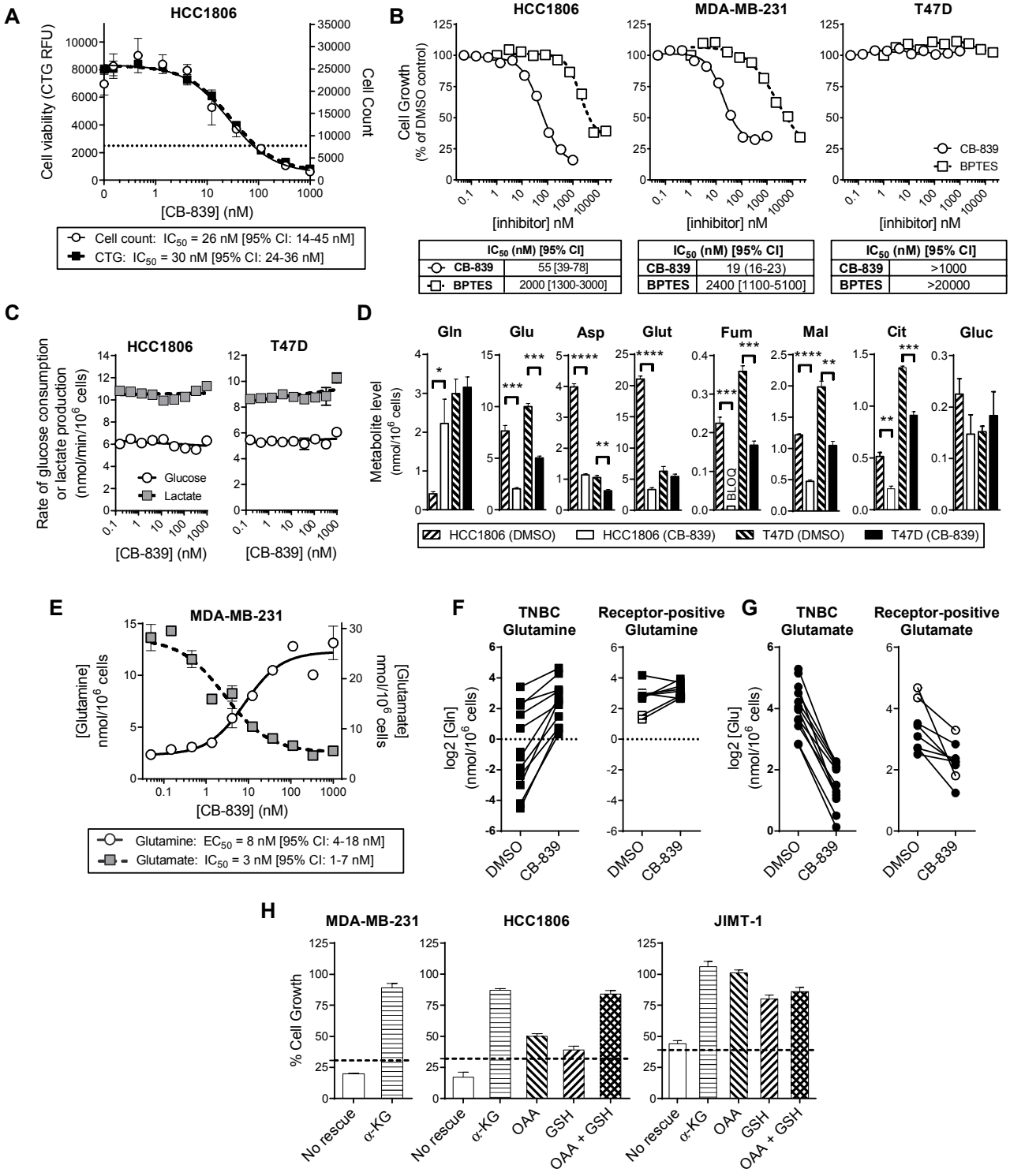
Gross et al., *"Antitumor Activity of the Glutaminase Inhibitor CB-839 in TNBC"*
Supplementary Figure S2



Supplementary Figure S2. CB-839 has potent anti-proliferative activity in TNBC cells that is associated with selective impairment of glutamine utilization. A, anti-proliferative effect of CB-839 on HCC1806 cells treated for 72 hours measured by Cell Titer Glo (CTG; left axis) or by cell counting (right axis). The mean and SEM of duplicate measurements are plotted; calculated IC_{50} and 95% CI values are shown. The dotted line represents the CTG signal or cell number at the time of compound addition. B, cell proliferation dose-response curves for HCC1806, MDA-MB-231, and T47D cells treated with CB-839 or BPTES for 72 hours. The mean and SEM of triplicate measurements averaged across at least three independent experiments (N≥9) are plotted; calculated IC_{50} and 95% CI values are shown. C, glucose consumption and lactate production by HCC1806 (left) and T47D cells (right) measured after 1 μM CB-839 treatment for 6 hours. Culture medium was analyzed for glucose and lactate with the YSI 2900 Biochemistry Analyzer. The mean and SEM from triplicate measurements are plotted. D, intracellular metabolite levels measured in HCC1806 and T47D cells treated with DMSO or 1 μM CB-839 for 4 hours. The level of fumarate in the CB-839 treated HCC1806 cells was below the limit of quantitation (BLOQ) of ~0.01 nmol per 10^6 cells. The mean and SEM of triplicate measurements are shown and statistical analysis was done by unpaired t-test: $*P \leq 0.05$;$**P \leq 0.01$;$***P \leq 0.001$;**** $P \leq 0.0001$. Gln=glutamine, Glu=glutamate, Asp=aspartate, Glut=glutathione, Fum=fumarate, Mal=malate, Cit=citrate, Gluc=glucose. E, dose response curves for intracellular glutamine and glutamate levels measured after treating MDA-MB-231 cells with CB-839 for 24 hours. The mean and SEM of duplicate measurements are plotted; calculated IC_{50} for glutamate decrease and EC_{50} for glutamine increase with associated 95% CI values are shown. F, difference in glutamine concentration between breast cancer cell lines [left, TNBC (N=12); right, receptor-positive (N=8)] treated with DMSO or CB-839 for 4 hours. G, difference in glutamate concentration between breast cancer cell lines [left, TNBC (N=12); right, receptor-positive (N=8)] treated with DMSO or CB-839 for 4 hours. For panels (F) and (G), the mean from duplicate measurements is plotted. Each cell line is depicted by two symbols (DMSO and CB-839 treated) with a connecting line showing the magnitude difference between the two treatment groups. The open symbols in the receptor-positive graphs (right) represent the two basal-like ER-/HER2+ cell lines JIMT-1 and HCC1954. H, anti-proliferative activity of 1 μM CB-839 on MDA-MB-231, HCC1806 and JIMT-1 cell lines treated for 72 hours in the absence or presence of cell permeable TCA cycle intermediates [alpha-ketoglutarate (α-KG), oxaloacetate (OAA)] and/or glutathione (GSH). The data is expressed as the percent proliferation in the presence of CB-839 relative to untreated controls (either DMSO, α-KG alone, GSH alone, OAA alone, or OAA and GSH). The mean and the SEM of duplicate experiments are plotted. The dotted line represents the cell signal at the time of compound of addition relative to the DMSO control at 72 hours.