

Supplementary Materials: Ellagic Acid Resensitizes Gemcitabine-Resistant Bladder Cancer Cells by Inhibiting Epithelial-Mesenchymal Transition and Gemcitabine Transporters

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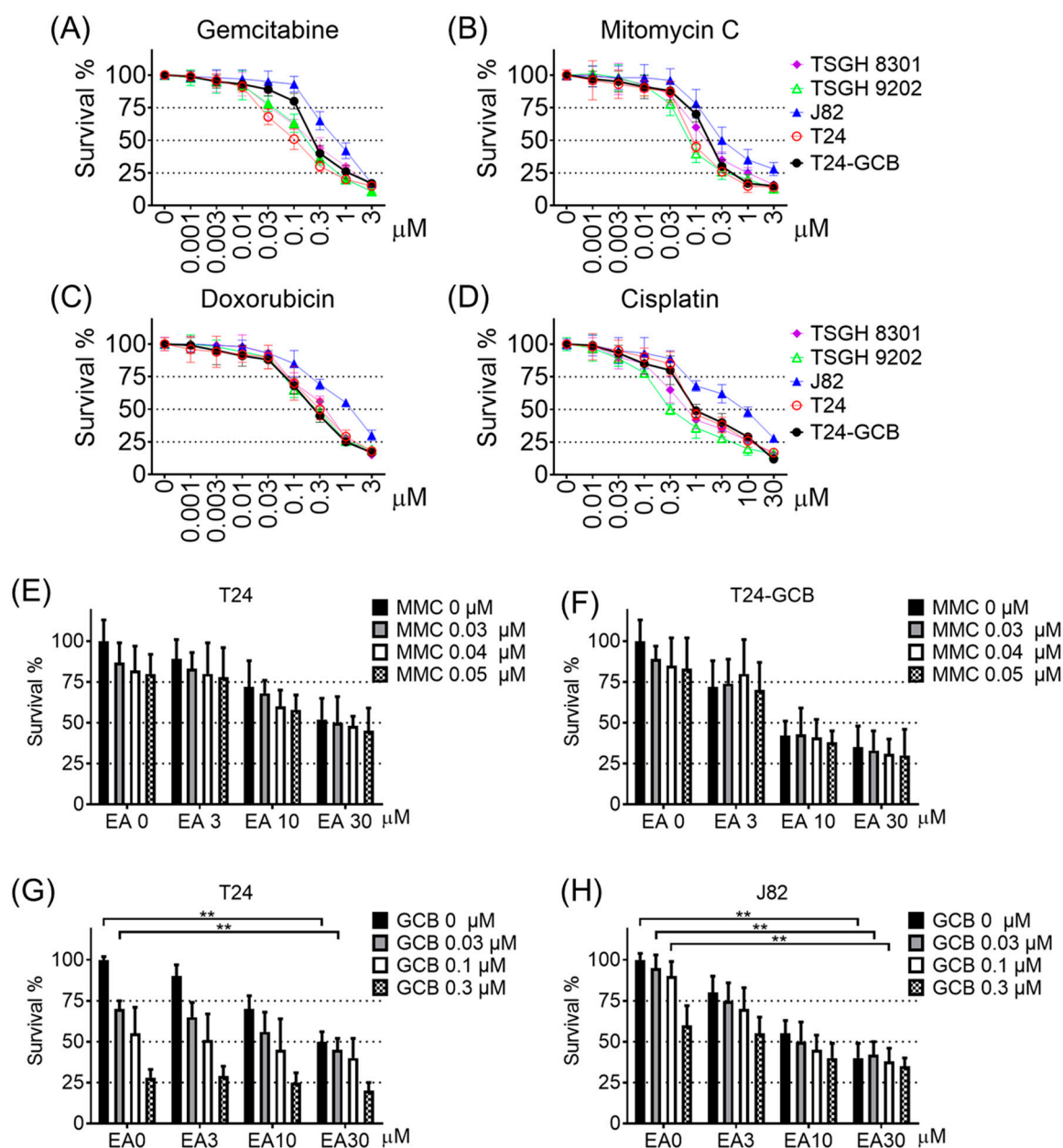


Figure S1. Cytotoxicities of (A) gemcitabine, (B) mitomycin C, (C) doxorubicin and (D) cisplatin compared among different bladder cancer cell lines, including TSGH-8301, TSGH-9202, J82, T24 and T24-GCB. The cytotoxicity of the combined treatment of EA and mitomycin C (MMC) was evaluated in (E) T24 and (F) T24-GCB cells. And the cytotoxicity of the combined treatment of EA and GCB was also evaluated in (G) T24 and (H) J82 cells. All statistical tests were analyzed with Student's t test with significance at * <0.05, ** <0.01 and *** <0.001.

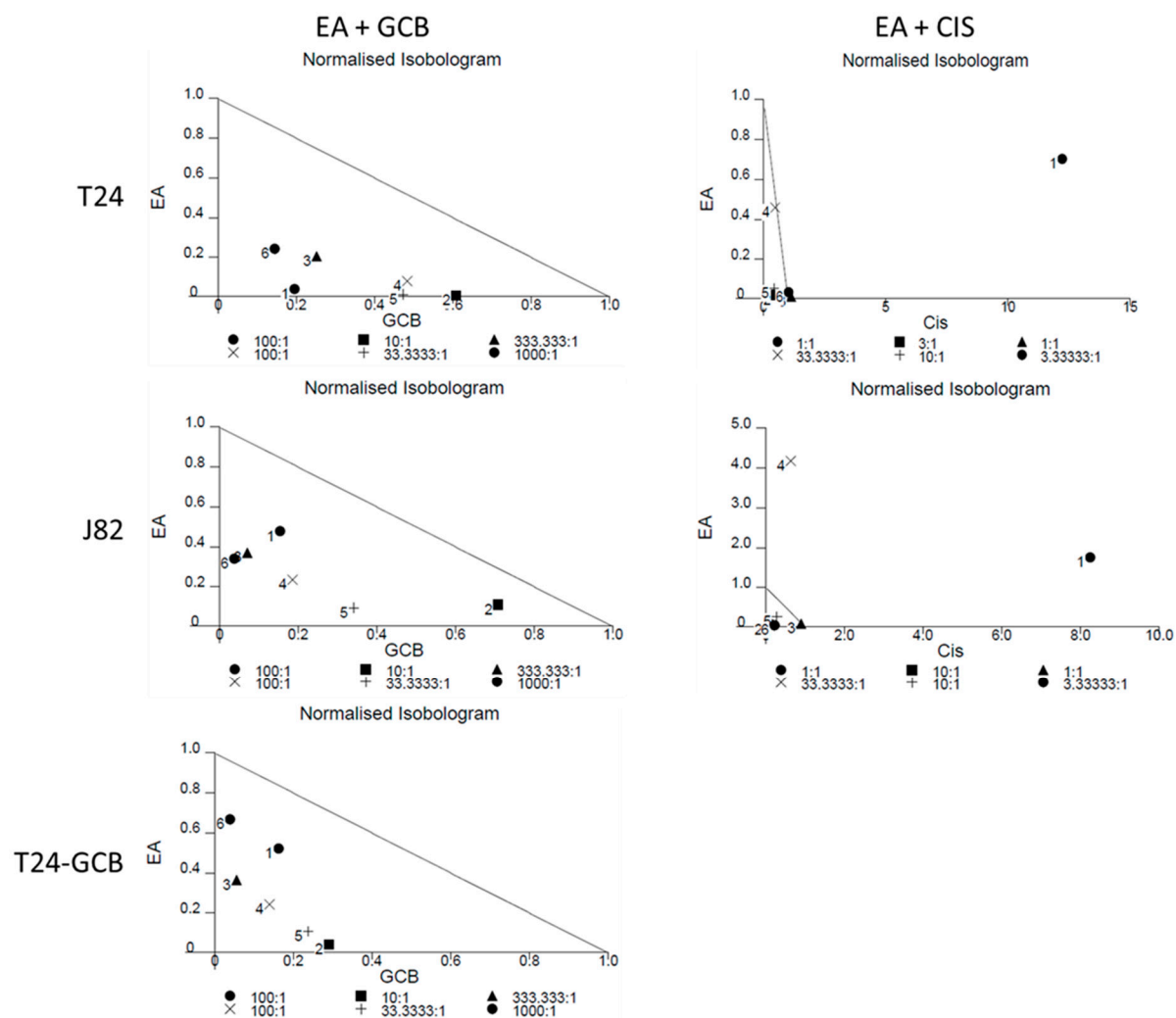


Figure S2. Isobologram analyses of combined effects between ellagic acid (EA) and gemcitabine (GCB) or EA and cisplatin (CIS) were compared in T24, J82 and T24-GCB cells.

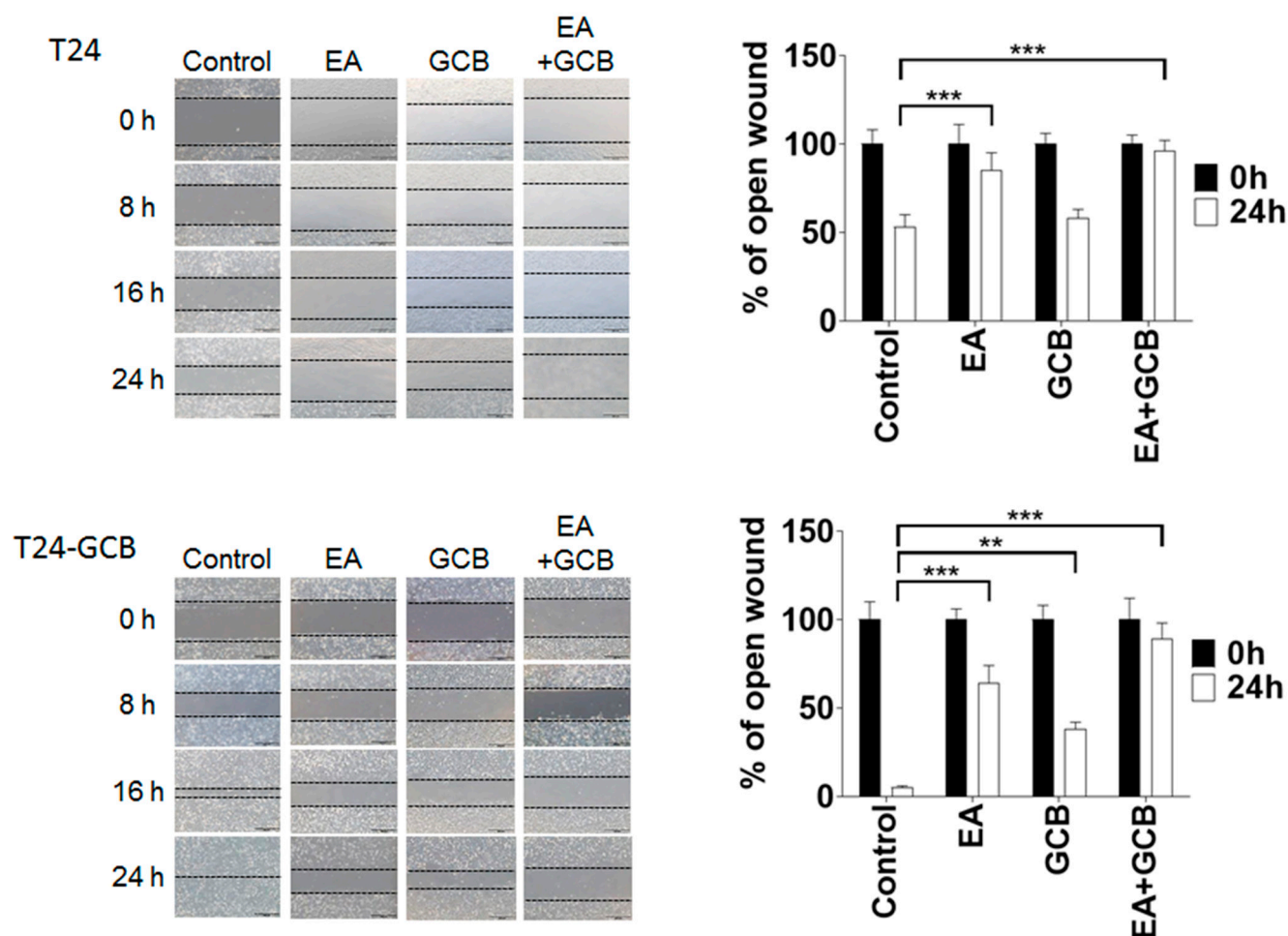


Figure S3. Cell motility of T24 and T24-GCB cells as determined by the wound-healing assay compared among the PBS control, EA, GCB and EA+GCB groups. Quantitative bar charts of migrated cells in the treated groups were showed on the right side. All statistical tests were analyzed with Student's t test with significance at ** <0.01 and *** <0.001.

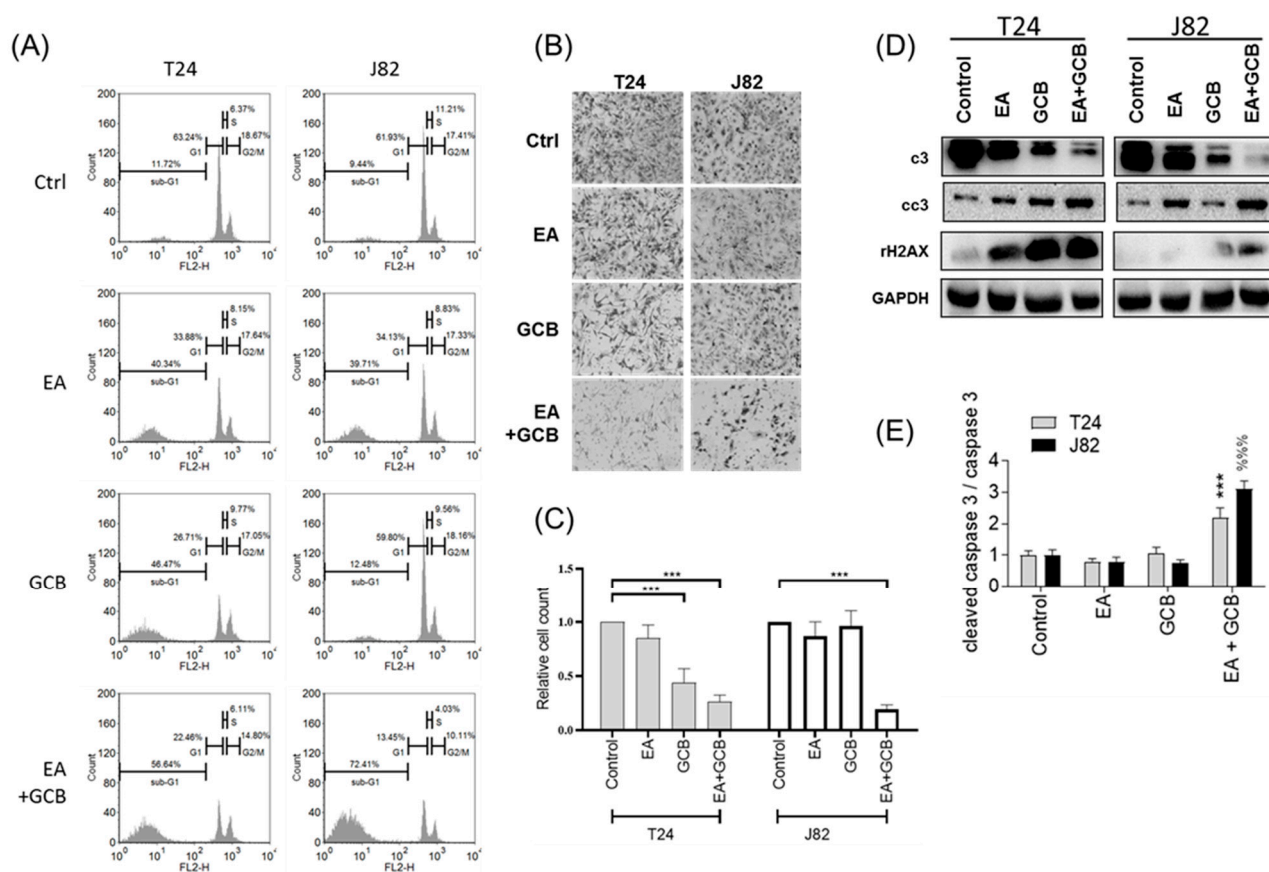


Figure S4. (A) Comparison of the cell cycle distribution of T24 and J82 cells among the PBS control, EA (30 μ M), GCB (0.1 μ M) and EA (30 μ M) + GCB (0.1 μ M) groups. (B) Results of the Matrigel-coated Transwell invasion assays compared among the same groups. (C) Quantitative bar chart of migrated cells among the treated groups. (D) Protein expression levels of procaspase 3 (C3) and cleaved caspase 3 (CC3) and γ H2AX in T24 and J82 cells were compared among the same groups. (E) Quantitative bar chart of C3 and CC3 expression among the treated groups. All statistical tests were analyzed with Student's t test with significance at ** <0.01 and *** <0.001 (or %%% <0.001).

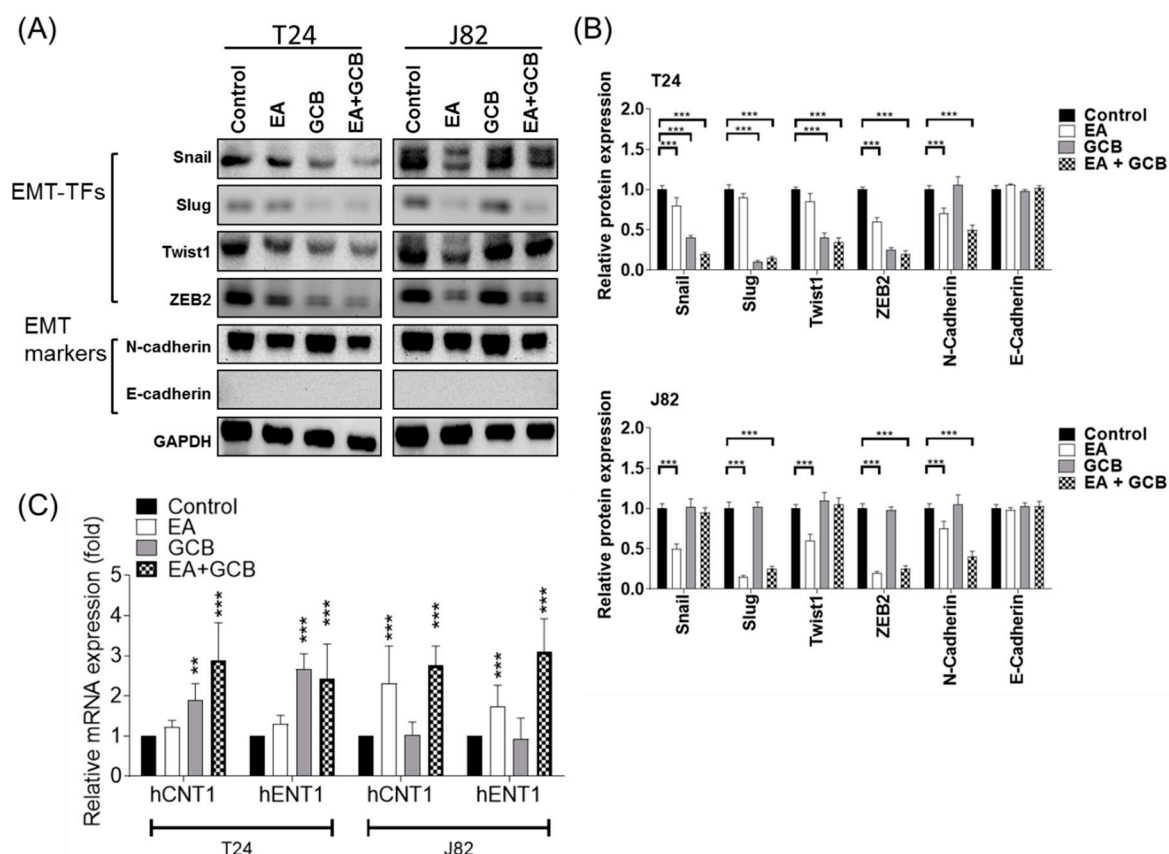
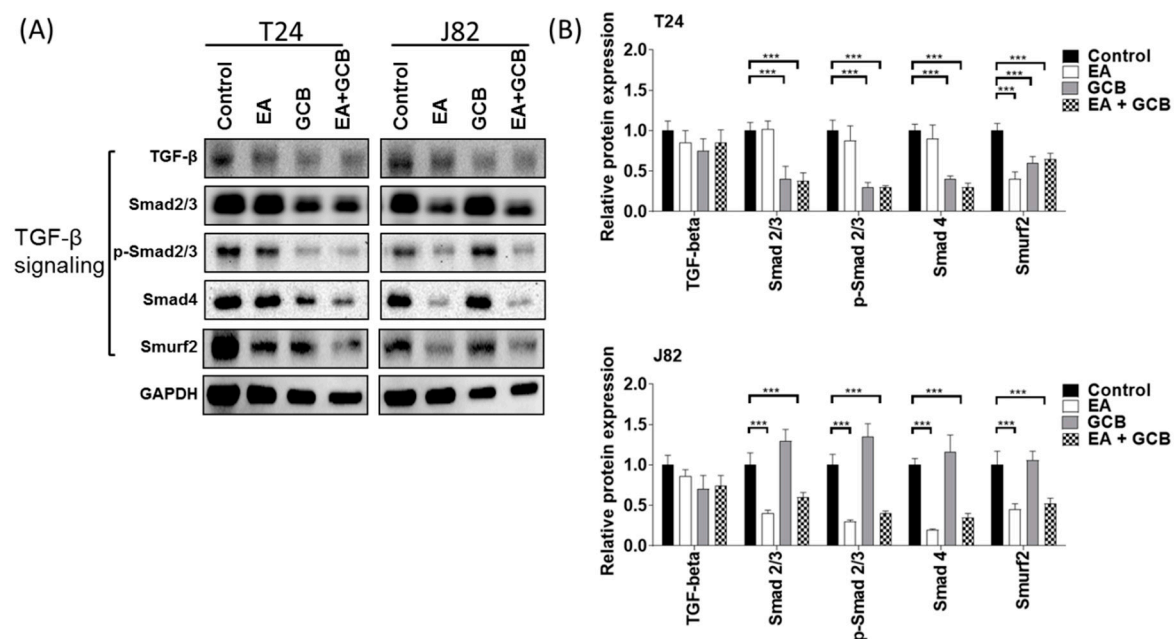


Figure S5. (A) Protein expression levels of EMT-TFs (Snail, Slug, Twist1 and ZEB2) and EMT markers (N-cadherin and E-cadherin) in T24 and J82 cells were compared among the PBS control, EA (30 μ M), GCB (0.1 μ M) and EA (30 μ M) + GCB (0.1 μ M) groups. (B) Quantitative bar chart of the protein expression of the indicated EMT-TFs among the treated groups. (C) Relative mRNA expression of hCNT1 and hENT1 compared among the same groups between T24 and J82 cells. All statistical tests were analyzed with Student's t test with significance at * <0.05, ** <0.01 and *** <0.001.



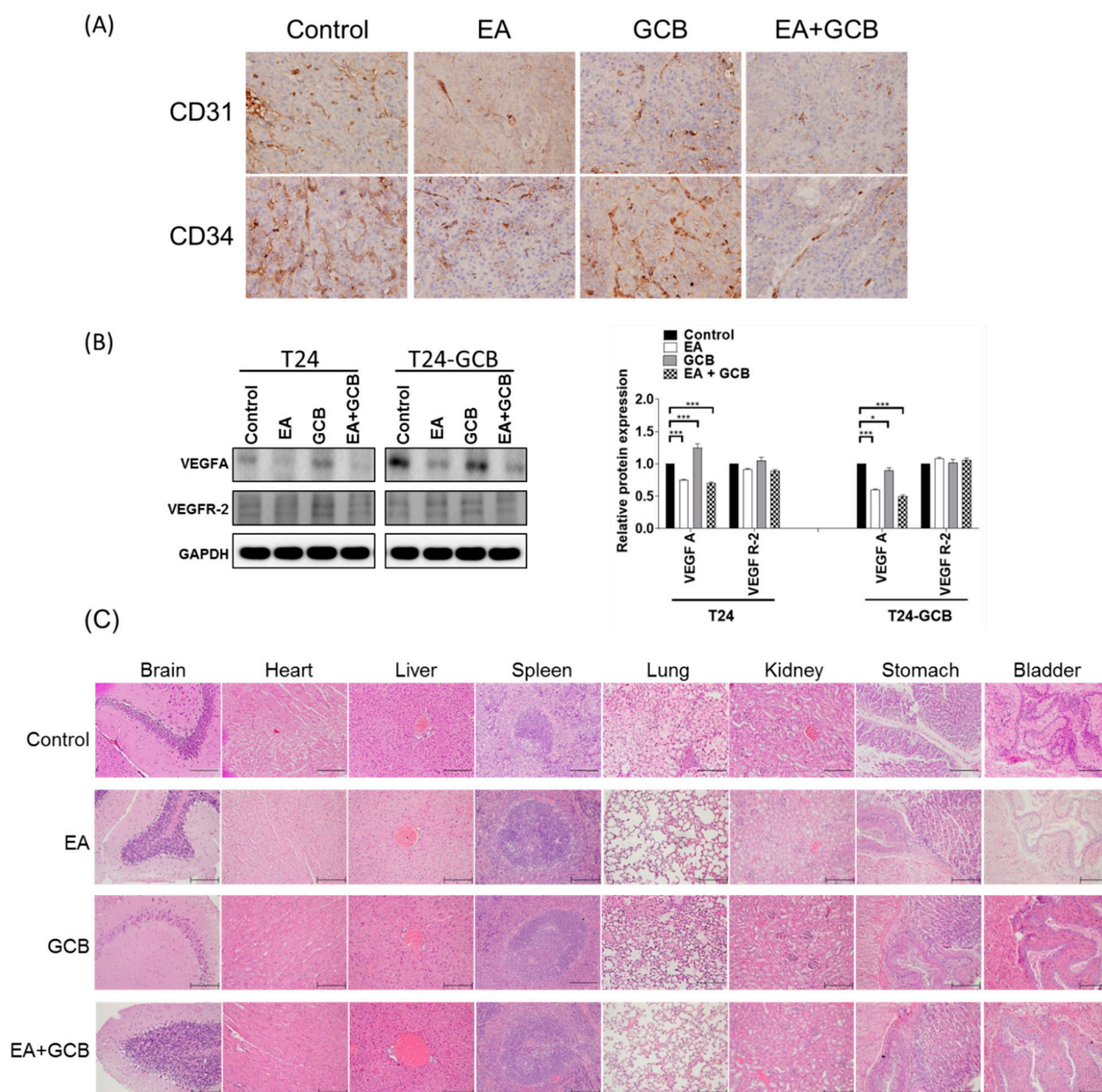
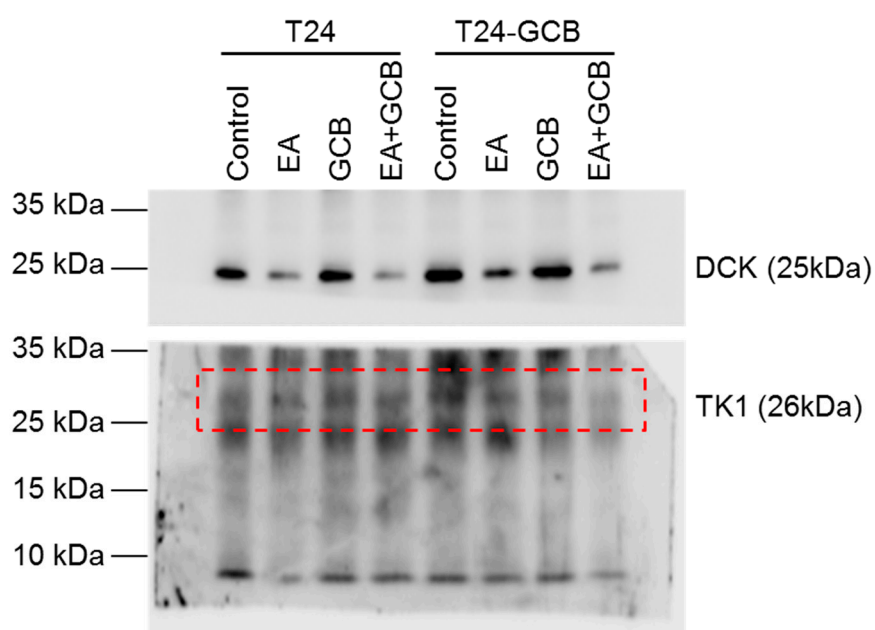
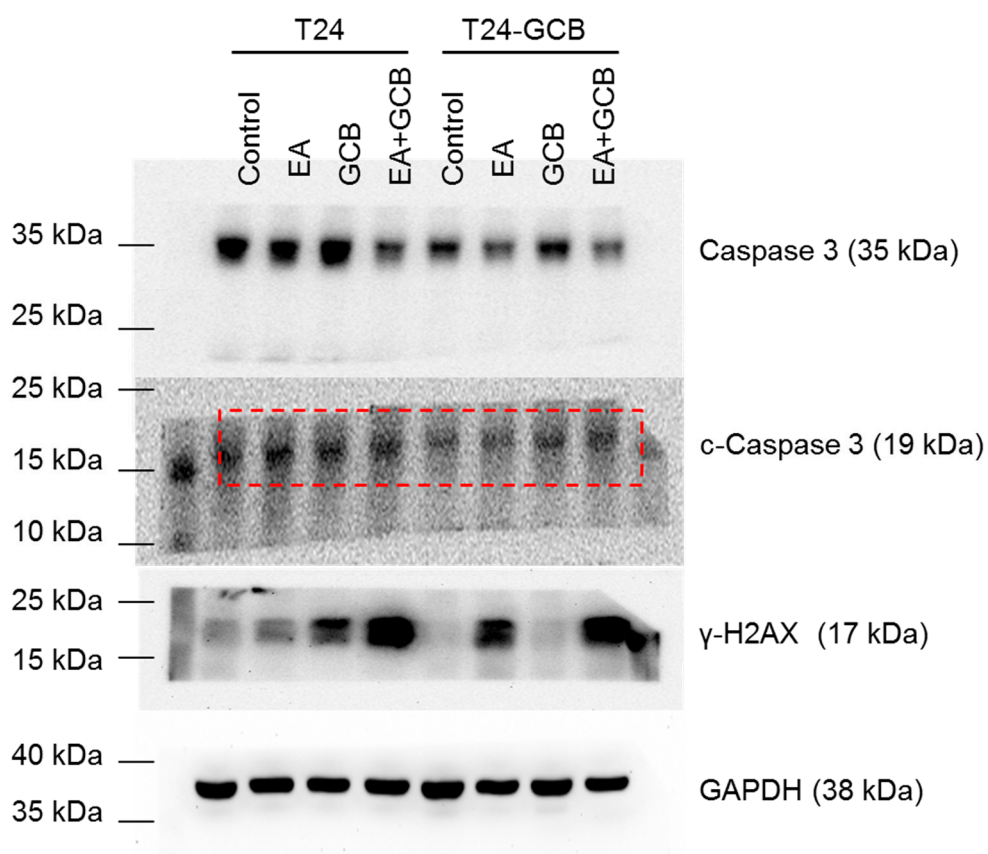
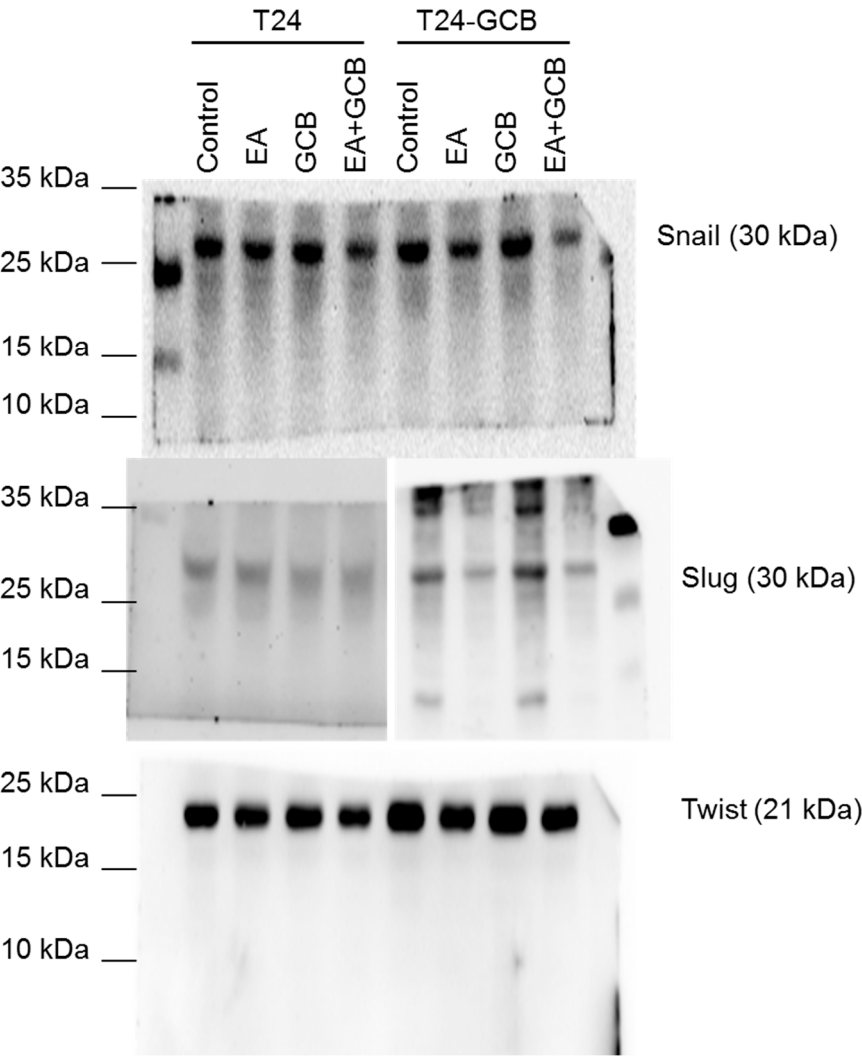
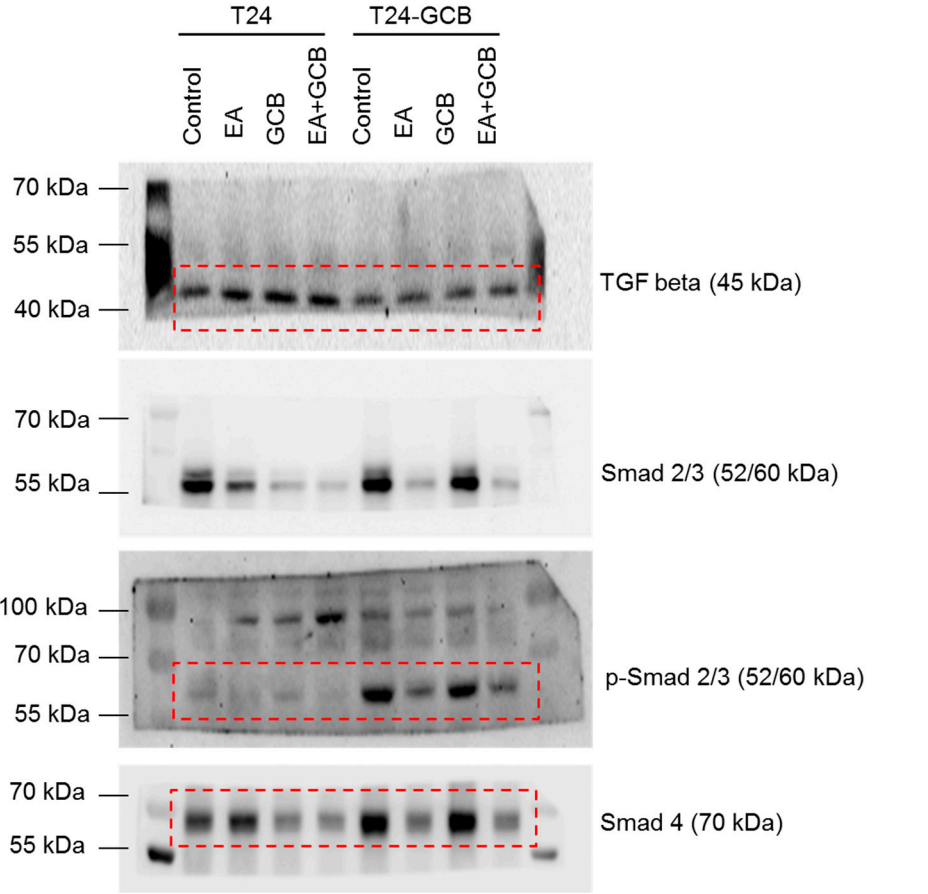
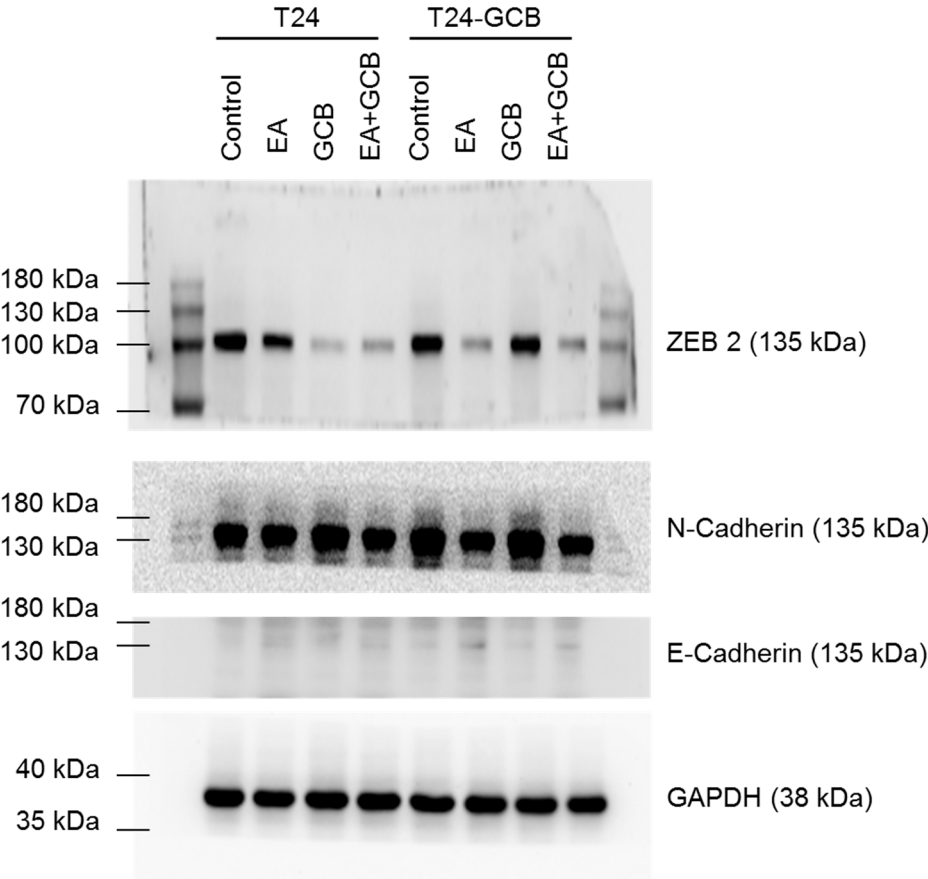
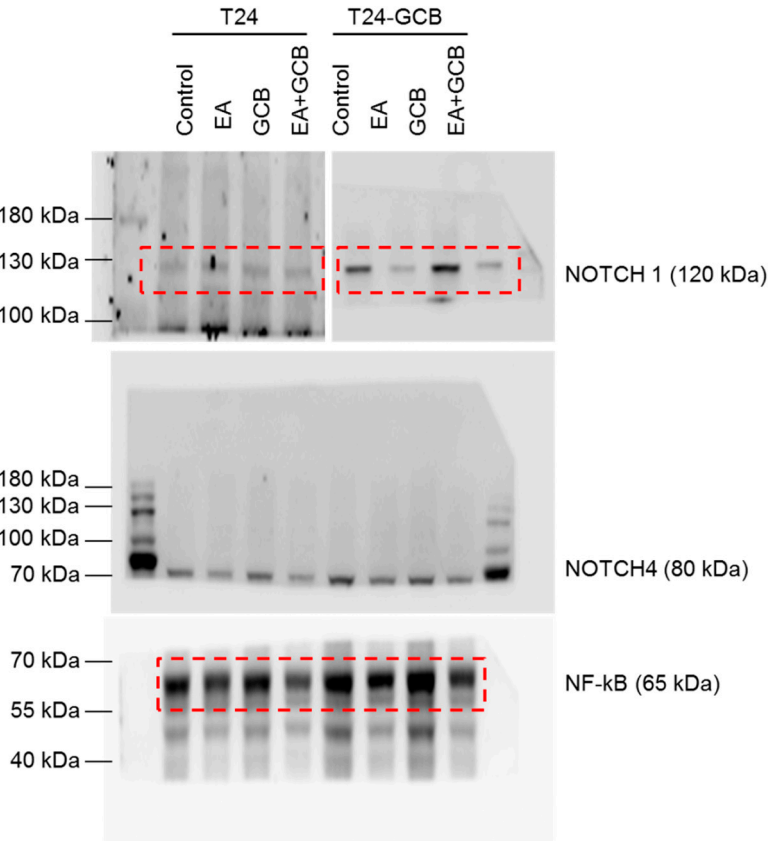
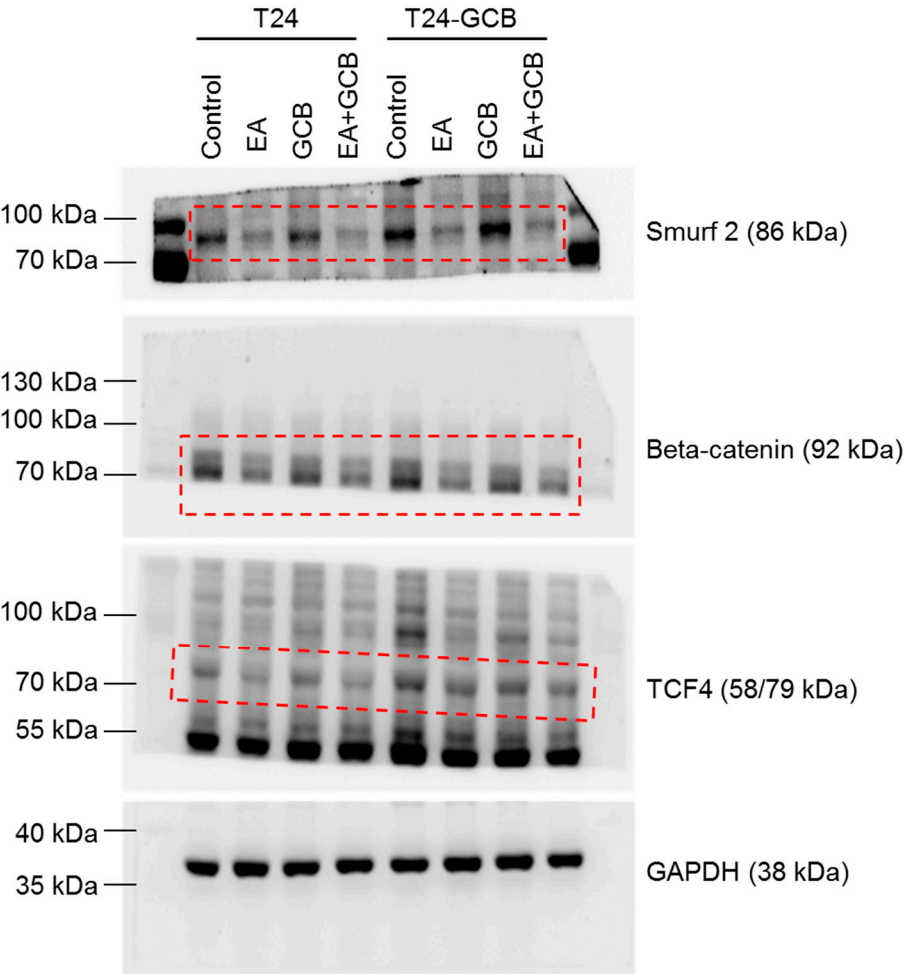


Figure S7. (A) Immunohistochemical staining of CD31 and CD34 in xenograft tumors from T24-GCB xenograft mice treated with the PBS control, EA, GCB and EA + GCB. (B) Protein expression levels of VEGFA and VEGFR-2 in T24 and T24-GCB cells compared among the PBS control, EA, GCB and EA+GCB groups. Quantitative bar charts of the expression of the indicated proteins among the treated groups were showed. All statistical tests were analyzed with Student's t test with significance at $* < 0.05$, $** < 0.01$ and $*** < 0.001$. (C) Alignment of H&E staining of the brain, heart, liver, spleen, lung, kidney, stomach and bladder of xenografted mice that received the four treatments, and no perceptible necrosis was observed.









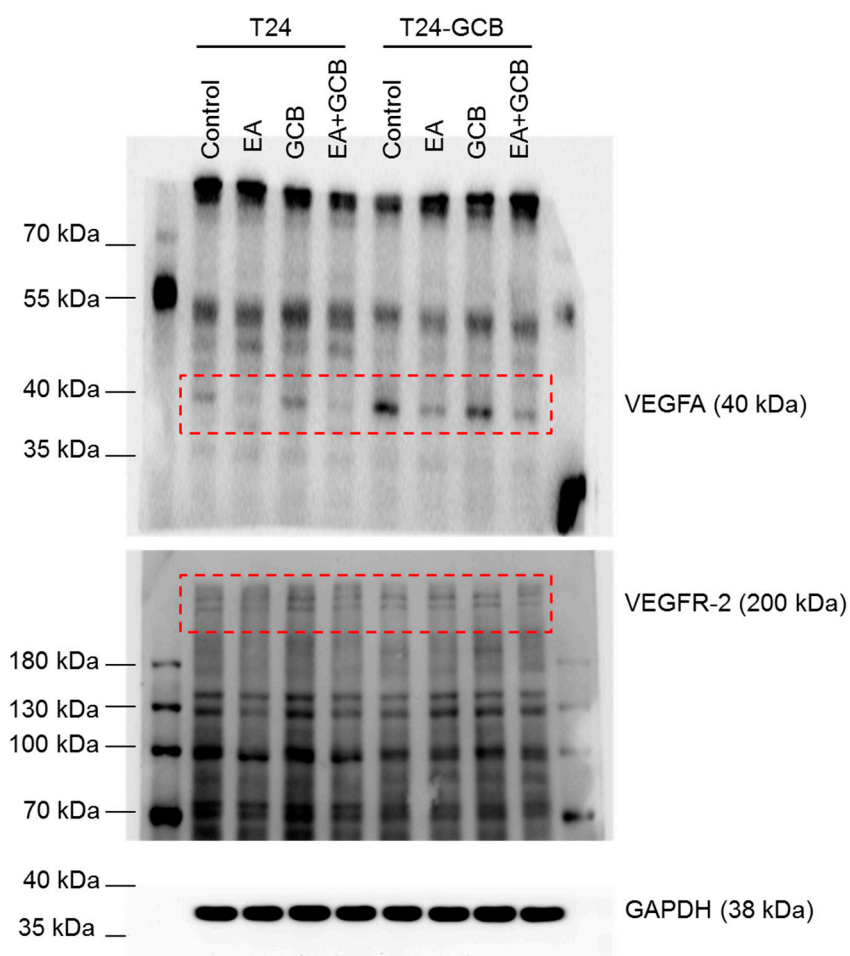


Figure S8. Original Western Blots.

Table S1. Lists of the primary antibodies.

Primary antibodies	Supplier	Cat. Number
caspase 3	Cell Signaling Technology	cat. no. #9662
cleaved-caspase 3	Cell Signaling Technology	cat. no. #9654
γ -H2AX	Cell Signaling Technology	cat. no. #9718
dCK	GeneTex	cat. no. GTX632065
TK1	GeneTex	cat. no. GTX113281
snail	Abcam, Cambridge	cat. no. ab53519
slug	Abcam, Cambridge	cat. no. ab27568
twist 1	Abcam, Cambridge	cat. no. ab50887
ZEB 2	Cell Signaling Technology	cat. no. #97885
N-cadherin	Abcam, Cambridge	cat. no. ab18203
E- cadherin	Abcam, Cambridge	cat. no. ab231303
TGF- β	Abcam, Cambridge	cat. no. ab92486
smad 2/3	Cell Signaling Technology	cat. no. #3102
p- smad 2/3	Cell Signaling Technology	cat. no. #8828
smad 4	Abcam, Cambridge	cat. no. ab236321
smurf 2	Cell Signaling Technology	cat. no. #12024
β -catenin	Cell Signaling Technology	cat. no. #8480
TCF-4	Cell Signaling Technology	cat. no. #2569
Notch 1	Cell Signaling Technology	cat. no. #3608
Notch 4	Cell Signaling Technology	cat. no. #2423
NF- κ B	Abcam, Cambridge	cat. no. ab231481
VEGF-A	Abcam, Cambridge	cat. no. ab1316
VEGFR-2	Abcam, Cambridge	cat. no. ab39256

GAPDH

Abcam, Cambridge

cat. no.ab9485

Table S2. Lists of the primer sequences.

Gene	Forward Primer	Reverse Primer
<i>NT5C</i>	GGACACGCAGGTCTTCATCTG	GCGGTACTTCTCACCCACACA
<i>NT5C2</i>	ACCTGCTGTATTACCCTTTCAGCTA	GCTCCACCGTTGATTCATGA
<i>NT5C3A</i>	AATCGGCGATGTACTAGAG	CATCTGCCATTCTTAAGTCTC
<i>NT5M</i>	CATCAGCATTGTTGGGAGTCAA	CAGCACAATCTGCTCCAGAA
<i>dCK</i>	AAACCTGAACGATGGTCTTTTACC	CTTTGAGCTTGCCATTGAGAGA
<i>TK1</i>	GGGGCAGATCCAGGTGATTG	GCATACTTGATCACCAGGCACTT
<i>TK2</i>	TTACCTTCGGACCAATCCTG	TGCTTCCGATTCTCTGGAGT
<i>CDA</i>	ATGGCCCAGAAGCGTCCTGCCTGCA	TCACTGGGTCTTCTGCAGGTCCTCA
<i>DCTD</i>	GTTGCCTTGTTCCCTTGTA	TCTTGCTGCACTTCGGTATG
<i>CMPK1</i>	GGGCATATTCTTTGCTTCCA	TGCATTTCAAGGTTCCACTG
<i>NME2</i>	GTGCAGCGCGGCCTGGTGGG	GACCCAGTCATGAGCACAAGAC
<i>DCTPP1</i>	AAATGGACATCAACCGGCGA	AGTCACAGGGAATGTCCGCA
<i>CTPS1</i>	GTGGGCAAATACACGAAGTT	TCCTCGAACACCAAATCCTC
<i>RRM1</i>	TACCAACCGCCCACTTT	TCTCAGCATCGGTACAAGGC
<i>RRM2</i>	GACACAAGGCATCGTTTCAA	TCTATGGCTTCCAAATTGCC
<i>hCNT1</i>	TCTGTGGATTGTTGCAATTTTCA	CGGAGCACTATCTGGGAGAAGT
<i>hENT1</i>	GCTGGGTCTGACCGTTGTAT	CTGTACAGGGTGCATGATGG
<i>hENT2</i>	ATGAGAACGGGATTCCCAGTAG	GCTCTGATTCCGGCTCCTT