Supplementary Materials for

MCM6 is a critical transcriptional target of YAP to promote gastric tumorigenesis and serves as a therapeutic target

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This PDF file includes:

Supplementary Materials and Methods

Figure S1 to S7

Table S1 to S10

References

SUPPLEMENTARY MATERIAL AND METHODS

RNA extraction and real-time quantitative PCR (qPCR). Total RNA was extracted by RNAiso Plus (Takara). Complementary DNA was synthesized by PrimeScript RT Master Mix (Takara) according to the manufacturer's instructions. qPCR was performed using TB Green Premix Ex Taq (Takara) in QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems). Relative expression differences were calculated using the $2^{-\Delta\Delta Ct}$ method with reference to β-actin. Primers sequences are provided in Table S3.

Transfection of small interfering RNA (siRNA) and plasmid. Lipofectamine RNAiMAX (Invitrogen) was used for siRNA transfection, whereas FuGENE HD (Promega) was used for transient plasmid transfection following the manufacturer's instructions. siYAP indicates a mixture of siYAP-1 and siYAP-2. All siRNA used are provided in Table S8.

Lentiviral production and transduction. For lentiviral production, lentiviral vector, psPAX2 (Addgene #12260), pMD2.G (Addgene #12259), and lipofectamine 3000 (Invitrogen) were mixed and added to HEK293T cells in 10 cm dish. The lentiviral particle-containing supernatant was harvested at 48 h post-transfection and passed through a 0.45 μm filter. After lentiviral transduction, cells were selected with puromycin (1 μg/mL). Plasmids and short hairpin RNA (shRNA) sequences used are listed in Table S9. pLVX-Flag-YAP-5SA lentiviral vector was kindly provided by Prof. Bin Zhao of Zhejiang University.

Cell viability and colony formation assays. Cell viability was measured by cell counting kit-8 (CCK8) from MedChemExpress. Results were presented as the means ± standard deviation (SD) from three independent experiments. For colony formation assay, cells were seeded in 6-well plates at a low density (500 to 1000 cells per well) and cultured in 2ml of complete medium for a week with medium change every two days. At the endpoint, colonies were stained with crystal violet and counted.

Reagents. Verteporfin was purchased from Cayman Chemical. Purpureaside C was purchased from MedChemExpress. MK-2206 2HCI, 5-FU, cisplatin, doxorubicin, and oxaliplatin were purchased from Selleck Chemicals.

Cell migration and invasion assays. Cell migration and invasion assays were conducted using 24-well Transwell Inserts (Corning) and Matrigel Invasion Chambers (Corning), respectively. Cells suspended in serum-free medium were added to the upper chamber, with 10% FBS-containing medium added to the bottom. After 24 h of incubation, cells that migrated or invaded to the lower surface of membrane were fixed with 4% PFA and stained with crystal violet (Sigma-Aldrich). Images of three randomly selected fields were taken under a 20x objective lens to count cell numbers.

Cell cycle and apoptosis assays. For cell cycle assay, cells were harvested and fixed in 70% ethanol at 4 °C overnight, followed by staining with propidium iodide (Sigma-Aldrich). For apoptosis assay, cells were harvested and stained with FITC Annexin V Apoptosis Detection Kit I (BD Biosciences). Samples were analyzed by BD LSRFortessa (BD Biosciences) and FlowJo software.

EdU proliferation assay. Cells were seeded onto sterile glass coverslips and overnight was allowed for cells to recover and attach to the coverslip. Afterward, cells were treated with 20 μM of 5-ethynyl-2'-deoxyuridine (EdU) and incubated at 37 °C for 4 h. Cells were stained using EdU Proliferation Kit (iFluor 488) (Abcam) according to the company protocol. Nuclei were stained with DAPI (Invitrogen).

Luciferase reporter assay. Forkhead response element (FHRE) luciferase reporter (Addgene, #1789) was used to monitor PI3K/Akt pathway activity [1]. To assess the activity of MCM6 promoter, wild-type or mutant MCM6 promoter region was cloned into the pGL3 luciferase reporter vector. Luciferase reporter was transiently co-transfected with pRL-TK Renilla luciferase vector (Promega #E2241) into GC cells using lipofectamine 3000 (Invitrogen). Luciferase activity was detected by the Dual-

luciferase reporter assay system (Promega). Luciferase reporter activity was presented as a ratio of Firefly: Renilla luminescence.

Immunohistochemistry (IHC) staining. Tissue sections were deparaffinized in xylene and rehydrated in graded ethanol, followed by antigen retrieval in Tris-EDTA. The endogenous peroxidase activity was quenched by 3% hydrogen peroxide. The sections were then blocked in fetal bovine serum for 1 h, incubated with primary antibodies at room temperature for 3 h, and incubated with secondary antibodies for 1 h. Signals were detected using DAB (Dako). GC tumors from tissue microarrays were stratified into high and low expression subgroups according to the criteria as follows: for YAP, tumors with ≥ 25% nuclear positive cells were considered as high expression cases, and those with < 25% positive cells were low expression cases; for MCM6, high expression cases were defined as ≥ 50% positive cells, while < 50% were for low expression cases. The IHC score was evaluated by two experienced pathologists. Antibodies used for IHC were listed in Table S10.

UV irradiation. Cells were seeded in 6-well plates and cultured for 48 h. After removing the medium, GC cells were exposed to UV irradiation in the UV strantalinker 2400 (Stratagene) and harvested at the indicated time points for western blot or functional assays.

F-actin labelling. GC cells were seeded on glass coverslips and fixed in 4% paraformaldehyde permeabilized with 0.1% Triton X-100, and blocked in 3% BSA. Phalloidin FITC Reagent (Abcam) was used to label F-actin. Fluorescent signals were visualized using Carl Zeiss LSM880 confocal microscopy.

TCGA (The Cancer Genome Atlas) and GSE (Genomic Spatial Event) data analyses. Gene expression profiling data of MCM6 in GC tumors and adjacent normal samples were analyzed under the stomach adenocarcinoma (STAD) of TCGA (https://xenabrowser.net/) and GSE63089 (https://www.ncbi.nlm.nih.gov/geo/). The

correlation analysis between YAP and MCM6 expression was performed using GEPIA (http://gepia.cancer-pku.cn/).

Expression microarray. Total RNA of AGS cells transfected with or without siYAP-1 were extracted and reverse transcribed into cDNA. Synthesized cDNA was then fragmented and labeled using the Quick Amp Gene Expression Labeling kit (Agilent). Next, labeled cDNA targets were hybridized to the Affymetrix GeneChip Human Gene 2.0 ST Array for microarray analysis. The procedure of expression microarray and analysis of the gene expression profiling were conducted by Macrogen, Korea.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 8.0.0 (GraphPad, La Jolla, CA), or SPSS version 26.0 (SPSS Inc, Chicago, IL). Results were presented as mean ± SD. The Student's t-test or Mann-Whitney U test was performed to compare the means between two groups, and one-way analysis of variance (ANOVA) with the Tukey post-hoc test was for statistical comparisons between multiple groups. Two-way ANOVA was applied for the combined effect evaluation. The difference in growth rates between groups was determined by repeated-measures ANOVA. Kaplan-Meier survival analysis and log-rank test were performed to evaluate the effect of MCM6 on survival. Clinicopathologic features were analyzed by the Pearson chi-square test or Fisher's exact test. Crude hazard ratios (HR) of disease-specific survival associated with MCM6 expression and other variables were estimated by the univariate Cox proportional hazards regression model and adjusted HR was further estimated by the multivariate Cox regression model. $P < 0.05$ was considered statistically significant.

SUPPLEMENTARY FIGURES

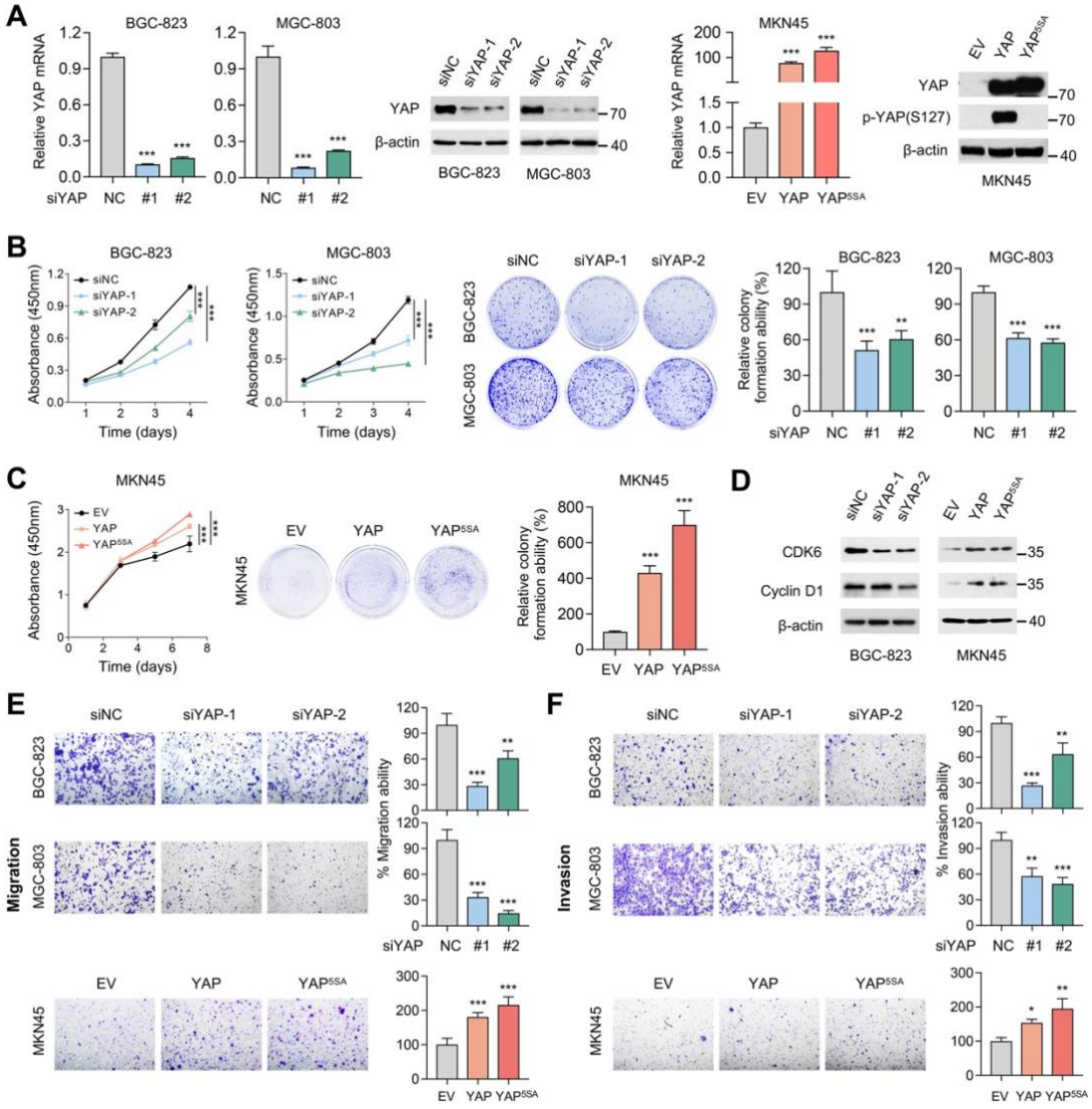


Figure S1. YAP promotes GC growth and metastasis *in vitro*. (Related to Figure 1)

(A) Two different YAP-siRNAs were used (siYAP-1 and siYAP-2). Quantitative PCR and western blot showed the successful knockdown of YAP expression in BGC-823 and MGC-803 cells. Successful overexpression of wild-type YAP (YAP) and a constitutively active YAP (YAP^{5SA}) was also confirmed. **(B-C)** YAP knockdown suppressed the abilities of cells to proliferate and form colony, while overexpressing YAP showed the opposite effects. **(D)** Western blot analysis of CDK6 and cyclin D1 in GC cells with or without YAP knockdown. **(E-F)** Representative images and quantification of GC cells that migrated (E) or invaded (F) with or without YAP modulated. Error bars in A-C, E and F represent mean ± standard deviation. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; analysis of variance test (ANOVA) (A-C, E and F). EV, empty vector; NC, negative control.

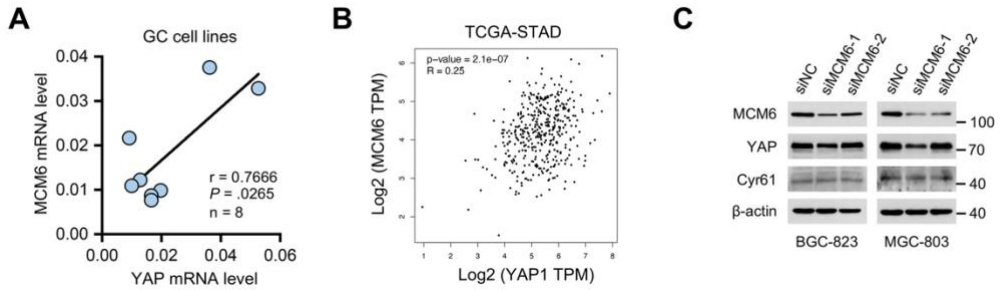


Figure S2. YAP modulates MCM6 expression in GC. (Related to Figure 1)

(A) Pearson correlation analysis of YAP and MCM6 mRNA expression in eight GC and two normal cell lines. **(B)** Pearson correlation analysis of YAP and MCM6 mRNA levels in GC tumors from TCGA-STAD dataset (http://gepia.cancer-pku.cn/). **(C)** Western blot showed no significant changes of YAP signatures in MCM6-deficient BGC-823 and MGC-803 cells. ***$P < 0.001$; Pearson r (A and B). NC, negative control; TCGA-STAD, The Cancer Genome Atlas-Stomach Adenocarcinoma.

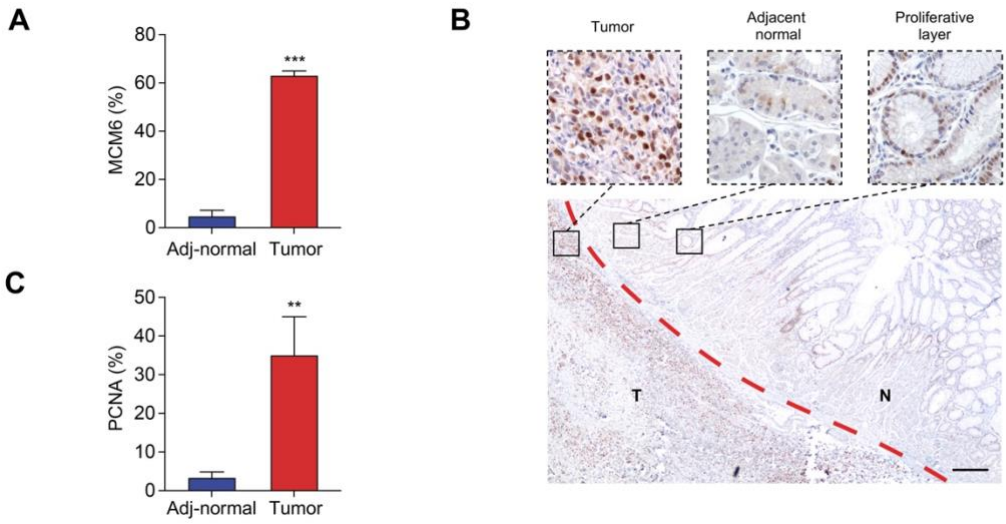


Figure S3. Expression pattern of PCNA in human GC. (Related to Figure 2)

(A) Quantification of MCM6-positive cells in GC tumors and adjacent normal tissues. **(B-C)** Representative IHC images (B) and quantification (C) of PCNA staining on resected GC tissues showed that PCNA-positive cells had similar expression pattern as MCM6-postive cells did in tumors (T) and adjacent normal (N) tissues. Scale bar, 100 μm. PCNA, proliferating cell nuclear antigen.

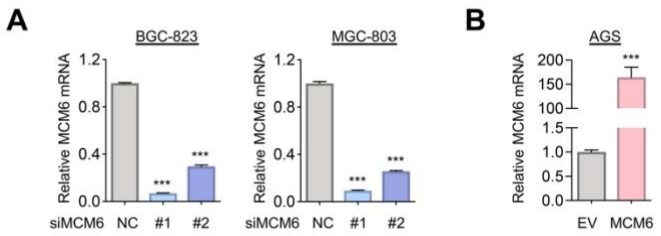


Figure S4. Knockdown or overexpression of MCM6 in GC cell lines. (Related to Figure 3)

(A-B) qPCR analyses verified successful MCM6 knockdown (A) and overexpression (B) for indicated GC cell lines. Error bars in A and B represent mean ± standard deviation from three independent experiments. ***$P < 0.001$; analysis of variance test (ANOVA) (A) or 2-tailed t test (B). EV, empty vector; NC, negative control.

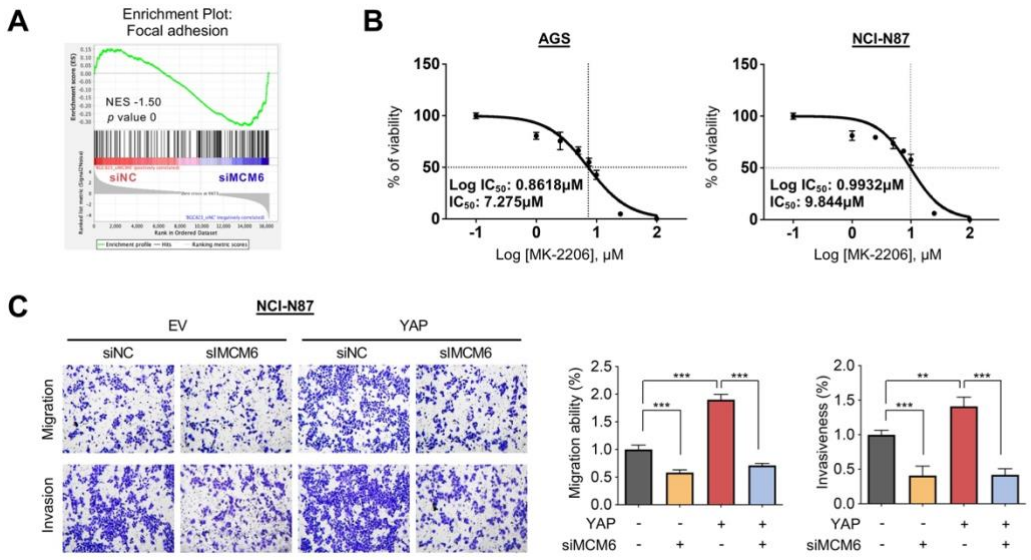
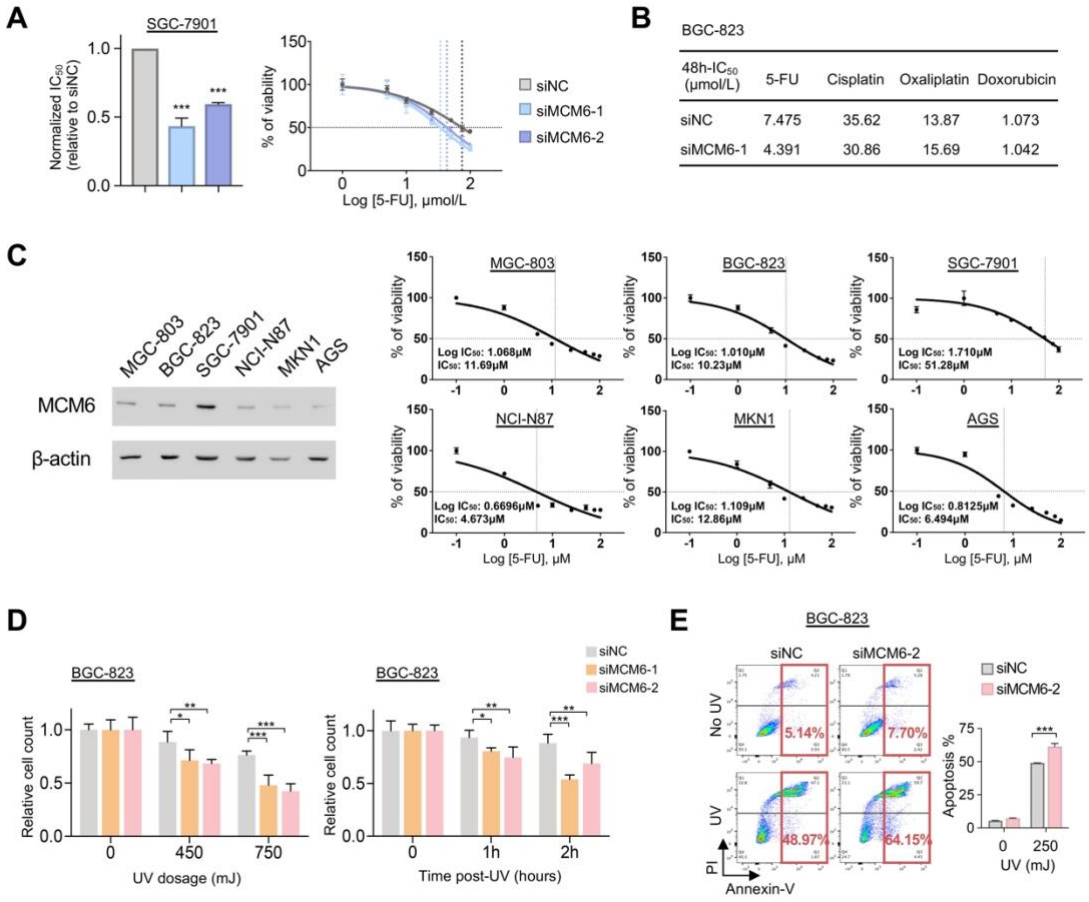


Figure S5. MCM6 activates PI3K/Akt signaling and focal adhesion in GC cells. (Related to Figure 4)

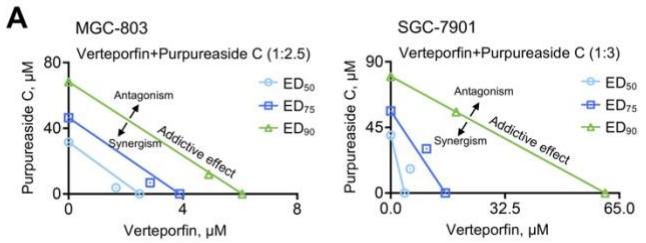
(A) GSEA analysis identified an enrichment of genes involved in focal adhesion in high-MCM6-expressing BGC-823 cells. **(B)** Determination of 48 h-IC_{50} values of MK-2206 in AGS and NCI-N87 cells by CCK8 assay. **(C)** Representative images and quantification of numbers of NCI-N87 cells that migrated and invaded. MCM6 knockdown partially abolished the tumor metastatic capability of YAP in GC cells. Error bars in B and C represent mean ± standard deviation. $^{**}P < 0.01$; $^{***}P < 0.001$; analysis of variance test (ANOVA) (C). EV, empty vector; GSEA, gene set enrichment analysis; IC_{50}, half-maximal inhibitory concentration; NC, negative control; NES, normalized enrichment score.



BGC-823				
48h-IC_{50} (μmol/L)	5-FU	Cisplatin	Oxaliplatin	Doxorubicin
siNC	7.475	35.62	13.87	1.073
siMCM6-1	4.391	30.86	15.69	1.042

Figure S6. Knockdown of MCM6 sensitizes cancer therapies in GC. (Related to Figure 6)

(A) Determination of IC_{50} values of 5-FU in SGC-7901 cells with or without MCM6 knockdown. **(B)** IC_{50} values of cisplatin, 5-FU, oxaliplatin, and doxorubicin in BGC-823 cells with or without MCM6 knockdown. **(C)** Western blot analysis of MCM6 protein expression (left) and determination of 48h-IC_{50} values of 5-FU (right) in a panel of GC cell lines. **(D)** Quantification of BGC-823 cells with or without MCM6 knockdown exposed to UV at different dosage or at different time points. **(E)** Apoptosis assay for BGC-823 cells with or without MCM6 knockdown after exposure to UV for 1 h. Error bars in A, D, and E represent mean ± standard deviation. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; analysis of variance test (ANOVA) (A, D, and E). 5-FU, fluorouracil; IC_{50}, half-maximal inhibitory concentration; NC, negative control; PI, propidium iodide.



B

Fraction affected (Fa)	Combination index (CI)	
	MGC-803	SGC-7901
0.5	0.799	1.822
0.75	0.887	1.195
0.9	0.985	1.001

Figure S7. Combinational treatment of purpureaside C and verteporfin in GC cells. (Related to Figure 7)

(A) Classical isobolograms showed no synergistic effect existed between purpureaside C and verteporfin in both MGC-803 and SGC-7901 cells. **(B)** A table summarized the CI values (calculated using the Chou-Talalay method) of this drug combination when achieved 50% (Fa = 0.5), 75% (Fa = 0.75), and 90% (Fa = 0.90) growth inhibition in MGC-803 and SGC-7901 cells. The CI value <1, = 1, and >1 indicates synergism, additive effect, and antagonism, respectively. CI, combination index; ED, effective dose; Fa, fraction affected.

SUPPLEMENTARY TABLES

Table S1. Characteristics of 264 GC patients in Hong Kong tissue microarray cohort.

No.	Sex	Age	Lauren subtype	Tumor grade	TNM stage	LNM	DSS time	DSS status	MCM6 (%)	MCM6 Tumor
1	male	52	diffuse	2	IV	positive	5	1	0	low
2	female	74	intestinal	2	III	positive	2.6	0	0	low
3	female	64	intestinal	1	I	negative	90	0	4	low
4	male	82	diffuse	3	III	positive	10.5	1	1	low
5	male	69	intestinal	2	II	negative	30.7	0	53	high
6	male	74	intestinal	2	I	negative	70.1	0	21	low
7	female	52	intestinal	2	IV	positive	9.4	1	51	high
8	male	83	diffuse	3	IV	positive	7.4	1	82	high
9	male	54	intestinal	3	IV	positive	83.9	0	89	high
10	male	72	intestinal	2	III	positive	48.7	1	0	low
11	female	55	diffuse	3	II	negative	87.6	0	0	low
12	female	67	intestinal	3	II	negative	89.2	0	11	low
13	male	58	intestinal	2	III	positive	55.7	0	13	low
14	male	35	diffuse	3	III	positive	67.4	0	2	low
15	male	79	intestinal	3	II	positive	15.6	0	1	low
16	female	75	diffuse	3	IV	positive	24.7	1	4	low
17	male	75	intestinal	2	III	positive	11.2	1	8	low
18	male	84	intestinal	2	I	negative	70.8	0	7	low
19	male	61	diffuse	3	III	positive	75.8	0	53	high
20	female	63	intestinal	2	IV	positive	13.8	1	23	low
21	male	72	intestinal	2	III	positive	40	1	0	low
22	male	53	diffuse	3	IV	positive	9	1	0	low
23	female	78	intestinal	2	I	negative	81.1	0	3	low
24	female	58	intestinal	2	III	positive	22.7	0	51	high
25	male	75	intestinal	1	I	negative	9	1	56	high
26	male	49	diffuse	3	IV	positive	7.5	1	54	high
27	male	50	diffuse	3	IV	positive	10.6	1	0	low
28	male	82	diffuse	3	IV	positive	17.4	1	52	high
29	female	73	diffuse	3	IV	positive	12.3	1	61	high
30	female	44	intestinal	2	III	positive	12.6	1	0	low
31	male	68	intestinal	2	II	positive	15.3	0	0	low

32	male	61	intestinal	3	IV	positive	11.4	1	11	low
33	female	88	intestinal	2	III	positive	0.7	0	18	low
34	female	82	intestinal	3	III	positive	4.1	0	62	high
35	male	80	intestinal	3	III	positive	11.3	1	59	high
36	male	77	intestinal	2	II	positive	73.2	0	31	low
37	male	88	intestinal	3	III	positive	9.4	1	72	high
38	male	64	intestinal	2	II	positive	76.5	0	54	high
39	male	73	intestinal	2	III	positive	7.2	0	33	low
40	male	68	diffuse	3	IV	positive	1.5	0	11	low
41	male	64	diffuse	3	IV	positive	6.7	1	51	high
42	male	62	intestinal	2	II	positive	75.4	0	22	low
43	female	78	intestinal	2	III	positive	20.3	1	0	low
44	male	83	diffuse	3	IV	positive	24.2	1	58	high
45	male	69	intestinal	2	IV	positive	3.9	1	51	high
46	male	75	intestinal	2	I	negative	56.4	0	11	low
47	male	74	diffuse	3	III	positive	2.3	0	72	high
48	female	66	intestinal	3	III	positive	43.6	1	58	high
49	male	59	diffuse	3	IV	positive	34.1	1	25	low
50	male	65	intestinal	2	II	positive	125.8	0	11	low
51	male	76	diffuse	3	III	positive	8.4	0	53	high
52	male	68	intestinal	2	I	negative	93.6	1	34	low
53	female	48	diffuse	3	I	negative	120.4	0	0	low
54	female	87	diffuse	3	III	positive	12	1	59	high
55	female	43	intestinal	3	IV	positive	6	1	32	low
56	male	73	intestinal	2	IV	positive	2	1	64	high
57	male	73	intestinal	2	II	negative	1.3	1	51	high
58	male	58	intestinal	3	I	negative	100.4	0	4	low
59	male	76	diffuse	3	IV	positive	12	1	0	low
60	male	61	intestinal	3	IV	positive	8.9	1	84	high
61	female	77	diffuse	3	IV	positive	0.8	0	38	low
62	female	86	intestinal	2	III	positive	20.1	1	61	high
63	male	42	diffuse	3	IV	positive	13.5	1	52	high
64	male	67	intestinal	1	I	negative	116.1	0	31	low
65	female	86	intestinal	3	III	positive	0.3	0	2	low
66	male	70	diffuse	3	IV	positive	2.3	0	8	low
67	male	54	intestinal	3	II	positive	114.6	0	35	low

68	male	71	diffuse	3	II	negative	29.2	1	13	low
69	female	42	diffuse	3	IV	positive	16.2	1	63	high
70	male	36	diffuse	3	III	positive	14.8	1	4	low
71	female	54	intestinal	2	III	positive	29.8	1	27	low
72	female	69	diffuse	3	IV	positive	11.9	1	3	low
73	male	63	diffuse	3	III	positive	55.1	0	1	low
74	male	72	intestinal	3	III	positive	10.8	0	42	low
75	female	38	diffuse	3	IV	positive	8.3	1	15	low
76	male	79	diffuse	3	I	negative	77.1	0	0	low
77	male	62	diffuse	3	III	positive	16.2	1	61	high
78	female	69	intestinal	2	III	positive	43	0	3	low
79	female	73	intestinal	2	IV	positive	6.3	1	52	high
80	female	50	diffuse	3	I	negative	102	0	16	low
81	male	57	intestinal	1	II	positive	108.8	0	2	low
82	male	71	intestinal	2	I	negative	107.3	0	0	low
83	male	58	intestinal	2	IV	positive	35.1	1	7	low
84	male	77	intestinal	2	III	positive	15.5	0	11	low
85	female	75	intestinal	3	I	negative	108.6	0	3	low
86	male	72	intestinal	2	IV	positive	1.8	0	39	low
87	male	77	intestinal	1	I	negative	106.3	0	0	low
88	male	54	intestinal	3	III	positive	0.5	0	8	low
89	male	84	intestinal	2	I	negative	45.8	0	51	high
90	female	50	diffuse	3	III	positive	32.6	0	22	low
91	female	62	intestinal	3	IV	positive	3.8	1	28	low
92	female	77	diffuse	3	IV	positive	28.6	1	3	low
93	male	77	diffuse	3	III	positive	116.2	1	0	low
94	male	72	intestinal	3	III	positive	34.9	1	5	low
95	female	46	diffuse	3	IV	positive	5.9	1	71	high
96	female	64	intestinal	2	III	positive	9.5	1	24	low
97	female	67	intestinal	3	IV	positive	9.3	1	3	low
98	female	73	diffuse	3	III	positive	9	1	43	low
99	female	74	diffuse	3	III	positive	7.8	1	54	high
100	female	78	diffuse	3	I	positive	16.1	1	92	high
101	male	56	diffuse	3	III	positive	143.4	0	52	high
102	female	64	diffuse	3	IV	positive	7.9	1	83	high
103	male	70	diffuse	2	IV	positive	11.7	1	12	low

104	female	76	intestinal	2	II	positive	6	1	96	high
105	female	83	intestinal	2	III	positive	19.1	1	71	high
106	male	72	intestinal	3	III	positive	17.2	1	0	low
107	male	48	intestinal	3	II	positive	133	0	67	high
108	female	42	diffuse	3	IV	positive	3.1	1	52	high
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111	male	66	diffuse	3	III	positive	22.7	1	53	high
112	male	82	intestinal	2	III	positive	24.5	1	34	low
113	female	81	diffuse	3	IV	positive	11.1	1	2	low
114	male	52	diffuse	3	IV	positive	2.7	1	19	low
115	male	48	intestinal	2	III	positive	22	1	55	high
116	male	77	diffuse	3	III	positive	76	1	12	low
117	female	72	intestinal	3	I	positive	20.6	1	24	low
118	male	59	diffuse	3	IV	positive	98.7	0	5	low
119	female	86	intestinal	2	III	positive	20	1	4	low
120	male	65	diffuse	3	III	positive	116.4	0	10	low
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125	male	62	diffuse	3	III	positive	55.1	1	56	high
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127	female	38	diffuse	3	IV	positive	8.3	1	0	low
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130	male	57	intestinal	1	II	positive	108.8	0	11	low
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132	male	77	intestinal	2	III	positive	15.5	0	37	low
133	female	62	diffuse	3	IV	positive	3.8	1	53	high
134	male	56	diffuse	3	IV	positive	10.4	1	8	low
135	male	64	intestinal	2	III	positive	32.3	1	51	high
136	male	50	diffuse	3	II	positive	17.2	1	9	low
137	female	73	diffuse	2	IV	positive	72.3	1	11	low
138	male	42	intestinal	3	II	positive	20.4	1	26	low
139	male	76	intestinal	1	II	positive	99.6	0	71	high

140	male	32	diffuse	3	III	positive	58.6	1	35	low
141	female	68	intestinal	3	I	negative	107.8	0	26	low
142	female	55	diffuse	3	IV	negative	106.9	0	14	low
143	female	53	diffuse	3	IV	positive	103.1	1	59	high
144	male	51	intestinal	2	IV	positive	15.2	1	88	high
145	male	64	intestinal	2	III	positive	53.5	1	33	low
146	male	70	intestinal	2	I	negative	102	0	46	low
147	male	58	intestinal	2	III	positive	96.1	0	91	high
148	male	82	intestinal	2	I	negative	39.1	0	14	low
149	female	50	diffuse	3	IV	positive	14.4	1	7	low
150	male	71	diffuse	3	II	negative	91.2	0	59	high
151	male	76	diffuse	3	I	negative	0	0	27	low
152	male	66	diffuse	3	IV	positive	4.9	1	61	high
153	male	37	intestinal	2	II	negative	90	0	42	low
154	male	76	diffuse	3	III	positive	10.6	1	83	high
155	male	65	intestinal	2	II	positive	11.8	0	48	low
156	male	76	diffuse	3	I	positive	6.9	1	78	high
157	male	77	intestinal	3	I	positive	78.1	1	89	high
158	female	71	diffuse	3	III	positive	17.9	1	42	low
159	male	73	diffuse	3	IV	positive	7.4	1	75	high
160	male	60	intestinal	3	I	negative	15.8	1	86	high
161	male	71	diffuse	3	IV	positive	40.2	1	69	high
162	male	35	diffuse	3	I	negative	88.7	0	74	high
163	male	69	diffuse	3	IV	positive	3.9	1	33	low
164	male	47	diffuse	3	III	positive	5.1	0	18	low
165	male	48	intestinal	2	IV	positive	2.3	0	55	high
166	male	44	diffuse	3	IV	positive	12.8	1	45	low
167	male	61	diffuse	3	IV	positive	8.8	1	4	low
168	female	44	diffuse	3	IV	positive	7.1	1	96	high
169	female	48	intestinal	2	I	negative	80.8	0	54	high
170	male	55	intestinal	2	IV	positive	52.4	1	77	high
171	male	82	intestinal	2	IV	positive	7.8	1	91	high
172	male	74	diffuse	3	III	positive	29.5	1	82	high
173	female	67	intestinal	2	I	negative	80.6	0	74	high
174	male	67	diffuse	3	IV	negative	2.4	1	7	low
175	male	56	intestinal	2	I	positive	29.7	0	82	high

176	male	61	diffuse	3	III	positive	7.4	1	11	low
177	female	57	intestinal	3	I	negative	15.9	0	37	low
178	male	66	intestinal	3	II	positive	1	0	74	high
179	female	60	diffuse	3	IV	positive	5.9	1	100	high
180	male	56	diffuse	3	IV	positive	78.9	0	3	low
181	male	65	intestinal	3	I	negative	79.1	0	25	low
182	female	73	diffuse	3	III	positive	19.3	1	77	high
183	male	69	diffuse	3	IV	positive	10.3	1	56	high
184	male	78	diffuse	3	IV	positive	6.2	1	67	high
185	female	65	intestinal	3	III	positive	41.7	1	100	high
186	male	70	diffuse	3	I	negative	28.5	1	55	high
187	male	71	intestinal	2	III	positive	3.3	1	51	high
188	male	57	diffuse	3	IV	positive	55.4	1	69	high
189	male	65	diffuse	3	IV	positive	24.9	1	76	high
190	male	75	intestinal	3	II	positive	30.7	1	19	low
191	male	52	intestinal	2	III	positive	27.5	1	92	high
192	male	54	diffuse	3	III	positive	73.3	0	45	low
193	male	60	intestinal	3	II	positive	69.4	0	74	high
194	male	37	intestinal	2	I	negative	71.2	0	100	high
195	female	80	diffuse	3	II	negative	26.6	0	66	high
196	female	66	intestinal	2	I	positive	75.2	0	67	high
197	male	66	intestinal	3	IV	positive	18.7	1	81	high
198	male	77	intestinal	2	I	negative	70.9	0	57	high
199	female	68	intestinal	3	I	negative	70.8	0	52	high
200	female	59	diffuse	3	II	negative	32.3	1	3	low
201	male	50	diffuse	3	IV	positive	9.8	1	49	low
202	female	75	intestinal	2	I	negative	51.6	1	67	high
203	male	52	diffuse	3	IV	positive	7	1	88	high
204	male	51	intestinal	2	IV	positive	20.9	1	39	low
205	male	56	intestinal	2	IV	positive	25.9	1	40	low
206	male	67	intestinal	2	I	negative	70.4	0	26	low
207	male	54	diffuse	3	I	positive	66.2	0	32	low
208	male	76	diffuse	3	I	positive	1.8	0	93	high
209	male	49	diffuse	3	IV	positive	5.7	0	4	low
210	male	51	intestinal	2	II	positive	68.7	0	92	high
211	male	58	intestinal	2	II	positive	68	0	45	low

212	female	49	intestinal	2	III	positive	54	0	93	high
213	male	55	diffuse	3	III	positive	62.7	0	72	high
214	male	88	intestinal	2	III	positive	17.4	0	46	low
215	female	48	diffuse	3	I	negative	25.2	0	43	low
216	male	62	diffuse	3	I	negative	64.9	1	61	high
217	male	65	intestinal	1	III	positive	64.4	0	77	high
218	female	51	diffuse	3	IV	positive	20.8	1	47	low
219	female	49	diffuse	3	III	positive	62.4	0	7	low
220	female	61	intestinal	2	II	positive	42	1	97	high
221	male	74	intestinal	2	I	negative	7.4	1	65	high
222	male	53	intestinal	2	IV	positive	29.5	1	92	high
223	male	57	diffuse	3	III	positive	20.6	1	13	low
224	female	50	intestinal	2	II	positive	60.9	0	11	low
225	male	76	intestinal	2	IV	positive	2.1	1	89	high
226	male	38	diffuse	3	III	positive	27.2	1	27	low
227	male	82	diffuse	3	III	positive	9.2	0	100	high
228	male	56	intestinal	2	IV	positive	61.1	0	72	high
229	male	51	diffuse	3	IV	positive	20.7	0	31	low
230	female	76	intestinal	2	I	negative	59.3	0	60	high
231	male	73	diffuse	3	III	positive	5.1	0	31	low
232	female	68	intestinal	2	I	negative	60.4	0	15	low
233	male	64	intestinal	2	I	positive	60.7	0	71	high
234	female	48	diffuse	3	I	negative	59.9	0	51	high
235	female	71	diffuse	3	I	negative	59.1	0	42	low
236	female	69	diffuse	3	IV	positive	6.3	1	96	high
237	male	52	diffuse	3	IV	positive	21.2	0	63	high
238	male	57	intestinal	3	III	positive	41	0	92	high
239	male	44	diffuse	3	IV	positive	47.1	0	0	low
240	male	77	diffuse	3	III	positive	0.8	1	6	low
241	male	52	diffuse	3	IV	positive	21.7	0	52	high
242	female	32	intestinal	2	IV	positive	18	0	89	high
243	female	48	diffuse	3	IV	positive	54.5	0	3	low
244	female	74	intestinal	2	I	negative	55	0	93	high
245	female	72	intestinal	2	III	positive	44.2	0	24	low
246	male	72	diffuse	3	III	positive	22.5	0	11	low
247	female	41	diffuse	3	III	positive	0.6	0	65	high

248	male	65	intestinal	2	I	negative	50.9	0	45	low
249	male	60	diffuse	3	I	negative	55.9	0	61	high
250	female	72	diffuse	3	IV	positive	21.8	1	0	low
251	male	72	diffuse	3	IV	positive	6	1	100	high
252	male	70	intestinal	2	III	positive	51.5	0	19	low
253	male	48	diffuse	3	III	positive	51.6	0	33	low
254	male	62	intestinal	2	I	negative	139.4	0	5	low
255	male	63	diffuse	3	III	positive	15.8	0	39	low
256	male	51	diffuse	3	I	negative	138.1	0	67	high
257	male	61	diffuse	3	II	positive	135.1	0	3	low
258	male	54	intestinal	3	III	positive	70.5	0	62	high
259	male	72	intestinal	2	I	negative	91.6	0	0	low
260	female	77	diffuse	3	IV	positive	49	0	0	low
261	female	34	intestinal	3	I	negative	138.1	0	42	low
262	male	69	intestinal	3	III	positive	127.8	0	98	high
263	female	66	intestinal	1	I	negative	68.7	1	58	high
264	female	32	diffuse	3	I	negative	89.7	0	11	low

DSS, disease-specific survival; LNM, lymph node metastasis.

Table S2. Antibodies used for western blot.

Antibodies	Source	Cat. No.	Working concentration
Rabbit monoclonal anti-MCM6	Abcam	ab201683	1:1000
Mouse monoclonal anti-β-actin	Immunoway	YM3028	1:1000
Rabbit monoclonal anti-YAP	Abcam	ab52771	1:1000
Rabbit monoclonal (D24E4) anti-YAP/TAZ	Cell Signaling	#8418	1:1000
Mouse monoclonal (AE005) anti-FLAG	ABclonal	AE005	1:2000
Rabbit monoclonal (92G2) anti-Cyclin D1	Cell Signaling	#2978	1:1000
Mouse monoclonal (DCS83) anti-CDK6	Cell Signaling	#3136	1:1000
Mouse monoclonal (12D10) anti-PCNA	Immunoway	YM3031	1:2000
Mouse monoclonal anti-p27	BD Biosciences	610241	1:1000
Rabbit anti-Cleaved caspase-7	Cell Signaling	#9491	1:1000
Mouse monoclonal (C9) anti-Caspase-9	Cell Signaling	#9508	1:1000
Rabbit anti-Cleaved PARP	Cell Signaling	#9541	1:500
Rabbit anti-Caspase-7	Cell Signaling	#9492	1:1000
Rabbit monoclonal (46D11) anti-PARP	Cell Signaling	#9532	1:1000
Rabbit anti-phosphorylated YAP (Ser127)	Cell Signaling	#4911	1:1000
Rabbit monoclonal (24E10) anti-E-cadherin	Cell Signaling	#3195	1:1000
Rabbit anti-N-cadherin	Immunoway	YT2988	1:500
Rabbit monoclonal (D21H3) anti-Vimentin	Cell Signaling	#5741	1:1000
Rabbit anti-Claudin-1	Cell Signaling	#4933	1:1000
Rabbit anti-phosphorylated PI3 Kinase p85 (Tyr458)/p55 (Tyr199)	Cell Signaling	#4228	1:1000
Rabbit anti-phosphorylated AKT (Ser473)	Cell Signaling	#9271	1:1000
Rabbit anti-phosphorylated GSK-3β (Ser9)	Cell Signaling	#9336	1:1000
Rabbit anti-AKT	Cell Signaling	#9272	1:1000
Rabbit monoclonal (27C10) anti-GSK-3β	Cell Signaling	#9315	1:1000
Rabbit monoclonal (D84C12) anti-c-Myc	Cell Signaling	#5605	1:1000
Rabbit anti-phosphorylated ATR (Ser428)	Cell Signaling	#2853	1:1000
Rabbit monoclonal (D6H9) anti-phosphorylated ATM (Ser1981)	Cell Signaling	#5883	1:1000
Rabbit monoclonal (133D3) anti-phosphorylated Chk1 (Ser345)	Cell Signaling	#2348	1:1000
Rabbit monoclonal (E1S3S) anti-ATR	Cell Signaling	#13934	1:1000
Rabbit monoclonal (D2E2) anti-ATM	Cell Signaling	#2873	1:1000
Mouse monoclonal (2G1D5) anti-Chk1	Cell Signaling	#2360	1:1000
Goat Anti-Rabbit Immunoglobulins/HRP	Dako	P0448	1:5000
Goat Anti-Mouse Immunoglobulins/HRP	Dako	P0447	1:5000

Table S3. Primer sequences for qPCR.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
For real-time qPCR		
MCM6	GAGGAACTGATTCGTCCTGAGA	CAAGGCCCGACACAGGTAAG
YAP	TAGCCCTGCGTAGCCAGTTA	TCATGCTTAGTCCACTGTCTGT
β-actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG
For ChIP-PCR		
MCM6	TCACACTGTGTTGCCCAGG	AATTAGTCGGGCGTGGTGG

Table S4. Top 15 compounds identified from high-throughput virtual screening.

No.	Compounds	CAS No.	MW	Rotatable bonds	No. HBA	No. HBD	TPSA (Å^2)	Docking score
1	Parishin C	174972-80-6	728.65	15	18	10	308.89	-13.394
2	Validamycin A	37248-47-8	497.49	7	14	12	253.02	-12.226
3	Secoisolariciresinol diglucoside	257930-74-8	686.70	15	16	10	257.68	-12.131
4	Isorhamnetin-3-O-neohespeidoside	55033-90-4	624.54	7	16	9	258.43	-11.918
5	**Purpureaside C**	108648-07-3	786.73	13	20	12	324.44	-11.804
6	Eriocitrin	13463-28-0	596.53	6	15	9	245.29	-11.724
7	1,4-b-D-Xylopentaose	49694-20-4	678.59	12	21	12	333.67	-11.688
8	α,β-Methylene ATP trisodium	1343364-54-4	505.21	11	16	4	236.9	-11.668
9	Ligustroflavone	260413-62-5	724.66	8	18	10	287.89	-11.606
10	ATP	56-65-5	507.18	8	14	7	279.13	-11.589
11	Uridine 5’-diphosphoglucose disodium salt	28053-08-9	566.30	11	18	7	274.99	-11.573
12	Xylotetraose	22416-58-6	546.47	10	17	10	274.75	-11.545
13	Uridine-5'-diphosphate disodium salt	27821-45-0	404.16	6	12	4	223.5	-11.539
14	Rebaudioside A	58543-16-1	967.01	12	23	14	374.13	-11.363
15	Oroxin B	114482-86-9	594.52	7	15	9	249.2	-11.346

MW: molecular weight; HBA: H-bond acceptors; HBD: H-bond donors; TPSA: topological polar surface area.

Table S5. Clinicopathologic features of MCM6 protein expression in GC from Hong Kong tissue microarray cohort.

Variables	Low MCM6 (n = 149)	High MCM6 (n = 115)	*P* value
Age, year, mean ± SD	63.1 ± 12.9	64.5 ± 12.5	0.353
Gender, n (%)			0.670
Male	96 (55.5)	77 (44.5)	
Female	53 (58.2)	38 (41.8)	
Lauren subtype, n (%)			0.997
Diffuse	70 (56.5)	54 (43.5)	
Intestinal	79 (56.4)	61 (43.6)	
Tumor grade, n (%)			0.938
I	5 (55.6)	4 (44.4)	
II	55 (57.9)	40 (42.1)	
III	89 (55.6)	71 (44.4)	
TNM stage, n (%)			0.684
I	32 (55.2)	26 (44.8)	
II	20 (62.5)	12 (37.5)	
III	50 (59.5)	34 (40.5)	
IV	47 (52.2)	43 (47.8)	
Lymph node metastasis, n (%)			0.423
Positive	113 (55.1)	92 (44.9)	
Negative	36 (61.0)	23 (39.0)	

TNM, tumor node metastasis.

Table S6. Cox regression analysis of potential poor prognostic factors for GC patients from Hong Kong cohort.

Variables	Univariate Cox regression analysis		Multivariate Cox regression analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (years)				
>60 vs. ≤60	1.433 (1.001-2.050)	0.049	1.837 (1.279-2.638)	0.001
Gender				
Male vs. Female	0.821 (0.580-1.163)	0.266	---	---
Lauren subtype				
Diffuse vs. Intestinal	1.904 (1.353-2.679)	0.000	1.479 (0.891-2.452)	0.130
Tumor grade				
III vs. I and II	1.277 (1.067-1.527)	0.008	0.956 (0.734-1.245)	0.739
TNM stage				
III and IV vs. I and II	2.434 (1.911-3.098)	0.000	2.024 (1.435-2.855)	0.000
Lymph node metastasis				
Positive vs. Negative	5.292 (2.914-9.612)	0.000	1.721 (0.752-3.943)	0.199
MCM6 protein expression				
High vs. Low	1.577 (1.125-2.212)	0.008	1.545 (1.093-2.184)	0.014

CI, confidence interval; HR, hazard ratio; TNM, tumor node metastasis.

Table S7. List of proteins have physical interactions with MCM6.

Interactor A	Interactor B	Experimental System	Type	Throughput	Scores	Publications (PMID)
MKI67	MCM6	Affinity Capture-MS	physical	High Throughput	68.7	26949251
CCNF	MCM6	Affinity Capture-MS	physical	High Throughput	6.119	33729478
FGD2	MCM6	Affinity Capture-MS	physical	High Throughput	4.790311538	32203420
DEPDC1B	MCM6	Affinity Capture-MS	physical	High Throughput	3.114018714	32203420
USP7	MCM6	Affinity Capture-MS	physical	High Throughput	2.49	19615732
TNFAIP3	MCM6	Affinity Capture-MS	physical	High Throughput	1.04	19615732
USP49	MCM6	Affinity Capture-MS	physical	High Throughput	1.04	19615732
MCM6	MCM3	Co-fractionation	physical	High Throughput	1	26344197
MCM6	MCM5	Co-fractionation	physical	High Throughput	1	26344197
MCM2	MCM6	Co-fractionation	physical	High Throughput	1	26344197
MCM4	MCM6	Co-fractionation	physical	High Throughput	1	26344197
MCM6	MCM7	Co-fractionation	physical	High Throughput	1	26344197
MCM6	MCMBP	Co-fractionation	physical	High Throughput	1	26344197
MCM2	MCM6	Affinity Capture-MS	physical	High Throughput	0.99986232	33961781

ASF1A	MCM6	Affinity Capture-MS	physical	High Throughput	0.999188517	28514442
MCM2	MCM6	Co-fractionation	physical	High Throughput	0.998	22939629
ASF1A	MCM6	Affinity Capture-MS	physical	High Throughput	0.996927062	33961781
H2AFX	MCM6	Affinity Capture-MS	physical	High Throughput	0.996572984	33961781
ASF1B	MCM6	Affinity Capture-MS	physical	High Throughput	0.9954816	28514442
MCM4	MCM6	Co-fractionation	physical	High Throughput	0.994	22939629
HIST1H2BA	MCM6	Affinity Capture-MS	physical	High Throughput	0.989445436	26186194
HIST1H3H	MCM6	Affinity Capture-MS	physical	High Throughput	0.987932919	33961781
HIST1H4B	MCM6	Affinity Capture-MS	physical	High Throughput	0.983718826	33961781
ASF1B	MCM6	Affinity Capture-MS	physical	High Throughput	0.982780246	33961781
HIST1H3A	MCM6	Affinity Capture-MS	physical	High Throughput	0.97702389	28514442
MCM6	MCM7	Co-fractionation	physical	High Throughput	0.973	22939629
HIST1H2BA	MCM6	Affinity Capture-MS	physical	High Throughput	0.967116608	28514442
HIST1H4I	MCM6	Affinity Capture-MS	physical	High Throughput	0.962819678	33961781
H3F3B	MCM6	Affinity Capture-MS	physical	High Throughput	0.954486055	33961781
HIST1H2AE	MCM6	Affinity Capture-MS	physical	High Throughput	0.946957461	33961781

MCM3	MCM6	Affinity Capture-MS	physical	High Throughput	0.944561808	28514442
H2AFX	MCM6	Affinity Capture-MS	physical	High Throughput	0.926195261	28514442
HIST1H4F	MCM6	Affinity Capture-MS	physical	High Throughput	0.920560389	33961781
HIST1H4A	MCM6	Affinity Capture-MS	physical	High Throughput	0.909759502	33961781
MCM10	MCM6	Affinity Capture-MS	physical	High Throughput	0.907069568	26186194
HIST1H2AB	MCM6	Affinity Capture-MS	physical	High Throughput	0.899304838	33961781
HIST1H3F	MCM6	Affinity Capture-MS	physical	High Throughput	0.899262594	33961781
HIST1H4C	MCM6	Affinity Capture-MS	physical	High Throughput	0.898491564	33961781
HIST1H4L	MCM6	Affinity Capture-MS	physical	High Throughput	0.893576767	33961781
HIST1H3A	MCM6	Affinity Capture-MS	physical	High Throughput	0.886145524	33961781
SSRP1	MCM6	Affinity Capture-MS	physical	High Throughput	0.881012864	28514442
HIST1H2BA	MCM6	Affinity Capture-MS	physical	High Throughput	0.878743859	33961781
SPIN2B	MCM6	Affinity Capture-MS	physical	High Throughput	0.873822839	26186194
MCM3	MCM6	Affinity Capture-MS	physical	High Throughput	0.872802882	33961781
HIST1H4A	MCM6	Affinity Capture-MS	physical	High Throughput	0.870251555	28514442
HIST1H3B	MCM6	Affinity Capture-MS	physical	High Throughput	0.851695182	33961781

MCM3	MCM6	Co-fractionation	physical	High Throughput	0.843	22939629
MCM5	MCM6	Co-fractionation	physical	High Throughput	0.838	22939629
MCM10	MCM6	Affinity Capture-MS	physical	High Throughput	0.822595333	28514442
MCM6	SF3B1	Co-fractionation	physical	High Throughput	0.821	22939629
ING5	MCM6	Affinity Capture-MS	physical	High Throughput	0.807339349	33961781
MCM6	SF3A1	Co-fractionation	physical	High Throughput	0.779	22939629
SPIN2B	MCM6	Affinity Capture-MS	physical	High Throughput	0.765446546	28514442
DCTN2	MCM6	Co-fractionation	physical	High Throughput	0.764	22939629
TAF10	MCM6	Affinity Capture-MS	physical	High Throughput	0.677452	17643375
MAPRE2	MCM6	Co-fractionation	physical	High Throughput	0.087790617	26344197
SDHA	MCM6	Affinity Capture-MS	physical	High Throughput	0.000446535	29128334
SOD1	MCM6	Affinity Capture-MS	physical	High Throughput	0.000296463	29128334
DLD	MCM6	Affinity Capture-MS	physical	High Throughput	0.000241786	29128334

Table S8. siRNA used in this study.

siRNA	Sequence (5'-3')	
siMCM6-1	Sense	GGAACAAUUUAACCAGCAATT
	Antisense	UUGCUGGUUAAAUUGUUCCTT
siMCM6-2	Sense	GCUCCCAAAGCCUCCUUAATT
	Antisense	UUAAGGAGGCUUUGGGAGCTT

siRNA	Source	Cat. No.
siYAP-1	QIAGEN	SI02662954
siYAP-2	QIAGEN	SI04438651
Negative control siRNA	QIAGEN	1027281

Table S9. shRNA and DNA plasmids.

shRNA knockdown		
shRNA	**Target sequence (5'-3')**	
shMCM6-1	TACAGCTAAGAGCCAATTTCTCAAGCACG	
shMCM6-2	GCATTCCAAGACCTGCCTACCAGACACAA	
Overexpression		
Gene	**Genbank No.**	**Source**
Empty vector (EV)	N/A	GeneChem
MCM6	NM_005915	GeneChem/31108-15

Table S10. Antibodies used for immunohistochemistry.

Antibodies	Source	Cat. No.	Working concentration	Antigen retrieval buffer
Rabbit monoclonal anti-MCM6	Abcam	ab201683	1:100	Tris-EDTA
Rabbit monoclonal anti-YAP	Abcam	ab52771	1:100	Tris-EDTA
Mouse monoclonal anti-PCNA	Immunoway	YM3031	1:200	Tris-EDTA
Rabbit anti-phosphorylated AKT (Ser473)	Cell Signaling	#9271	1:100	Tris-EDTA
Rabbit anti-Cleaved PARP	Cell Signaling	#9541	1:100	Tris-EDTA

References

1. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999; 96: 857-68.