Supplementary Materials

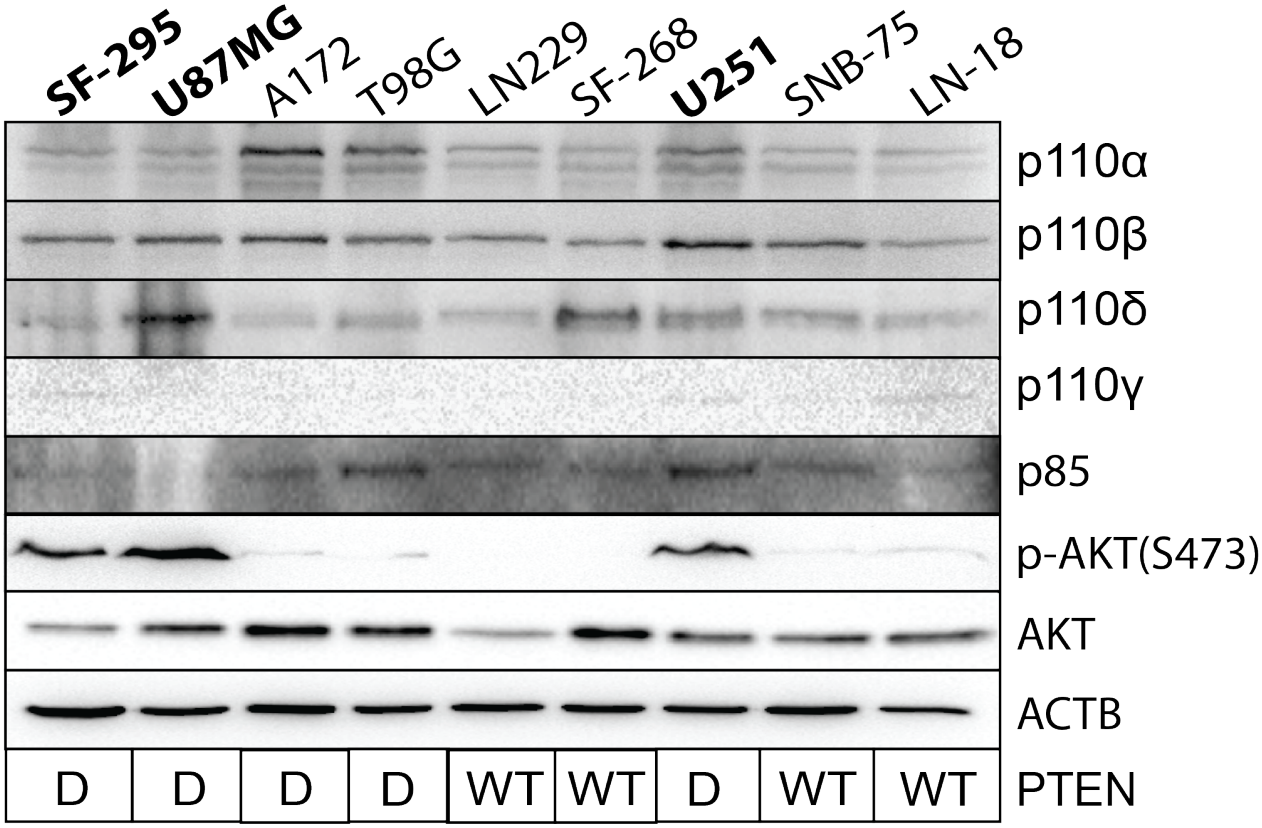

Fig. S1. Immunoblotting of PI3K and AKT proteins in GBM cell lines. This is one set of experiments shown in **Fig. 3B**. The intensities of each protein were quantified using Image J. PTEN status in each cell line determined by previous studies is shown. D: deficient; WT: wild type. Deficient PTEN is defined as either PTEN gene deletion or inactivating PTEN mutations.

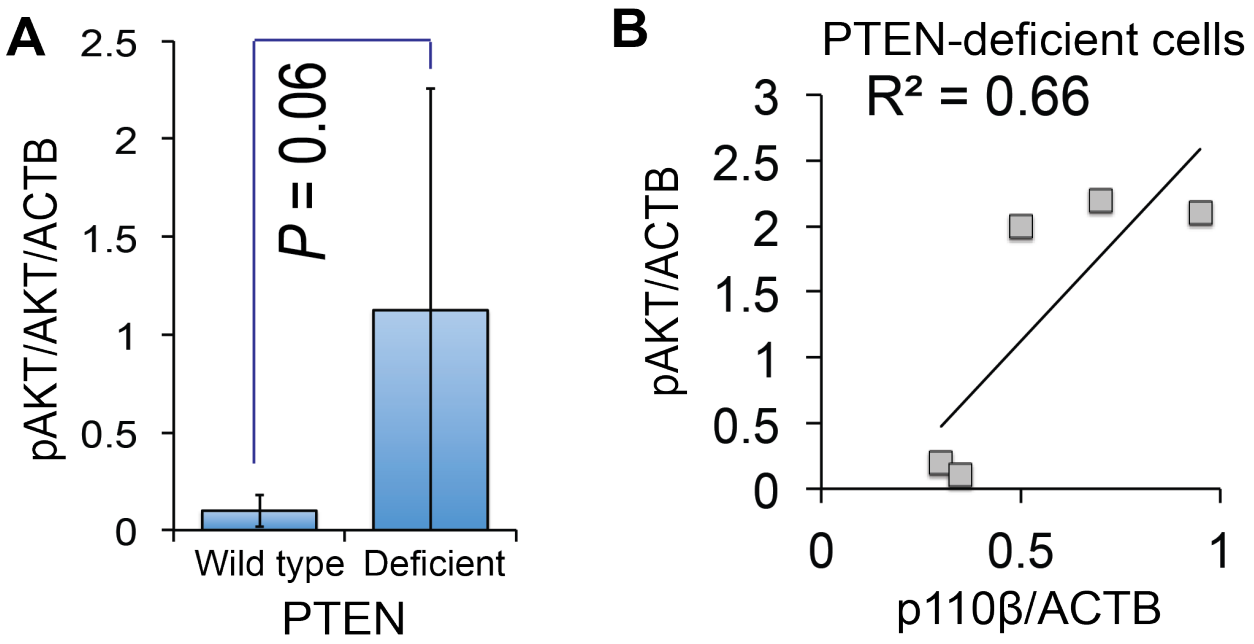

Fig. S2. PTEN and p110β/pAKT in GBM cell lines. To explore the relationship of PTEN and p110β/pAKT, nine GBM cell lines were divided into two groups: PTEN-wild type and PTEN-deficient, based on data shown in **Figure S1**. The means of intensities of pAKT/ACTB (A) in each group were plotted. Correlation of pAKT/ACTB and p110β/ACTB in PTEN-deficient GBM cell lines were determined using a linear regression model and *t*-test was used to determine the difference between means of two groups (B). R square refers to the correlation between two measurements. Compared to results in all GBM cell lines (R^2 = 0.79; **Fig. 3B**), the correlation of p110β and pAKT decreased in PTEN-deficient GBM cells.

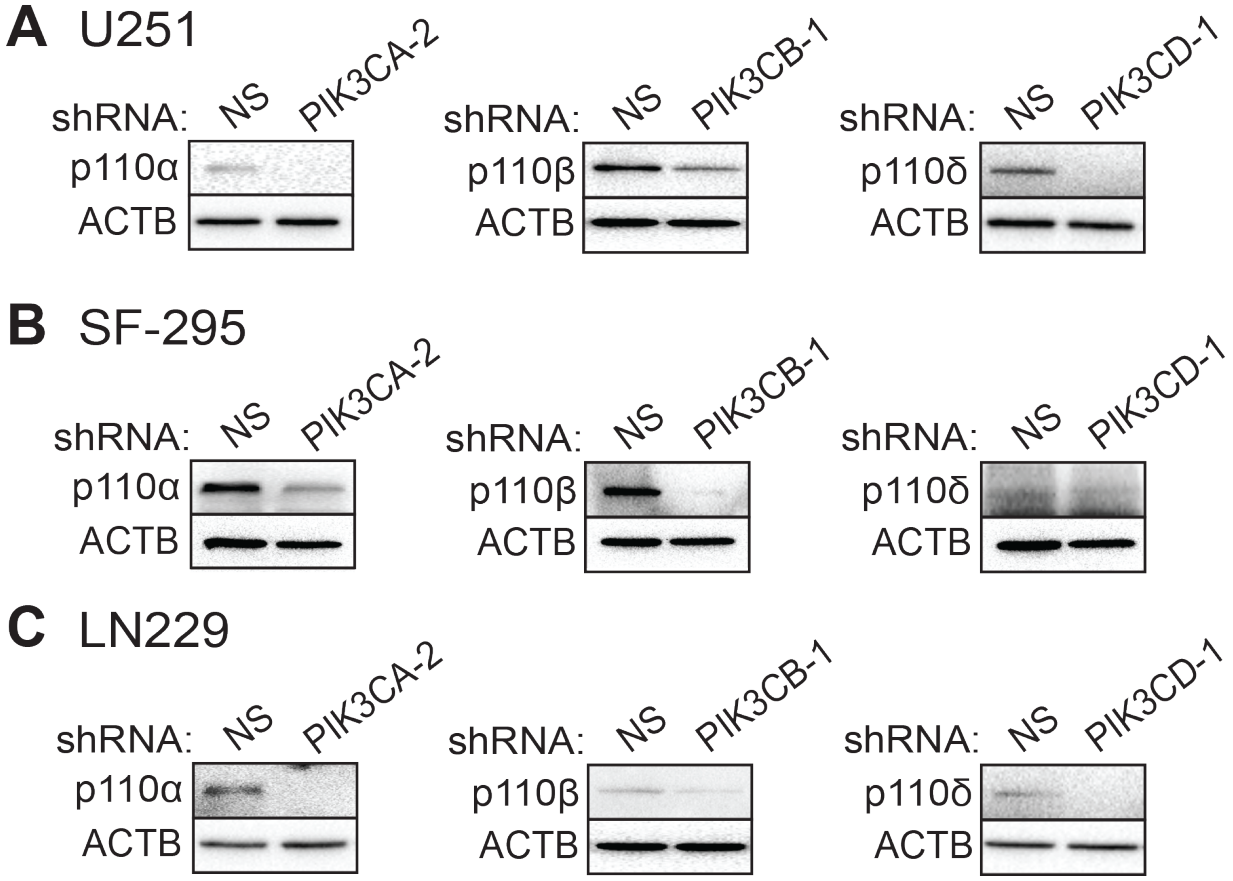

Fig. S3. Knockdown of p110 proteins in GBM cell lines. GBM cells were transduced with viruses of NS shRNA or shRNAs of PIK3CA, PIK3CB, or PIK3CD or transfected with plasmids encoding above shRNAs. Levels of p110α, p110β, and p110δ were determined in U251 (A), SF-295 (B), and LN229 (C) cells using immunoblotting. β-actin (ACTB) is the loading control.

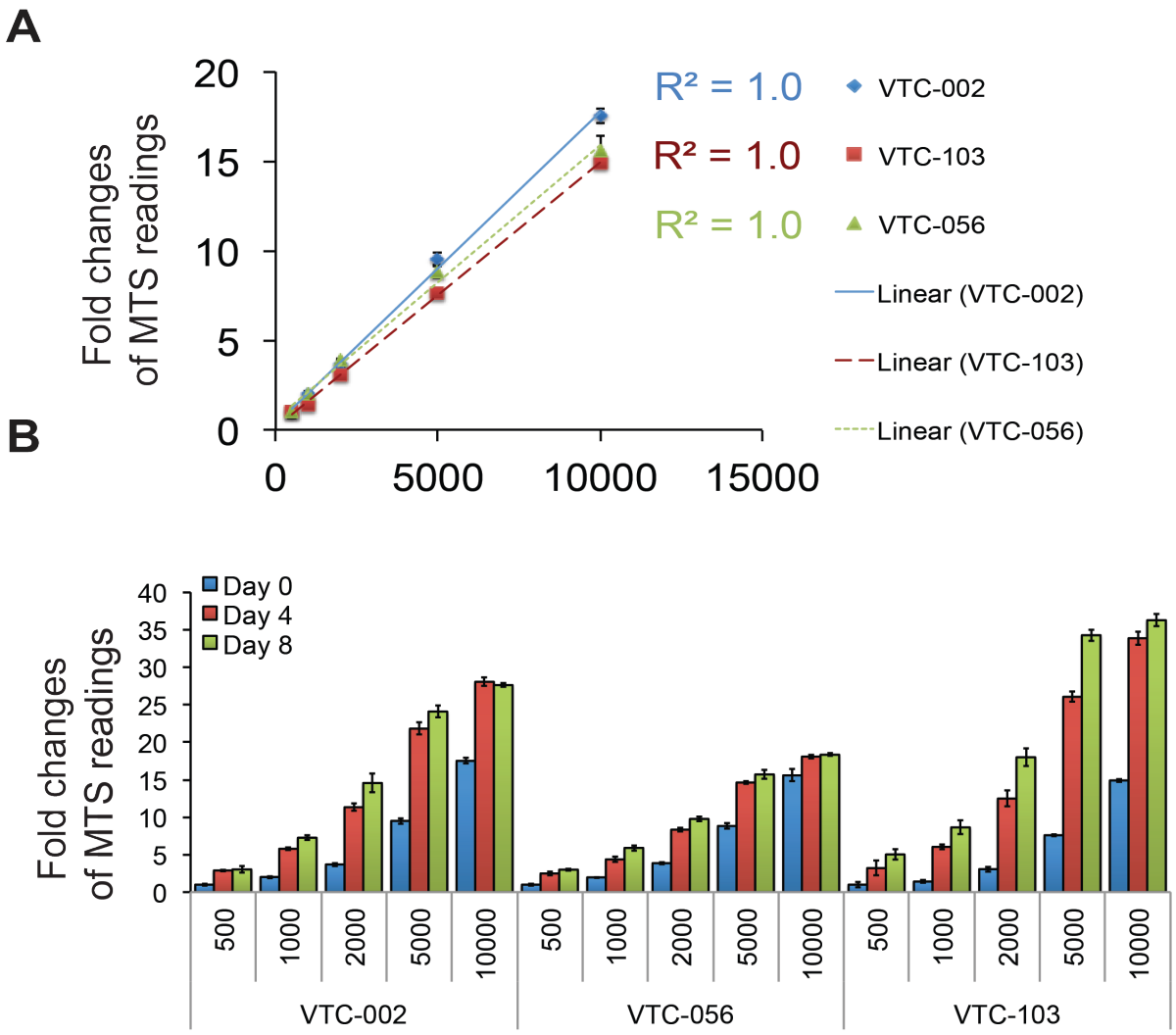

Fig. S4. Growth rates of primary GBM cells expressing high or low levels of p110β. (A) Correlation of MTS readings and cell numbers. The cell numbers of p110β-high VTC-002 and VTC-103 cells and p110β-low VTC-056 cells were determined using the Trypan blue exclusion assay. Cells at different densities were plated in a 96-well plate and immediately analyzed using the MTS assay. The MTS readings were plotted against cell numbers. R squares were determined by linear regression analysis. (B) Growth curves of GBM primary cells. VTC-002, VTC-056, and VTC-103 cells were plated at different cell densities. Cells were then incubated in culture media for 0, 4, and 8 days. Cell numbers were determined using the MTS assay. Fold changes of MTS readings are shown. At all cell densities tested, p110β-high VTC-002 and VTC-103 cells divided faster than p110β-low VTC-056 cells. At high cell densities, the growth of VTC-056 cells was halted, whereas VTC-002 and VTC-103 continued to propagate.

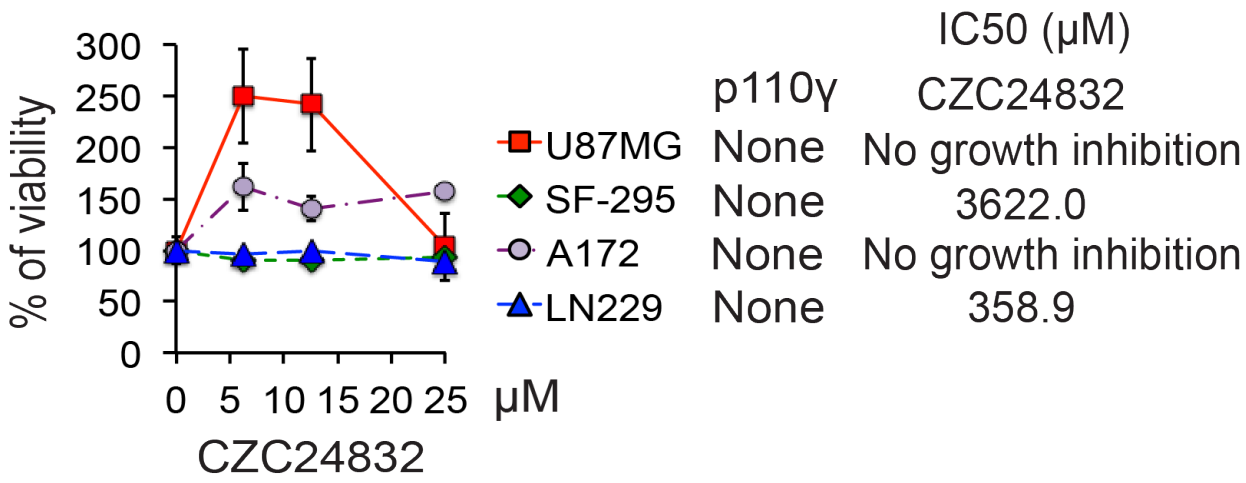

Fig. S5. Cytotoxicity of p110γ inhibitor CZC24832 to GBM cell lines. Four GBM cell lines were treated with CZC24832 at various doses for 4 days. Cell viability was determined using the MTS assay. Levels of p110γ and IC50s of CZC24832 in different cells are shown. CZC24832 showed a growth promotion in U87MG and A172 cells; hence, IC50s were undetectable.

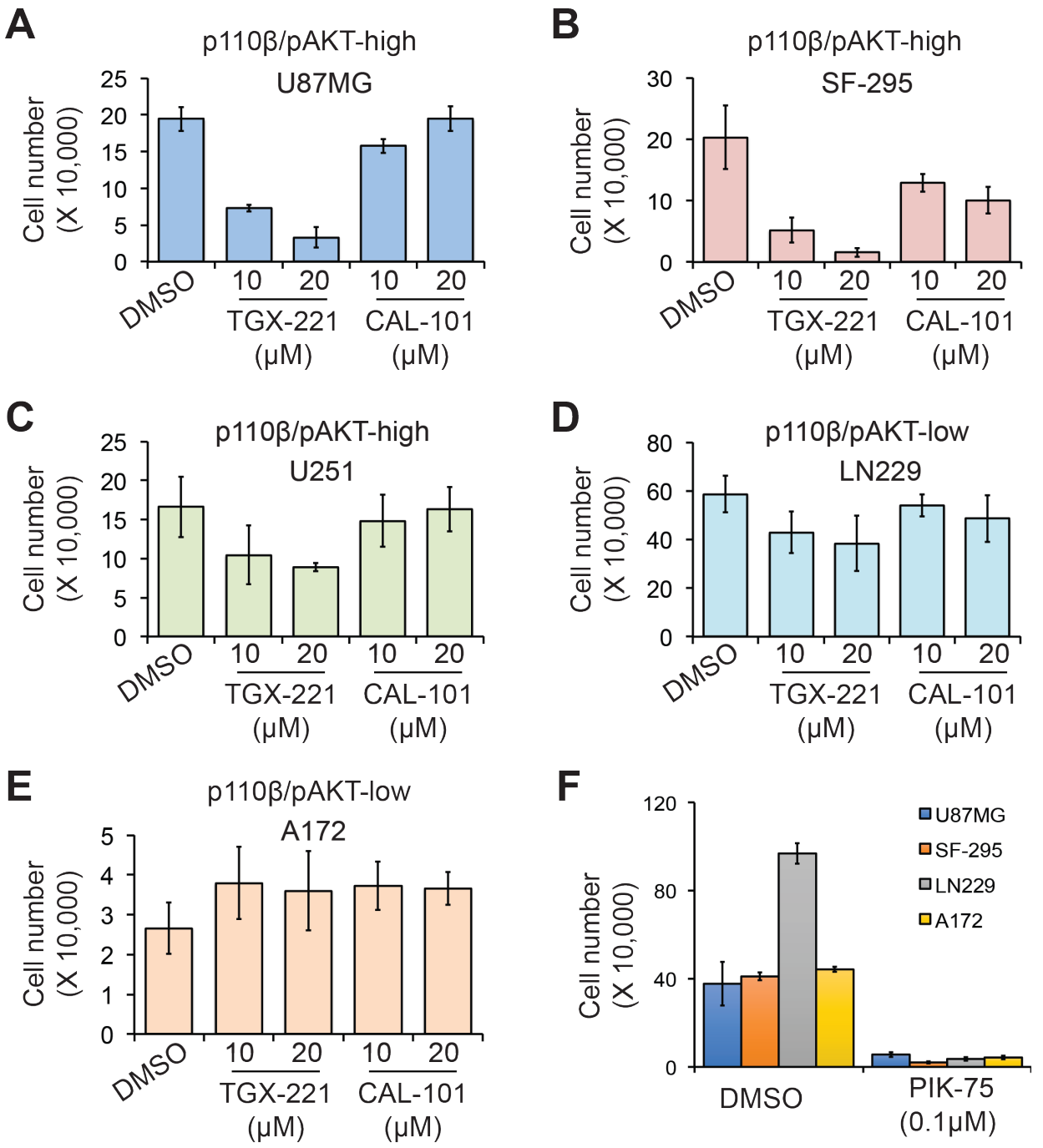

Fig. S6. Trypan blue exclusion assay in GBM cell lines treated with PI3K isoform-selective inhibitors. GBM cell lines U87MG (A), SF-295 (B), U251 (C), LN229 (D), and A172 (E) with different levels of p110β were seeded at 1 to 5 x 10^4 cells per well in a 12-well plate. Cells were then treated with DMSO (vehicle control), TGX-221 (10 or 20 μM) or CAL-101 (10 or 20 μM) for 4 days. Single cells were suspended in culture media and counted using the Trypan blue exclusion assay. The numbers of live cells are shown. (F) PIK-75-treated GBM cells. U87MG, SF-295, LN229 and A172 cells were treated with DMSO or 0.1 μM PIK-75. Live cells were counted as described above. Results shown in this figure are consistent with those in **Figure 5**.

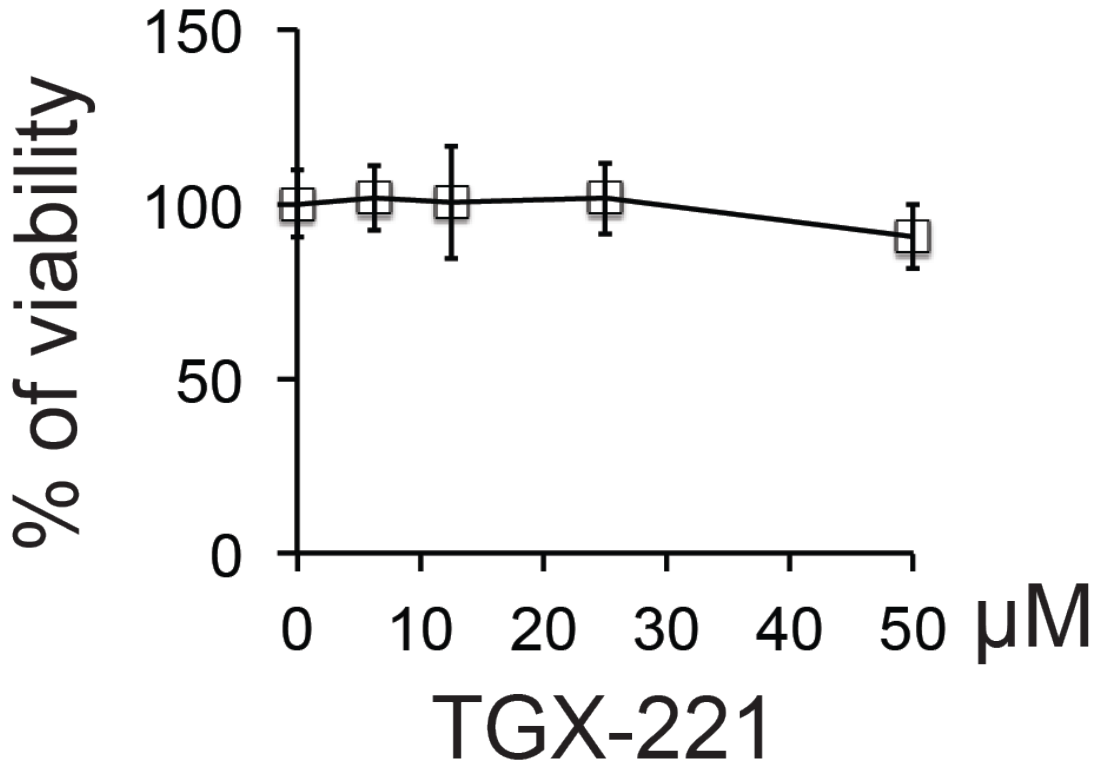

Fig. S7. The effect of TGX-221 on the growth of VTC-034/GSCs. p110β-high/pAKT-low/pGSK3β-low VTC-034/GSCs were treated with TGX-221 at different doses ranging from 0 to 50 μM. Cell viability was determined by the MTS assay. TGX-221 did not significantly inhibit the growth of VTC-034/GSCs.

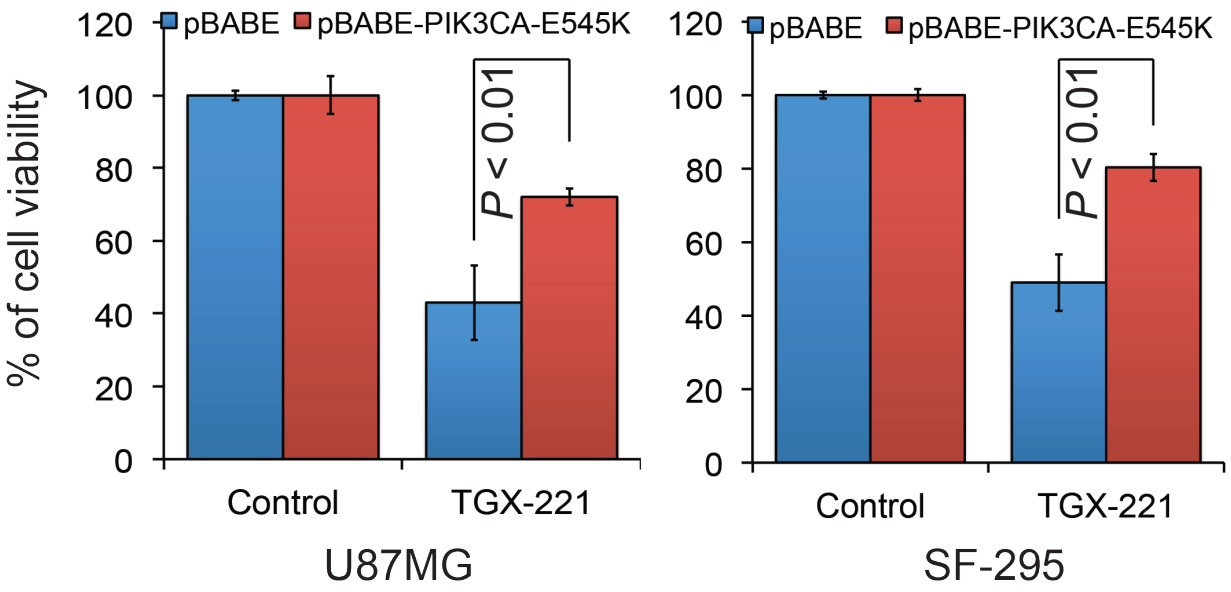

Fig. S8. The effect of a constitutively active PIK3CA mutant on TGX-221-induced growth inhibition in p110β-high U87MG and SF-295 cells. U87MG and SF-295 cells were transiently transfected with pBABE (empty vector) or pBABE-PIK3CA-E545K that encodes a mutant PIK3CA that constitutively activates AKT. Cells were then treated with 25 μM TGX-221 for 4 days. Cell viability was determined by the MTS assay. Student t test was used to determine the difference between two groups.

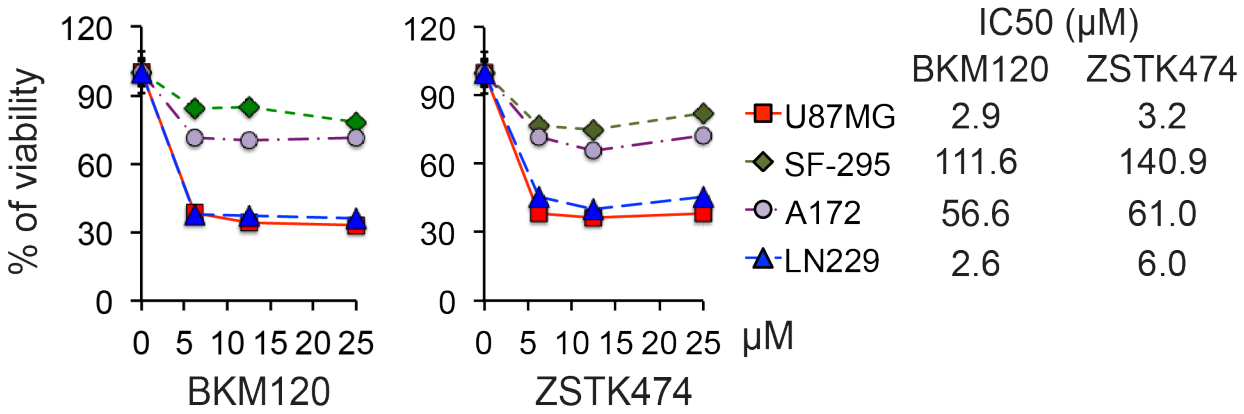

Fig. S9. Cytotoxicity of pan PI3K inhibitors to GBM cell lines. Four GBM cell lines were treated with pan PI3K inhibitors BKM120 or ZSTK474 at various doses for 4 days. Cell viability was determined using the MTS assay. IC50s are shown.

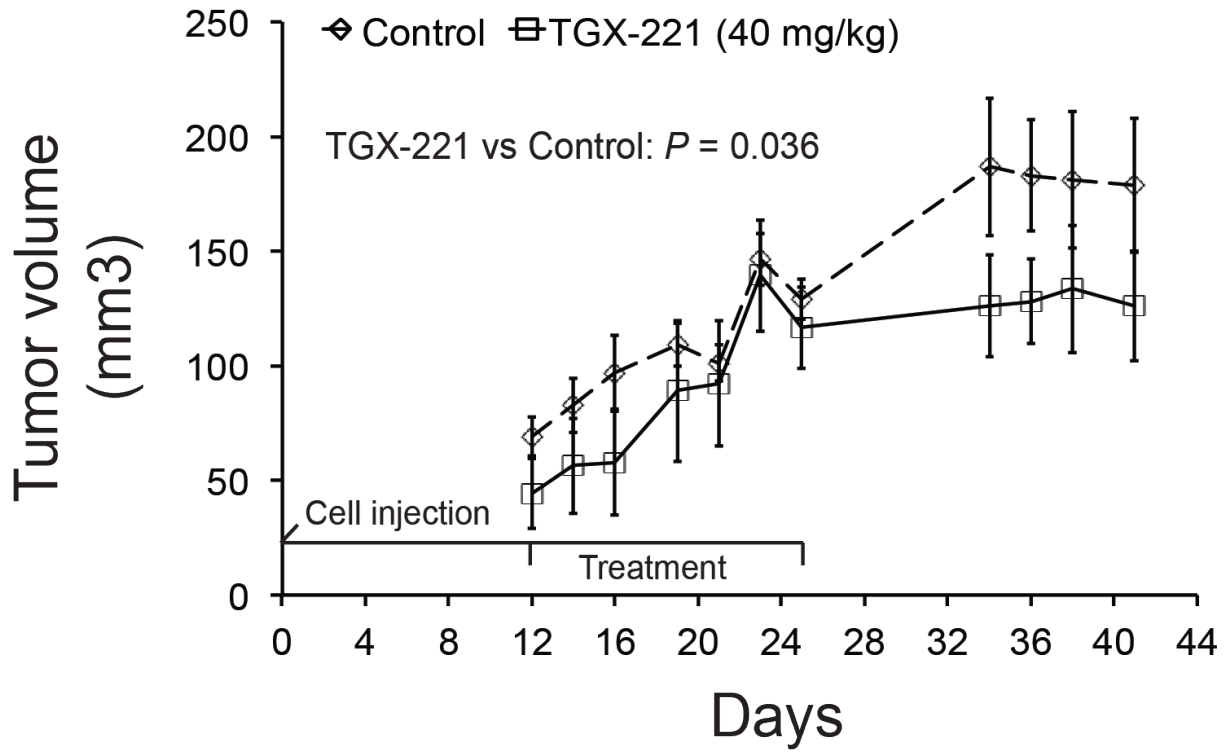

Fig. S10. TGX-221 inhibits the growth of SF-295 tumors in mice. 10^6 SF-295 cells were subcutaneously injected into the mice. 12 days after injection, mice were treated with TGX-221 (40 mg/kg) daily through intraperitoneal injection. Treatment duration time was 14 days. Tumor sizes were measured every other day till 18 days after TGX-221 treatment. Student *t* test was used to determine the difference between two groups. Tumors in TGX-221 treatment group showed a significant growth retardation post drug treatment, compared to tumors in the control group.

Table S1. Correlation of p110β and pAKT protein levels and survival of GBM patients.

p110β/pAKT	Sample ID	Days to death	Average /SE	*P* (*t*-test)
Low	VTC-001	257	263±42.4	**0.011**
	VTC-004	392		
	VTC-037	319		
	VTC-056	181		
	VTC-064	166		
High	VTC-084	46	77±29	
	VTC-103	135		
	VTC-061	50		

Note: Based on protein levels of p110β and pAKT in primary cells (**Fig. 3A**), patients whose tumor specimens were analyzed were divided into two groups. Patient survival is determined by the average days to death. Student *t*-test was used to determine the difference between means of groups.

Table S2. IC50s of PI3K inhibitors in GBM cell lines.

PI3K Inhibitors	IC50s (μM)			
	U87MG	SF-295	A172	LN229
MLN1117(α)	62.8	30.1	88.3	12.9
HS173 (α)	0.5	N/A	0.6	N/A
IC87114 (δ)	9.4	N/A	11.6	N/A
PKI587 (PI3K/MTOR)	0.1	0.2	0.3	0.1
LY294002 (PI3K/MTOR)	26.3	14.3	19.9	11.7

Note: Low IC50s are highlighted in red. N/A: not available.

Table S3. IC50s of PI3K inhibitors in astrocytes.

PI3K Inhibitors	IC50s (μM) in astrocytes
TGX221(β)	100.4
GSK2636771(β)	291.0
PIK-75(α)	0.1
BYL719(α)	7.1
MLN1117(α)	8.1
HS173 (α)	0.3
CAL101(δ)	122.0
PI3065(δ)	11.8
IC87114 (δ)	21.1
CZC24832(γ)	21.2
BKM120(pan)	4.4
ZSTK474(pan)	9.0
PKI587 (PI3K/MTOR)	0.2
LY294002 (PI3K/MTOR)	21.9

Note: IC50s were calculated based on the viability of cells treated with various doses of drugs for 4 days. Low IC50s are highlighted in red.