Table S1: Supplementary table 1 is an excel file having 3 spread sheets. Sheet 1: Sample 1 partial denotes proteins picked up by partially coated gold nanoparticles. Sheet 2: Sample 2 cover, denotes proteins picked up by fully coated gold nanoparticles. Sheet 3 represents a comparison of proteins picked up by partially and fully coated gold nanoparticles. UBAP2 protein is highlighted in the spreadsheet as yellow.

Table S2. Statistical analysis of UBAP2 staining in 101 cases of pancreatic tissues (Separate Excel sheet).

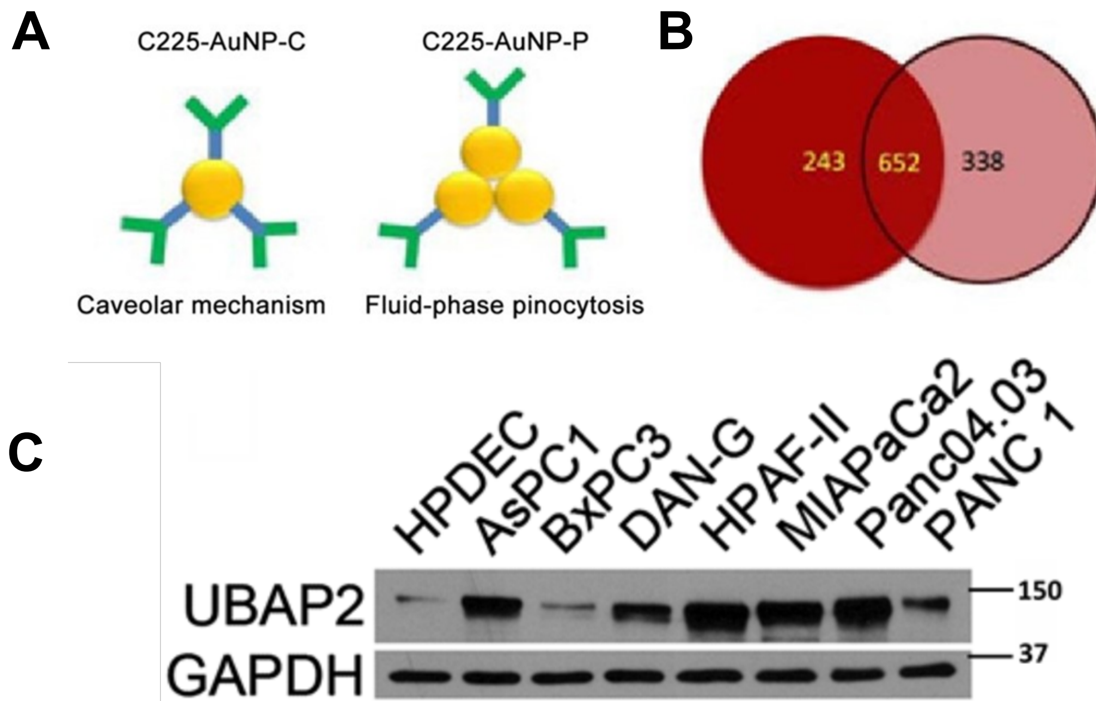

Figure S1. Schematic representation of the two anti-EGFR antibody gold nanoparticle conjugates used in the study and expression of UBAP2 protein in pancreatic cancer cell lines. (**A**) Schematic representation of the two anti-EGFR antibody gold nanoparticle conjugates used in the study. (**B**) Schematic representation of the results of proteomics/bioinformatics on nanoparticle-bound proteins from PANC-1 lysates. 652 proteins were common to C225-AuNP-C and C225-AuNP-P. 243 unique proteins were bound to C225-AuNP-P and 338 unique proteins were bound to C225-AuNP-C. (**C**) Expression of UBAP2 was determined by Western Blot. HPDEC and BxPC3 cells are pancreatic cell lines with wild-type *KRAS* (1st and 3rd columns). All other cell lines contain an oncogenic *KRAS* mutation. GAPDH is shown as a loading control. GAPDH is shown as a loading control. Ladder represents molecular weight in KDa.

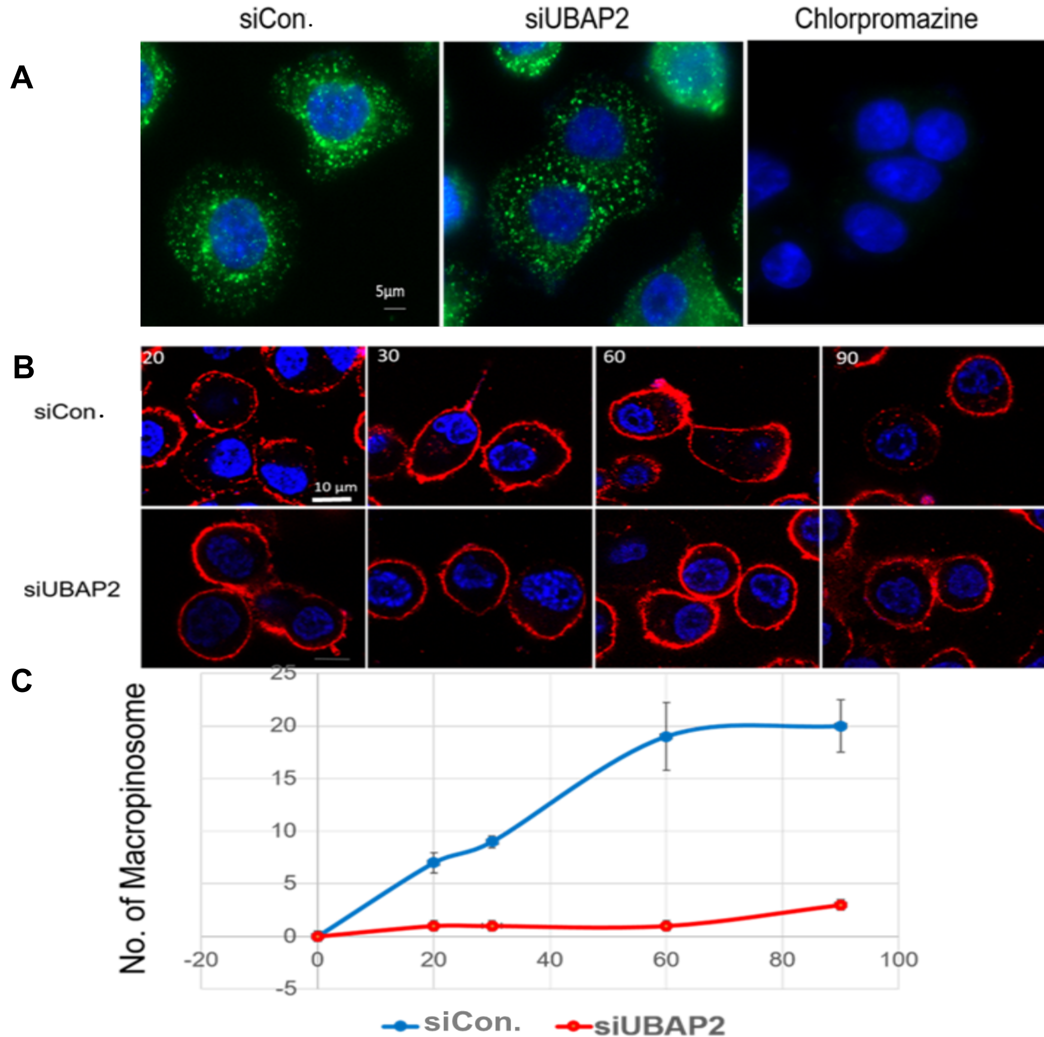

Figure S2. UBAP2 affects dextran uptake but not other modes of endocytosis. (**A**) Transferrin uptake in the UBAP2-silenced AsPC1 cells. 48 h post transfection, cells were starved for 30 min at 37° C and pulsed with Alexa fluor-488 conjugated transferrin (50 µg/ml) for 40 min. Chlorpromazine (right panel) is shown as a positive control for endocytosis inhibition. (**B**) Kinetics of dextran uptake assay was analyzed by microscopy in siUBAP2 cells. Cells with centrally-located macropinosomes were counted. **(C)** Dextran kinetics is represented in the graph. Values are means ±SD.

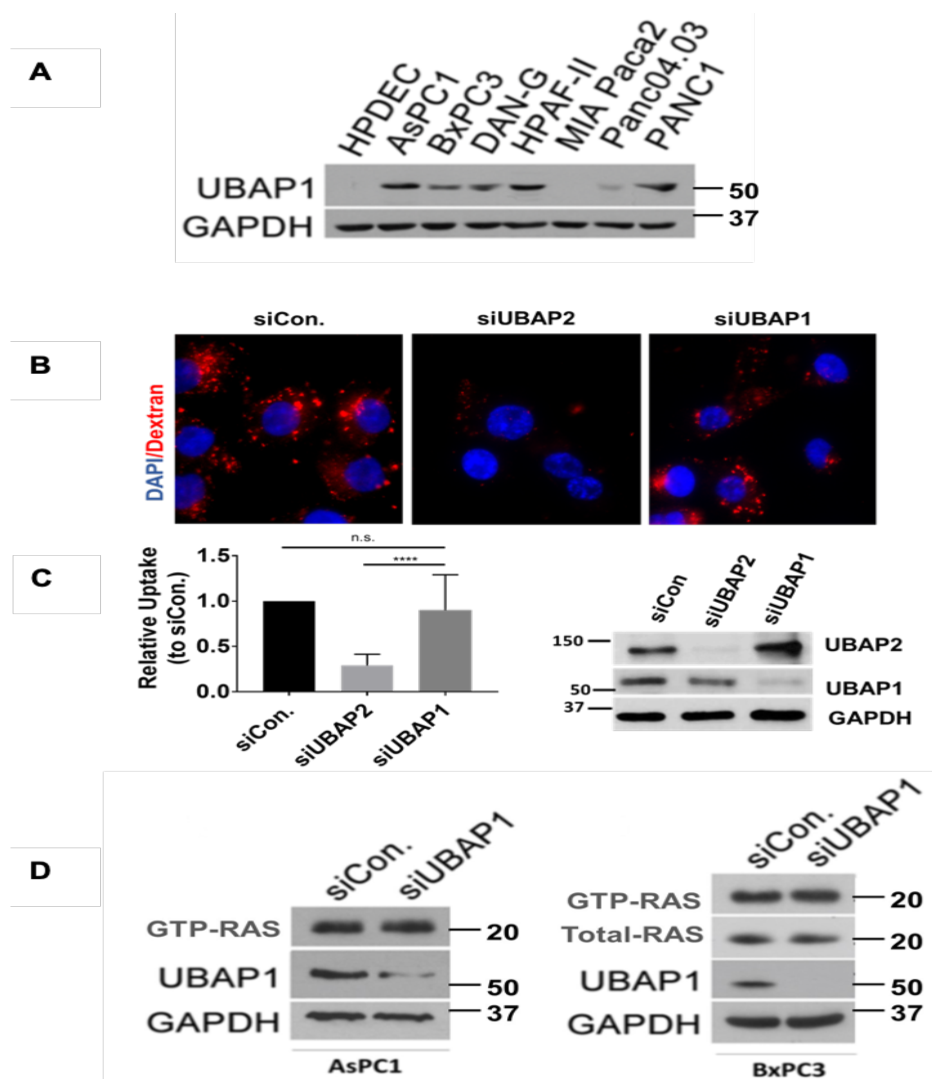

Figure S3: UBAP2 but not UBPA1 regulates macropinocytosis activity in AsPC1 cells. (A) Expression of UBAP1 was determined by Western Blot. UBAP1 expression is not correlated to KRAS mutation in PDAC cell lines with oncogenic *KRAS* mutations *KRAS* (1st and 3rd columns). GAPDH is shown as a loading control. Ladder represents molecular weight in KDa. **(B)** Dextran uptake images of AsPC1 cells under siCon, siUBAP2, or siUBAP1 respectively. **(C)** Relative Quantification of AsPC1 macropinocytosis indices. Relative Control was set to a value of 1 for analyses. (*Right*) Validation of protein knockdown in AsPC1 cells via western blot analysis in the same set of samples used for dextran uptake. Statistical analyses on all comparisons used the Student's T-Test. (****; P < 0.0001). (**D**) UBAP1 depletion does not affect KRAS activation in AsPC1 and BxPC3 cells. Ladder represents molecular weight in KDa.

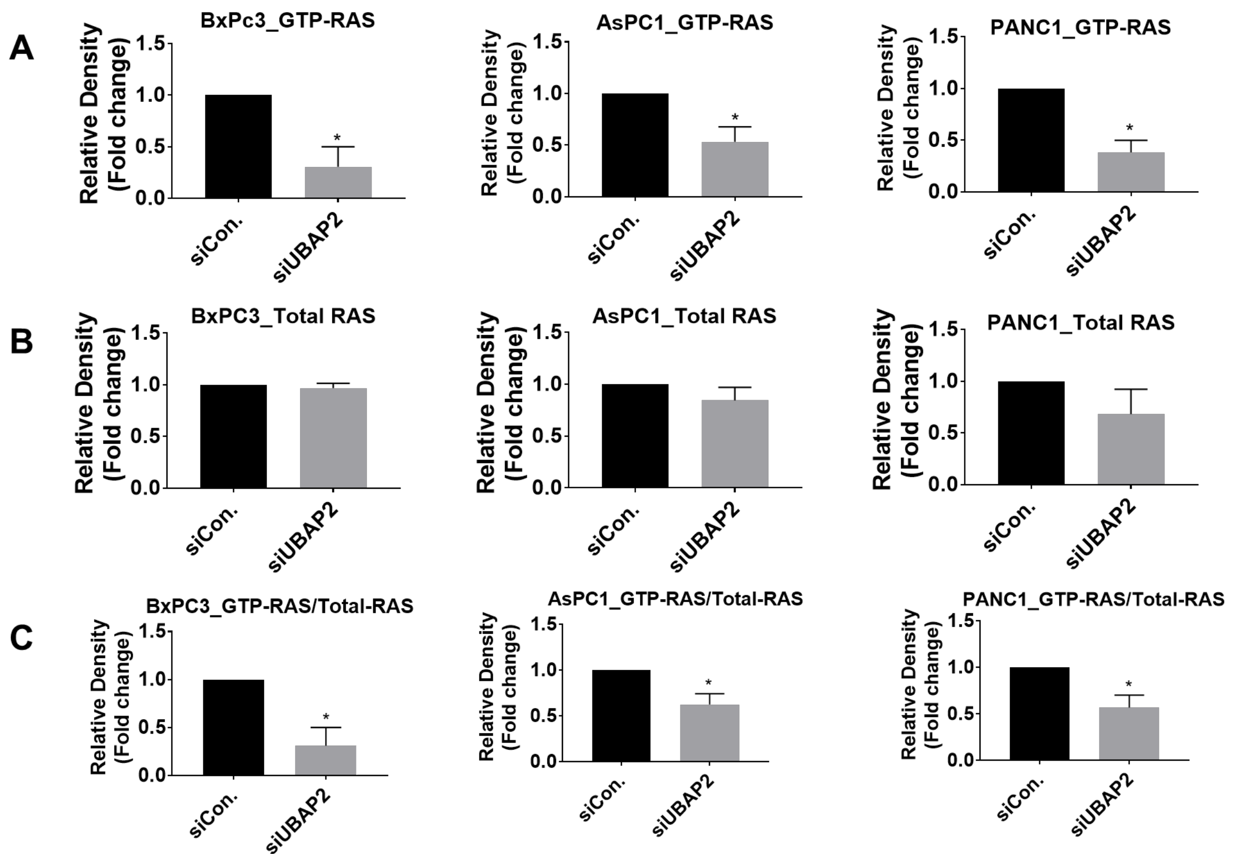

Figure S4. Densitometric analysis of activated RAS (GTP-RAS), Total-RAS and GTP-RAS/ Total -RAS ratio in UBAP2 silenced BxPC3, AsPC1 and PANC1 cells from figure 2A and 2B. (**A**) UBAP2 knockdown using siRNA (siUBAP2) decreased the activated RAS (GTP-RAS) in BxPC3 (wild type), AsPC1 and PANC-1 (Mutant) cells using a pulldown-based RAS activation assay. (**B**) UBAP2 knockdown using siRNA (siUBAP2) did not change the Total-RAS levels in BxPC3 (wild type), AsPC1 and PANC-1 (Mutant) cells. (**C**) UBAP2 knockdown using siRNA (siUBAP2) decreased the GTP-RAS/Total-RAS in BxPC3 (wild type), AsPC1 and PANC-1 (Mutant) cells using pulldown-based RAS activation assay. For measuring the GTP-RAS/Total - RAS ratio, first the Total-RAS was normalized to GADPH (loading control) thereafter the GTP-RAS was divided by Total-RAS.

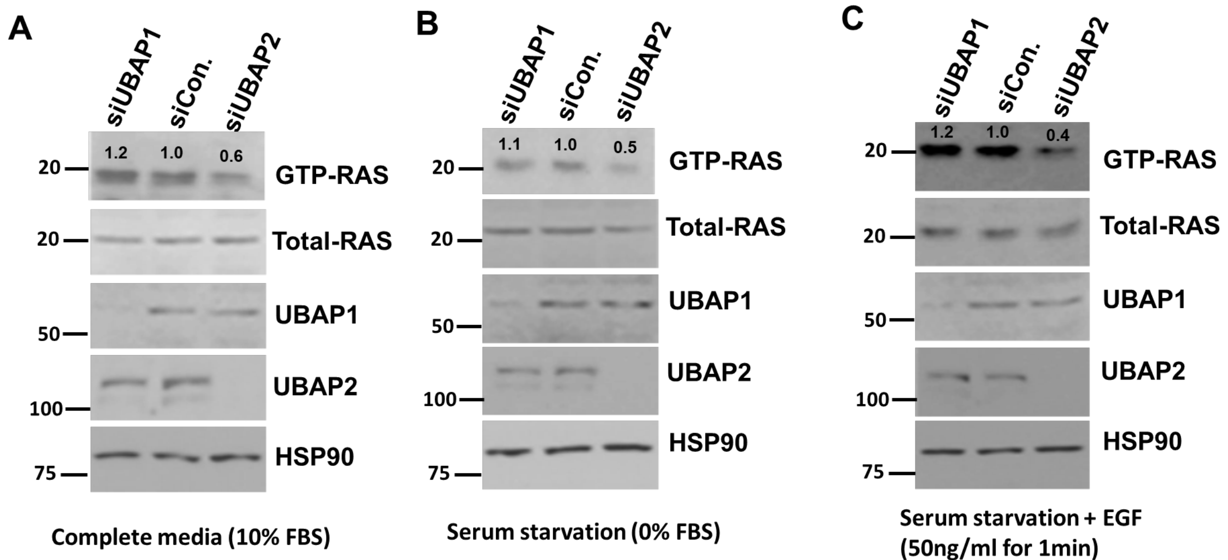

Figure S5. UBAP2 but not UBAP1 affects GTP-RAS level in normal, serum starvation and serum starvation with EGF stimulation (50ng/ml for 1 min) in BxPC3. BxPC3 cells were transfected with either scrambled siRNA (siCon), siUBAP1, or siUBAP2. For serum starvation, 36h post-transfection cells were placed in 0% FBS media for overnight. For serum starvation and EGF stimulation, 36h post-transfection cells were placed in 0% FBS media for overnight and stimulated with EGF (50ng/ml) for 1 min. 48h post-transfection, cell lysate was collected and use for RAS activation assay and immunoblotting**.** (**A**) Expression of GTP-RAS (as determined using pulldown-based activation assay), Total-RAS, UBAP1, UBAP2 in BxPC3 cells transfected with either siUBAP1 or siCon or siUBAP2 in normal serum (10% FBS) condition. HSP90 was used as loading control. (**B**) Expression of GTP-RAS (as determined using pulldown-based activation assay), Total-RAS, UBAP1, UBAP2 in BxPC3 cells transfected with either siUBAP1 or siCon or siUBAP2 in serum starved (0% FBS) condition. HSP90 was used as loading control. (**C**) Expression of GTP-RAS (as determined using pulldown-based activation assay), Total-RAS, UBAP1, UBAP2 in BxPC3 cells transfected with either siUBAP1 or siCon or siUBAP2 in serum starved (0% FBS) plus EGF stimulation condition. HSP90 was used as loading control. Numbers above GTP-RAS panel indicates band intensity quantified using Image J. Values were first normalized with respect to Total-RAS and then respective control was set to 1. Ladder represents molecular weight in KDa.

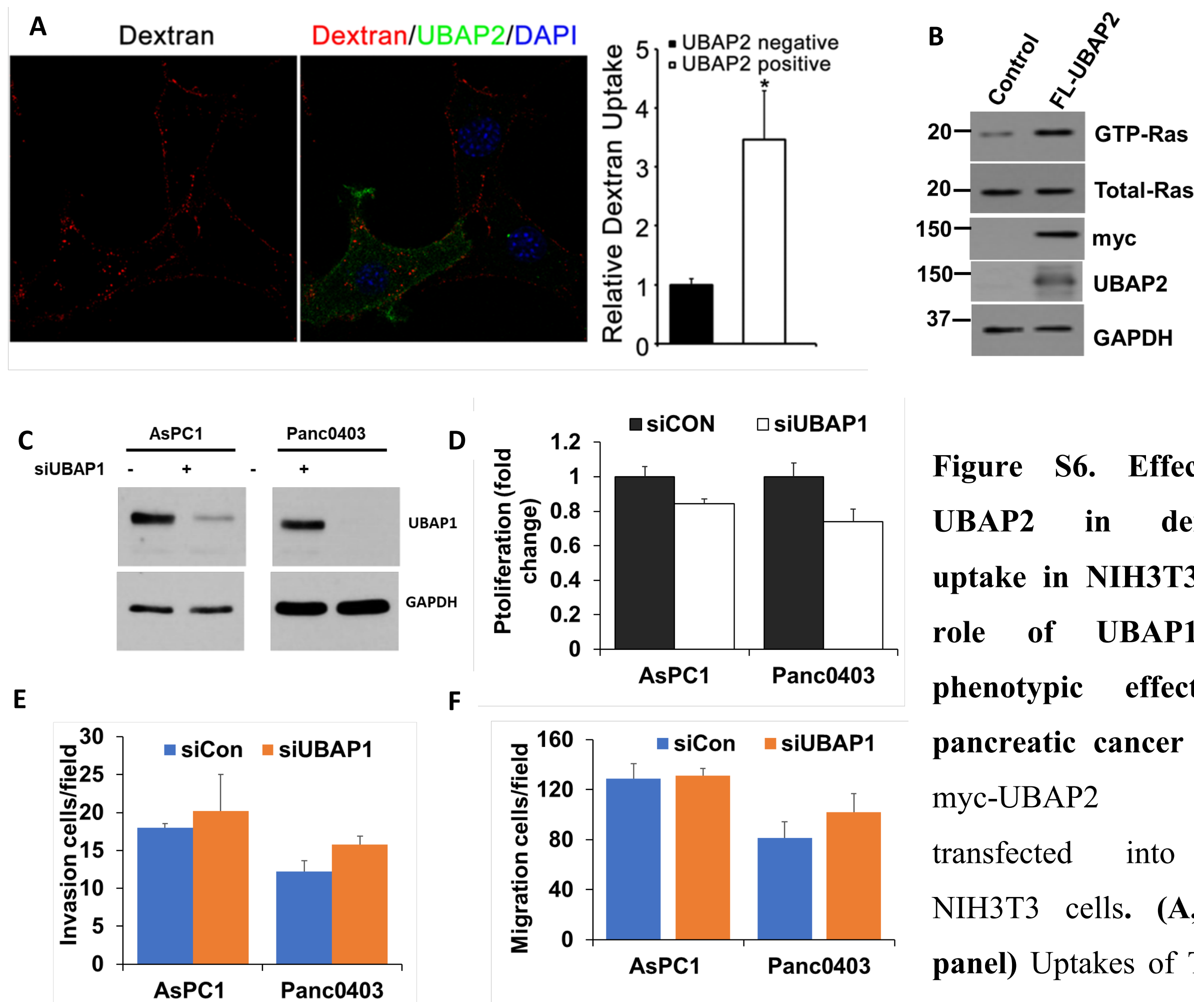

Figure S6. Effect of UBAP2 in dextran uptake in NIH3T3 and role of UBAP1 in phenotypic effect in pancreatic cancer cells. myc-UBAP2 was transfected into the NIH3T3 cells**. (A, left panel)** Uptakes of TMR-dextran (red) were visualized via fluorescence microscopy. Dextran uptake was increased in UBAP2 positive cells (green). (right panel) quantification of relative dextran uptakes; *, $p<0.05$. (**B**) Expression of GTP-RAS (as determined using pulldown-based activation assay), Total-RAS, myc, UBAP2 in myc-UBAP2 transfected NIH3T3 cells. GAPDH was used as loading control. Each experiment was repeated at least 3 times. Ladder represents molecular weight in KDa. **(C)** Silencing of UBAP1 does not affect proliferation **(D),** invasion **(E)** and migration (F) in AsPC1 and Panc0403 cells. Cells were transfected with either siUBAP1 or siCon. To analyze cell proliferation, cells were seeded 20k/w to 24-well plate 36h post-transfection. Cell numbers were counted 3 days after seeding. To analyze invasion **(E)** and migration **(F)** cells were starved for 48h after siRNA transfection. Cells were then seeded 50k/w to chambers and induced for 8h (migration) or 16h (invasion) with 2% FBS. Experiments were repeated 3-4 times. Ladder represents molecular weight in KDa.

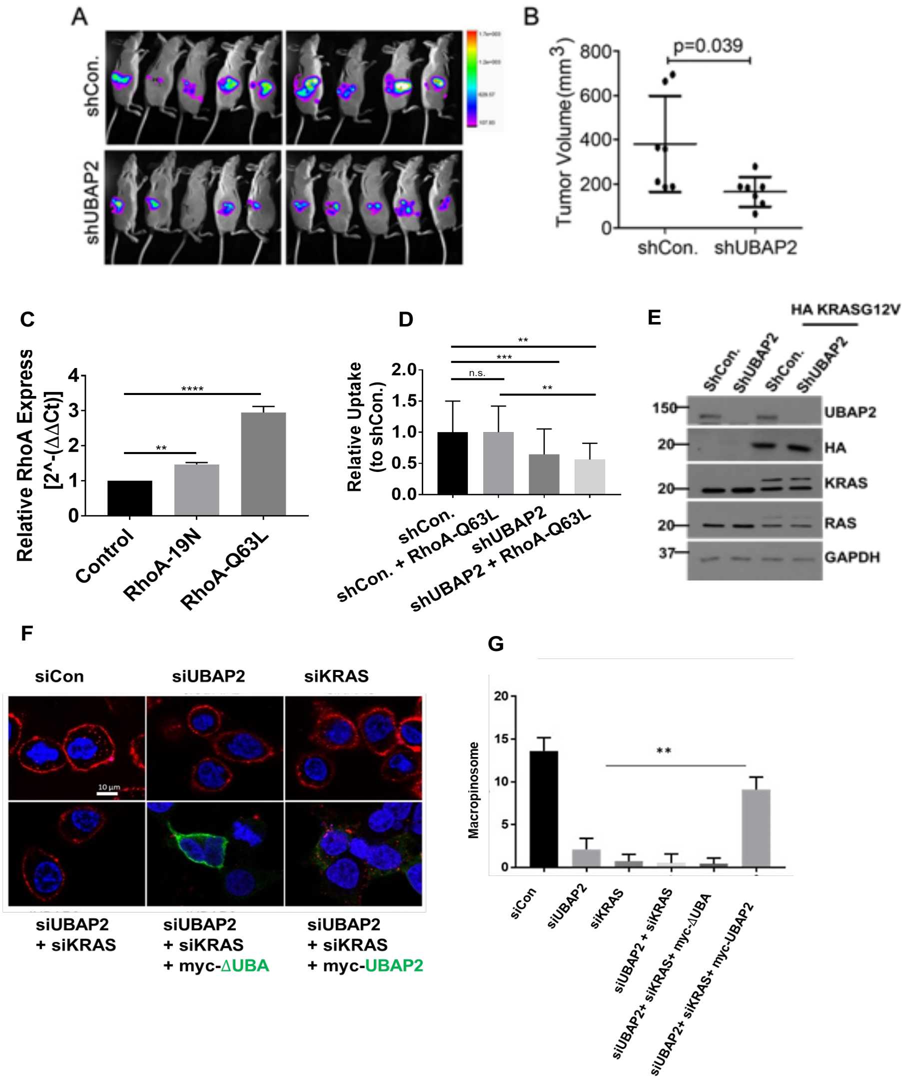

Figure S7. UBAP2 silencing in orthotopic tumor xenografts growth *in vivo and effect of RhoA in macropinocytosis in vitro*. (A) Tumor growth were monitored by bioluminescence using a Xenogen-IVIS system. **(B)** Final tumor volume. Each group contains 7 mice. Values are means ±SD. **(C)** Relative expression of plasmids Rho-A19N and RhoA- Q63L in the shAsPC1 cell lines. (**D**) Cells were transiently transfected with RhoA-Q63L and subjected for dextran. Statistical analyses on all comparisons used the Student's T-Test. (****; P < 0.0001. (**E**) Immunoblot analysis of stable AsPC1 knockdown or control cells at 48h after transfection with HA-KRAS-G12V. Expression of endogenous KRAS, total RAS proteins were also depicted. (**F**), myc-UBAP2 FL and myc ΔUBA was transfected into AsPC1 cells. Uptakes of dextran (red) were visualized by fluorescence microscopy. **(G),** Quantitation of macropinosome numbers were shown.

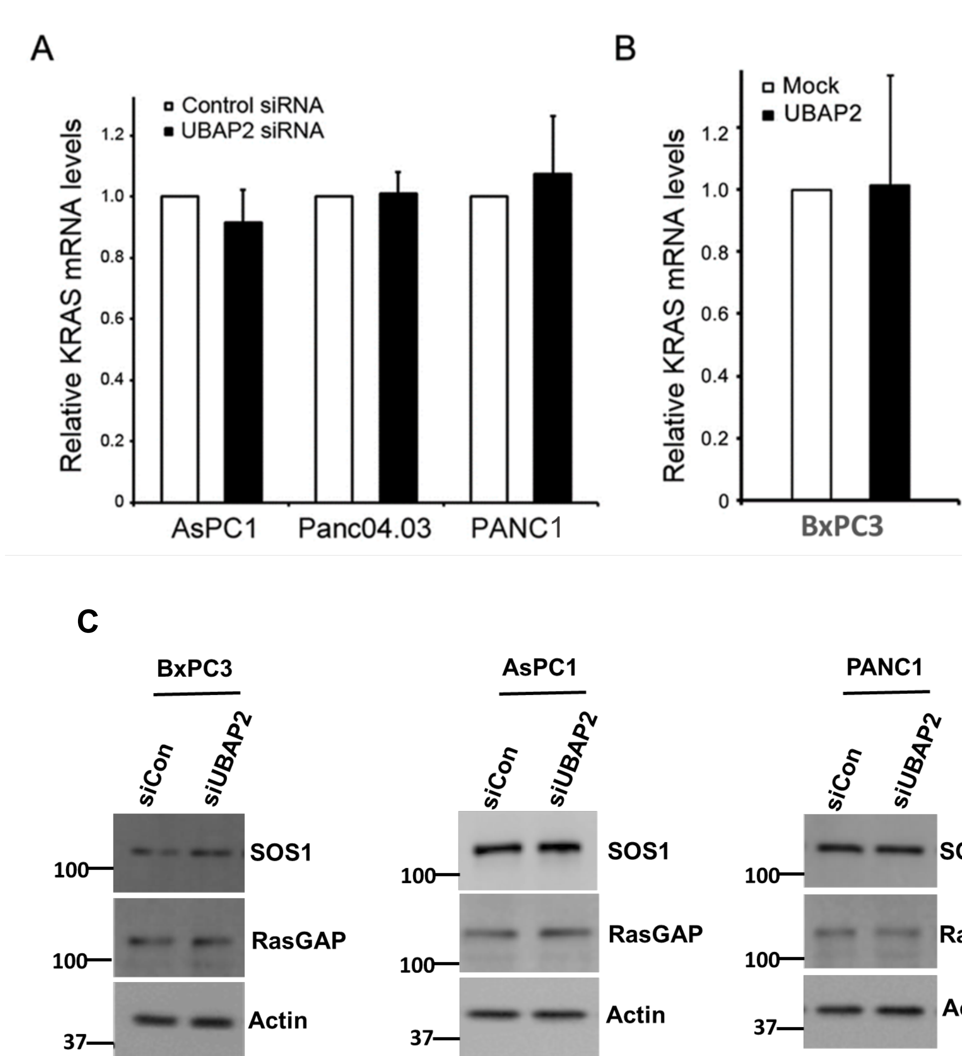

Figure S8. UBAP2 does not regulate KRAS transcription, GAP and GEF expression. (**A**), The indicated pancreatic cancer cells were transfected with UBAP2 or control siRNA, respectively. KRAS mRNA levels were determined by qPCR and normalized to GAPDH expression. (**B**), BxPC3 cells were transfected with pcDNA3.1-myc-UBAP2 (UBAP2) and pcDNA3.1 (Mock) respectively and mRNA levels were detected by qPCR. Each experiment was repeated at least 3 times. (**C**), UBAP2 was silenced in both wild type cell line (BxPC3 cell) and mutant cell lines (AsPC1 and PANC1 cells). 48hrs after transfection cells were lysed and immunoblotted for detection SOS1 (GEF) and Ras GAP (GAP). Actin was used as loading control. Each experiment was repeated at least 3 times. Ladder represents molecular weight in KDa.