Supplementary Figure S1

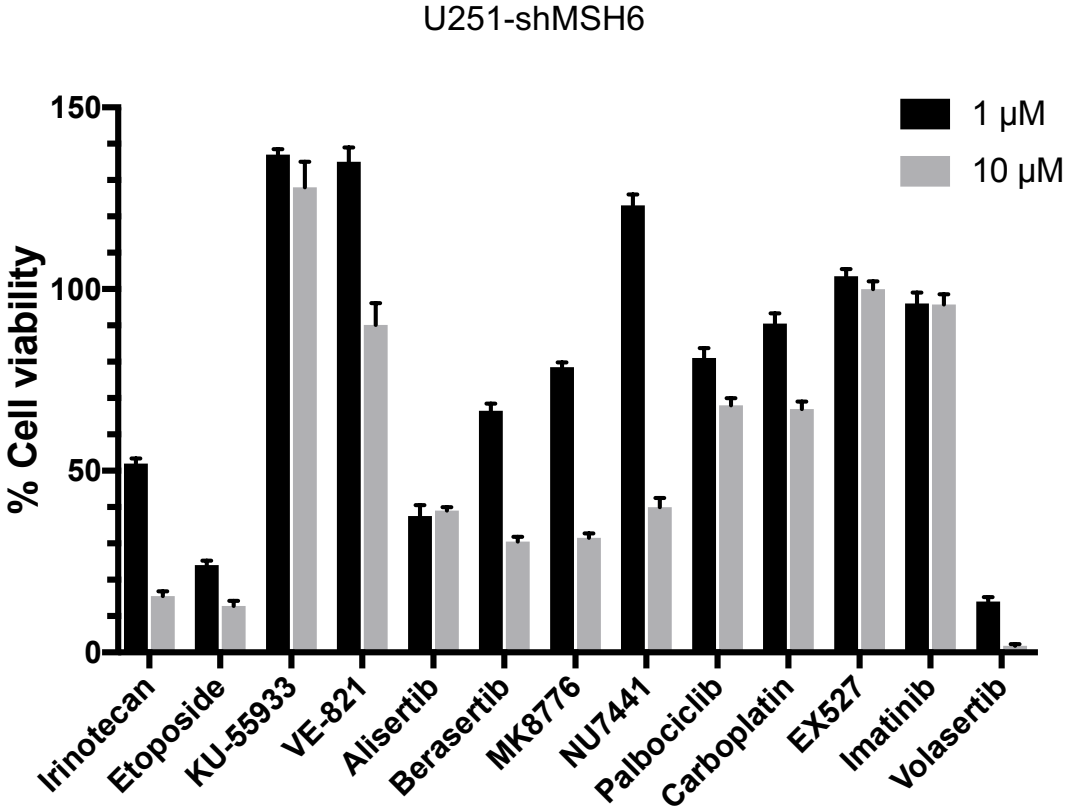


Figure S1. A compound screen identifies Volasertib as potently cytotoxic to MSH6-deficient glioblastoma cells. A compound screening of 13 DNA damage response modulators in U251-shMSH6 cells at 1 and 10 mM. Cell viability was measured by CellTiter Glo on day 6.

Supplementary Figure S2

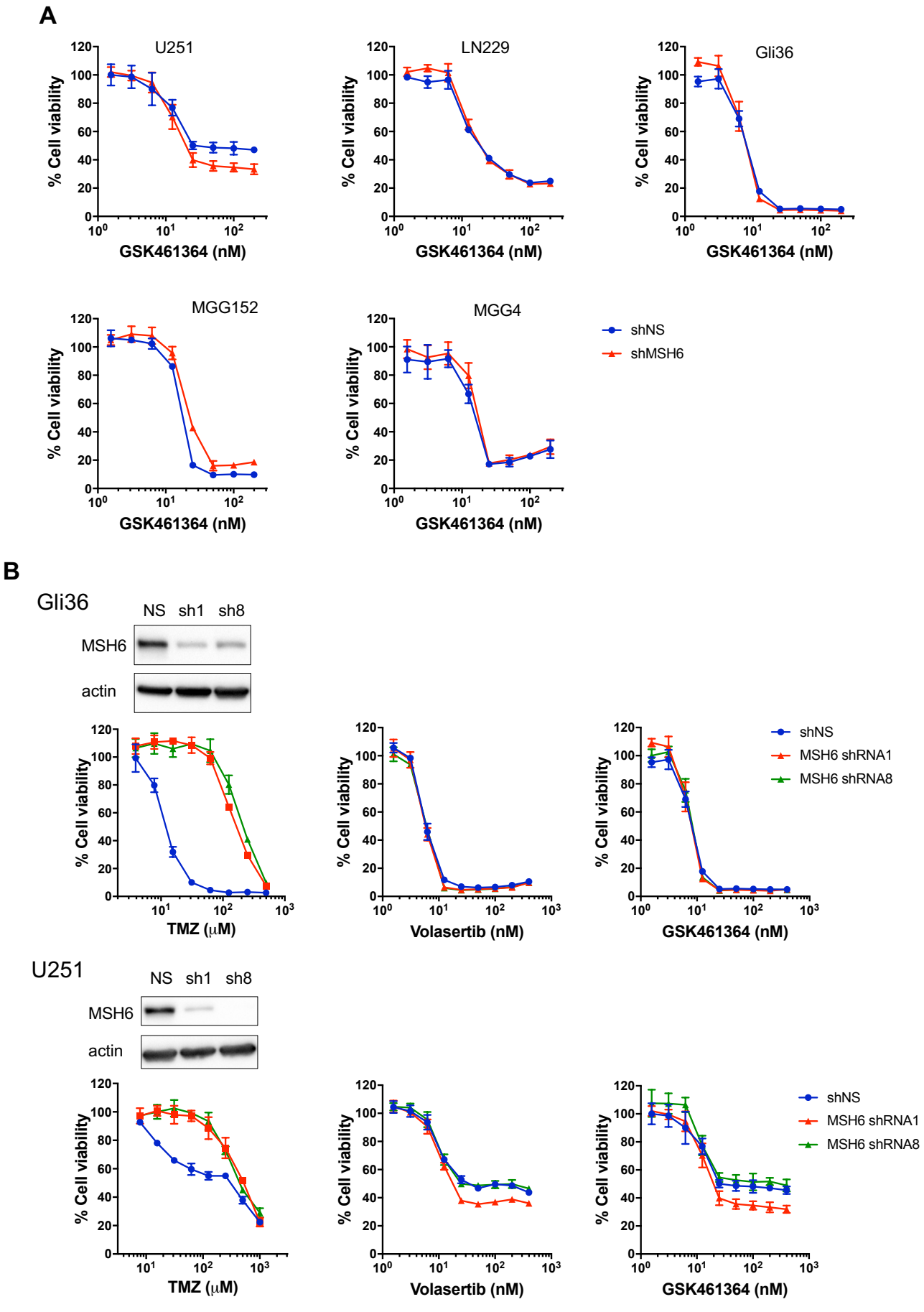


Figure S2. The effects of temozolomide and PLK1 inhibitors on glioblastoma cells with and without MSH6 knockdown.
(A) GSK461364 dose response in control (shNS, blue) and MSH6-knockdown (shMSH6, red) glioblastoma cells. Cell viability was measured by CellTiter Glo assay at 72 hours. (B) Immunoblot for MSH6 and actin in Gli36 and U251 glioblastoma cells engineered with control (shNS) and 2 shRNA sequences directed at MSH6 (sh1 that was used in main figures and sh8). Cell viability was measured by CellTiter Glo assay to determine dose response of TMZ (at day 6), Volasertib (day 3) and GSK461364 (day 3) in these cells. shNS, blue; MSH6 shRNA1, red; MSH6 shRNA8, green.

Supplementary Figure S3

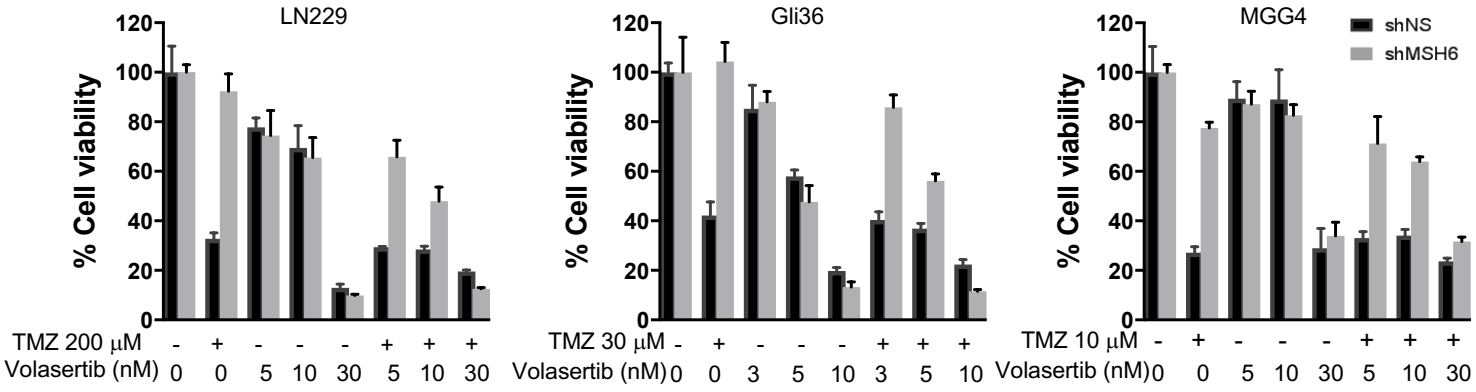


Figure S3. Combination of Volasertib and temozolomide on glioblastoma cells with and without MSH6 knockdown. Control (shNS) and MSH6-knockdown (shMSH6) glioblastoma cells were treated with indicated doses of Volasertib combined with TMZ (200 mM for LN229, 30 mM for Gli36, 10 mM for MGG4). Cell viability was evaluated by CellTiter Glo on day 6.

Supplementary Figure S4

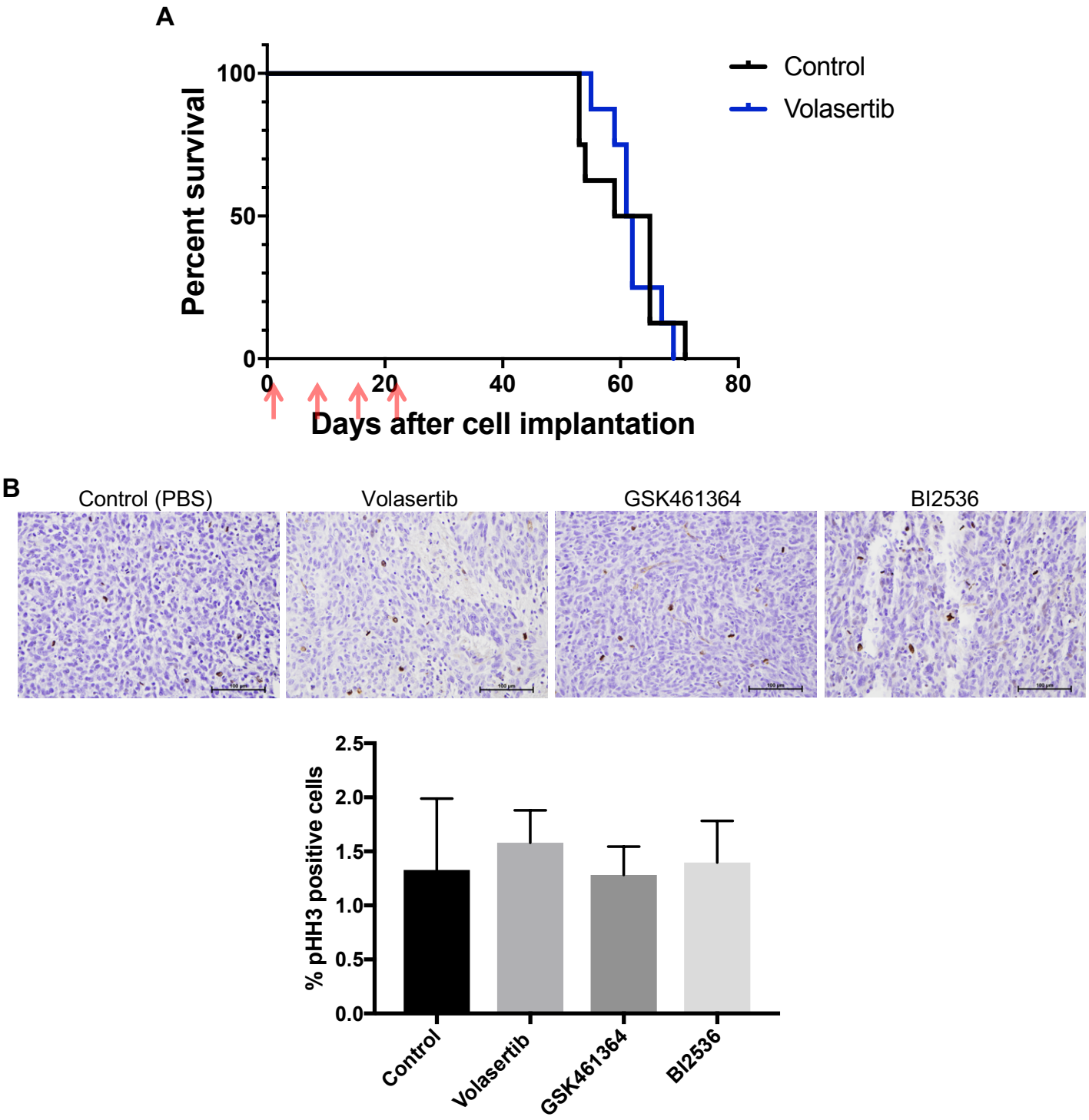


Figure S4. PLK1 inhibitor effects in orthotopic glioblastoma models. (A) Kaplan-Meier survival curves of nude mice bearing intracerebral MGG4-shMSH6 (MSH6 knockdown) tumors treated with PBS (control, N=8, black) or Volasertib (N=8, blue). Red arrows indicate dosing (i.v.) of PBS or Volasertib. P=0.87 (Log-rank test). (B) Nude mice bearing intracerebral LN229shMSH6 (MSH6 knockdown) tumors were treated on day 27 post-implantation with a single dose of 40mg/kg Volasertib (N=3, i.v.), GSK461364 (N=3, i.p.), BI2536 (N=3, i.v.) or PBS (Control, N=3, i.p.). Brains were collected at 24 hours after dosing, and pHH3 immunohistochemistry was performed (brown). Scale bars, 100 mm. Quantification of immunopositivity is shown at the bottom.

Supplementary Figure S5

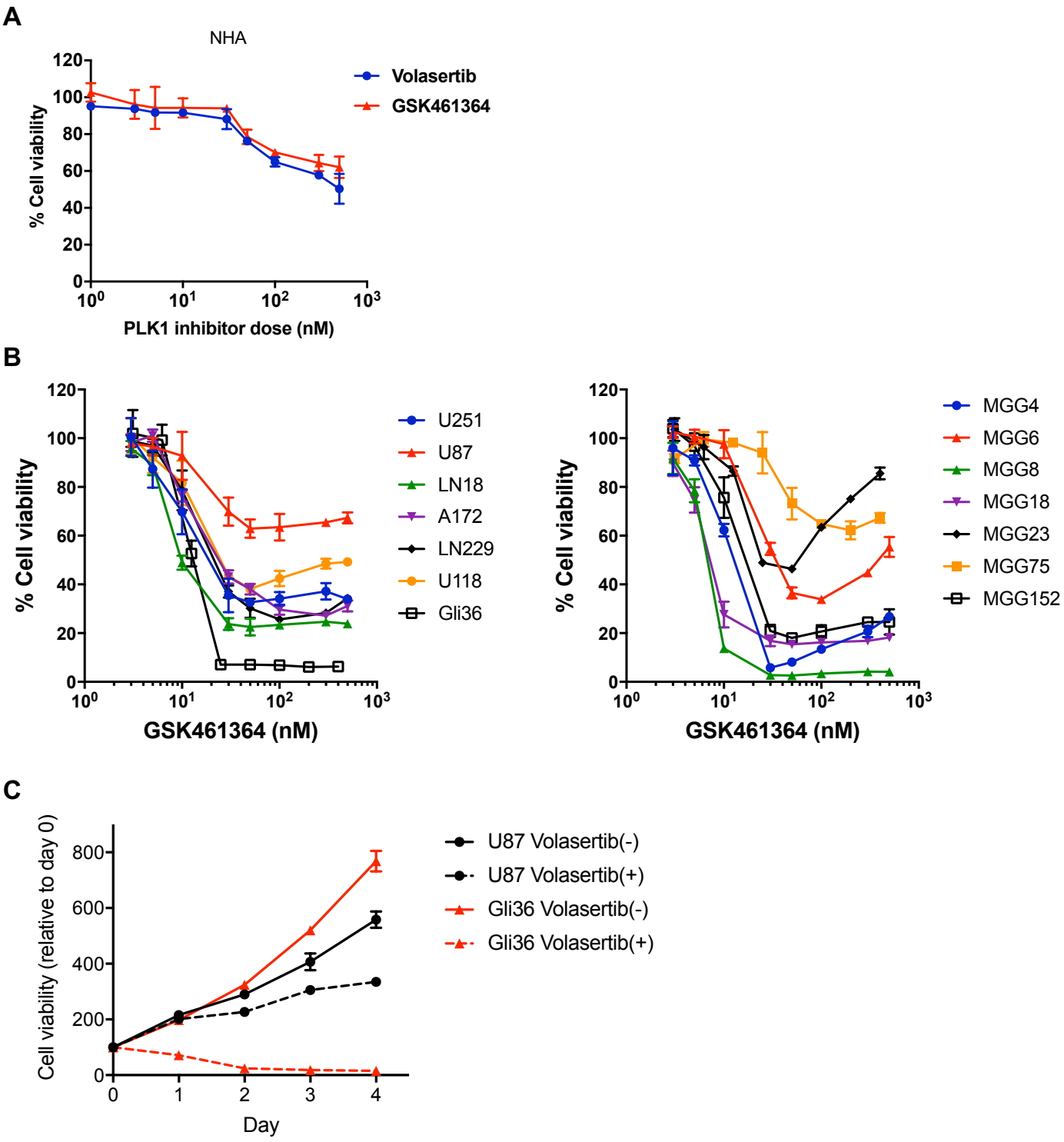
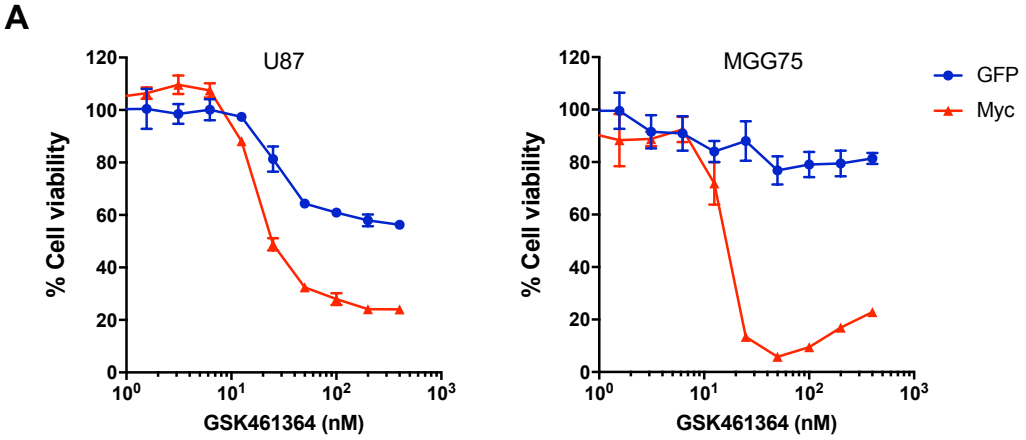
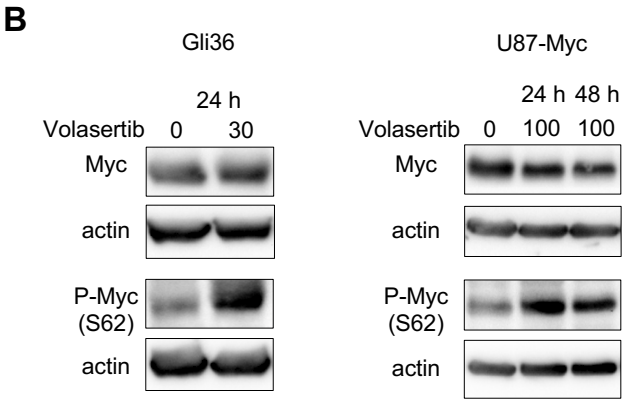
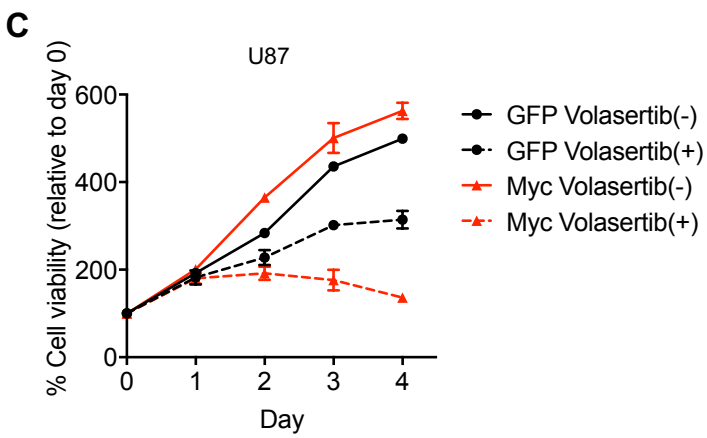


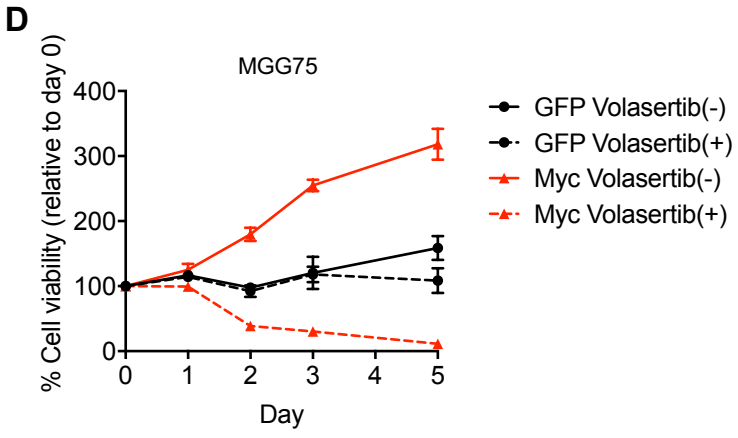
Figure S5. *In vitro* PLK1 inhibitor effects on normal astrocytes and glioblastoma cells.
(A) Dose response of Volasertib and GSK461364 in normal human astrocytes (NHA). Cell viability was measured by CellTiter Glo assay at 72 hours. (B) A cohort of glioblastoma cell lines (left), and patient-derived glioma sphere lines (right) were treated with specified concentrations of GSK461364. Cell viability was measured by CellTiter Glo at 72 hours. (C) Time course assay evaluating cell viability daily from day 1 to day 5 with U87 and Gli36 cells with and without Volasertb exposure (30 nM). Data were standardized to those on day 0 when treatment began.

Supplementary Figure S6









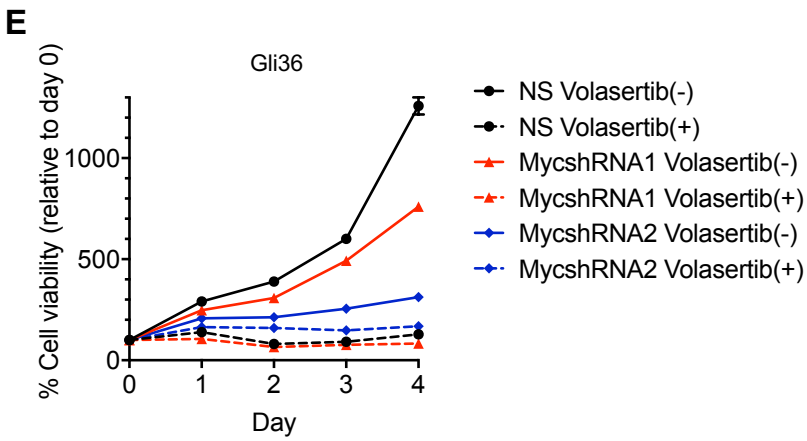


Figure S6. Myc expression and PLK1 inhibitor effects.
(A) U87 and MGG75 cells stably transduced with GFP (blue) or Myc (red) cDNA were treated with different concentrations of GSK461364. Cell viability was evaluated by CellTiter Glo at 72 hours. (B) Immunoblot of Myc and p-Myc (Serine 62) in Gli36 (left) and U87-Myc (right) cells treated with Volasertib (30 nM, for 24 hours for Gli36; 100 nM for 48 hours for U87-Myc) compared to control treatment. Actin was used as a loading control. (C-E) Time course cell viability assay. Genetically engineered cells were treated with and without Volasertib on Day 0 and cell viability was evaluated daily. Cell viability values were standardized to those on day 0. (C) U87-GFP (black) and U87-Myc (red) cells. Volasertib, 30 nM. (D) MGG75-GFP (black) and MGG75-Myc (red) cells. Volasertib, 30 nM. (E) Gli36-NS (black), Gli36-MycshRNA1 (red), and Gli36-MycshRNA2 (blue) cells. Volasertib, 20 nM.