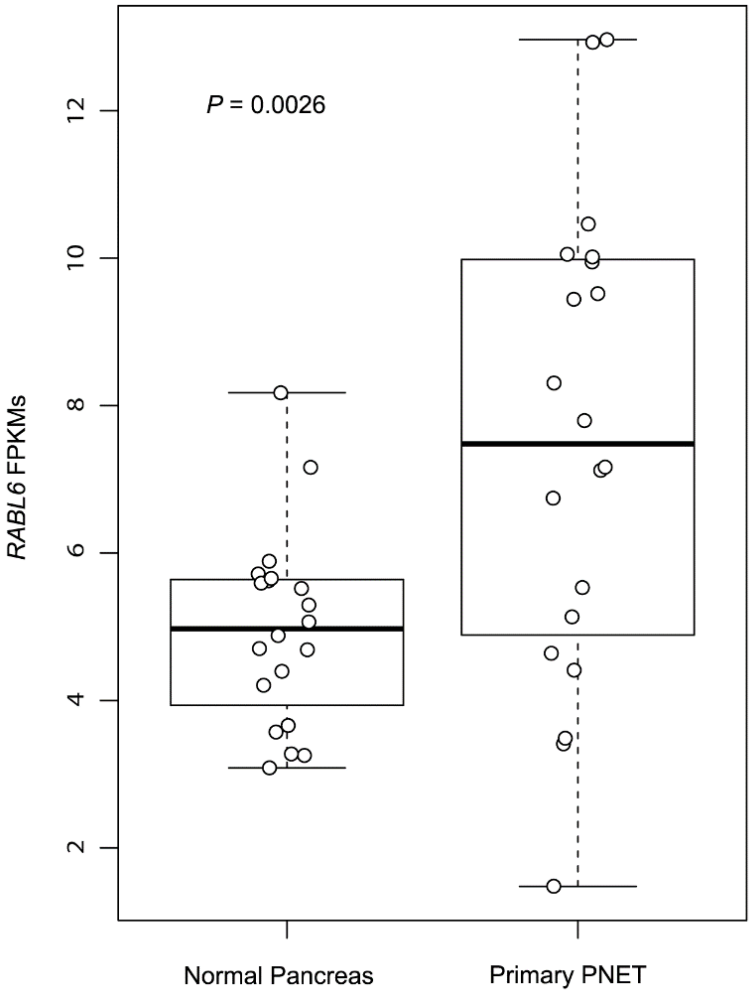
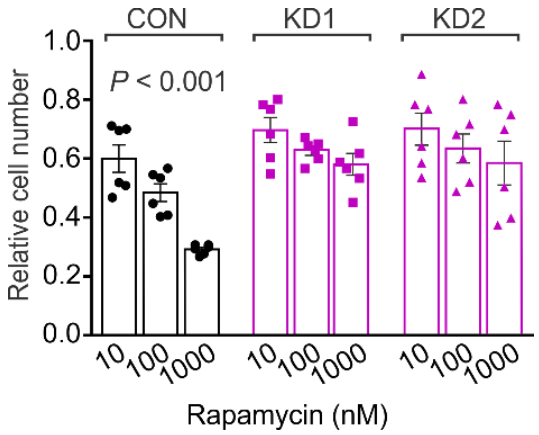
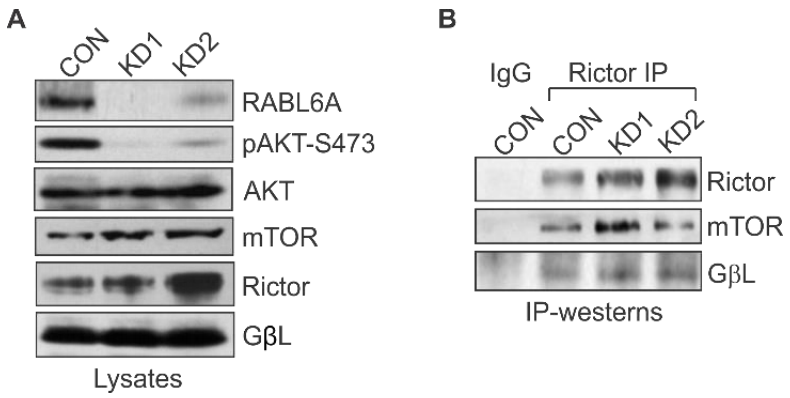


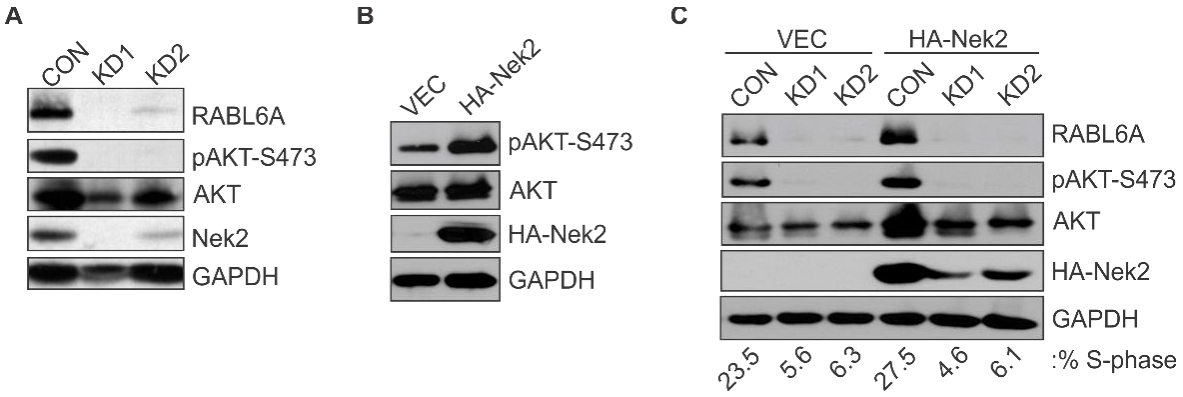
Supplemental Figure 1. *RABL6* mRNA expression from RNA-Seq analyses in normal human pancreas and primary PNETs. Box plots exhibit the quartile FPKM values of the gene *RABL6* (all isoforms) in 20 paired tumor and normal patient tissue samples. For normal pancreas samples, the first quartile value is 3.93, median is 4.97, third quartile is 5.64 and the whiskers are 3.09 and 8.18. For primary PNETs, the first quartile value is 4.89, median is 7.48, third quartile is 9.98 and the whiskers are 1.48 and 12.97. Statistical significance was assessed by paired Student's *t* test.



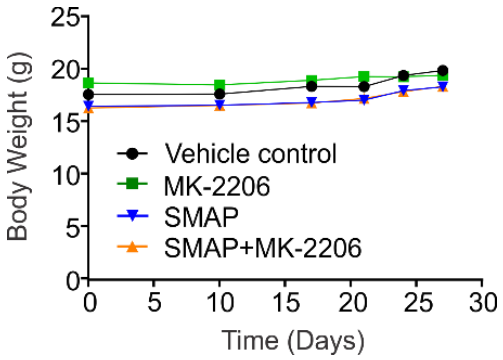
Supplemental Figure 2. RABL6A sensitizes PNET cells to the mTOR inhibitor, rapamycin. BON-1 cells expressing CON, KD1 or KD2 shRNAs were exposed for five days to increasing concentrations of the mTOR inhibitor, Rapamycin, and relative cell proliferation assayed using Cell-Quant™ (Genecopoeia). Results were normalized to values for untreated control cells within each group. Mean +/- SEM for data from 2 independent experiments, each performed in triplicate. Statistical significance ($P < 0.001$ for CON versus all points for KD1 and KD2) was determined by two-way ANOVA and adjusted for multiple comparisons using the Bonferroni method.



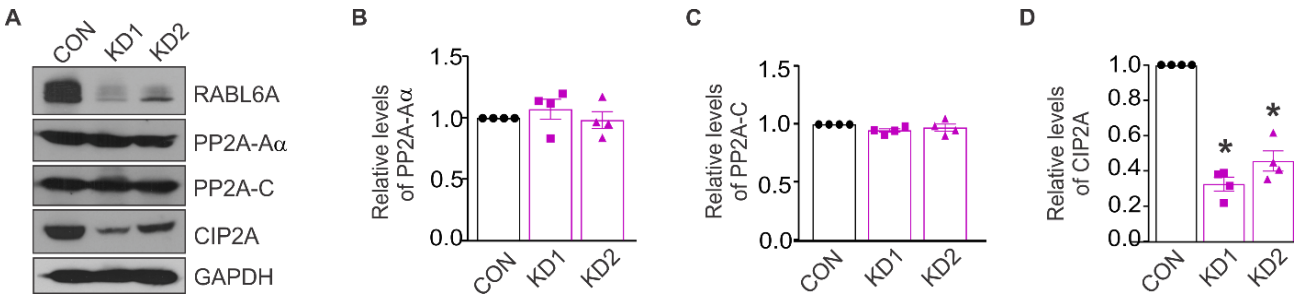
Supplemental Figure 3. Endogenous mTORC2 complexes remain intact in RABL6A knockdown cells. BON-1 cells expressing vector (CON) or RABL6A shRNAs (KD1 and KD2) were treated with a cell permeable cross-linking reagent and lysates prepared for western blot or IP-western analyses. **(A)** Western blots of whole cell lysates show expression of endogenous mTORC2 components (mTOR, Rictor and GβL) is largely unchanged by RABL6A depletion in BON-1 cells. **(B)** IP of mTORC2 complexes with Rictor antibodies versus IgG control. Intact mTORC2 kinase complexes, as detected by coprecipitation of mTOR and GβL, were seen in RABL6A depleted cells. Representative results from two independent experiments are shown.



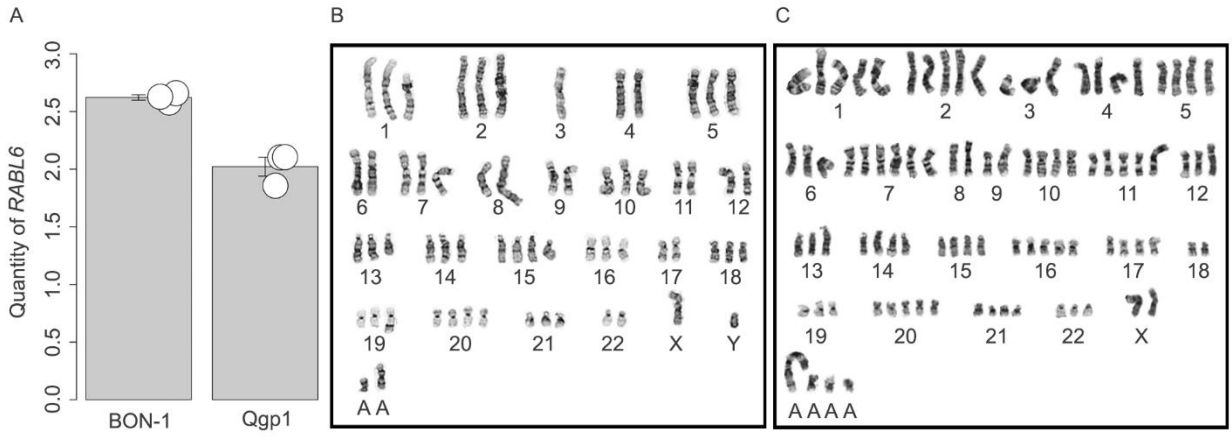
Supplemental Figure 4. Nek2 kinase overexpression fails to rescue the effects of RABL6A loss on AKT-S473 phosphorylation and cell proliferation in PNET cells. BON-1 cells expressing vector control (CON) or shRNAs targeting RABL6A (KD1 and KD2) were examined by western analyses. **(A)** Loss of RABL6A in BON-1 KD1 and KD2 cells reduces levels of endogenous Nek2. Data are representative of 2 independent experiments. **(B)** BON-1 cells overexpressing HA-tagged Nek2 (HA-Nek2) display increased phosphorylation of AKT-S473. Data are representative of 2 independent experiments. **(C)** BON-VEC control and BON-HA-Nek2 cells were infected with CON, KD1 or KD2 viruses. Westerns show that AKT-S473 phosphorylation was not restored by HA-Nek2 overexpression (detected using HA antibodies) in RABL6A deficient cells. Likewise, HA-Nek2 failed to override the cell cycle arrest induced by RABL6A loss, as indicated by the inability of cells to enter S phase. Data are representative of 3 experimental repeats.



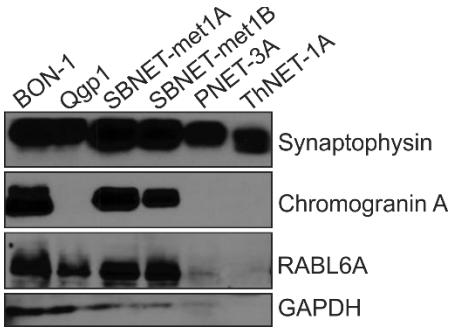
Supplemental Figure 5. Mouse body weights are unchanged over the course of the drug treatment study. Mouse body weights were measured at the indicated times just before and during treatment with vehicle control or the different indicated drugs. Data represent the means +/- SEM. The number of animals for each group is n=7 (Vehicle), n=6 (SMAP), n=5 (MK-2206), and n=6 (SMAP+MK-2206).



Supplemental Figure 6. Relative expression of PP2A subunits (A and C) and the PP2A inhibitor, CIP2A, in RABL6A depleted BON-1 cells. BON-1 cells expressing vector control (CON) or shRNAs targeting RABL6A (KD1 and KD2) were examined by western analyses. **(A)** Representative western blots showing expression levels of RABL6A, PP2A Aα subunit, PP2A C subunit, and CIP2A (cancerous inhibitor of protein phosphatase 2A). GAPDH served as the loading control. Data are representative of 4 independent experiments. Graphs showing relative levels of **(B)** PP2A-Aα subunit, **(C)** PP2A-C subunit, and **(D)** CIP2A in CON versus RABL6A knockdown cells. Data were quantified by ImageJ. Data are represented as the mean +/- SEM (*, $P \leq 0.005$ compared to CON cells, two-way ANOVA and adjusted for multiple comparisons using the Bonferroni method).



Supplemental Figure 7. Quantitative PCR of *RABL6* in BON-1 and Qgp1 PNET cell lines. **(A)** Quantity of *RABL6* DNA was determined relative to the *GAPDH* gene on chromosome 12. Given the numerous aneuploidies seen by karyotyping of both BON-1 **(B)** and Qgp1 **(C)** cell lines, *GAPDH* was chosen for normalization. That is because chromosome 12 displays minor changes compared to chromosome 7 (which contains *ACTIN*) and chromosome 11 (which contains the *β-GLOBIN* gene). Despite only two identifiable copies of chromosome 9, the level of *RABL6* DNA was higher than that of *GAPDH* suggesting additional copies of *RABL6* exist in both PNET cell lines.



Supplemental Figure 8. Comparable expression of endogenous RABL6A in PNET cell lines compared to primary human NET-derived cells. Western blots of RABL6A in whole cell lysates from BON-1, Qgp1, and primary cells derived from patient tumors, including a metastatic small bowel NET (SBNET-met1), pancreatic NET (PNET-3A), and thyroid NET (ThNET-1A). 'A' refers to cells grown in ultra-low attachment (spheroid) conditions; 'B' refers to cells initially grown under adherent conditions before being switched to ultra-low attachment dishes. Markers of NET cells, synaptophysin and chromogranin A, were also evaluated. GAPDH served as loading control. Note: Patient-derived spheroids were unable to be sustained beyond 8-12 weeks in culture, and in some cases the number of spheroids was low as seen for PNET-3A and ThNET-1A. Fresh tumor samples were resected from human patients at University of Iowa Hospitals and Clinics and processed within one to two hours following surgery. Tumors were cleaned with sterile PBS, minced and subjected to 15 min collagenase type-1 (Sigma) digestion at room temperature while rocking with quick vortexing every 5 min. Cells were filtered through a 70 µm cell strainer and cultured on ultra-low attachment or regular attachment dishes in BON-1 media. Depending on growth rates, cells were passaged every 1-3 weeks by dilution or mild trypsinization, and media replaced twice per week.

Table S1. Partial list of potential phosphorylation sites in RABL6A compiled from protein databases[A]

Human RABL6A			Mouse RABL6A	Rat RABL6A
Modified residue	**Position**	**Sequence[B]**	**Position**	**Position**
Phosphoserine	S402	PDDRLDRsFLEDTTP	S414	S406
Phosphoserine	S425	AKAAQQDsDSDGEAL	S436	S428
Phosphoserine	S427	AAWWDSDsDGEALGG	S438	S430
Phosphothreonine	T468	PVPSQDItLSSEEEA	S480	S472
Phosphoserine	S470	PSQDITLsSEEEAEV	S482	S474
Phosphoserine	S471	SQDITLSsEEEAEVA	S483	S475
Phosphoserine	S492	APAPQQCsEPETKWS	S502	S494
Phosphoserine	S525	PPWPGGVsVRTGPEK	T534	S526
Phosphoserine	S577	MDDPDFEsEGSDTQR	S575	S580
Phosphoserine	S580	PDFESEGsDTQRRAD	S578	S583
Phosphoserine	S596	FPVRDDPsDVTDEDE	S594	S599
Phosphothreonine	T599	RDDPSDVtDEDEGPA	T597	T602
Phosphoserine	S640	EEAGPKEsSEEGKEG	S637	S643
Phosphoserine	S641	EAGPKESsEEGKEGK	S638	S644
Phosphotyrosine	Y726	RHPGGGDyEEL	Y722	Y728

[A]Phosphosite.org and UniProtKB databases. The majority of sites listed here had HTP values greater than 15 at phosphosite.org. HTP, high throughput papers; the number of records in which the modified site was assigned using only proteomic discovery mass spectrometry.

[B]The modified residue is highlighted in red and lowercase text.

Table S2. Antibodies and conditions used for western analyses in this study

Antibody	Source	Catalogue Number	Working Dilution
RABL6A	Quelle lab	(Tompkins et al., Cell Cycle 2006)	1.5 µg/ml
AKT	Cell Signaling	4691	1:1,000
p-AKT (S473)	Cell Signaling	4060	1:1,000
p-AKT (T308)	Cell Signaling	9275	1:1,000
ERK	Cell Signaling	4695	1:1,000
p-ERK (T202/Y204)	Cell Signaling	9106	1:1,000
PRAS40	Cell Signaling	2997	1:1,000
p-PRAS40 (T246)	Cell Signaling	2610	1:1,000
p70 S6 Kinase	Cell Signaling	2708	1:2,000
p-p70 S6 Kinase (T389)	Cell Signaling	9206	1:500
SGK1	Millipore	07-315	1:1,000
p-SGK1 (S422)	Santa Cruz	SC-16745	1:200
PKCα	Cell Signaling	2056	1:1,000
p-PKCα (S657)	Santa Cruz	SC-12356	1:200
mTOR	Cell Signaling	2983	1:1,000
Rictor	Cell Signaling	9476	1:1,000
GβL	Cell Signaling	3274	1:1,000
Nek2	Santa Cruz	SC-55601	1:200
CIP2A	Santa Cruz	SC-80659	1:200
Vinculin	Sigma	V9131	1:2000
GAPDH	Abcam	Ab8245	1:10,000
β-Actin	Novus Biologicals	NB600-503	1:1,000
Synaptophysin	Abcam	32127	1:20,000
p-RB1 (Ser807/811)	Cell Signaling	9308S	1:1,000
Total RB1	Cell Signaling	9309S	1:1,000
PP2A A subunit	Cell Signaling	2041	1:1,000
PP2A C subunit	Abcam	Ab106262	1:1,000
p-FoxO1 (T24) / p-FoxO3a (T32)	Cell Signaling	9464	1:1,000
HA (3F10)	Roche	11867423001	0.2 µg/ml
HRP-coupled anti-mouse, anti-rat and anti-rabbit	Sigma	NA931, NA935, and NA934	1:2,000