Supplementary Data

Cooperative blockade of PKCα and JAK2 drives apoptosis in glioblastoma

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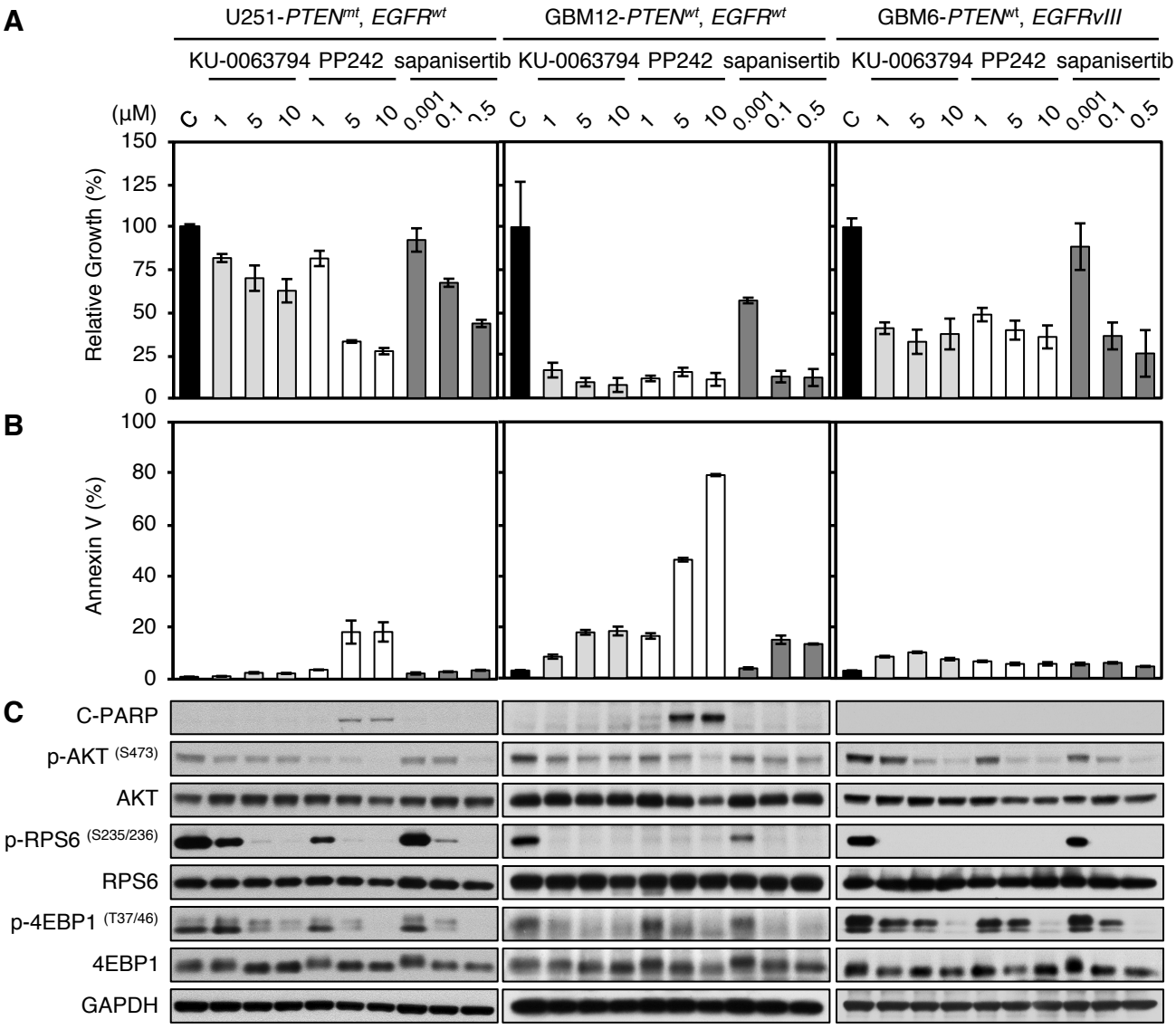
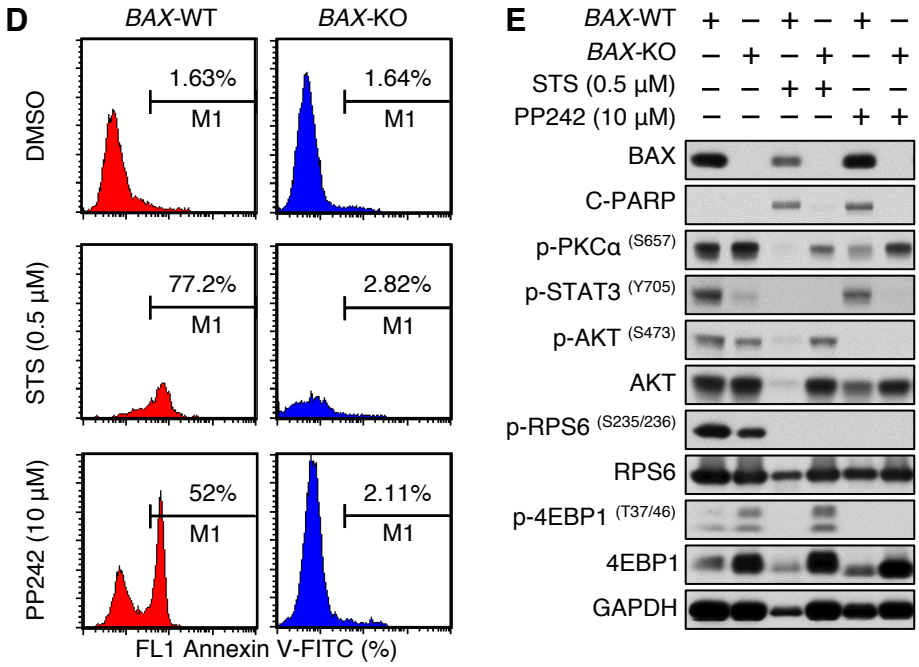
Supplementary Figure S1.

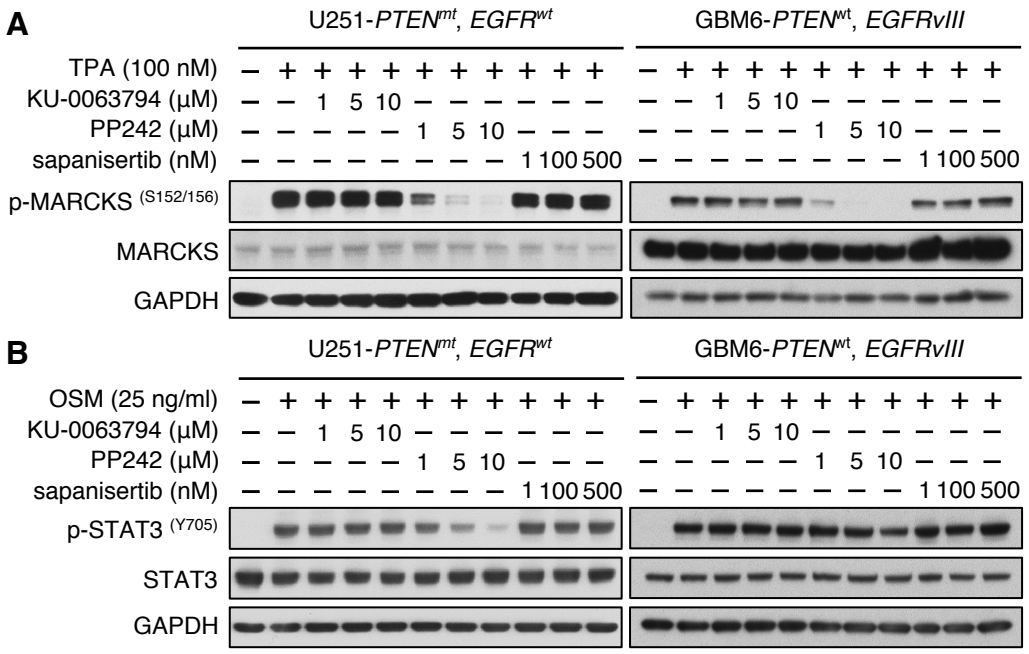


Supplementary Figure S1 (Continued).



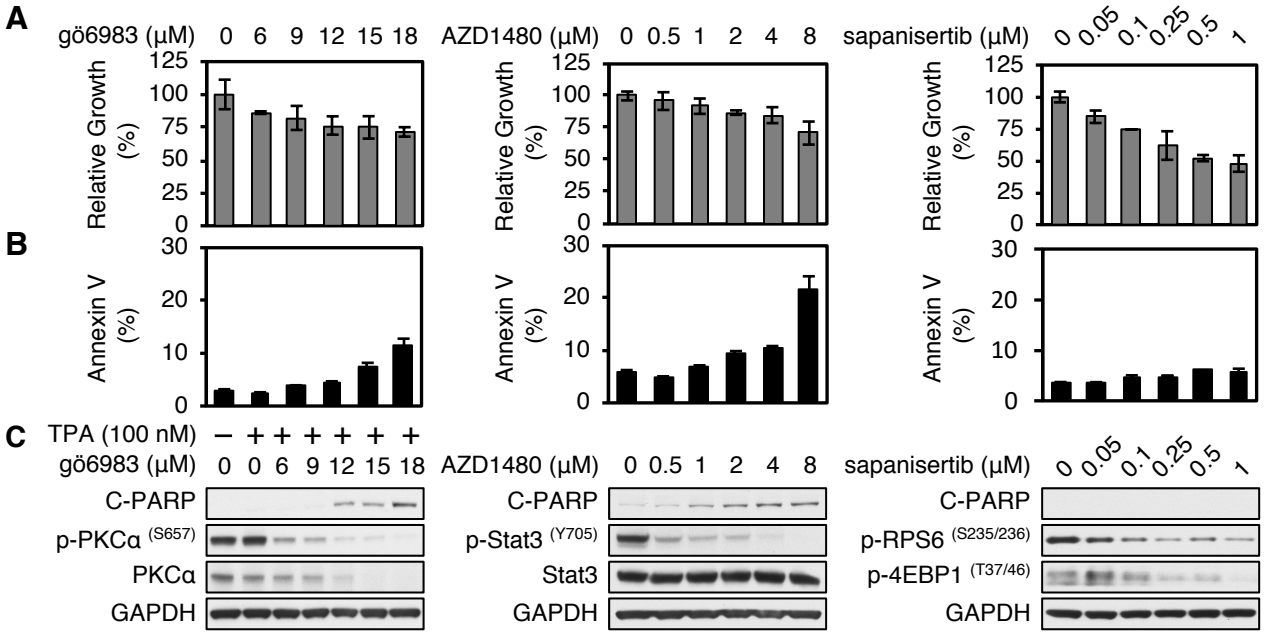
Supplementary Figure S1. PP242 induced apoptosis through a mitochondrial-dependent pathway. U251, GBM12, and GBM6 cells were treated with KU-0063794, PP242, and sapanisertib at indicated doses for 72 hours. *PTEN* and *EGFR* status in glioma cell lines are shown (wt, wild type; mt, mutant). (**A)** Proliferation was measured by WST-1 assay. Data shown represent mean ± SD (percentage of growth inhibition relative to DMSO-treated control) of triplicate measurements. (**B)** Apoptosis was analyzed by flow cytometry for annexin V. Data shown represent mean ± SD (percentage of apoptotic cells relative to DMSO-treated control) of triplicate measurements. (**C)** An aliquot of each lysate was analyzed by western blot with antibodies indicated. Blot representative of two independent experiments is shown. (**D)** *BAX* wild-type and *BAX*-deficient MEFs were treated with 10 μM PP242 for 72 hours or with 0.5 μM staurosporine (STS) for 24 hours. Apoptotic cells were analyzed by flow cytometry for annexin V-FITC. Percentages of apoptotic cells is indicated. (**E)** An aliquot of each lysate was analyzed by western blotting with antibodies indicated.

Supplementary Figure S2.



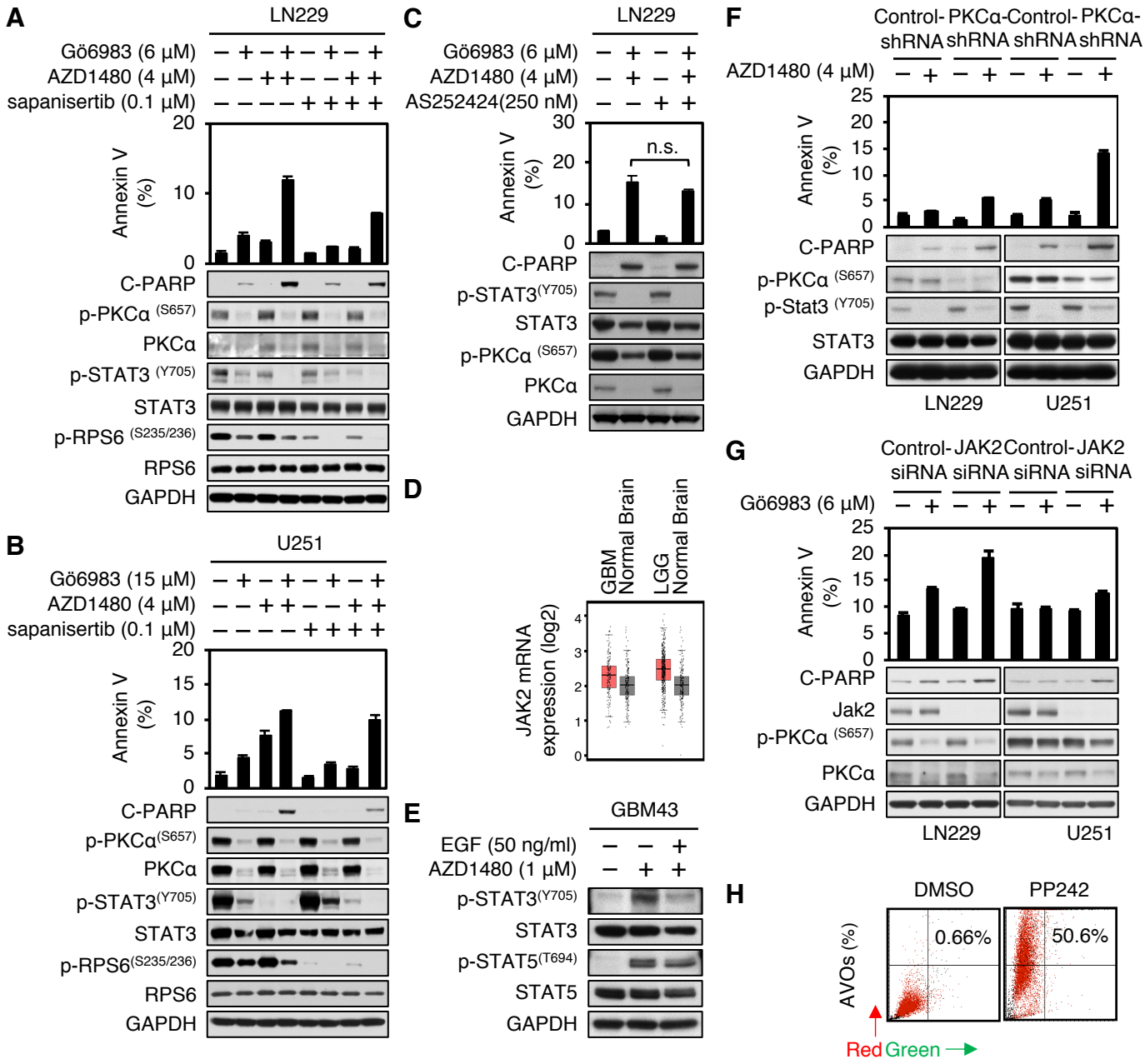
Supplementary Figure S2. PP242 showed potent blockade of p-MARCKS, but not p-STAT3^{Y705} in GBM6 cells. **(A and B)** U251 and GBM6 cells were treated with KU-0063794, PP242, and sapanisertib at indicated doses for 24 hours. *PTEN* and *EGFR* status in glioma cell lines are shown (wt, wild type; mt, mutant). Cells were harvested, lysed, and analyzed by western blot with antibodies indicated. Thirty minutes prior to harvest, PKC was activated through addition of tetradecanoyl phorbol acetate (TPA) 100 nM, while JAK2 was activated by adding oncostatin M (OSM) 25 ng/ml.

Supplementary Figure S3.



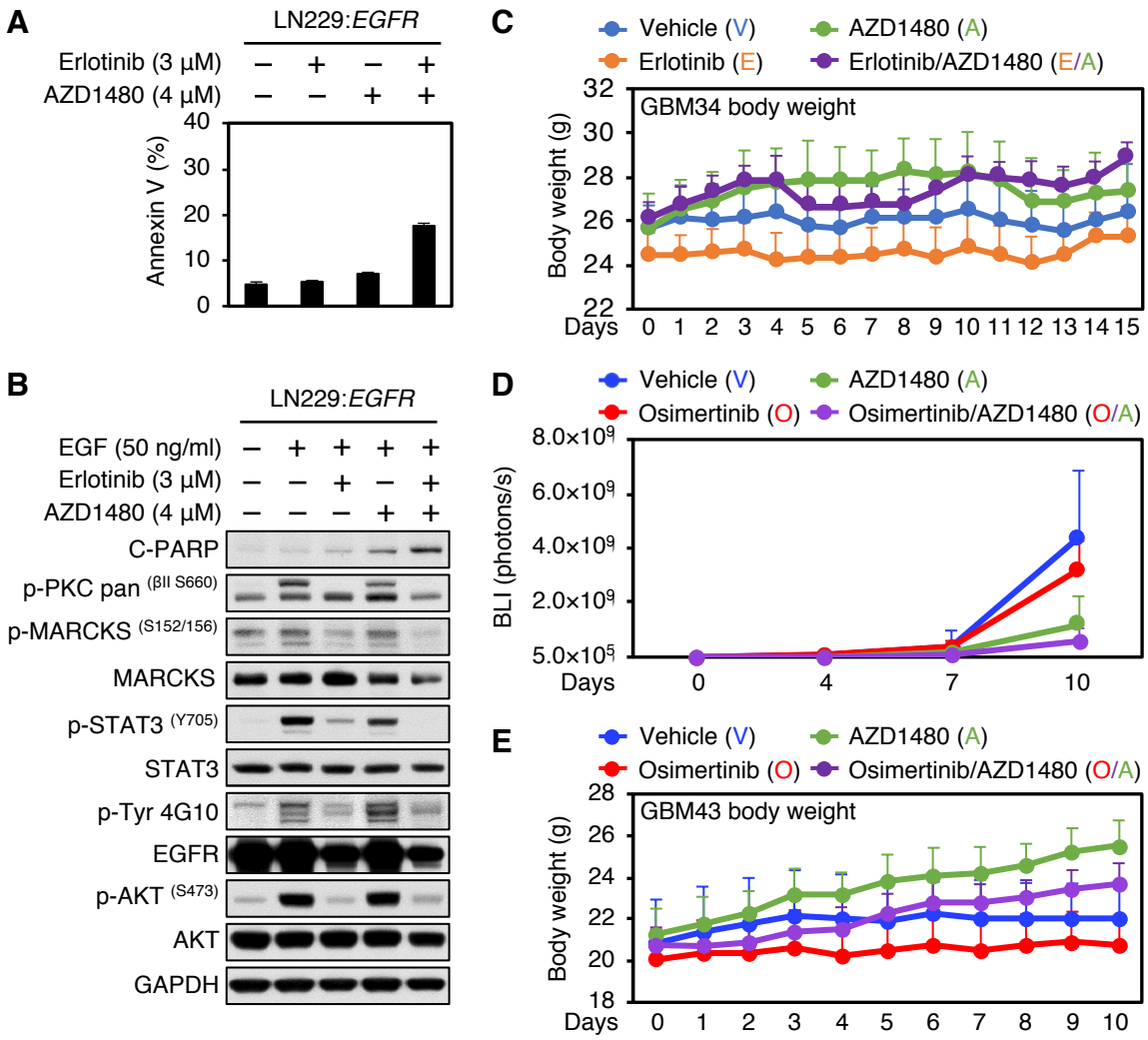
Supplementary Figure S3. Effects of PKC, JAK2, and mTOR inhibitors on cell proliferation and apoptosis. U251 parent cells were treated with PKC inhibitor gö6983, JAK2 inhibitor AZD1480, or mTOR inhibitor sapanisertib at indicated doses for 72 hours. (**A)** Proliferation was measured by WST-1 assay. Data shown represent mean ± SD (percentage of growth relative to DMSO-treated control) of triplicate measurements. (**B)** Apoptosis was analyzed by flow cytometry for annexin V. Data shown represent mean ± SD (percentage of apoptotic cells relative to DMSO-treated control) of triplicate measurements. (**C)** An aliquot of each lysate was analyzed by western blot with antibodies indicated. Blot representative of two independent experiments is shown. TPA (100 nM) was added 30 minutes before harvest.

Supplementary Figure S4.

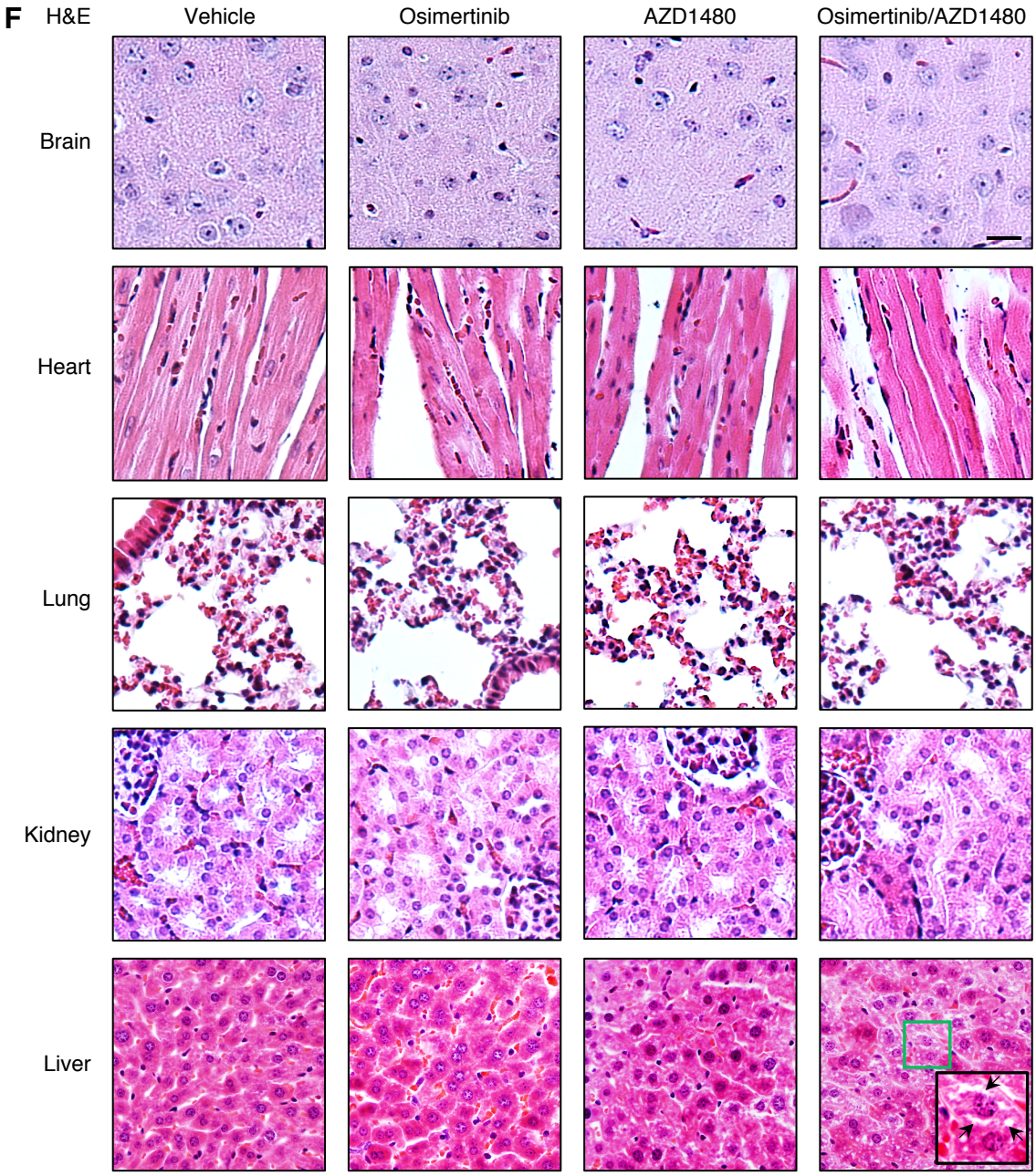


Supplementary Figure S4. Cooperative blockade of PKCα and JAK2 drives apoptosis. **(A and B)** LN229 and U251 parent cells were treated with PKC inhibitor gö6983, JAK2 inhibitor AZD1480, TORKi sapanisertib, gö6983 plus AZD1480, gö6983 plus sapanisertib, AZD1480 plus sapanisertib, or gö6983 plus AZD1480 and sapanisertib at indicated doses for 72 hours. Apoptosis was analyzed by flow cytometry for annexin V. Data shown represent mean ± SD (percentage of apoptotic cells relative to DMSO-treated control) of triplicate measurements (**top panel**). An aliquot of each lysate was analyzed by western blot with antibodies indicated. Blot representative of two independent experiments is shown (**bottom panel**). (**C)** LN229 cells were treated with gö6983 plus AZD1480, p110γ inhibitor AS252424 or gö6983 plus AZD1480 and AS252424 at indicated doses for 72 hours. Apoptosis was analyzed by flow cytometry for annexin V. Data shown represent mean ± SD (percentage of apoptotic cells relative to DMSO-treated control) of triplicate measurements (Student's t test, p = 0.0064, DMSO versus gö6983 plus AZD1480; p = 0.0056, AS252424 versus gö6983 plus AZD1480; p = 0.0573, gö6983 plus AZD1480 versus gö6983 plus AZD1480 and AS252424) (**top panel**). An aliquot of each lysate was analyzed by western blot with antibodies indicated. Blot representative of two independent experiments is shown (**bottom panel**). (**D)** Levels of JAK2 mRNA expression levels from 163 GBM patients, 518 low-grade glioma (LGG) patients and 207 normal brain tissue samples were downloaded from GEPIA (dataset: TCGA and GTEx). Expression levels of JAK2 mRNA were compared using the online tool at http://gepia.cancer-pku.cn ($p = 2.77x10^{-6}$, GBM versus Normal brain; $p = 1.99x10^{-23}$, LGG versus Normal brain). (**E)** GBM43 cells were treated 1 μM AZD1480 for 24 hours. Cells were harvested, lysed, and analyzed by western blot with antibodies indicated. EGF (50 ng/ml) was added 15 minutes before harvest. (**F)** LN229 and U251 parent cells stably expressing shRNA against PKCα were treated with or without JAK2 inhibitor AZD1480 at indicated doses for 72 hours. Apoptosis was analyzed by flow cytometry for annexin V. Data shown represent mean ± SD (percentage of apoptotic cells relative to DMSO-treated control) of triplicate measurements (**top panel**). An aliquot of each lysate was analyzed by western blot with antibodies indicated (**bottom panel**). (**G)** LN229 and U251 parent cells were treated with PKC inhibitor gö6983 at indicated doses or gö6983 plus siRNA against JAK2 for 72 hours. Apoptosis was analyzed by flow cytometry for annexin V. Data shown represent mean ± SD (percentage of apoptotic cells relative to DMSO-treated control) of triplicate measurements (**top panel**). An aliquot of each lysate was analyzed by western blot with antibodies indicated (**bottom panel**). (**H)** U251 parent cells were treated with PP242 (5 μM) for 48 hours and stained with acridine orange (1 μg/ml) for 15 minutes. 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 fluorescent (510-530 nm) staining of cytoplasm and nucleolus. Autophagy was quantified by the accumulation of acidic vesicular organelles (AVOs). Percentage of AVOs is indicated.

Supplementary Figure S5.



Supplementary Figure 5 continued.



Supplementary Figure S5. Efficacy and tolerability of combination therapy in mice. **(A)** LN229:*EGFR* cells were treated with erlotinib, AZD1480, or erlotinib plus AZD1480 at indicated doses for 72 hours. Apoptosis was analyzed by flow cytometry for annexin V. Data shown represent mean ± SD (percentage of apoptotic cells relative to DMSO-treated control) of triplicate measurements. (**B)** Cell lysates were analyzed by western blot with antibodies indicated. EGF (50 ng/ml) was added 15 minutes before harvest. (**C)** Body weights of mice xenograft with GBM34 were measured every day for 15 days. Data shown are mean ± SD of n = 7 mice in each group. (**D)** GBM43 cells expressing firefly luciferase were injected intracranially into BALB/$c^{nu/nu}$ mice. After tumor establishment, mice were sorted into four groups and treated daily by oral gavage, with vehicle, osimertinib (25 mg/kg), AZD1480 (30 mg/kg), or osimertinib (25mg/kg) plus AZD1480 (30mg/kg). Bioluminescence imaging of tumor-bearing mice was obtained at days shown (day 0 was start of treatment). Dynamic measurements of bioluminescence intensity (BLI) in treated tumors over time, and quantified as maximum photons/s/cm^2 squared/steradian. Data shown represent mean of photon flux ± SD from n = 6 mice per group. (**E)** Body weights of mice xenograft with GBM43 were measured every day for 10 days. Data shown are mean ± SD of n = 6 mice per group. **F,** Mice in each group were sacrificed when they reached endopoint. Organs were removed and from the mice. Sections were stained with hematoxylin and eosin (H&E). Representative H&E staining sections of brain, heart, lung, kidney, and liver are shown. An enlarged image of region marked with a green square is shown. Arrows indicate position of microvesicular steatosis. Scale bar = 10 μm.