Supporting Information

Guo et al. 10.1073/pnas.0906606106

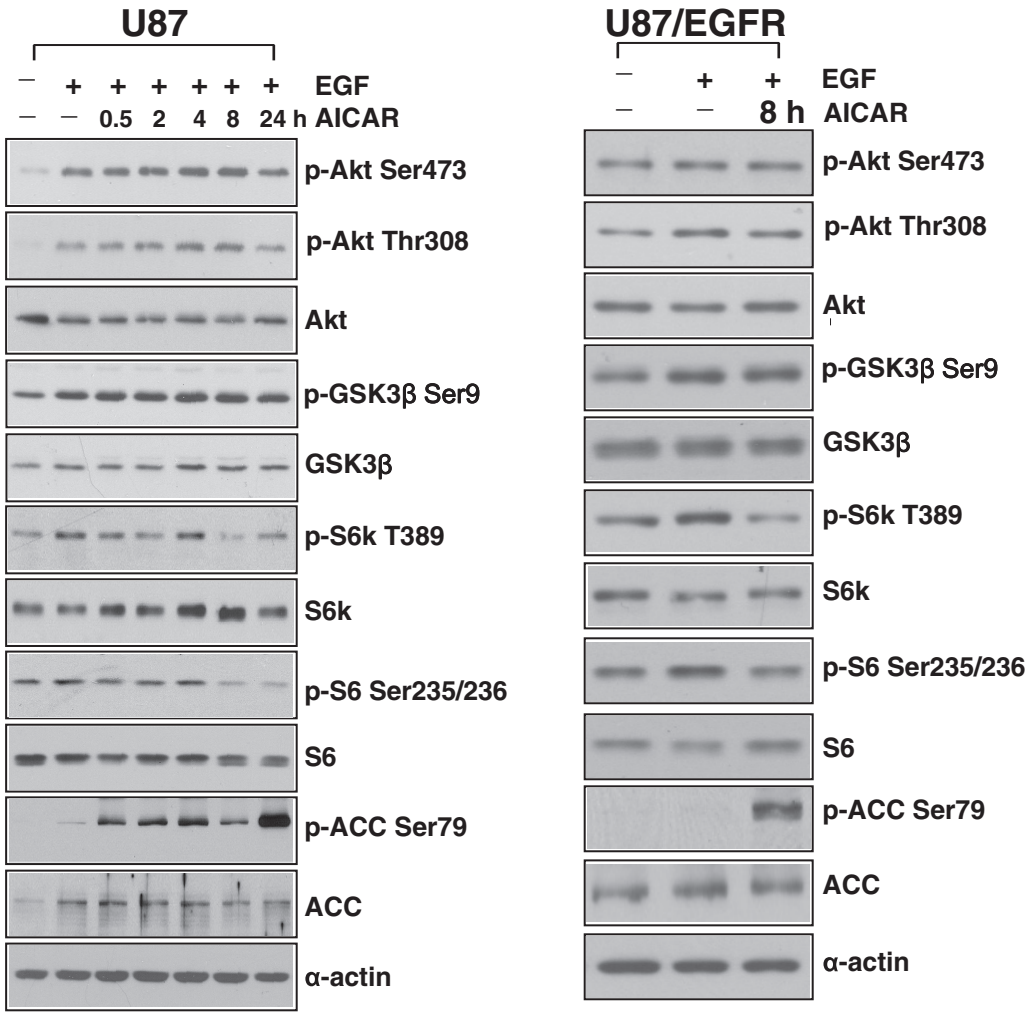

Fig. S1. AICAR treatment does not promote Akt Ser-473 or Akt Thr-308 phosphorylation. U87 or U87-EGFR cells, were treated with AICAR (0.5 mM) for up to 24 h, then EGF (10 ng/mL) for 15 min, and the effect on signaling proteins was determined by immunoblot analysis with indicated antibodies.

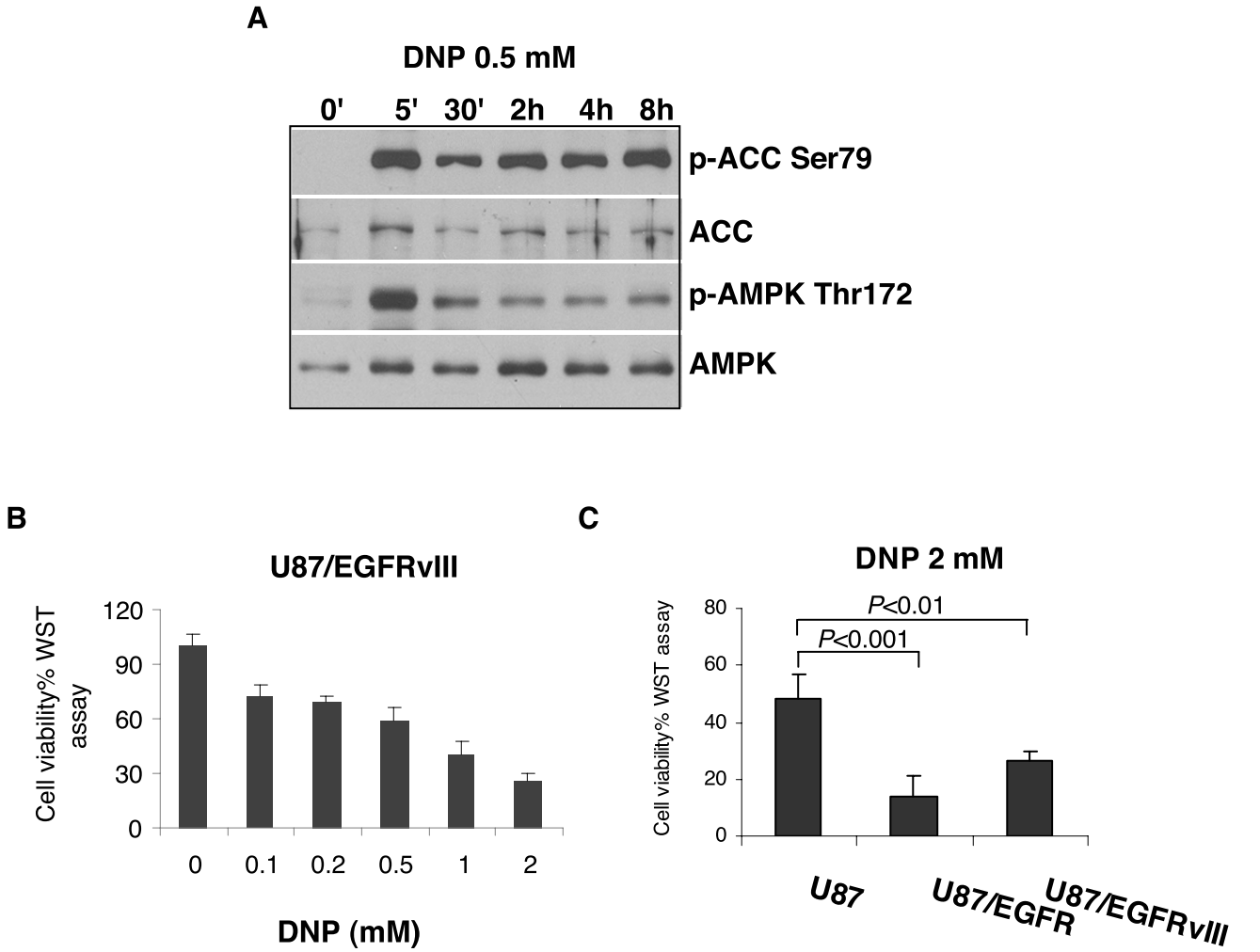

Fig. S2. Activation of AMPK through an alternative mechanism inhibits glioblastoma growth with preferential inhibition in EGFR-activated tumor cells. 2, 4-dinitrophenol (DNP) activates AMPK by lowering ATP via inhibition of oxidative phosphorylation, thus increasing the AMP/ATP ratio. (*A*) Western blot analysis of effect of DNP on AMPK and ACC. U87-EGFRvIII cells were plated and 24 h later treated with DNP (0.5 mM) for up to 8 h. AMPK activation was determined by probing cellular lysates with antibodies against AMPK Thr-172 and ACC Ser-79 phosphorylation. (*B*) U87-EGFRvIII cells were plated and 24 h later, treated with a range of concentrations of DNP in 1% FBS conditions for 3 days. Relative cell number was assessed using the WST assay (Chemicon). (*C*) DNP is significantly more effective at blocking the growth of EGFRvIII or EGFR expressing glioblastoma cells. U87, U87/EGFR, and U87/EGFRvIII cell lines were treated for 3 days with DNP (2 mM) and relative proliferation was measured by the WST assay (Chemicon). Control cells were treated by ethanol, the diluent, alone (1:1,000).

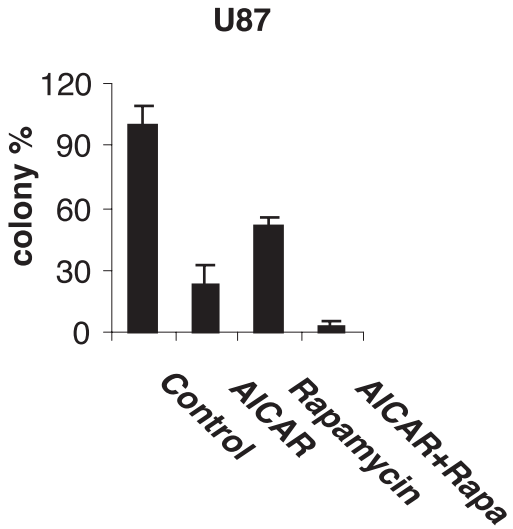

Fig. S3. Effect of AICAR and rapamycin treatment on U87 glioblastoma cell colony growth. U87 glioblastoma cells were seeded at a density of 500 cells per 60-mm dish, 24 h later, medium was changed and AICAR (0.5 mM) and/or rapamycin (1 nM) was added. The medium, including AICAR or rapamycin, was replenished every 3 days. Three weeks after initial treatment, cultures were fixed with 4% formaldehyde (Sigma) in PBS and stained with a 0.5% crystal violet solution (Sigma) in 25% methanol. Colonies of more than 50 cells were counted. Control cells were treated with the diluent, ethanol (1:1,000).

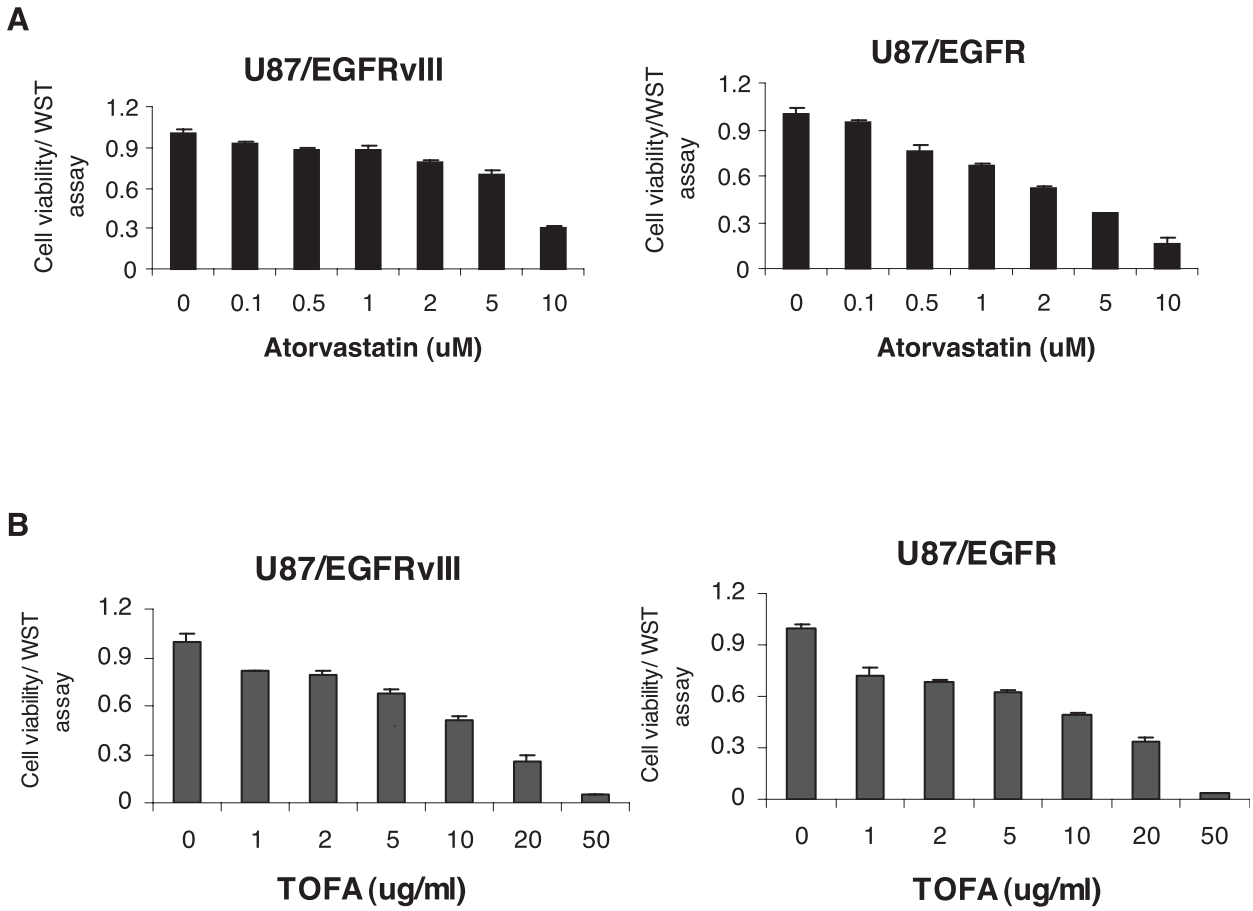

Fig. S4. Dose–response of U87-EGFRvIII and U87-EGFR glioblastoma cells to atorvastatin and TOFA. U87-EGFRvIII, and U87-EGFR cells were plated in 96-well dishes for 24 h and then treated with atorvastatin (*A*) or TOFA (*B*) for 3 days in 1% FBS at doses indicated. Relative cell number/viability was measured using the WST assay (Chemicon). Ethanol (1:1,000) as control for atorvastatin; DMSO (1:1,000) as control for TOFA treatment.

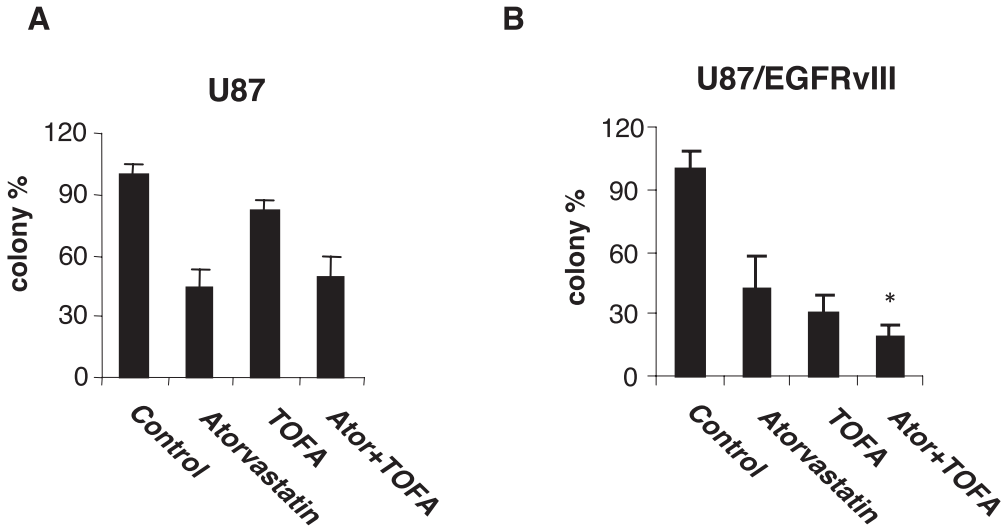

Fig. S5. Effect of inhibition of HMG-CoA reductase and ACC on glioblastoma cell colony growth. U87 and U87-EGFRvIII glioblastoma cells were seeded at a density of 500 cells per 60-mm dish, and 24 h later, medium was changed and atorvastatin (Ator 1 μM) and/or TOFA (10 μg/mL) was added. The medium, including atorvastatin (Ator) and TOFA, was replenished every 3 days. Three weeks after initial treatment, cultures were fixed with 4% formaldehyde (Sigma) in PBS and stained with a 0.5% crystal violet solution (Sigma) in 25% methanol. Colonies of more than 50 cells were counted. The experiment was repeated 3 times. Colony number was normalized with control group, into which DMSO alone was added, *P* value was determined using student *t* Test. (*A* and *B*) Evidence for enhanced efficacy by combining atorvastatin and TOFA in blocking clonogenic growth of U87-EGFRvIII cells (*B*), *, $P < 0.05$ compared with TOFA treatment. No such combination benefit is detected in non-EGFRvIII expressing U87 cells (*A*). Control cells treated by DMSO (1:1,000).

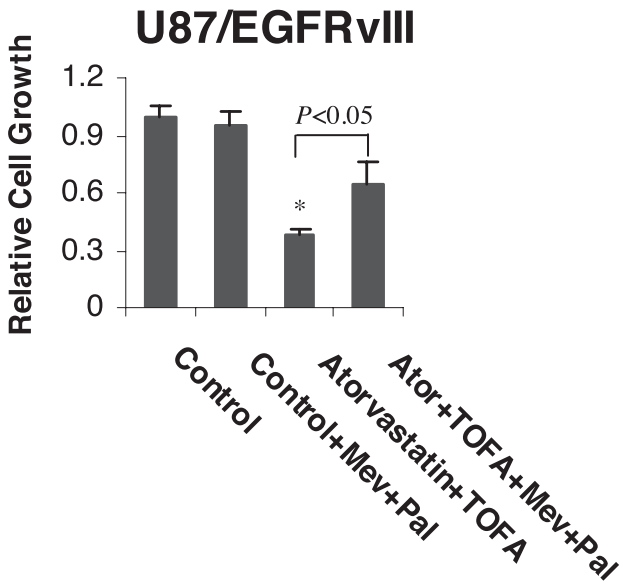

Fig. S6. Addition of mevalonate (Mev) and palmitate (Pal) rescued cell growth inhibited by atorvastatin (1 μM) and TOFA (10 μg/mL). U87-EGFRvIII cells were plated, 24 h later they were treated with either atorvastatin and TOFA at indicated doses, mevalonate and palmitate (100 μM) or atorvastatin (Ator) and TOFA plus mevalonate and palmitate. Cells were treated for 3 days and relative cell growth was determined by the WST assay. *, $P < 0.0001$ compared with control cells treated DMSO (1:1,000).

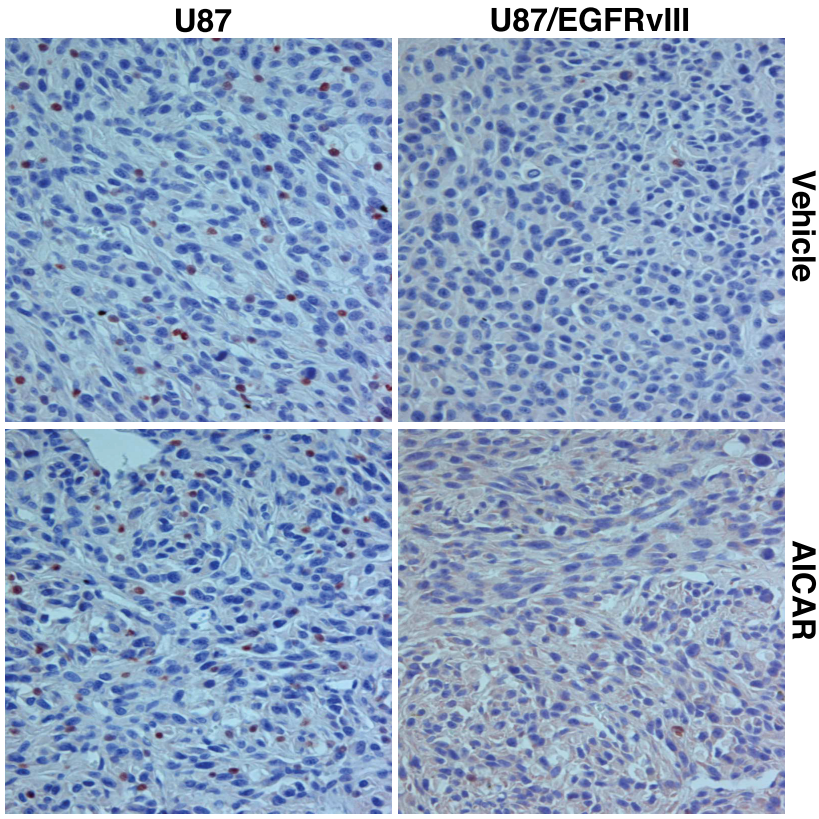

Fig. S7. AICAR treatment did not induce apoptotic cell death in vivo. TUNEL immunohistochemical staining was performed on AICAR-treated or vehicle (PBS)-treated xenografts. No increase in TUNEL positive cells was detected after treatment with AICAR (quantitation not shown as no difference was seen). EGFRvIII expressing tumors have lower baseline apoptosis.