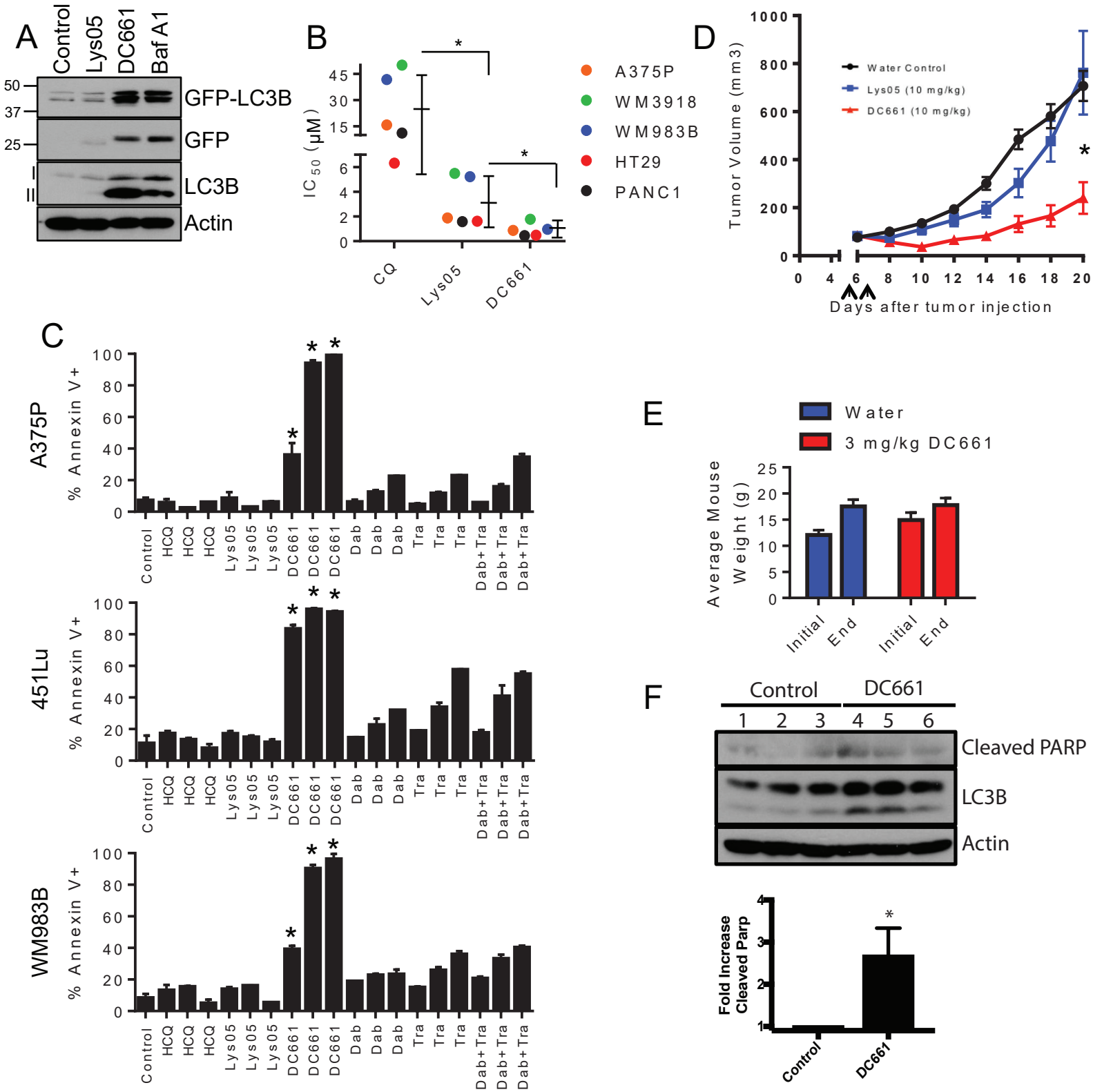
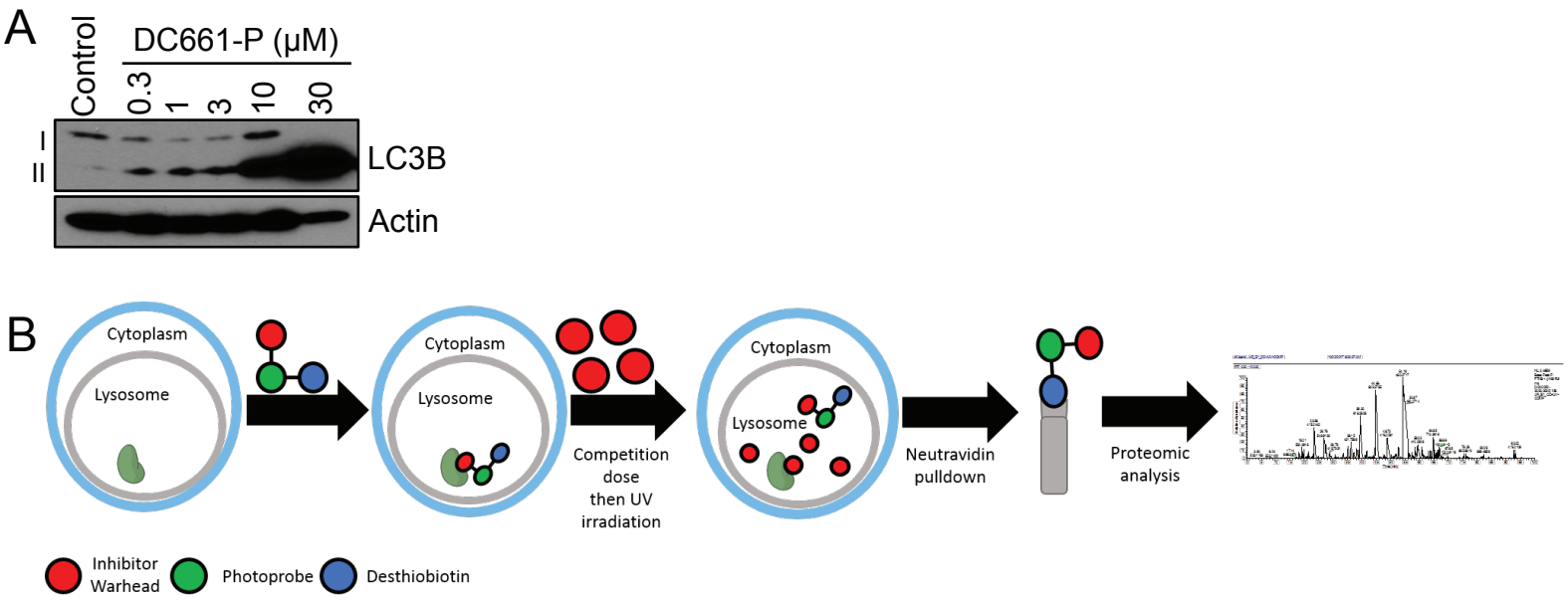
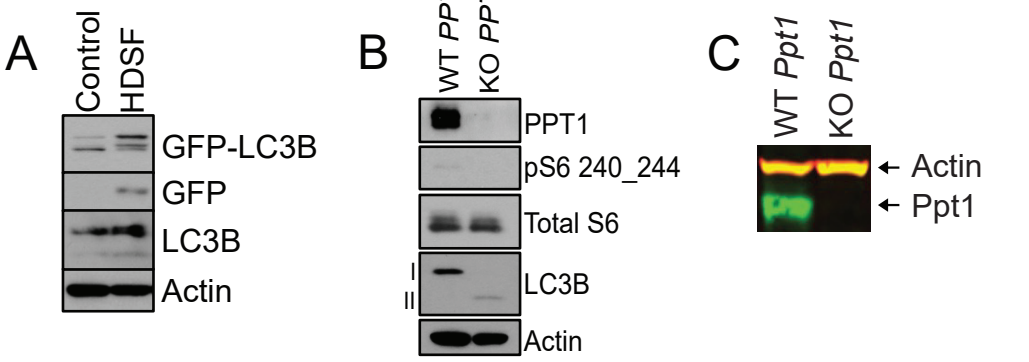
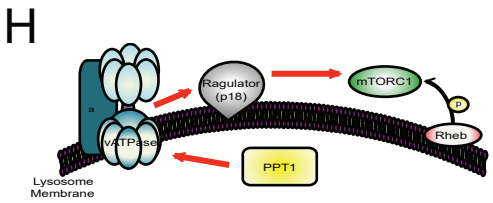


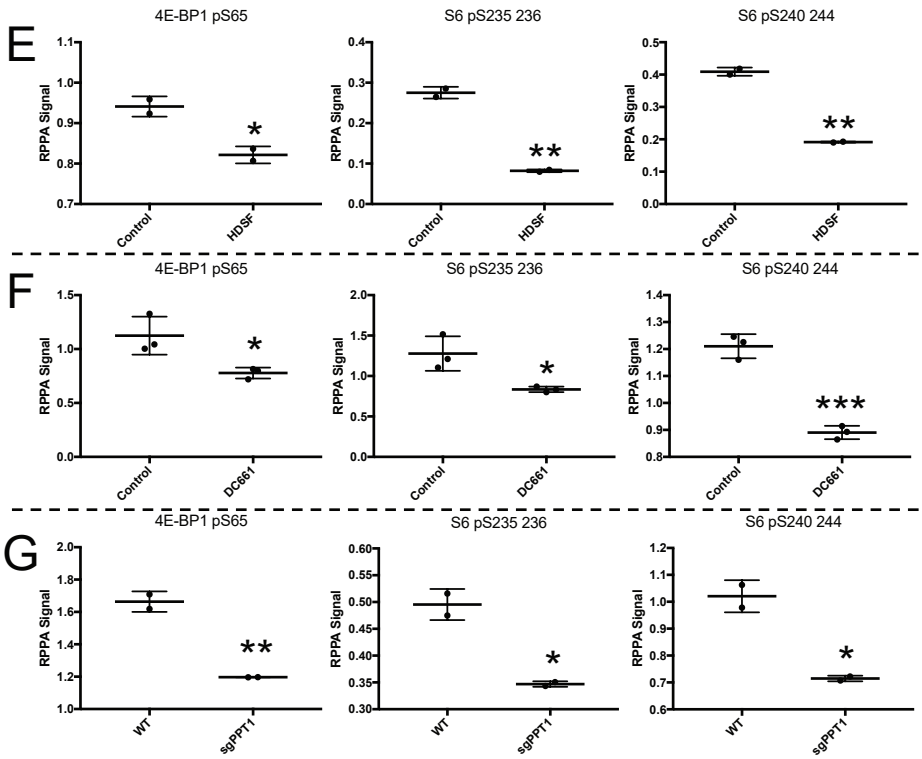
Supplemental Figure S1: (A) C8161 (melanoma) cells expressing GFP-LC3B were treated with Lys05 (3 μM), DC661 (3 μM) or bafilomycin A1 (100 nM, Baf A1) for 6 hours. Lysate was subsequently immunoblotted. (B) IC_{50} graphs for HCQ, Lys05 and DC661 across a panel of melanoma (A375P, WM3918, WM983B), pancreatic cancer (PANC1) and colorectal cancer (HT29) cell lines generated from 72 hr MTT assays. (C) A375P, 451Lu, and 1205Lu cells were treated with HCQ (3 μM), Lys05 (3 μM) DC661 (3 μM), dabrafenib (3 μM), trametinib (100 nM) or the combination of dabrafenib and trametinib (0 – 72 hr). Cells were subsequently stained with Annexin-V and analyzed by flow cytometry. (D) HT29 (colorectal cancer) cells were injected subcutaneously into the flanks of NSG mice. Once tumors were palpable, mice were treated with 10 mg/kg of Lys05, DC661, or vehicle control via intraperitoneal (i.p.) injection twice (as indicated by arrows on x-axis). Shown are tumor volumes (n = 6 in each arm). (E) Shown are starting and ending average mouse weight from mice treated daily with 3 mg/kg DC661 i.p. (F) Immunoblotting of tumor lysate generated from mice treated daily with 3 mg/kg DC661 i.p. Shown below is densitometry of cleaved Parp. * indicates $p<0.05$. Students T test was used.



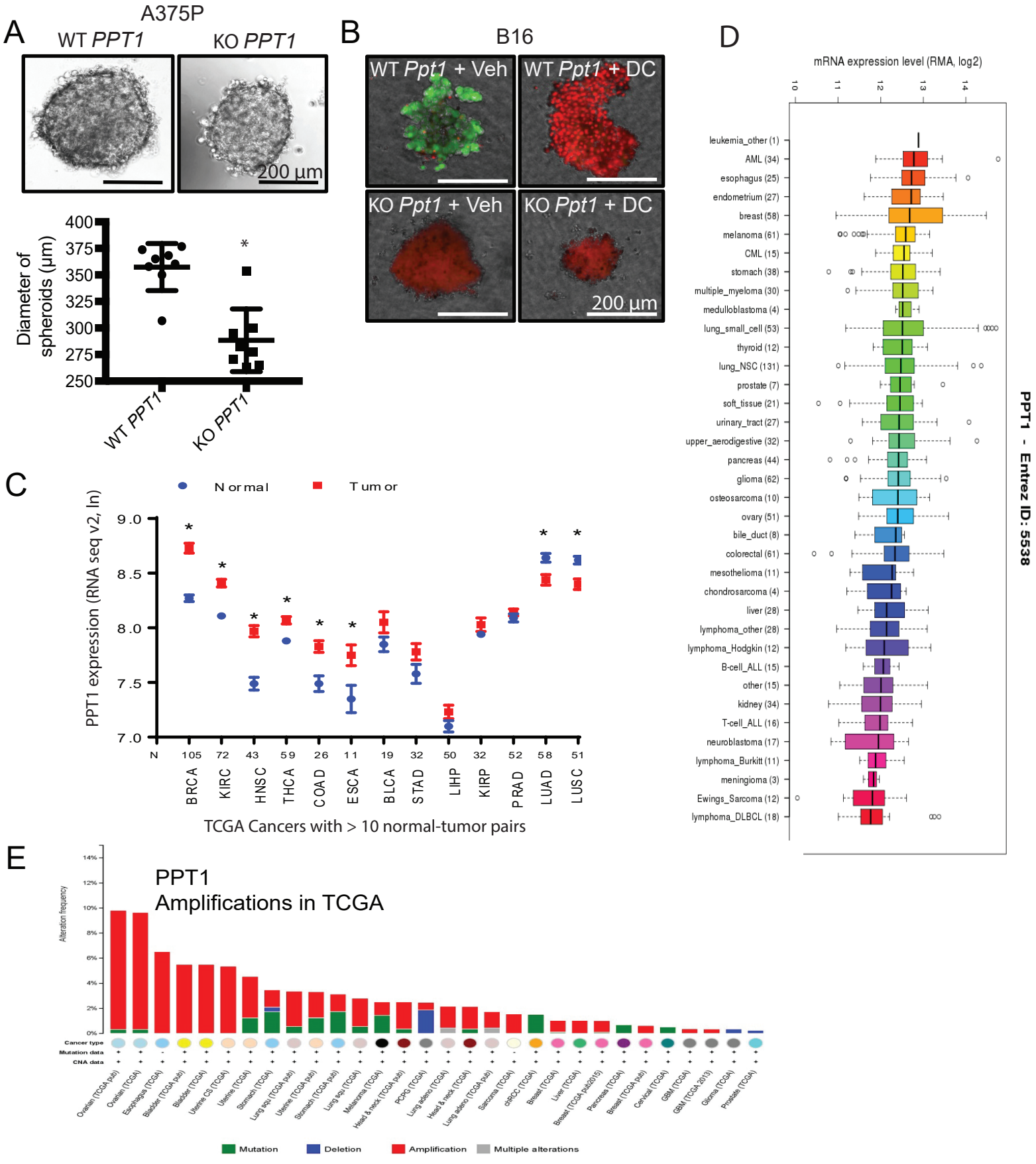
Supplemental Figure S2: (A) Schematic of the pulldown strategy to identify the protein target of CQ-derivatives. (B) A375P cells were treated with DC661-photoprobe (DC661-P) (0 – 30 μM, 24 hrs). Lysate was subsequently immunoblotted. (C) A375P cells were treated with Lys05-photoprobe (Lys05-P, 6 hrs) or (D) CQ-photoprobe (CQ-P, 6 hrs) and lysate was subsequently immunoblotted. Results shown are representative of at least 2 experiments.







Supplemental Figure S3: (A) C8161 cells expressing GFP-LC3B were treated with HDSF (40 μM, 6 hrs) and lysate was subsequently immunoblotted. (B) Lysate generated from basal WT PPT1 or KO PPT1 cells was immunoblotted. (C) Lysate from B16 WT *Ppt1* and KO *Ppt1* cells was immunoblotted (D) Lysate generated from WM3918 (melanoma) cells treated with HDSF (40 μM, 6 hrs), WT *PPT1* and KO *PPT1* cells, and A375P (melanoma) cells treated with DC661 (3 μM, 6hrs) was subjected to RPPA analysis. Shown is a heat map representing increases (red) or decreases (green) in protein or phospho-protein expression. (E) Graphs of changes in the RPPA signal for 4E-BP1 pS65, S6 pS235 236, and S6 pS240 244 in either WM3918 cells treated with HDSF, (F) A375P cells treated with DC661, or (G) relative expression in WT *PPT1* cells versus KO *PPT1* cells. (H) Schematic of the lysosome membrane visualizing the complexes dependent upon PPT1 activity. Standard deviation was shown for (E), (F), and (G). * indicates $p<0.05$. Students T test was used.



Supplemental Figure S4: (A) Spheroids were formed from A375P WT PPT1 and KO PPT1 cells and implanted into collagen. Diameters of spheroids were quantified and shown below. (B) Spheroids were formed from B16 WT *Ppt1* and KO *Ppt1* cells and implanted into collagen alongside primary fibroblasts. 3D cultures were then treated (3 µM, 24 hrs), stained with a live (green)/ dead (red) viability assay and imaged by microscopy. (C) PPT1 expression (TCGA RNA seqV2) for cancers for which > 10 pairs of normal and tumor tissue were available. BRCA: breast cancer; KIRC: clear cell renal cell carcinoma; HNSC: head and neck squamous cell carcinoma; THCA: thyroid cancer; COAD: colon cancer; ESCA: esophageal carcinoma; BLCA: bladder cancer; STAD: gastric cancer; LIHC: hepatocellular carcinoma; KIRP: Papillary renal cell carcinoma; PRAD: prostate adenocarcinoma; LUAD: non-small cell lung cancer adenocarcinoma; LUSC: non-small cell lung cancer squamous cell. Mean +/- SEM is presented; *p<0.05 paired t-test. (D) PPT1 expression (RNA seq v2, Log2) from Broad Novartis CCLE database. (E) Amplifications of PPT1 across multiple tumor types from the TCGA.