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Supplementary Materials for

Akt and Autophagy Cooperate to Promote Survival of Drug-Resistant Glioma

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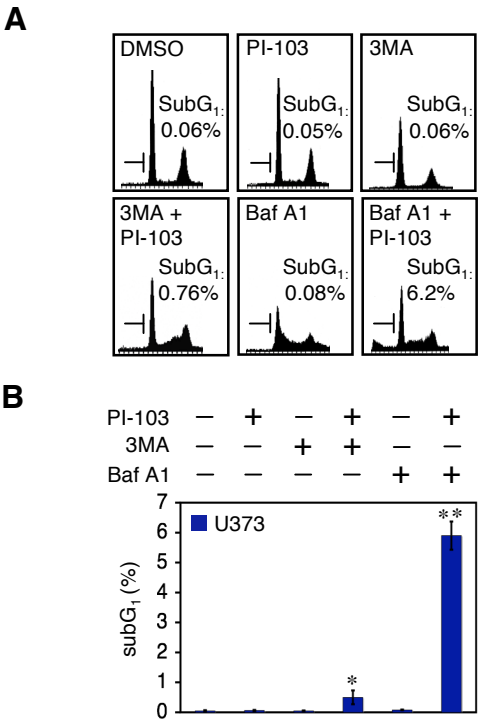

Fig S1. Blockade of autophagosome maturation synergizes with inhibition of PI3K and mTOR to induce apoptosis in *PTEN*^{mt} glioma. (**A,B**) U373 *PTEN*^{mt} cells transduced with GFP-LC3 were treated with DMSO (vehicle) or PI-103 (1 μM) for 24 hr, then treated with either 3 methyl adenine (3MA-5 mM), or with Baf A1 (10 nM) for 48 hr. Cells were fixed and analyzed by flow cytometry to measure subG1 fraction using Modifit-LT software. Percentage of cells in sub-G1 fractions is shown, mean ± s.e. for triplicate samples ($p < 0.05$ by Student's t test for PI-103 plus 3MA versus DMSO; $p < 0.0001$ for PI-103 plus Baf A1 versus DMSO).

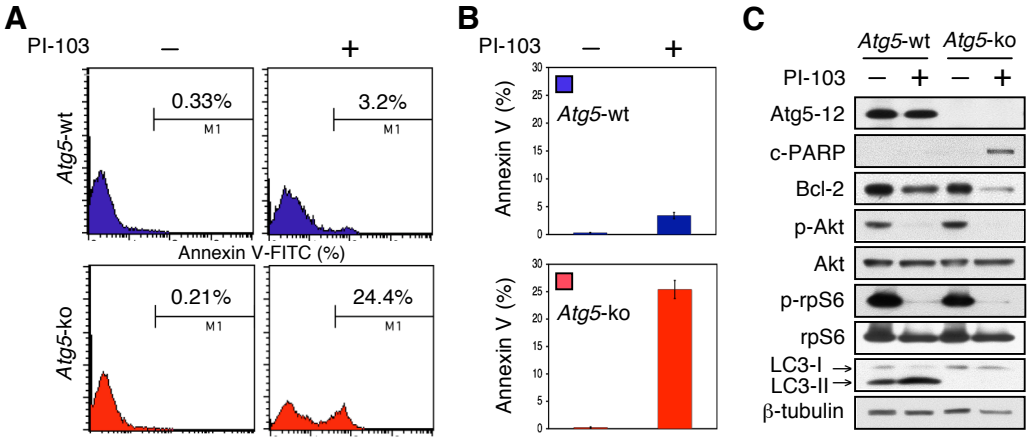

Fig S2. PI-103 induces apoptosis in *Atg5* mutant mouse embryo fibroblasts. Mouse embryo fibroblasts wild-type or mutant for *Atg5* were treated with 1 μM PI-103 or DMSO for 48 hr. Cells were analyzed by flow cytometry for the apoptotic marker annexin V. (**A**) Percentages of apoptotic cells were indicated. (**B**) Data show error among triplicate measurements for each value. (**C**) An aliquot of cells was lysed and analyzed by immunoblot using antibodies indicated.

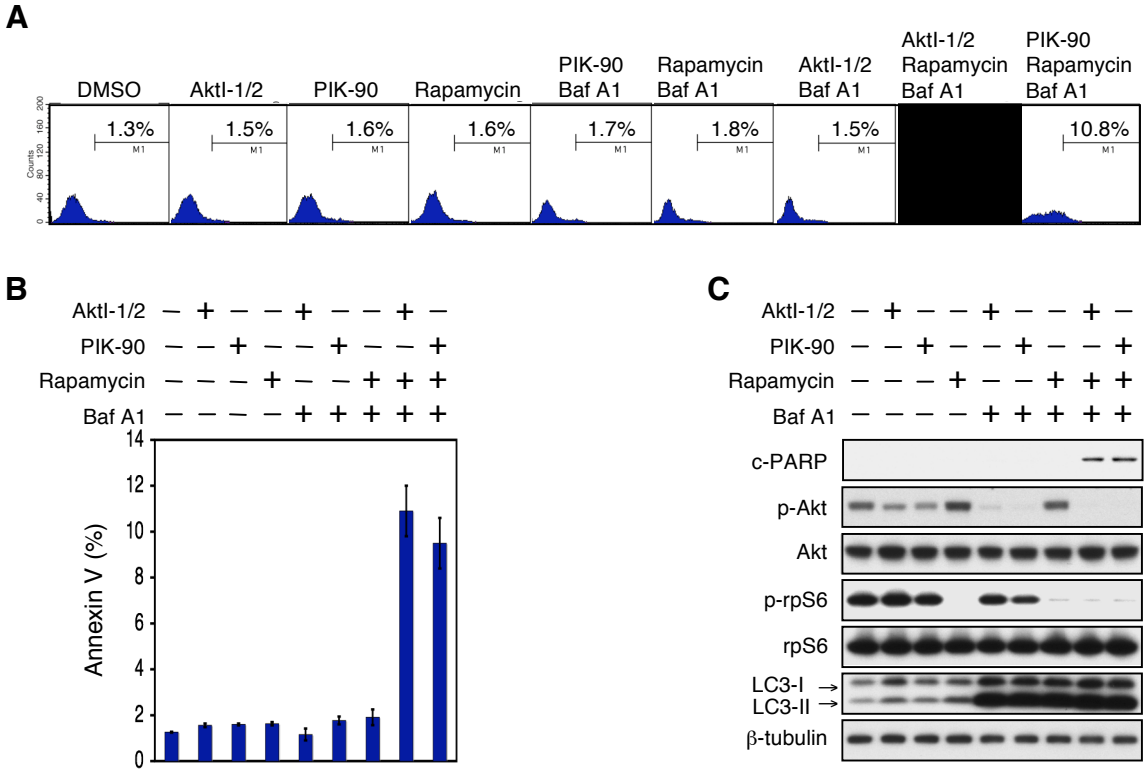

Fig S3. Apoptosis in response to inhibition of mTOR and autophagy requires concomitant blockade of signaling through PI3K/Akt. U373 *PTEN*^{mt} cells were treated with DMSO (vehicle), AktI-1/2 (1 μM), PIK-90 (1 μM), or Rapamycin (100 nM) for 24 hr, then treated with Baf A1 (10 nM) for 48 hr. Cells were analyzed by flow cytometry for apoptotic marker annexin V, or by immunoblot using antibodies indicated. (**A**) Cells were stained with annexin V-FITC and subjected to flow cytometric analysis. Percentage of apoptotic cells were indicated (M1). (**B**) Data show error between triplicate measurements for each value. (**C**) An aliquot of cells was lysed and analyzed by immunoblot using antibodies indicated.

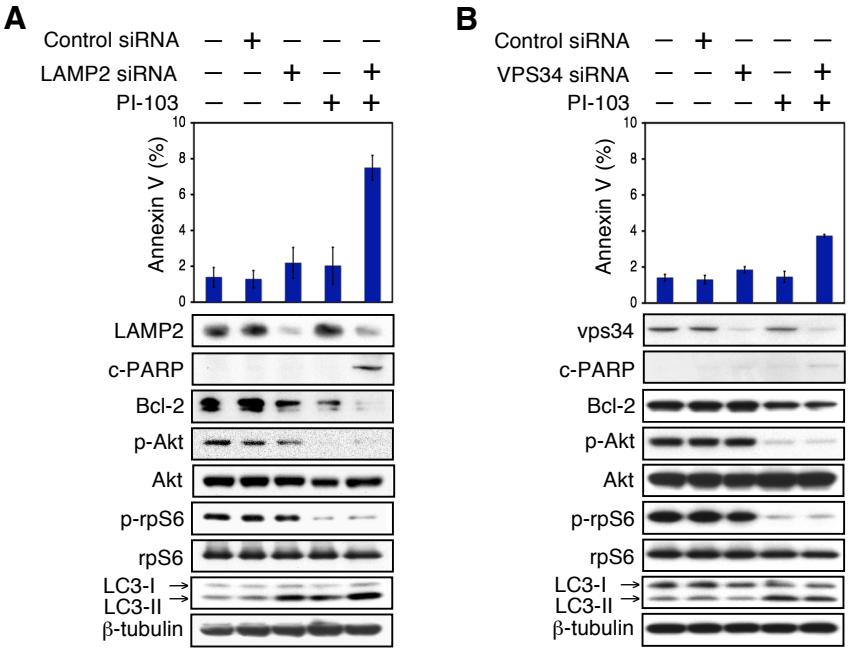

Fig S4. Blockade of LAMP2 and Vps34 synergizes with inhibition of PI3K and mTOR to induce apoptosis in U373 *PTEN*^{mt} glioma. (**A**) Knockdown of LAMP2 cooperates with PI-103 to induce apoptosis in U373 *PTEN*^{mt} glioma. To exclude off-target effects of Baf A1 independent of lysosomal trafficking, we transfected cells with siRNA directed against lysosome-associated membrane protein-2 (LAMP-2) or control siRNA for 24 hr. Cells were then treated with PI-103 (1 μM) for 48 hr, analyzed by flow cytometry for the apoptotic marker annexin V (top panel) or lysates analyzed by immunoblot (bottom panel). (**B**) To determine whether knock-down of the lipid kinase Vps34 contributed to autophagy, we transfected U373 glioma cells with siRNA against Vps34 or with control siRNA. Cells were then treated with PI-103 (1 μM) for 48 hr and analyzed by flow cytometry and immunoblot. Knockdown of Vps34 only slightly reduced phosphorylation of mTOR target p-rpS6, modestly blocked autophagy as single agent, and induced a small degree of apoptosis when combined with PI-103.

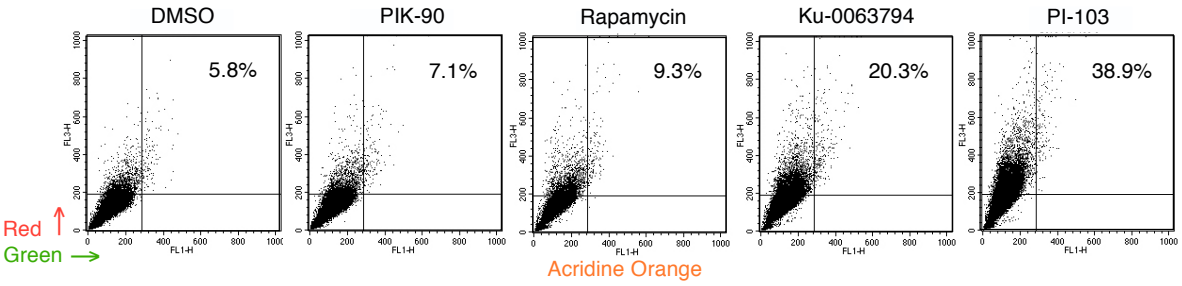

Fig S5. The ATP-competitive mTOR inhibitor Ku-0063794 induced autophagy more potently than does the allosteric mTORC1 inhibitor rapamycin and the PI3Kα inhibitor PIK-90 in *PTEN*^{mt} glioma. Cells were treated with DMSO, PIK-90 (1 μM) Rapamycin (100 nM), Ku-0063794 (5 μM), or PI-103 (1 μM) for 48 hr and stained with acridine orange (1 μg/ml) for 15 min. Cells were analyzed by flow cytometry. Red fluorescence (650 nm) indicated volume of the cellular acidic compartment (Y-axis as indicated by red arrow), while X-axis (green arrow) denotes green fluorescenct (510-530 nm) staining of cytoplasm and nucleolus (16, 47, 48). Autophagy was quantified by the accumulation of acidic vesicular organelles. Percentage of AVOs is indicated.