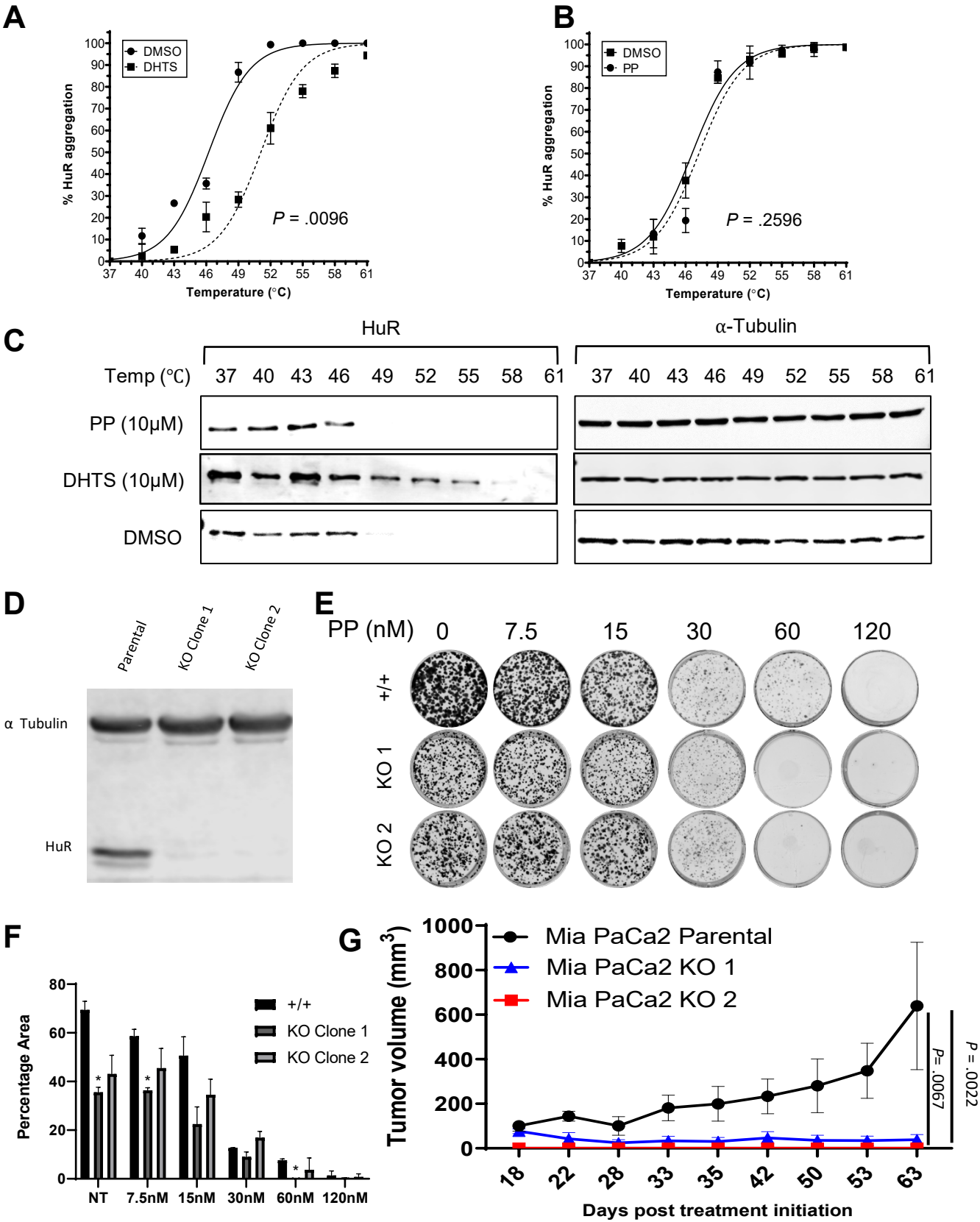
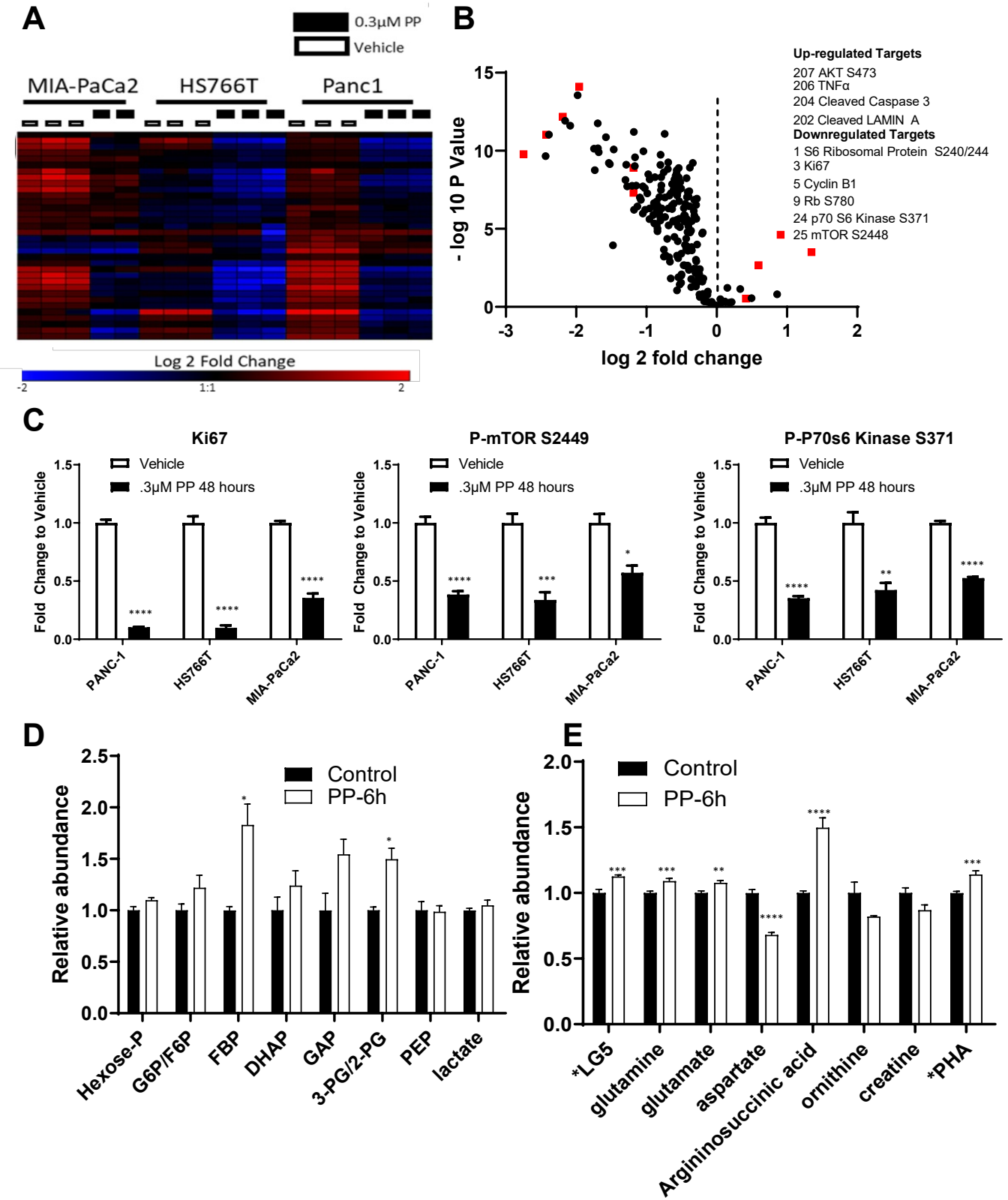
Supplemental Figure 1



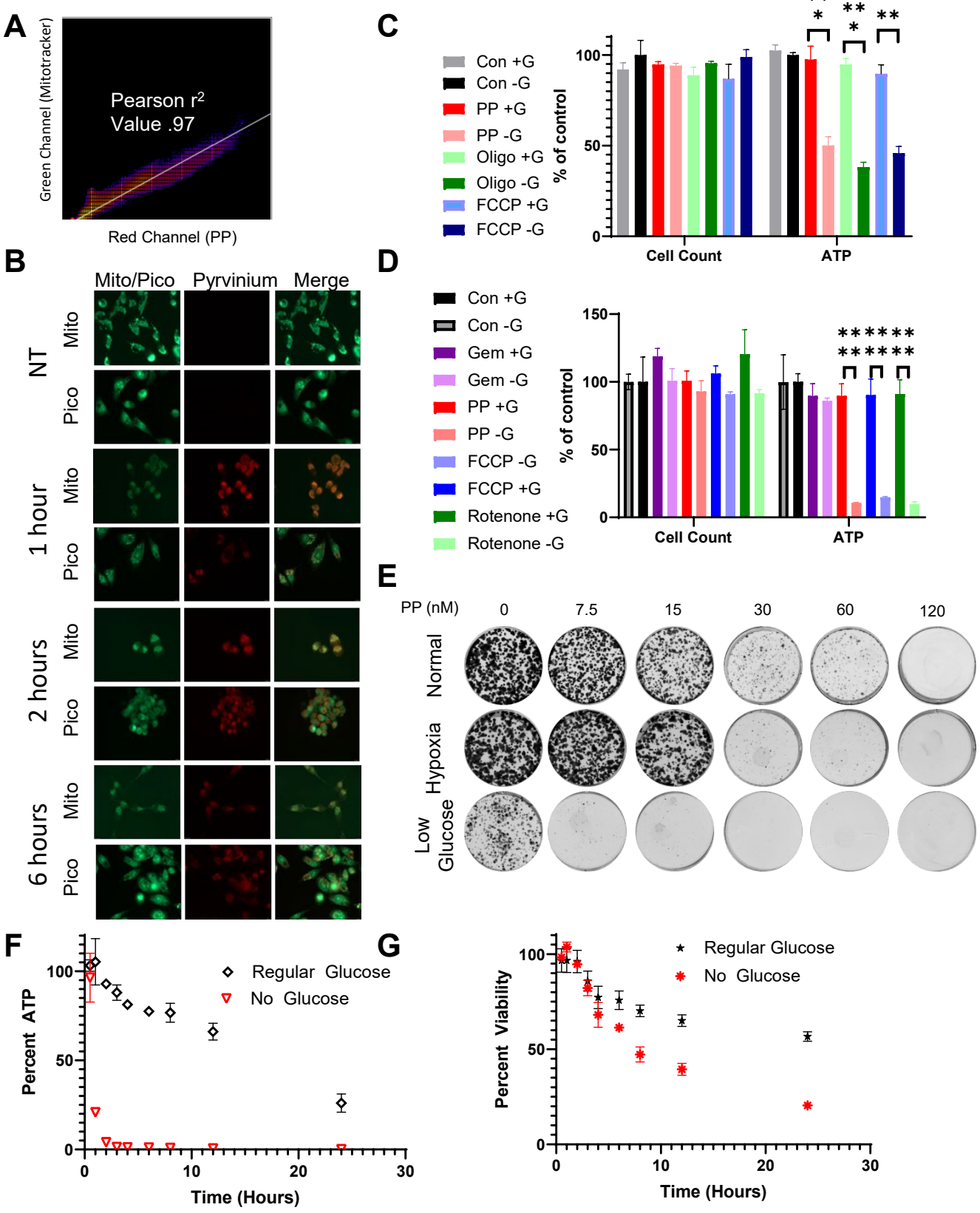
Supplemental Figure 1 Pyrvinium Pamoate does not target HuR: A,B Panc1 cells were treated with 10uM of dihydrotranshinone I (DHTS) (**A**) or PP (**B**) for 4 hours and HuR temperature dependent stability was quantified by Western blot (n=3) **C)** Representative Western blots from B and C **D)** Parental MIA-PaCa2 cells and knockout clones Immunostaining of HuR and α-tubulin as a loading control, the knockout cells displayed no HuR staining **E)** +/+ and HuR KO clones were treated with PP at varying concentrations for a ten day colony formation assay in (n=3) **F)** Quantification of E **G)** Parental cells and knockout clones were engrafted in nude mice (5 million cells per flank), parental clones were able to form tumors whereas knockout clones were not

SUPPLEMENTAL FIGURE 2



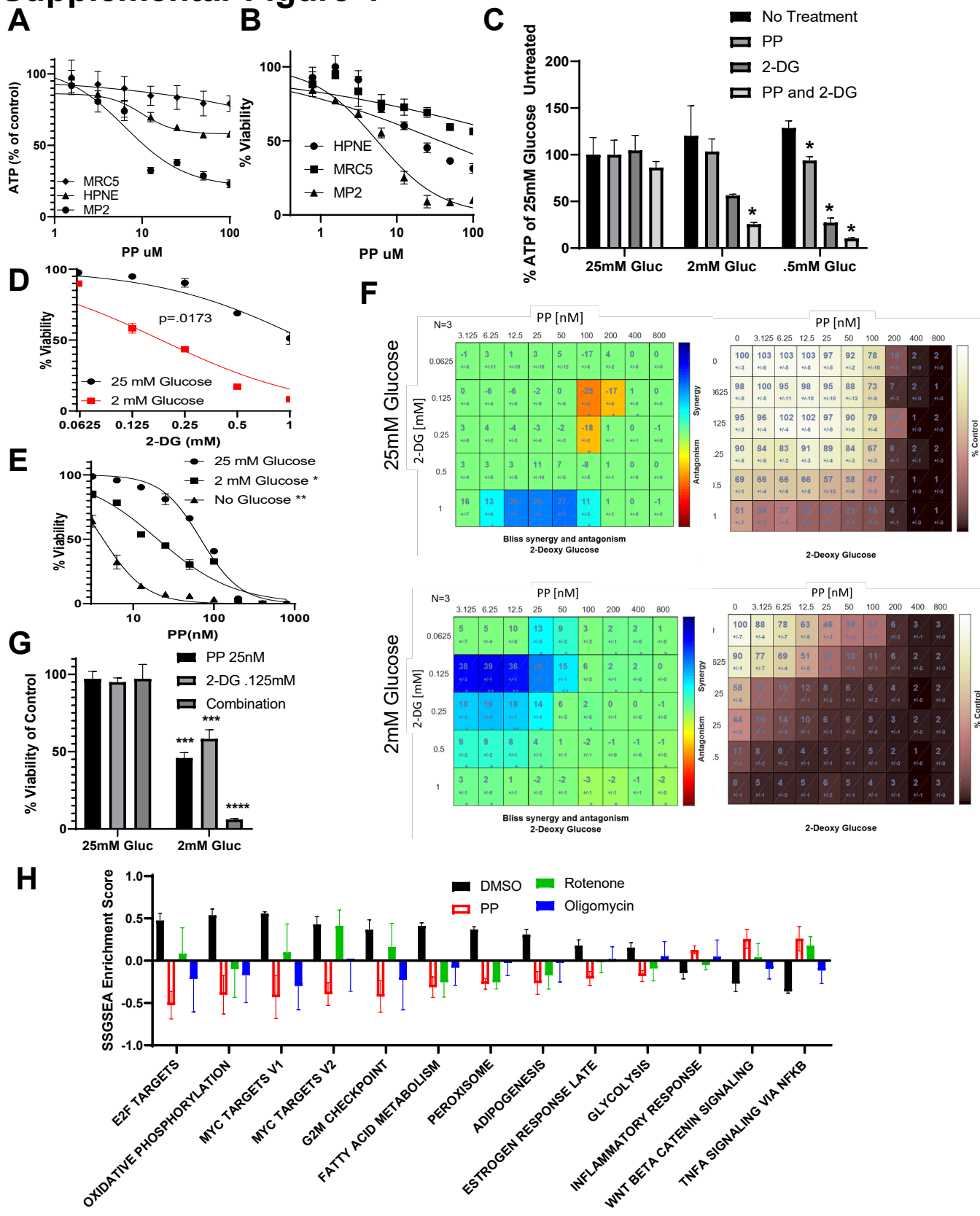
Supplemental Figure 2 Supplemental Phosphoproteomic and metabolomic data: A) MIA-PaCa2, HS766T and PANC-1 cells were treated with .3uM PP for 48 hours and then analyzed utilizing a reverse phase protein microarray, this heatmap displays strong clustering between cell lines and treatment groups **B)** These data indicate the average expression of each protein across the three cell lines after they were normalized to their own controls samples. Significantly affected targets relevant to cell cycle, cellular metabolism and apoptosis are highlighted in red, and called out in text with a corresponding number with 1 being the protein with the greatest decrease in average across all three cell lines, and 207 being the greatest increase across all three cell lines. **C)** These data indicate the average expression of representative target proteins across the three cell lines after they were normalized to their own controls samples. **D)** These data indicate the metabolites involved in glycolysis from the same samples as Figure 1 **E)** There were not drastic changes in proline or arginine metabolites with PP treatment *LG5 stands for L-Glutamate-5-semialdehyde from the same samples as Figure 1

SUPPLEMENTAL FIGURE 3



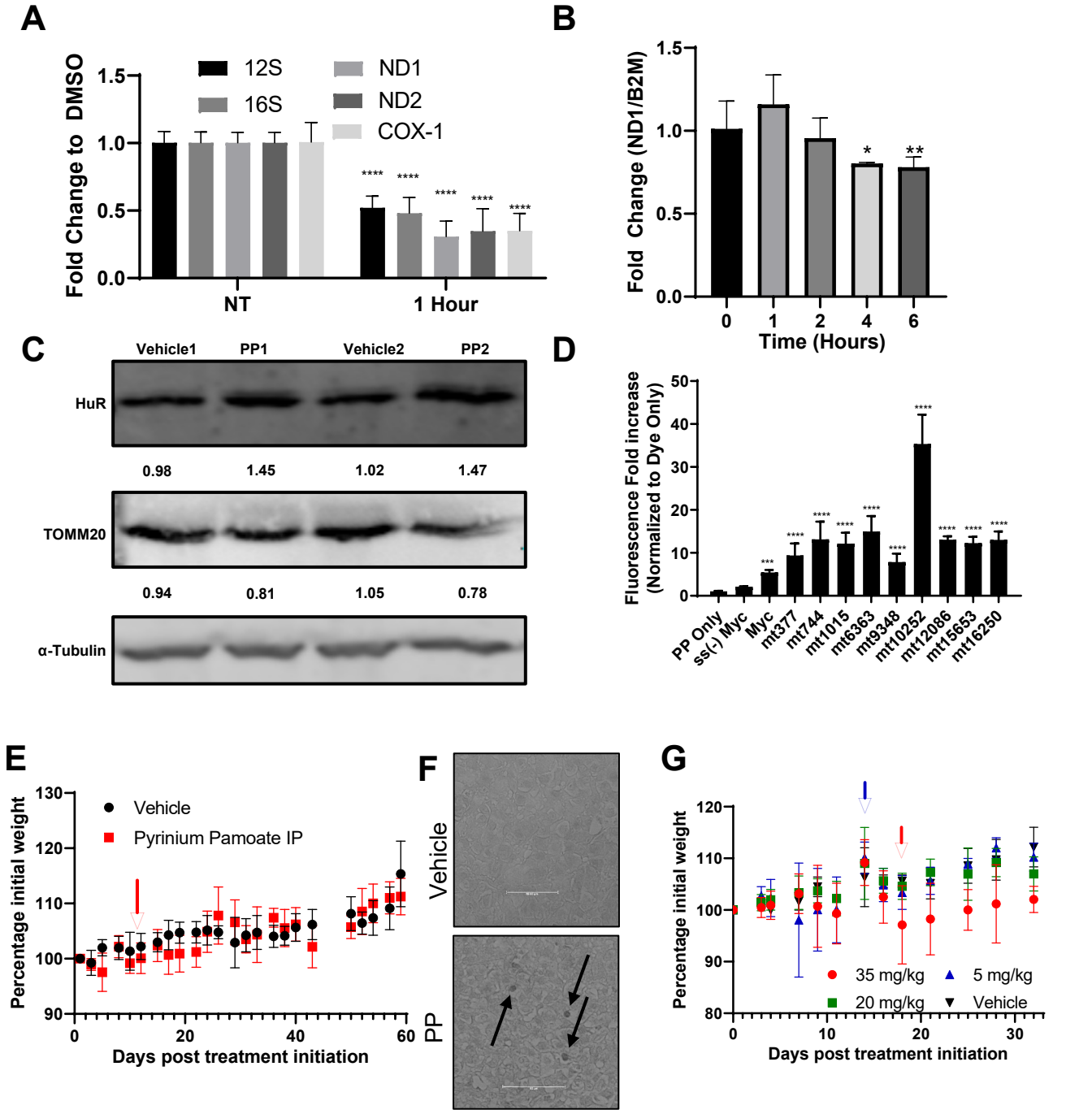
Supplemental Figure 3 Pyrvinium Pamoate and Oxidative Phosphorylation: A) Quantification of PP and mitotracker colocalization from figure 2A **B)** Treatment of MIA-PaCa2 cells with mitotracker (stain mitochondria green) or picogreen (stain nuclear and mitochondrial DNA green) and PP (red) for one, 2 and 6 hours displayed sustained colocalization of PP with mitotracker and not with nuclear DNA **C)** MIA-PaCa2 Cells were pretreated in no glucose no FBS conditions for 24 hours prior to 1 hour treatment of PP .3uM or oligomycin 10uM or FCCP 1uM +/- 25mM glucose demonstrating no change in cell count as quantified by picogreen but significant changes in ATP levels as indicated by Cell Titer Glo **D)** PANC1 Cells were pretreated in no glucose no FBS conditions for 24 hours prior to 1 hour treatment of PP .3uM or rotenone .5uM or FCCP 1uM or gemcitabine 1 uM +/- 25mM glucose demonstrating no change in cell count as quantified by picogreen but significant changes in ATP levels as indicated by Cell Titer Glo **E)** MIA-PaCa2 cells were treated with varying concentrations of PP for 10 days in hypoxic (1% oxygen), or low glucose (2mM glucose) conditions as compared to normal conditions **F)** Time course of ATP production inhibition of MIA-PaCa2 cells treated with .3µM PP in no glucose and 25mM glucose conditions as determined by Cell-Titer Glo **G)** Cell viability as measured by double stranded DNA content using Pico Green for the same time period

Supplemental Figure 4



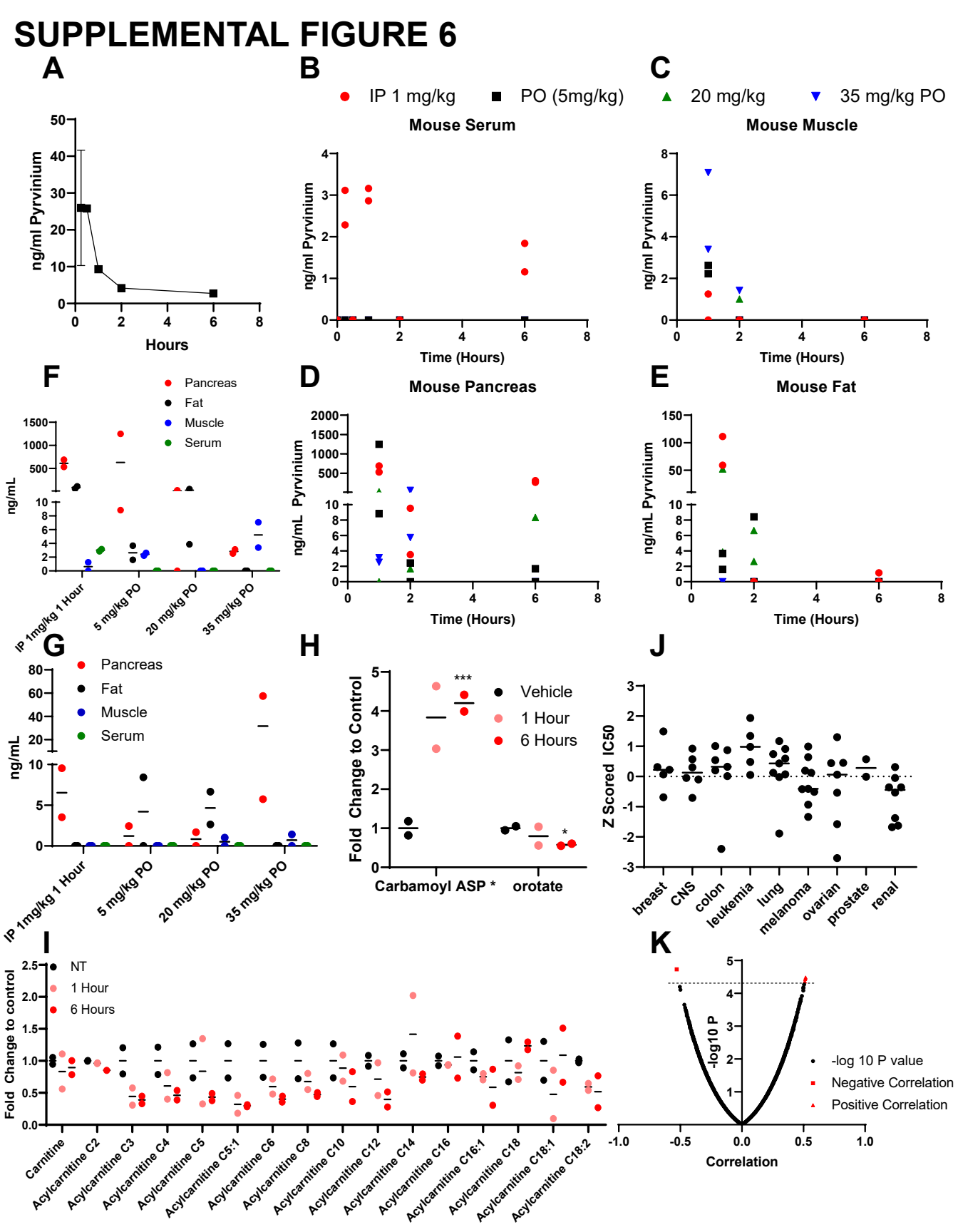
Supplemental Figure 4 PP and other mitochondrial modulators: A) Mia-Paca2, HPNE (Human Pancreatic Normal Epithelial) and MRC5 (Immortalized Fibroblasts) were plated at high density (20,000 cells per well 96 well plate) and pretreated in glucose replete conditions, and ATP levels were assessed 5 hours after PP treatment **B)** Mia-Paca2, HPNE (Human Pancreatic Normal Epithelial) and MRC5 (Immortalized Fibroblasts) were plated at high density (20,000 cells per well 96 well plate) treated with PP and viability was assessed after 48 hours **C)** Cells were plated at 5,000 cells per well and treated with varying concentrations of PP, 2-DG or combination for 2 hours and ATP levels were assessed **D)** MIA-PaCa2 cells demonstrated a significant increase in sensitivity to 2-Deoxy Glucose at low-Glucose (2mM) conditions as compared to 25mM glucose conditions as measured with a 5 day Pico Green cell viability assay **E)** MIA-PaCa2 cells were significantly more sensitive to PP at 2mM and no glucose conditions as compared to 25mM glucose conditions as measured with a 5 day Pico Green cell viability assay (statistics are comparisons to 25mM Glucose) **F)** Bliss synergy calculation of PP and 2Deoxy glucose in media with 25mM or 2mM glucose (n=3) along with accompanying cell viability charts as measured with a 5 day Pico Green cell viability assay **G)** PP and 2-DG efficacy solo or in combination at specific concentrations at either 25mM or 2mM glucose concentrations **H)** The hallmark pathways that PP significantly altered from **Figure 3B** in comparison to changes made by rotenone and oligomycin

Supplemental Figure 5



Supplemental Figure 5 PP and mitochondrial RNA and DNA, mouse weights and TUNEL staining: A) MIA-PaCa2 cells were treated with PP at .3µM for 1 hour and mitochondrial targets were assessed using QOCR **B)** MIA-PaCa-2 cells were treated with .3µM PP for the indicated times and mitochondrial DNA content was assessed by comparing ND1 (mitochondrial DNA) to B2M (nuclear DNA) **C)** MIA-PaCa-2 cells were treated with PP .3µM for 48 hours and displayed a modest reduction in TOMM20 a marker of overall mitochondrial mass as determine by immunostaining here α-Tubulin is a control, this western depicts two independent experiments with two separate controls and treatment groups **D)** PP was incubated with either single stranded DNA as a negative control and multiple known and proposed mitochondrial G-Quadruplexes and fluorescence intensity was determined **E)** Weights of mice treated with IP PP, arrow indicates a mouse that was found dead from **F)** Representative images for TUNEL staining from tumors from IP treated mice (from Figure 5B) **G)** Weights of mice treated with oral PP, arrows indicate mice that were found dead

SUPPLEMENTAL FIGURE 6



Supplemental Figure 6 In vivo Pharmacokinetics, and public data: A) Mice were treated with PP IV 1mg/kg and plasma samples were analyzed **B,C,D,E)** Mice were treated with PP 1mg/kg IP, and PO at 5, 20 and 35mg/kg and serum and tissue samples were collected and analyzed at the indicated time points in **B)** Serum **C)** Muscle **D)** Pancreas **E)** Fat **F)** All data across tissues and treatment types at 1 hour **G)** All data across tissue and treatment types at 2 hours **H,I)** Mice were treated with PP IP 1mg/kg and plasma was collected and analyzed for metabolomics at 1 and 6 hours, this data indicated significant changes in **H)** Carbamoyl Aspartate and orotate levels and **I)** acylcarnitine levels **J,K)** Z scored efficacy data (with positive being more efficacious i.e. lower IC50) of PP across the NCI60 was downloaded from CellMinerCDB **J)** PP efficacy was assessed in regards to tissue of origin **K)** PP efficacy was correlated across microarray expression, and the top most significantly correlated ($p<10^{-3}$) genes are highlighted here which are Negatively correlated – ABCB1 Positively Correlated - MDH2, NDUFB11, HARS2

SUPPLEMENTAL TABLE 1

Drug	MIA-PaCa2 +/+	MIA-PaCa2 -/-	(+/+)/(-/-)
Pyrvinium Pamoate	0.225	0.280	0.802
Oxaliplatin	30.690	3.301	9.297
Iriniotican	34.002	4.768	7.131
Paclitaxel	0.015	0.002	6.964
Gemcitabine	0.072	0.010	7.363

Supplemental Table 1: Increased efficacy of agents upon inhibition of HuR via CRISPR knockout: In a three-day Pico Green cell viability assay we demonstrated that CRISPR knockout cells were more sensitive to standardly used PDAC agents as compared to +/+ controls, PP however was not less effective as expected.

SUPPLEMENTAL TABLES 2A,B

Serum Collection Schedule			
Animal Number (per group)	**Pre-Dose and 1 hr**	**0.5 and 2 hr**	**0.25 and 6 hr**
Animal numbers	1-2, 7-8, 13-14, 19-20		
Animal numbers		3-4, 9-10, 15-16, 21-22	
Animal numbers			5-6, 11-12,17-18, 23-24

Fat, Muscle, and Pancreas Schedule			
Animal Number (per group)	**1 hr**	**2 hr**	**6 hr**
Animal numbers	1-2, 7-8, 13-14, 19-20		
Animal numbers		3-4, 9-10, 15-16, 21-22	
Animal numbers			5-6, 11-12,17-18, 23-24

Supplemental Table 2A,B: A) This table indicates the scheduling of animals for pharmacokinetic studies for collection of Serum **B)** This table indicates the scheduling of the same animals from A for pharmacokinetic studies for collection of tissues