

Supporting Information

An immuno-biochip selectively captures tumor-derived exosomes and detects exosomal RNA for cancer diagnosis

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Table S1 Characteristics of human subjects

Patient ID	NSCLC subtype	Stage	Sex	Age (y)	Exosome diameter (nm)	Exosome number concentration (10 ¹² exosomes/mL)
1	Normal	NA	M	70-74	85	1.44
2	Normal	NA	M	65-69	115	4.18
3	Normal	NA	F	70-74	101	4.23
4	Normal	NA	M	75-79	109	2.56
5	Normal	NA	M	70-74	76	1.09
6	Normal	NA	F	75-79	80	5.04
7	Normal	NA	F	55-59	96	5.33
8	Normal	NA	F	75-79	138	4.07
9	Normal	NA	F	50-54	64	5.62
10	Normal	NA	F	75-79	123	3.2
11	Adenocarcinoma	2A	F	60-64	94	7.56
12	Adenocarcinoma	2B	F	75-79	71	6.81
13	Adenocarcinoma	2B	F	70-74	58	3.24
14	Adenocarcinoma	1A	M	70-74	72	1.48
15	Adenocarcinoma	1A	M	70-74	86	8.51
16	Adenocarcinoma	1A	F	70-74	73	9.09
17	Adenocarcinoma	1A	F	70-74	71	4.45
18	Adenocarcinoma	1A	F	50-54	84	2.7
19	Adenocarcinoma	1B	F	55-59	73	7.97
20	Squamous cell carcinoma	2A	M	60-64	109	8.58
21	Adenocarcinoma	2A	F	55-59	86	5.18
22	Squamous cell carcinoma	2B	M	65-69	70	3.3
23	Squamous cell carcinoma	2B	M	75-79	113	5.8
24	Adenocarcinoma	3A	F	50-54	92	5.25
25	Adenocarcinoma	4	F	75-79	102	3.28
26	Adenocarcinoma	4	M	55-59	119	3.08
27	Adenocarcinoma	3A	M	70-74	68	2.5
28	Adenocarcinoma	3B	F	75-79	118	7.87
29	Adenocarcinoma	3A	F	55-59	114	3.91
30	Adenocarcinoma	4	M	70-74	103	5.86

Note: Subjects 1-10 are normal controls. NA: not applicable; M: male; F, female.

Figure S1. Image analysis. (a) A signal cut-off level was selected in Image J to collect signals from bright spots (yellow arrows). Pixels with higher intensity than the cut-off level, as shown in (b), were considered as signals from tumor-derived exosomal miR-21 or TTF-1 mRNA. The sum intensity of these pixels, I_{signal} , was calculated by MATLAB for each image. (c) ImageJ was also used to determine the highest pixel intensity in the dark areas (red arrow) as the background level. The mean intensity of all pixels with lower intensity than the background level, $I_{\text{background}}$, was calculated as a normalization factor to overcome chip-to-chip variation. The expression of exosomal RNA was calculated as $I_{\text{signal}}/I_{\text{background}}$. For each biomarker, at least 100 images were collected and analyzed.

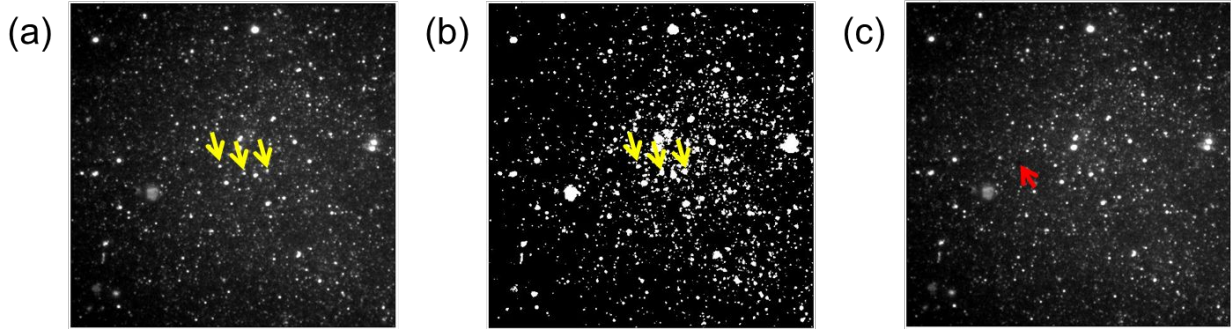


Figure S2. Negative controls showed little fluorescence on immuno-biochip. (a) When the biochip surface was modified with IgG control antibodies, little fluorescence from MB-miR-21 and MB-TTF-1 was observed when A549 exosomes were applied at 10^{10} exosomes/mL. (b) The immuno-biochip was modified with anti-EGFR antibodies. When CLP containing MB-miR-21-scramble and MB-TTF-1-scramble were used to detect EGFR+ exosomal miR-21 and TTF-1 mRNA from A549 cells, almost no fluorescence signals were observed. A549 exosomes were applied at 10^{10} exosomes/mL.

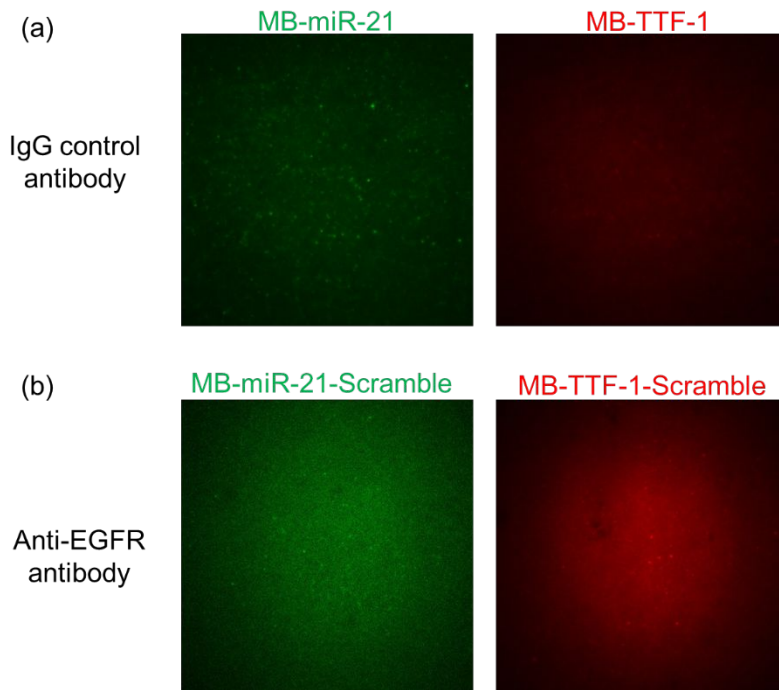


Figure S3. Quantification of EGFR+ or PD-L1+ exosomal miR-155 and miR-210 expression in cell culture-conditioned media by immuno-biochip. (a) A representative set of TIRF microscopy images of EGFR+ or PD-L1+ exosomal miR-155 and miR-210 from A549 and BEAS-2B cells. (b) Higher level of miR-155 was observed in EGFR+ exosomes from A549 cancer cells than those from BEAS-2B normal cells, however, similar expression of miR-210 was detected in EGFR+ exosomes from both A549 cells and BEAS-2B cells. (c) Higher levels of miR-155 and miR-210 were observed in PD-L1+ exosomes from A549 cancer cells than those from BEAS-2B normal cells.

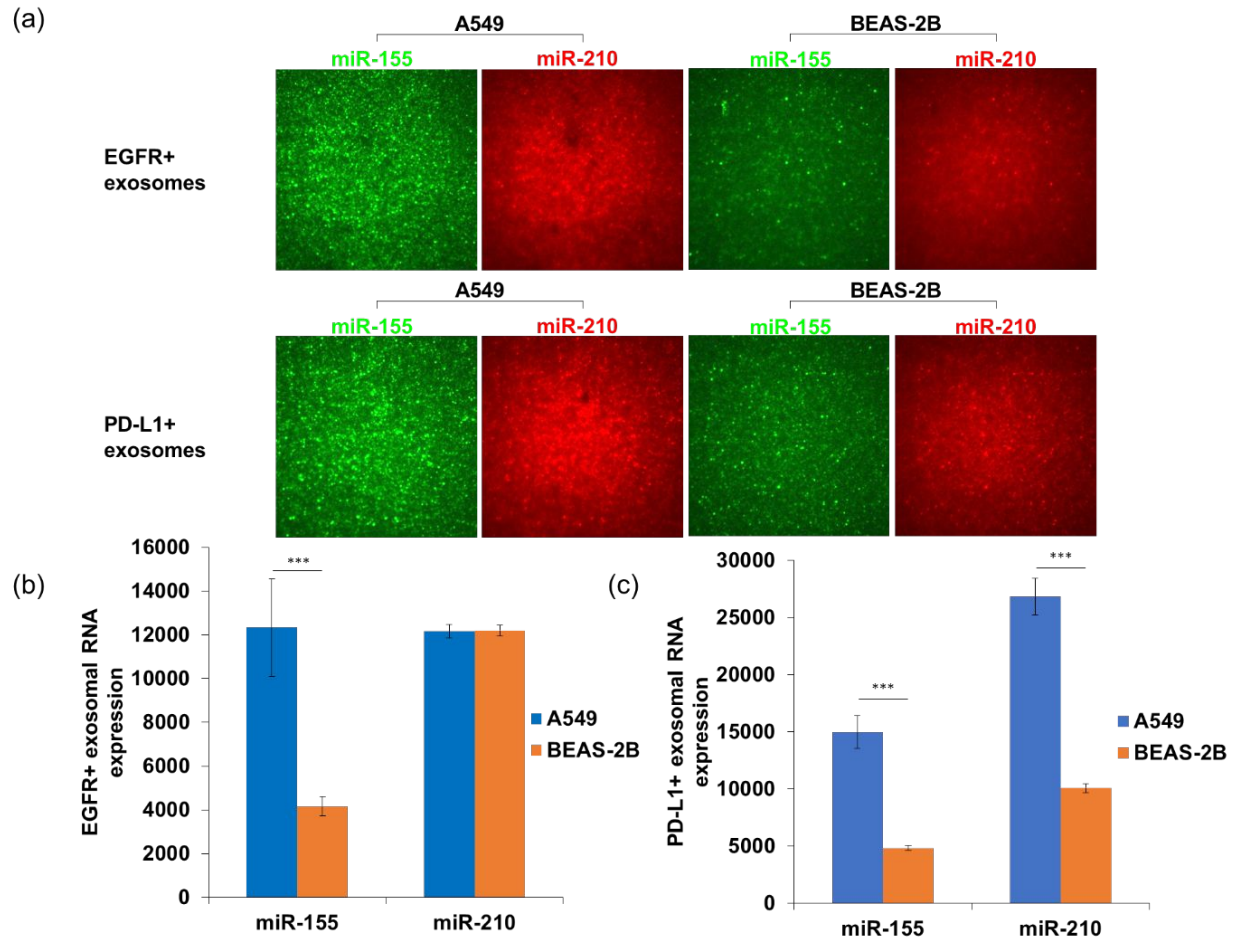


Figure S4. Representative TIRF microscopy images of EGFR+ exosomal miR-21 from A549 cells at exosome concentrations of 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} exosomes/mL.

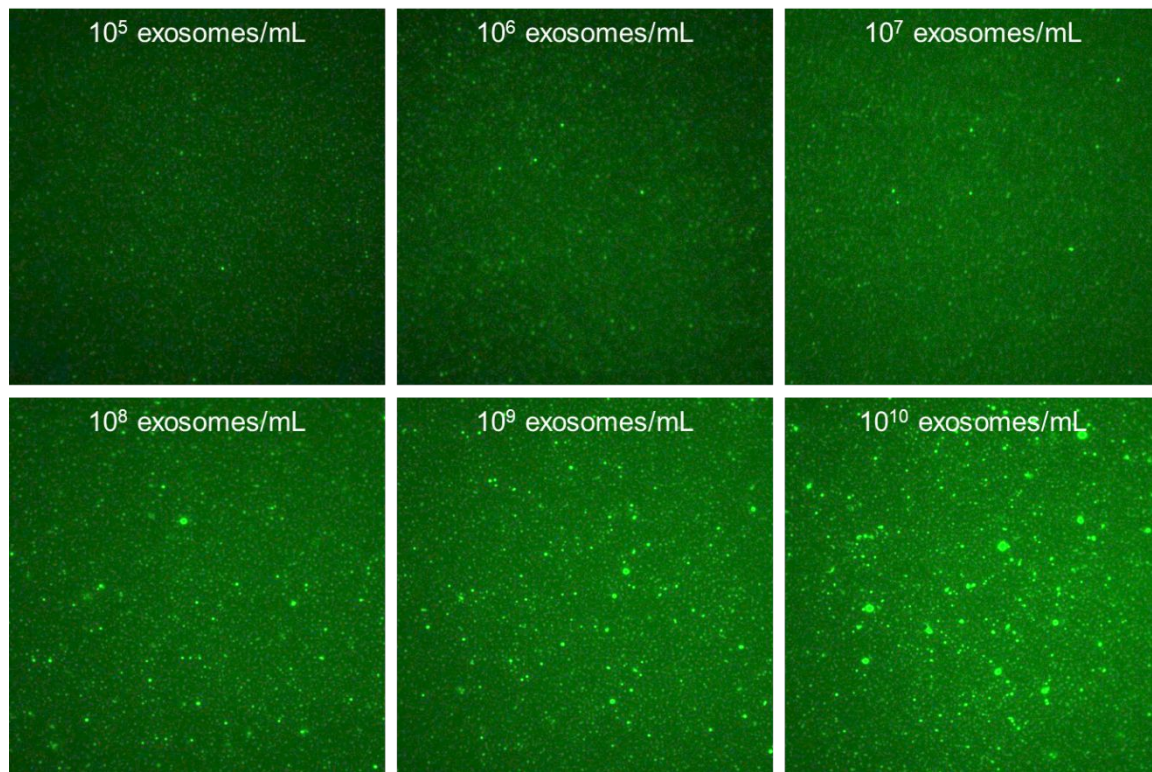
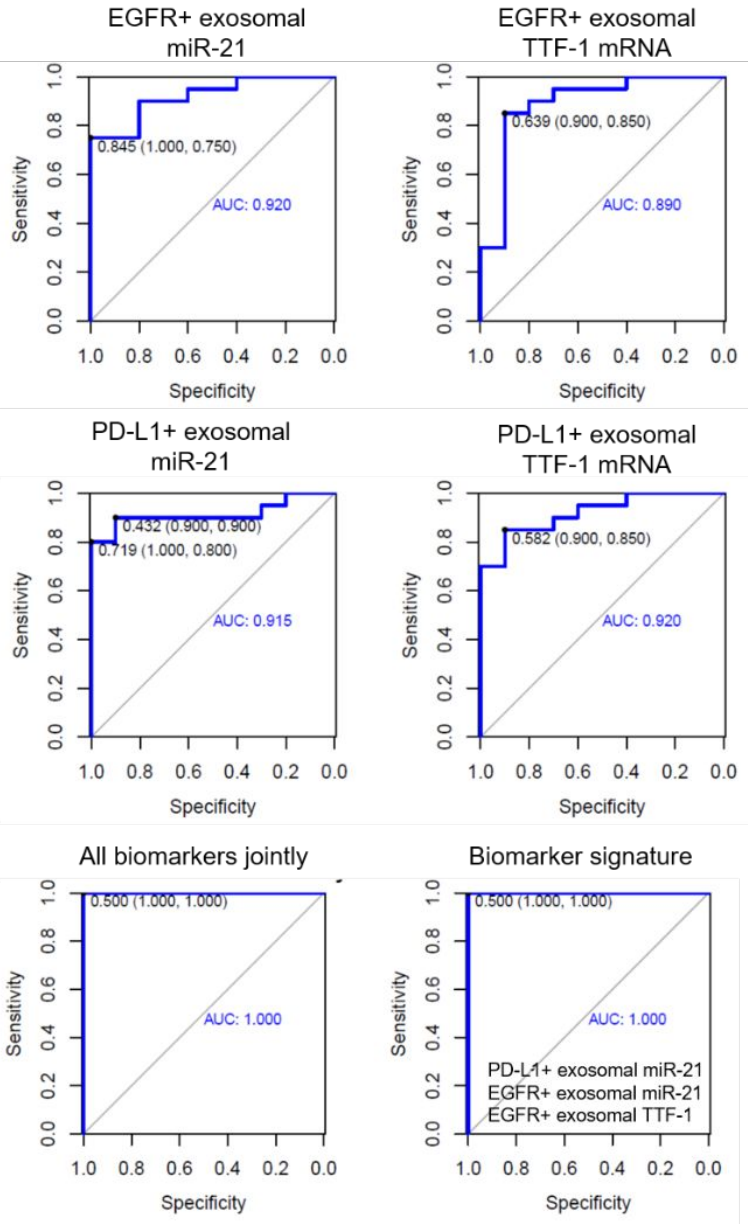
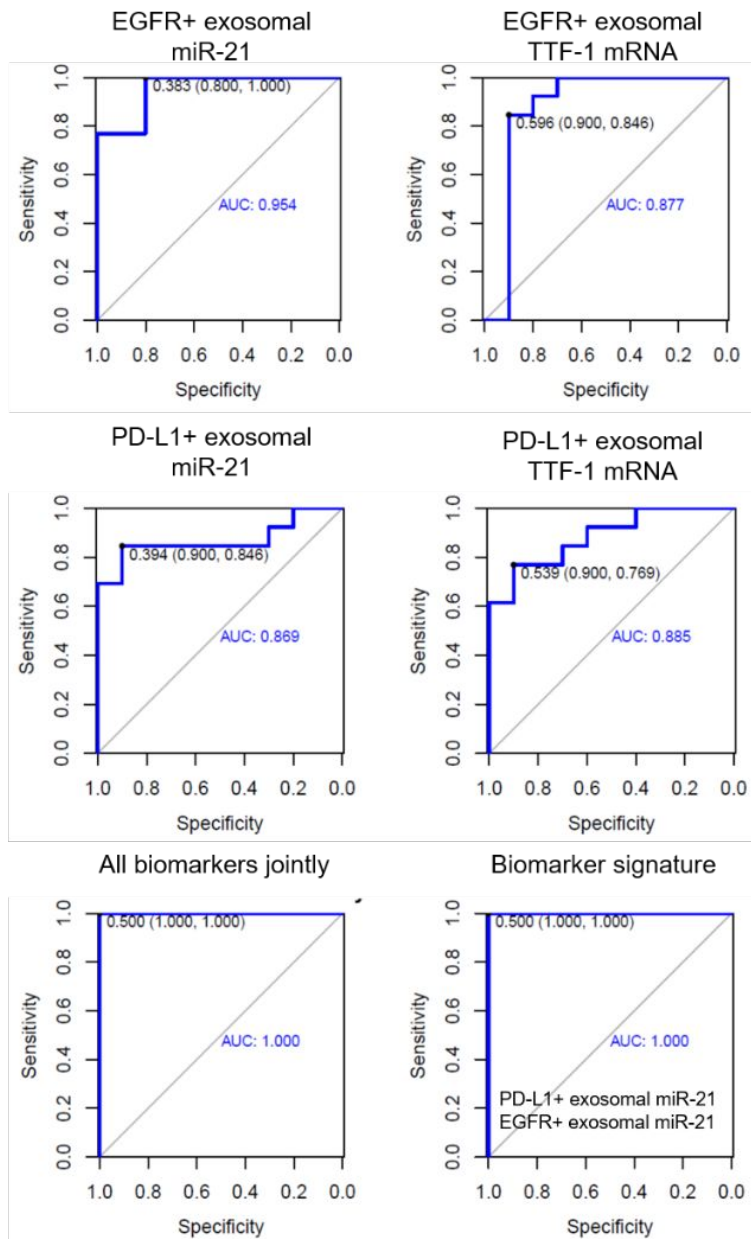


Figure S5. Diagnostic value of serum EGFR+ or PD-L1+ exosomal miR-21 and TTF-1 mRNA measured by immuno-biochip. Shown are receiver operating characteristic (ROC) curves with areas under curve (AUC) for EGFR+ exosomal miR-21, EGFR+ exosomal TTF-1 mRNA, PD-L1+ exosomal miR-21, PD-L1+ exosomal TTF-1 mRNA to distinguish (a) normal controls from all NSCLC patients; (b) normal controls from early stage NSCLC patients; (c) normal controls from late stage NSCLC patients, and (d) early stage NSCLC patients from late stage NSCLC patients. Curves are shown for the four biomarkers individually and jointly, and for a combination of 1~4 of the markers (biomarker signature; Table 1) with best diagnostic value.

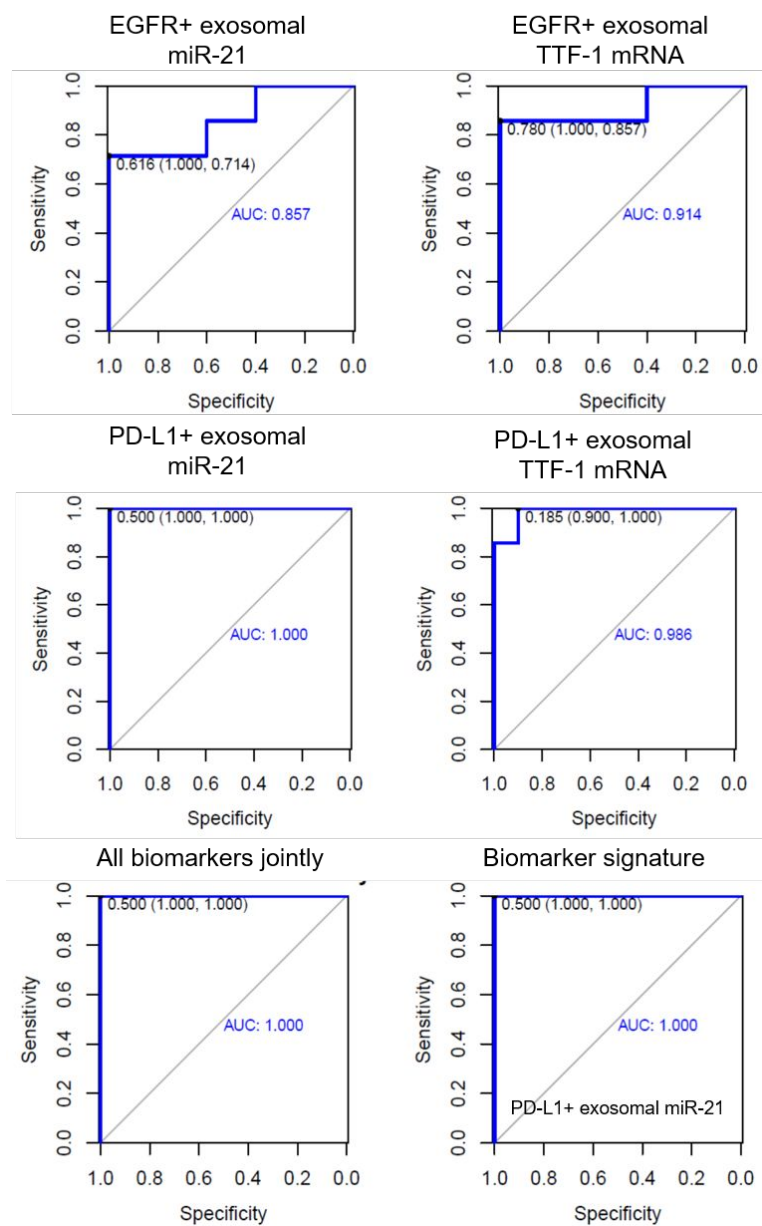
(a) Normal vs NSCLC



(b) Normal vs Early Stage NSCLC



(c) Normal vs Late Stage NSCLC



(d) Early Stage NSCLC vs Late Stage NSCLC

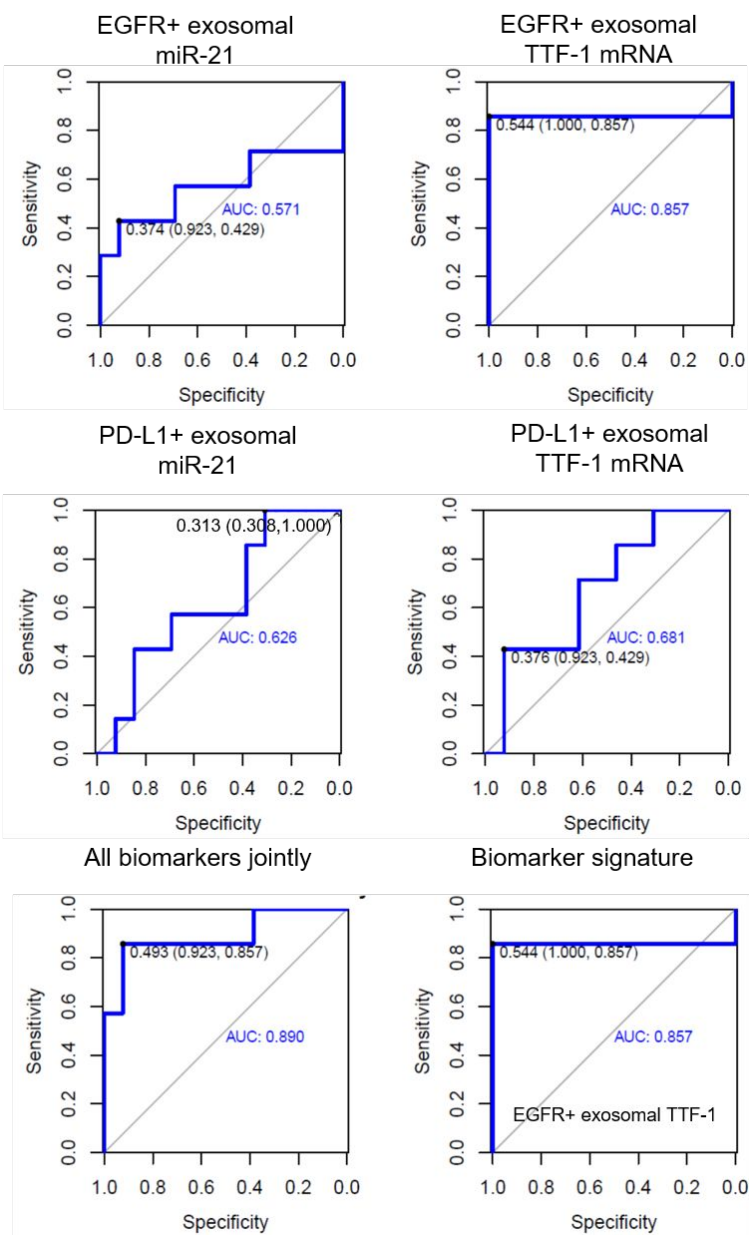
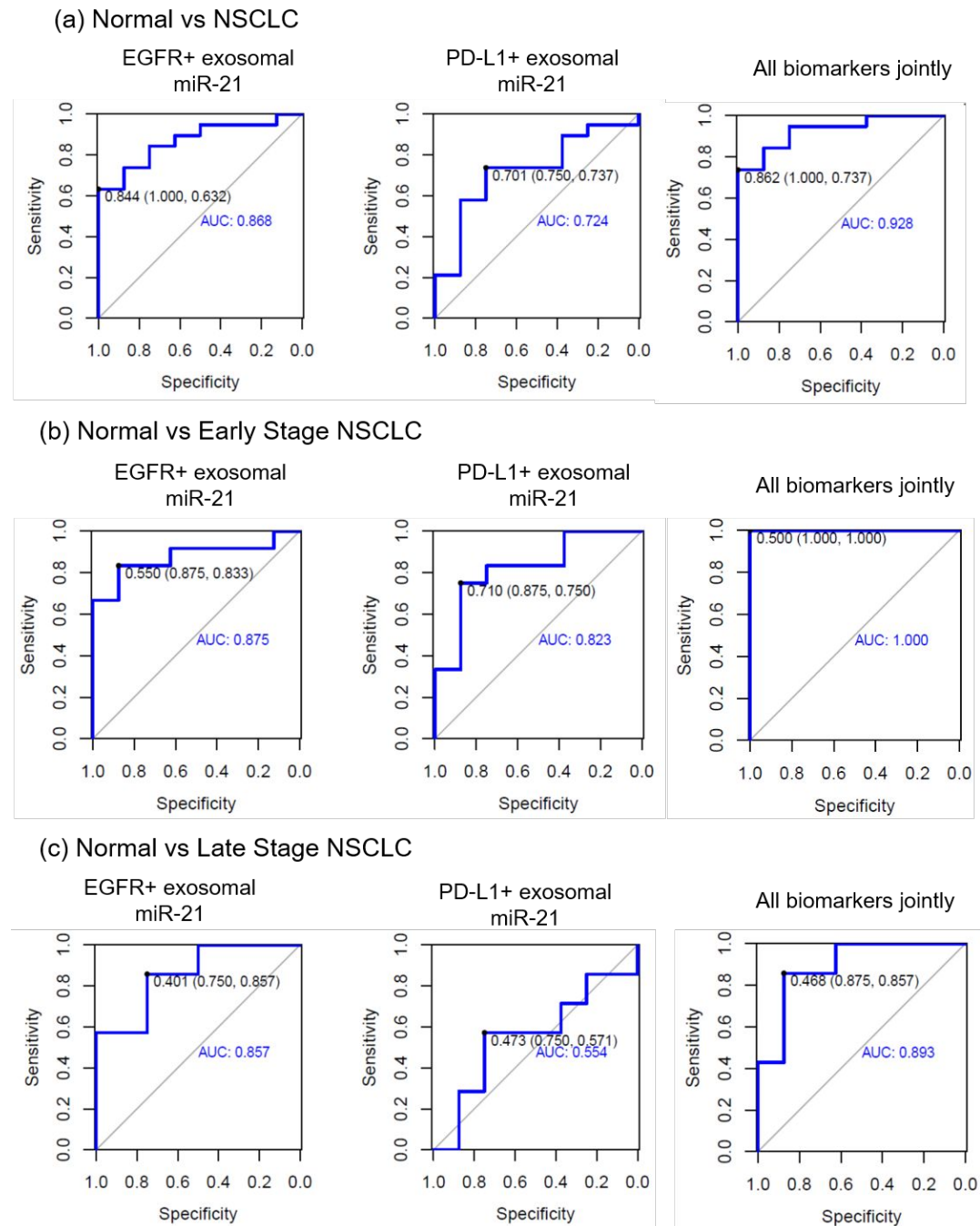


Figure S6. Diagnostic value of serum EGFR+ or PD-L1+ exosomal miR-21 measured by IMS-PCR workflow. Shown are receiver operating characteristic (ROC) curves with areas under curve (AUC) for EGFR+ exosomal miR-21 and PD-L1+ exosomal miR-21 to distinguish (a) normal controls from all NSCLC patients; (b) normal controls from early stage NSCLC patients; (c) normal controls from late stage NSCLC patients and (d) early stage NSCLC patients from late stage NSCLC patients. Curves are shown for the two biomarkers individually and jointly.



(d) Early Stage NSCLC vs Late Stage NSCLC

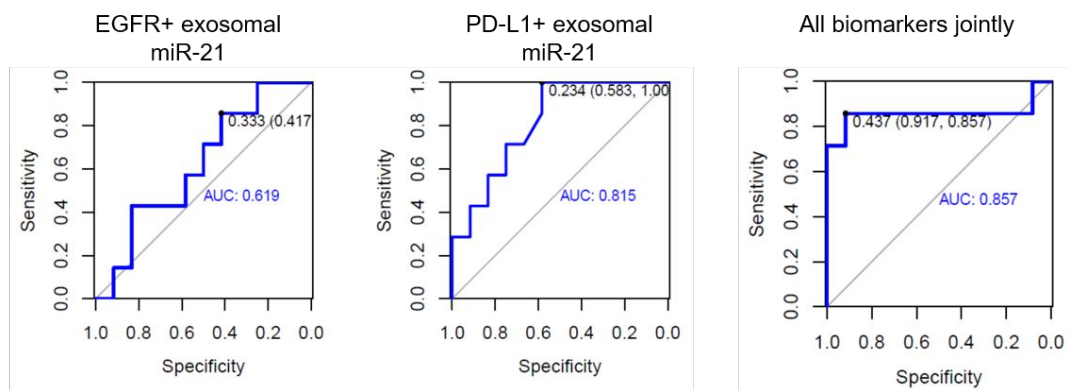


Figure S7. Diagnostic value of whole serum miR-21 measurements by PCR. Shown are receiver operating characteristic (ROC) curves with areas under curve (AUC) for whole serum miR-21 to distinguish (a) normal controls from all NSCLC patients; (b) normal controls from early stage NSCLC patients; (c) normal controls from late stage NSCLC patients and (d) early stage NSCLC patients from late stage NSCLC patients.

