Figure S1. Carbonic Anhydrase Family Member Expression and Patient Survival Correlations. Carbonic anhydrase family member expression screened and correlated with patient survival using The Cancer Genome Atlas (TCGA) dataset and plotted with GlioVis.
Figure S2. CA9 and CA12 Expression Stratified by GBM Subtype and Correlated with Patient Survival in TCGA Dataset. CA9 and CA12 expression was screened for mRNA expression, stratified by GBM subtype, and correlated with patient outcomes using the TCGA database. These data were plotted using GlioVis.
Figure S3. SLC-0111 is More Effective Than Other CA Inhibitors in Combination with Temozolomide. To determine if there was a benefit of carbonic anhydrase inhibitor addition to temozolomide in vitro, we performed a screen of GBM PDX (A) 1016 and (B) D456 cells treated with TMZ alone or in combination with 50uM of the indicated carbonic anhydrase inhibitors. Cell growth was measured after seven days using cell titer. ****, p<0.0001 with comparison to cells treated with TMZ alone. (C) 1016 and D456 PDX derived cells express low levels of MGMT in vitro as determined (C) via Western and via qRT-PCR (data not shown).
Figure S4. Deletion of TP53 Impairs But Does Not Eliminate the Growth Inhibitory Effects of SLC-0111 in Combination with Temozolomide. To determine the potential impact of TP53 status on the efficacy of SLC-0111, U87 cells that are wildtype for TP53 (A) and those with complete loss of TP53 generated via CRISPR (B) were treated with SLC-0111 alone or in combination with temozolomide. Cell growth was measured after seven days using cell titer. **, p<0.01; ***, p<0.001; ****, p<0.0001 with comparison to vehicle.
Figure S5. Cells Refractory to Temozolomide Do Not Become Sensitive to Temozolomide with SLC-0111 Addition. To determine the potential impact of TMZ resistance on the efficacy of SLC-0111, U251 cells that are (A) sensitive or (B) generated to be resistant to TMZ were treated with SLC-0111 alone or in combination with TMZ. Cell growth was measured after seven days using cell titer. **, p<0.01 ****, p<0.0001 with comparison to vehicle.
**Figure S6. IDH Mutant Cells are Sensitive to SLC-0111 in vitro.** To determine the potential impact of IDH status on the efficacy of SLC-0111, IDHmut HK-322 BTICs were treated with 25 µM SLC-0111 alone or in combination with 120 µM TMZ. Cell growth was measured after seven days using cell titer. **, p<0.01 with comparison to vehicle. There was no combinatorial benefit for addition of SLC-0111 to temozolomide in these IDH mutant cells in vitro.
**Figure S7. SLC-0111 and TMZ Alter GBM Cell Cycle.** 1016 and D456 GBM PDX were treated with SLC-0111 and/or TMZ and incubated in 2% O₂ for 7 days. Representative flow plots for 1016 (A) and D456 (B) with EdU incorporation as a marker for S phase. (C) 1016 GBM PDX were treated as above for 48 hours and stained with propidium iodide for single variable cell cycle analysis. *, p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 with ANOVA. * comparison to the vehicle. # comparison to TMZ.
Figure S8. Influence of SLC-0111 and TMZ Combinatorial Therapy on Metabolism and Intracellular pH.
(A) Flow cytometry overlays of cells exposed to vehicle or SLC-0111 or TMZ alone or in combination and exposed to the pH indicator carboxy-SNARF-1. Boxplots demonstrate decreased (B) serine and (C) leucine with SLC-0111 and TMZ combinatorial therapy in subcutaneous 1016 GBM PDX. 1016 GBM PDX were treated with SLC-0111 and/or TMZ for 4 or 48 hours in 2% O₂ before performing a metabolic stress test for estimation of cellular mitochondrial function and basal extracellular acidification (D) Energetic profile of cells using basal cellular OCR and ECAR. (E) 1016 GBM PDX were treated for 48 hours in 2% O₂ following measurement of intracellular and extracellular lactate concentration. Representative histograms from 1016 GBM PDX cells treated for 48 hours and measured intracellular pH using carboxy-SNARF-1 pH indicator. *, p<0.05; ****, p<0.0001 with ANOVA comparison to vehicle.
Figure S9. CD133+ Cell Surface Expression Plots after Treatments. Bulk tumor cells isolated from D456 and 1016 GBM PDX were treated with SLC-0111 and TMZ or DMSO as a vehicular control incubated in 2% O₂ for at least five days. Representative flow plots of cell surface expression of stem cell marker CD133+. This marker was probed using flow cytometry with an antibody specific for the glycosylated form of CD133.
Figure S10. BMP in Combination with SLC-0111 as an Alternative Treatment is Effective in vitro. (A) D456 and 1016 GBM PDX or (B) immortalized human astrocytes were treated with SLC-0111 and 25 ng/mL BMP and incubated in 21% O₂ or 2% O₂ for 7 days. Cell growth was determined using cell titer assay. Human astrocytes were also treated similarly as GBM PDX and incubated in 21% O₂ for 7 days. *, p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 with ANOVA comparison to the vehicle.