Supplemental Figure 1: Ascorbate induces apoptosis and inhibits proliferation of gastric cancer cells. (A) Quantification of cell apoptosis in the indicated cells treated with ascorbate (4mM, 2h) were determined by Annexin V/propidium iodide (PI) assays. (B) The intracellular ATP level in the indicated cells treated with ascorbate for 1h was measured. (C) Immunoblotting of γ-H2AX in MGC803 cells after treatment with ascorbate for 2h. β-Actin was used as a loading control. (D) Paraffin-embedded tumor sections were stained with anti-Ki67 and cleaved caspase 3 antibody (scale bar: 50 μm), the proliferation and apoptotic index was quantified. Data in A, B and D (n = 4 for A, B and n = 6 for D) are presented as mean ±S.D. *P < 0.05 versus control.

Supplemental Figure 2: Ascorbate acts as pro-oxidant in gastric cancer. (A) Quantification of ROS contents in the presence and absence of ascorbate (1mM or 2mM for 1h) in the indicated cells as detected by the fluorescent probe DCF-DA. (B) Relative levels of NADPH/NADP+ in the indicated cells in the presence and absence of ascorbate at 1mM for 1h. (C, D) Pretreatment with NAC or catalase attenuated pro-apoptosis effects of ascorbate in the indicated cells. (E) Apoptosis analysis of SGC7901 cells treated with DFO (200μM) and DTPA (1mM) for 3h followed by 2h exposure to ascorbate (4mM) in the continued presence of these chelators. (F) Apoptosis analysis of SGC7901 cells in the presence

or absence of red blood cells (RBC) at 25% hematocrit treated with ascorbate at 2mM for 2h. Data in A, B, C, D, E and F (n = 3) are presented as mean ±S.D. *P < 0.05 versus control. NS, non-significant.

Supplemental Figure 3: GLUT1 mediates anti-tumor activity of ascorbate in gastric cancer cells. (A) Concentrations of glucose and lactate were determined with the SBA40C Biosensor. The glucose uptake was determined as the concentration in the fresh medium minus that in the cell culture medium for 24h, while the lactate production was calculated as the concentration in the cell culture medium for 24h minus that in the fresh medium. (B) qPCR analysis of HK2, Aldolase, PFK1, PKM2 and LDH-A in the indicated cells. (C) Relative levels of DCF-DA, glutathione and ATP in HGC27 cells in the presence and absence of ascorbate at 1mM for 1h. (D) Immunoblotting of γ-H_2AX in HGC27 cells treated with ascorbate at 2mM and 4mM for 2h. β-Actin was used as a loading control. (E-H) qPCR analysis of SVCT1, SVCT2, Glut3 and Glut4 mRNA level in the indicated cells. (I) Immunoblotting of SVCT1, SVCT2, GLUT3 and GLUT4 in the indicated cells. β-Actin was used as a loading control. (J) qPCR analysis of Glut1 mRNA level in the indicated cells. Data in A, B, C, E, F, G, H and J (n = 3) are presented as mean ±S.D. *P < 0.05 versus control.

Supplemental Figure 4: Efficiency of GLUT1 manipulation. (A) Western blot analysis of knockdown efficiency of GLUT1. The membranous proteins were isolated. β-Actin and Na^+/K^+ ATPase was used as cytoplasmic and membranous loading control, respectively. (B) Flow cytometry analysis of GLUT1 fluorescence in the indicated cells. (C) Colony formation of HGC27 cells with enforced GLUT1 expression after ascorbate treatment. (D) Immunoblotting of γ-H_2AX in HGC27 cells with GLUT1 overexpression after treatment with ascorbate for 2h. β-Actin was used as a loading control. (E-F) Mass spectra analysis of ascorbate or DHA in the culture medium after manipulation of GLUT1 expression. Data in B, C, E and F are presented as mean ±S.D. (n = 3). *P < 0.05 versus control. NS, non-significant.

Supplemental Figure 5: GLUT1 is overexpressed in gastric cancer tissues and predicts poor prognosis. (A) Representative staining of GLUT1 in two paired non-tumor tissues and tumor tissues and two paired primary tumor and lymph node metastasis. Scale bar: 100μm. Kaplan-Meier analysis of overall survival based on GLUT1 expression in patients staged as I-II (B) and III-IV (C). (D-E) Kaplan-Meier analysis of overall survival and disease free survival based on membranous GLUT1 expression in all 209 patients.

Supplemental Figure 6: Chemotherapeutic drugs induce ROS and DNA damage. (A) Intracellular glutathione level of the indicated cells treated with irinotecan (40μM, 6h), alone or in combination with ascorbate (1mM, 1h) was measured with spectrophotometric analysis. (B) Representative histograms (left panel) and quantification (right panel) of ROS contents after treatment with irinotecan (40μM, 6h), alone or in combination with ascorbate (1mM, 1h) in the indicated cells as detected by the fluorescent probe DCF-DA. (C) Immunoblotting of γ-H_2AX in indicated cells after treatment with oxaliplatin (50μM), alone or in combination with ascorbate (2mM) for 2h. β-Actin was used as a loading control. (D) Immunoblotting of γ-H_2AX in indicated cells after treatment with irinotecan (50μM), alone or in combination with ascorbate (2mM) for 2h. β-Actin was used as a loading control.

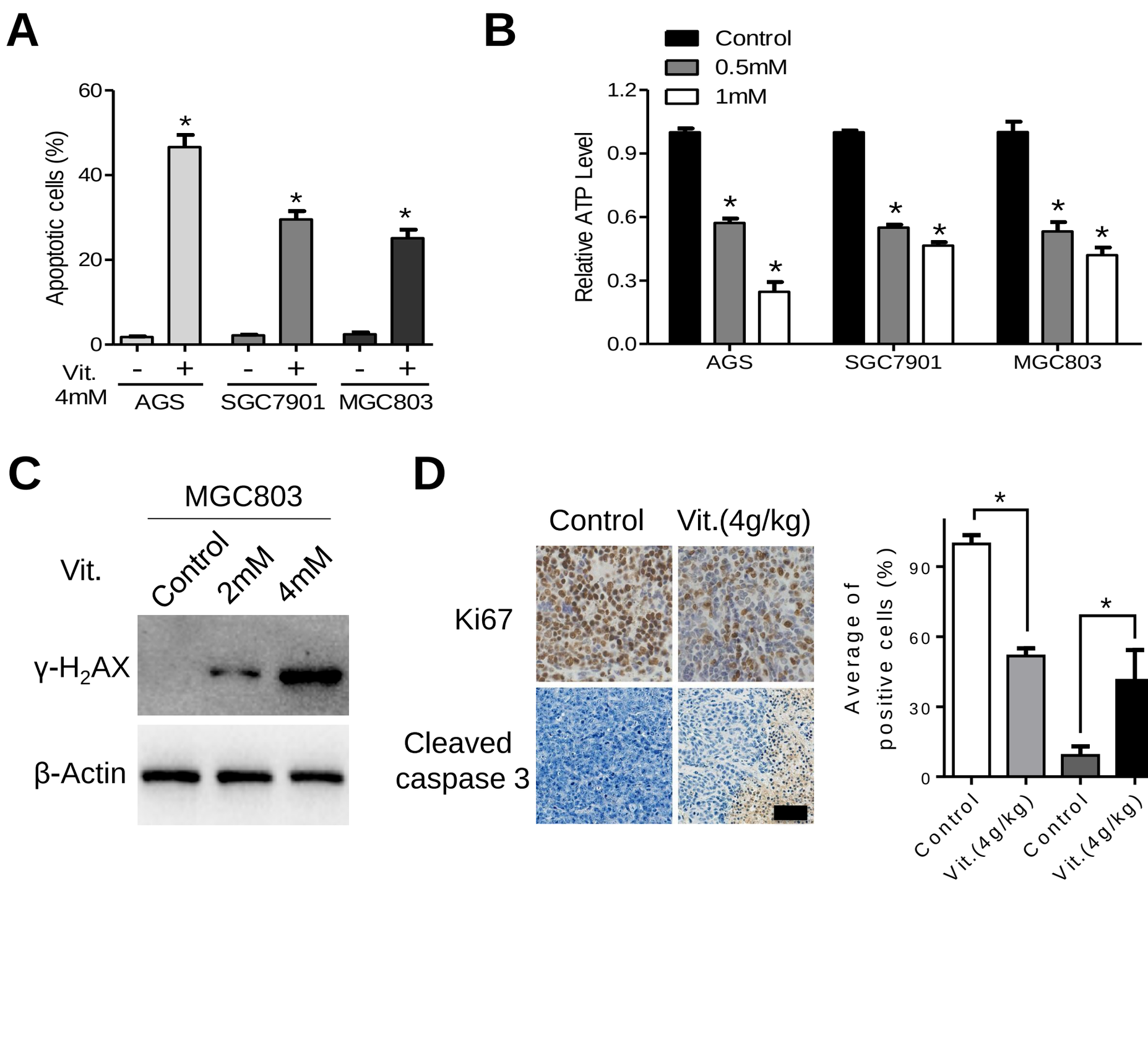
Supplemental Figure 7: Ascorbate synergize with irinotecan in gastric cancer cells. (A) Cell viability of AGS and SGC7901 cells treated with irinotecan alone or combined with ascorbate (1mM,2h) at indicated concentrations was detected by MTS. (B) The combination index (CI) of irinotecan and ascorbate treatment in AGS and SGC7901 cells was analyzed using a median dose-effect method with CalcuSyn software (Biosoft). CI = 1 indicates an additive effect, CI < 1 indicates a synergistic effect and CI > 1 indicates an antagonist effect. (C)

Representative images (left panel) and quantification (right panel) of colony formation assays in AGS and SGC7901 cells treated with irinotecan (2.5μM) and ascorbate (25μM). The predicted value was calculated by multiplying the relative colony numbers in the irinotecan-treated and ascorbate-treated sample. The combination effect is considered additive when the observed value is equal to the predicted value. When observed value is less than the predicted value, the combination effect is considered as synergistic. (D) Representative images (left panel) and quantification (right panel) of Annexin V/PI assays in the indicated cells treated with irinotecan (50μM, 24h) and ascorbate (2mM, 2h). (E) The volume of the tumors and the weight of mice were measured and recorded, and a tumor growth curve was created for each group. (F) Paraffin-embedded tumor sections were stained with anti-Ki67 or cleaved caspase 3 antibody (scale bar: 50μm), the proliferation and apoptosis index was quantified. Data in A, C, D, E and F are presented as mean ±S.D. (n = 4 for A, C, D and n=6 for E, F). *P < 0.05 versus corresponding control.

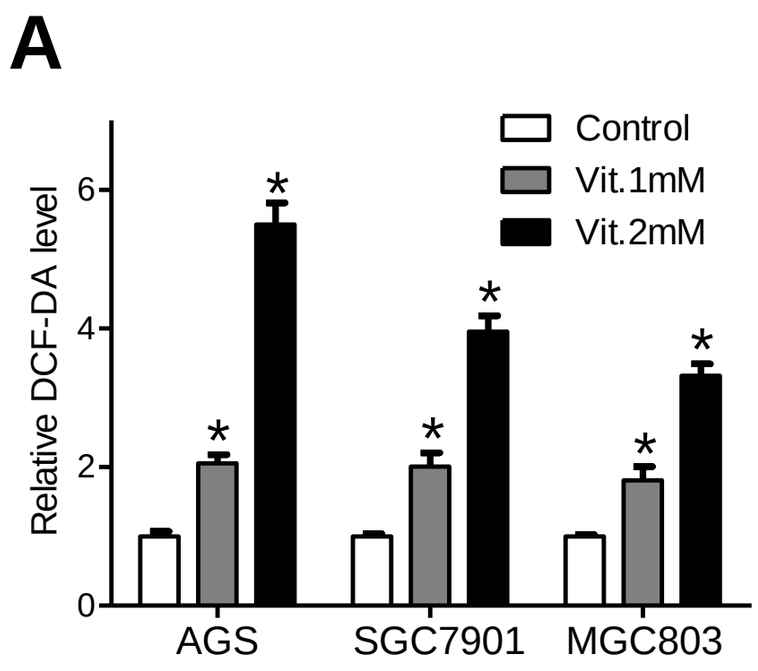
Supplemental Figure 8: Ascorbate synergize with oxaliplatin or irinotecan in MGC803 cells. (A) Representative images (left panel) and quantification (right panel) of colony formation assays in MGC803 cells treated with oxaliplatin (2.0μM) and ascorbate (25μM). (B)

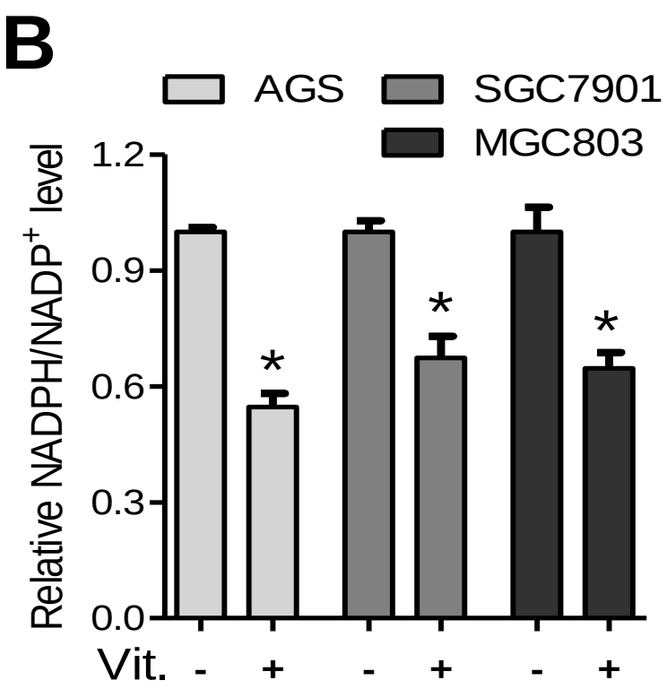
Representative images (left panel) and quantification (right panel) of colony formation assays in MGC803 cells treated with irinotecan (2.5μM) and ascorbate (25μM). The predicted value was calculated by multiplying the relative colony numbers in the irinotecan-treated and ascorbate-treated sample. The combination effect is considered additive when the observed value is equal to the predicted value. When observed value is less than the predicted value, the combination effect is considered as synergistic. Data in A and B are presented as mean ±S.D. (n = 3). *P < 0.05 versus corresponding control.

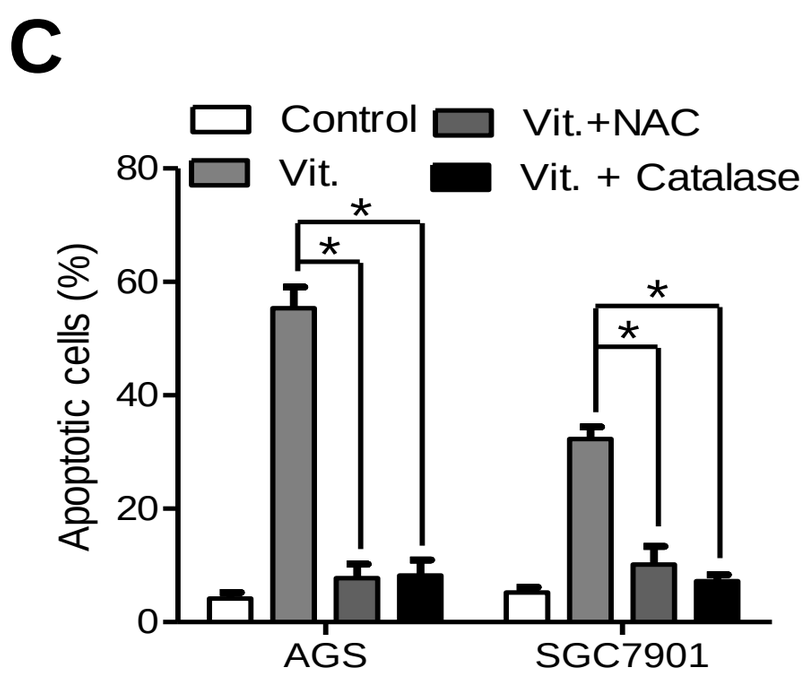
Supplementary Figure 1

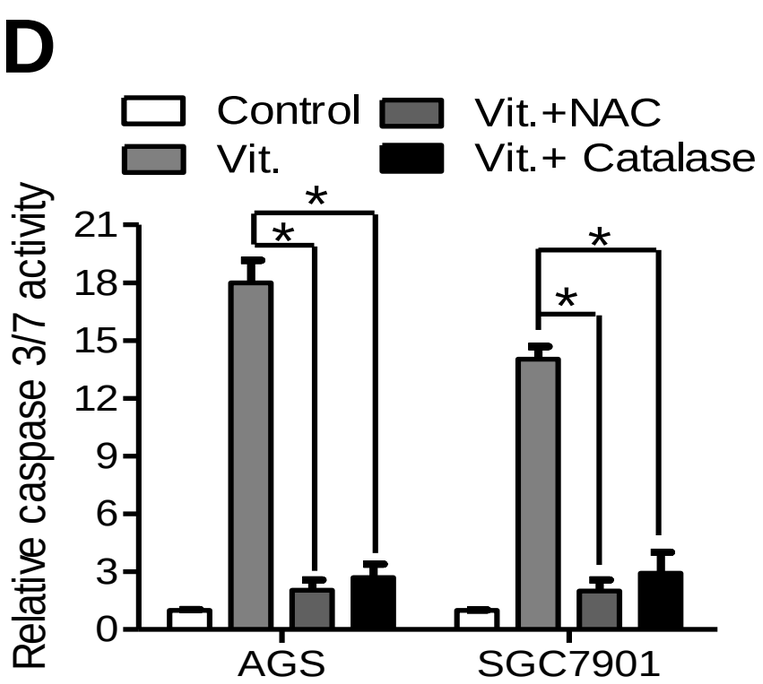


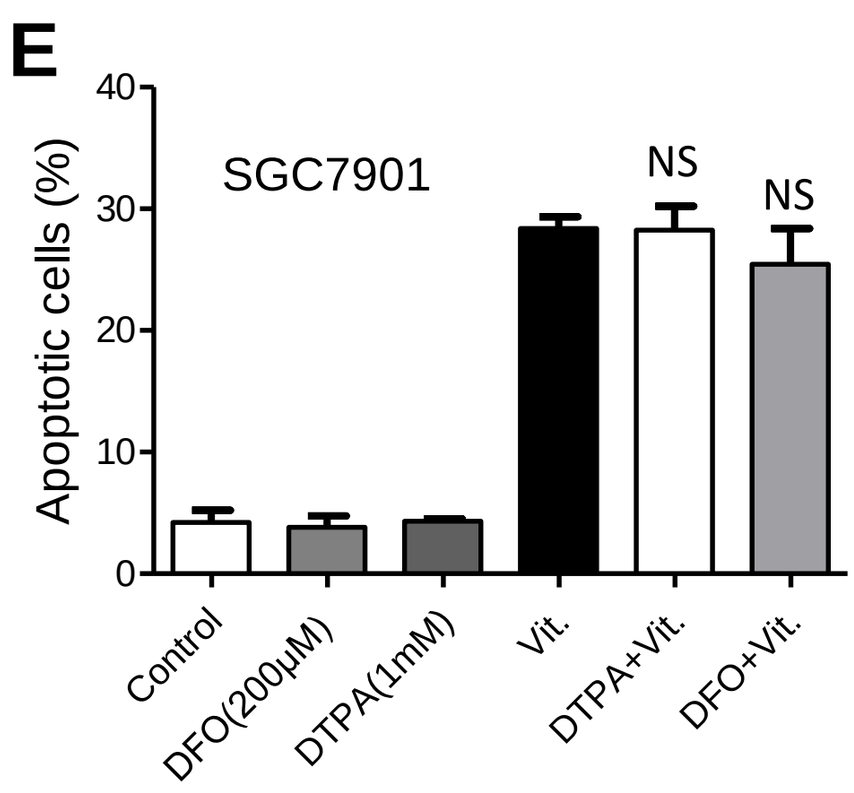
Supplementary Figure 2

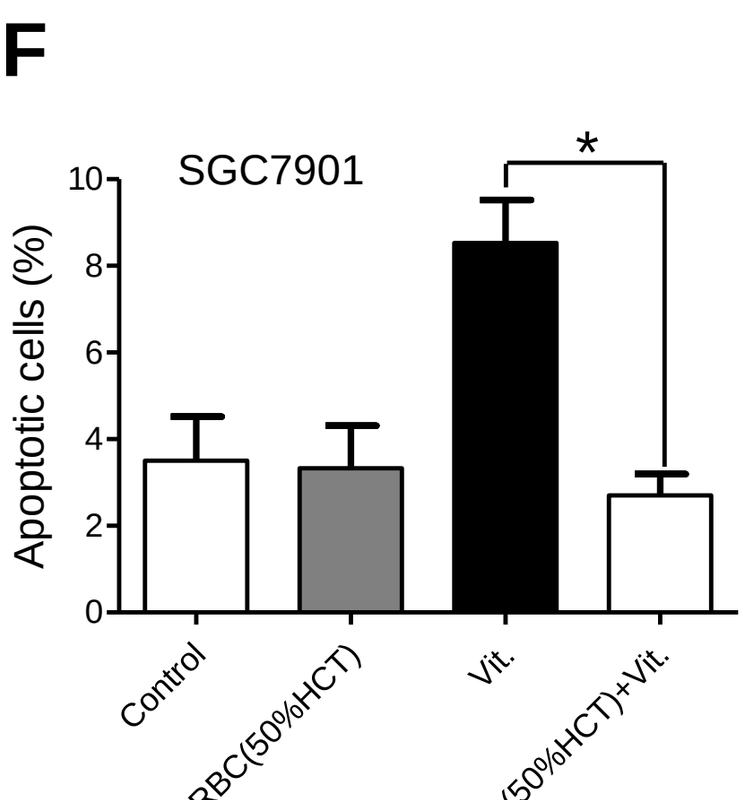




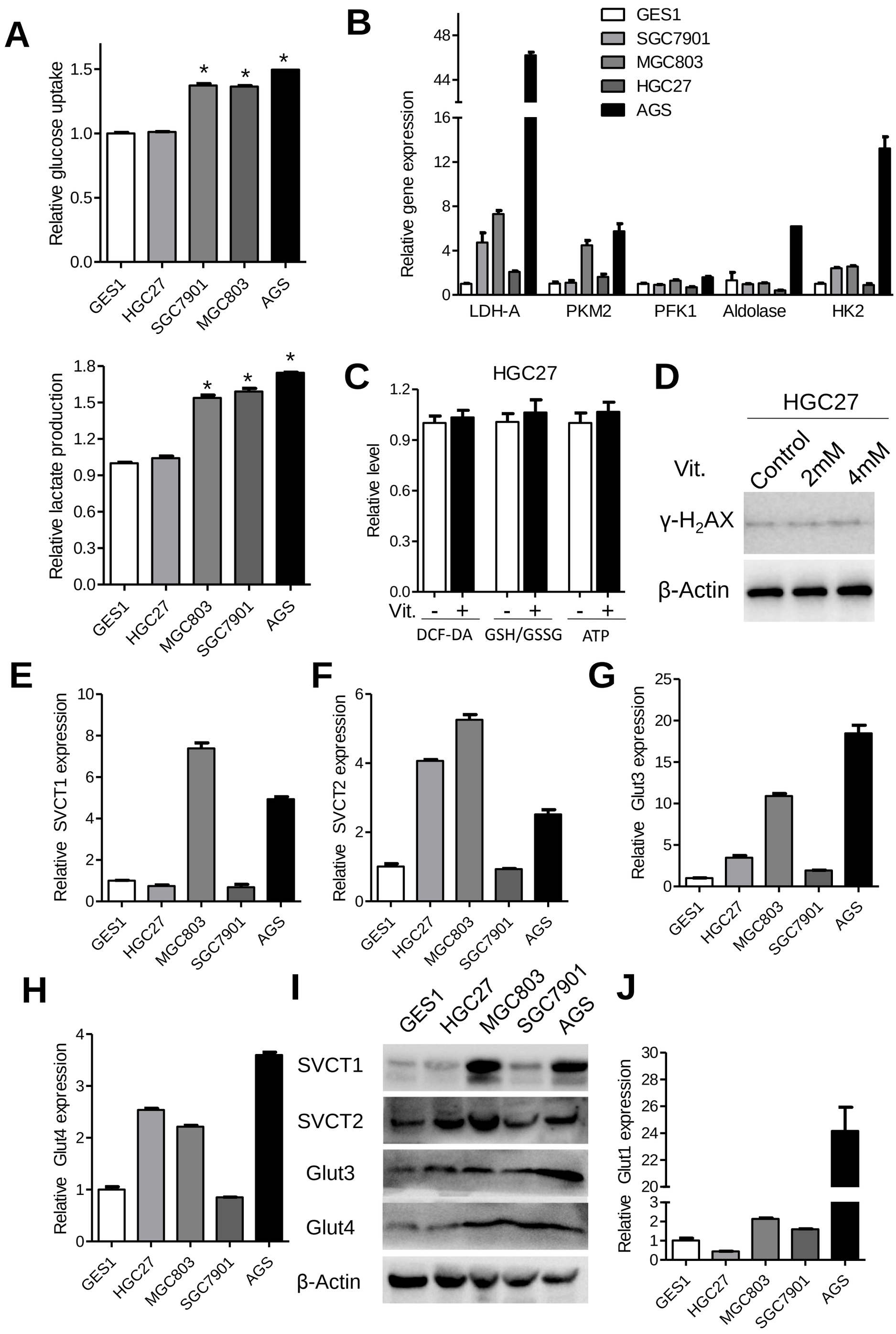




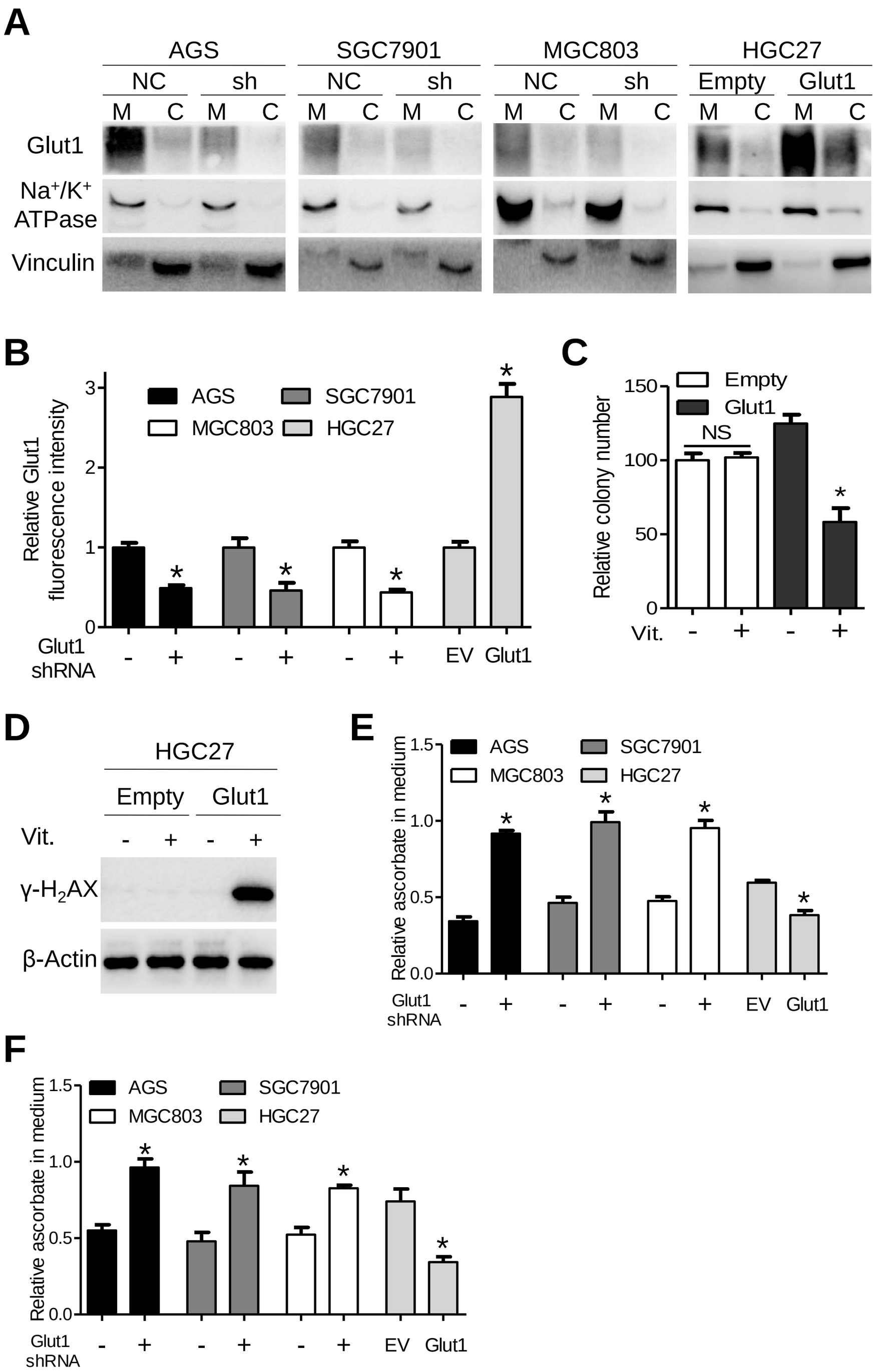




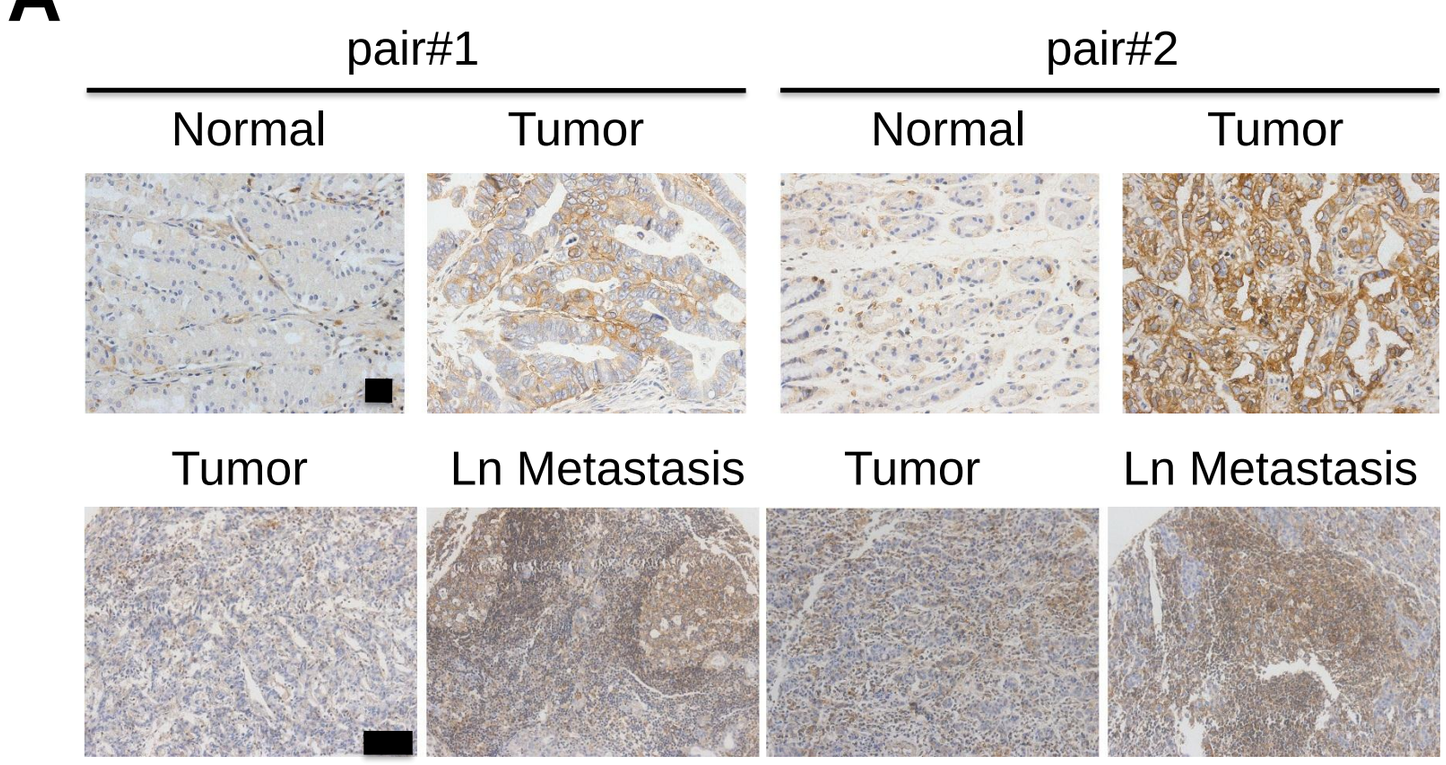
Supplementary Figure 3

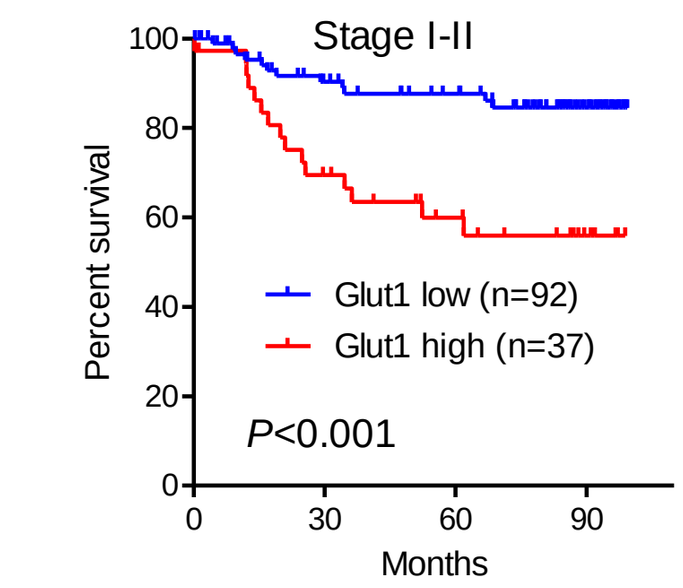


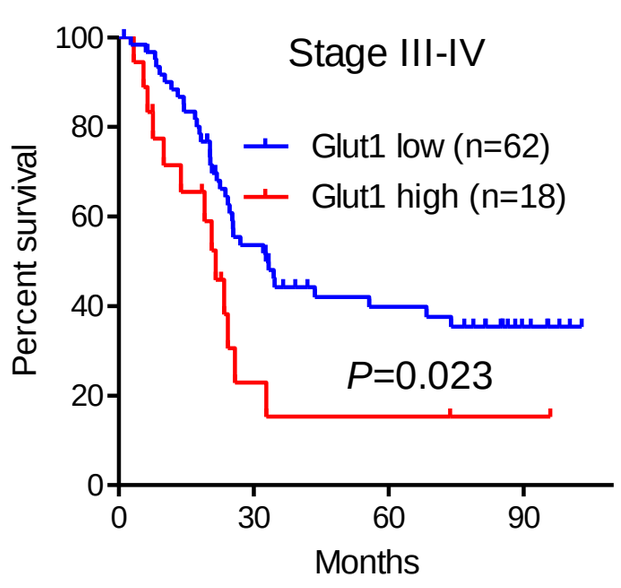
Supplementary Figure 4

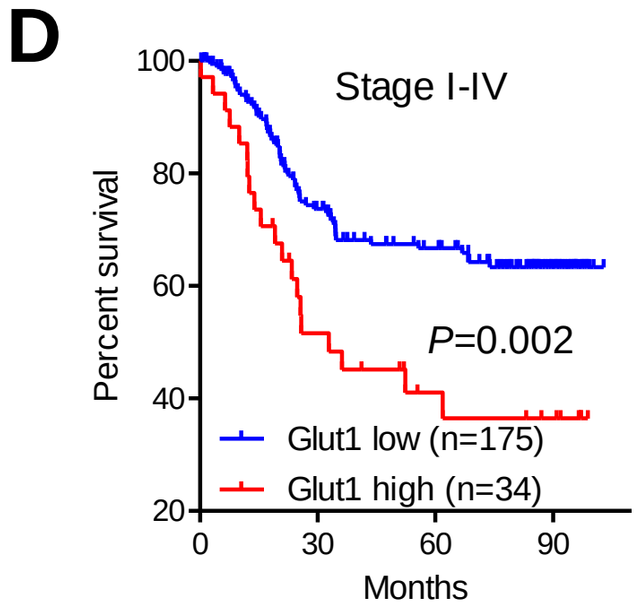


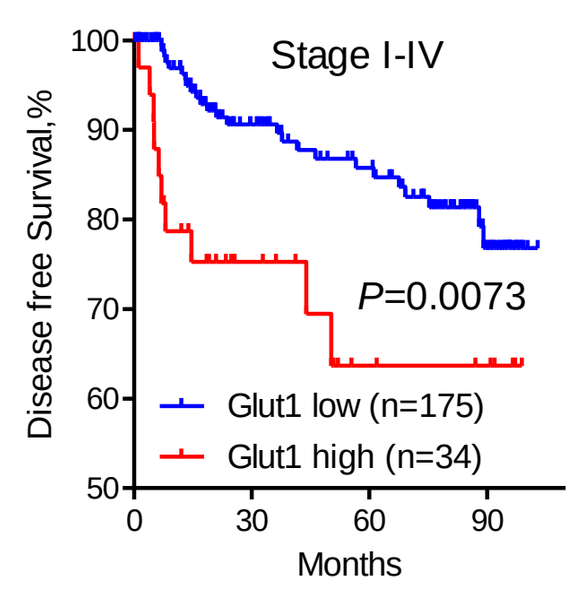
Supplementary Figure 5



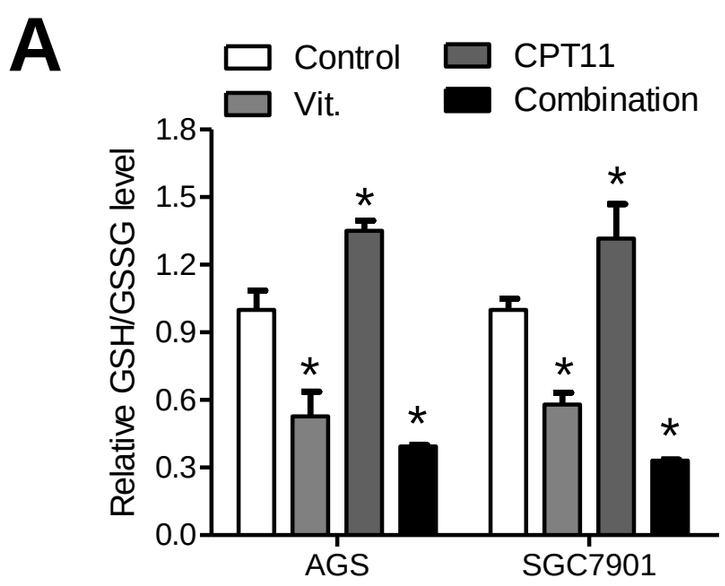


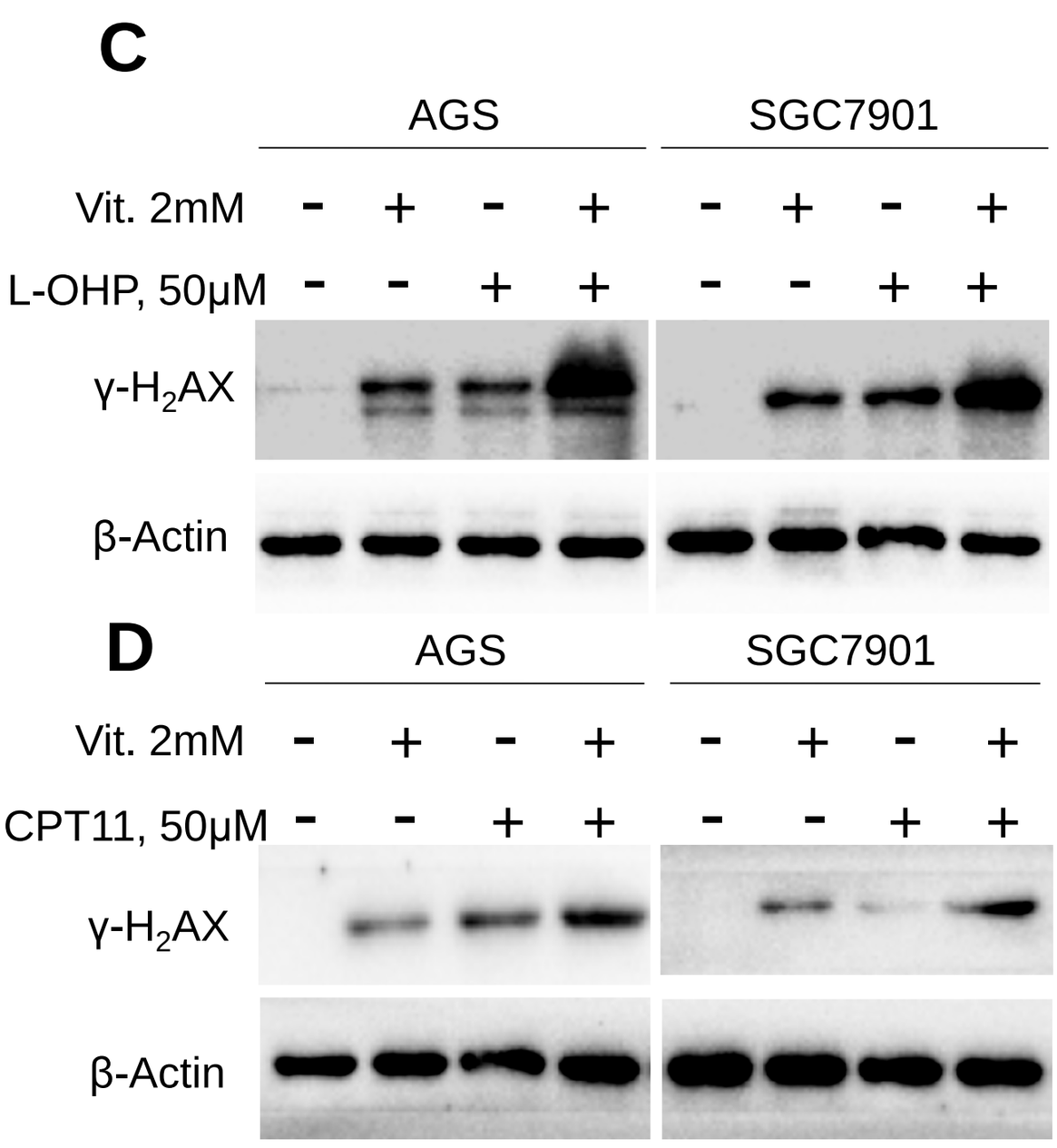


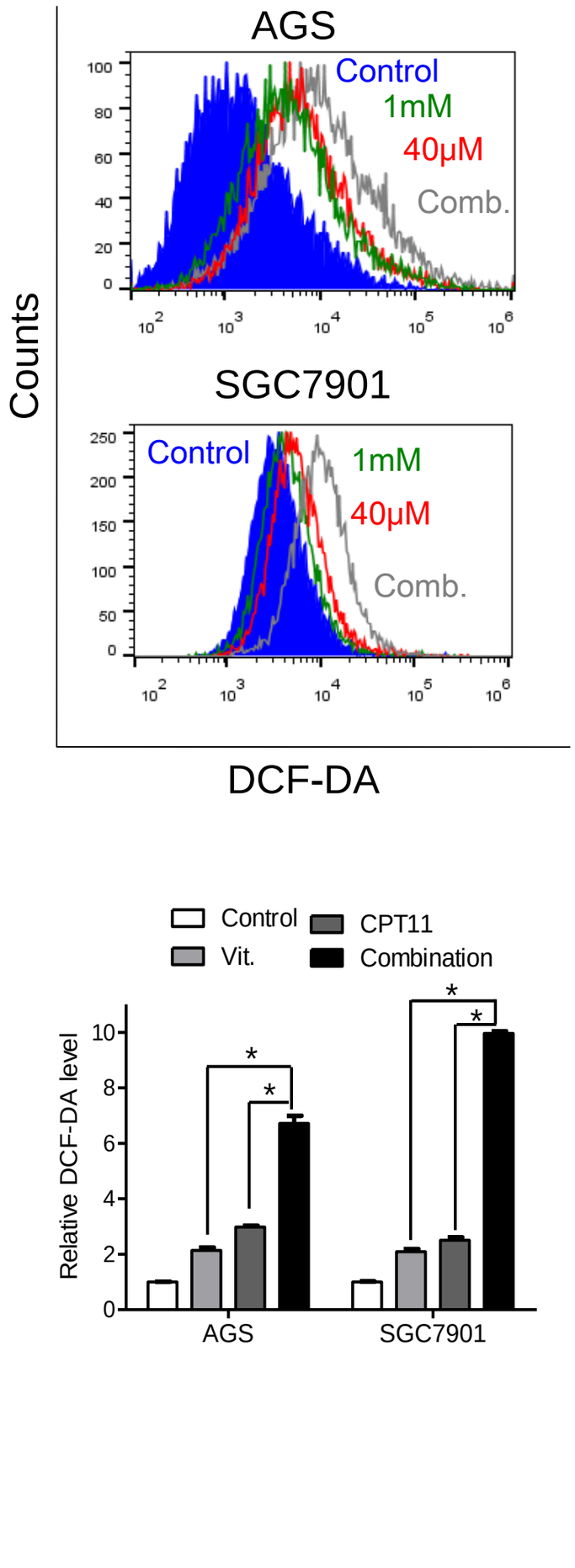




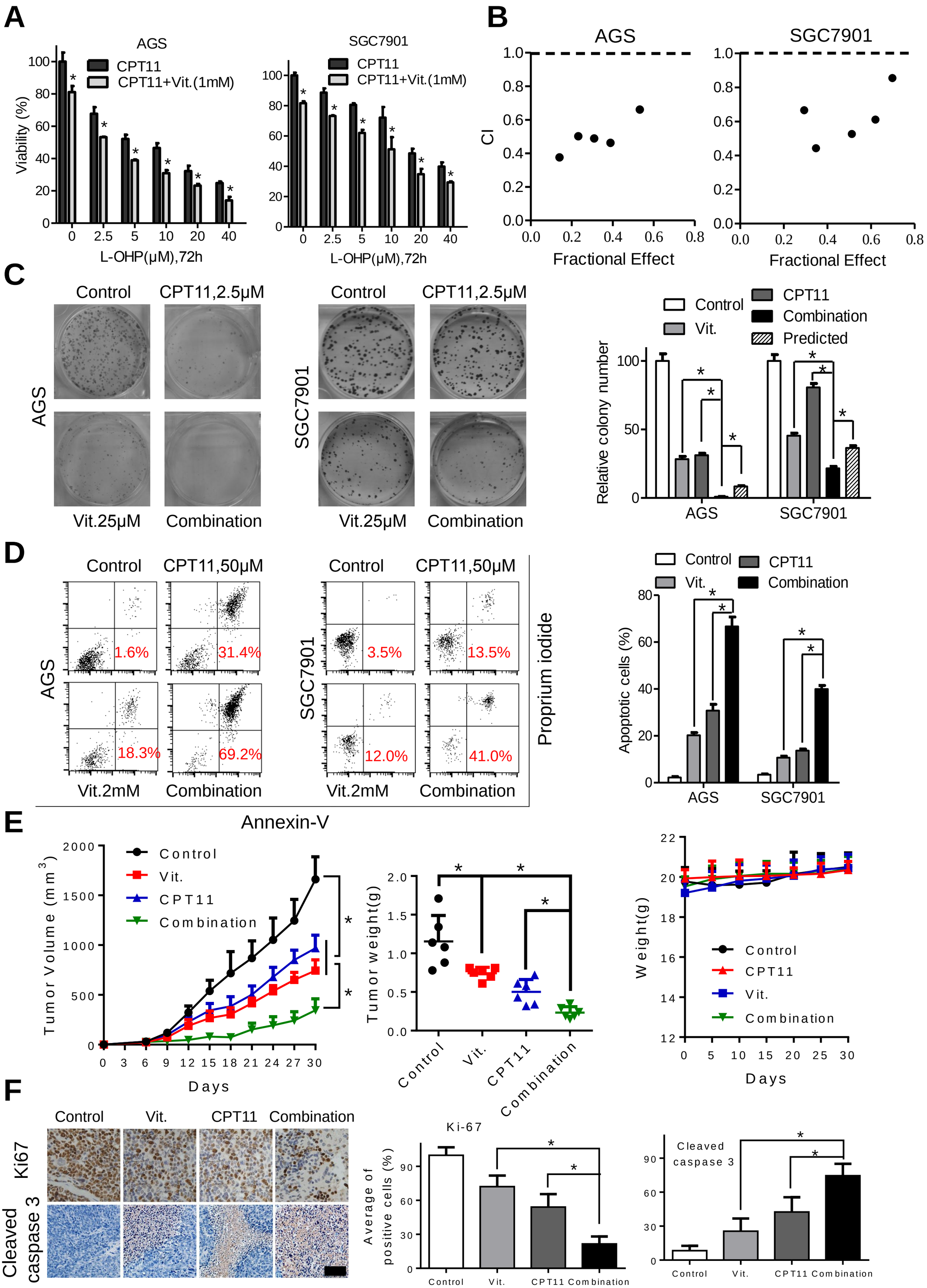
Supplementary Figure 6



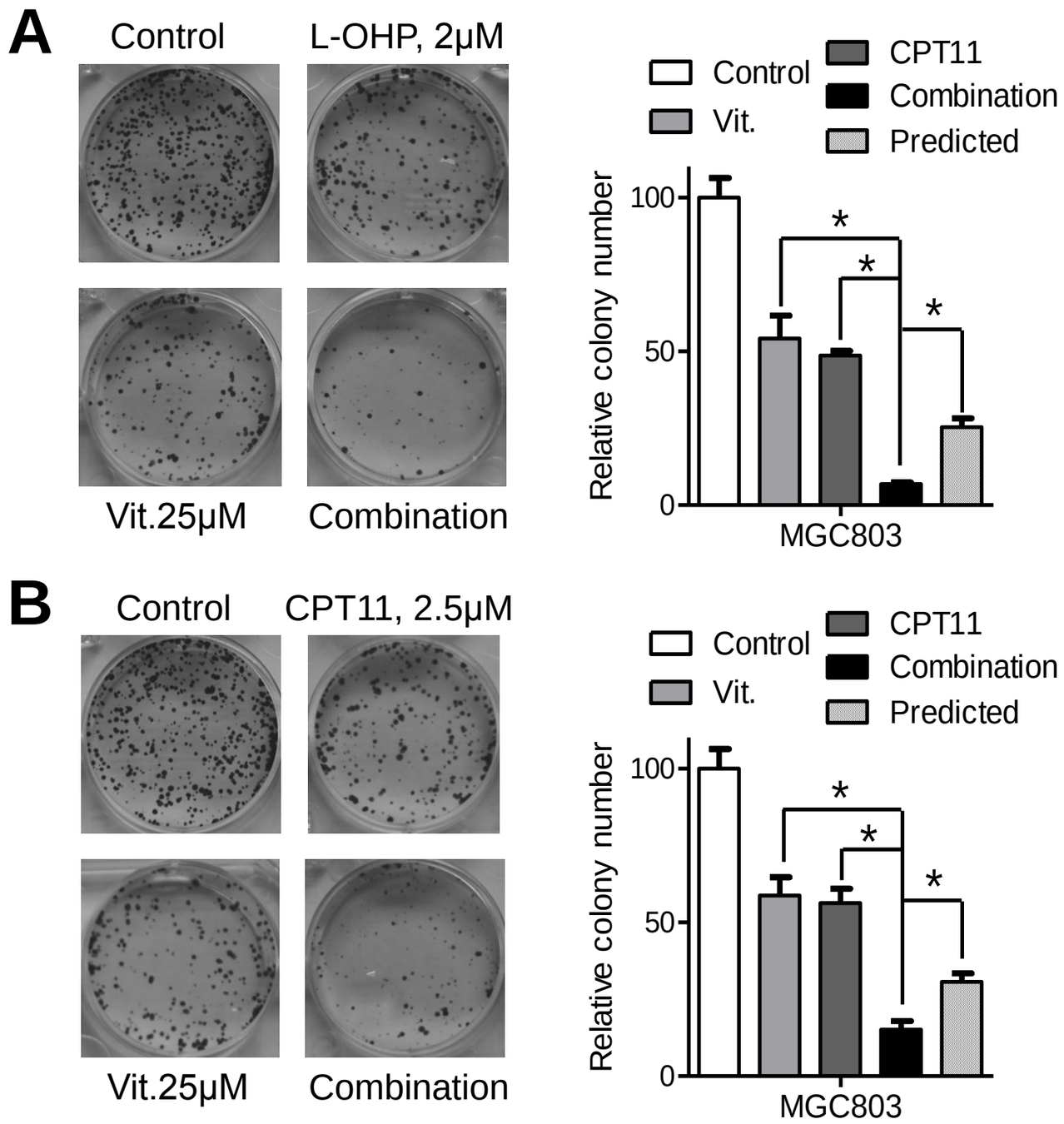




Supplementary Figure 7



Supplementary Figure 8



Supplemental Table S1. Primers sequence for qPCR

Gene	Sequence (5'-3')
Glut1-F	TCCCTGCAGTTTGGCTACAA
Glut1-R	AAGGCCAGCAGGTTCATCAT
HK2-F	AGCACCTGTGACGACAGCAT
HK2-R	ATCACATTTCGGAGCCAGGT
LDH-A -F	GGTGTGAATGTGGCAGGTGT
LDH-A -R	ACCATTGTTGACACGGGATG
PFK1-F	AGGCTCCATTCTTGGGACAA
PFK1-R	TGACCATGGGGACACAGAAC
PKM2-F	GGCCAGGGAGGGACTTTTAT
PKM2-R	TCACAGGCCTTCATTCGCTT
Aldolase -F	CACTCGTACCCAGCCCTTTC
Aldolase -R	ACGCCTCCAATGCACTTTTT
SVCT1 -F	TTCCAGGCACGAACCGATG
SVCT1 -R	AGTGGCGCTGAACATTCCC
SVCT2 -F	CTTCACTCTTCCGGTGGTGAT
SVCT2 -R	TTTCCGTAGTGTAGATCGCCA
Glut3 -F	GCTGGGCATCGTTGTTGGA
Glut3 -R	GCACTTTGTAGGATAGCAGGAAG
Glut4 -F	TGGGCGGCATGATTTCCTC
Glut4 -R	GCCAGGACATTGTTGACCAG
β-Actin -F	CATGTACGTTGCTATCCAGGC
β-Actin -R	CTCCTTAATGTCACGCACGAT

Supplemental Table S2. The correlation between clinicopathological parameters and Glut1 expression

	Glut1 expression		*P*
	Low, n(%)	High, n(%)	
Age			
< 60	105 (77.8)	30(22.2)	0.074
≥60	49(66.2)	25(33.8)	
Gender			
Male	96(70.1)	41(29.9)	0.069
Female	58(80.6)	14(19.4)	
Tumor size			
< 5cm	100(80.6)	24(19.4)	0.007*
≥5cm	54(63.5)	31(36.5)	
Differentiation status			
Well or Moderate	26(53.1)	23(46.9)	0.000*
Poor and others	128(80.0)	32(20.0)	
Lymph node invasion			0.727
Absent	109(72.7)	41(27.3)	
Present	45(76.3)	14(23.7)	
Venous invasion			0.084
Absent	81(79.4)	21 (20.6)	
Present	73(68.2)	34(31.8)	
Perineural invasion			0.003*
Absent	38(59.4)	26(40.6)	
Present	116(80.0)	29(20.0)	
TNM stage			0.338
I-II	92(71.3)	37(28.7)	
III-IV	62(77.5)	18(22.5)	

*$P < 0.05$.

Supplemental Table S3. Univariate and multivariate analyses of various potential prognostic factors in GC patients

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (<60/≥60)	0.71-1.81	0.611	-	-
Gender (male/female)	0.69-1.76	0.690	-	-
Differentiation (well, moderate/poor)	0.83-2.65	0.184	-	-
Tumor size (≥5cm/<5cm)	0.82-2.02	0.269	-	-
Lymph node invasion (present/absent)	0.80-2.07	0.306	-	-
Venous invasion (present/absent)	1.34 -3.57	0.002*	0.66-1.91	0.671
Perineural invasion (present/absent)	1.25-4.14	0.007*	0.97-3.65	0.062
TNM Stage(III-IV/I-II)	2.54-6.55	0.000*	2.23-6.40	0.000*
Glut1 protein (high/low)	1.21-3.07	0.006*	1.70-4.66	0.000*

HR: hazard ratio; CI: confidence interval; *$P < 0.05$.