Supporting Information

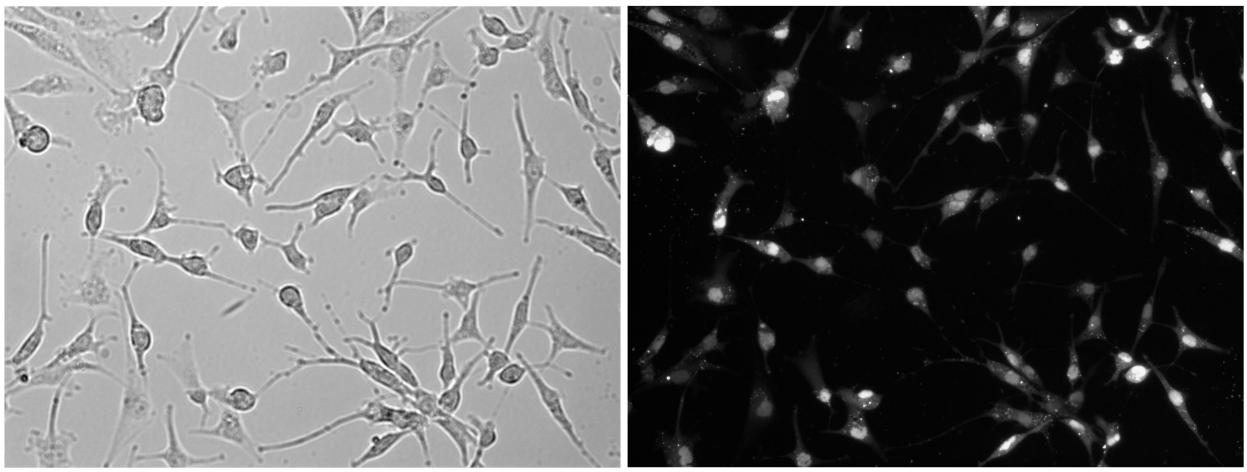

Fig. S1. In order to assess siRNA transfection efficiency, A172 cells were transfected with BLOCK-iT^{TM} fluorescein-labeled siRNA. Bright field microscopy (left) shows the total number of cells in the field, and fluorescence microscopy (right) reveals the uptake of siRNA. Under the transfection conditions employed, siRNA was taken up by essentially 100% of the cells, although the extent of uptake varied from one cell to another. According to the manufacturer, the siRNA oligos localize primarily to the nucleus upon uptake, and this pattern was observed for the A172 cells.

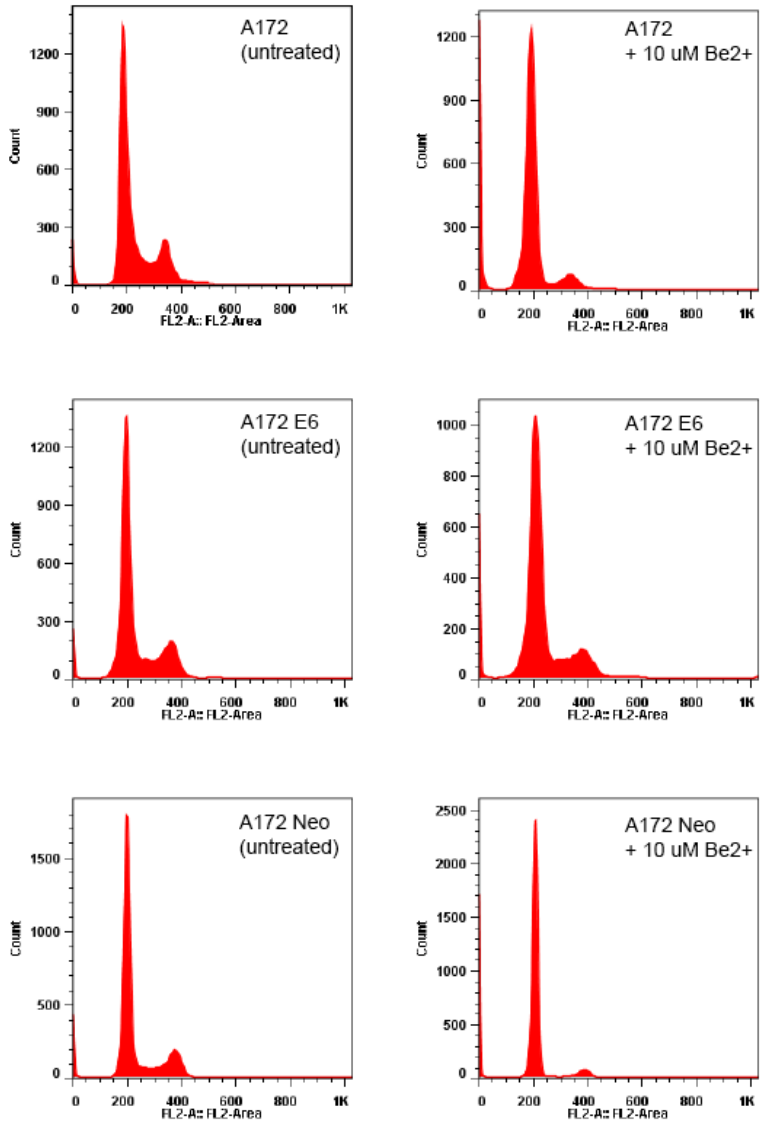

Fig. S2. Representative examples of flow cytometry raw data. Cells were grown in the presence or absence of 10 µM $BeSO_4$ for 72 hr prior to fixation, propidium iodide (PI) staining, and cell cycle analysis by flow cytometry. Histograms show PI fluorescence intensity on the x-axis and cell counts on the y-axis. Bins were assigned to quantify the G1 phase (first peak, 2n DNA content), the G2 phase (second peak, 4n DNA content), and S phase (intermediate DNA content between G1 and G2). For each sample, 20,000 events were collected. The percentage of cells in a given phase was calculated as [(counts in phase of interest / total counts in G1 + S + G2) times 100]. Mean percentage ± standard deviation (n=3/group) is reported in Fig. 3.

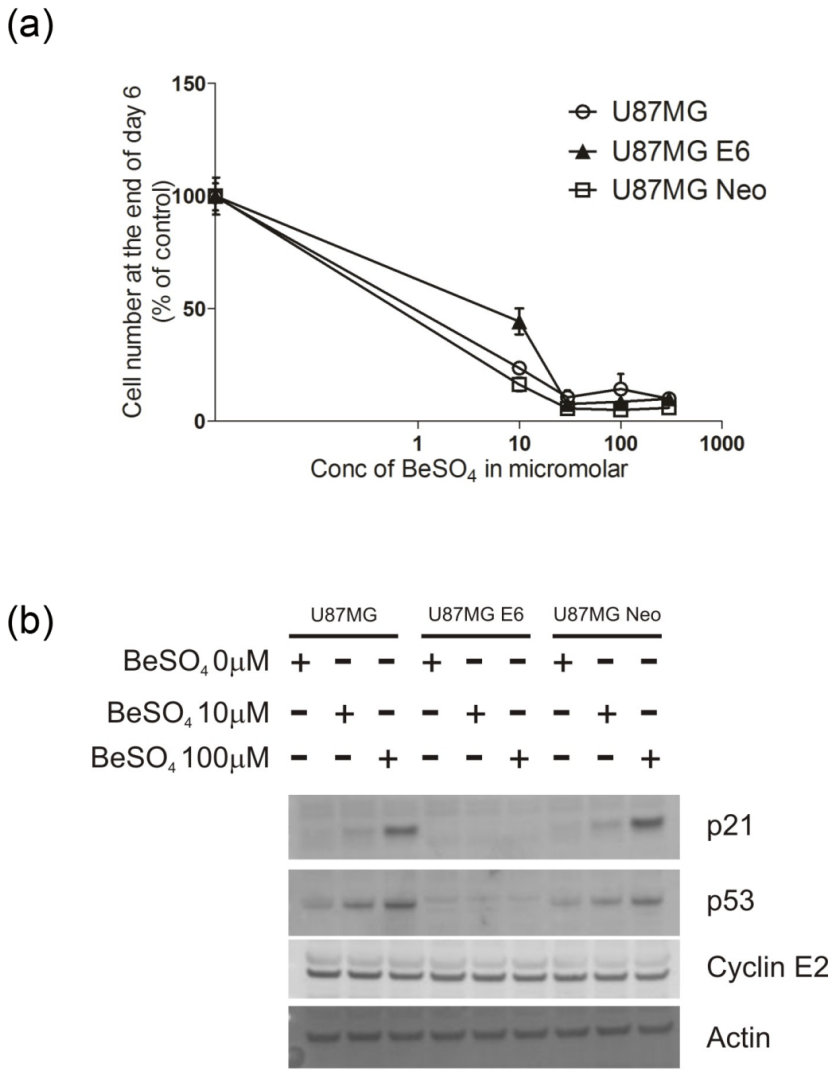

Fig. S3. $BeSO_4$ induces p53-dependent inhibition of growth and up-regulation of p21 in U87MG glioma cells. (a) U87MG cells, E6-expressing U87MG cells, and U87MG Neo vector control cells were cultured in RPMI in the presence of 0, 10, 30, 100, or 300 μM $BeSO_4$ and counted at the end of 6 days. Counts are expressed as percent of untreated (0 μM $BeSO_4$) control cell number (mean ± SD) for each cell line. Cells were passaged on Day 3 to maintain sub-confluency. (b) E6-expressing U87MG cells and the control cell lines were cultured in RPMI in the presence of 0, 10, or 100 μM $BeSO_4$ for 48 hr, then protein expression was analyzed by Western blotting.

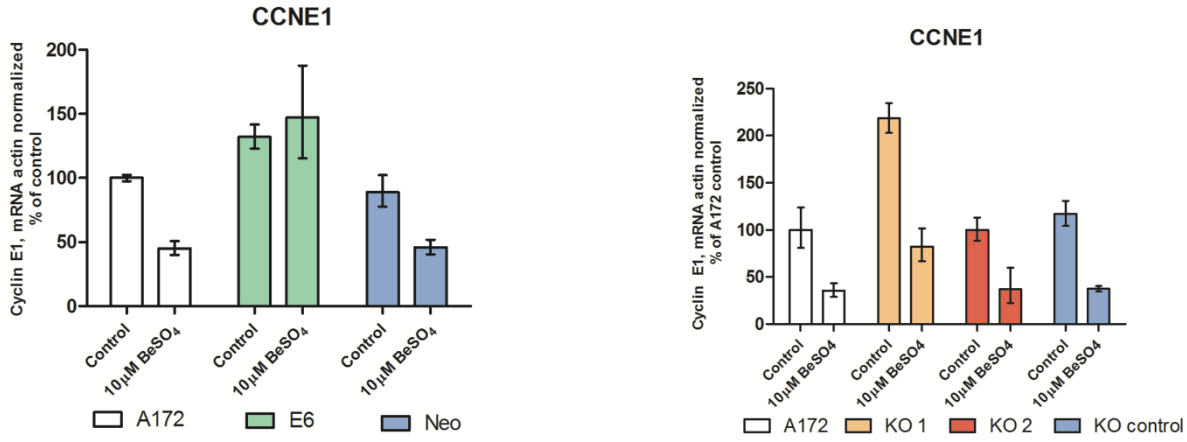

Fig. S4. Effect of $BeSO_4$ on cyclin E1 mRNA expression. Untransfected A172 cells, E6-transfected A172 cells with suppressed p53, or Neomycin vector A172 cells (left panel) or untreated A172 cells, cells treated with anti-p53 siRNA KO 1 or KO 2, or cells treated with the non-targeted KO Control siRNA sequence (right panel) were grown in 0 or 10 μM $BeSO_4$ for 48 hr and mRNA levels of cyclin E1 (CCNE1) and the housekeeping gene actin were measured in each sample using quantitative RT-PCR. Actin-normalized quantities of CCNE1 mRNA are expressed as percent of untreated control A172 in each experiment. Error bars bracket a confidence interval that is equivalent to the mean ± SD.

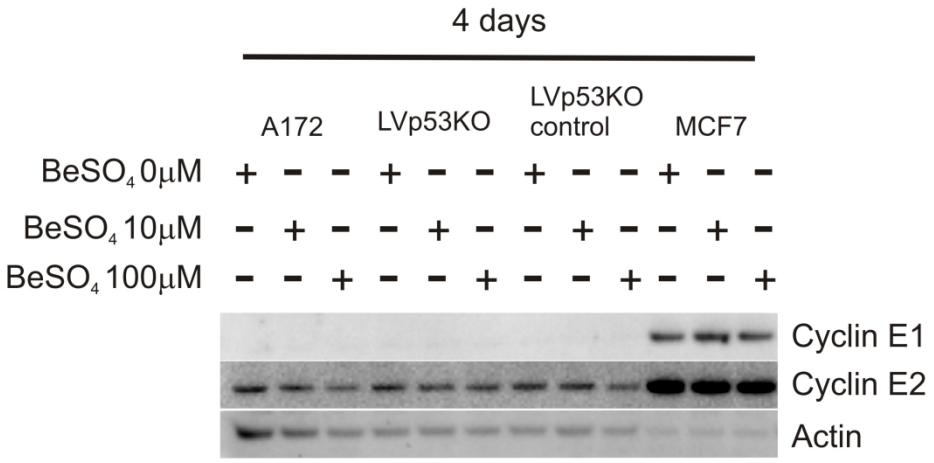

Fig. S5. Expression of cyclin E isoforms in A172 and MCF-7 cells. Untransfected control A172 cells, a lentivirus-infected A172 sub-clone expressing anti-p53 shRNA (LVp53KO), a lentivirus-infected A172 sub-clone expressing a negative control sequence shRNA, or normal MCF-7 (human breast cancer) cells were cultured in RPMI in the presence of 0, 10, or 100 μM $BeSO_4$ for 4 days, then protein expression was analyzed by Western blotting with cyclin E1, cyclin E2, and actin antibodies. Cyclin E1 protein was expressed at low levels in A172 cells, such that it was difficult to quantify this protein in this cell type by Western blotting. Samples from MCF-7 cells, which express cyclin E1 at higher levels, are shown for comparison.