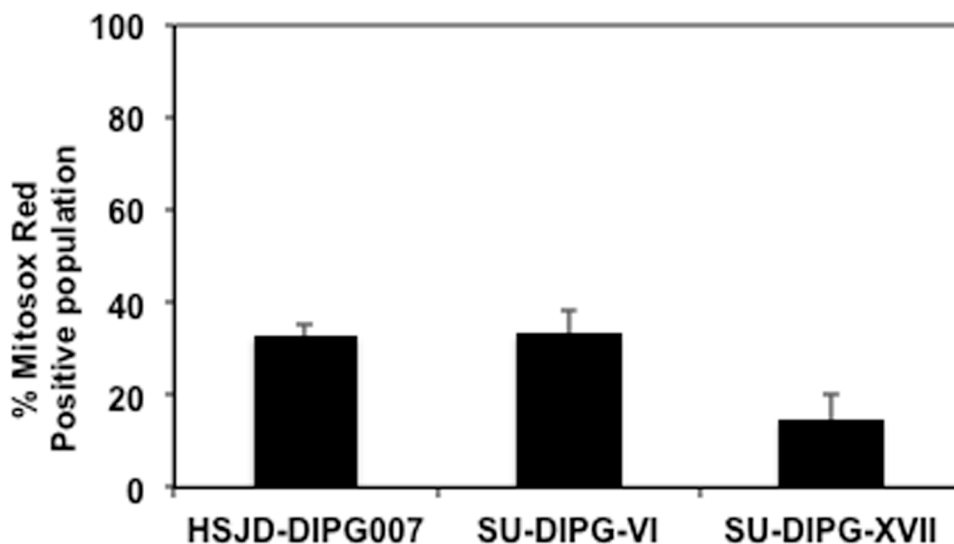
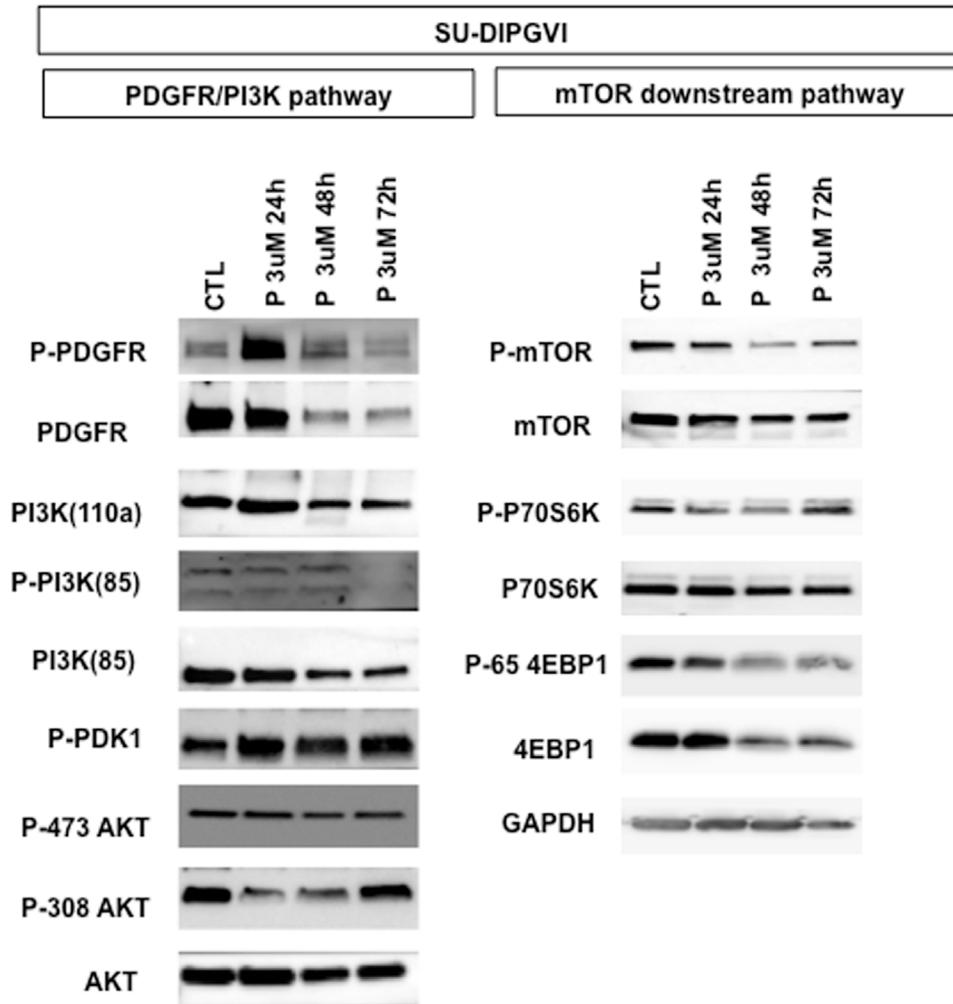


Dual targeting of mitochondrial function and mTOR pathway as a therapeutic strategy for diffuse intrinsic pontine glioma

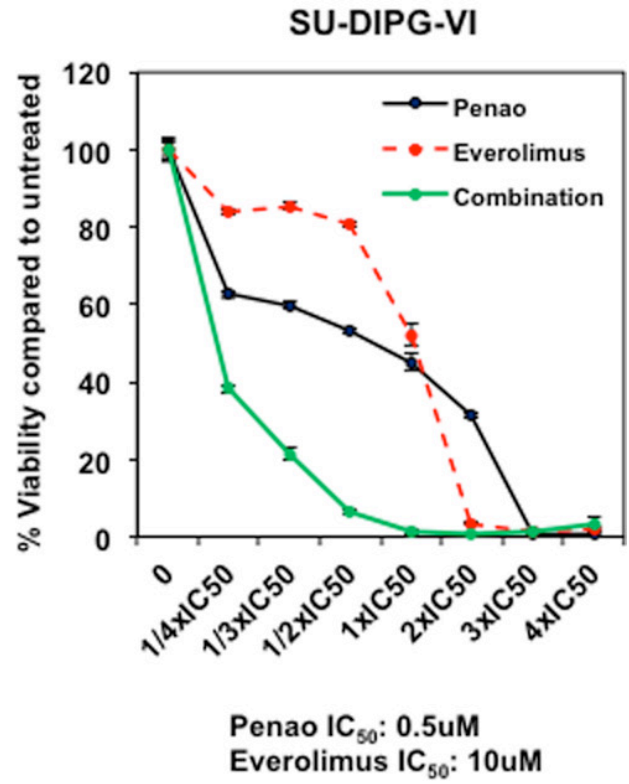
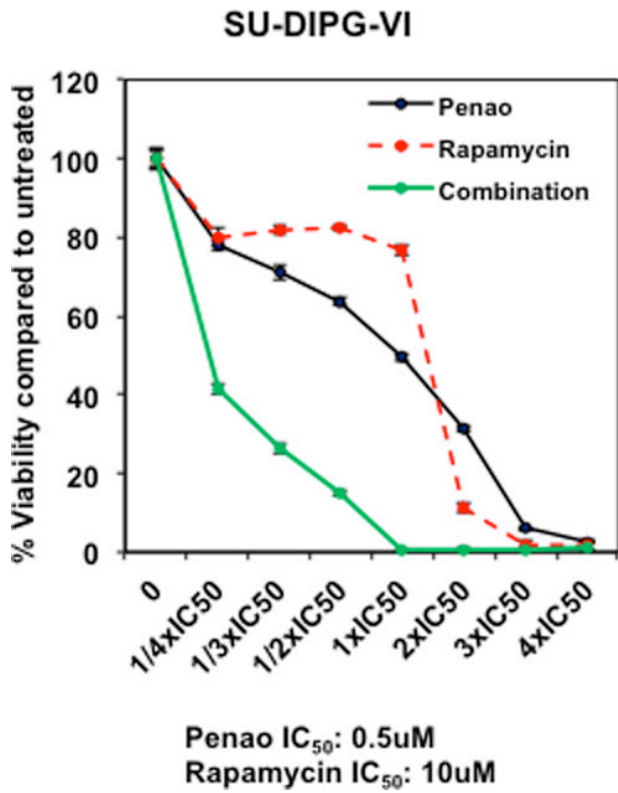
SUPPLEMENTARY MATERIALS



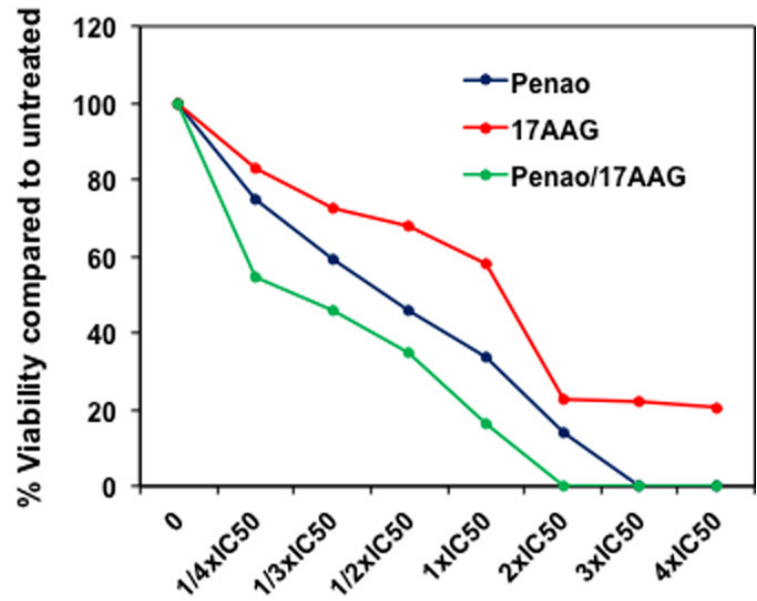
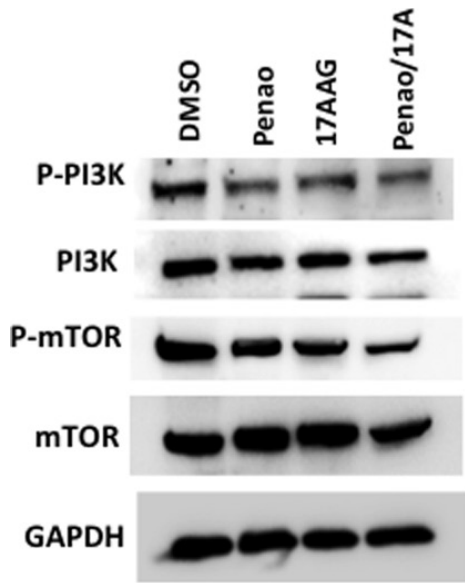
Supplementary Figure 1: Flow cytometric analysis of neurosphere-forming DIPG cells for production of mitochondrial ROS. DIPG cells were stained with MitosoxRed and analysed with FACS Canto B. Data represent average and SD of 3 determinations.



Supplementary Figure 2: PENAO affects PDGFR/PI3K/mTOR pathway in SU-DIPG-VI cells. Representative western blot images of key players involved in PDGFR α /PI3K/mTOR pathway in SU-DIPG-VI neurospheres treated with PENAO as a function of time. DIPG cells were treated with 3 μ M of PENAO for 24 h, 48, 72 h and subsequently lysed pellets were examined by western blotting.



Supplementary Figure 3: Cytotoxic efficacy of PENA0 can be enhanced with Rapalogue drugs. Cytotoxic efficacy of PENA0 combined with Rapamycin and Everolimus tested at IC₅₀ fractions in neurosphere-forming SU-DIPG-VI cells. Viability was assessed by resazurin assay and presented as percentage viability compared to untreated cells. Experiment was replicated 2 times each time $N = 10$.



Penao IC₅₀: 3uM
 17AAG IC₅₀: 80nM

Supplementary Figure 4: Cytotoxic efficacy of PENAO can be enhanced with HSP90 inhibitor 17AAG. Cytotoxic efficacy of PENAO combined with 17AAG tested at IC₅₀ fractions in neurosphere-forming HSJD-DIPG007 cells. Viability was assessed by resazurin assay and presented as percentage viability compared to untreated cells. Experiment was replicated 2 times each time $N = 10$. Western blot analysis of PI3K and mTOR upon treatment with PENAO/17AAG combination.