Supplementary Information

Supplementary Figures

Figure S1

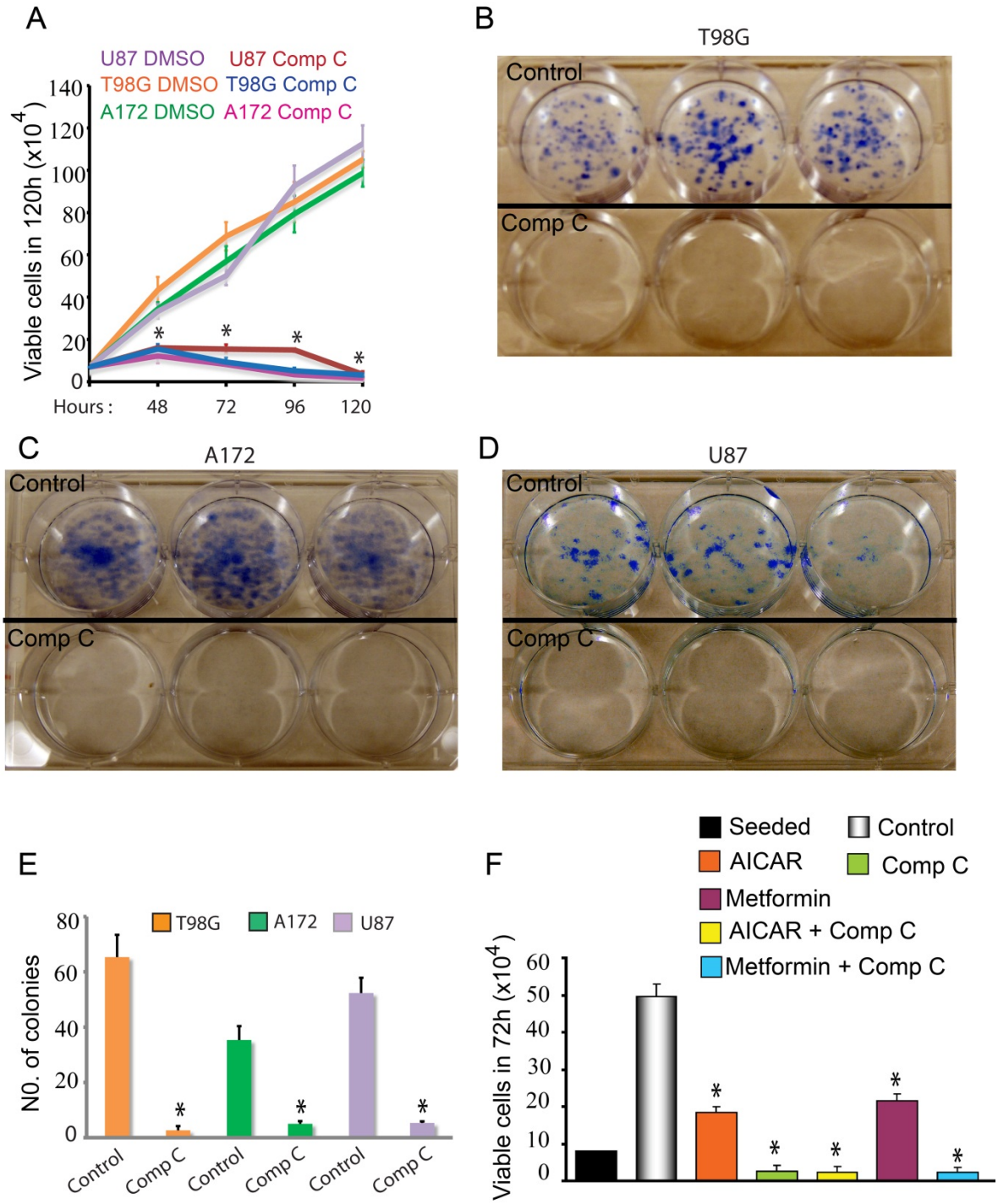

Figure S1. Antiproliferative effects of Compound C in glioma cells. (A) Proliferation analysis showing the effect of continuous treatment of glioma cells with Compound C or DMSO (control). Photomicrographs (B-D) and quantitation (E) of colony growth of three glioma cell lines grown in the presence of Compound C (5μM) or vehicle (DMSO). (F) Viability assay showing the antiproliferative actions of Compound C alone or in combination with AICAR (1mM), metformin (10mM). * $P \leq 0.001$. Data shown is representative of three to five independent experiments.

Figure S2

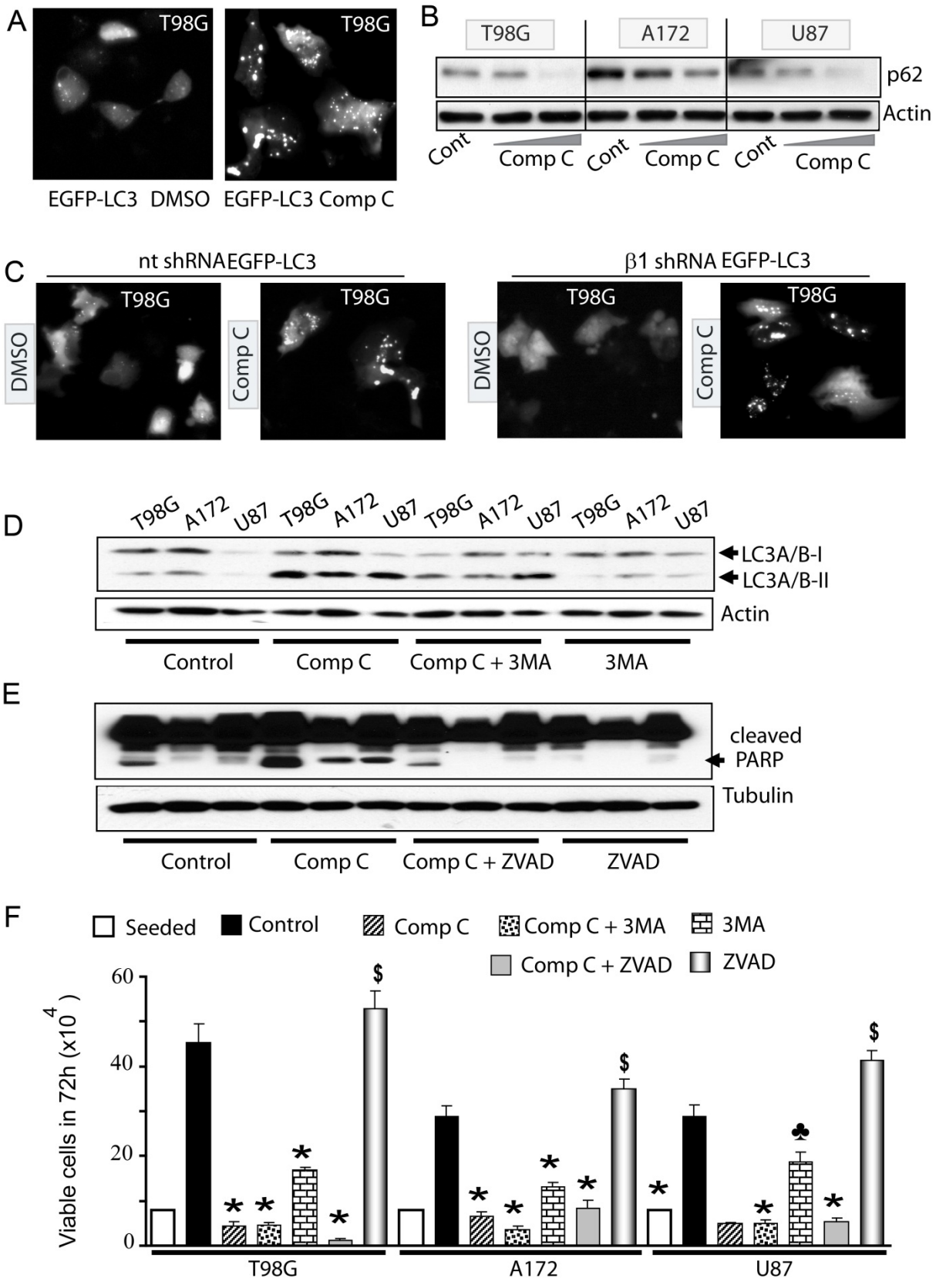
A
T98G
T98G
EGFP-LC3 DMSO
EGFP-LC3 Comp C
B
T98G
A172
U87
p62
Actin
Cont
Comp C
Cont
Comp C
Cont
Comp C
C
nt shRNA EGFP-LC3
β1 shRNA EGFP-LC3
T98G
T98G
T98G
T98G
DMSO
Comp C
DMSO
Comp C
D
T98G A172 U87 T98G A172 U87 T98G A172 U87 T98G A172 U87
LC3A/B-I
LC3A/B-II
Actin
Control
Comp C
Comp C + 3MA
3MA
E
cleaved PARP
Tubulin
Control
Comp C
Comp C + ZVAD
ZVAD
F
Seeded
Control
Comp C
Comp C + 3MA
3MA
Comp C + ZVAD
ZVAD
Viable cells in 72h (x10^4)
60
40
20
0
T98G
A172
U87

Figure S2. Compound C induces AMPK-independent autophagy and the antiproliferative effects of Compound C are not blocked by autophagy and apoptosis inhibitors. (A) Immunofluorescence microscopy of glioma cells transfected with EGFP-LC3 and treated with either DMSO (control) or Compound C (5μM). Note, accumulation of LC3 positive autophagic puncta in EGFP-LC3 transfected glioma cells treated with Compound C. (B) Immunoblots showing degradation of autophagy substrate p62 in Compound C treated cells. (C) Immunofluorescence microscopy of control (nt) and AMPK β1-silenced glioma cells transfected with EGFP-LC3 and treated with either DMSO (control) or Compound C (5μM). Note, accumulation of LC3 positive autophagic puncta in both control (nt) and AMPK β1-silenced EGFP-LC3 transfected glioma cells treated with Compound C. (D, E) Immunoblots showing the effects of the autophagy inhibitor 3MA (5mM; D) and pan-Caspase inhibitor ZVAD (25μM; E) in reducing autophagy and apoptosis, respectively, alone or in the presence of Compound C. (F) Glioma cell viability assay showing the effects of Compound C alone or in the presence of the autophagy inhibitor 3MA or pan-Caspase inhibitor ZVAD. * $P \leq 0.005$.; $ ≤ 0.002; ♣ ≤ 0.001. Data is representative of two to three independent experiments.

Figure S3

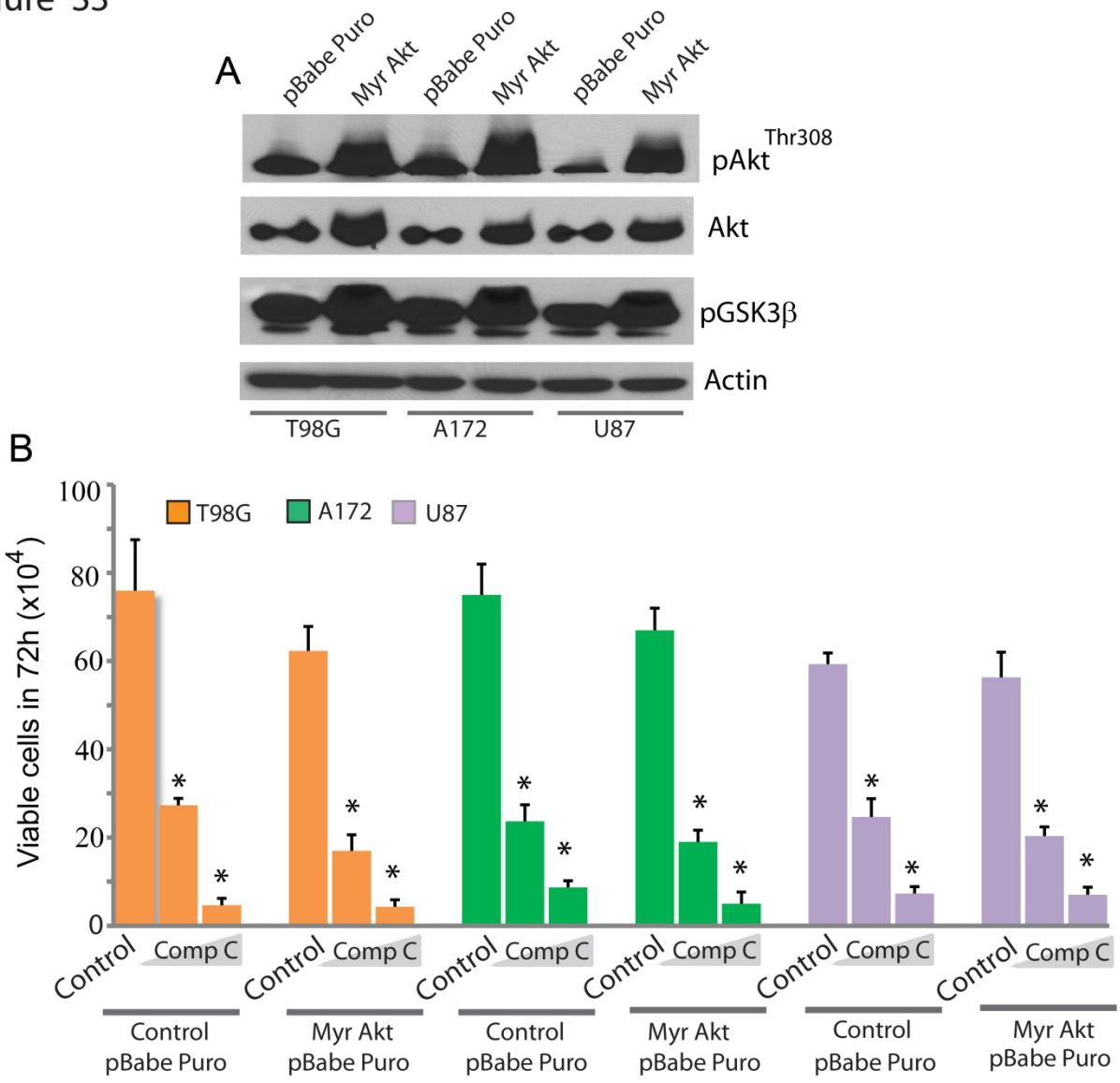

Figure S3. Constitutively active Akt fails to block Compound C's antiproliferative action. (A) Immunoblot analysis of three glioma cells transfected with pBABE puro (empty vector) or pBABE puro Myr-Akt showing levels of total Akt, phosphorylated Akt and phosphorylated GSK3β. Actin was used as a protein loading control. (B) Viability assay of three glioma cells transfected with empty vector or Myr-Akt, treated with DMSO (control) or Compound C (5μM and 10μM). * $P \leq 0.001$. Data is representative of two independent experiments.

Figure S4

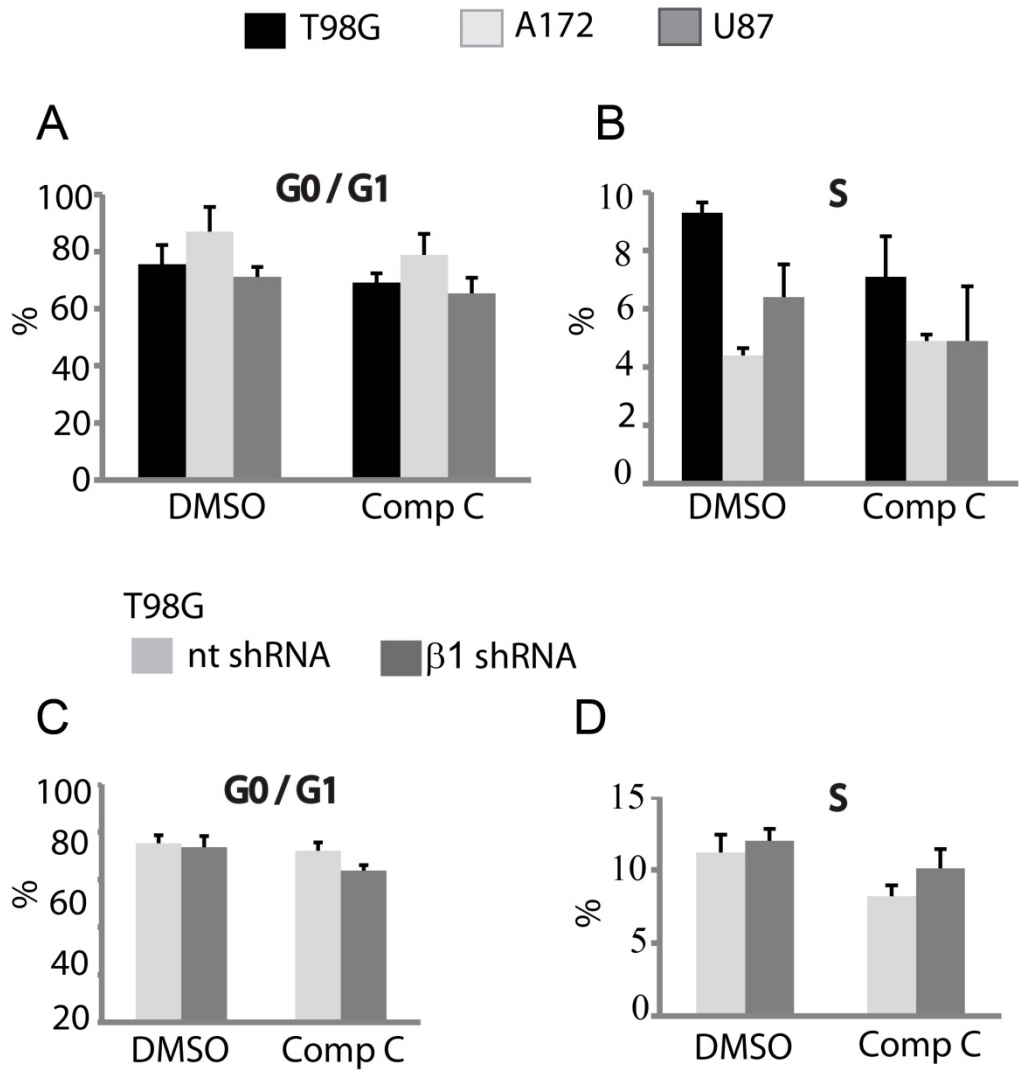

Figure S4. Effects of Compound C on glioma cell cycle. Quantitation from a flow cytometry-based cell cycle analysis of T98G, A172 and U87 glioma cells (A, B) treated with DMSO (control) and Compound C. Cells in G0/G1 and S phases are shown. (C, D) Similar analysis was done on control (nt) and AMPK β1-silenced T98G glioma cells. Data shown is representative of two independent experiments.