Supplementary Materials

Supplemental figure 1

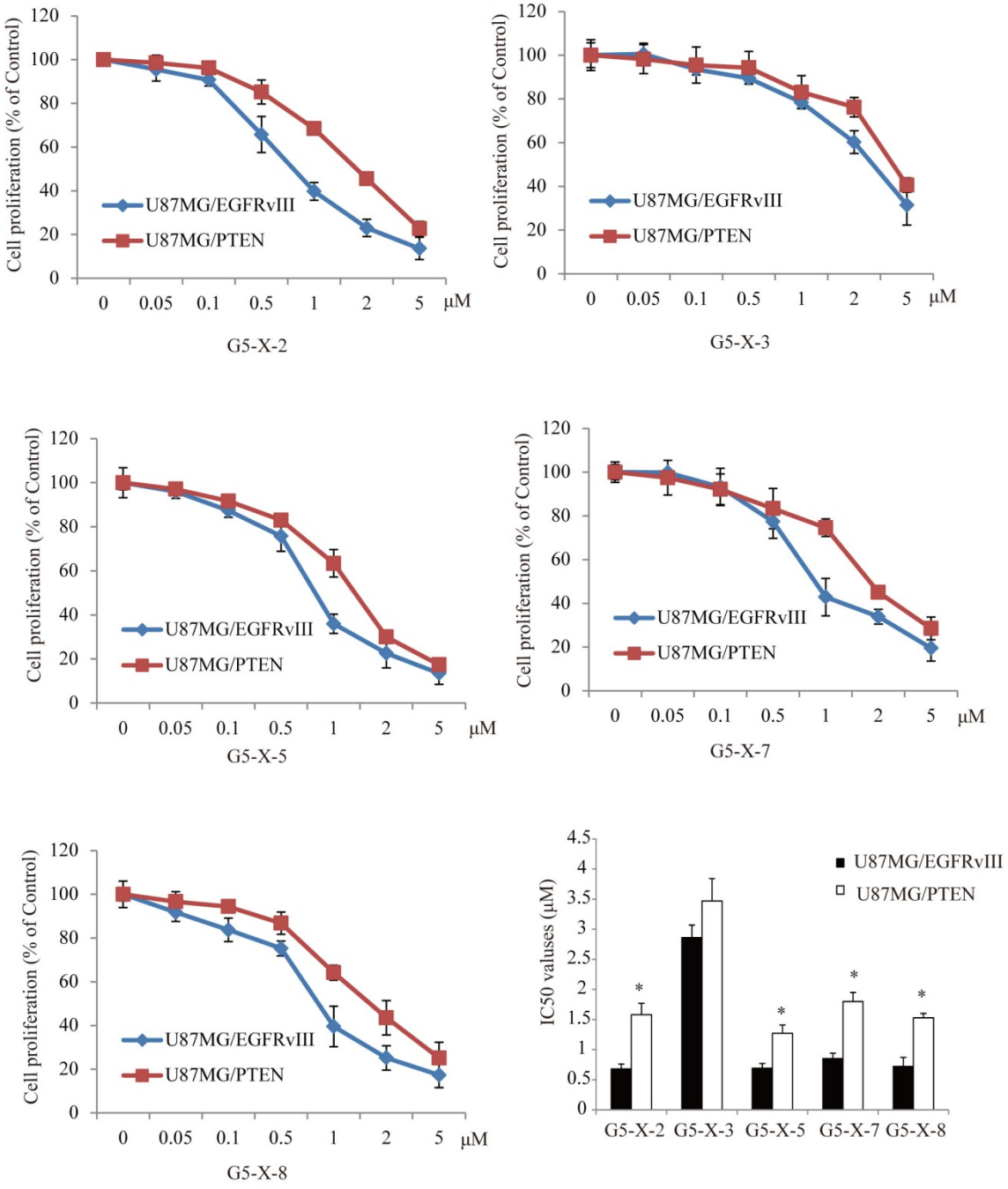


Figure S1. Effect of G5-7 derivatives (G5-X-2, -3, -5, -7, -8) on the proliferation of U87MG/EGFRvIII and U87MG/PTEN cells. Cells were treated with different doses of G5-7

for 96 hours and then were analyzed with the MTT cell proliferation assay. Data are shown as mean and S.E.M., (n = 3 experiments, * P<0.05, ** P<0.01, U87MG/EGFRvIII vs. U87MG/PTEN, two-way ANOVA)

Supplemental figure 2

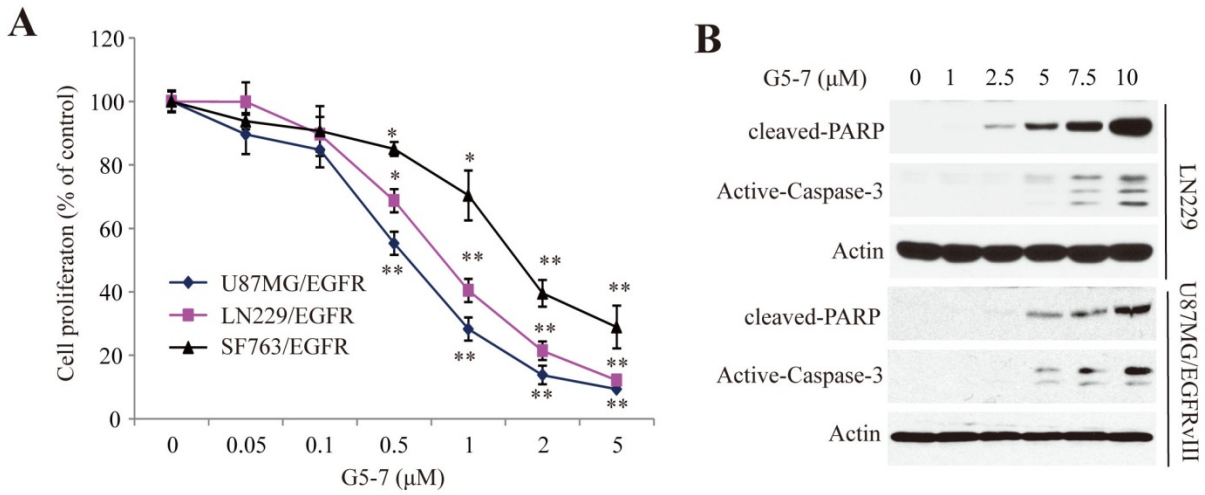


Figure S2. G5-7 suppresses human GBM in vitro. A. G5-7 inhibits EGFR overexpressed GBM cells in a dose-dependent manner. The cells were treated with different dosage of G5-7 for 96 hours followed by an MTT assay. Except for U87MG/EGFR, the proliferation of EGFR stable transfected LN229 and SF273 cells were inhibited in a dose-dependent manner by G5-7. Data were expressed as mean ± S.E.M. (n = 3 experiments, * P<0.05, ** P<0.01, one-way ANOVA). **B.** G5-7 induces apoptosis in cancer cells. LN229 and U87MG/EGFRvIII cells were treated with different doses of G5-7 for 24 hours. The cell lysates were analyzed by different antibodies. G5-7 triggered apoptosis in a dose-dependent manner.

Supplemental figure 3

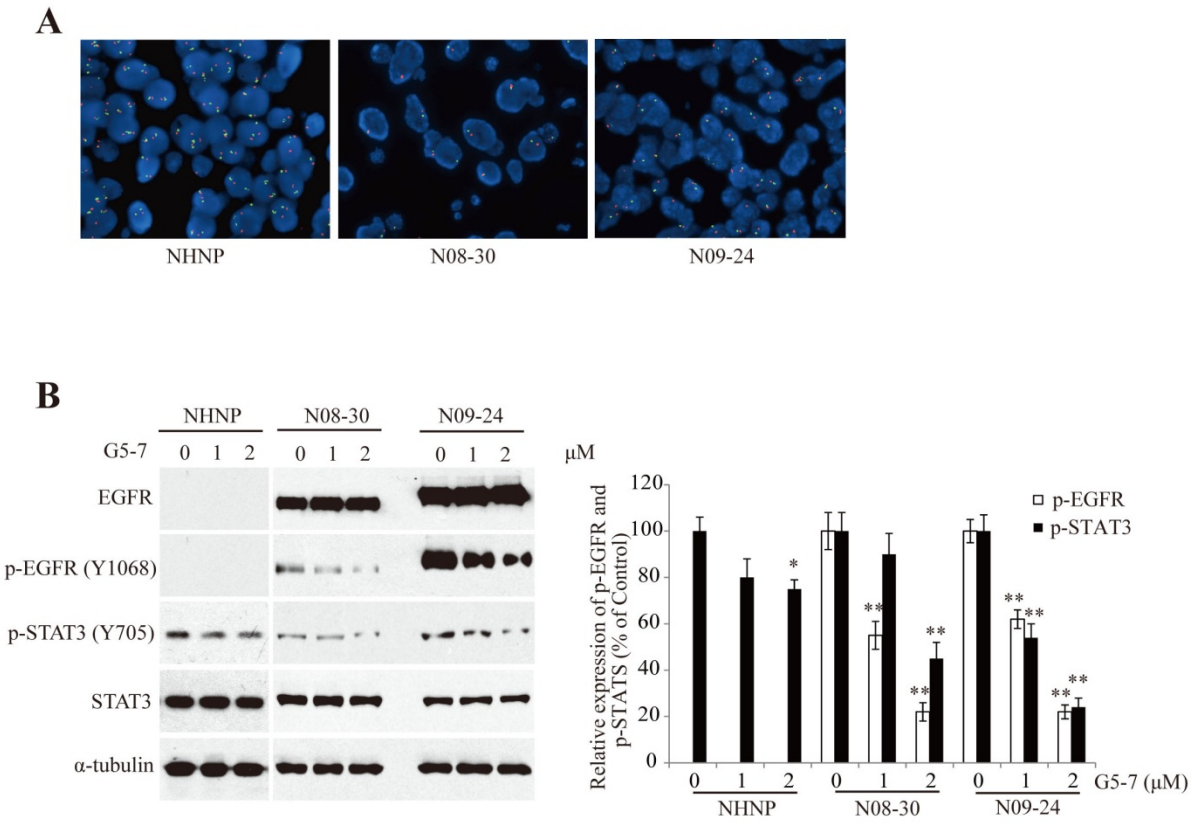


Figure S3. Effect of G5-7 on N09-24 and N08-30 neurospheres. A. PTEN status in NHNP, N09-24, and N08-30 neurospheres. FISH assay on the PTEN locus (10q23.3) showed that both of the tumor samples used to create the neurosphere cell lines had *PTEN* loss compared with NHNP. The red probe is for the PTEN locus and the green probe is for the CEP10 (centromere). **B.** G5-7 inhibits EGFR and STAT3 phosphorylation in N09-24 and N08-30 neurospheres. Different doses of G5-7 incubated with the neurosphere for 6 hours. The lysates were analyzed by immunoblotting with various antibodies. P-EGFR and p-STAT3 were quantitatively analyzed against control samples (n = 3 experiments, * $P<0.05$, ** $P<0.01$, one-way ANOVA).

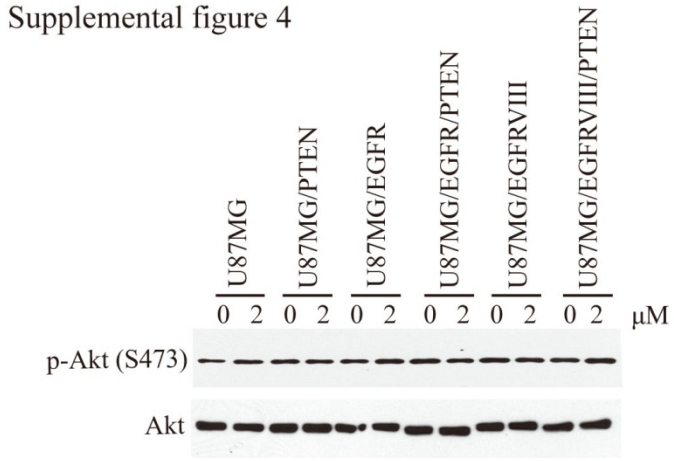


Figure S4. Effect of G5-7 on p-Akt expression in U87MG isogenic cell lines. Cells treated with G5-7 (2 μM) for 6 hours, the lysates were analyzed by immunoblotting with p-Akt (S473) antibody.

Supplemental figure 5

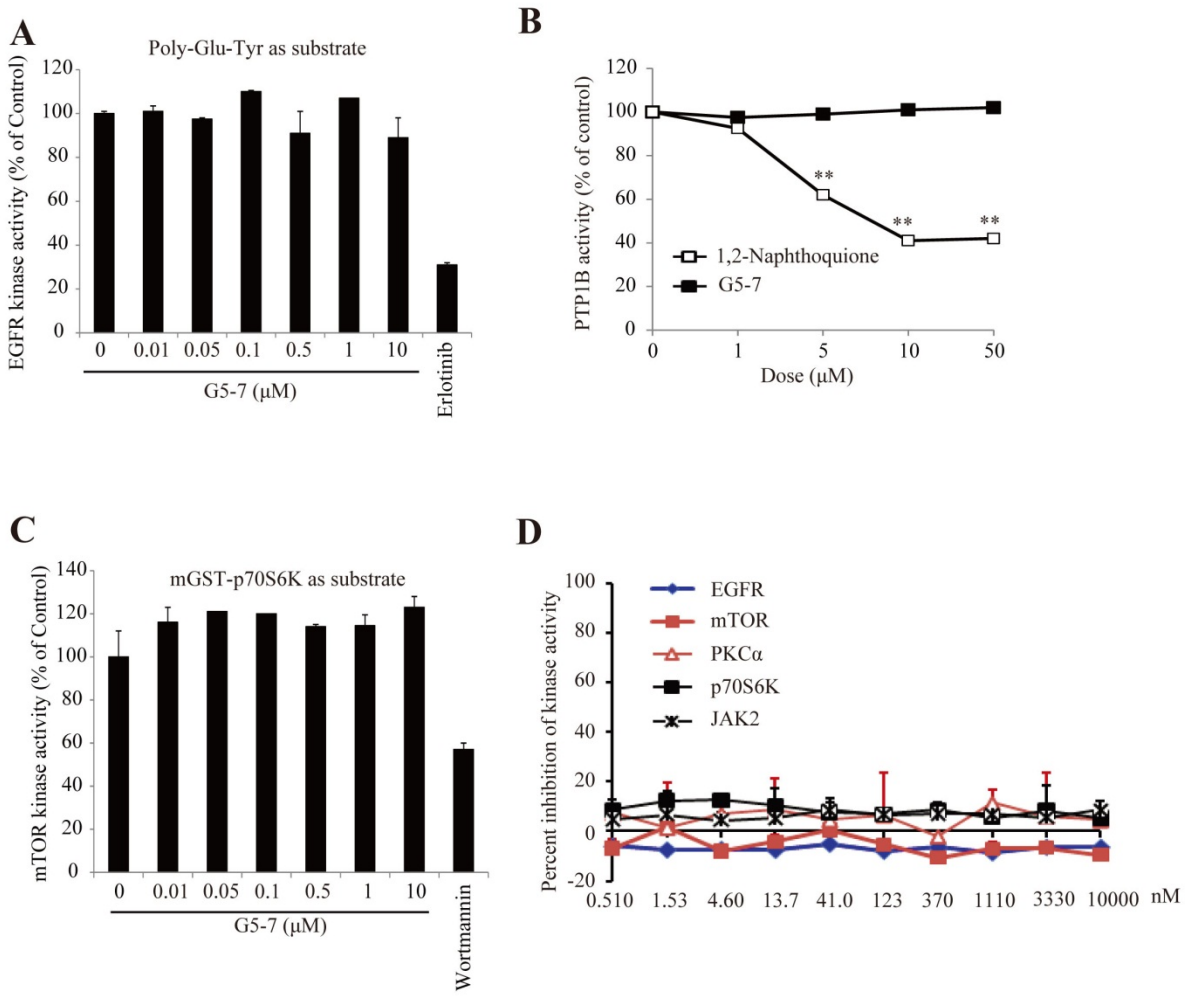


Figure S5. G5-7 does not directly block PTP1B, EGFR, and mTOR kinase activity. A. EGFR in vitro kinase assay. The phosphorylated PGT was measured by ELISA with anti-p-Tyr-HRP antibody. **B**. In vitro PTP1B assay. PTP1B protein was pre-incubated with different concentrations of G5-7 or 1, 2-Naphthoquione for 20 minutes on ice. The reaction was initiated after addition of 4 mM pNPP and lasted 30 minutes at 30 ºC. Data were shown as mean ± S. E. M. (n = 3 experiments, * P<0.05, ** P<0.01, one-way ANOVA). **C.** mTOR in vitro kinase assay. The phosphorylation of $p70^{S6K}$ was measured by ELISA with anti-p-p70S6K 389 antibody. **D**. G5-7 does not inhibit the kinase domain's activity from EGFR, JAK2, mTOR, PKCα and p70S6K kinases. The kinases were selected for the in vitro kinase assay (Invitrogen,

SelectScreen^{TM} Kinase Profiling) with different concentrations of G5-7 and 10 μM ATP. Data were shown as mean ± S. E. M. (n = 3 experiments, one-way ANOVA).

Supplemental figure 6

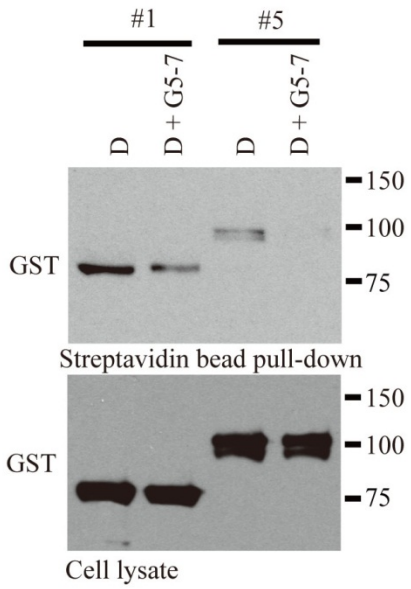


Figure S6. Confirmation of the binding of G5-7 and JAK2. Biotin-streptavidin pull-down assay. Following transfecting GST-JAK2 (A.A.1-543, fragment #1) or GST-JAK2 (A.A.1-806, fragment #5) into HEK293 cells, cell lysate were collected. Then biotin-labeled G5-7 (D, final concentration 5 μM) and G5-7 (final concentration 50 μM) or vehicle was added into equal amount of lysate (total protein 1 mg). Biotin-labeled G5-7-associated proteins were pulled down with NeutrAvidin beads and analyzed by immunoblotting analysis.

Supplemental figure 7

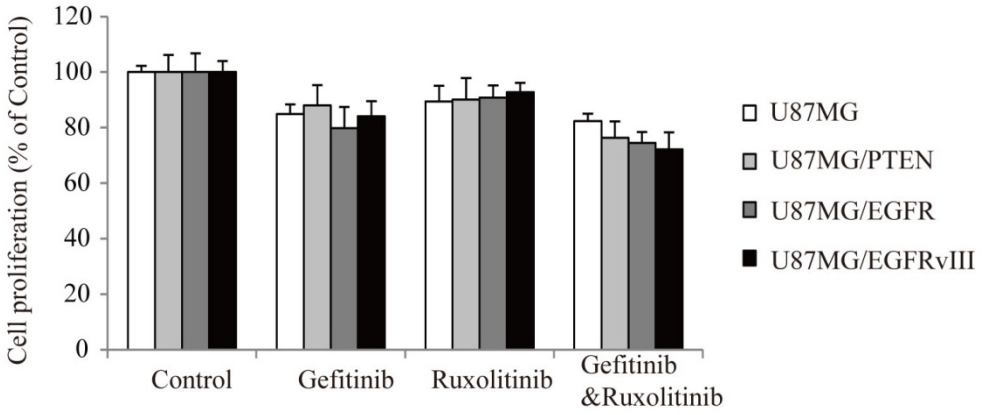


Figure S7. Effect of gefitinib and ruxolitinib on proliferation of U87MG/EGFRvIII and U87MG/PTNE cells. Cells were treated with gefitinib (10 μM) or ruxolitinib (200 nM) or their combination for 96 hours followed by an MTT assay.

Supplemental figure 8

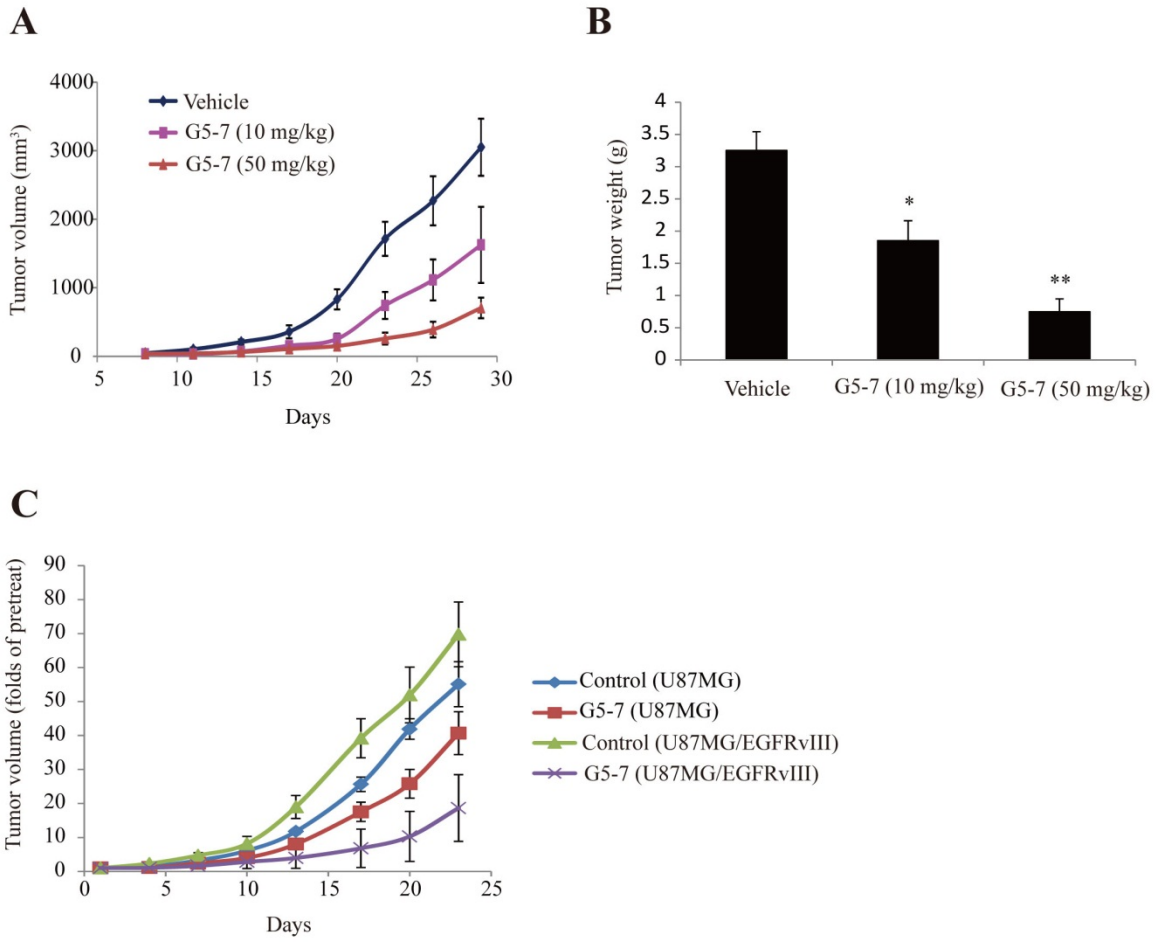


Figure S8. G5-7 blocks tumor growth of U87MG/EGFRvIII in subcutaneous model.

A & B. G5-7 significantly inhibits the growth of U87MG/EGFRvIII xenograft tumors. U87MG/EGFRvIII cells were inoculated subcutaneously in the flank of nude mice, and after the tumors were formed, the nude mice were orally injected with vehicle (0.5% methylcellulose) or G5-7 at doses of 10 and 50 mg/kg (n=7-8/group) for 21 days. Data represent mean ± S. E. M. (*P< 0.05, **P< 0.01, one-way ANOVA, n=3). **C.** G5-7 selectively inhibits the growth of U87MG/EGFR xenograft tumors compared to that of U87MG xenograft tumors (n=7-8/group). Data represent mean ± S. E. M.

Supplemantary figure 9

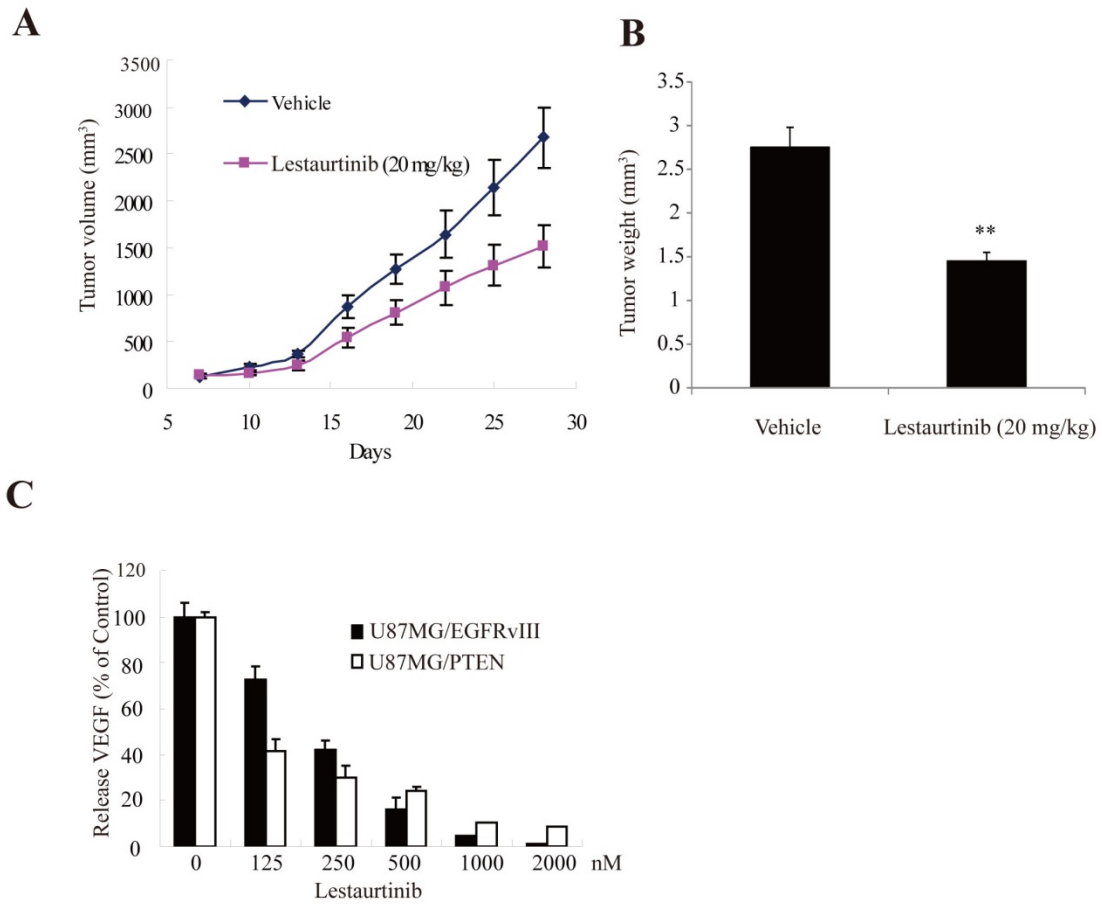


Figure S9. Lestaurtinib inhibits U87MG/EGFRvIII tumor growth in subcutaneous model. **A & B.** Lestaurtinib blocks the growth of U87MG/EGFRvIII subcutaneous xenograft tumors in mice. U87MG/EGFRvIII cells were inoculated subcutaneously in the flank of nude mice. After the tumors were formed, the nude mice were orally injected with vehicle (Gelucire: propylene glycol as 3:1) or lestaurtinib at a dose of 20 mg/kg (n=6/group) for 21 days. Data of tumor volume in (A) and tumor weight in (B) represent mean ± S. E. M. (*P< 0.05, **P< 0.01, one-way ANOVA, n = 3). **C.** Lestaurtinib inhibits VEGF secretion in a dose-dependent manner. U87MG/EGFRvIII and U87MG/PTEN cell lines were treated with different doses of lestaurtinib for 24 hours and the VEGF released in the medium was measured with a human VEGF ELISA kit.

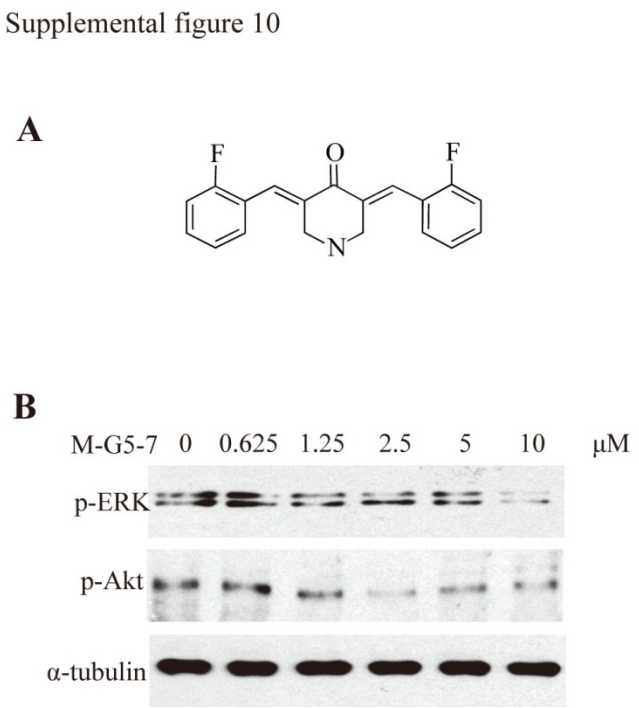
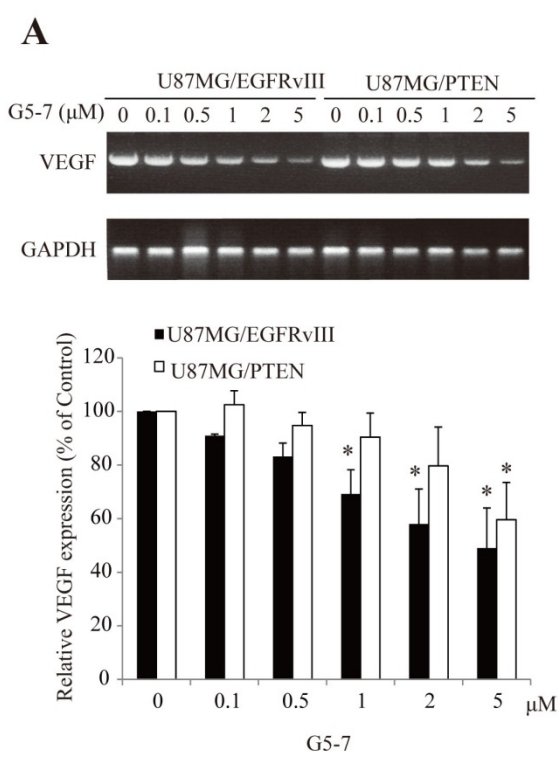


Figure S10. Effect of the metabolite of G5-7 (M-G5-7) on p-Akt and p-ERK expression in U87MG/EGFRvIII cells. A. Structure of M-G5-7. **B.** U87MG/EGFRvIII cells treated with

M-G5-7 at indicated concentrations for 6 hours, the lysates were analyzed by immunoblotting with various antibodies as indicated.

Supplemental figure 11



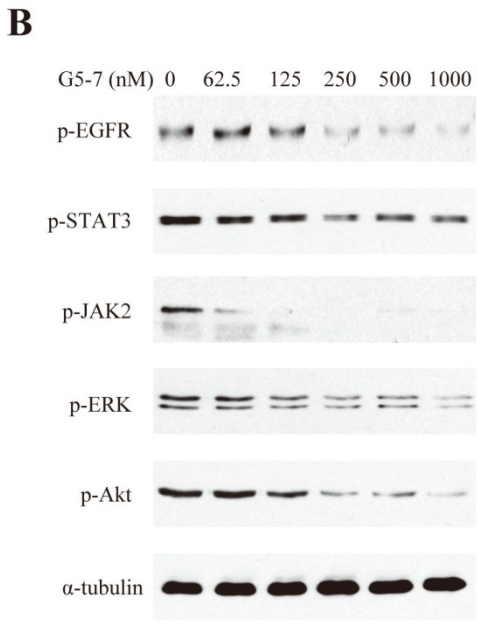


Figure S11. Regulatory effect of G5-7 on VEGF mRNA level and invasion/migration related signaling. A. G5-7 suppresses VEGF expression at mRNA level in U87MG/EGFRvIII and U87MG/PTEN cells. The cells were treated with indicated concentrations of G5-7 for 24 hours, and then RT-PCR analysis was employed. Quantification was carried out with Image J software. Data represent mean ± S. E. M. (*P< 0.05, one-way ANOVA, n=3). **B.** G5-7 dose-dependently suppresses JAK2/EGFR/STAT3 signaling, as well as Akt and ERK activation. HUVEC cells were treated with indicated concentrations of G5-7 for 6 hours; the lysates were analyzed by immunoblotting with various antibodies as indicated.

Supplemental figure 12

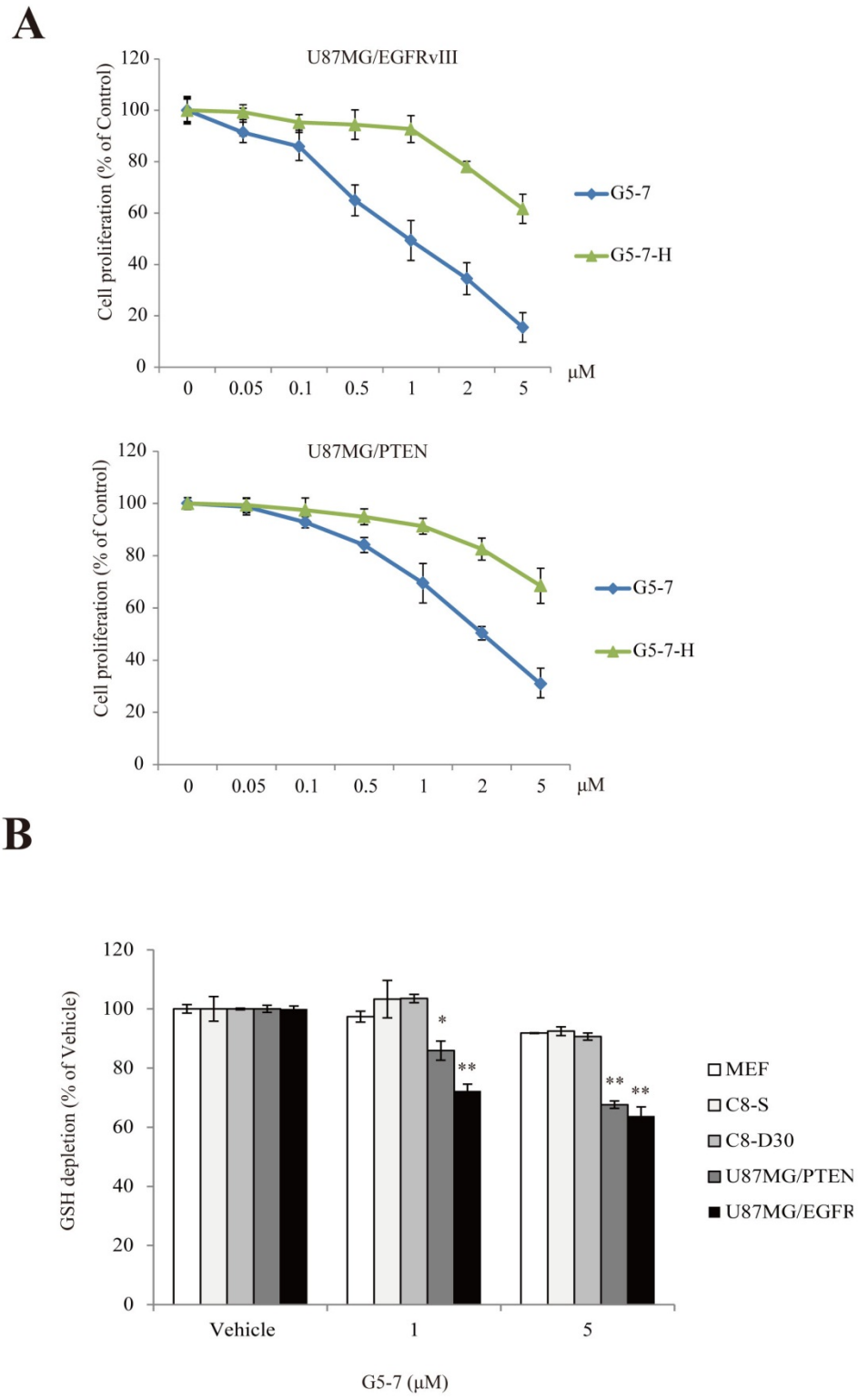


Figure S12. Effect of G5-7 on GSH depletion in different kinds of cells and effect of G5-7-H on cell proliferation in U87MG/EGFRvIII and U87MG/PTEN cells. A. Effect of G5-7-H one cell proliferation in U87MG/EGFRvIII and U87MG/PTEN cells. The cells were treated with different dosage of G5-7 for 96 h followed by an MTT assay. **B**. U87MG/EGFRvIII, U87MG/PTEN, C8-S, C8-D30 and MEF cells were treated with the indicated concentrations of G5-7 for 5 hours, followed by GSH depletion analysis. Data were expressed as mean ± S.E.M. (n = 3 experiments, * $P<0.05$, ** $P<0.01$, vs. Vehicle, one-way ANOVA).

Supplemental figure 13

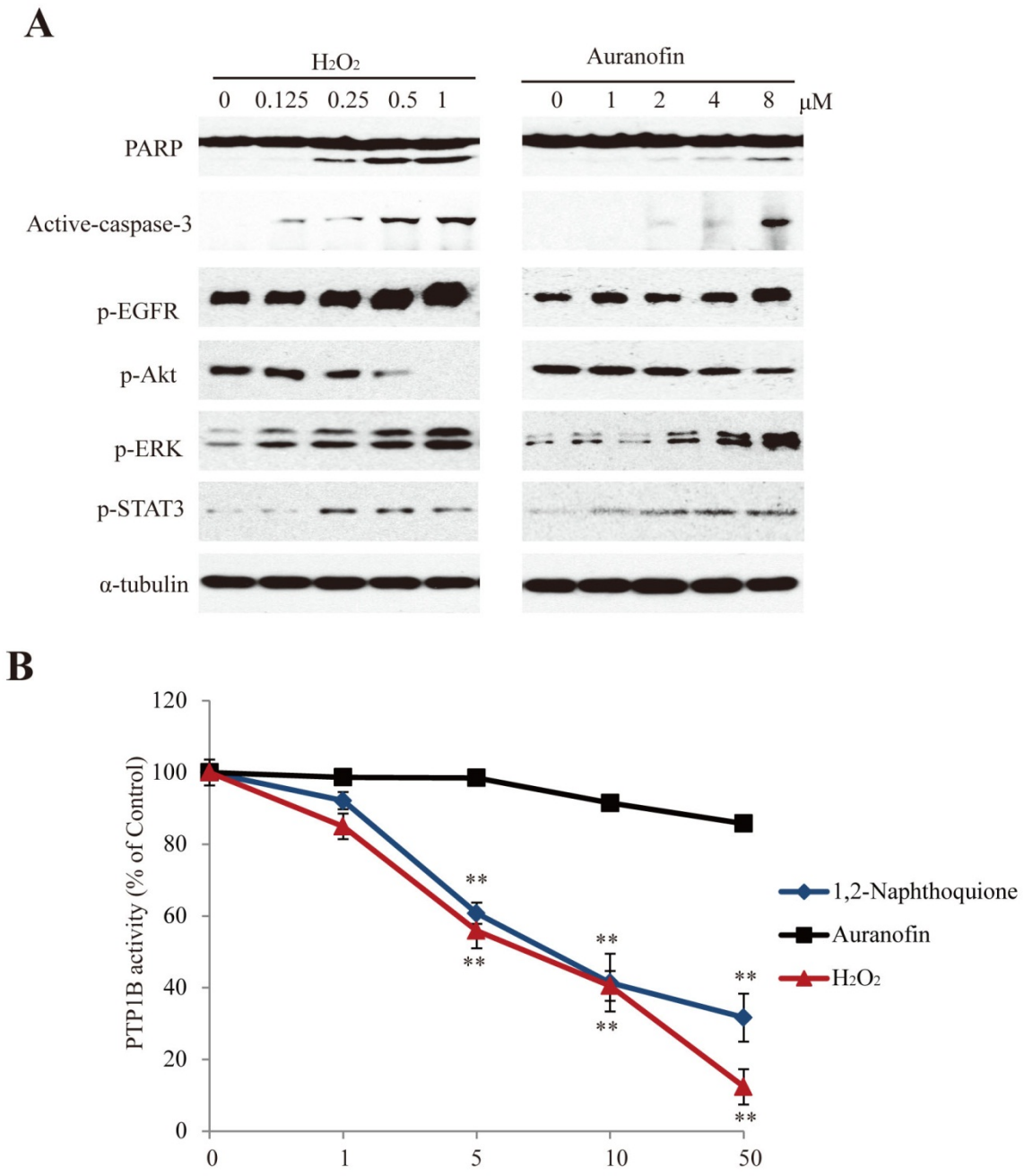


Figure S13. Effect of hydrogen peroxide (H_2O_2) and Auranofin on p-EGFR, p-Akt, p-ERK, p-STAT3 expression in U87MG/EGFRvIII cells and on PTP1B activity in vitro. A. U87MG/EGFRvIII cells treated with H_2O_2 or auranofin at indicated concentrations 6 hours, the

lysates were analyzed by immunoblotting with various antibodies as indicated. **B.** in vitro PTP1B activity assay. (n = 3 experiments, * $P<0.05$, ** $P<0.01$, one-way ANOVA).

Supplemental figure 14

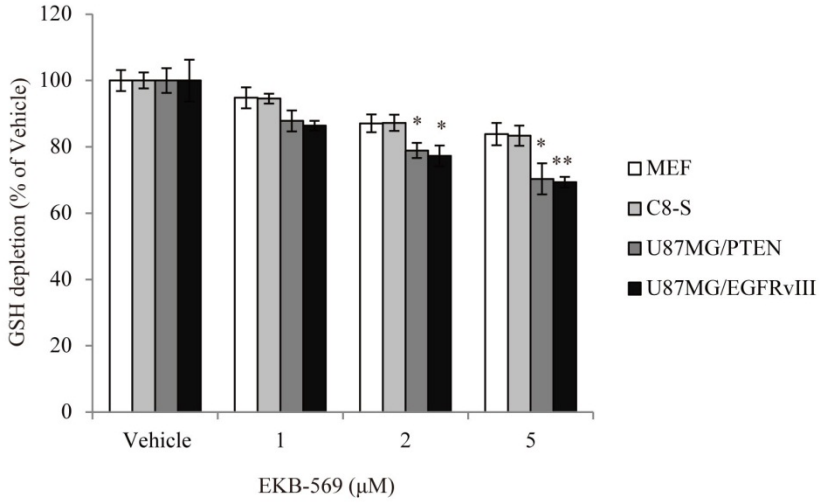


Figure S14. Effect of EKB-569 on GSH depletion in different kinds of cells.

U87MG/EGFRvIII, U87MG/PTEN, C8-S, and MEF cells were treated with the indicated concentrations of EKB-569 for 5 hours, followed by GSH depletion analysis. Data were expressed as mean ± S.E.M. (n = 3 experiments, * P<0.05, ** P<0.01, vs. Vehicle, one-way ANOVA)

Table S1. Chemical structures of G5-7 derivatives.

Supplemental table 1 Chemical structures of G5-7 derivatives.

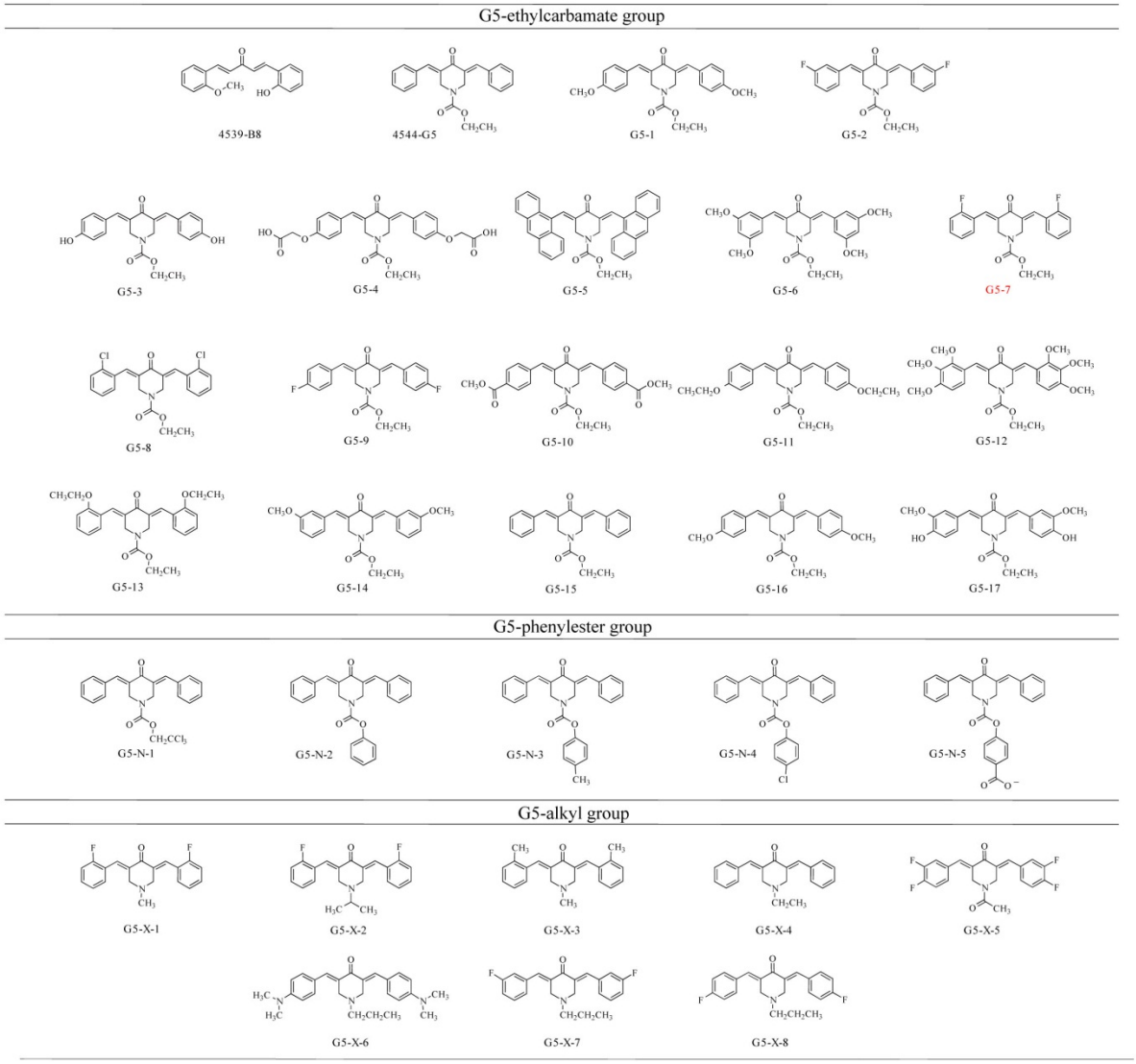


Table S2. The kinase profiling of G5-7.

A panel of kinases was selected for the in vitro kinase profile screening (Invitrogen, SelectScreen^{TM} Kinase Profiling). These assays employed the kinase domain recombinant proteins with 10 μM G5-7, 10 μM ATP or 100 μM ATP, if it is the available lowest concentration, and FRET (Fluorescence Resonance Energy Transfer)-peptide as substrates. The kinase assays were performed according to Invitrogen instructions.

Supplemental table 2

Kinase tested	% of inhibition	SD	Kinase tested	% of inhibition	SD
ABL1	-6	1	MAPK3 (ERK1)	-3	11
ADRBK1 (GRK2)	-6	2	MAPK8 (JNK1)	-3	11
AKT1 (PKB α)	11	4	MARK1 (MARK)	-7	7
ALK	-8	2	MET (cMet)	-4	1
AMPK A1/B1/G1	9	11	MINK1	38	14
AURKA (Aurora A)	1	3	MUSK	12	12
AXL	-7	5	NEK1	-5	4
BRAF	4	1	NTRK1 (TRKA)	-6	4
CDC42 BPA (MRCKA)	8	13	NTRK2 (TRKB)	-5	3
CDK1/cyclin B	-1	3	PAK1	0	13
CDK5/p25	6	4	PDGFRA (PDGFR α)	7	16
CHEK1 (CHK1)	3	17	PDK1	13	5
CLK1	-3	1	PHKG1	14	22
CSF1R (FMS)	4	3	PIM1	17	11
CSNK1A1 (CK1 α1)	-6	8	PKN1 (PRK1)	10	4
DNA-PK	0	5	PLK1	-6	2
DYRK1A	-7	3	PRKACA (PKA)	0	0
EEF2K	8	5	PRKCA (PKC α)	18	9
EGFR (ErbB1)	-4	3	PRKG1	-12	9
EPHA1	-5	5	PTK2 (FAK)	-3	3
EPHB1	-1	5	RAF1 (cRAF) Y340D Y341D	-4	9
ERBB2 (HER2)	1	3	RET	-4	2
FER	2	4	ROCK1	15	4
FGFR1	5	5	RPS6KA1 (RSK1)	1	3
FLT1 (VEGFR1)	-1	2	RPS6KA5 (MSK1)	12	11
FRAP1 (mTOR)	-10	2	RPS6KB1 (p70S6K)	10	8
FRK (PTK5)	-14	3	SGK (SGK1)	6	11
FYN	8	1	SRC	2	7
GRK4	7	1	SRPK1	1	0
GSK3A (GSK3 α)	-1	2	SRPK2	0	6
HCK	-52	26	STK4 (MST1)	1	4
HIPK1 (Myak)	-9	1	ZAP70	-1	2
IGF1R	-19	4	CAMK1 (CaMK1)	3	5
IKBKB (IKK β)	-5	0	CHUK (IKK α)	-2	12
INSR	-6	6	DAPK1	-5	7
JAK1	2	3	IRAK1	0	11
KIT	14	7	LRRK2	8	4
LCK	-9	6	PI4KA (PI4K α)	1	2
LTK (TYK1)	0	3	PIK3C2A (PI3K-C2 α)	-6	19
MAP2K1 (MEK1)	0	2	PIK3CA/PIK3R1 (p110 α/p85 a)	-7	5
MAP2K2 (MEK2)	-8	3	PIK3CD/PIK3R1 (p110 δ/p85a)	16	7
MAPK14 (p38 α)	9	10	PIK3CG (p110 γ)	11	2

Table S3. Complete blood count of vehicle and G5-7-treated mice.

C57BL/6J mice were orally treated with G5-7 (50 mg/kg) for 30 days and the blood was collected for complete blood count (CBC). The parameters of blood chemistry and biochemistry in G5-7-treated animals are within normal ranges, comparable to vehicle-treated mice.

Supplemental table 3

Parameter	Vehicle	G5-7 (50 mg/kg)	Normal range
WBC ($\times 10^3/\mu l$)	3.15±1.16	3.98 ±1.3 1	1.8 -10.7
Neutrophis ($\times 10^3/\mu l$)	0.32±0.09	0.68 ±0.2 9	0.1 -2.4
Neutrophis (% in WBC)	10.8±1.23	16.8 ±3.06	6.6 -38.9
Lymphocytes ($\times 10^3/\mu l$)	2.58±1.02	3.23 ±1.07	0.9 -9.3
Lymphocytes (% in WBC)	80.40±3.92	79.01 ±2.7	55.8 -91.6
Monocytes ($\times 10^3/\mu l$)	0.04±0.01	0.14 ±0.05	0.0 -0.4
Monocytes (% in WBC)	1.20±0.19	3.38 ±0.58	0.0 -7.5
Eosinophils ($\times 10^3/\mu l$)	0.13±0.01	0.03 ±0.0 1	0.0 -0.2
Eosinophils (% in WBC)	5.70±3.19	0.74 ±0.3	0.0 -3.9
Basophils ($\times 10^3/\mu l$)	0.02±0.01	0±0	0.0 -0.2
Basophils (% in WBCl)	0.80±0.07	0±0	0.0 -2.0
RBC ($\times 10^6/\mu l$)	10.80±0.66	8.00 ±0.38	6.36 -9.42
Hemoglobin (g/dl)	15.50±0.31	11.9 ±0.26	11.0 -15.1
Hematocrit (%)	60.67±1.40	38.8 ±1.22	35.1 -45.4
MCV (fl)	57.60±1.03	48.67 ±2.80	45.4 -60.3