

Figure S1 TEM image of BALF-EVs. (A) Image of negatively stained EVs. (B) Image of sectioned EVs. TEM, transmission electron microscopy; BALF, bronchoalveolar lavage fluid; EV, extracellular vesicle.

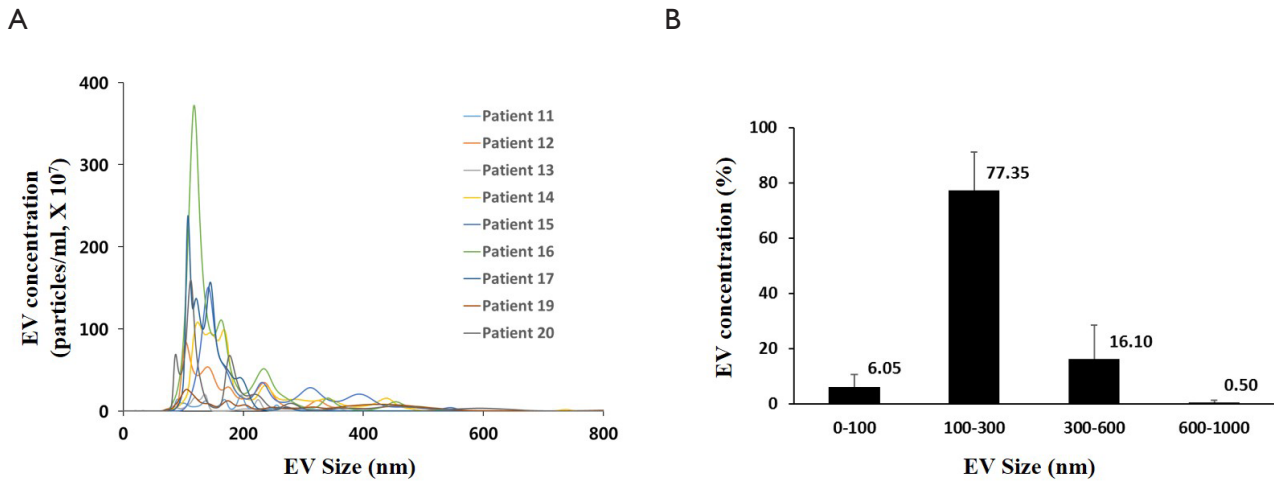


Figure S2 Size distribution of BALF-EV. (A) Sizes of purified EVs were determined using Nanosight NS300 particle-tracking analysis (n=9). Average size distribution from three separate measurements is plotted in concentration (particles/mL) according to their size. (B) Size distributions of the total EV populations (n=9). BALF, bronchoalveolar lavage fluid; EV, extracellular vesicle.

Table S1 Characteristics of BALF-EV

Patient	BALF-EV mean size (nm)	BALF-EV concentration (particles/mL, $\times 10^{11}$)
11	176.0	0.147
12	188.1	0.694
13	292.8	0.113
14	205.2	1.060
15	229.7	1.054
16	166.4	1.810
17	142.6	1.032
19	266.8	0.328
20	195.6	0.780

BALF, bronchoalveolar lavage fluid; EV, extracellular vesicle.

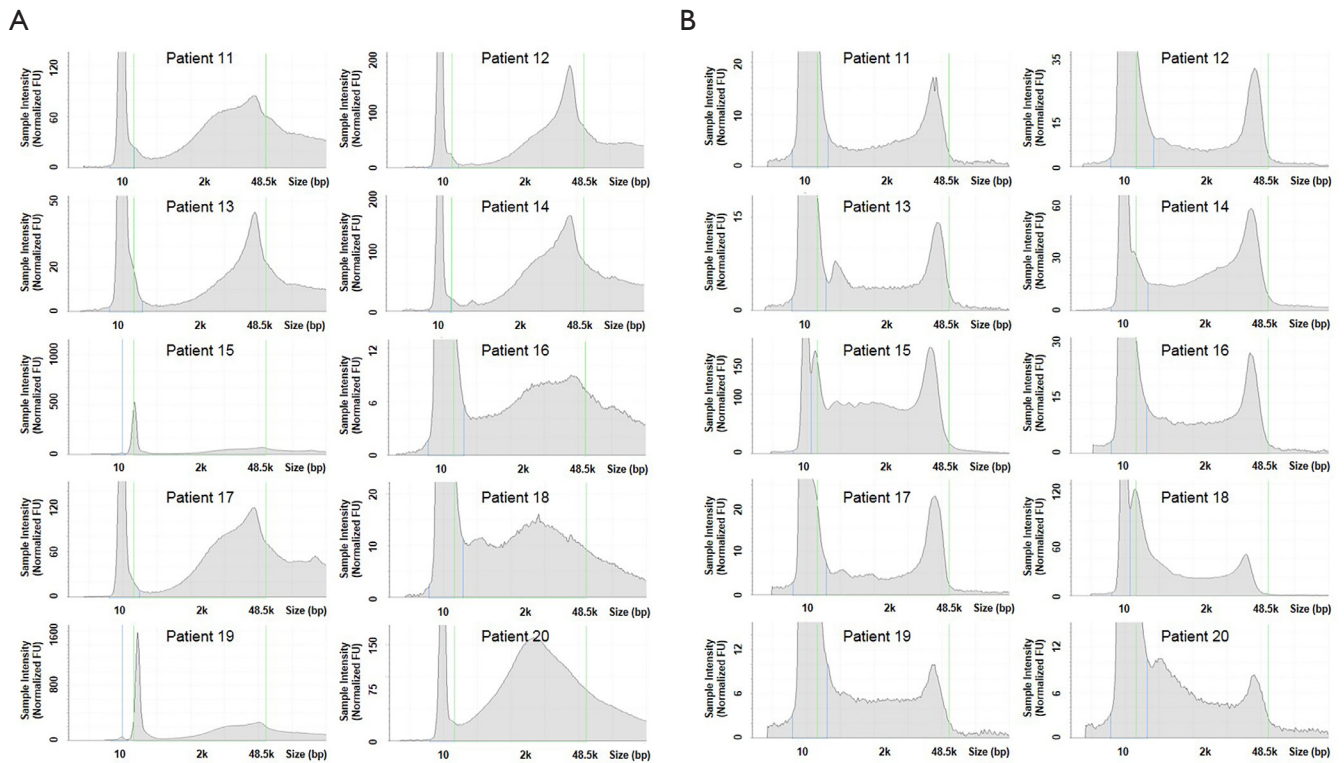


Figure S3 Electropherograms of tissue and BALF EV DNAs. (A) Electropherograms show the size and amount of tissue DNA determined using the TapeStation (n=10). (B) Electropherograms show the size and amount of BALF EV DNA determined using the TapeStation (n=10). The x-axis represents DNA size in base pairs (bp), and the y-axis represents sample intensity (fluorescence units, FU). BALF, bronchoalveolar lavage fluid; EV, extracellular vesicle.

Table S2 DNA characteristics of tissue and BALF-EV

Patient	Tissue DNA Conc. (ng/ μ L)	Tissue DNA total amount (ng)	Tissue DNA size (main/median size, bp)	EV DNA Conc. (ng/ μ L)	EV DNA total amount (ng)	EV DNA size (main/median size, bp)
11	26	1170	13,919/7,858	0.465	20.9	13,291/7,164
12	29	1305	12,065/9,628	0.987	44.4	12,496/7,932
13	5.4	205.2	16,470/10,729	0.491	22.1	14,199/7,484
14	34	1,292	12,893/8,427	3.68	165.6	9,576/6,195
15	20	760	17,632/8,829	16	720	8,099/4,236
16	2.03	77	12,203/6,595	1.22	54.9	8,706/5,568
17	28	1,064	14,882/8,623	0.724	32.6	11,955/7,597
18	1.52	58	2,746/5,034	5.3	238.5	6,609/2,368
19	12	456	10,994/7,592	0.53	23.9	11,234/4,954
20	14	532	2,464/5,274	0.37	16.7	12,133/4,504

BALF, bronchoalveolar lavage fluid; EV, extracellular vesicle; bp, base pairs.

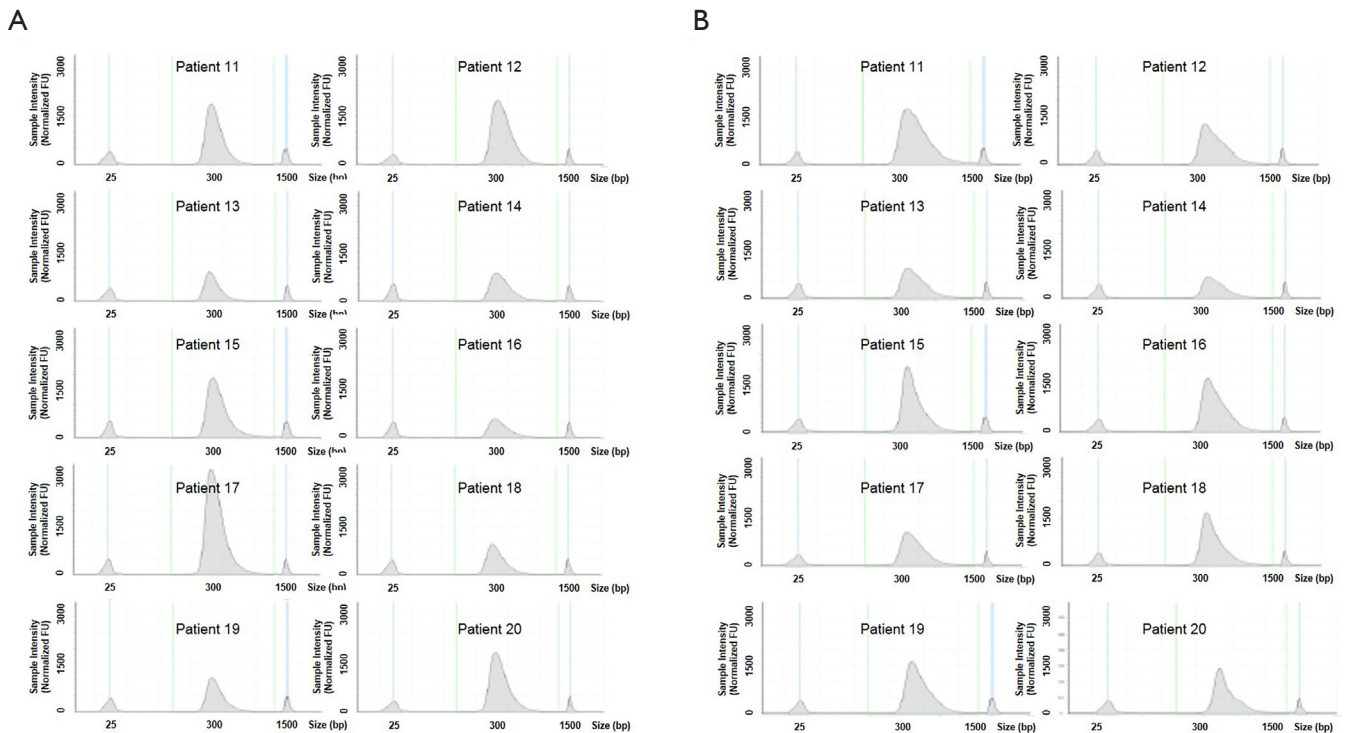


Figure S4 Quality of NGS library. (A) Electropherograms show the library of tissue DNA determined using the TapeStation (n=10). (B) Electropherograms show the library of BALF EV DNA determined using the TapeStation (n=10). The x-axis represents DNA size in base pairs (bp), and the y-axis represents sample intensity (fluorescence units, FU). NGS, next-generation sequencing; BALF, bronchoalveolar lavage fluid; EV, extracellular vesicle.

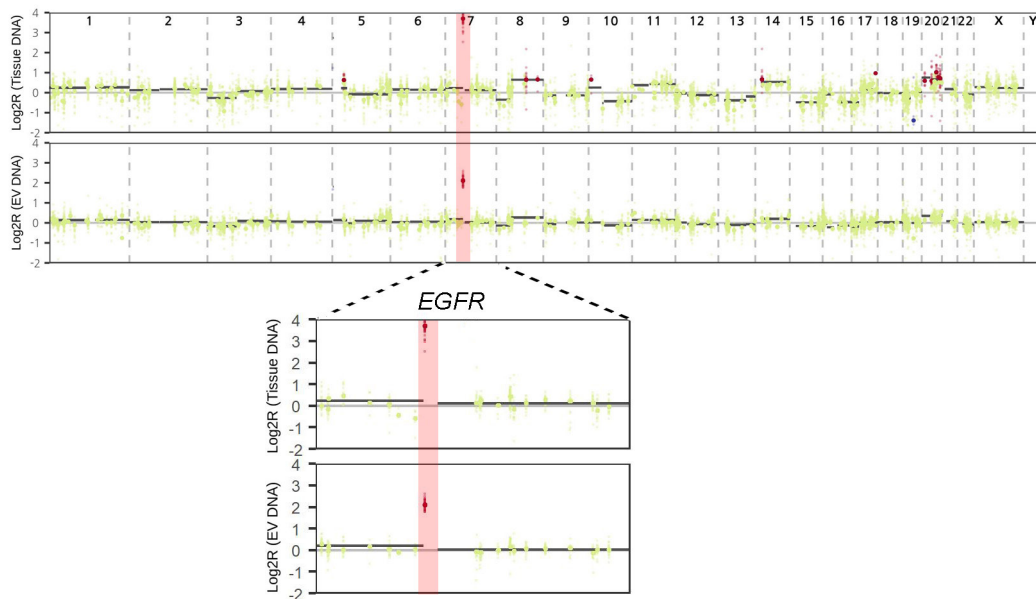


Figure S5 Representative case of EGFR amplification. In case 20, EGFR amplifications were detected both in tissue and BALF EV DNAs. The log₂ ratio of amplification was higher in tissue DNA than in the BALF EV DNA. BALF, bronchoalveolar lavage fluid; EV, extracellular vesicle.