0,032	hsa-miR-191	2,36	0,068	hsa-miR-216a	1,47	0,005	hsa-miR-500a	0,66	0,273
hsa-miR-373*	19,74	0,010	hsa-miR-197	2,34	0,023	hsa-miR-196b	1,47	0,313	hsa-miR-888	0,65	0,373
hsa-miR-572	12,00	0,123	hsa-miR-140-5p	2,29	0,173	hsa-miR-140-3p	1,46	0,515	hsa-miR-193b	0,64	0,072
hsa-miR-487b	8,23	0,060	hsa-miR-376c	2,29	0,045	hsa-miR-338-3p	1,44	0,271	hsa-miR-130a	0,64	0,076
hsa-miR-886-3p	7,80	0,002	hsa-let-7i	2,29	0,040	hsa-miR-19a	1,42	0,217	hsa-miR-934	0,61	0,026
hsa-miR-128	7,80	0,013	hsa-miR-218	2,25	0,294	hsa-miR-665	1,42	0,218	hsa-miR-152	0,60	0,027
hsa-miR-431	6,87	0,008	hsa-miR-127-3p	2,25	0,005	hsa-miR-199a-3p	1,41	0,053	hsa-miR-595	0,60	0,447
hsa-miR-31*	5,64	0,003	hsa-miR-93	2,19	0,131	hsa-miR-21	1,40	0,054	hsa-miR-423-5p	0,59	0,095
hsa-miR-495	5,03	0,020	hsa-miR-27b	2,17	0,013	hsa-miR-382	1,40	0,527	hsa-miR-451	0,55	0,122
hsa-miR-18a*	4,97	0,068	hsa-miR-26b	2,17	0,056	hsa-miR-222	1,37	0,004	hsa-miR-450a	0,49	0,262
hsa-miR-1979	4,89	0,003	hsa-miR-425*	2,17	0,182	hsa-miR-32	1,36	0,142	hsa-miR-34c-3p	0,44	0,043
hsa-miR-148a	4,85	0,004	hsa-miR-940	2,16	0,023	hsa-let-7d*	1,36	0,314	hsa-miR-34a	0,41	0,015
hsa-miR-886-5p	4,75	0,047	hsa-miR-337-3p	2,15	0,178	hsa-let-7b	1,35	0,251	hsa-miR-181c	0,38	0,077
hsa-miR-31	4,64	0,005	hsa-miR-126*	2,15	0,069	hsa-miR-433	1,34	0,602	hsa-miR-210	0,36	0,122
hsa-miR-154*	4,61	0,017	hsa-miR-374a	2,14	0,126	hsa-miR-328	1,32	0,521	hsa-miR-424	0,34	0,011
hsa-miR-720	4,56	0,004	hsa-miR-103	2,12	0,070	hsa-miR-660	1,32	0,366	hsa-miR-193a-3p	0,34	0,010
hsa-miR-18a	4,52	0,016	hsa-miR-137	2,12	0,030	hsa-miR-148b	1,31	0,192	hsa-miR-542-5p	0,25	0,006
hsa-miR-361-5p	4,35	0,023	hsa-miR-425	2,10	0,052	hsa-miR-615-3p	1,30	0,633	hsa-miR-503	0,17	0,089
hsa-miR-381	4,29	0,174	hsa-miR-130b	2,10	0,173	hsa-miR-505	1,28	0,197	hsa-miR-205	0,15	0,037
hsa-miR-223	4,22	0,027	hsa-miR-369-5p	2,09	0,171	hsa-miR-132	1,27	0,595	hsa-miR-146a	0,13	0,005
hsa-miR-29b	4,13	0,000	hsa-let-7g	2,08	0,105	hsa-miR-22*	1,27	0,540			
hsa-let-7f	4,13	0,029	hsa-miR-24	2,07	0,040	hsa-miR-92a	1,26	0,109			
hsa-miR-142-3p	3,94	0,038	hsa-miR-365	2,04	0,039	hsa-miR-181b	1,26	0,305			
hsa-let-7d	3,92	0,028	hsa-miR-377	1,99	0,096	hsa-miR-483-3p	1,25	0,747			
hsa-miR-376a	3,80	0,026	hsa-miR-23b	1,97	0,057	hsa-miR-101	1,23	0,429			
hsa-miR-598	3,69	0,079	hsa-miR-30b	1,96	0,065	hsa-miR-326	1,21	0,191			
hsa-miR-142-5p	3,68	0,009	hsa-miR-126	1,94	0,060	hsa-miR-497	1,20	0,434			
hsa-miR-410	3,65	0,147	hsa-miR-221	1,93	0,011	hsa-miR-574-3p	1,16	0,486			
hsa-miR-301a	3,41	0,036	hsa-miR-652	1,91	0,025	hsa-miR-345	1,14	0,565			
hsa-miR-155	3,36	0,048	hsa-let-7c	1,90	0,002	hsa-miR-455-5p	1,12	0,672			
hsa-miR-18b	3,35	0,011	hsa-miR-134	1,90	0,003	hsa-miR-484	1,11	0,432			
hsa-miR-376b	3,33	0,012	hsa-miR-27a	1,87	0,033	hsa-miR-185	1,10	0,287			
hsa-miR-15b	3,20	0,042	hsa-miR-622	1,86	0,030	hsa-miR-590-5p	1,09	0,803			
hsa-miR-106a	3,14	0,036	hsa-miR-20b*	1,84	0,312	hsa-miR-154	1,09	0,821			
hsa-miR-100	3,12	0,002	hsa-miR-29a	1,80	0,064	hsa-miR-217	1,07	0,833			
hsa-miR-766	3,10	0,090	hsa-miR-582-5p	1,77	0,292	hsa-miR-671-5p	1,06	0,621			
hsa-miR-329	3,02	0,027	hsa-miR-181a	1,77	0,019	hsa-miR-877	1,04	0,957			
hsa-miR-33b	3,01	0,109	hsa-miR-30d	1,76	0,150	hsa-miR-486-5p	1,01	0,904			
hsa-miR-299-5p	2,95	0,165	hsa-miR-16	1,76	0,165	hsa-miR-324-5p	1,01	0,904			
hsa-miR-33a	2,94	0,006	hsa-miR-374b	1,73	0,140	hsa-miR-224	1,00	0,986			
hsa-miR-17	2,93	0,106	hsa-miR-409-3p	1,71	0,161	hsa-miR-25	0,98	0,843			
hsa-let-7a	2,92	0,016	hsa-miR-99b	1,71	0,032	hsa-miR-30c-2*	0,95	0,942			
hsa-miR-30e*	2,82	0,078	hsa-miR-323-3p	1,71	0,092	hsa-miR-22	0,94	0,708			
hsa-miR-98	2,80	0,002	hsa-miR-214	1,68	0,055	hsa-miR-188-5p	0,93	0,475			
hsa-miR-30c	2,76	0,031	hsa-miR-125a-5p	1,67	0,096	hsa-miR-490-3p	0,92	0,867			
hsa-miR-423-3p	2,75	0,068	hsa-miR-151-3p	1,67	0,011	hsa-miR-324-3p	0,92	0,791			
hsa-miR-411	2,68	0,048	hsa-miR-19b	1,63	0,051	hsa-miR-378	0,91	0,634			
hsa-miR-150	2,64	0,263	hsa-miR-15a	1,61	0,117	hsa-miR-452	0,90	0,888			
hsa-miR-21*	2,60	0,036	hsa-let-7e	1,61	0,030	hsa-miR-532-5p	0,89	0,791			
hsa-miR-370	2,60	0,032	hsa-miR-186	1,57	0,055	hsa-miR-663	0,84	0,414			
hsa-miR-30a	2,59	0,052	hsa-miR-204	1,57	0,563	hsa-miR-337-5p	0,84	0,687			
hsa-miR-151-5p	2,58	0,022	hsa-miR-26a	1,57	0,162	hsa-miR-10b	0,83	0,287			
hsa-miR-20a	2,55	0,033	hsa-miR-136	1,54	0,003	hsa-miR-361-3p	0,78	0,580			
hsa-miR-106b	2,54	0,061	hsa-miR-602	1,54	0,051	hsa-miR-199a-5p	0,76	0,729			
hsa-miR-331-3p	2,50	0,032	hsa-miR-887	1,54	0,284	hsa-miR-99a	0,76	0,103			
hsa-miR-29c	2,50	0,073	hsa-miR-299-3p	1,53	0,469	hsa-miR-215	0,76	0,560			
hsa-miR-107	2,48	0,004	hsa-miR-125b	1,53	0,244	hsa-miR-144	0,75	0,308			
hsa-miR-127-5p	2,43	0,137	hsa-miR-509-3p	1,51	0,576	hsa-miR-346	0,74	0,473			
hsa-miR-194	2,42	0,291	hsa-miR-193a-5p	1,51	0,267	hsa-miR-192	0,72	0,363			
hsa-miR-28-5p	2,41	0,023	hsa-miR-342-3p	1,50	0,165	hsa-miR-320a	0,71	0,075			

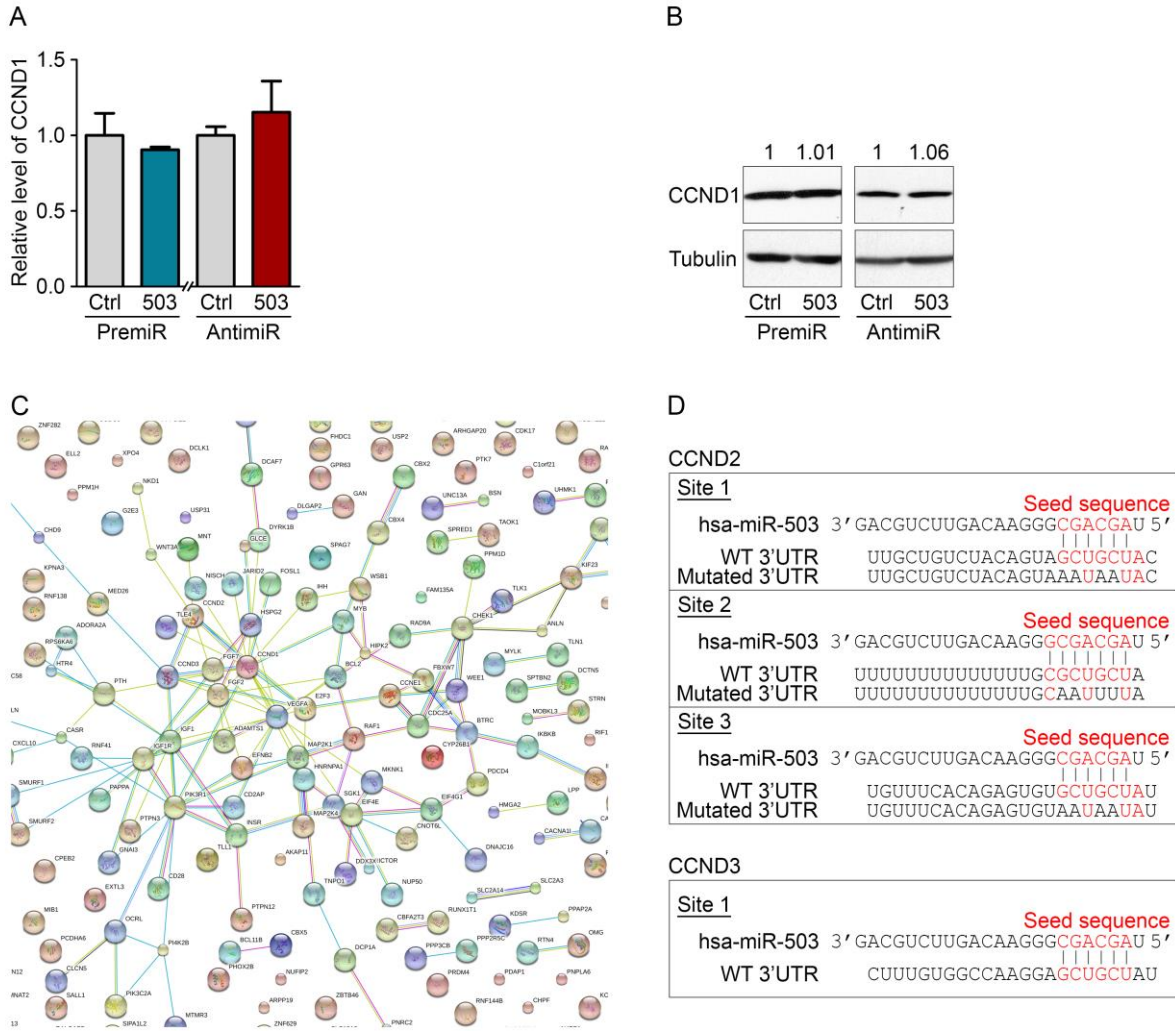
**Figure S2**

(A) Table of miRNA common between HUVECs and exosomes or only detected in exosomes or in producing HUVECs. (B) Table of the 204 miRNAs detected in exosomes from HUVECs cultured in basal or tumoral displaying a variation lower than 2 between replicates and an individual Ct value lower than 40.



**Figure S3**

(A) MiR-503 levels, evaluated by qRT-PCR, in MDA-MB-231 cells transfected with pre-miR-control or pre-miR-503 and with anti-miR-control or anti-miR-503. (B) Boyden invasion assay of MDA-MB-231 cells transfected with pre-miR-control or pre-miR-503 and with anti-miR-control or anti-miR-503. (C) BrdU incorporation in MDA-MB-231 cells transfected with pre-miR-control or pre-miR-503 and with anti-miR-control or anti-miR-503. (D) MiR-503 levels, evaluated by qRT-PCR, in HUVECs transfected with pre-miR-control or pre-miR-503.



**Figure S4**

(A) mRNA levels, quantified by qRT-PCR, of CCND1 in MDA-MB-231 cells transfected with pre-miR-503 or pre-miR-control and with anti-miR-control or anti-miR-503 and starved for 36 h. (B) Western blotting of CCND1 in MDA-MB-231 cells transfected with pre-miR-503 or pre-miR-control and with anti-miR-control or anti-miR-503 and starved for 36 h. (C) Bioinformatics analysis of miR-503 target genes predicted by Targetscan using String 9.1 software. (D) Predicted miR-503 binding sites within the 3'-UTRs of CCND2 and CCND3.