SUPPORTING INFORMATION

Simultaneous Detection of Tumor Derived Exosomal Protein-MicroRNA Pairs with an Exo-PROS Biosensor for Cancer Diagnosis

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Table S1. Characteristics of normal controls and nonsmall cell lung cancer patients

No	Histopathological type	Race	Sex	Age	Stage	Exosome size (nm)	Exosome concentration ($\times 10^{12}$ particles/mL)
1	Normal, low risk	White	M	70-74		112	10.64
2	Normal, low risk	White	M	65-69		112	4.14
3	Normal, low risk	White	M	75-79		107	2.1
4	Normal, low risk	White	F	55-59		122	24.51
5	Normal, low risk	White	F	75-79		126	21.12
6	Normal, low risk	White	F	50-54		124	7.82
7	Normal, low risk	White	F	75-79		111	8.08
8	Normal, low risk	White	F	55-59		99	13.92
9	Normal, low risk	White	M	55-59		102	11.69
10	Normal, low risk	White	F	60-64		148	18.03
11	Normal, low risk	White	M	60-64		136	25.07
12	Normal, low risk	White	F	75-79		121	26.82
13	Normal, low risk	White	M	55-59		149	26.28
14	Normal, low risk	White	M	55-59		120	19.82
15	Normal, low risk	White	F	70-74		108	27.28
16	Normal, high risk	White	M	65-69	No pulmonary diseases	108	32.18
17	Normal, high risk	White	M	40-44	Pneumonia; asthma; chronic bronchitis; emphysema/COPD	131	20.06
18	Normal, high risk	White	M	70-74	Chronic bronchitis; emphysema/COPD	150	18.46
19	Normal, high risk	White	F	45-49	Pneumonia; Emphysema/COPD	143	13.67
20	Normal, high risk	White	M	65-69	Asthma; emphysema/COPD	87	8.37
21	Normal, high risk	White	F	50-54	No pulmonary diseases	139	19.37
22	Normal, high risk	White	M	50-54	Chronic bronchitis; emphysema/COPD	146	10.58
23	Normal, high risk	White	M	55-59	No pulmonary diseases	112	20.24
24	Normal, high risk	White	F	55-59	No pulmonary diseases	102	10.73
25	Normal, high risk	White	F	55-59	Emphysema/COPD	139	24

26	Normal, high risk	White	M	50-54	No pulmonary diseases	154	17.4
27	Normal, high risk	White	M	60-64	Chronic bronchitis; emphysema/COPD	140	21.2
28	Normal, high risk	White	F	60-64	Asthma; chronic bronchitis	136	14.66
29	Normal, high risk	Unknown	M	55-59	Emphysema/COPD	141	9.06
30	Normal, high risk	White	F	70-74	Pneumonia	155	20.14
31	Normal, high risk	White	M	65-69	Emphysema/COPD	164	16.9
32	Normal, high risk	White	M	50-54	Emphysema/COPD	162	8.44
33	Normal, high risk	White	F	50-54	Asthma; chronic bronchitis; emphysema/COPD	117	18.24
34	Normal, high risk	White	F	50-54	Emphysema/COPD	90	10.02
35	Normal, high risk	White	M	55-59	Asthma; chronic bronchitis; emphysema/COPD	165	29.28
36	Adenocarcinoma	White	M	70-74	1A	139	15.41
37	Adenocarcinoma	White	M	70-74	1A	131	7.47
38	Adenocarcinoma	White	F	70-74	1A	126	21.92
39	Adenocarcinoma	White	F	70-74	1A	99	8.66
40	Adenocarcinoma	White	F	50-54	1A	122	7.35
41	Adenocarcinoma	White	F	55-59	2B	126	15.18
42	Squamous cell carcinoma	White	M	60-64	2A	105	33.3
43	Adenocarcinoma	White	F	55-59	1B	108	27.52
44	Squamous cell carcinoma	White	M	65-69	2A	111	19.33
45	Squamous cell carcinoma	White	M	75-79	2B	120	19.05
46	Squamous cell carcinoma	White	M	55-59	2A	128	30.28
47	Adenocarcinoma	White	F	55-59	1A	128	24.16
48	Adenocarcinoma	White	M	65-69	1A	144	22.14
49	Adenocarcinoma	White	F	65-69	2A	115	19.66
50	Adenocarcinoma	White	F	70-74	2B	136	26.72
51	Adenocarcinoma	White	F	60-64	1B	118	29.72
52	Squamous cell carcinoma	White	M	65-69	2A	146	22.09
53	Adenocarcinoma	White	F	70-74	2B	124	23.59
54	Squamous cell carcinoma	White	M	55-59	1A	143	23.02
55	Adenocarcinoma	White	F	65-69	1B	140	24.68

56	Adenocarcinoma	White	F	70-74	2B	97	7.32
57	Adenocarcinoma	White	F	75-79	1A	66	2.05
58	Adenocarcinoma	White	F	55-59	1A	88	12.16
59	Adenocarcinoma	White	M	60-64	1A	102	10.24
60	Adenocarcinoma	White	F	60-64	2B	105	12.53
61	Adenocarcinoma	White	M	55-59	2B	77	6.95
62	Squamous cell carcinoma	White	M	75-79	1A	85	7.68
63	Small cell carcinoma	White	F	70-74	2B	86	6.19
64	Small cell carcinoma	White	F	75-79	1B	85	18.89
65	Small cell carcinoma	White	F	70-74	1A	74	6.05
66	Adenocarcinoma	White	M	55-59	4	109	16.04
67	Adenocarcinoma	White	M	70-74	3A	144	13.59
68	Adenocarcinoma	White	M	75-79	3A	114	25.04
69	Adenocarcinoma	White	F	75-79	3B	107	14.42
70	Adenocarcinoma	White	F	55-59	4	87	6.97
71	Adenocarcinoma	White	M	65-69	3B	104	6.31
72	Adenocarcinoma	White	F	60-64	4	111	29.84
73	Adenocarcinoma	White	M	50-54	4	110	10.06
74	Adenocarcinoma	White	F	75-79	4	123	16.79
75	Adenocarcinoma	White	F	70-74	4	134	8.28
76	Squamous cell carcinoma	White	F	55-59	4	109	22.31
77	Squamous cell carcinoma	White	F	55-59	3B	122	23.85
78	Adenocarcinoma	White	M	55-59	3A	138	22.92
79	Squamous cell carcinoma	White	M	60-64	3A	114	7.7
80	Squamous cell carcinoma	White	M	50-54	3A	136	19.03
81	Adenocarcinoma	White	M	50-54	4	104	27.09
82	Adenocarcinoma	White	M	60-64	3B	113	29.34
83	Adenocarcinoma	White	M	65-69	3A	127	25.32
84	Squamous cell carcinoma	White	M	55-59	4	105	19.35
85	Adenocarcinoma	White	F	55-59	3B	128	18.07
86	Adenocarcinoma	White	F	55-59	3B	66	5.60
87	Adenocarcinoma	White	F	60-64	3A	72	4.26
88	Adenocarcinoma	White	F	55-59	3B	74	3.13
89	Adenocarcinoma	White	M	60-64	4A	109	12.88

90	Squamous cell carcinoma	White	F	70-74	4B	88	1.96
91	Small cell carcinoma	White	M	60-64	4B	69	4.80
92	Small cell carcinoma	White	M	60-64	4	70	10.93
93	Small cell carcinoma	White	F	70-74	4A	81	8.85
94	Small cell carcinoma	White	F	65-69	4B	74	8.21
95	Small cell carcinoma	White	M	55-59	4B	64	5.53

Table S2. Characteristics of normal controls and breast cancer patients

No	Histopathological type	Race	Sex	Age	Stage	ER	PR	HER2
1	Normal	Black	F	40-44				
2	Normal	White	F	35-39				
3	Normal	White	F	40-44				
4	Normal	White	F	45-49				
5	Normal	White	F	55-59				
6	Normal	White	F	55-59				
7	Normal	White	F	65-69				
8	Normal	White	F	65-69				
9	Normal	White	F	70-74				
10	Normal	White	F	75-79				
11	Normal	White	F	70-74				
12	Normal	White	F	50-54				
13	Normal	White	F	50-54				
14	Normal	White	F	60-64				
15	Normal	White	F	60-64				
16	Normal	White	F	75-79				
17	Normal	White	F	60-64				
18	Normal	White	F	45-49				
19	Normal	Asian	F	65-69				
20	Normal	White	F	75-79				
21	Ductal carcinoma in situ	White	F	60-64	0	Positive	Positive	NA
22	Ductal carcinoma in situ	White	F	55-59	0	Positive	Negative	NA
23	Ductal carcinoma in situ	White	F	65-69	0	Positive	Positive	NA
24	Ductal carcinoma in situ	White	F	30-34	0	Negative	Negative	NA
25	Ductal carcinoma in situ	White	F	70-74	0	Negative	Negative	NA
26	Ductal carcinoma in situ	Asian	F	60-64	0	Positive	Positive	NA
27	Ductal carcinoma in situ	White	F	40-44	0	Positive	Positive	NA
28	Ductal carcinoma in situ	White	F	60-64	0	Negative	Negative	NA
29	Ductal carcinoma in situ	White	F	75-79	0	Positive	Positive	NA
30	Intraductal carcinoma, noninfiltrating	White	F	45-49	0	Positive	Positive	NA
31	Intraductal carcinoma, noninfiltrating	White	F	55-59	0	Positive	Positive	NA
32	Intraductal carcinoma, noninfiltrating	White	F	40-44	0	Positive	Positive	NA
33	Intraductal carcinoma, noninfiltrating	Black	F	75-59	0	Positive	Positive	NA
34	Ductal carcinoma in situ	White	F	70-74	0	Positive	Positive	NA

35	Intraductal carcinoma, noninfiltrating	White	F	55-59	0	Positive	Positive	NA
36	Infiltrating duct carcinoma	White	F	45-49	1	Positive	Positive	Borderline
37	Infiltrating duct carcinoma	White	F	55-59	1	Negative	Positive	Negative
38	Infiltrating duct carcinoma	White	F	55-59	1	Negative	Negative	Positive
39	Infiltrating duct carcinoma	White	F	65-69	1	Positive	Positive	Negative
40	Infiltrating duct carcinoma	White	F	65-69	1	Positive	Positive	Negative
41	Infiltrating duct carcinoma	White	F	75-79	1	Negative	Negative	Negative
42	Infiltrating duct carcinoma	White	F	65-69	1	Negative	Negative	Negative
43	Infiltrating duct carcinoma	White	F	35-39	2	Negative	Negative	Positive
44	Infiltrating duct carcinoma	White	F	65-69	2	Negative	Negative	Negative
45	Infiltrating duct carcinoma	Black	F	40-44	2	Negative	Positive	Negative
46	Infiltrating duct carcinoma	White	F	35-39	2	Positive	Positive	Negative
47	Infiltrating duct carcinoma	White	F	40-44	2	Positive	Positive	Negative
48	Infiltrating duct carcinoma	White	F	70-74	2	Positive	Positive	Negative
49	Infiltrating duct carcinoma	White	F	30-34	2	Negative	Negative	Negative
50	Infiltrating duct carcinoma	White	F	55-59	2	Negative	Negative	Positive
51	Infiltrating duct carcinoma	White	F	75-79	3	Positive	Positive	Negative
52	Infiltrating duct carcinoma	White	F	45-49	3	Positive	Positive	Negative
53	Infiltrating duct carcinoma	White	F	45-49	3	Positive	Positive	Negative
54	Infiltrating duct carcinoma	White	F	60-64	3	Negative	Negative	Positive
55	Infiltrating duct carcinoma	Black	F	45-49	3	Negative	Negative	Positive
56	Infiltrating duct carcinoma	White	F	60-64	3	Negative	Negative	Negative
57	Infiltrating duct carcinoma	White	F	55-59	3	Positive	Positive	Negative
58	Infiltrating duct carcinoma	Asian	F	35-39	3	Positive	Positive	Negative
59	Infiltrating duct carcinoma	White	F	55-59	3	Negative	Negative	Negative
60	Infiltrating duct and lobular carcinoma	White	F	50-54	3	Positive	Negative	Positive
61	Infiltrating duct carcinoma	Black	F	75-79	3	Positive	Negative	Positive
62	Infiltrating duct and lobular carcinoma	Black	F	55-59	3	Negative	Negative	Negative
63	Infiltrating duct carcinoma	White	F	65-69	3	Positive	Negative	Negative
64	Infiltrating duct carcinoma	White	F	55-59	3	Negative	Negative	Negative
65	Invasive carcinoma of no special type	White	F	60-64	3	Negative	Negative	Negative
66	Infiltrating duct carcinoma	White	F	50-54	4	Negative	Negative	Positive
67	Infiltrating duct carcinoma	White	F	70-74	4	Positive	Positive	Negative
68	Infiltrating duct carcinoma	Black	F	40-44	4	Positive	Positive	Negative
69	Infiltrating duct carcinoma	White	F	35-39	4	Positive	Positive	Negative
70	Infiltrating duct carcinoma	White	F	40-44	4	Positive	Positive	Positive
71	Infiltrating duct carcinoma	White	F	45-49	4	Positive	Positive	Negative
72	Infiltrating duct carcinoma	White	F	55-59	4	Negative	Negative	Negative

73	Infiltrating duct carcinoma	White	F	55-59	4	Positive	Positive	Negative
74	Infiltrating duct carcinoma	White	F	65-69	4	Positive	Positive	Negative
75	Infiltrating duct carcinoma	White	F	65-69	4	Positive	Positive	Negative
76	Infiltrating duct carcinoma	White	F	75-79	4	Negative	Positive	Negative
77	Infiltrating duct carcinoma	Black	F	70-74	4	Negative	Negative	Positive
78	Infiltrating duct carcinoma	White	F	70-74	4	Negative	Negative	Negative
79	Infiltrating duct carcinoma	White	F	55-59	4	Negative	Negative	Positive
80	Infiltrating duct carcinoma	White	F	50-54	4	Positive	Positive	Negative

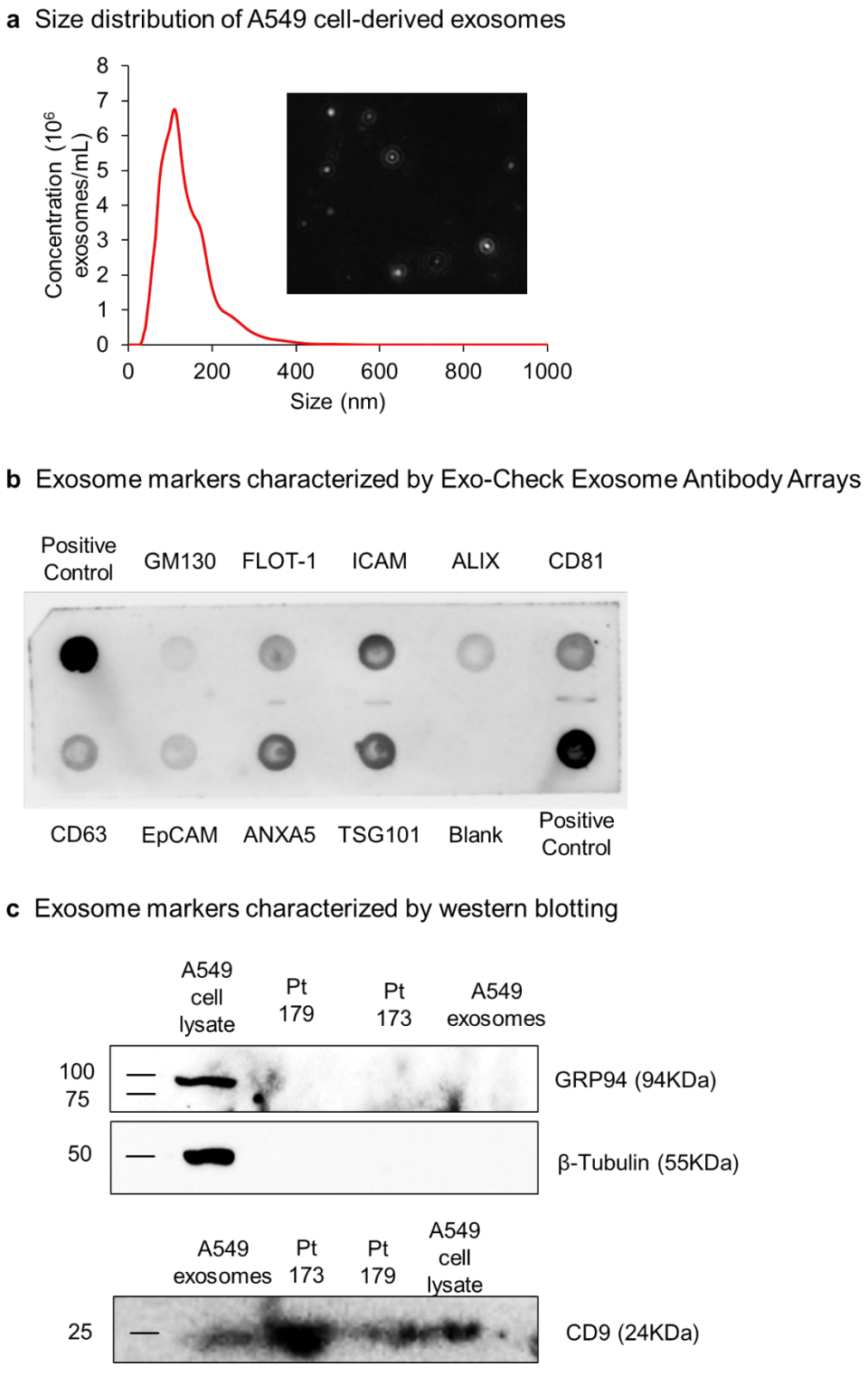
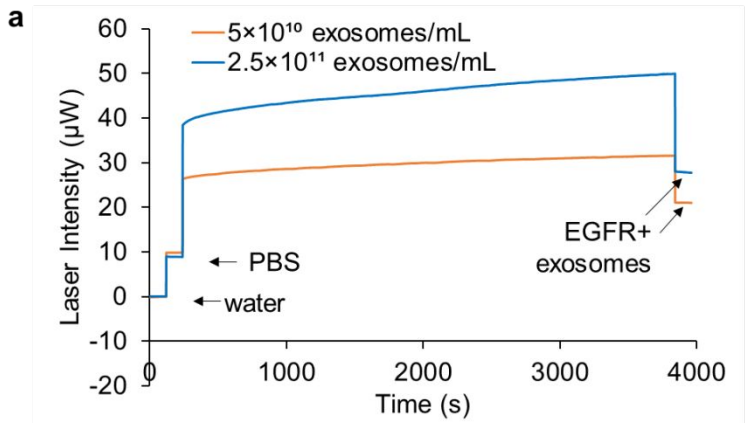
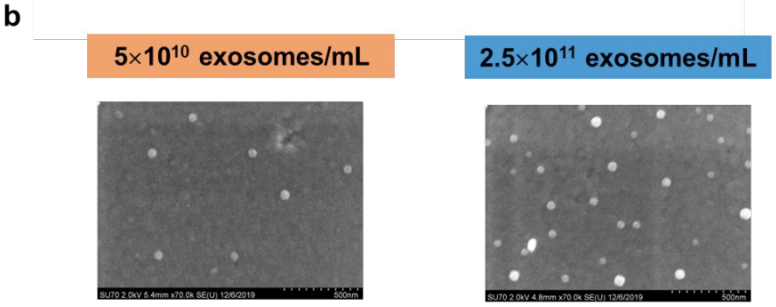
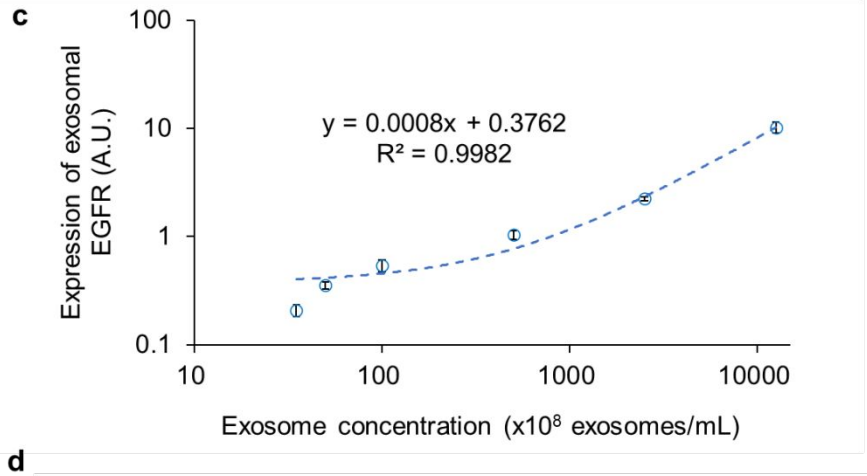


Figure S1. Characterization of exosomes. (a) Representative size distribution of exosomes from A549 NSCLC cells. (b) Exosome markers characterized by Exo-Check Exosome Antibody Arrays. Exosomes were isolated from the serum sample of a late stage nonsmall cell lung cancer

(NSCLC) patient (patient ID: 129). Strong expression of exosome positive markers such as FLOT-1, ICAM, CD81 and TSG101, and little expression of exosome negative marker GM130 (cis-Golgi marker) demonstrated the high purity and minimal cellular contamination of isolated exosomes. (c) Exosome markers characterized by western blotting. Exosomes were isolated from serum samples from 2 NSCLC patients (patient IDs: 173 and 179) and cell culture medium of A549 NSCLC cells. A549 cell lysate was used as the positive control. No expression of negative markers including GRP94 and β-tubulin was detected in exosomes but in A549 cell lysate. Strong expression of positive marker, CD9 was detected in all samples. The results demonstrated high purity of exosomes. Western blotting conditions: 20 μg protein per sample was loaded. Primary antibodies against GRP94 (Santa Cruz Biotechnology, sc-393402, clone H-10), β-tubulin (Sigma, T8328, clone AA2) and CD9 (System Bioscience, EXOAB-CD9A-1) were added at 1:500, 1:1000 and 1:1000 respectively. Secondary antibodies (Santa Cruz Biotechnology, sc-516102) were added at 1:2000 for GRP94 and CD9 and 1:5000 for β-tubulin.


a
5×10¹⁰ exosomes/mL
2.5×10¹¹ exosomes/mL
Laser Intensity (μW)
60
50
40
30
20
10
0
-10
-20
0
1000
2000
3000
4000
Time (s)
PBS
water
EGFR+
exosomes


b
5×10¹⁰ exosomes/mL
2.5×10¹¹ exosomes/mL


c
Expression of exosomal EGFR (A.U.)
100
10
1
0.1
y = 0.0008x + 0.3762
R² = 0.9982
10
100
1000
10000
Exosome concentration (x10⁸ exosomes/mL)

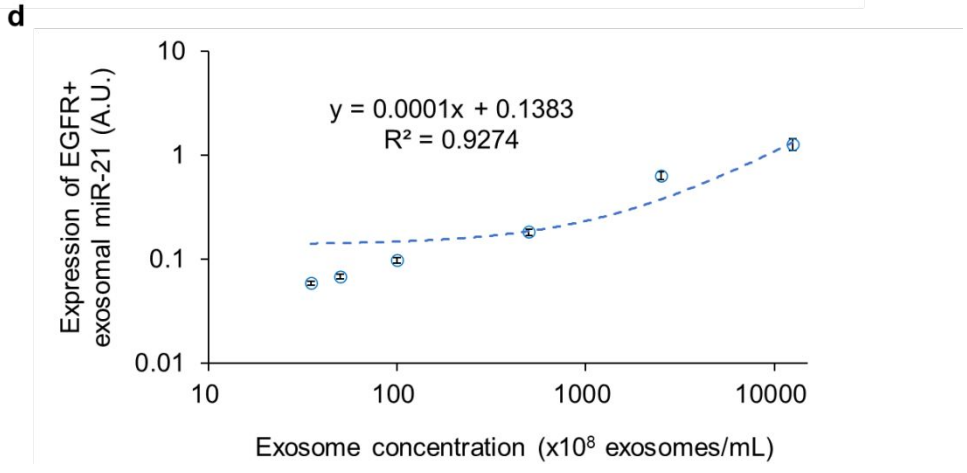

d
Expression of EGFR+ exosomal miR-21 (A.U.)
10
1
0.1
0.01
y = 0.0001x + 0.1383
R² = 0.9274
10
100
1000
10000
Exosome concentration (x10⁸ exosomes/mL)

Figure S2. Limit of detection and linear range of Exo-PROS assay in detecting exosomal EGFR-miR-21 pair. The expression of EGFR and miR-21 in A549 cell-derived exosomes at concentrations of 0 (blank control) to 1.25×10^{12} exosomes/mL were measured by Exo-PROS assay. (a) Representative SPR curves that measured the levels of EGFR in A549 cell-derived exosomes at concentrations of 5×10^{10} and 2.5×10^{11} exosomes/mL. (b) SEM images of EGFR+ exosomes captured on the biochip surface. Approximately 5-fold more exosomes were captured on the biochip when exosomes were applied at 5-fold higher concentration. (c, d) The LODs for EGFR and EGFR+ exosomal miR-21 were 3.5×10^{9} exosomes/mL and 5×10^{9} exosomes/mL, respectively. Exo-PROS assay showed about 3-log linear range in detecting exosomal EGFR-miR-21 pair.

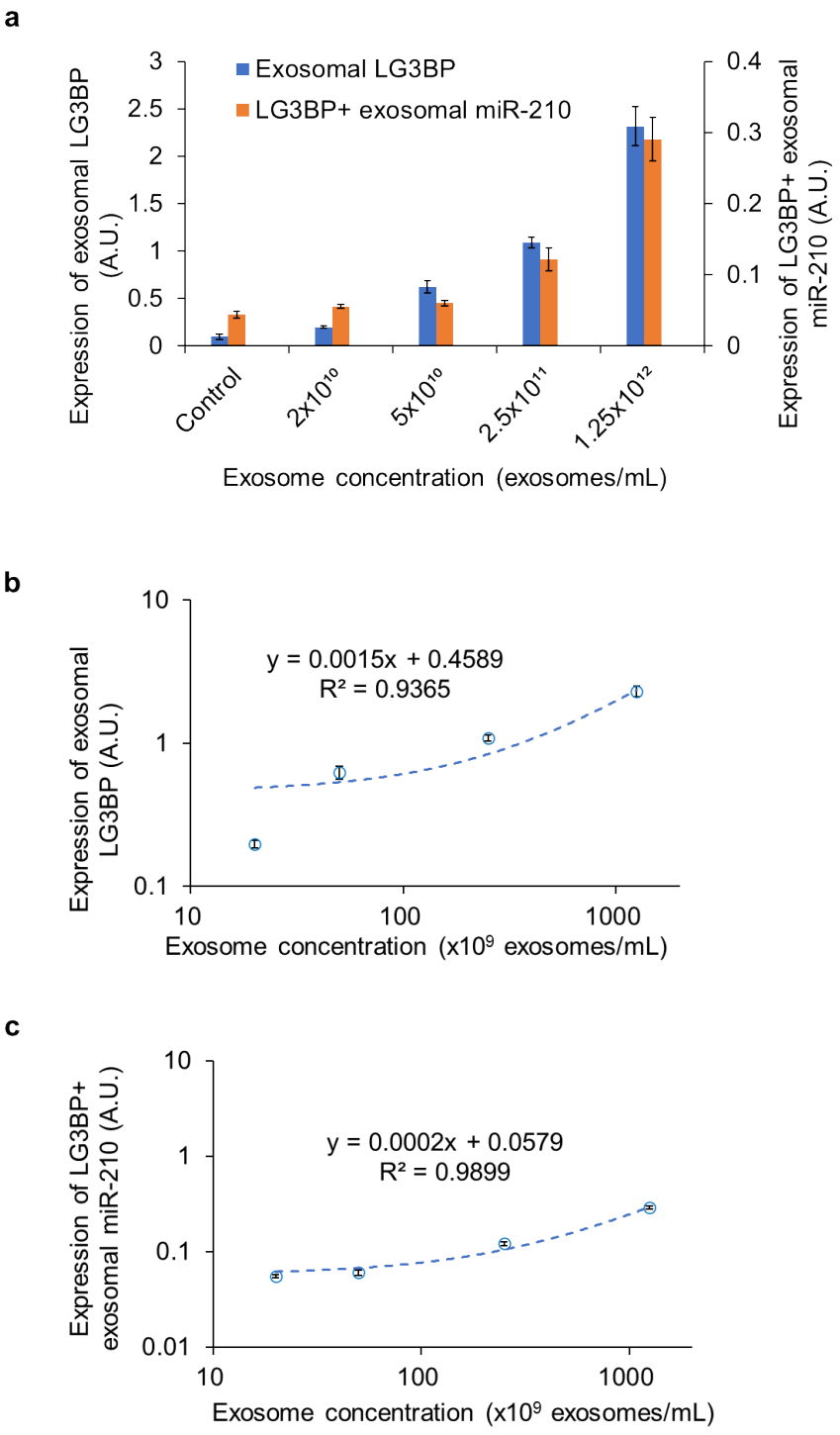


Figure S3. Limit of detection and linear range of Exo-PROS assay in detecting exosomal LG3BP-miR-210 pair. The expression of LG3BP (a, b) and miR-210 (a, c) in A549 cell-derived exosomes at concentrations of 0 (blank control) to 1.25×10^{12} exosomes/mL were measured by Exo-PROS assay. The LODs for LG3BP and LG3BP+ exosomal miR-210 were 2×10^{10} and 5×10^{10} exosomes/mL, respectively. Exo-PROS assay showed 2-log linear range in detecting exosomal LG3BP-miR-210 pair.

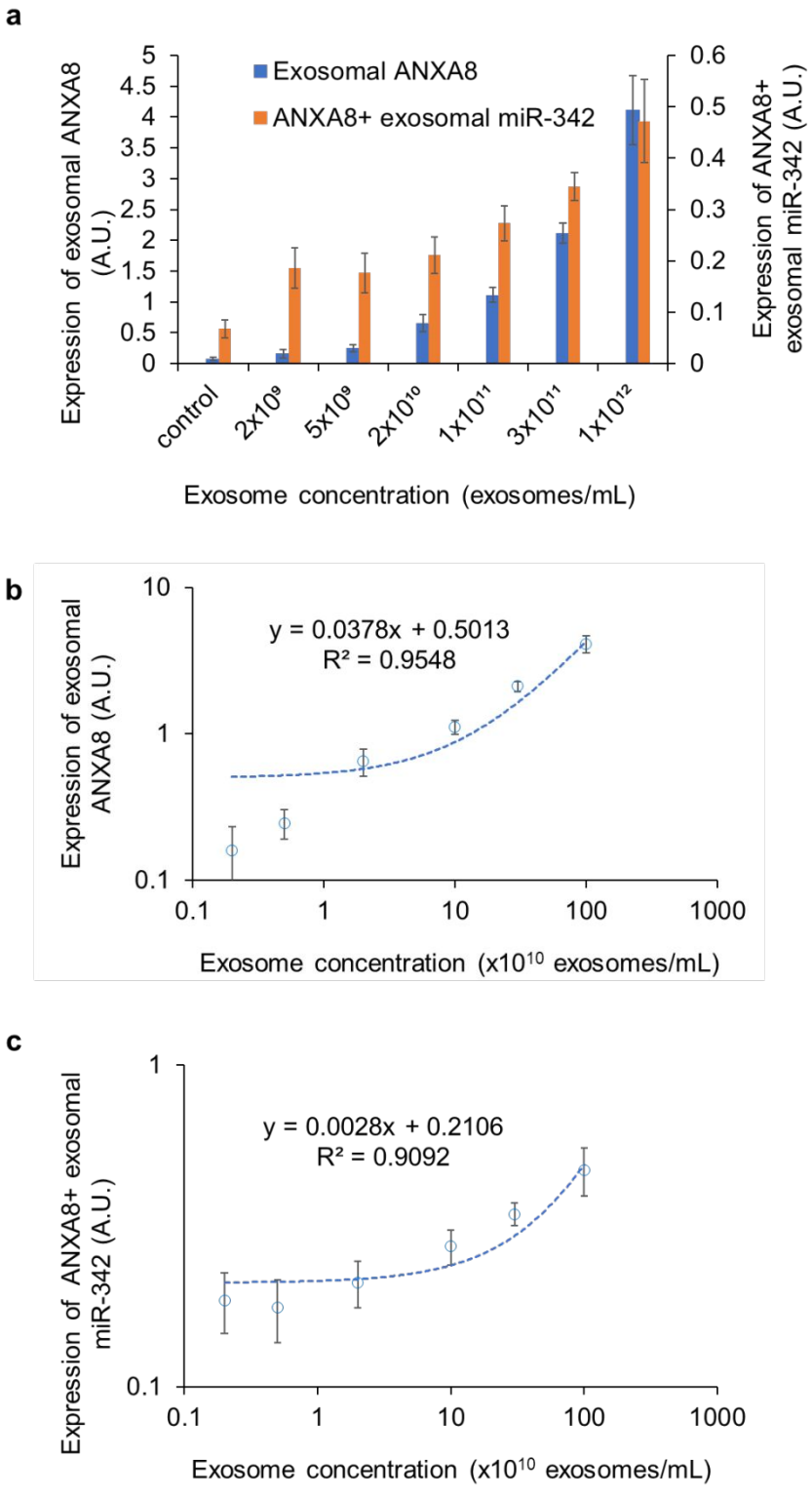


Figure S4. Limit of detection and linear range of Exo-PROS assay in detecting exosomal ANXA8-miR-342 pair. The expression of ANXA8 (a, b) and miR-342 (a, c) in MDA-MB-231 cell-derived exosomes at concentrations of 0 (blank control) to 10^{12} exosomes/mL were measured by Exo-PROS assay. The LODs for ANXA8 and ANXA8+ exosomal miR-342 were

5×10^9 and 2×10^{10} exosomes/mL, respectively. Exo-PROS assay showed 2-log linear range in detecting exosomal ANXA8-miR-342 pair.

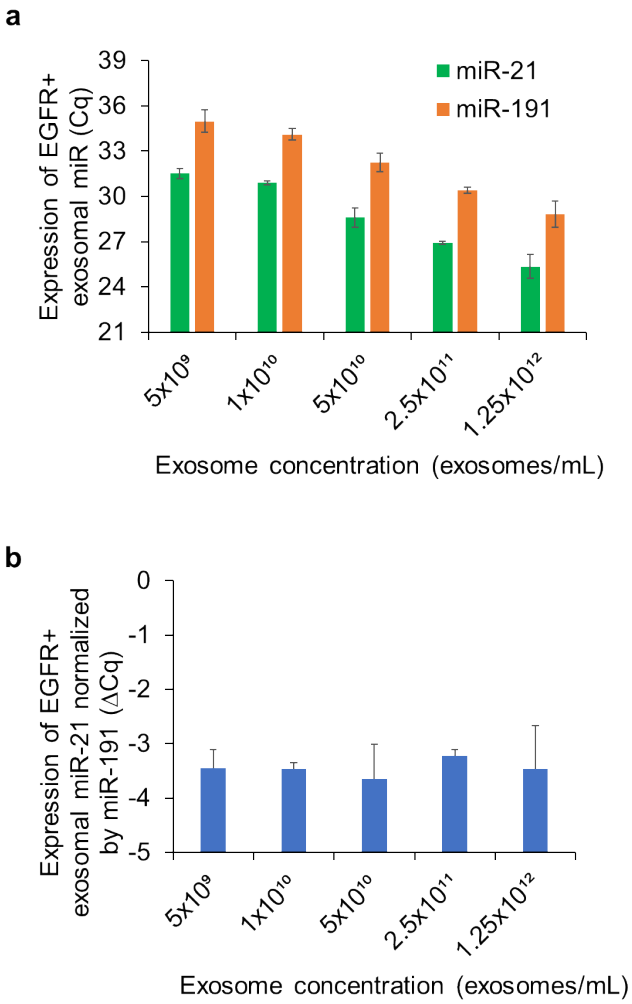


Figure S5. Characterization of EGFR+ exosomal miR-21 using IMS-PCR workflow. A549 cell-derived exosomes were prepared at concentrations of 5×10^9 to 1.25×10^{12} exosomes/mL. (a) IMS-PCR workflow was used to measure the expression of EGFR+ exosomal miR-21 and miR-191 (endogenous control). The levels of miR-21 and miR-191 increased with the increase of exosome concentration. (b) The expression of EGFR+ exosomal miR-21 was normalized by miR-191. $\Delta Cq = Cq_{,miR\text{-}21} - Cq_{,\ miR\text{-}191}$. No significant difference between samples was seen, demonstrating that the measurements of miRNAs were accurate.

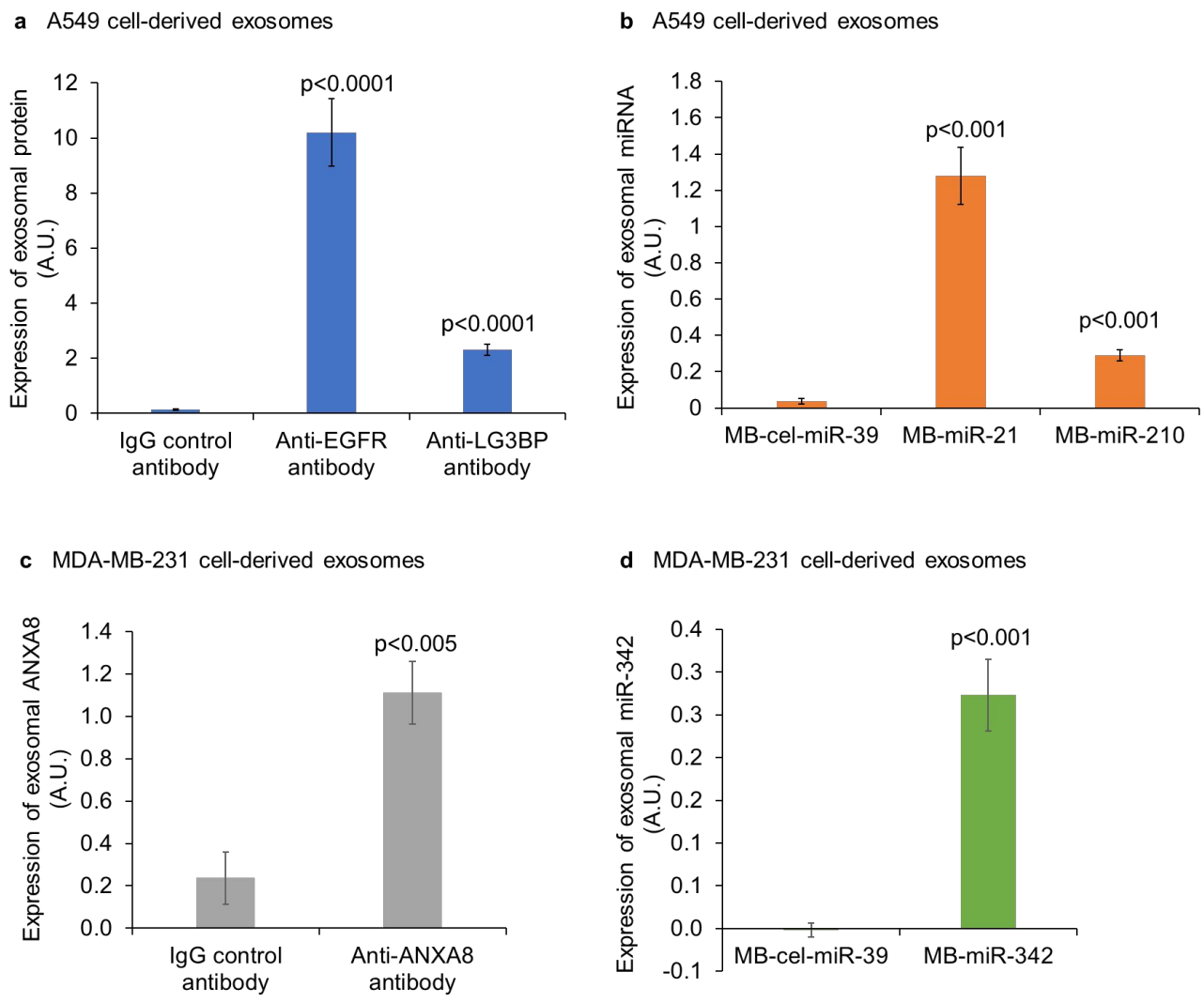


Figure S6. Specificity of Exo-PROS assay. (a, b) A549 cell derived exosomes were applied at the concentration of 1.25×10^{12} exosomes/mL. The expression of exosomal EGFR and LG3BP was measured when the surface of the biochip was modified with anti-EGFR, anti-LG3BP and anti-IgG control antibodies. The expression of EGFR+ exosomal miR-21 and LG3BP+ exosomal miR-210 was measured using miR-21 sensing MBs, miR-210 sensing MBs and cel-miR-39 control MBs. (c, d) MDA-MB-231 cell derived exosomes were applied at the concentration of 10^{11} exosomes/mL. The expression of exosomal ANXA8 was measured when the surface of biochip was modified with anti-ANXA8 and anti-IgG control antibodies. The expression of ANXA8+ exosomal miR-342 was measured using miR-342 sensing MBs and cel-miR-39 control MBs. Little signals were observed from the anti-IgG control antibodies and cel-miR-39 control MBs, demonstrating that Exo-PROS assay has great specificity.

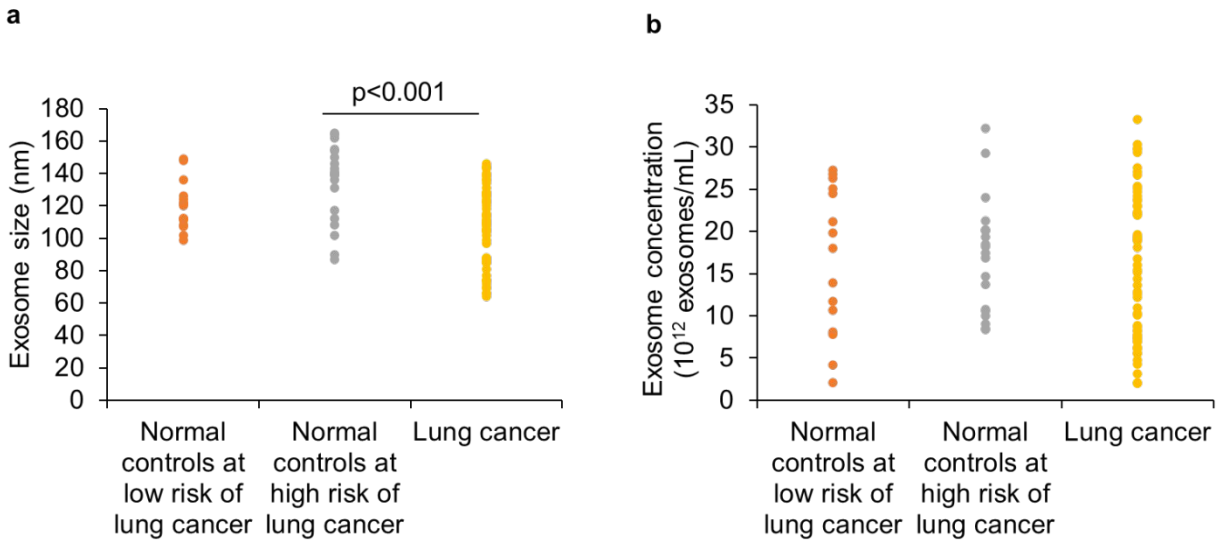
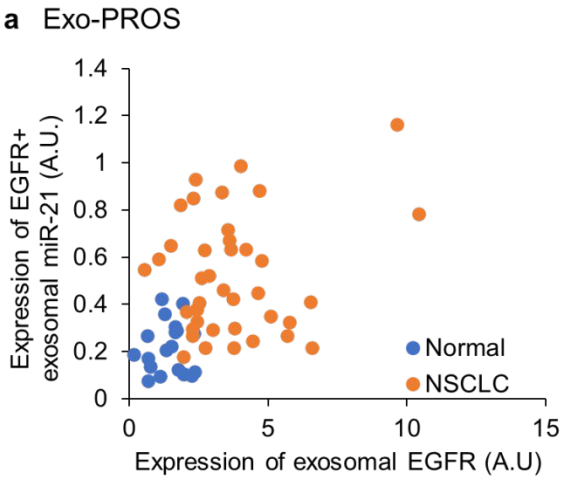
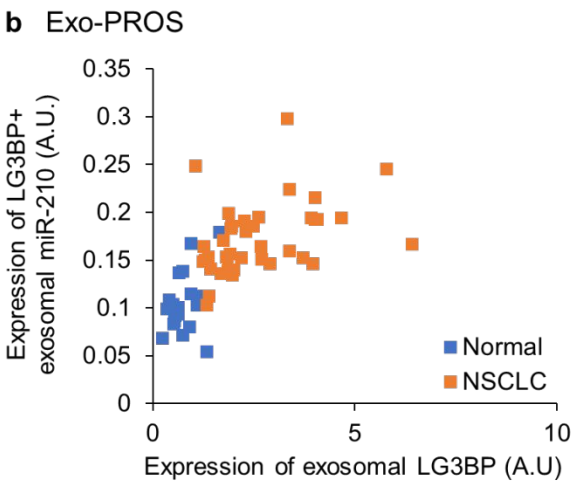
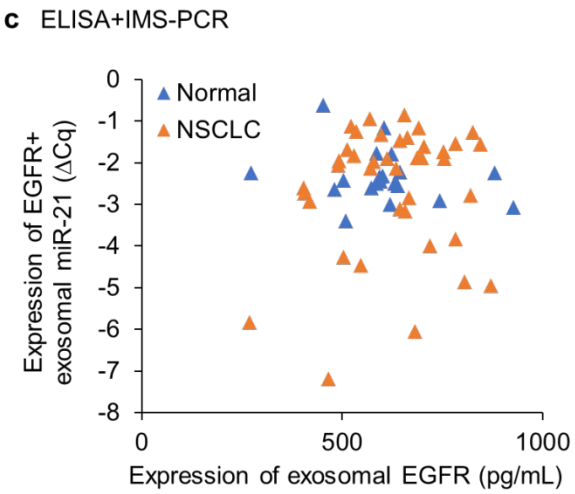
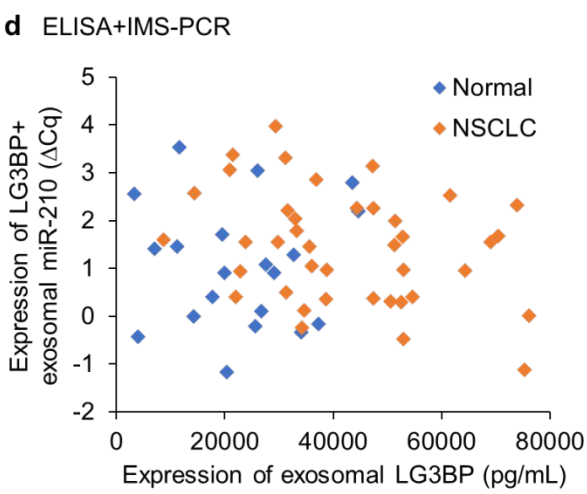


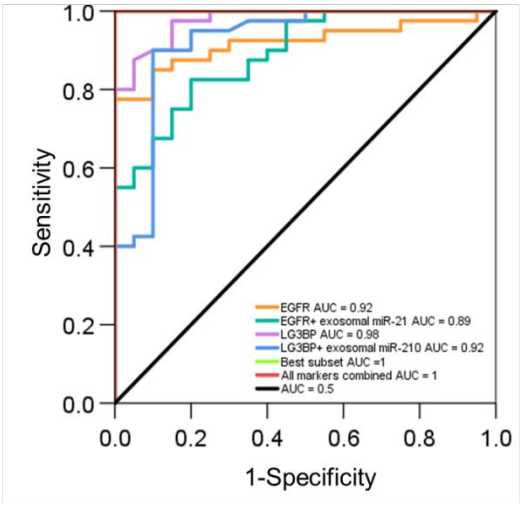
Figure S7. Characterization of exosomes isolated from serum samples. No significant difference was observed in the size and number concentration of serum-derived exosomes between normal controls at low risk of lung cancer (n=15) and lung cancer patients (n=60). Exosomes from normal controls at high risk of lung cancer (n=20) had larger size than those from lung cancer patients (n=60) (134 nm vs. 108 nm; $p<0.001$) but had similar number concentration as those from lung cancer patients. Exosomes were isolated from 50 µL serum, resuspended in 50 µL PBS and characterized by nanoparticle tracking analysis.

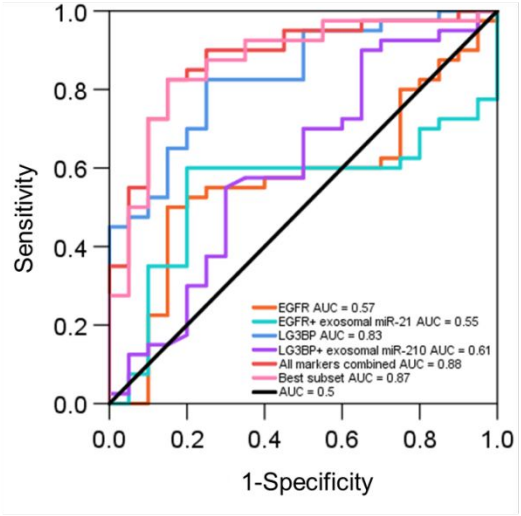








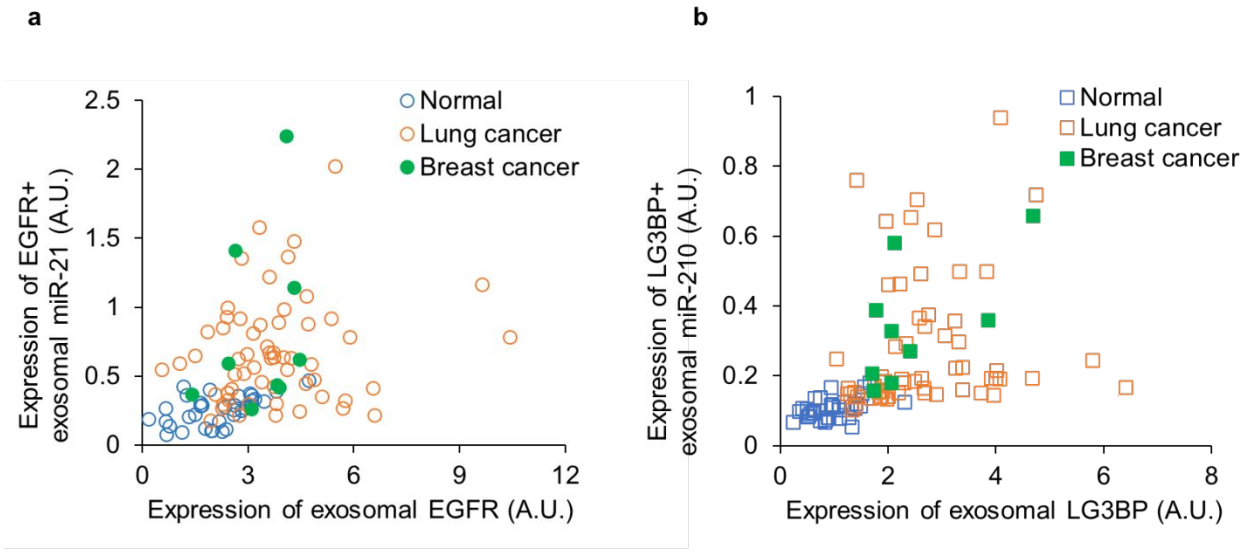


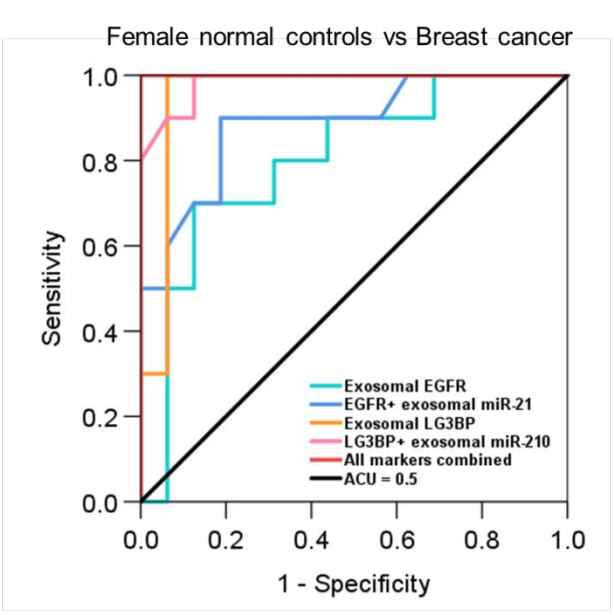


g

Assay	EGFR alone			miR-21 alone			LG3BP alone			miR-210 alone			All markers combined		
Normal vs NSCLC	SEN	SPE	AUC	SEN	SPE	AUC	SEN	SPE	AUC	SEN	SPE	AUC	SEN	SPE	AUC
Exo-PROS	0.78	1.00	0.92	0.83	0.80	0.89	0.98	0.85	0.98	0.90	0.90	0.92	1.00	1.00	1.00
ELISA+IMS-PCR	0.50	0.85	0.57	0.60	0.80	0.55	0.83	0.75	0.83	0.55	0.70	0.61	0.83	0.85	0.88

Figure S8. Diagnostic performance comparison between Exo-PROS assay and IMS-PCR workflow. Exosomes were isolated from 50 μL sera from normal controls (n=20) and NSCLC patients (n=40). The normal controls included 15 normal controls at low risk of lung cancer (ID: 1-15 in Table S1) and 5 normal controls at high risk of lung cancer (ID: 16-20 in Table S1). NSCLC patients included 20 early stage NSCLC patients (stage I/II; ID: 36-55 in Table S1) and 20 late stage NSCLC patients (stage III/IV; ID: 66-85 in Table S1). The levels of TEX EGFR-miR-21 pair and LG3BP-miR-210 pair were measured by Exo-PROS assay (a, b), ELISA and IMS-PCR workflow (c, d). The Exo-PROS assay detected significantly higher levels of TEX EGFR-miR-21 pair and LG3BP-miR-210 pair in sera from NSCLC patients than normal controls ($p<0.0001$). For ELISA and IMS-PCR workflow, only exosomal LG3BP showed significantly higher levels in NSCLC patients than normal controls ($p<0.0001$). No significant difference in the expression of exosomal EGFR, miR-21 and miR-210 was seen between NSCLC patients and normal controls. (e, f, g) ROC analysis was performed to determine the sensitivity (SEN), specificity (SPE) and AUC of each biomarker, best subset of the biomarkers and all markers combined. For Exo-PROS assay, the best subset of the biomarkers was TEX EGFR-miR-21 pair and LG3BP+ exosomal miR-210, which achieved diagnostic sensitivity of 1, specificity of 1 and AUC of 1. For EGFR and IMS-PCR workflow, the best subset of biomarkers was TEX LG3BP-miR-210 pair and EGFR+ exosomal miR-21, which provided diagnostic sensitivity of 0.83, specificity of 0.85 and AUC of 0.87. For each biomarker or combined biomarkers, Exo-PROS assay showed better diagnostic performance in distinguishing NSCLC patients from normal controls than ELISA and IMS-PCR workflow.





d

Biomarker	EGFR alone			miR-21 alone			LG3BP alone			miR-210 alone			All markers combined		
	SEN	SPE	AUC	SEN	SPE	AUC	SEN	SPE	AUC	SEN	SPE	AUC	SEN	SPE	AUC
Female normal controls vs Breast cancer	0.70	0.88	0.80	0.90	0.81	0.89	1.00	0.94	0.96	1.00	0.88	0.98	1.00	1.00	1.00

Figure S9. Exo-PROS assay detected TEX EGFR-miR-21 and LG3BP-miR-210 pairs in sera from both lung cancer patients and breast cancer patients. Exosomes were isolated from 50 μL sera from normal controls (n=35 including 15 normal controls at low risk of lung cancer and 20 smokers at high risk of lung cancer), lung cancer patients (n=60, stage I-IV) and breast cancer patients (n=10, stage 0-IV). The levels of TEX EGFR-miR-21 and LG3BP-miR-

210 pairs were measured by Exo-PROS assay (a, b). The Exo-PROS assay detected significantly higher levels of TEX EGFR-miR-21 pair and LG3BP-miR-210 pair in sera from both lung cancer patients and breast cancer patients than normal controls ($p<0.05$). (c, d) ROC analysis was performed to determine the sensitivity (SEN), specificity (SPE) and AUC of each biomarker and all four markers combined in distinguish breast cancer patients (n=10) from female normal controls (n=16). Exo-PROS assay showed high diagnostic accuracy in distinguishing breast cancer patients from female normal controls.