FIG S1. Effect of PX-866 on proliferation of human glioma cell lines. Four glioma cell lines were plated in 96-well plates at a density of 5,000 per well. Cells were treated with increasing concentrations of PX-866 in triplicate wells for 72 h under serum-fed conditions, and cell viability was assessed by sulforhodamine B assay described in Materials and Methods. The value for DMSO-treated cells was set to 100% (Y axis), and the number of cells for all drug doses were normalized as percentage of the number of cells in DMSO. There was a dose-dependent decrease in cell viability in all cell lines tested.
FIG S2: Autophagy detection in malignant glioma cells treated with PX-866. A) fluorescence-activated cell sorting analysis using acridine orange. U87-MG cells treated with or without PX-866 for 72 hours were stained with acridine orange (1 ug/mL) and then subjected to flow cytometric analysis. FL1-H, green color intensity; FL3-H, red color intensity. Top of the grid was considered as AVO. B) Immunobloting of cells treated with PX-866 for 72 h shows LC3-II expression as an indication of autophagy in the (o) untreated and PX-866-treated cells. High levels of LC3-II were observed in a dose dependent manner in PX-866-treated cell lines An Increase in LC3-11/LC3-1 ratio has been observed in PX-866 treated cells..
FIG S3. A) Analysis of PX-866 effects on tumor xenograft proliferation. Nude athymic mice with established U87 xenografts and intracranial tumors were treated with vehicle control, PX-866 (2 mg/kg/d) per gavage for 5 days for 4 weeks. Tumors were harvested, formalin fixed, and stained for Ki-67 as a marker of proliferation. Areas of viable tumor were examined for positive cells (brown). PX-866 treatment decreases Ki-67 staining in both subcutaneous and intracranial tumors indicative of decreased proliferation in treated tumors. B) Immunohistochemical analysis of tumor xenografts treated with PX-866. Nude athymic mice with established U87 xenografts and intracranial tumors were treated with vehicle control, PX-866 (2 mg/kg/d) per gavage for 5 days for 4 weeks. Tumors were harvested and formalin fixed. Tumor sections were stained with antibodies to phosphorylated Akt, S6 and mTOR. Representative microscopic fields of each immunohistochemical reaction with the overall semiquantitative grading scale (x400). In the treated tissues, the reactivity for the total antigens often exhibited markedly stronger immunoreactivity than the phosphorylated forms, indicating that the phosphorylated forms were effectively diminished in quantity versus nontreated controls.