Supplemental Data

On-target inhibition of glutaminase diminishes cell autonomous tumorigenesis

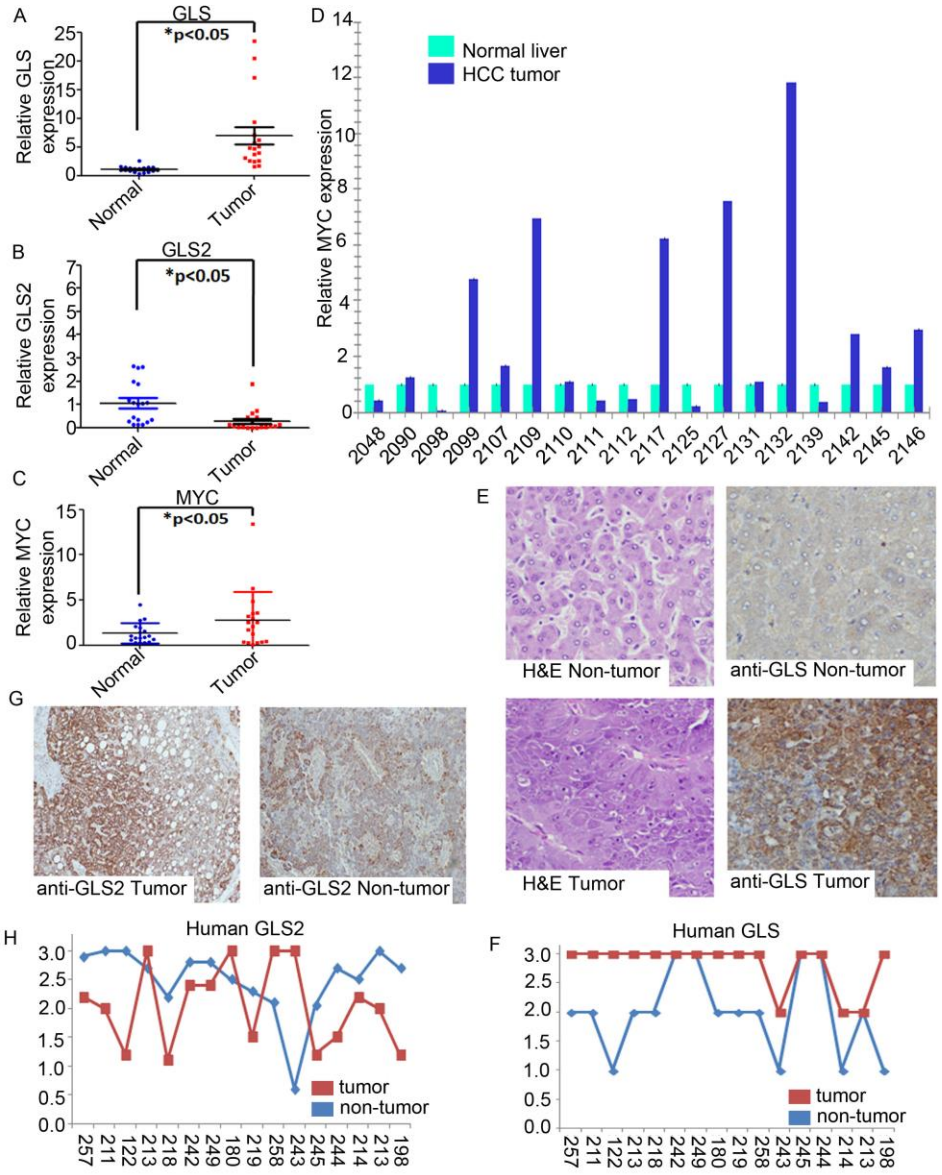

Figure S1. Immunohistochemistry and qPCR of GLS and GLS2 in human HCC samples. A-B) Dot plots of GLS and GLS2 expression in human HCC tumors from Figure 1. C-D) Dot plot and qPCR of MYC levels in human HCC tumors used in Figure 1. E) Representative Hematoxylin & Eosin (H&E) and anti-GLS IHC staining of human non-tumor and HCC tumor tissues. F) Anti-GLS IHC weighted score (y-axis) of human HCC tumor and non-tumor liver tissues (n=16). Numbers at the bottom correspond to different patient samples. G) Representative anti-GLS2 IHC staining of human non-tumor and HCC tumor tissue. H) Anti-GLS2 IHC weighted score (y-axis) of human HCC tumor and non-tumor liver tissues (n=16)

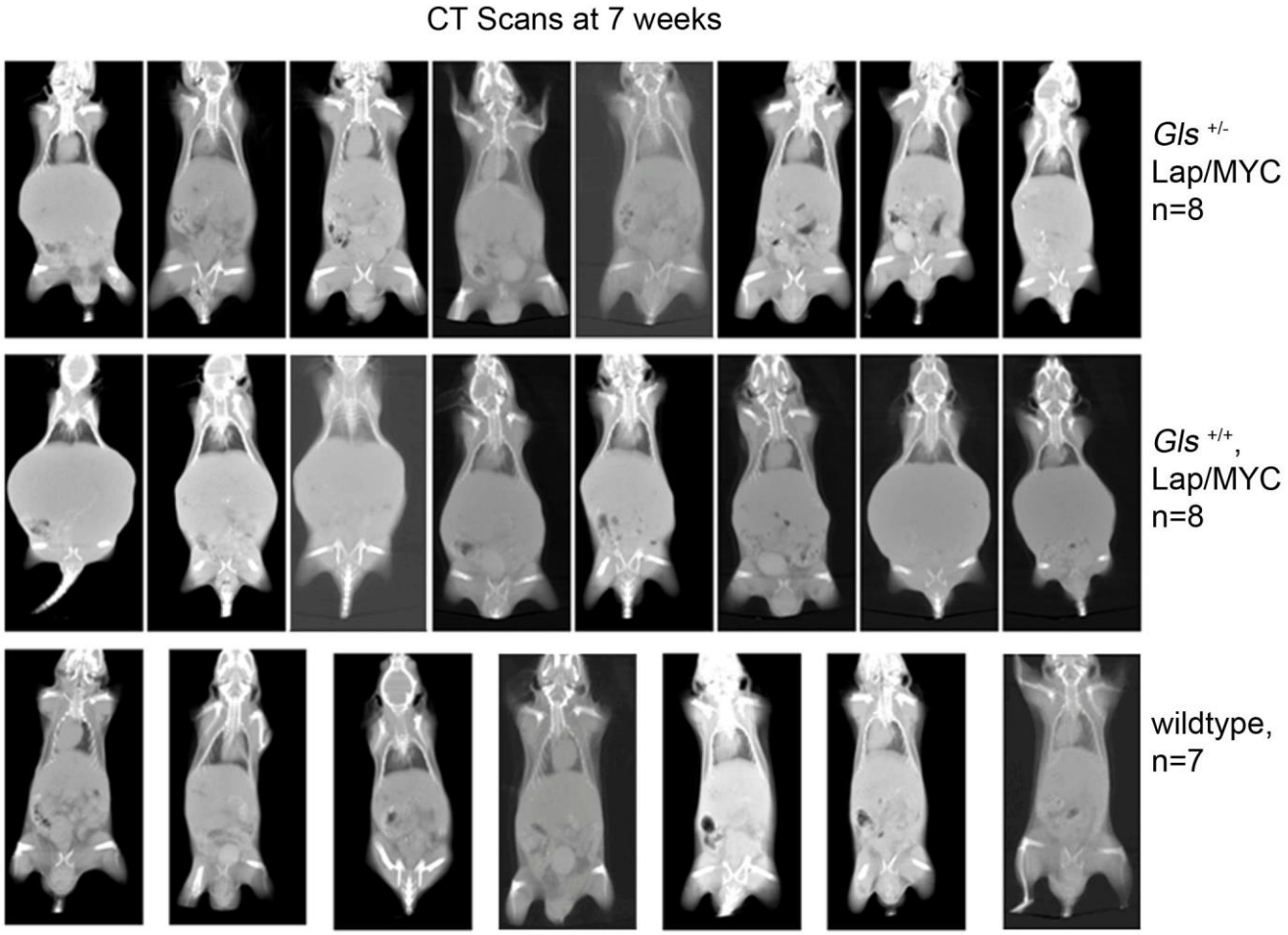

Figure S2. **CT scans of LAP/MYC and GLS double transgenic mice at 7 weeks.** CT scans were obtained on 7-week old normal (wild-type), LAP/MYC:*Gls*$^{+/+}$ or LAP/MYC:*Gls*$^{+/-}$ mice.

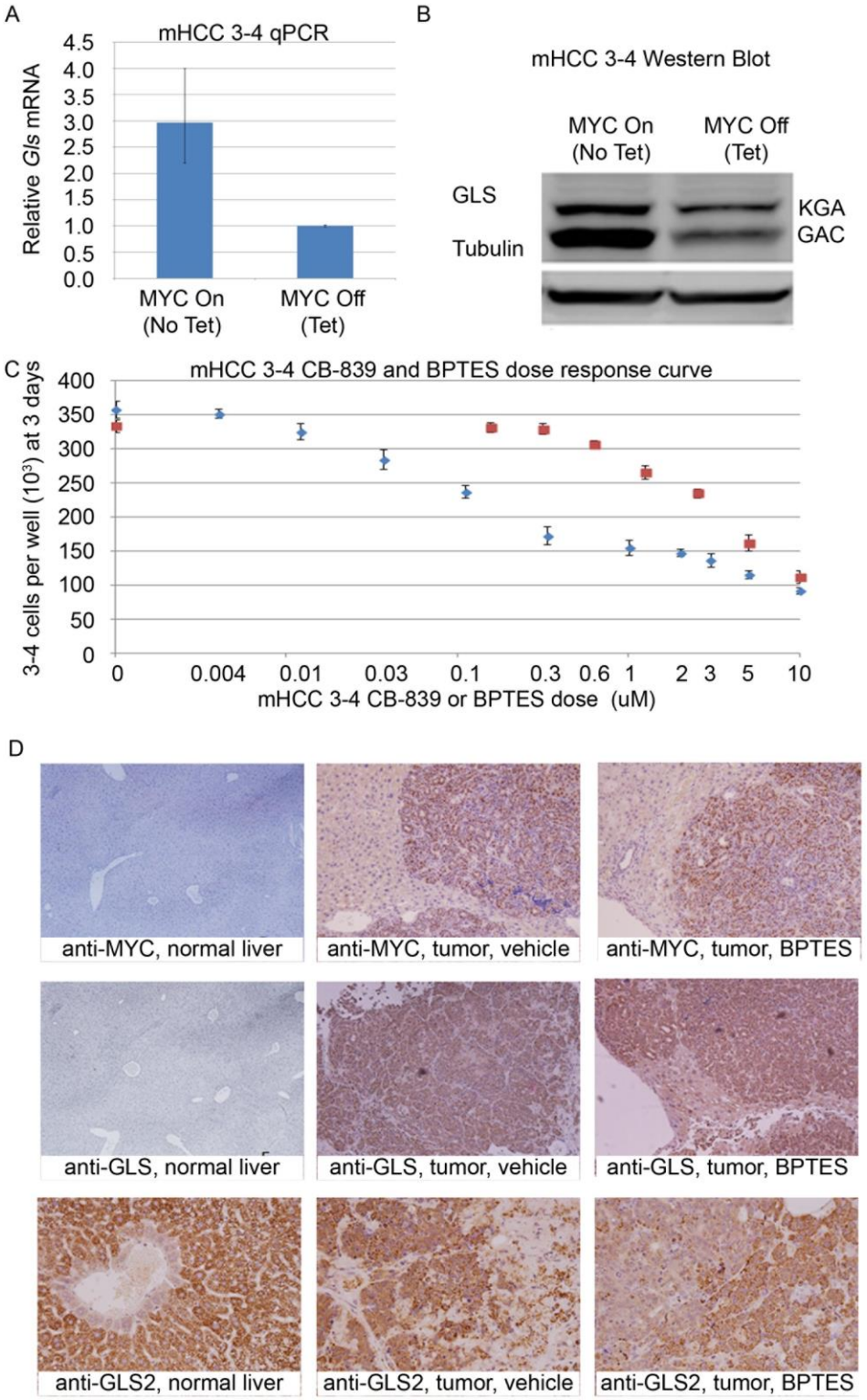

Figure S3. Effects of BPTES on mouse hepatocellular carcinoma *in vitro* and *in vivo*. A) Response of *Gls* mRNA level at 24 hours after ectopic *MYC* inactivation in LAP/MYC mouse hepatocellular carcinoma 3-4 cell line (mHCC 3-4). Values are shown as mean ± standard deviation (SD), n=3. B) Immunoblot of GLS (GAC and KGA isoform) protein at 24 hours after ectopic *MYC* inactivation in LAP/MYC mouse hepatocellular carcinoma 3-4 cell line (mHCC 3-4). C) Dose response growth curve of mHCC 3-4 cells grown in different doses (0-10 μM) of CB-839 or BPTES for 72 hours. Values are shown as mean ± standard deviation (SD), n=3. D) Immunostaining of normal mouse livers (7 weeks) or DMSO versus BPTES-treated LAP/MYC tumors (7 weeks) for MYC, GLS, or GLS2 protein.

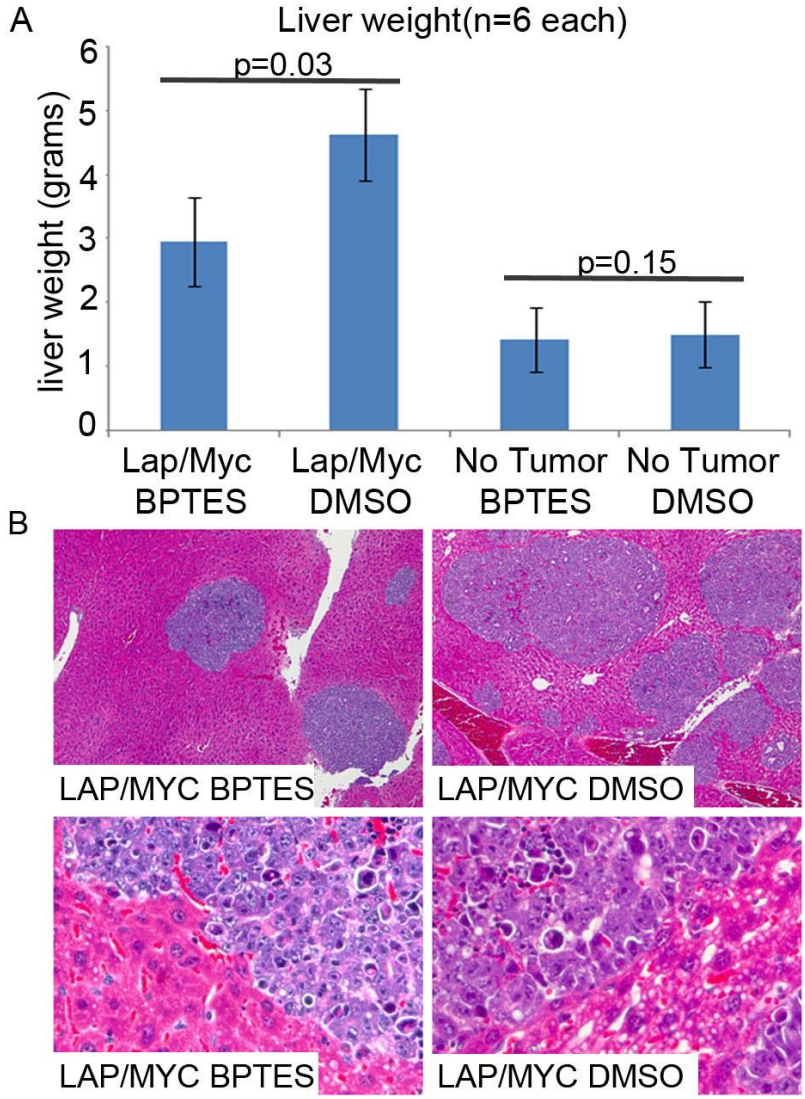

Figure S4 A) Liver weight at ~5-5.5 weeks of age of LAP/MYC mice or sibling controls treated with BPTES or DMSO (n=6 each). B) Representative hematoxylin and eosin staining of LAP/MYC liver tumor sections from mice treated with BPTES or DMSO. Statistics are Student's t-test.

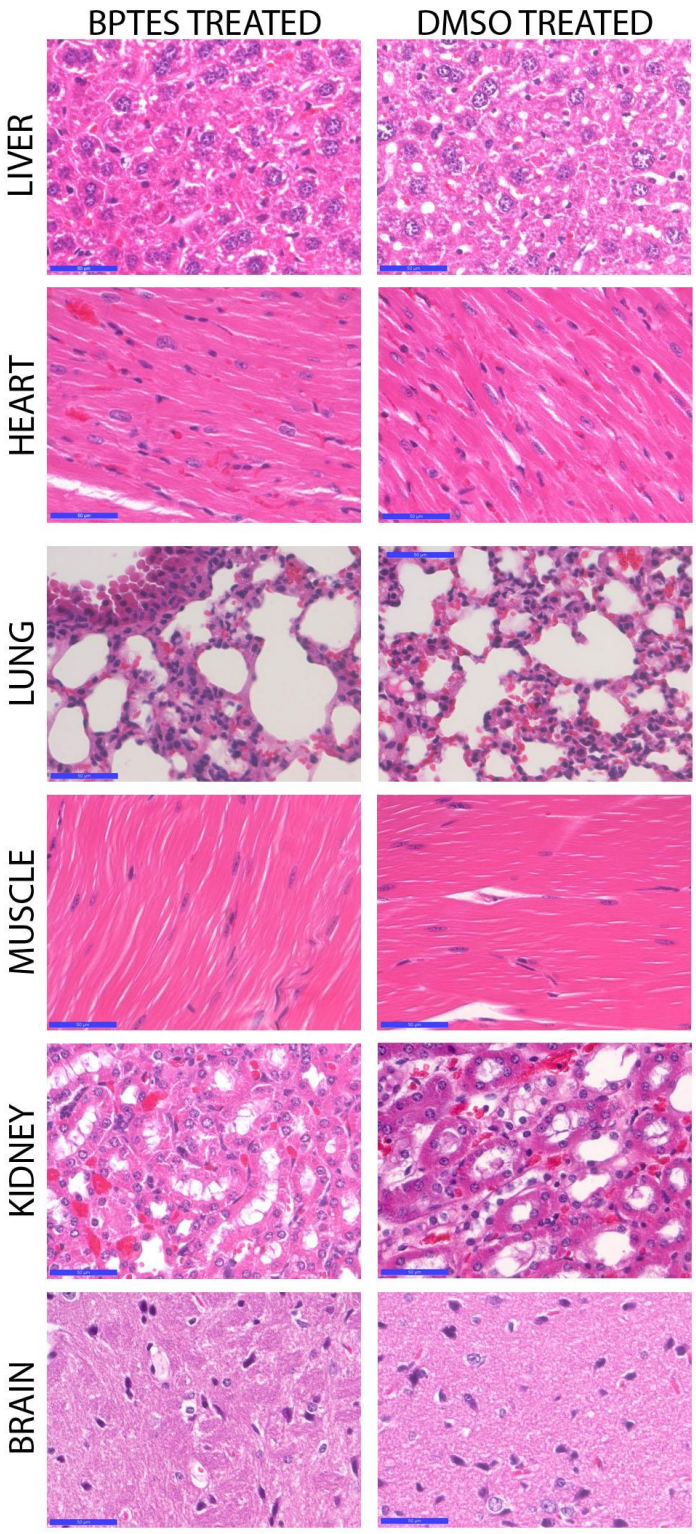

Figure S5. Hematoxylin and eosin stained sections of tissue from the liver, heart, lung muscle , kidney and brain of mice treated for 10 consecutive days with DMSO vehicle or BPTES . Slides were reviewed by a pathologist and found to have no histopathologies.

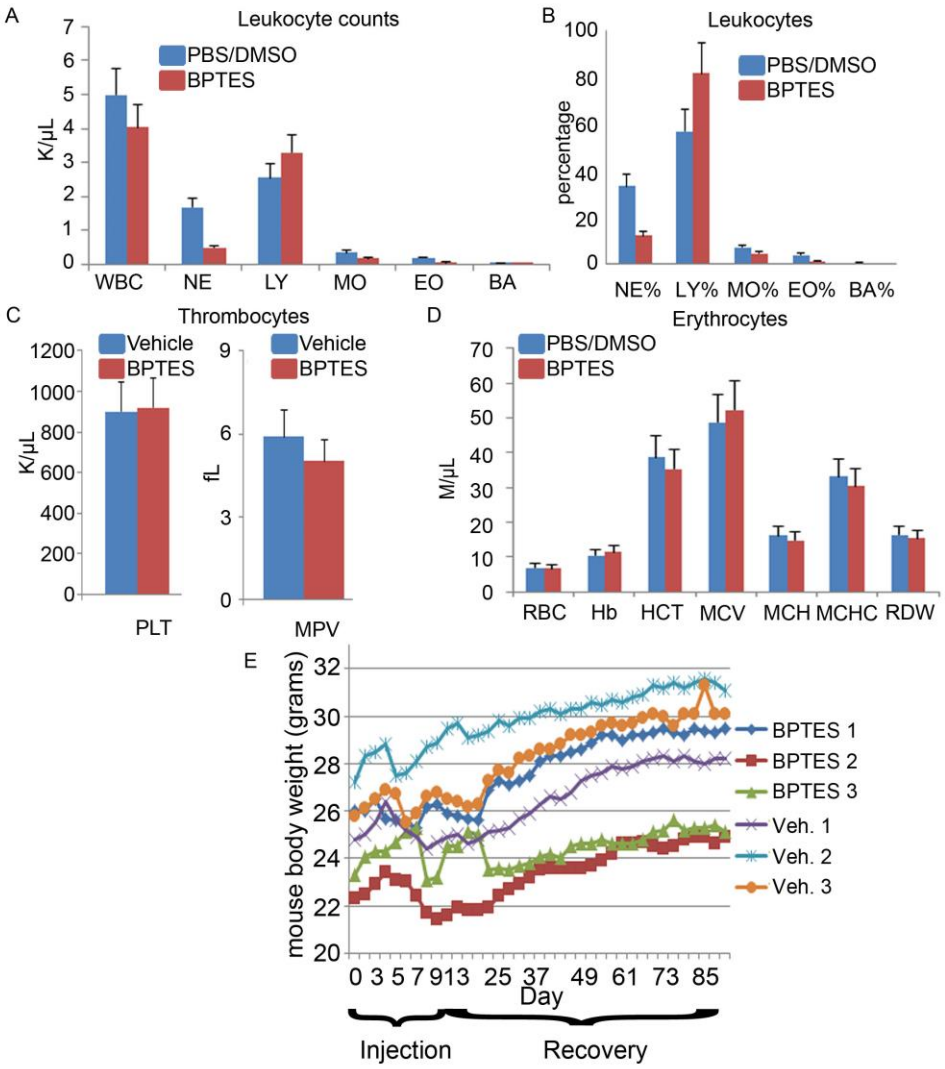

Figure S6. Toxicity profile of BPTES. A) Leukocytes counts in units of K/µl (thousand per microliter). WBC=White Blood Cell or leukocyte count (normal range= 1.8-10.7 K/µl); NE=Neutrophil (normal range=0.1-2.4 K/µl); Ly=Lymphocytes (normal range=0.9-9.3 K/µl); MO= Monocytes, normal range=0.0-0.4 K/µl; EO= Eosinophil (normal range=0.0-0.2 K/µl), BA= (normal range 0.0-0.2 K/µl). Values are shown as mean ± standard deviation, n=4. B) Leukocyte differential percentage. Values are shown as mean ± standard deviation, n=4. C) Thrombocyte count; PLT=platelet or thrombocyte (normal range= 592-2972 K/µl); MPV=Mean platelet volume (normal range= 5-20 fL). Values are shown as mean ± standard deviation, n=4. D) Erythrocytes RBC= Red blood cell or erythrocyte count (normal range 6.36-9.42 M(10^6)/µl); Hb= Hemoglobin concentration (normal range = 11-15.1 g/dL); HCT= hematocrit (normal range= 45.4-60.3 fL); MCV= mean corpuscular volume (erythrocyte; normal range=(45.4-60.3 fL), MCH=Mean corpuscular hemoglobin (normal range= 14.1-19.3 pg); MCHC= Mean corpuscular hemoglobin concentration (normal range=30.2-34.2 g/dL), RDW= Red cell distribution width (12.4-27%). Values are shown as mean ± standard deviation, n=4. E) Effect of BPTES on 129SvEv/j-C57BL6/J mouse body weight. Mice were injected with BPTES every other day for 10 days starting at 9 weeks of age. Their weights were tracked over 91 days. Each graph represents a single mouse.

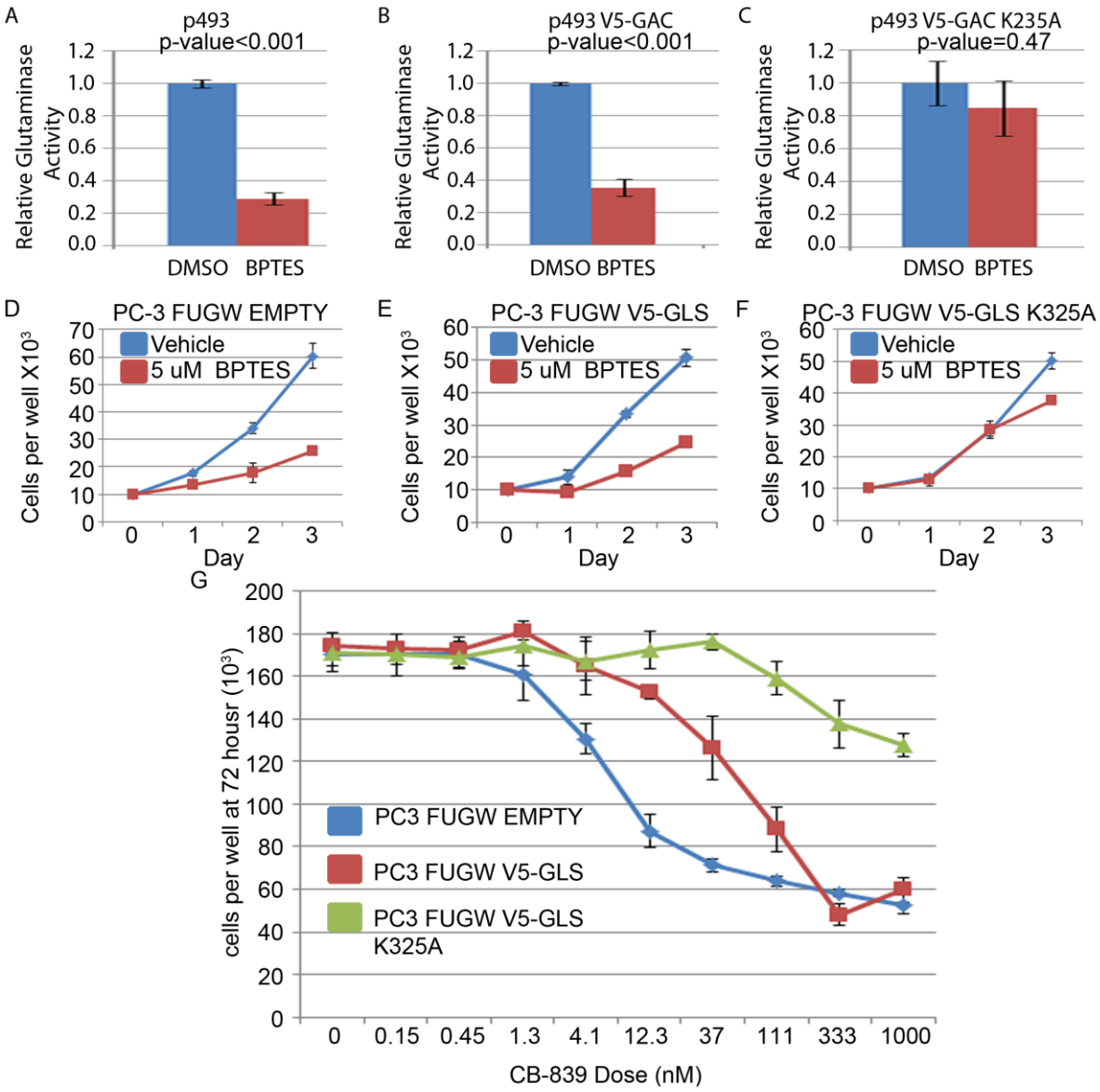

Figure S7. BPTES treatment of PC-3 cell lines. A-C) Cell lysate glutaminase activity with lysates incubated in DMSO or 10 µM BPTES from p493, p493 V5-GLS or p493 V5-GLS K325A cells. D) BPTES (5 µM) inhibited growth of PC-3 FUGW Empty cell line. E) 5 µM BPTES inhibited growth of PC-3 FUGW V5-GLS. F) Overexpression of the BPTES-resistant mutant V5-GLS K325A significantly rescued the growth inhibition of PC-3 cells treated with 5 µM BPTES. G) CB-839 dose response curves for PC-3 FUGW Empty, PC-3 FUGW V5-GLS and PC-3 FUGW V5-GLS K325A measured as cells per well treated for 72 hours with 0 to 1000 nM CB-839. Error bars represent standard deviation for n=3 biological replicates. Statistics are Student's t-test.

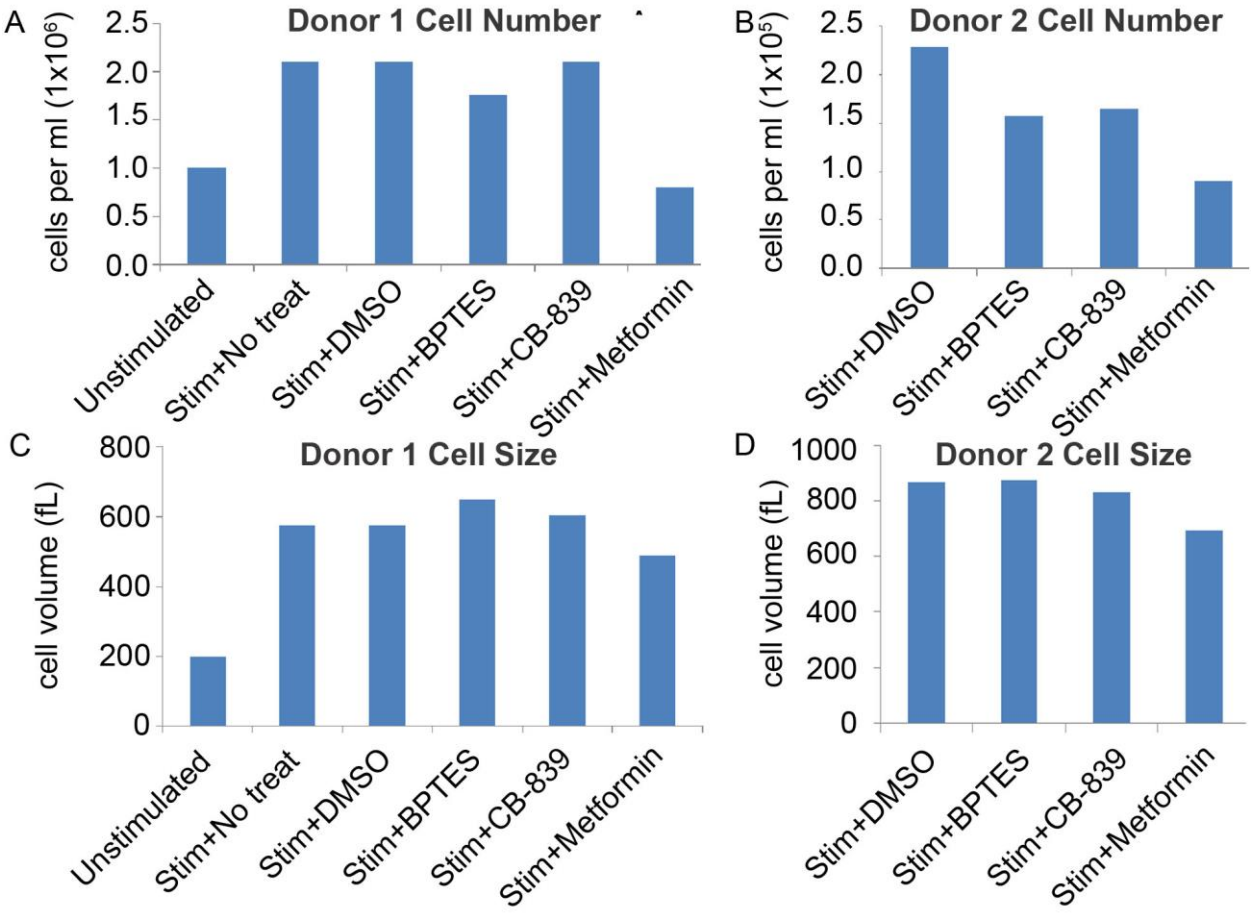

Figure S8. BPTES does not block CD3/CD28 stimulation of lymphocytes obtained from two human anonymized donors. A and B) Number of cells per ml following CD3/CD28 stimulation of human T lymphocytes with following treatment with media only (no treatment), a DMSO vehicle control, BPTES, CB-839 or metformin from two different human lymphocyte donors. C and D) Cell volume of CD3/CD28 stimulation of human T lymphocytes with following treatment with media only (no treatment), a DMSO vehicle control, BPTES (10 μM), CB-839 (10 μM), or metformin (1 mM) from two different human lymphocyte donors.

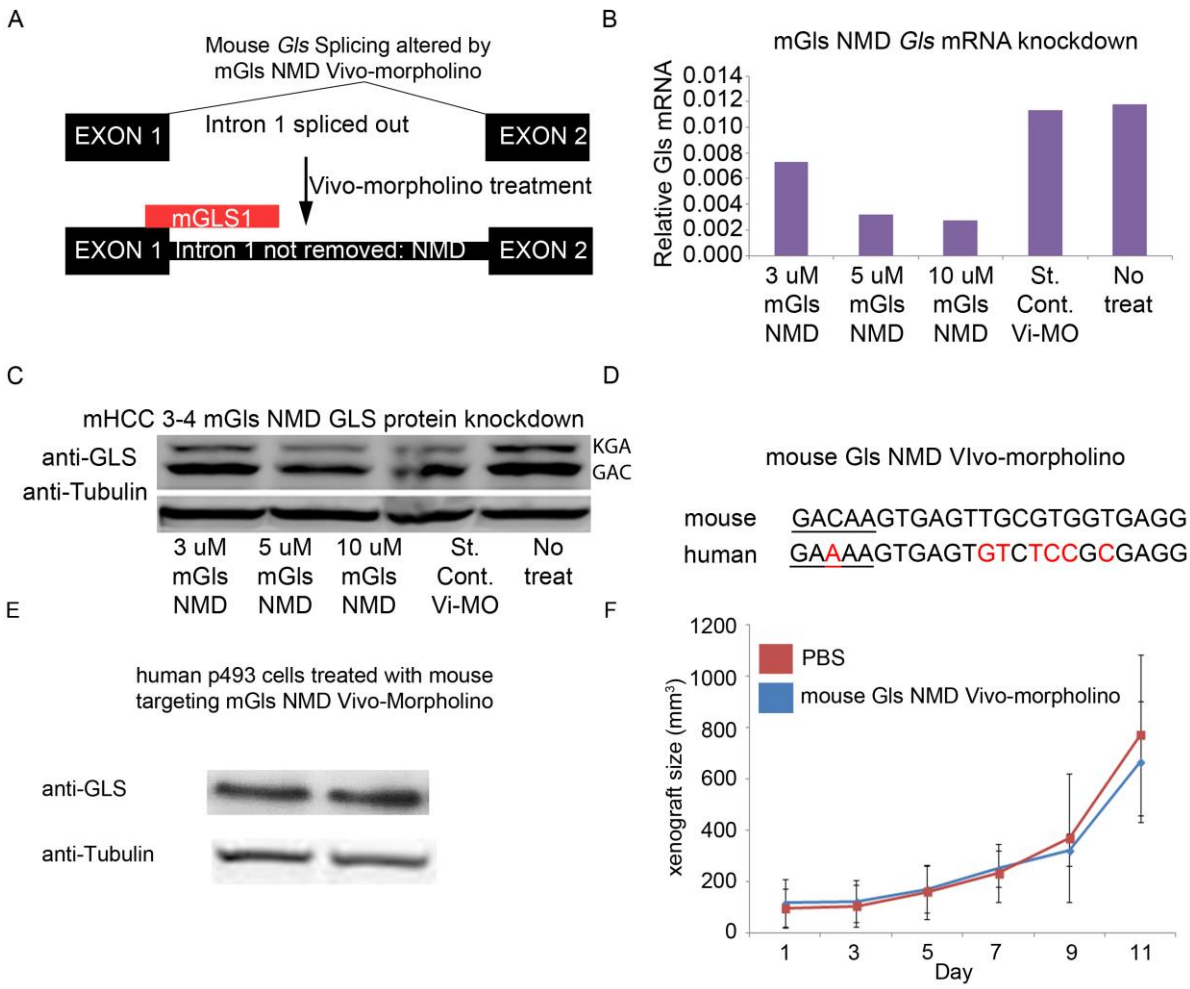

Figure S9. Mouse Gls NMD *Vivo*-morpholino. A) Mouse Gls NMD *Vivo*-morpholino (mGls NMD) triggered non-sense mediated decay of mouse *Gls* pre-mRNA by blocking the exon 1 splice donor complex. B) Knockdown of mouse *Gls* mRNA in mHCC 3-4 cells with different doses of mGls NMD *Vivo*-morpholino, standard control, or PBS. Values are means with standard errors; n=3, each. C) Western blot shows knockdown of mouse Gls protein normalized to tubulin in mHCC 3-4 cells when treated with 3 µM, 5 uM or 10 µM mGls NMD *Vivo*-morpholino (mGls NMD), Standard Control *Vivo*-morpholino (Cont. Vi-MO) or PBS (No treat). Antibody Anti-GLS GTX81012 , GeneTex. D) Mismatches (marked in red) between the human and mouse *Gls* exon 1/intron 1 boundary sequence provide species specificity of mGls NMD *Vivo*-morpholino. E) Treatment of human P493 cells with mouse targeting mGls NMD *Vivo*-morpholino does not affect human GLS protein level (KGA band, Ab nova antibody M01, clone 5C4). F) Human P493 xenografts did not show change in growth when treated with mouse mGls NMD *Vivo*-morpholino. Values are shown as mean ± standard deviation, n=5.