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## **Supplemental information**

**Matrix-assisted laser desorption ionization**

**mass spectrometry profiling of plasma**

**exosomes evaluates osteosarcoma metastasis**

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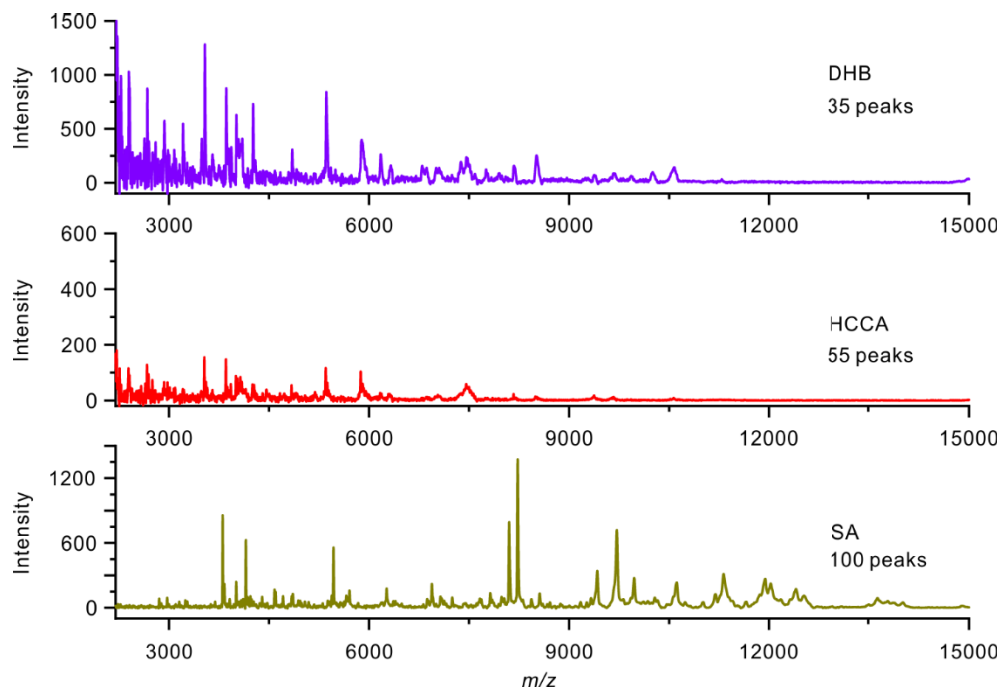


Figure S1. MALDI-TOF mass spectra of exosomes isolated from 143B cells acquired using DHB, HCCA, and SA matrices, respectively, related to Figure 1.

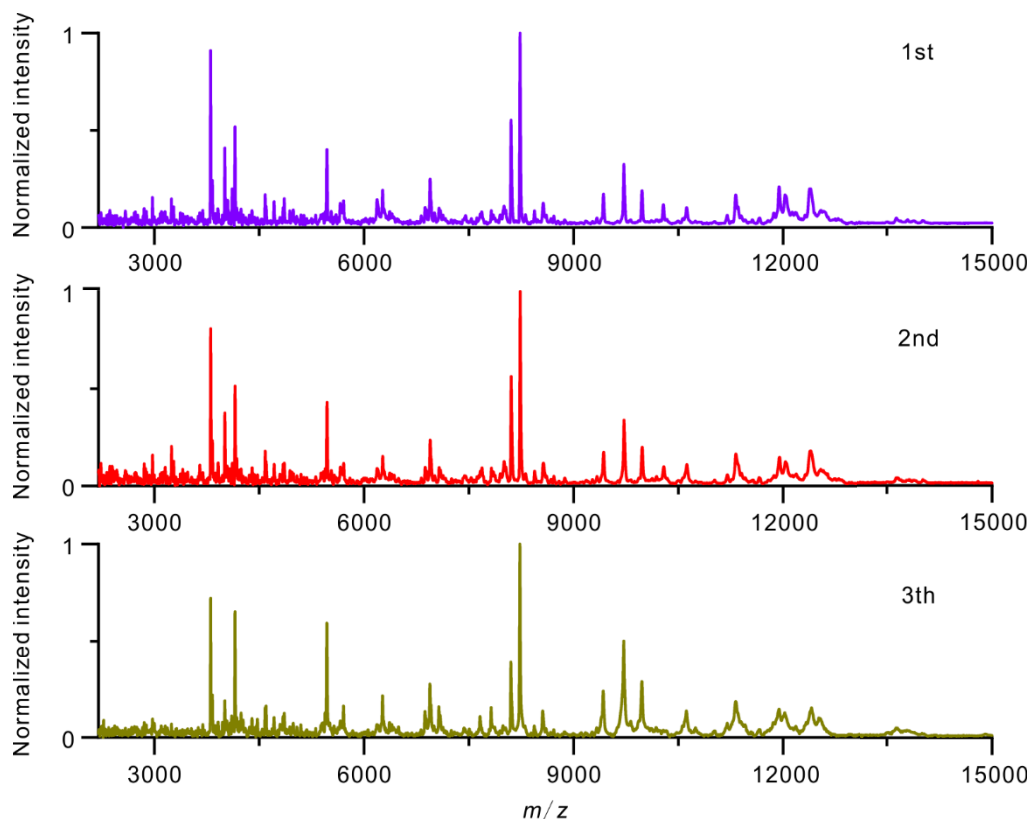
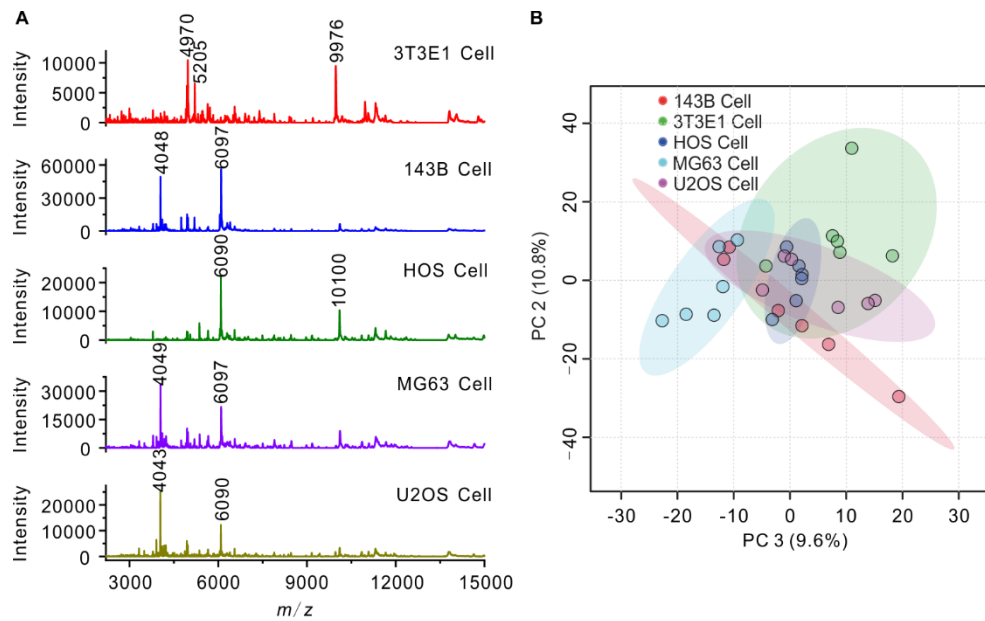
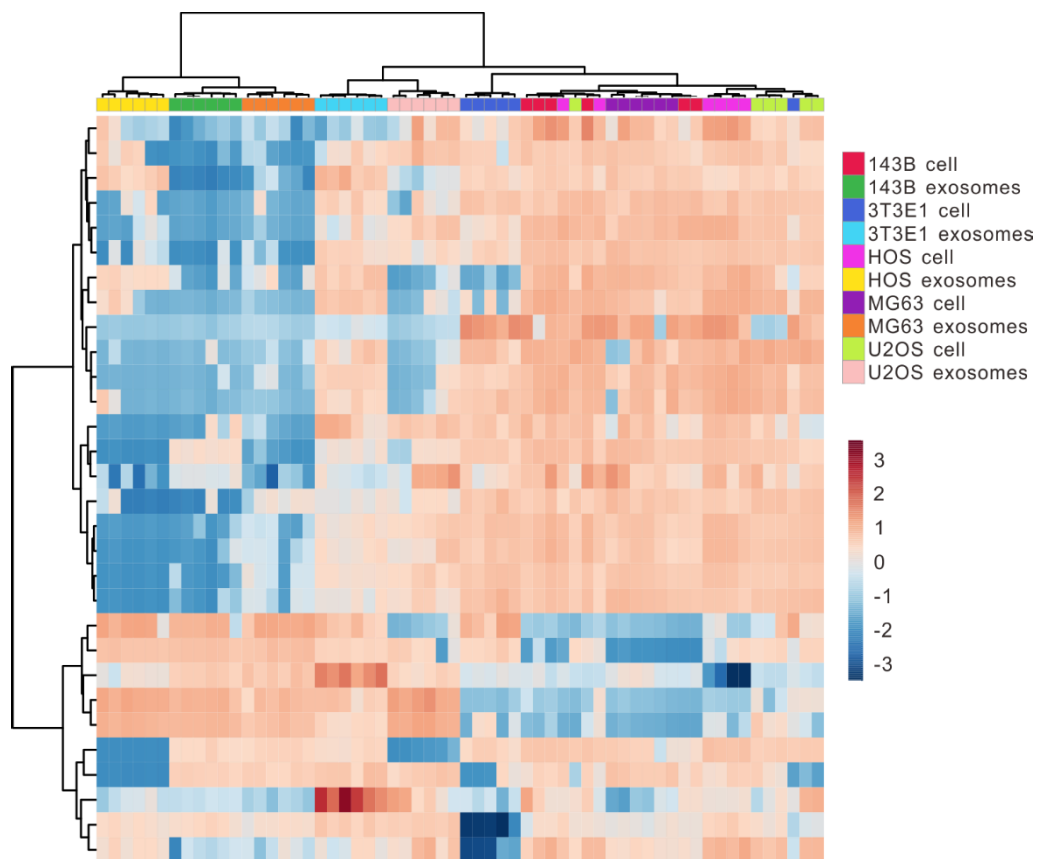


Figure S2. MALDI-TOF mass spectra of exosomes derived from 3 different batches of 143B cells, related to Figure 1.



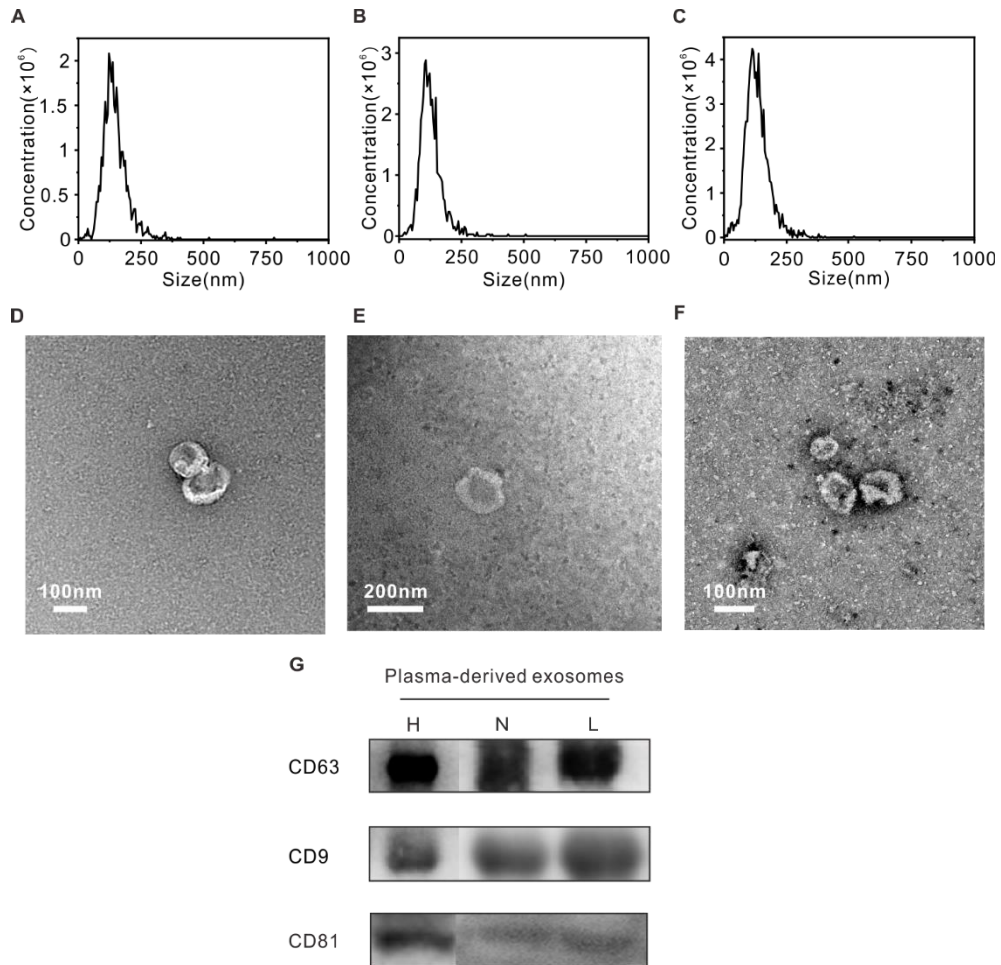
**Figure S3. MALDI-TOF mass spectra of different cell lines, and PCA analysis of the MALDI mass spectra, related to Figure 2.**

(A) MALDI-TOF mass spectra of 3T3E1, 143B, HOS, MG63 and U2OS cells. Main peaks of cells were labeled in the figure. (B) PCA score plot based on the MALDI-TOF mass spectra of the cells. Six replicates were performed on each cell line.



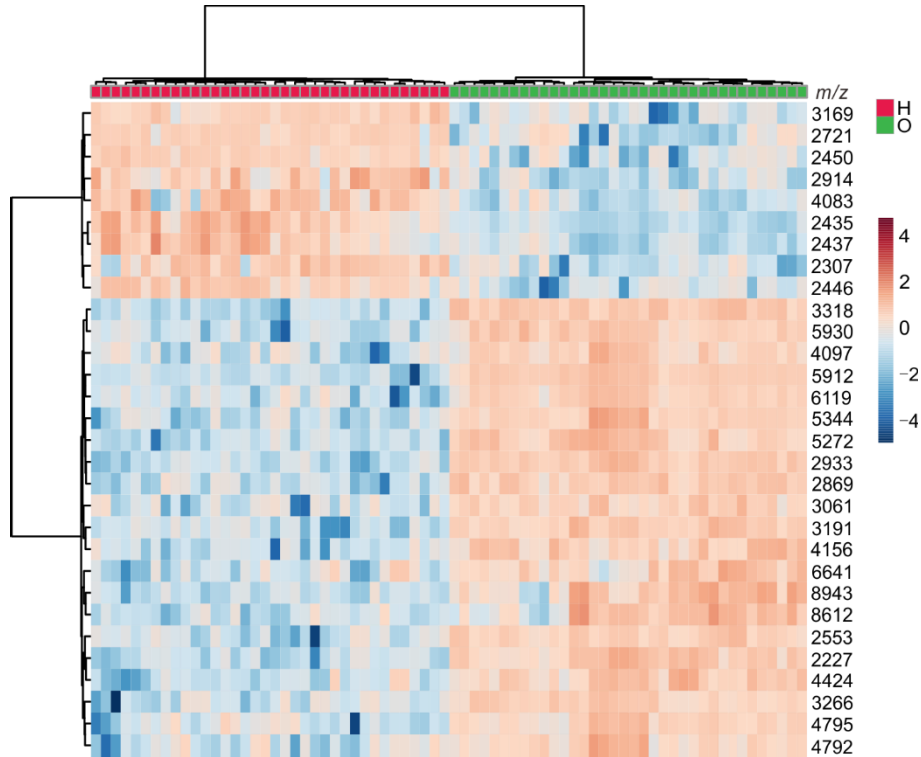
**Figure S4. Hierarchical clustering of the MALDI mass spectra of different cell lines and the corresponding exosomes, related to Figure 2.**

Hierarchical clustering of the MALDI-TOF mass spectra of 3T3E1, 143B, HOS, MG63 and U2OS cells and the corresponding exosomes in a heatmap using Euclidean distance measure and Ward linkage. Six replicates were performed on each sample.



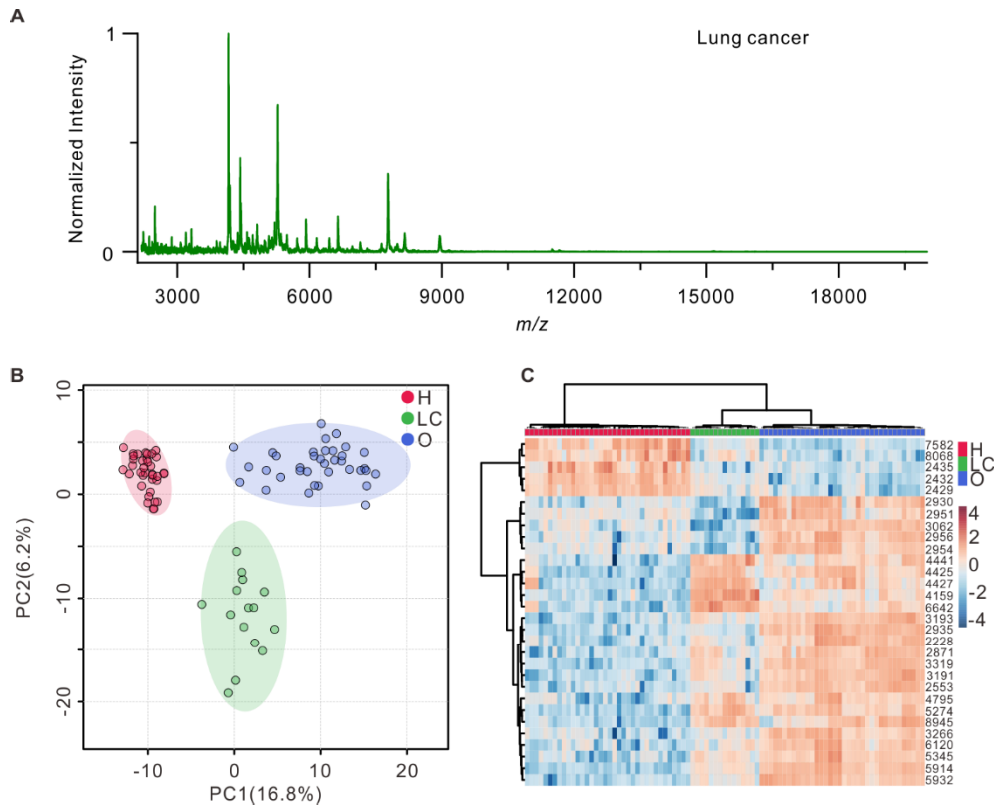
**Figure S5. NTA, TEM, and Western blot analyses of plasma-derived exosomes, related to Figure 3 and 4.**

NTA measurements (200-times dilution) and TEM micrographs of plasma-derived exosomes isolated from (A and D) healthy volunteers (H), (B and E) osteosarcoma non-lung metastasis patients (N), and (C and F) osteosarcoma lung metastasis patients (L), and (G) Western blot analysis on the samples. (D) and (F) Scale bar: 100 nm. (E) Scale bar: 200 nm.



**Figure S6. Hierarchical clustering of the MALDI mass spectra of plasma-derived exosomes, related to Figure 3.**

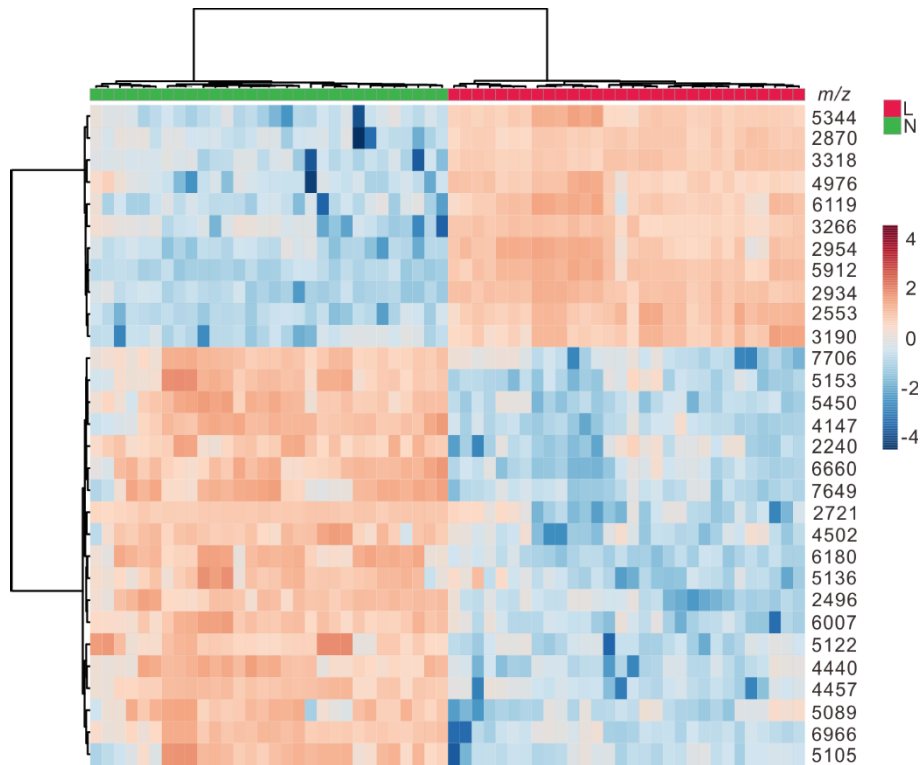
Hierarchical clustering of the MALDI-TOF data of plasma-derived exosomes isolated from 12 healthy volunteers (H) and 12 osteosarcoma patients (O) shown as heatmap using Euclidean distance measure and Ward linkage. Three replicates were performed on each sample.



**Figure S7. MALDI-TOF mass spectra of plasma-derived exosomes for the discrimination of different cancers, related to Figure 3.**

(A) MALDI-TOF mass spectrum of plasma-derived exosomes isolated from a lung cancer patient. (B) PCA score plot based on the MALDI-TOF profile of plasma-derived exosomes. (C) Hierarchical clustering of the MALDI-TOF profile of plasma-derived exosomes shown as heatmap using Euclidean distance measure and Ward linkage. The cohorts include 12 healthy controls (H), 12 osteosarcoma patients (O), and 5 lung cancer patients (LC). Three replicates were performed on each sample.





**Figure S8. Hierarchical clustering of the MALDI mass spectra of plasma-derived exosomes, related to Figure 4.**

Hierarchical clustering of the MALDI-TOF data of plasma-derived exosomes isolated from 10 osteosarcoma non-lung metastasis (N) and 10 lung metastasis (L) patients shown as heatmap using Euclidean distance measure and Ward linkage. Three replicates were performed on each sample.

Table S1 Common platforms for exosome characterization, related to Figure 1, Figure 4,  
and Figure S5

Number	category	Methods
1	size characterization	dynamic light scattering (DLS)
		nanoparticle tracking analysis (NTA)
		tunable resistive pulse sensing (TRPS)
2	subgroup characterization	Western blot
		enzyme-linked immunosorbent assay (ELISA)
		nano-flow cytometry
3	proteomic characterization	mass spectrometry
4	genomic and transcriptomic characterization	high throughput sequencing

Table S2 Information of Clinical samples, related to Figure 3-5, and S6-S8

Sample No.	Age	Gender	Category	Stage
1	15	Male	Osteosarcoma	Lung metastasis
2	13	Female	Osteosarcoma	Lung metastasis
3	25	Male	Osteosarcoma	Lung metastasis
4	19	Female	Osteosarcoma	Lung metastasis
5	34	Male	Osteosarcoma	Lung metastasis
6	13	Male	Osteosarcoma	Lung metastasis
7	17	Female	Osteosarcoma	Lung metastasis
8	58	Male	Osteosarcoma	Lung metastasis
9	16	Male	Osteosarcoma	Lung metastasis
10	38	Male	Osteosarcoma	Lung metastasis
11	35	Female	Osteosarcoma	Lung metastasis
12	20	Male	Osteosarcoma	Lung metastasis
13	25	Male	Osteosarcoma	Lung metastasis
14	19	Male	Osteosarcoma	Lung metastasis
15	16	Male	Osteosarcoma	Lung metastasis
16	15	Female	Osteosarcoma	Lung metastasis
17	38	Male	Osteosarcoma	Lung metastasis
18	18	Male	Osteosarcoma	Lung metastasis
19	46	Female	Osteosarcoma	Lung metastasis
20	62	Male	Osteosarcoma	Lung metastasis
21	69	Female	Osteosarcoma	Non-lung metastasis
22	19	Male	Osteosarcoma	Non-lung metastasis
23	16	Female	Osteosarcoma	Non-lung metastasis
24	24	Female	Osteosarcoma	Non-lung metastasis
25	17	Male	Osteosarcoma	Non-lung metastasis
26	12	Male	Osteosarcoma	Non-lung metastasis
27	16	Male	Osteosarcoma	Non-lung metastasis
28	11	Female	Osteosarcoma	Non-lung metastasis
29	6	Male	Osteosarcoma	Non-lung metastasis
30	33	Female	Osteosarcoma	Non-lung metastasis
31	25	Female	Osteosarcoma	Non-lung metastasis
32	38	Male	Osteosarcoma	Non-lung metastasis
33	14	Male	Osteosarcoma	Non-lung metastasis
34	20	Male	Osteosarcoma	Non-lung metastasis
35	19	Female	Osteosarcoma	Non-lung metastasis
36	30	Male	Osteosarcoma	Non-lung metastasis
37	14	Male	Osteosarcoma	Non-lung metastasis
38	17	Male	Osteosarcoma	Non-lung metastasis
39	14	Male	Osteosarcoma	Non-lung metastasis
40	5	Male	Osteosarcoma	Non-lung metastasis

41	67	Female	Lung cancer	NA
42	58	Female	Lung cancer	NA
43	55	Female	Lung cancer	NA
44	51	Female	Lung cancer	NA
45	60	Male	Lung cancer	NA
46	14	Male	Healthy	NA
47	10	Male	Healthy	NA
48	32	Female	Healthy	NA
49	56	Male	Healthy	NA
50	45	Female	Healthy	NA
51	19	Male	Healthy	NA
52	17	Male	Healthy	NA
53	26	Male	Healthy	NA
54	30	Female	Healthy	NA
55	39	Female	Healthy	NA
56	25	Male	Healthy	NA
57	50	Male	Healthy	NA