

Supplementary figures

## **Multi-miRNAs panel of tumor-derived extracellular vesicles as promising diagnostic biomarkers of early-stage breast cancer**

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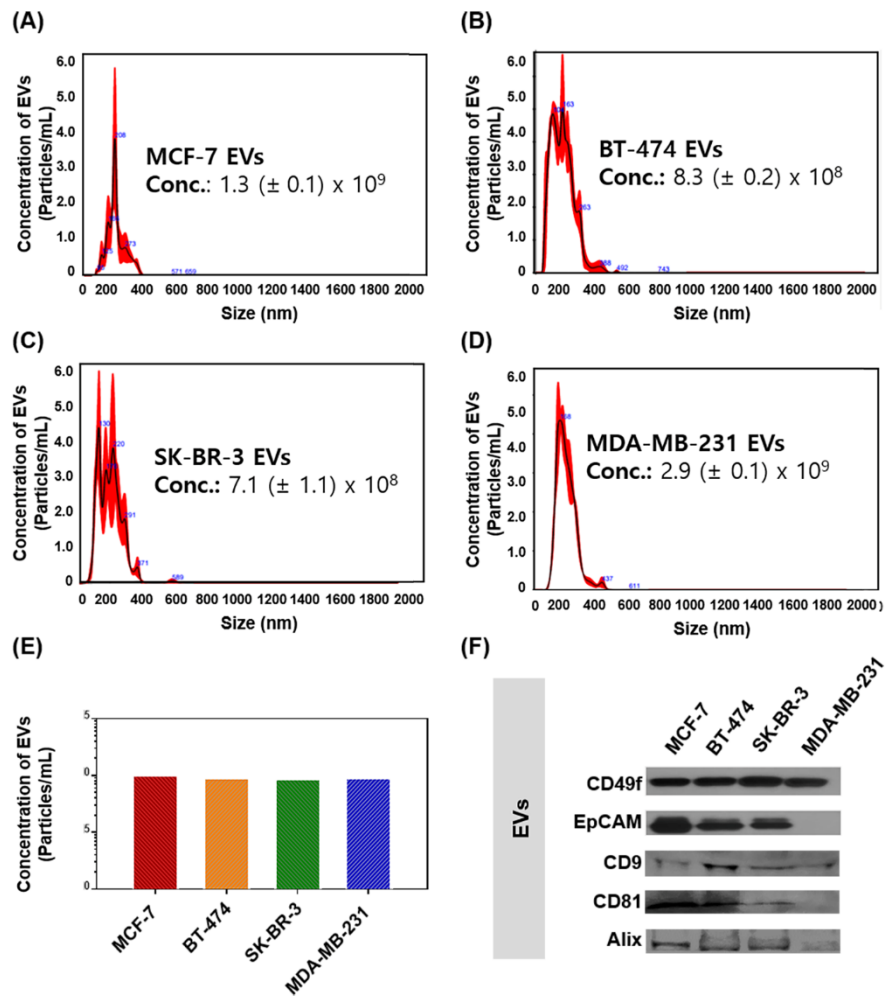
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**Figure S1.** Nanoparticle tracking analysis (NTA) quantification of extracellular vesicles (EVs) released from media of breast cancer cell lines. (A) NTA distribution of MCF-7 EVs. (B) NTA distribution of BT-474 EVs. (C) NTA distribution of SK-BR-3 EVs. (D) NTA distribution of MDA-MB-231 EVs. (E) Measurement of EVs amounts by NTA. (F) Western blot analysis of EpCAM, CD49f, CD9, CD81, and Alix on EVs.


(A)

	miR-484	miR-let7a	miR-16
miR-16	0.92*	0.27	0.00
miR-21	0.99*	0.91	0.96*
miR-9	0.82	0.05	0.46
miR-429	0.99**	0.73	0.99**
miR-96	0.50	0.43	0.53
miR-155	0.25	0.19	0.28
miR-128	0.25	0.99*	0.00

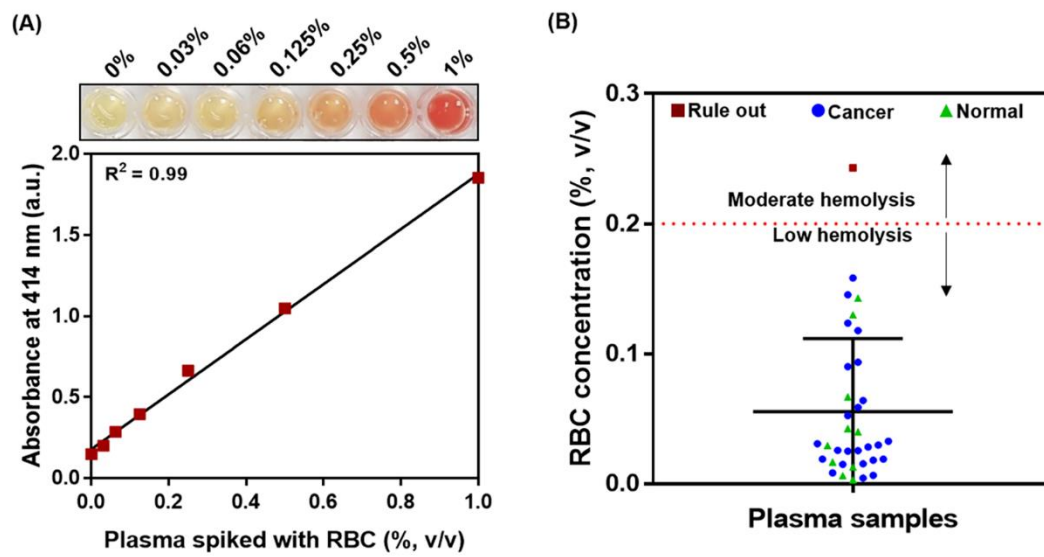
(B)

	miR-484	miR-let7a	miR-16
miR-16	0.03*	0.73	1.00
miR-21	0.02*	0.09	0.04*
miR-9	0.28	1.00	1.00
miR-429	0.001**	0.14	0.01**
miR-96	0.49	0.57	0.47
miR-155	0.50	0.57	0.47
miR-128	0.50	0.001**	1.00

(non-correlation) 0  1 (correlation)  
Pearson *r*

(non-significant) 1  0 (significant)  
*P*-value

**Figure S2.** Evaluation of endogenous controls for comparing microRNA (miRNA) expression levels between breast cancer cells (BT-474, MCF-7, SK-BR-3, and MDA-MB-231) and their EVs. (A) Heat map of correlations between miRNA expression in cell lines and cell-derived EVs for miR-16, miR-21, miR-9, miR-429, miR-96, miR-155, miR-128, and miR-let-7a normalized by miR-484, miR-let7a, and miR-16. A darker red color represents a stronger positive correlation (Pearson correlation coefficients closer to 1). (B) A darker green color represents significant correlations. The miR-16, miR-21, miR-9, and miR-429 of extracellular vesicles normalized by miR-484 were significantly correlated with those of breast cancer cells. \* $p < 0.05$ , \*\* $p < 0.01$ .



**Figure S3.** Evaluation of hemolysis of plasma samples prior to isolation of EVs to minimize the hemolysis-induced miRNA expression inhibition. (A) Standard curve of hemolysis at 414 nm of absorbance. (B) Condition of rule-out by RBC concentration in 32 representative plasma samples from total population.