

GDF15 Promotes Cell Growth, Migration, and Invasion in Gastric Cancer by Inducing STAT3 Activation

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Supplementary Table S1. Patient information analyzed for mRNA expression of GDF15.

Patient No.	Age (yr)	Gender	Type	<i>H. Pylori</i>
1	81	Male	Adenocarcinoma, intestinal	-
2	96	Female	Adenocarcinoma, diffuse	-
3	69	Male	Adenocarcinoma, intestinal	-
4	80	Female	Adenocarcinoma, diffuse	+

Supplementary Table S2. Baseline characteristics of the CNUH cohort.

Patient No.	GDF15 Expression	Age	Gender	Live or Death	DAYS	OS (mon)	OS (yr)	Location	Size	Differentiation	Lymphatic Invasion	Venous Invasion	T Stage
1	2	50	male	live	1912	63.73	5.3	lower	≤ 5cm	undifferentiated	invasion	invasion	1
2	2	68	female	live	2385	79.50	6.6	lower	≤ 5cm	differentiated	no invasion	no invasion	1
3	3	71	female	death	401	13.37	1.1	middle	> 5cm	undifferentiated	invasion	no invasion	4
4	2	72	male	live	1243	41.43	3.5	lower	≤ 5cm	differentiated	no invasion	invasion	1
5	2	75	female	live	1826	60.87	5.1	middle	≤ 5cm	undifferentiated	no invasion	no invasion	1
6	3	61	male	death	243	8.10	0.7	lower	≤ 5cm	differentiated	no invasion	no invasion	2
7	3	60	male	death	194	6.47	0.5	middle	≤ 5cm	differentiated	invasion	invasion	1
8	1	71	male	live	1925	64.17	5.3	middle	≤ 5cm	differentiated	no invasion	no invasion	1
9	2	64	male	live	1673	55.77	4.6	middle	≤ 5cm	undifferentiated	no invasion	no invasion	1
10	2	46	female	death	394	13.13	1.1	middle	> 5cm	undifferentiated	no invasion	no invasion	3
11	2	50	male	live	1698	56.60	4.7	middle	≤ 5cm	differentiated	no invasion	invasion	1
12	0	38	male	death	8	0.27	0.0	lower	≤ 5cm	differentiated	invasion	invasion	1
13	1	53	female	live	1951	65.03	5.4	middle	≤ 5cm	differentiated	no invasion	no invasion	1
14	1	68	male	live	1356	45.20	3.8	lower	≤ 5cm	differentiated	no invasion	no invasion	1
15	1	55	male	live	2093	69.77	5.8	middle	≤ 5cm	differentiated	no invasion	no invasion	3
16	1	45	male	live	1518	50.60	4.2	lower	≤ 5cm	undifferentiated	no invasion	no invasion	1
17	2	66	male	live	1572	52.40	4.4	middle	≤ 5cm	differentiated	invasion	no invasion	1
18	3	61	male	death	572	19.07	1.6	lower	≤ 5cm	differentiated	no invasion	no invasion	1
19	2	60	male	death	927	30.90	2.6	middle	> 5cm	undifferentiated	no invasion	no invasion	3
20	1	68	female	live	1551	51.70	4.3	lower	≤ 5cm	differentiated	no invasion	no invasion	1
21	2	43	female	live	1843	61.43	5.1	lower	≤ 5cm	differentiated	no invasion	invasion	1
22	2	69	male	death	883	29.43	2.5	lower	≤ 5cm	differentiated	no invasion	no invasion	3
23	0	62	male	death	980	32.67	2.7	middle	> 5cm	undifferentiated	no invasion	no invasion	2
24	2	75	male	death	67	2.23	0.2	lower	≤ 5cm	undifferentiated	invasion	invasion	1

25	3	54	male	death	353	11.77	1.0	lower	> 5cm	undifferentiated	no invasion	no invasion	3
26	1	63	female	death	827	27.57	2.3	lower	≡ 5cm	undifferentiated	no invasion	no invasion	3
27	2	50	male	live	1491	49.70	4.1	middle	≡ 5cm	undifferentiated	invasion	no invasion	1
28	0	68	male	live	1600	53.33	4.4	middle	≡ 5cm	undifferentiated	no invasion	no invasion	1
29	2	69	male	live	1856	61.87	5.2	lower	≡ 5cm	differentiated	no invasion	no invasion	1
30	2	74	female	death	1529	50.97	4.2	middle	≡ 5cm	undifferentiated	no invasion	no invasion	3
31	1	54	male	death	199	6.63	0.6	lower	≡ 5cm	undifferentiated	no invasion	no invasion	1
32	2	60	male	live	1240	41.33	3.4	middle	≡ 5cm	undifferentiated	no invasion	invasion	1
33	0	40	female	live	1793	59.77	5.0	middle	≡ 5cm	undifferentiated	invasion	invasion	1
34	1	66	male	live	1936	64.53	5.4	middle	≡ 5cm	differentiated	no invasion	no invasion	1
35	0	68	male	death	1434	47.80	4.0	middle	≡ 5cm	differentiated	invasion	invasion	1
36	0	60	female	live	1734	57.80	4.8	middle	≡ 5cm	undifferentiated	no invasion	no invasion	1
37	1	69	female	live	1739	57.97	4.8	lower	> 5cm	differentiated	no invasion	no invasion	2
38	2	62	male	death	873	29.10	2.4	middle	≡ 5cm	differentiated	no invasion	no invasion	1
39	3	59	male	live	1503	50.10	4.2	middle	≡ 5cm	undifferentiated	no invasion	no invasion	1
40	3	70	male	death	607	20.23	1.7	lower	≡ 5cm	differentiated	no invasion	no invasion	1
41	2	68	female	death	1168	38.93	3.2	lower	≡ 5cm	differentiated	no invasion	no invasion	1
42	0	54	male	live	2227	74.23	6.2	lower	≡ 5cm	undifferentiated	no invasion	no invasion	2
43	1	73	female	death	452	15.07	1.3	lower	> 5cm	undifferentiated	no invasion	no invasion	3
44	1	55	male	death	523	17.43	1.5	middle	> 5cm	undifferentiated	no invasion	no invasion	3
45	2	60	male	live	1541	51.37	4.3	lower	≡ 5cm	differentiated	no invasion	no invasion	1
46	2	86	male	death	46	1.53	0.1	middle	≡ 5cm	undifferentiated	no invasion	no invasion	3
47	3	39	male	death	846	28.20	2.4	middle	≡ 5cm	undifferentiated	no invasion	invasion	3
48	1	63	male	death	1167	38.90	3.2	upper	≡ 5cm	undifferentiated	no invasion	no invasion	3
49	2	63	male	death	1349	44.97	3.7	lower	> 5cm	undifferentiated	no invasion	no invasion	3
50	2	70	female	live	2443	81.43	6.8	lower	≡ 5cm	differentiated	invasion	no invasion	1
51	3	49	male	live	2025	67.50	5.6	middle	≡ 5cm	undifferentiated	no invasion	no invasion	2
52	2	58	female	live	1794	59.80	5.0	lower	> 5cm	undifferentiated	no invasion	no invasion	2
53	3	77	male	death	283	9.43	0.8	middle	≡ 5cm	undifferentiated	no invasion	invasion	3
54	3	59	male	live	2108	70.27	5.9	middle	≡ 5cm	undifferentiated	no invasion	no invasion	2
55	3	66	male	death	204	6.80	0.6	middle	≡ 5cm	differentiated	no invasion	no invasion	3
56	3	48	male	death	457	15.23	1.3	middle	> 5cm	undifferentiated	no invasion	no invasion	3
57	3	82	female	death	180	6.00	0.5	middle	≡ 5cm	undifferentiated	no invasion	no invasion	3
58	2	44	female	live	1771	59.03	4.9	lower	≡ 5cm	differentiated	invasion	no invasion	1
59	3	68	male	death	446	14.87	1.2	middle	> 5cm	undifferentiated	no invasion	no invasion	3
60	3	40	male	live	2018	67.27	5.6	middle	> 5cm	undifferentiated	no invasion	no invasion	1
61	1	43	male	live	2172	72.40	6.0	middle	≡ 5cm	undifferentiated	no invasion	no invasion	2
62	3	60	male	death	1409	46.97	3.9	upper	> 5cm	differentiated	no invasion	no invasion	3
63	1	54	male	live	1671	55.70	4.6	middle	≡ 5cm	differentiated	invasion	invasion	1
64	1	33	male	death	459	15.30	1.3	middle	≡ 5cm	undifferentiated	no invasion	no invasion	3
65	3	67	female	death	438	14.60	1.2	lower	≡ 5cm	differentiated	no invasion	no invasion	2
66	1	74	male	death	536	17.87	1.5	middle	≡ 5cm	undifferentiated	no invasion	no invasion	1
67	3	50	male	death	42	1.40	0.1	lower	> 5cm	undifferentiated	no invasion	no invasion	3
68	1	79	male	death	413	13.77	1.1	lower	≡ 5cm	undifferentiated	no invasion	no invasion	3
69	1	69	male	live	1435	47.83	4.0	lower	≡ 5cm	undifferentiated	no invasion	no invasion	1
70	0	46	female	death	653	21.77	1.8	middle	≡ 5cm	undifferentiated	no invasion	no invasion	3
71	2	70	male	death	1221	40.70	3.4	middle	> 5cm	differentiated	no invasion	no invasion	3
72	3	77	female	death	370	12.33	1.0	lower	≡ 5cm	differentiated	no invasion	no invasion	2
73	2	63	female	live	1984	66.13	5.5	lower	> 5cm	undifferentiated	no invasion	no invasion	1
74	1	50	female	death	294	9.80	0.8	upper	≡ 5cm	undifferentiated	no invasion	no invasion	3
75	1	50	female	death	1598	53.27	4.4	lower	≡ 5cm	differentiated	no invasion	no invasion	2
76	2	59	male	live	2252	75.07	6.3	lower	> 5cm	undifferentiated	no invasion	no invasion	2

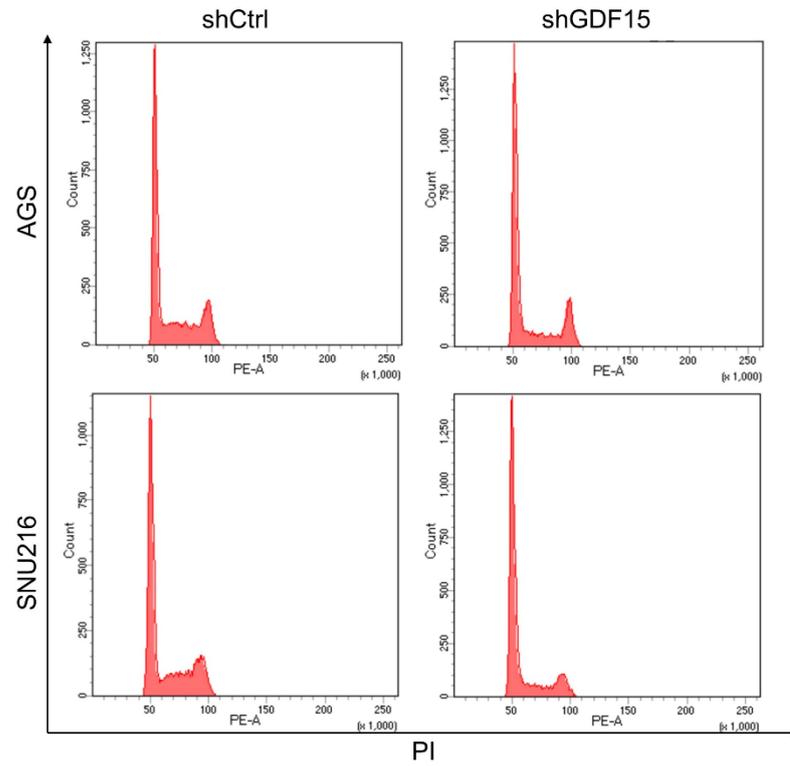
77	2	63	male	death	369	12.30	1.0	middle	> 5cm	undifferentiated	no invasion	no invasion	3
78	0	67	male	death	470	15.67	1.3	middle	> 5cm	undifferentiated	no invasion	no invasion	3
79	3	66	male	death	144	4.80	0.4	lower	> 5cm	undifferentiated	no invasion	invasion	3
80	1	58	male	death	795	26.50	2.2	middle	≤ 5cm	undifferentiated	no invasion	no invasion	3
81	0	64	female	death	618	20.60	1.7	upper	> 5cm	undifferentiated	invasion	invasion	2
82	2	64	female	death	519	17.30	1.4	middle	> 5cm	undifferentiated	no invasion	no invasion	3
83	2	60	male	death	600	20.00	1.7	middle	≤ 5cm	undifferentiated	invasion	no invasion	1
84	3	62	male	death	562	18.73	1.6	upper	> 5cm	undifferentiated	no invasion	no invasion	2
85	1	63	female	death	344	11.47	1.0	lower	> 5cm	undifferentiated	no invasion	no invasion	3
86	1	55	male	live	1924	64.13	5.3	lower	≤ 5cm	undifferentiated	no invasion	no invasion	1
87	2	66	male	live	2089	69.63	5.8	middle	≤ 5cm	differentiated	no invasion	no invasion	1
88	3	54	female	live	2055	68.50	5.7	lower	≤ 5cm	differentiated	no invasion	no invasion	2
89	0	63	male	live	1828	60.93	5.1	lower	≤ 5cm	undifferentiated	invasion	invasion	1
90	1	36	male	live	1782	59.40	5.0	middle	≤ 5cm	undifferentiated	no invasion	no invasion	2
91	3	36	male	death	520	17.33	1.4	upper	> 5cm	undifferentiated	invasion	invasion	3
92	1	43	female	death	1511	50.37	4.2	lower	≤ 5cm	undifferentiated	invasion	invasion	1
93	1	52	male	live	1591	53.03	4.4	middle	≤ 5cm	undifferentiated	no invasion	no invasion	1
94	3	67	male	death	758	25.27	2.1	lower	≤ 5cm	undifferentiated	no invasion	no invasion	3
95	3	61	male	death	576	19.20	1.6	middle	≤ 5cm	undifferentiated	no invasion	no invasion	1
96	2	51	male	death	578	19.27	1.6	middle	> 5cm	undifferentiated	no invasion	no invasion	3
97	2	60	male	death	364	12.13	1.0	middle	≤ 5cm	differentiated	invasion	invasion	1
98	0	51	male	death	684	22.80	1.9	lower	≤ 5cm	differentiated	no invasion	no invasion	3
99	0	29	female	live	1888	62.93	5.3	middle	≤ 5cm	undifferentiated	invasion	invasion	1
100	2	61	male	death	748	24.93	2.1	lower	> 5cm	differentiated	no invasion	no invasion	1
101	1	58	female	live	2029	67.63	5.6	middle	≤ 5cm	differentiated	invasion	invasion	1
102	1	72	female	death	880	29.33	2.4	lower	> 5cm	undifferentiated	no invasion	no invasion	2
103	1	69	female	death	1584	52.80	4.4	lower	≤ 5cm	differentiated	invasion	invasion	1
104	3	66	male	death	762	25.40	2.1	lower	≤ 5cm	undifferentiated	no invasion	no invasion	3
105	1	53	male	live	1957	65.23	5.4	middle	≤ 5cm	differentiated	invasion	invasion	1
106	0	38	male	death	1515	50.50	4.2	middle	≤ 5cm	undifferentiated	no invasion	no invasion	2
107	2	66	male	live	1560	52.00	4.3	lower	≤ 5cm	differentiated	no invasion	no invasion	2
108	3	67	female	death	329	10.97	0.9	lower	> 5cm	undifferentiated	no invasion	no invasion	3
109	2	57	male	live	1905	63.50	5.3	lower	≤ 5cm	differentiated	invasion	invasion	1
110	2	57	female	live	1884	62.80	5.2	lower	≤ 5cm	undifferentiated	no invasion	no invasion	1
111	2	54	male	live	1832	61.07	5.1	lower	≤ 5cm	undifferentiated	invasion	invasion	1
112	3	54	male	death	1358	45.27	3.8	lower	> 5cm	differentiated	no invasion	no invasion	3
113	1	44	male	death	1518	50.60	4.2	upper	≤ 5cm	differentiated	no invasion	no invasion	1
114	2	80	male	death	1154	38.47	3.2	middle	≤ 5cm	differentiated	no invasion	no invasion	2
115	3	66	male	live	1804	60.13	5.0	lower	≤ 5cm	undifferentiated	no invasion	no invasion	3
116	3	42	female	death	531	17.70	1.5	lower	≤ 5cm	differentiated	no invasion	no invasion	1
117	2	39	male	live	1758	58.60	4.9	lower	≤ 5cm	differentiated	no invasion	no invasion	1
118	1	41	female	death	1009	33.63	2.8	middle	≤ 5cm	undifferentiated	no invasion	no invasion	3
119	3	68	male	death	107	3.57	0.3	upper	> 5cm	undifferentiated	no invasion	no invasion	3
120	2	43	male	live	1828	60.93	5.1	lower	≤ 5cm	differentiated	no invasion	no invasion	1
121	2	41	female	live	1833	61.10	5.1	middle	≤ 5cm	undifferentiated	invasion	invasion	1
122	2	55	male	death	217	7.23	0.6	middle	> 5cm	undifferentiated	no invasion	no invasion	3
123	2	61	male	live	1560	52.00	4.3	upper	≤ 5cm	differentiated	no invasion	no invasion	1
124	2	63	male	live	1764	58.80	4.9	middle	≤ 5cm	differentiated	invasion	invasion	1
125	2	52	female	live	1526	50.87	4.2	middle	≤ 5cm	differentiated	no invasion	no invasion	1
126	2	70	female	death	284	9.47	0.8	lower	≤ 5cm	differentiated	no invasion	no invasion	1
127	1	44	female	live	1622	54.07	4.5	middle	≤ 5cm	undifferentiated	no invasion	no invasion	1
128	2	59	female	live	1790	59.67	5.0	lower	≤ 5cm	undifferentiated	no invasion	no invasion	1

129	3	60	male	death	740	24.67	2.1	middle	≅ 5cm	differentiated	no invasion	no invasion	3
130	1	47	male	live	1789	59.63	5.0	lower	≅ 5cm	differentiated	invasion	invasion	1
131	3	41	male	live	1586	52.87	4.4	middle	≅ 5cm	differentiated	invasion	invasion	1
132	1	54	male	live	1751	58.37	4.9	lower	≅ 5cm	differentiated	invasion	invasion	1
133	2	58	male	death	342	11.40	1.0	middle	≅ 5cm	differentiated	no invasion	no invasion	1
134	1	72	male	death	682	22.73	1.9	middle	≅ 5cm	differentiated	invasion	invasion	1
135	0	57	female	live	1737	57.90	4.8	lower	≅ 5cm	differentiated	no invasion	no invasion	1
136	0	37	female	live	1587	52.90	4.4	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
137	1	59	male	death	458	15.27	1.3	middle	≅ 5cm	differentiated	no invasion	no invasion	1
138	2	66	male	death	7	0.23	0.0	middle	≅ 5cm	differentiated	no invasion	no invasion	1
139	0	49	male	live	1285	42.83	3.6	lower	≅ 5cm	differentiated	invasion	invasion	1
140	1	47	male	live	1714	57.13	4.8	lower	≅ 5cm	differentiated	invasion	invasion	1
141	2	69	female	death	1107	36.90	3.1	middle	≅ 5cm	differentiated	invasion	invasion	1
142	2	65	male	live	1662	55.40	4.6	lower	≅ 5cm	differentiated	no invasion	no invasion	1
143	3	66	male	death	1137	37.90	3.2	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
144	3	53	male	live	1671	55.70	4.6	lower	≅ 5cm	undifferentiated	no invasion	no invasion	1
145	1	69	male	death	7	0.23	0.0	middle	≅ 5cm	differentiated	invasion	invasion	1
146	2	59	male	death	168	5.60	0.5	middle	≅ 5cm	differentiated	no invasion	no invasion	1
147	0	41	male	live	1380	46.00	3.8	lower	≅ 5cm	undifferentiated	no invasion	no invasion	1
148	0	49	male	death	586	19.53	1.6	middle	≅ 5cm	differentiated	invasion	invasion	1
149	3	47	male	live	1584	52.80	4.4	middle	≅ 5cm	differentiated	invasion	invasion	1
150	3	67	male	live	1625	54.17	4.5	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
151	1	58	female	live	1369	45.63	3.8	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
152	2	39	male	death	1062	35.40	3.0	lower	≅ 5cm	undifferentiated	no invasion	no invasion	1
153	1	42	male	live	1419	47.30	3.9	middle	≅ 5cm	differentiated	no invasion	no invasion	1
154	1	69	male	live	1474	49.13	4.1	lower	≅ 5cm	differentiated	invasion	invasion	1
155	0	60	male	live	1211	40.37	3.4	lower	≅ 5cm	differentiated	no invasion	no invasion	1
156	1	72	male	death	566	18.87	1.6	middle	≅ 5cm	differentiated	no invasion	no invasion	1
157	1	59	male	death	875	29.17	2.4	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
158	1	64	male	death	326	10.87	0.9	lower	≅ 5cm	undifferentiated	no invasion	no invasion	1
159	0	45	male	death	1030	34.33	2.9	lower	≅ 5cm	differentiated	invasion	invasion	1
160	3	62	female	death	861	28.70	2.4	lower	≅ 5cm	differentiated	no invasion	no invasion	1
161	2	66	male	live	1404	46.80	3.9	lower	≅ 5cm	differentiated	no invasion	no invasion	1
162	3	50	male	live	1218	40.60	3.4	middle	≅ 5cm	differentiated	no invasion	no invasion	1
163	3	76	female	live	1504	50.13	4.2	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
164	2	59	female	live	1213	40.43	3.4	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
165	3	69	male	live	1430	47.67	4.0	lower	≅ 5cm	undifferentiated	no invasion	no invasion	1
166	2	58	male	live	1280	42.67	3.6	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
167	2	63	male	death	1003	33.43	2.8	lower	≅ 5cm	differentiated	no invasion	no invasion	1
168	3	47	female	live	1097	36.57	3.0	upper	≅ 5cm	undifferentiated	no invasion	no invasion	1
169	0	78	female	live	1344	44.80	3.7	upper	≅ 5cm	differentiated	no invasion	no invasion	1
170	3	45	male	live	1052	35.07	2.9	middle	≅ 5cm	undifferentiated	invasion	no invasion	1
171	2	40	male	death	1033	34.43	2.9	middle	≅ 5cm	differentiated	invasion	invasion	1
172	1	68	male	death	473	15.77	1.3	lower	≅ 5cm	differentiated	invasion	invasion	1
173	2	63	male	death	453	15.10	1.3	middle	≅ 5cm	differentiated	invasion	invasion	1
174	3	66	male	death	712	23.73	2.0	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1
175	3	56	male	live	1330	44.33	3.7	middle	≅ 5cm	differentiated	no invasion	no invasion	1
176	2	60	male	live	1161	38.70	3.2	lower	≅ 5cm	differentiated	no invasion	no invasion	1
177	1	67	male	live	1293	43.10	3.6	middle	≅ 5cm	differentiated	no invasion	no invasion	1
178	1	69	male	death	564	18.80	1.6	middle	≅ 5cm	undifferentiated	no invasion	no invasion	1

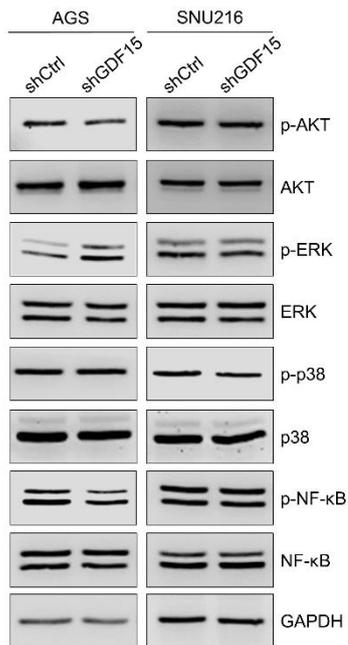
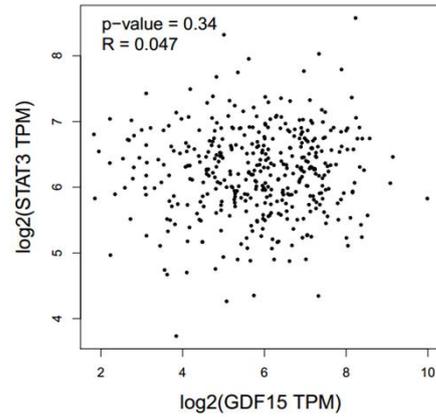
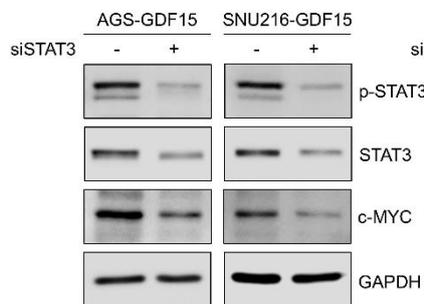
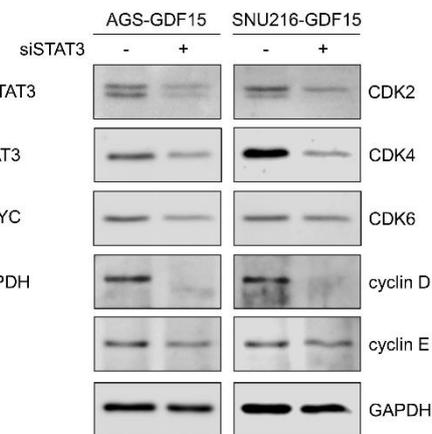
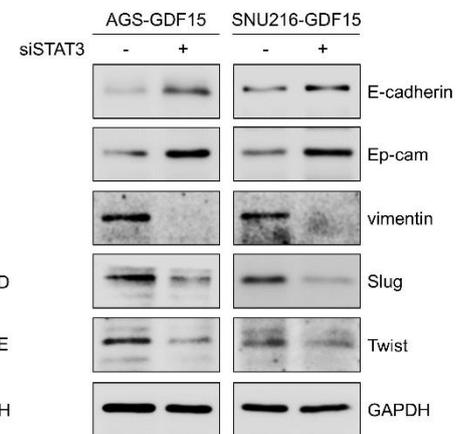
* OS: Overall Survival.

Supplementary Table S3. Primer sequences for RT-qPCR.

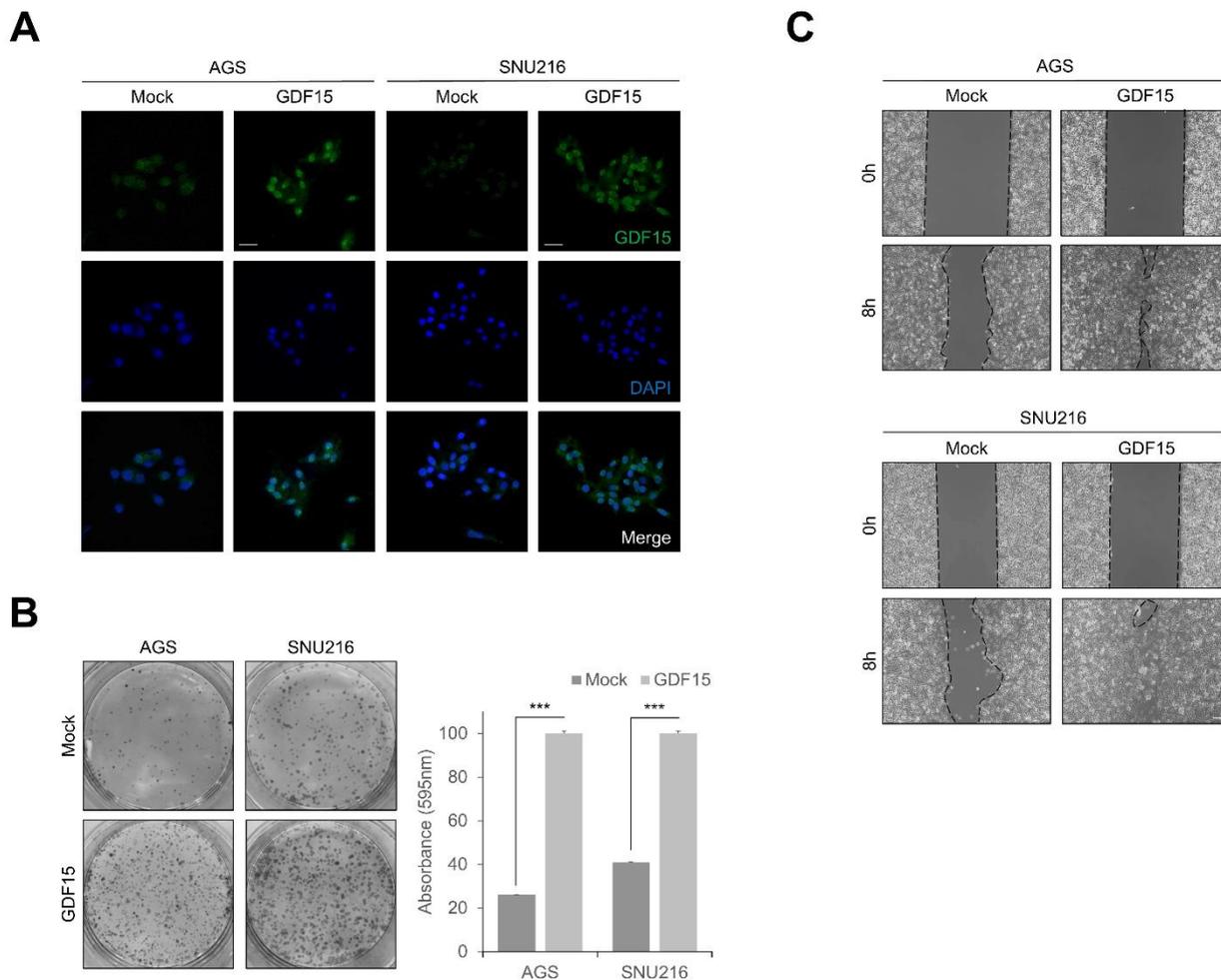
Gene	Forward (5'-3')	Reverse (3'-5')
GDF15	CTCCAGATTCCGAGAGTTGC	ACCTGCACCTGCGTATCTCT
GAPDH	TTGATTTTGGAGGGATCTCG	GAGTCAACGGATTTGGTCGT



Supplementary Figure S1. Cell cycle analysis by FACS of GDF15-knockdown cells after propidium iodide (PI) staining. Knockdown of GDF15 significantly increased the proportion of cells in the G0/G1 phase compared with the control, whereas it decreased the proportion of cells in S phase and G2/M phase.

A**B****C****D****E**

Supplementary Figure S2. (A) Western blotting analysis of components of several cancer-related pathways. (B) The correlation of GDF15 and STAT3 expression in GEPIA dataset. (C) Transiently transfected with control and STAT3 siRNA in GDF15-overexpressing cells. Western blotting analysis showing decreased phosphorylation of STAT3 and c-MYC. (D) Knockdown of STAT3 significantly downregulated cell cycle markers in GDF15-overexpressing cells. (E) Western blot analysis showed that STAT3 knockdown in GDF15 overexpressing cells increased expression of epithelial markers and decreased expression of mesenchymal markers.



Supplementary Figure S3. (A) Immunofluorescence staining of GDF15 in GDF15-overexpressing cells. Scale bar, 100 μ m. (B) The colonogenic assay was performed on a 6-well culture plate in GDF15-overexpressing cells. Crystal-violet-stained cells were dissolved in 70% alcohol, and absorbance at 595 nm was measured using a spectrophotometer. Data are presented as the mean \pm SD and were evaluated using Student's t-test ($n = 3$). (C) Gap closure assay in GDF15-overexpressing cells at 0h and 8h. ($n = 3$) Scale bar, 100 μ m. Three independent experiments were carried out in triplicate. *** $p < 0.001$.