Supplementary materials

Verteporfin blocks Clusterin which is required for survival of gastric cancer stem cell by modulating HSP90 function

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Supplementary Figures:

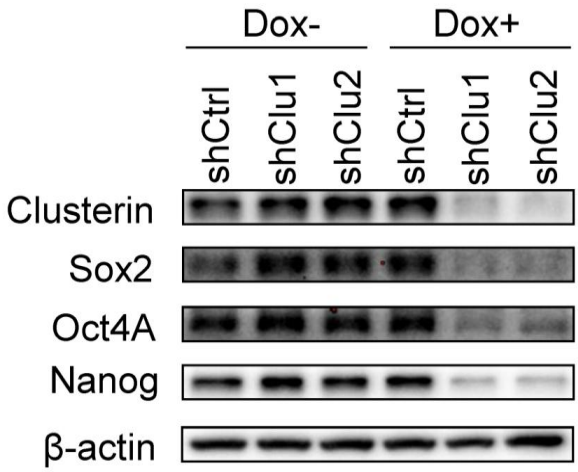

Figure S1. Expression of stem cell-related markers (Sox2, Oct4A, Nanog) in GCSC-shCtrl, -shClu1 and -shClu2 lines. The cell lysates of GCSC-shCtrl, -shClu1 and -shClu2 in Figure 2e were prepared at D12. The expression levels of Clusterin, Sox2, Oct4A, Nanog, and β-actin in these cells were examined by western blot..

Supplementary Tables:

Table S1

MS-based quantification of proteins in supernatant of gastric cancer stem cell culture.

Protein name	Accession number	iTRAQ quantification					
		D0(115)	D1(116)	D2(117)	D3(118)	D4(119)	D5(121)
Clusterin	P10909	0.31	1.00	2.27	3.13	4.47	7.11
Annexin A1	P04083	0.57	1.00	1.91	3.82	4.53	8.86
Cochlin	O43405	0.93	1.00	1.59	4.17	3.84	4.39
Prosaposin	P07602	0.78	1.00	1.97	3.68	3.85	5.68
Cathepsin B	P07858	1.40	1.00	1.62	3.60	3.44	4.32
Serum albumin	P02768	1.40	1.00	2.20	2.64	3.16	4.45
Serotransferrin	P02787	1.64	1.00	2.52	3.22	3.61	5.34
Insulin	P01308	3.19	1.00	2.47	5.21	3.57	3.42
Hemopexin	P02790	0.85	1.00	1.49	1.32	2.20	2.70
Glucose-6-phosphate isomerase	P06744	0.31	1.00	2.27	3.13	4.47	7.11
Transgelin-2	P37802	1.28	1.00	2.86	7.38	5.48	7.27
Protein S100-A10	P60903	0.96	1.00	1.09	6.43	2.14	6.87
Plectin	Q15149	2.80	1.00	2.06	5.89	3.34	8.77
Profilin-1	P07737	2.00	1.00	2.43	3.94	4.52	9.81
Sulfhydryl oxidase 1	O00391	0.96	1.00	2.13	8.94	6.29	9.01

Table S2

Association between clusterin expression and clinicopathologic characteristics of patients with GC (n = 90)

Clinicopathologic characteristics	N	Clusterin expression no. (%)		*P* value
		High-expression[a]	Low-expression[b]	
Age (y)				
≤ 65	46	35(76.1)	11(23.9)	0.965
> 65	44	34(77.3)	10(22.7)	
Gender				
Female	30	23(76.7)	7(23.3)	0.449
Male	60	46(76.7)	14(23.3)	
T-stage				
T1 and T2	15	8(53.3)	7(46.7)	0.0004*
T3 and T4	73	59(80.8)	14(19.2)	
Lymph nodes metastasis(N)				
N_0	22	12(54.5)	10(45.5)	0.026*
N_1	68	57(83.8)	11(16.2)	
Distant metastasis(M)				
M_0	80	61(76.2)	19(23.8)	0.945
M_1	10	8(80.0)	2(20.0)	
TNM stage				
I and II	18	11(61.1)	7(38.9)	0.013*
III and Ⅳ	72	58(80.6)	14(19.4)	

* $P < 0.05$

[a]High-expression: Clusterin expression score ≥4;

[b]Low-expression: Clusterin expression score <4.

Table S3

Identification of proteins co-immunoprecipitated with clusterin from GCSC lysate.

Protein name	Accession number	Peptide no.
Clusterin	P10909	12
Heat shock protein HSP 90β	P08238	10
Protein phosphatase 1B	O75688	10
Splicing factor 3B subunit 2	Q13435	8
Tubulin alpha-1C chain	Q9BQE3	6
RNA-binding protein FUS	P35637	5
Heterochromatin protein 1-binding protein 3	Q5SSJ5	4
PEST proteolytic signal-containing nuclear protein	Q8WW12	4
60S ribosomal protein L12	P30050	4
Heterogeneous nuclear ribonucleoprotein Q	O60506	4
Brain acid soluble protein 1	P80723	4
Small nuclear ribonucleoprotein-associated protein N	P63162	4
SRA stem-loop-interacting RNA-binding protein	Q9GZT3	3
Cytochrome b-c1 complex subunit 6	P07919	3
Pyruvate kinase PKM	P14618	3
Small nuclear ribonucleoprotein Sm D3	P62318	3
40S ribosomal protein S10	P46783	3
Serine/arginine-rich splicing factor 3	P84103	3
Transformer-2 protein homolog beta	P62995	3
Tubulin beta chain	P07437	3
Plasminogen activator inhibitor 1 RNA-binding protein	Q8NC51	3

Table S4

Clinicopathological features and clusterin expression in 90 informative GC patients

No	Clusterin[a]	Age	Gender[b]	Survival	Censor[c]	T[d]	LN Met[e]	M[f]	Stage
1	8	79	1	21	0	T4	1	0	III
2	12	52	1	24	0	T3	1	0	III
3	4	58	1	98	1	T3	1	1	III
4	4	50	1	98	1	T3	1	0	IV
5	12	54	1	98	1	T3	1	0	III
6	9	48	1	43	0	T3	0	0	III
7	9	57	1	No data	1	T4	1	0	III
8	8	77	1	6	0	T3	1	1	III
9	8	59	1	3	0	T4	1	1	III
10	6	84	1	97	1	T4	1	0	III
11	4	77	1	9	0	T2	1	0	II
12	8	73	1	9	0	T2	0	0	III
13	6	62	0	97	1	T1	0	0	II
14	4	61	1	11	0	T3	1	0	II
15	4	75	0	63	0	T2	1	0	III
16	8	58	1	43	0	T3	1	0	III
17	8	71	1	1	0	T3	1	0	III
18	8	41	1	8	0	T4	1	0	IV
19	3	69	0	96	1	T3	0	0	III
20	6	67	0	74	0	T3	1	0	III
21	4	67	1	12	0	T3	1	0	III
22	8	65	1	96	1	T3	0	0	III
23	4	60	1	22	0	T3	1	0	III
24	4	72	0	32	0	T3	1	1	IV
25	4	53	0	19	0	T4	1	0	II
26	8	53	1	55	0	T4	0	0	II
27	4	73	0	23	0	T2	1	0	III
28	1	37	1	No data	1	T1	0	0	II
29	2	48	0	8	0	T3	1	0	III
30	2	71	1	17	0	T3	1	0	III
31	3	77	0	92	1	T3	0	0	IV
32	3	48	1	No data	1	T3	1	0	III
33	3	72	1	17	0	T3	1	0	III
34	2	70	1	92	1	T2	0	0	III
35	6	77	0	20	0	T3	1	0	III
36	8	45	1	No data	1	T3	1	0	IV
37	3	80	0	92	1	T1	0	0	II

38	8	72	1	4	0	T3	1	0	II
39	8	49	1	20	0	T3	1	0	IV
40	8	79	1		1	T3	0	0	IV
41	4	63	0	91	1	T3	0	0	II
42	0	65	1	22	0	T3	1	0	II
43	8	78	1	91	1	T3	1	0	II
44	12	57	0	17	0	T3	1	1	III
45	2	45	1	23	0	T3	1	1	II
46	4	50	0	17	0	T3	1	0	III
47	1	76	1	9	0	T3	1	0	III
48	8	65	1	65	0	T3	0	0	III
49	8	71	1	17	0	T3	1	1	III
50	6	75	0	85	0	T3	1	0	III
51	8	32	1	3	0	No data	1	1	IV
52	3	58	1	No data	1	T3	0	0	III
53	12	73	0	20	0	T4	1	0	III
54	6	58	0	90	1	T3	1	0	II
55	3	62	1	90	1	T1	0	0	II
56	8	73	0	15	0	T3	1	0	III
57	12	68	1	23	0	T3	1	0	III
58	6	52	1	22	0	T3	1	0	III
59	6	50	1	28	0	T3	1	0	IV
60	3	74	0	7	0	T3	1	1	III
61	16	67	0	3	0	T4	1	0	III
62	12	53	1	90	1	T3	1	0	III
63	8	65	1	90	1	T3	1	0	II
64	8	68	0	12	0	T3	1	0	III
65	8	52	0	48	0	T3	1	0	III
66	4	47	1	38	0	T4	1	1	III
67	6	72	1	69	0	T4	1	0	III
68	6	62	1	89	1	T2	1	0	III
69	6	66	1	89	1	T1	1	0	III
70	3	67	0	89	1	T3	0	0	III
71	6	59	0	89	1	T1	1	0	III
72	6	58	0	89	1	T3	1	0	III
73	6	77	1	34	0	T3	1	0	III
74	1	57	0	89	1	T1	1	0	II
75	12	78	1	89	1	T3	1	0	III
76	2	76	1	89	1	T3	1	0	III
77	9	56	1	89	1	T3	1	0	II
78	8	51	1	89	1	T3	1	0	III
79	3	55	1	89	1	T2	0	0	II
80	12	52	1	89	1	T3	1	0	III
81	2	61	1	89	1	T3	1	0	III

82	8	72	1	89	1	T3	1	0	III
83	8	67	0	69	0	No data	0	0	IV
84	4	62	1	11	0	T3	1	0	III
85	6	74	1	89	1	T4	0	0	III
86	4	81	0	43	0	T3	0	0	III
87	8	81	0	3	0	T4	1	0	III
88	3	62	1	40	0	T1	0	0	III
89	12	62	1	17	0	T4	1	0	IV
90	6	77	0	47	0	T3	0	0	III

[a]Clusterin expression score detected by IHC;

[b]Gender: 0=female, 1=male;

[c]Censored: 0=dead at the end of the follow-up, 1=still alive at the end of the follow-up;

[d]T: T stage;

[e]Lymph-node metastasis: 0=no LN metastasis, 1=with LN metastasis;

[f]M, distant metastasis: 0=no distant metastasis, 1=with distant metastasis.