Supplementary Figure Legends

Supplementary Figure 1. Fibulin-3 expression in lung tumors and epigenetic silencing of *fibulin-3* in lung cancer cell lines. (A) Additional pictures of fibulin-3 immunostaining in lung tumors on the tissue microarray (200×). Fibulin-3 was detected in the cytoplasm of the positive tumors. Scale bar, 50 μm. **(B)** *Fibulin-3* expression was determined by real-time RT-PCR in 6 lung cancer cell lines, 2 prostate cancer cell lines, and 2 breast cancer cell lines with or without 5-aza-dC treatment. The results are the average of three independent experiments normalized to *GAPDH* expression.

Supplementary Figure 2. Fibulin-3 expression suppressed long-term survival of lung cancer cells.

(A) A549, **(B)** H1299, and **(C)** H460 cells were transfected with V5-tagged fibulin-3 or the control pcDNA vector. *Left*, western blot analysis of fibulin-3 at 48 hr after transfection. *Middle*, cells were plated out at 48 hr after fibulin-3 transfection and selected for G418 resistance. Colonies were visualized by crystal violet staining 11 to 14 days later. *Right*, quantification of colony numbers. Scale bar, 1 cm.

Supplementary Figure 3. Fibulin-3 suppressed lung cancer cell invasion.

(A) Representative pictures of Matrigel analysis of parental, fibulin-3-negative (C1, C2, and C3) and fibulin-3-expressing (F1, F2, F3 and F4) H1299 cells (200×). **(B)** Representative pictures of Matrigel analysis of parental, fibulin-3-negative (C1 and C2) and stable fibulin-3-expressing A549 cells (F1, F2 and F3) (200×). Scale bar, 25 μm.

Supplementary Figure 4. Correlation of *fibulin-3* promoter methylation and MMP-7 expression.

Representative fibulin-3 and MMP-7 immunostaining pictures of lung tumors with and without

fibulin-3 promoter methylation are shown (200×). Scale bar, 50 μm.

Supplementary Figure 5. *Fibulin-3* and *MMP-7* expression and DNA methylation in the TCGA databases. (A) Heatmaps of *fibulin-3* and *MMP-7* mRNA expression in the TCGA lung cancer (LUNG) RNAseq (IlluminaHiSeq; N=965) dataset. Red: high expression; black: average expression; green: low expression. C1 and C2, which represent sample type and significance, respectively, are the first and second criteria for sorting the data. **(B)** Heatmaps of *fibulin-3* and *MMP-7* DNA methylation in the TCGA lung cancer (LUNG) HumanMethylation27 (Illumina 27K platform; N=312) dataset. Red: high methylation; white: average methylation; blue: low methylation. C1 and C2, which represent sample type and significance, respectively, are the first and second criteria for sorting the data.

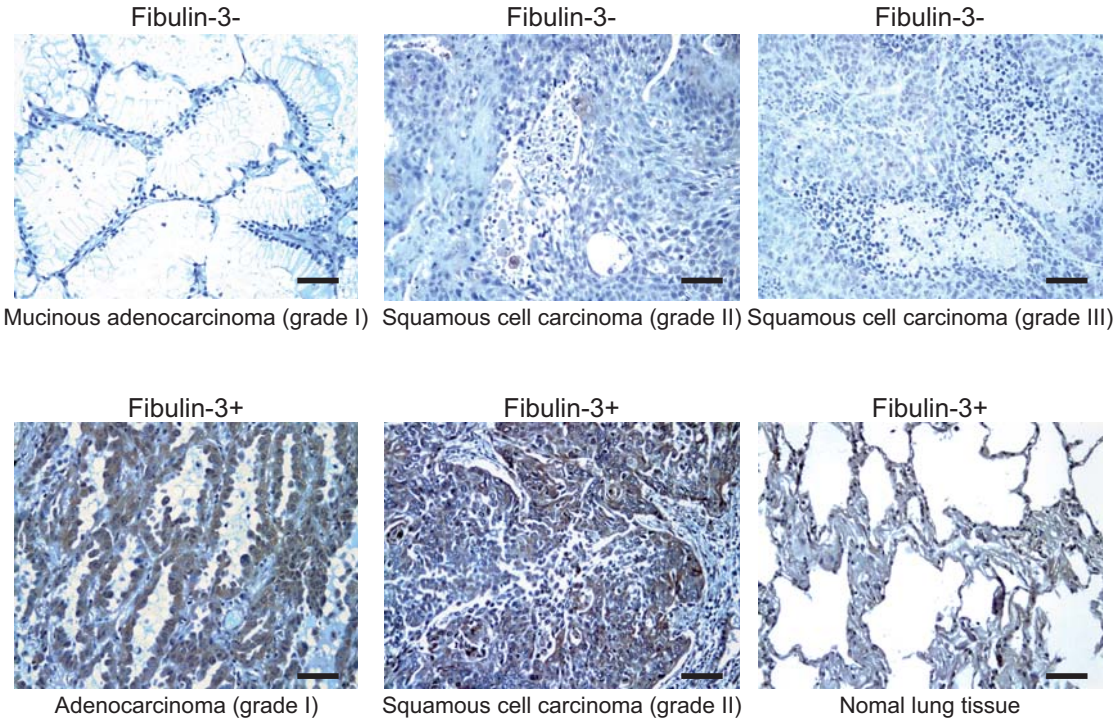
Supplementary Figure 6. Downregulation of *c-Myc* and *cyclin D1* by fibulin-3 in lung cancer cells.

H1299 and A549 cells were transfected with *fibulin-3* or control pcDNA vector. *c-Myc* and *cyclin D1* mRNA expression at indicated time points after transfection was analyzed by real-time RT-PCR with *GAPDH* as the internal control. The results were normalized to cells without *fibulin-3* transfection (0 hr), which were defined as 1.0.

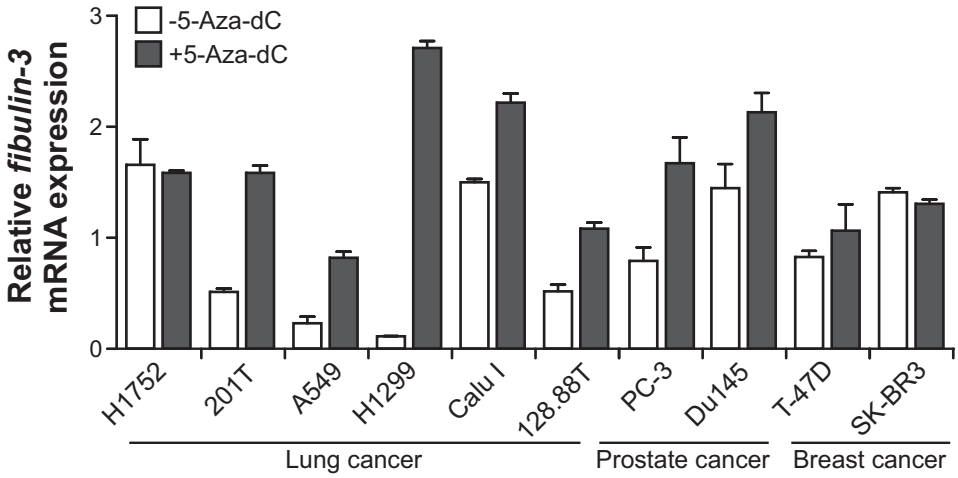
Supplementary Figure 7. Fibuin-3 inhibited lung tumor growth and metastasis in mice. (A) Pictures of parental and fibulin-3-expressing H1299 xenograft tumors at 27 days after inoculation. Scale bar, 5 mm. **(B)** Comparison of the body weight between the mice with parental and fibulin-3-expressing H460 tumors before sacrifice. The difference between the two groups is statistically significant (*P<0.05, Student's t test).

Supplementary Figure 1

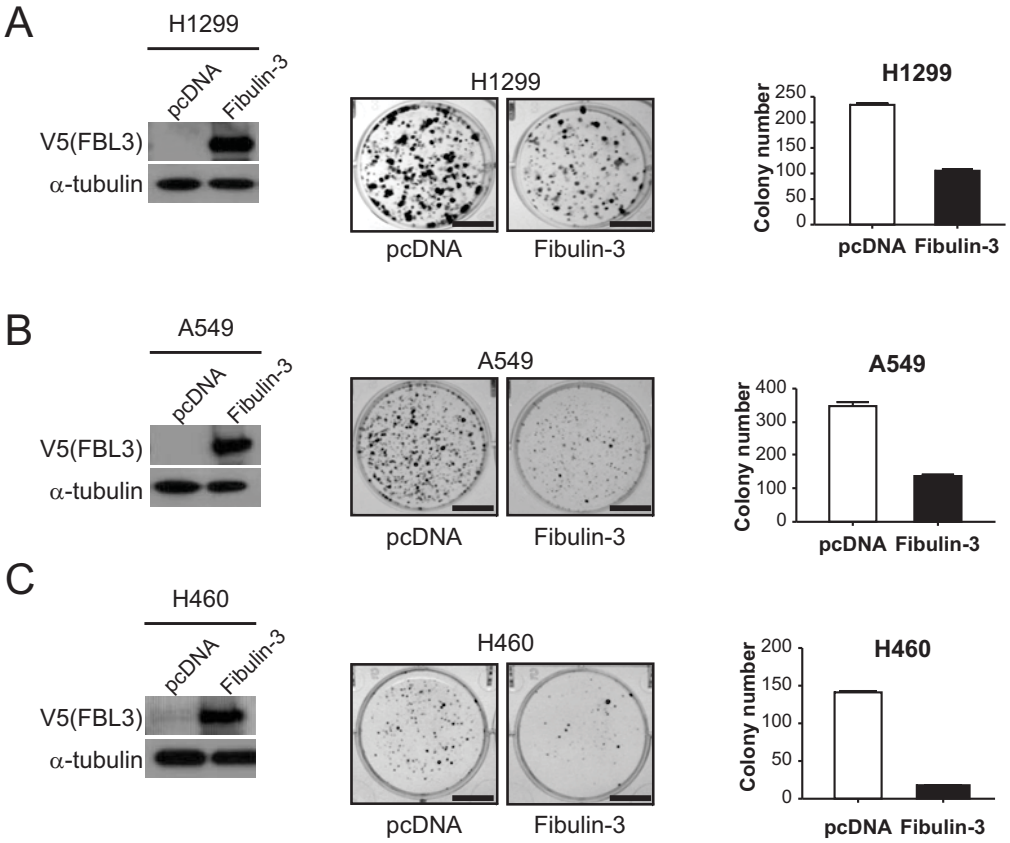
A



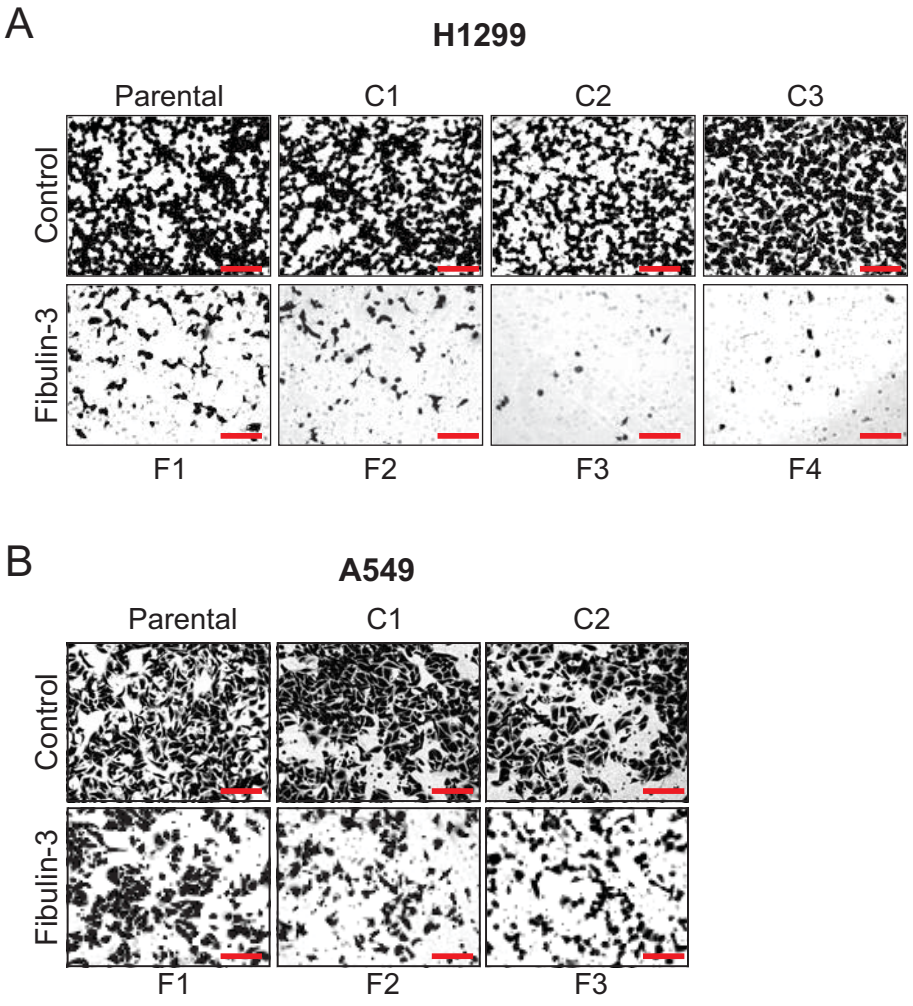
B



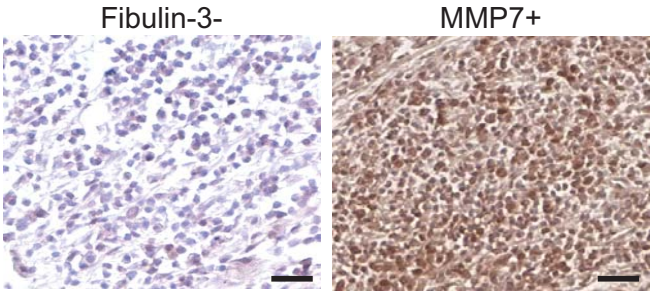
Supplementary Figure 2



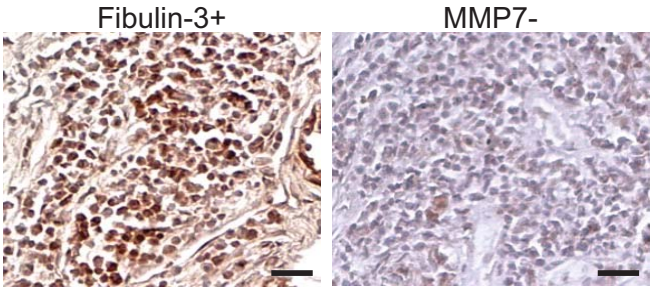
Supplementary Figure 3



Supplementary Figure 4

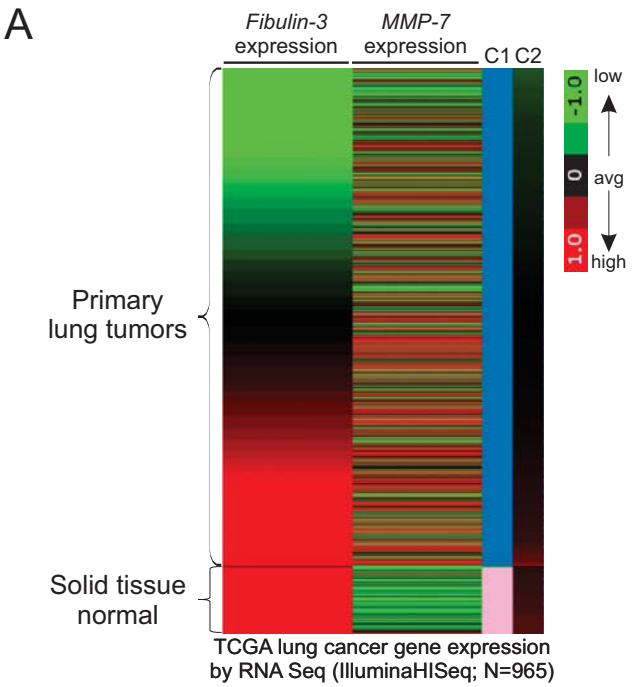


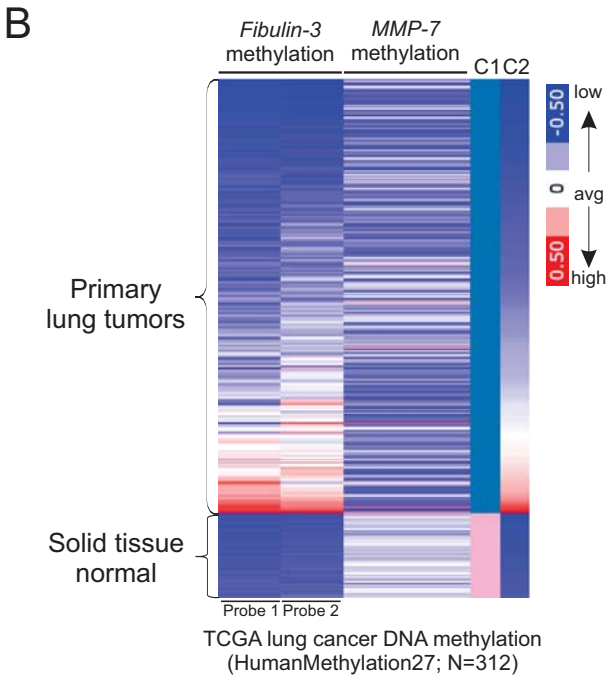
Patient #19 (*Fibulin-3* methylated)



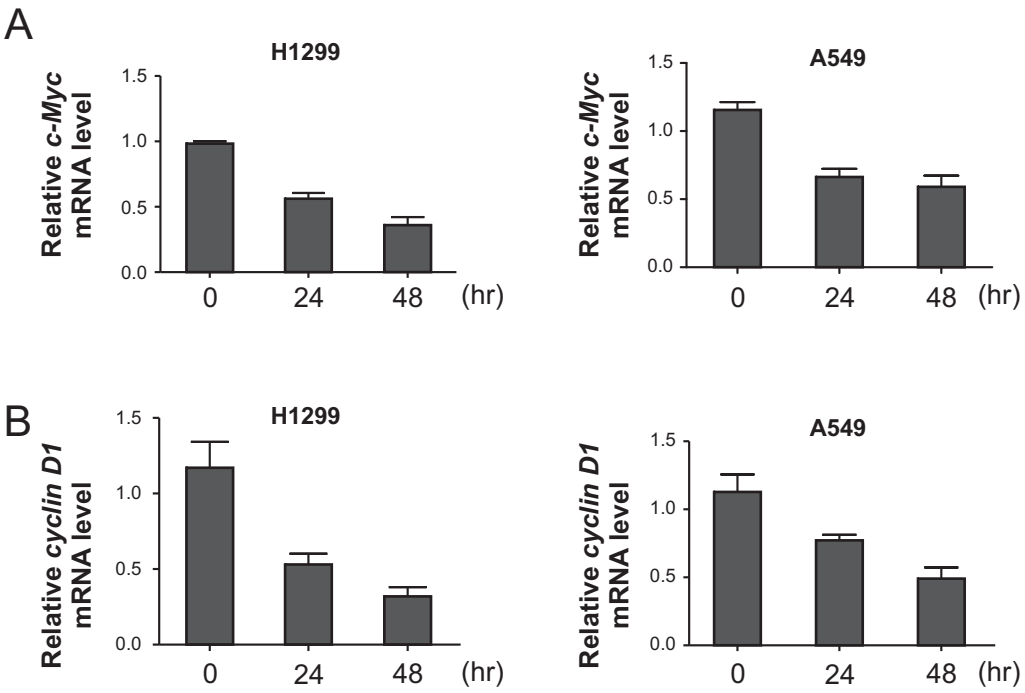
Patient #29 (*Fibulin-3* unmethylated)

Supplementary Figure 5



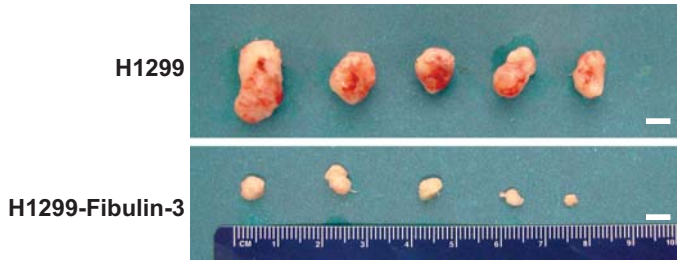


Supplementary Figure 6



Supplementary Figure 7

A



B

Cell lines	Body Weight (gm)	
	Initial	Final
H460	20.62±0.78	19.45±1.23
H460-Fibulin-3	20.89±0.63	24.7±1.96
P value	>0.05	0.024*

Supplementary Table 1. Fibulin-3 and MMP-7 expression in NSCLC and normal lung specimens

#	Sex	Age	Diagnosis	Grade	Fibulin-3 expression in normal	Fibulin-3 expression in tumor	MMP-7 expression in tumor
1	M	58	Squamous cell carcinoma	I		-	-
2	F	66	Squamous cell carcinoma	I		+	+
3	M	58	Adenocarcinoma	I		-	+
4	M	62	Papillary adenocarcinoma	I		-	-
5	F	45	Adenocarcinoma	I		-	-
6	M	60	Adenocarcinoma	I		+	-
7	M	66	Mucinous adenocarcinoma	I		-	-
8	F	53	Adenocarcinoma	I		+	-
9	F	55	Mucinous adenocarcinoma	I		-	+
10	M	68	Adenocarcinoma	I	+	+	+
11	M	72	Adenocarcinoma	I	+	+	-
12	M	65	Adenocarcinoma	I	+	-	-
13	F	64	Adenocarcinoma	I	+	-	+
14	F	42	Adenocarcinoma	I	+	-	-
15	F	56	Adenocarcinoma	I	+	-	+
16	F	69	Adenocarcinoma	I	+	-	-
17	M	66	Adenocarcinoma	I	+	-	+
18	M	56	Adenocarcinoma	I	+	-	-
19	M	65	Adenocarcinoma	I	+	+	-
20	F	60	Adenocarcinoma	I	-	+	-
21	M	46	Lung carcinoid	I	+	-	-
22	F	53	Adenocarcinoma	I	+	+	-
23	M	74	Squamous cell carcinoma	I	-	-	+
24	M	79	Squamous cell carcinoma	I	+	+	-
25	F	59	Squamous cell carcinoma	I	-	-	+
26	M	61	Squamous cell carcinoma	I	+	+	-
27	F	74	Large cell-neuroendocrine	I	+	+	-
28	M	69	Squamous cell carcinoma	I	+	-	+
29	M	72	Large cell tumor	I	+	+	-
30	F	64	Squamous cell carcinoma	I	+	-	+
31	F	46	Squamous cell carcinoma	II		-	-
32	M	70	Squamous cell carcinoma	II		-	-
33	M	61	Squamous cell carcinoma	II		-	-
34	M	58	Squamous cell carcinoma	II		+	+
35	F	67	Squamous cell carcinoma	II		-	+
36	M	53	Squamous cell carcinoma	II		+	-
37	F	65	Squamous cell carcinoma	II		-	+
38	M	78	Squamous cell carcinoma	II		-	+
39	F	49	Squamous cell carcinoma	II		-	+
40	M	59	Squamous cell carcinoma	II		-	-
41	M	62	Squamous cell carcinoma	II		-	+

42	F	72	Adenocarcinoma	II		-	+
43	F	35	Adenocarcinoma	II		+	-
44	M	47	Adenocarcinoma	II		+	+
45	F	54	Adenocarcinoma	II		-	-
46	F	46	Adenocarcinoma	II		+	-
47	F	58	Adenocarcinoma	II		-	+
48	M	47	Papillary adenocarcinoma	II		-	-
49	F	37	Adenocarcinoma	II		-	+
50	F	63	Adenocarcinoma	II		-	+
51	F	55	Mucinous adenocarcinoma	II		+	-
52	M	42	Adenocarcinoma	II	+	-	-
53	M	61	Adenocarcinoma	II	+	-	+
54	M	55	Adenocarcinoma	II	+	-	-
55	M	54	Adenocarcinoma	II	+	-	+
56	F	51	Adenocarcinoma	II	+	-	+
57	M	61	Adenocarcinoma	II	+	+	+
58	F	60	Adenocarcinoma	II	+	-	-
59	M	40	Adenocarcinoma	II	-	-	+
60	F	59	Adenocarcinoma	II	+	-	-
61	F	80	Squamous cell carcinoma	II	+	-	+
62	M	69	Squamous cell carcinoma	II	-	+	-
63	F	66	Squamous cell carcinoma	II	-	+	+
64	M	72	Squamous cell carcinoma	III		+	+
65	M	58	Squamous cell carcinoma	III		-	+
66	M	53	Squamous cell carcinoma	III		-	-
67	M	41	Squamous cell carcinoma	III		+	-
68	M	54	Squamous cell carcinoma	III		-	-
69	M	50	Squamous cell carcinoma	III		-	+
70	F	49	Squamous cell carcinoma	III		-	+
71	M	51	Squamous cell carcinoma	III		-	-
72	M	65	Squamous cell carcinoma	III		+	+
73	M	72	Squamous cell carcinoma	III		-	-
74	M	51	Squamous cell carcinoma	III		-	+
75	M	53	Squamous cell carcinoma	III		+	+
76	M	61	Squamous cell carcinoma	III		-	-
77	M	47	Squamous cell carcinoma	III		-	-
78	F	47	Squamous cell carcinoma	III		-	+
79	M	61	Squamous cell carcinoma	III		-	-
80	M	56	Squamous cell carcinoma	III		-	+
81	M	52	Adenocarcinoma	III		-	+
82	M	65	Adenocarcinoma	III		-	-
83	F	49	Adenocarcinoma	III		-	+
84	M	59	Adenocarcinoma	III		-	-
85	F	50	Adenocarcinoma	III		+	-
86	M	35	Adenocarcinoma	III		-	+

87	M	62	Adenocarcinoma	III		+	-
88	F	50	Squamous cell carcinoma	III		-	+
89	M	52	Adenocarcinoma	III	+	-	+
90	F	54	Adenocarcinoma	III	+	-	+
91	M	69	Adenocarcinoma	III	+	-	+
92	F	54	Adenocarcinoma	III	+	+	+
93	M	65	Adenocarcinoma	III	-	+	-
94	F	36	Adenocarcinoma	III	+	+	+
95	F	58	Adenocarcinoma	III	+	-	+
96	M	66	Adenocarcinoma	III	+	+	-
97	M	58	Adenocarcinoma	III	+	-	+
98	M	60	Adenocarcinoma	III	-	-	-
99	M	49	Adenocarcinoma	III	-	-	+
100	F	66	Large cell tumor	III	+	-	+
101	F	60	Large cell-neuroendocrine	III	+	-	+

Supplementary Table 2. Primers for RT-PCR

Gene	Forward primer (5-3′)	Reverse primer (5-3′)	PCR product (bp)
Fibulin-3	ATAATGAACAGCCTCAGCAGG	TGTTGTAGCACTGCTGAGCAC	513
MMP-1	CACAGCTTTCCTCCACTGCTGCT	GGCATGGTCCACATCTGCTCTTG	397
MMP-2	ACCTGGATGCCGTCGTGGAC	TGTGGCAGCACCAGGGCA	448
MMP-7	AAACTCCCGCGTCATAGAAAT	CCCTAGACTGCTACCATCCG	397
MMP-9	TGGCATCCGGCACCTCTATGGT	GCCACTTGTCGGCGATAAGGAA	371
MMP-13	TCAGCAGGTTGATGCGGAGCTG	TGACGCGAACAATACGGTTACTC	449
TIMP-1	AGCAGGGCCTGCACCTGTGTC	TTCAGAGCCTTGGAGCTGGTC	477
TIMP-2	ATGAGATCAAGCAGATAAAGATG	GGTCCTCGATGTCGAGAAACTC	450
TIMP-3	GCTCATCGTGCTCCTGGGCAG	CTCGGTACCAGCTGCAGTAGC	572
GAPDH	CTCAGACACCCTGGGGAAGGTGA	ATGATCTTGAGGCTGTTGTCATA	427
c-Myc	CCTCAACGTTAGCTTCACCAA	TTTGATGAAGGTCTCGTCGTC	373
Cyclin D1	CCCTCGGTGTCCTACTTCAAA	CCAGGTTCCACTTGAGCTTGT	339