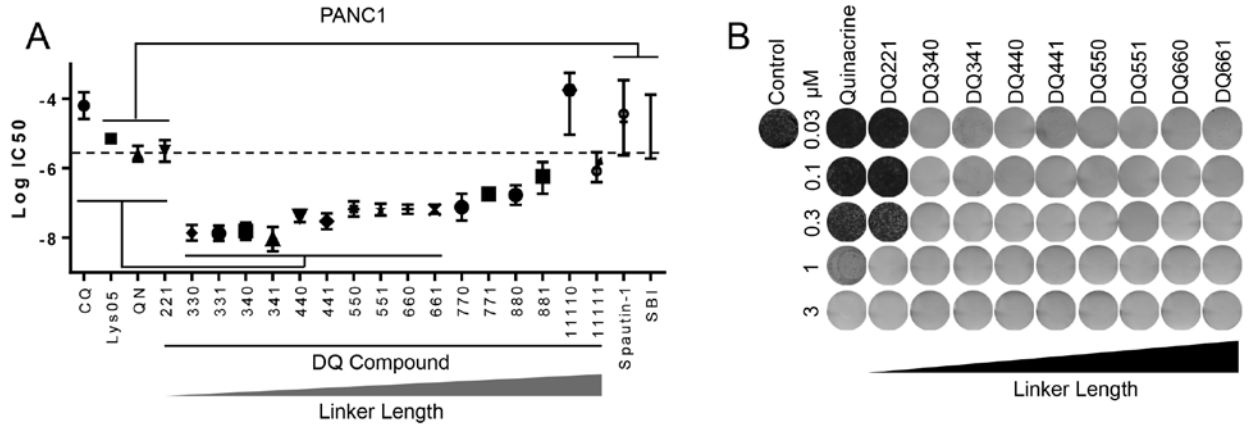
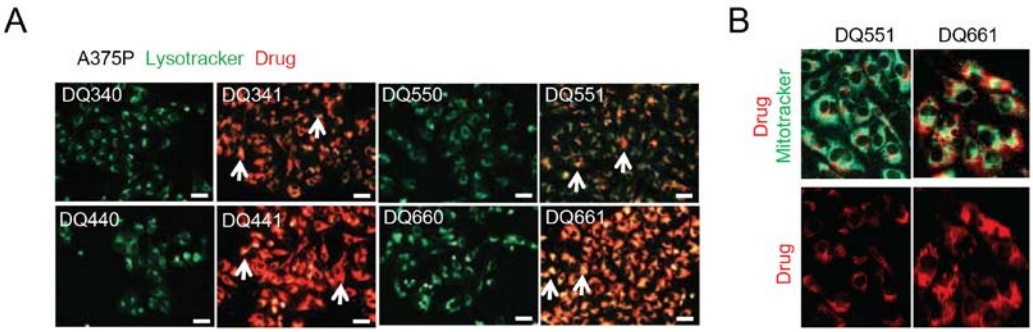
Supplemental Figures S1 – S7, and Supplemental Table S1
Rebecca et al.

Supplemental Figure S1



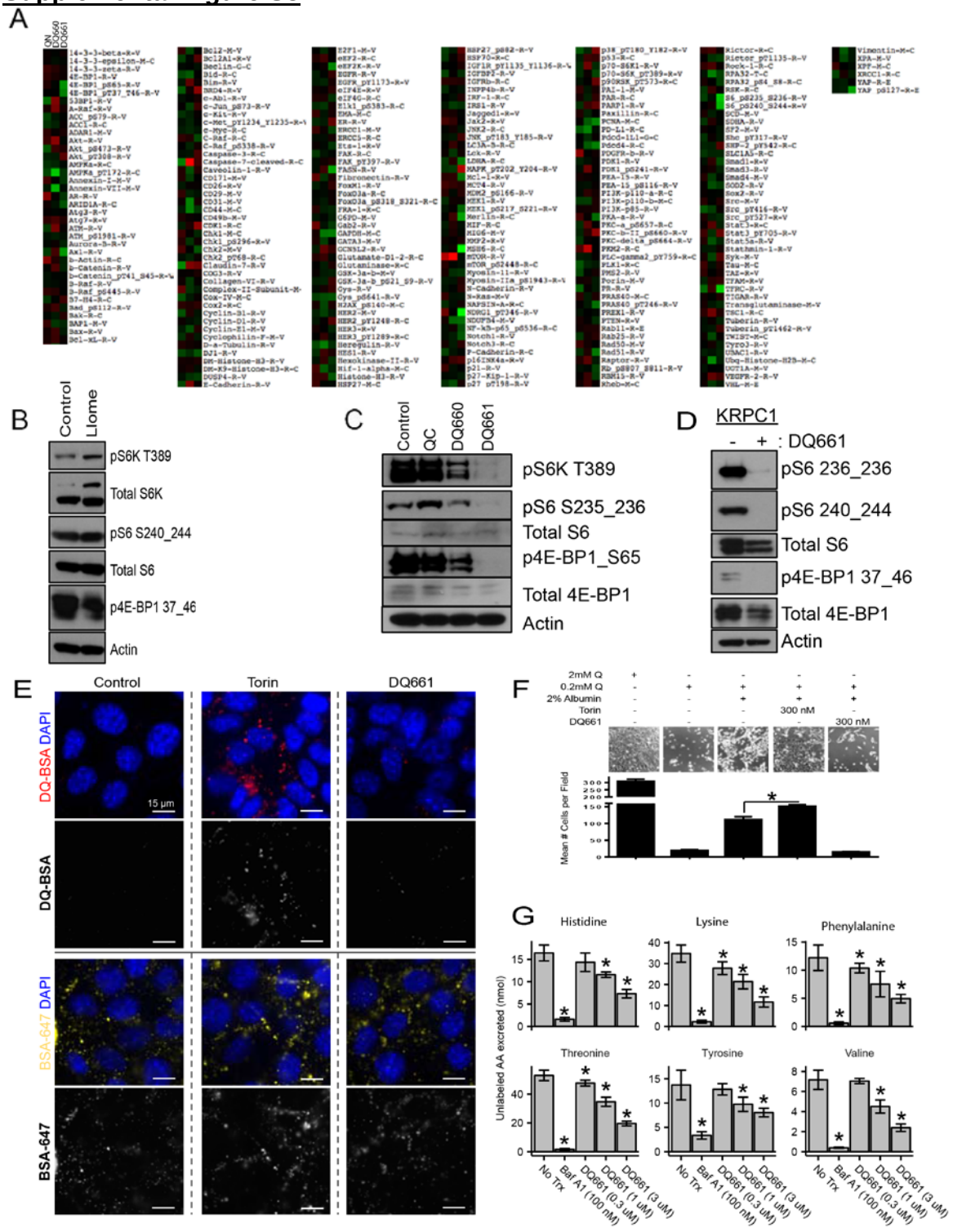
Supplemental Figure S1 related to Figure 1:
(A) PANC1 (pancreatic cancer) cells were treated with compounds shown (72 hr, 1 nM – 30 μM). Displayed are calculated Log IC_{50} values generated from GraphPad Prism. **(B)** PANC1 cells were treated with compounds shown (2 weeks, 30 nM - 3 μM) in colony formation assays. Cells were subsequently stained with crystal violet and imaged.

Supplemental Figure S2



Supplemental Figure S2 related to Figure 2:
(A) A375P cells were treated with DQs shown (6 hr, 3 μM) and co-stained with LysoTracker far-red. Fluorescent microscopy images were acquired and arrows indicate co-localization of DQ (RFP channel) and LysoTracker. Scale bars are 50 μm. **(B)** A375P cells were treated as in **(A)** and co-stained with MitoTracker far-red. **(C)** PANC1 cells were treated with compounds shown (6 hr, 3 μM). Lysates were immunoblotted and change in p62 densitometry levels were quantified and are depicted in the graph to the right; *p<0.05

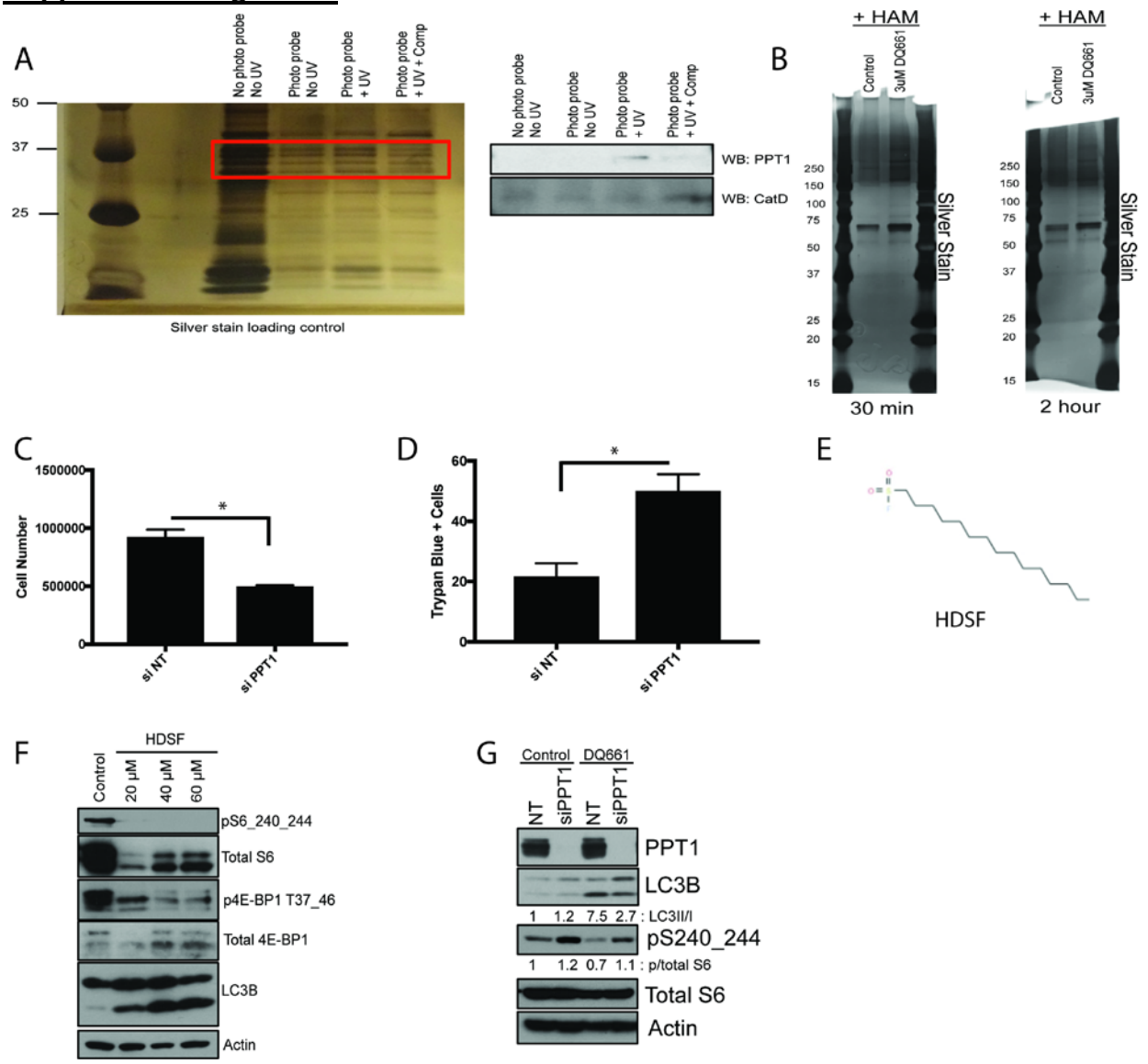
Supplemental Figure S3



Supplemental Figure S3 related to Figure 3:
(A) A375P cells were treated with QC, DQ660, or DQ661 (6 hr, 3 µM) and lysate was analyzed by RPPA. Shown are calculated fold-changes for the 6 hr and 24 hr timepoints relative to vehicle-treated control. Results reflect the average of 3 biological replicates.

(B) A375P cells were treated with Llome (30 min, 2 mM) and lysate was immunoblotted. **(C)** Western blotting validation of RPPA results, demonstrating only DQ661 possesses anti-mTORC1 activity. **(D)** KRPC cells treated with DQ661 (6 hr, 3 μM) and lysate was immunoblotted. **(E)** Fluorescence microscopy of G43 (immunotherapy-resistant *KPC*) cells grown in glutamine-limiting conditions and treated for 6 hr with vehicle control, Torin-1 (100 nM), or DQ661 (3 μM) in the presence of DQ-BSA (red) or BSA-647 (yellow) (1 μg/mL). **(F)** G43 cells were grown in glutamine limiting conditions in the presence or absence of Torin 1 or DQ661 (72 hr, 300 nM). **(G)** Effect of bafilomycin (100nM) or DQ661 (0.3, 1, 3 μM) on excretion of serum protein-derived amino acids in KRPC-A cells. * $p<0.05$ by t-test for (D), (F) and (G).

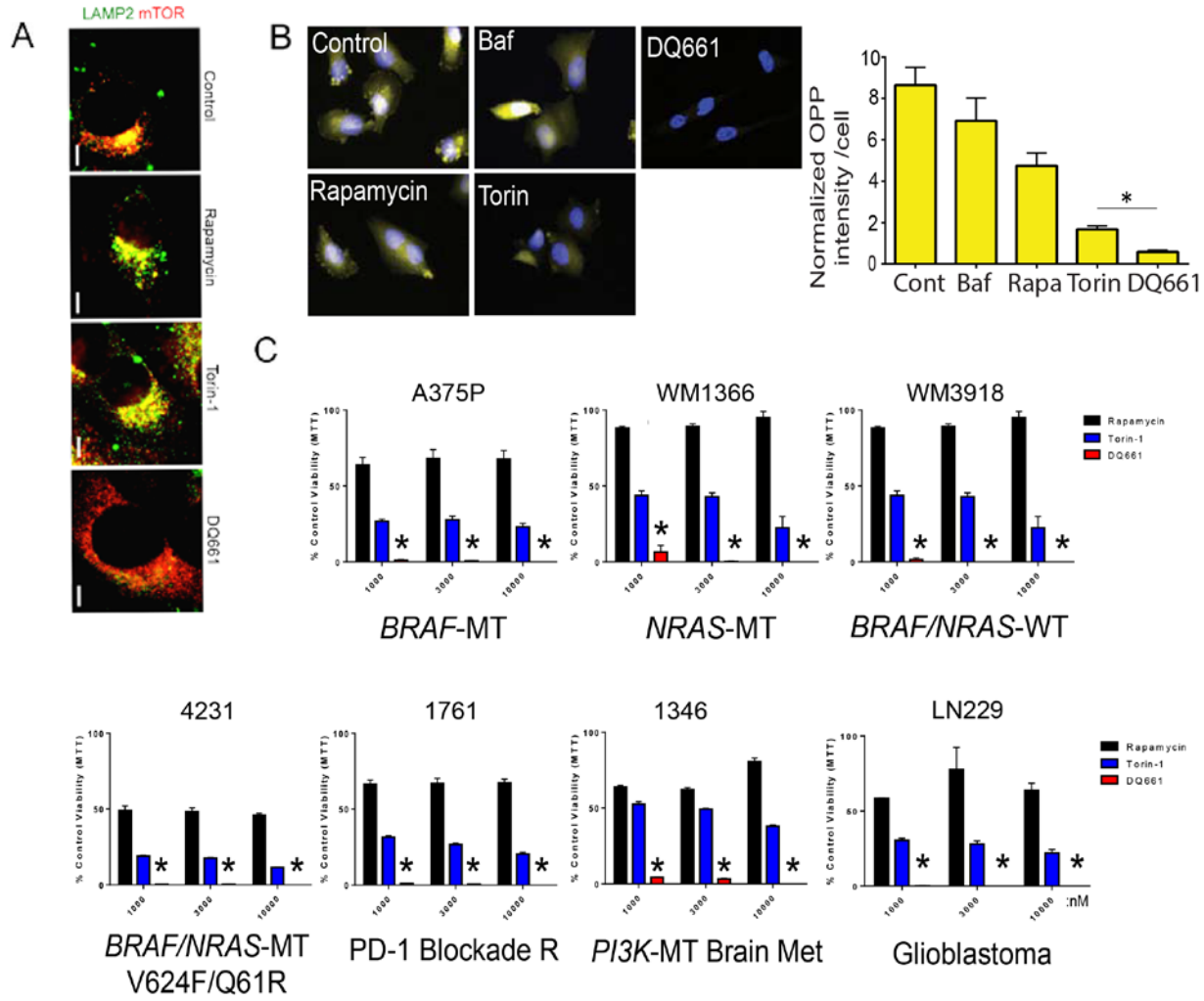
Supplemental Figure S4



Supplemental Figure S4 related to Figure 4:

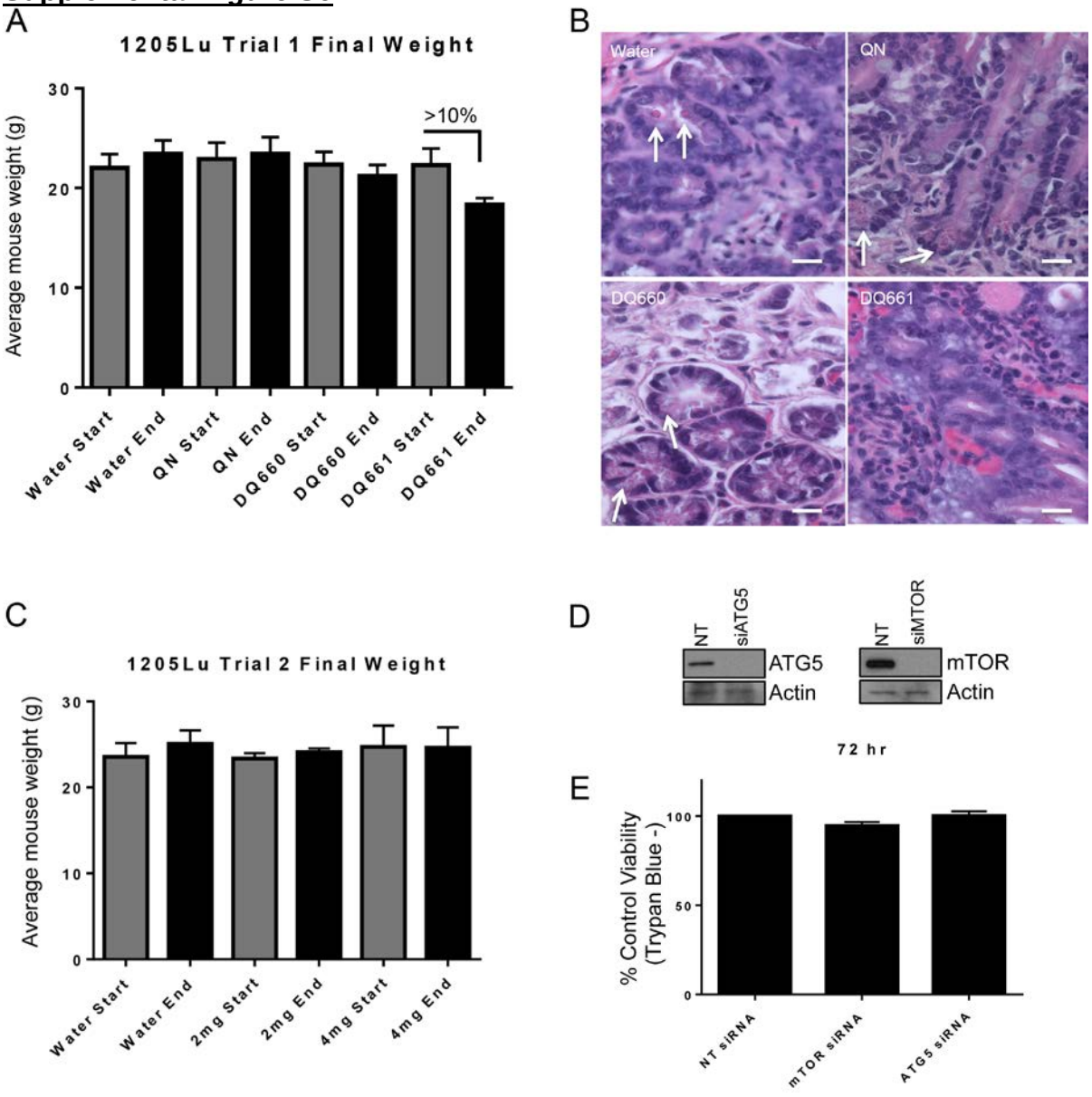
(A) Silver stained gel (for loading control) and immunoblot demonstrating pull-down of PPT1 with DQ661-photoprobe. **(B)** A375P cells were treated as shown and an ABE assay was performed. Lysate was run on and silver stained to reveal changes in global palmitoylation. **(C)** A375P cells were treated with siPPT1 and cells were counted after 24 hrs transfection. **(D)** A375P cells were treated identical to (C) and trypan blue exclusion was quantified. Results reflect at least 2 independent experiments. **(E)** Chemical structure of HDSF. **(F)** A375P cells were treated as shown for 4 hours. Lysates were immunoblotted. **(G)** A375P cells were transfected with NT or siPPT1 for 48 hr prior to treatment with DQ661 (6 hr, 3 µM). Lysate was subsequently immunoblotted.

Supplemental Figure S5



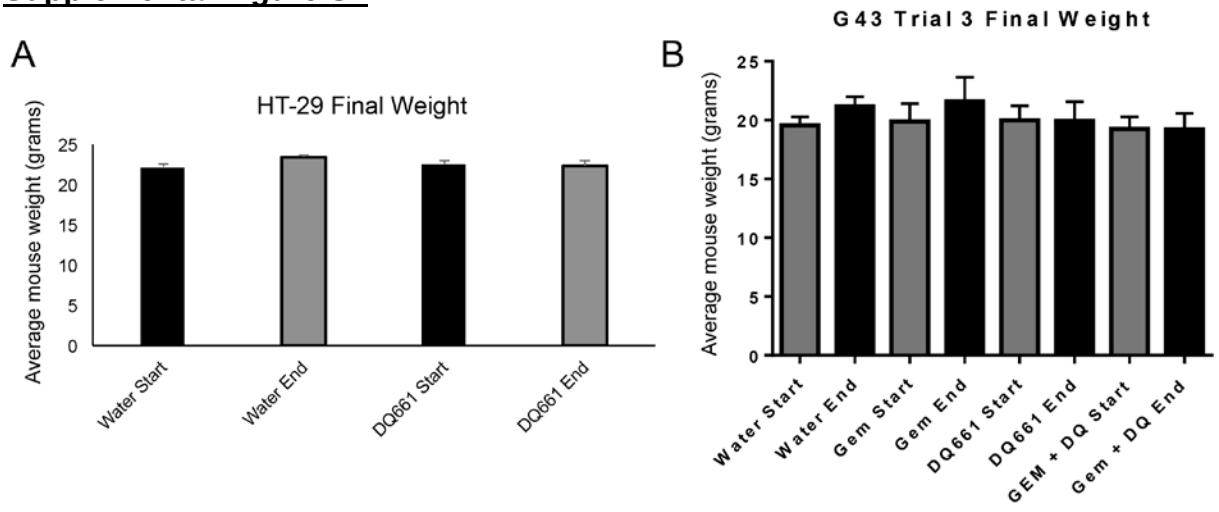
Supplemental Figure S5 related to Figure 5:
(A) Immunofluorescence against mTOR (red) and LAMP2 (green) in A375P cells treated with vehicle control, rapamycin (6hr, 3 μM), torin-1 (6 hr, 3 μM) or DQ661 (6 hr, 3 μM). Scale bars represent 8.5 μm. **(B)** 1205Lu (melanoma) cells were treated with rapamycin (3 μM), torin-1 (3 μM), baf (100 nM), or DQ661 (3 μM) for 3 hr. Blue is DAPI and yellow fluorescence reflects de novo synthesized protein. Graph to the right represented as mean ± STDERR. *N* = 30 cells per condition. *p<0.001. Scale bars: 30 μm. **(C)** Cells treated with rapamycin, torin-1, or DQ661 (1 - 10μM, 72 hrs, MTT). **(E)** A375P cells were treated with quinacrine, DQ661 or Siramesine (1 - 10μM, 72 hrs, MTT). Scale bars: 30 μm in (H).

Supplemental Figure S6



Supplemental Figure S6 related to Figure 6:
(A) Average mouse weights at the start and end of treatment with vehicle control (water), QN (8 mg/kg), DQ660 (8 mg/kg) or DQ661 (8 mg/kg). **(B)** Microscopy of H&E of gastrointestinal sections isolated from mice treated with either vehicle control (Water), QN (8 mg/kg), DQ660 (8 mg/kg) or DQ661 (8 mg/kg). Arrows denote presence of paneth cells. Magnification 40X. **(C)** Average mouse weights at the start and end of treatment with vehicle control (water), DQ661 (2 mg/kg) or DQ661 (4 mg/kg). **(D)** 1205Lu cells were transfected with NT, siMTOR or siATG5 prior to immunoblotting lysate. **(E)** 1205Lu cells from **(D)** were allowed to grow up to 72 hours. Viability by Trypan blue exclusion analysis was determined at 72 hours post transfection.

Supplemental Figure S7



Supplemental Figure S7 related to Figure 7:
(A) Average mouse weights at the end and start of treatment with water or DQ661 (4 mg/kg) in the colorectal model or **(B)** water, gemcitabine (one dose of 120 mg/kg), DQ661 (4 mg/kg) or the combination of gemcitabine (one dose of 120 mg/kg) plus DQ661 (4 mg/kg) in the pancreatic model.

Compound	MW	pKa	CLogP	tPSA	nROTB	Linker Atoms
DQ 221	600.54	7.996	10.3455	70.48	10	7
DQ 330	614.571	9.02	10.3715	79.27	12	9
DQ 331	628.598	9.07	10.9955	70.48	12	9
DQ 340	628.598	9.43	10.265	79.27	13	10
DQ 341	642.625	9.383	10.8445	70.48	13	10
DQ 440	642.625	9.74	10.1575	79.27	14	11
DQ 441	656.652	9.693	10.6935	70.48	14	11
DQ 550	670.679	10.009	11.2155	79.27	16	13
DQ 551	684.706	9.963	11.7515	70.48	16	13
DQ 660	698.733	10.229	12.2735	79.27	18	15
DQ 661	712.76	10.182	12.8095	70.48	18	15
DQ 770	726.787	10.241	13.3315	79.27	20	17
DQ 771	740.814	10.195	13.8675	70.48	20	17
DQ 880	754.841	10.293	14.3895	79.27	22	19
DQ 881	768.868	10.246	14.9255	70.48	22	19
DQ 11110	839.003	10.302	17.5635	79.27	28	25
DQ 11111	853.03	10.256	18.0995	70.48	28	25

Supplemental Table S1:
Physical properties were calculated using ChemBio Draw Ultra 2014 edition.