

Attenuating Hypoxia Driven Malignant Behavior in Glioblastoma with a Novel Hypoxia-Inducible Factor 2 Alpha Inhibitor

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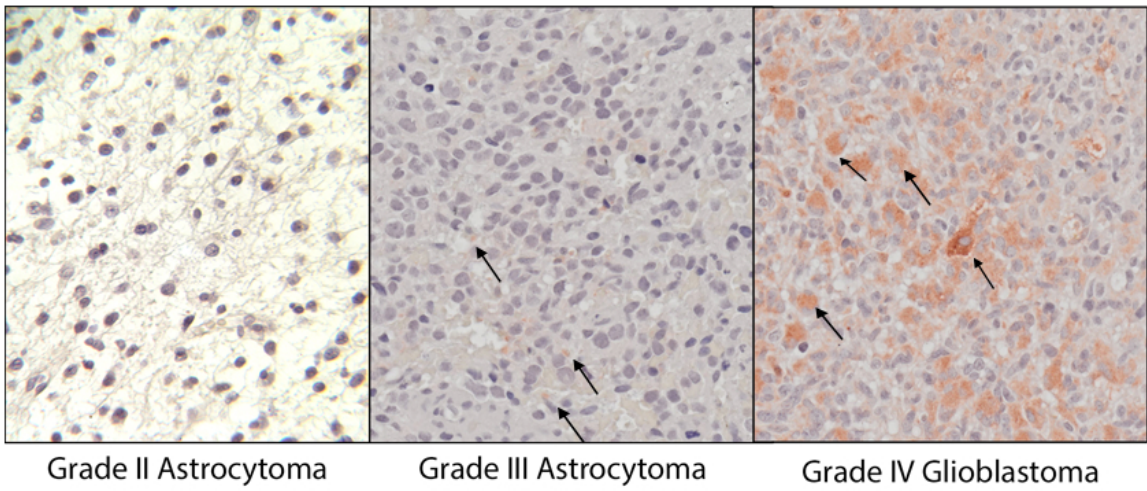
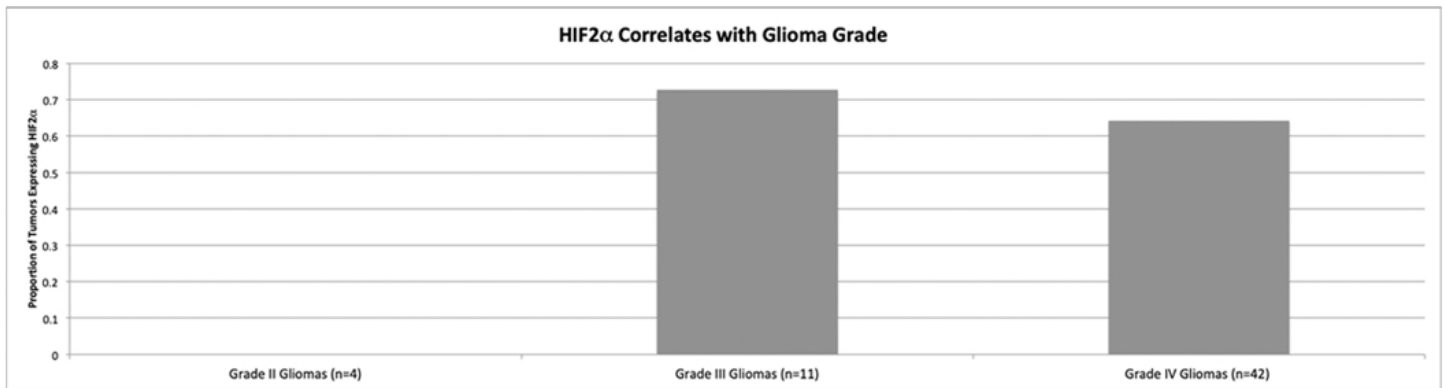
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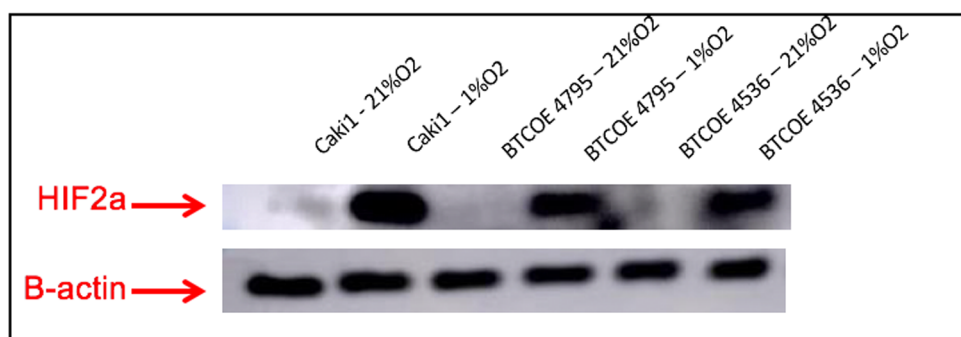
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Supplementary Figure 1 Bar graph summarizing the presence of HIF2 α in a cohort of increasing grades of gliomas (upper panel). Immunohistochemistry on human glioma samples of increasing grade for HIF2a demonstrating preferential staining in high grade gliomas with images at 40x magnification (lower panel).



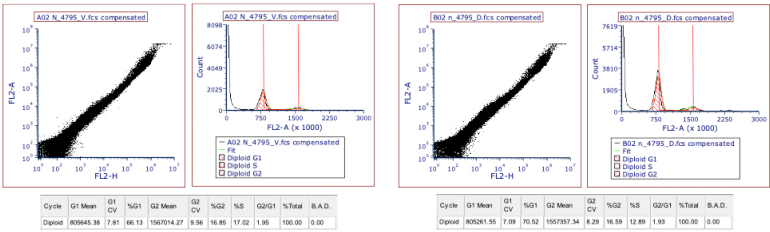
Supplementary Figure 2 Western blot with protein isolates from Caki-1 (renal cell carcinoma cell line), BTCOE 4795 and BTCOE 4536 (patient-derived glioblastoma cell lines) stained for HIF2 α expression in both conditions of normoxia (21% O₂) and hypoxia (1% O₂) for 72 hours with staining for β -Actin as a loading control. The gel for this blot was cut to facilitate primary antibody staining for HIF2 α and β -Actin respectively and the bands from the corresponding molecular weights were cropped to create the figure. Hypoxia upregulates HIF2 α protein expression in all three cell lines.

BTCOE 4795 Cell Cycle Analysis

Normoxia

Vehicle

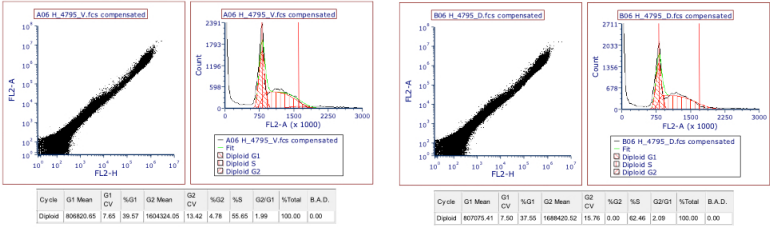
PT2385



Hypoxia

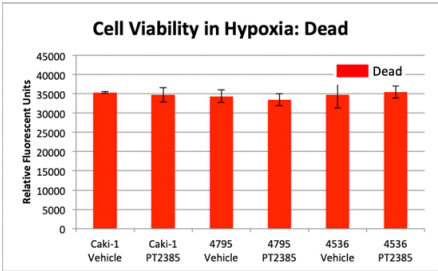
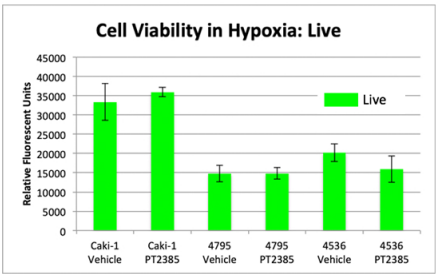
Vehicle

PT2385



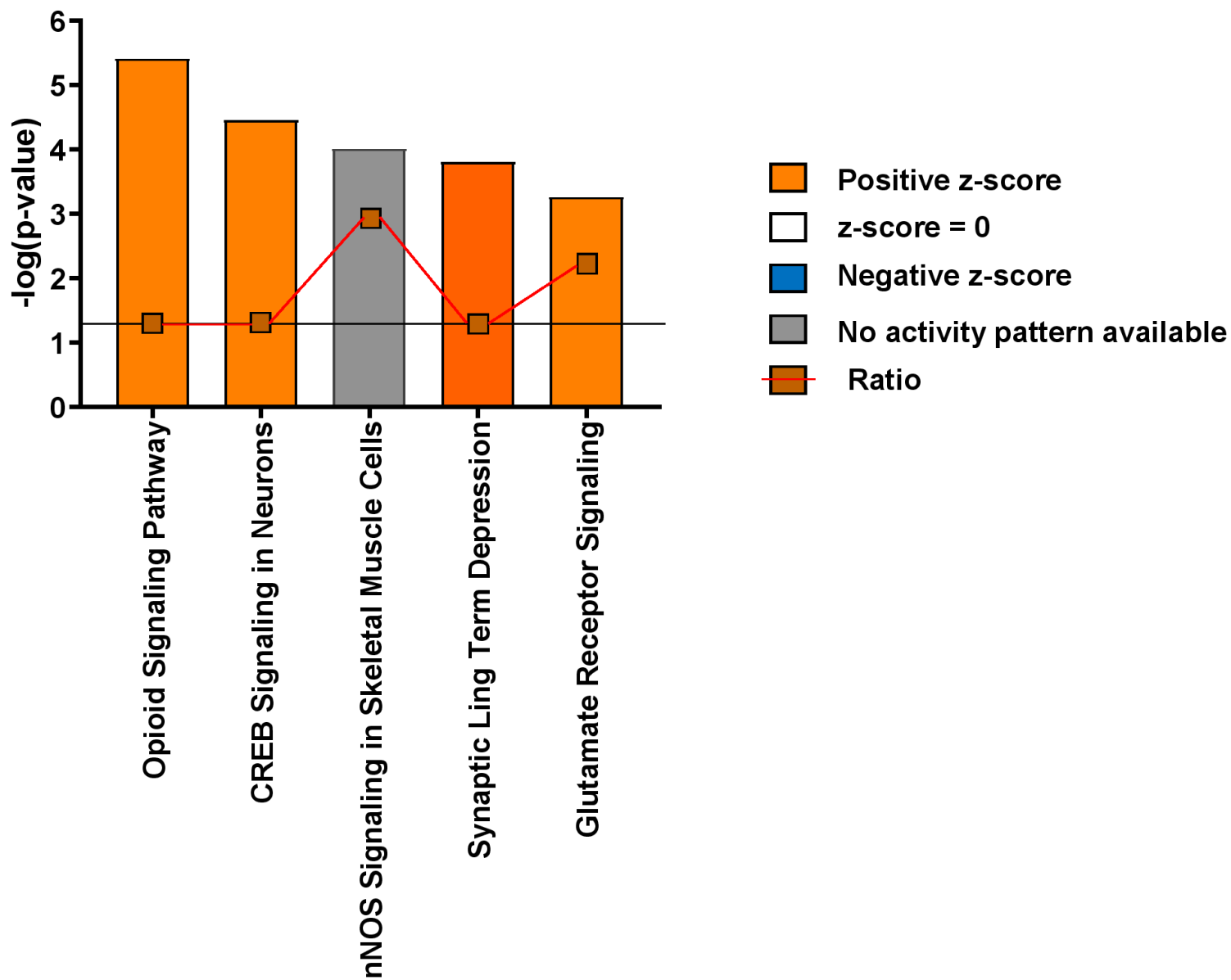
A)

BTCOE 4795 Cell Viability Analysis

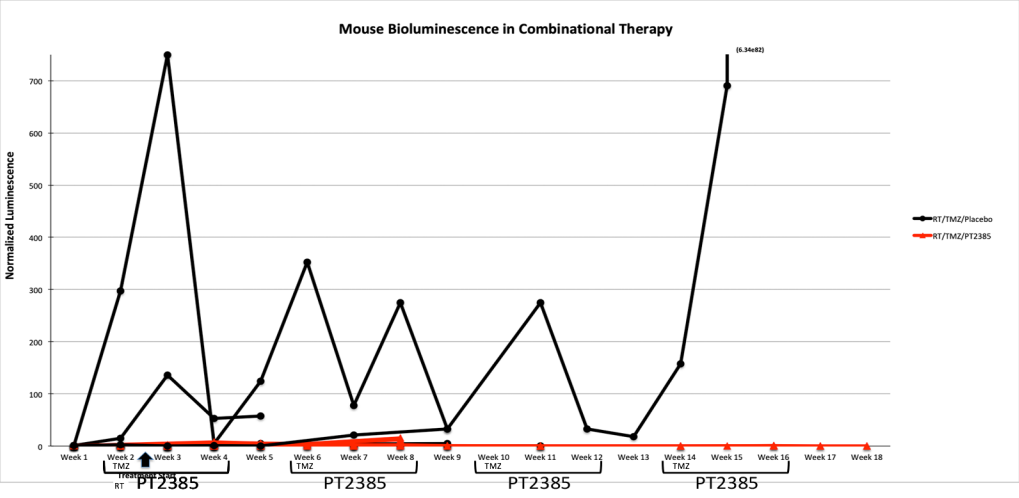
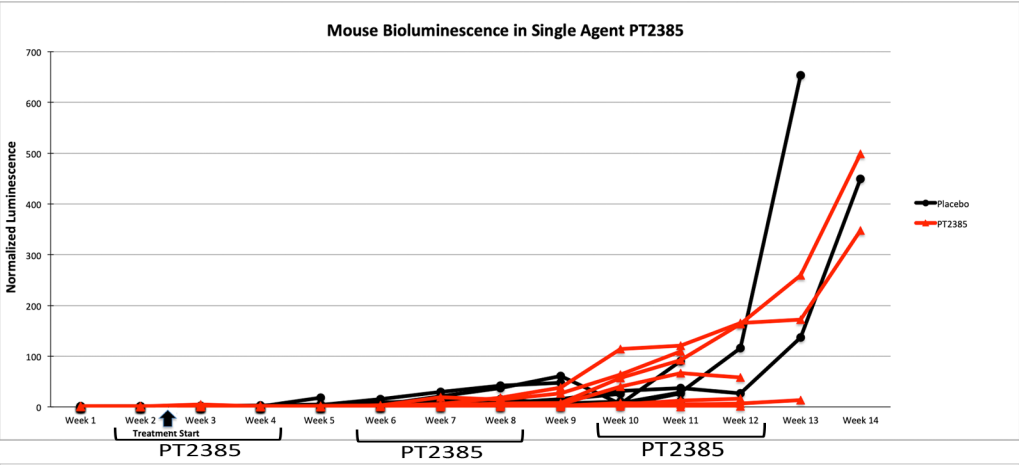


B)

Supplementary Figure 3 A) Cell cycle analysis scatter plot and histograms for summative graph depicted in Figure 3A. **B)** Numerically data for relative fluorescence measurements in the cell viability assay images represented in Figure 3B.



Supplementary Figure 4 Ingenuity Pathway Analysis (IPA) revealed upregulation of four pathways in the PT2385 (10 mg/kg PO BID PT2385 in 21 days on/7 days off cycles) treated animals compared to placebo including: opioid signaling, CREB signaling, synaptic long-term depression, and glutamate receptor signaling. The functional pathway analyses were generated through the use of IPA (QIAGEN Inc., <https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis>)³⁰.



Supplementary Figure 5 Individual normalized bioluminescent values for mice undergoing the single agent study (upper panel) and the combinational study (RT/TMZ/PT2385) in the lower panel.

Supplementary Table 1

Cell Line	Age/Sex	Location	Specimen Source
BTCOE 4536	71 Female	Right Temporal	Glioblastoma – index resection
BTCOE 4795	43 Male	Right Frontal	Glioblastoma – first recurrence resection

Supplementary Table 2

Pathway	Representative Genes	Fold Enrichment	p-value	False Discovery Rate
Voltage-gated potassium channel activity	KCNH1, HCN1, KCNS1, KCNAB3, KCNC3, KCNE2, KCNH7, CNGA4, KCNG4, KCNH5	10.94	2.75E-07	3.86E-04
Potassium channel	KCNH1, HCN1, KCNS1, KCNC3, KCNE2, KCNH7, KCNG4, KCNK12, KCNH5	8.29	1.24E-05	0.016
Anterior/Posterior pattern specification	ARC, HOXA2, HOXB2, HOXB8, HOXB5, VANG12, HOXA10, HOXB9, ZBTB16, GLI2	8.04	3.96E-06	0.0066