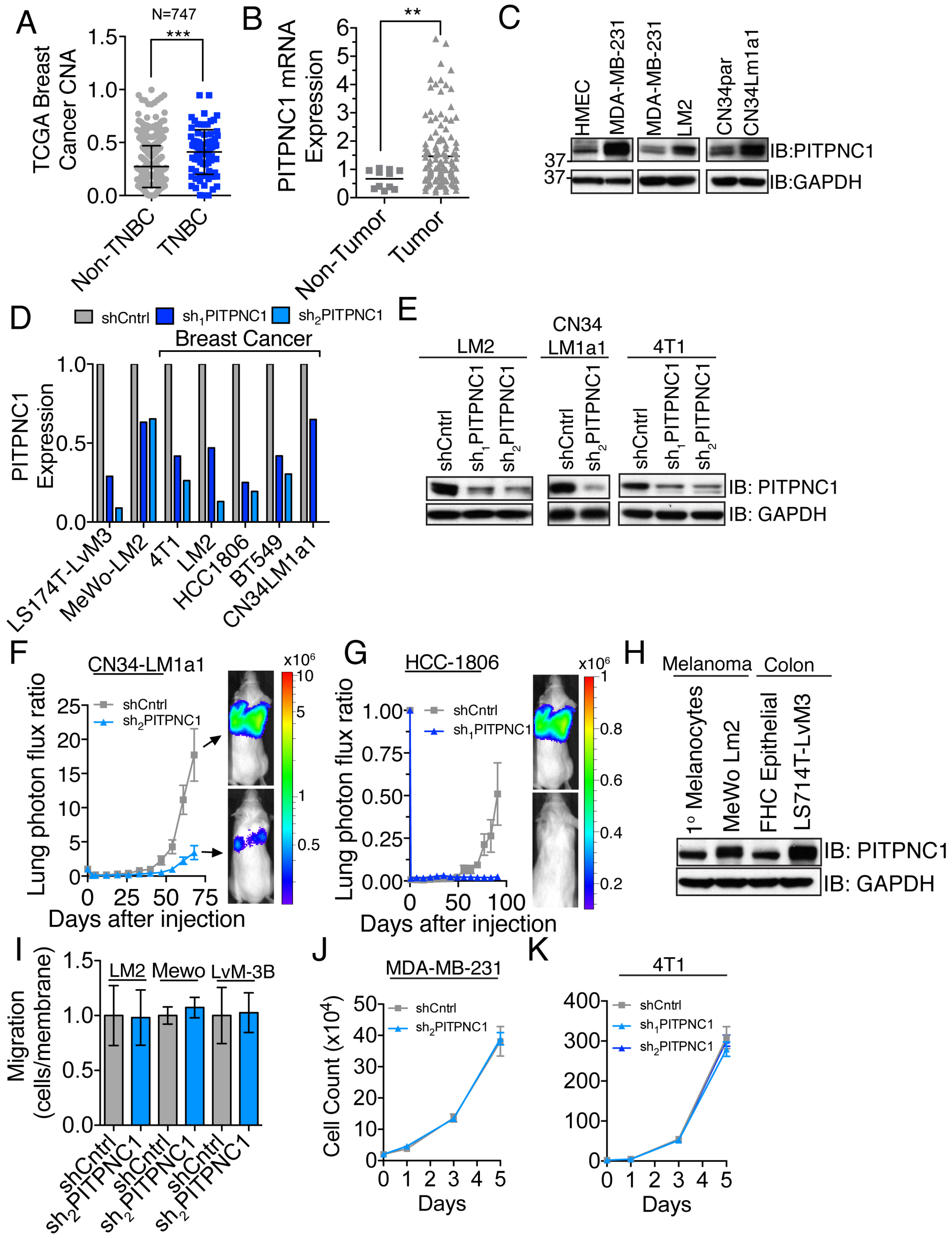
SUPPLEMENTAL INFORMATION



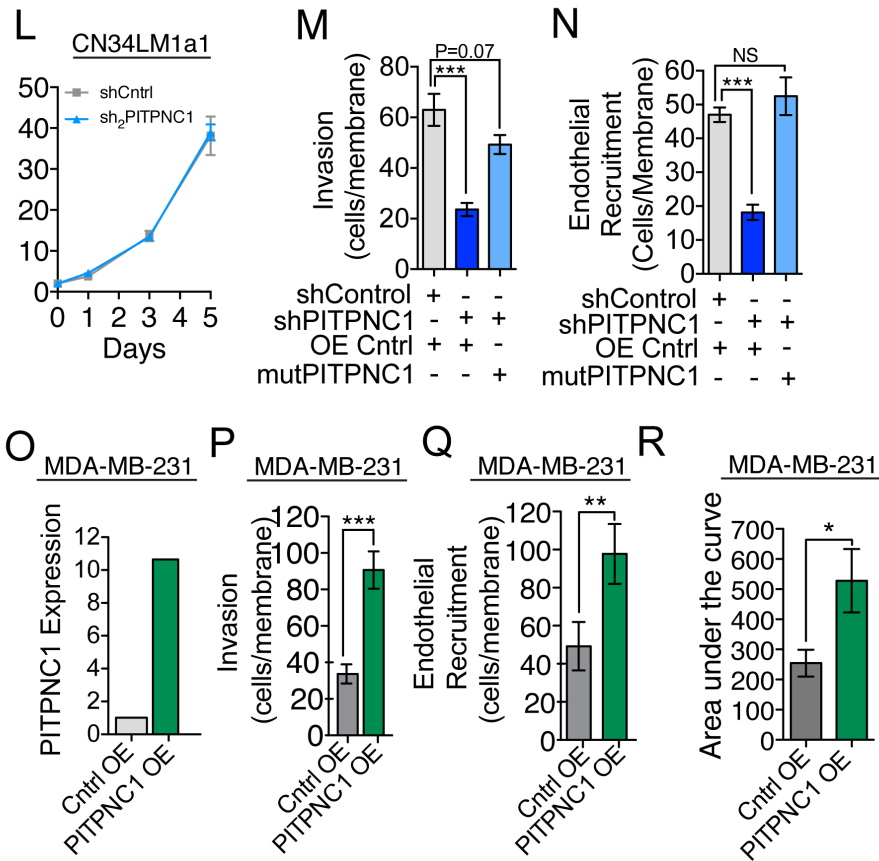


Figure S1 | PITPNC1 regulates metastatic progression in melanoma, breast, and colon cancers, Related to Figure 1

(A) DNA copy number alterations in 747 breast cancer patients presenting with either a triple negative cancer subtype or not (Ciriello et al., 2012).

(B) PITPNC1 expression analysis of human breast cancers (stages I-IV) and normal epithelial breast tissue was performed using TissueScan qPCR Array Breast Cancer Panel I, II, and III (Origene).

(C) Western blotting analysis for endogenous PITPNC1 in cellular lysates from three sets of breast cancer cell lines using anti-PITPNC1 and anti-GAPDH as loading control: human mammary epithelial cells (HMEC) versus MDA-MB-231, MDA-MB-231 versus LM2 and CN34par versus CN34LMa1.

(D) LS174T-LvM3 colon cancer cells, metastatic MeWo melanoma cells, 4T1 murine breast cancer cells and human metastatic breast cancer LM2, HCC1806, BT549 and CN34Lm1a1 cells were transduced with lentivirus expressing a control hairpin or hairpins targeting PITPNC1. PITPNC1 expression levels were determined by qRT-PCR.

(E) Western blotting analysis of endogenous PITPNC1 levels in cellular lysates from LM2, CN34Lm1a1, and 4T1 cells expressing PITPNC1 knockdown or control short hairpins.

(F,G) Bioluminescence imaging plot of lung metastatic colonization by 100,000 Cn34Lm1a1 (F) and HCC1806 (G) in control or PITPNC1 knockdown cells. N=5/group. Right, Lungs were extracted and stained by H&E.

(H) Western blotting analysis for endogenous levels of PITPNC1 in cellular lysates from primary melanocytes versus MeWo-LM2 cell and FHC colon epithelial cells versus LS174t-LvM3 cells using anti-PITPNC1 and anti-GAPDH as loading control.

(I) Trans-well migration of 20,000 LM2, MeWo-LM2, or LS174T-LvM3 cells expressing PITPNC1 knockdown or control hairpin. Values were normalized to those of shControl cells. N=4/group.

(J,K,L) Proliferation of 20,000 MDA-MB-231, 4T1 and CN34Lm1a1 expressing PITPNC1 knockdown or control hairpin. N=3/group.

(M,N) Matrigel invasion (M) and endothelial recruitment assays (N) of LM2 cells transduced with either shControl or shPITPNC1. The specific effect of shPITPNC1 was evaluated by further transduction with a mutant from of PITPNC1 wherein the shRNA target sequence was silently mutated rendering the mutant PITPNC1 untargetable by the shRNA. N=4/group.

(O) MDA-MB-231 cells were retrovirally transduced with PITPNC1 over-expression or control vectors. PITPNC1 expression levels were determined by qRT-PCR.

(P,Q) Matrigel invasion (P) and endothelial recruitment assays (Q) by MDA-MB-231 cells retrovirally transduced with PITPNC1 over-expression or control vectors. N=4.

(R) Area under curve calculation from Figure 1I.

Error bars represent S.E.M.

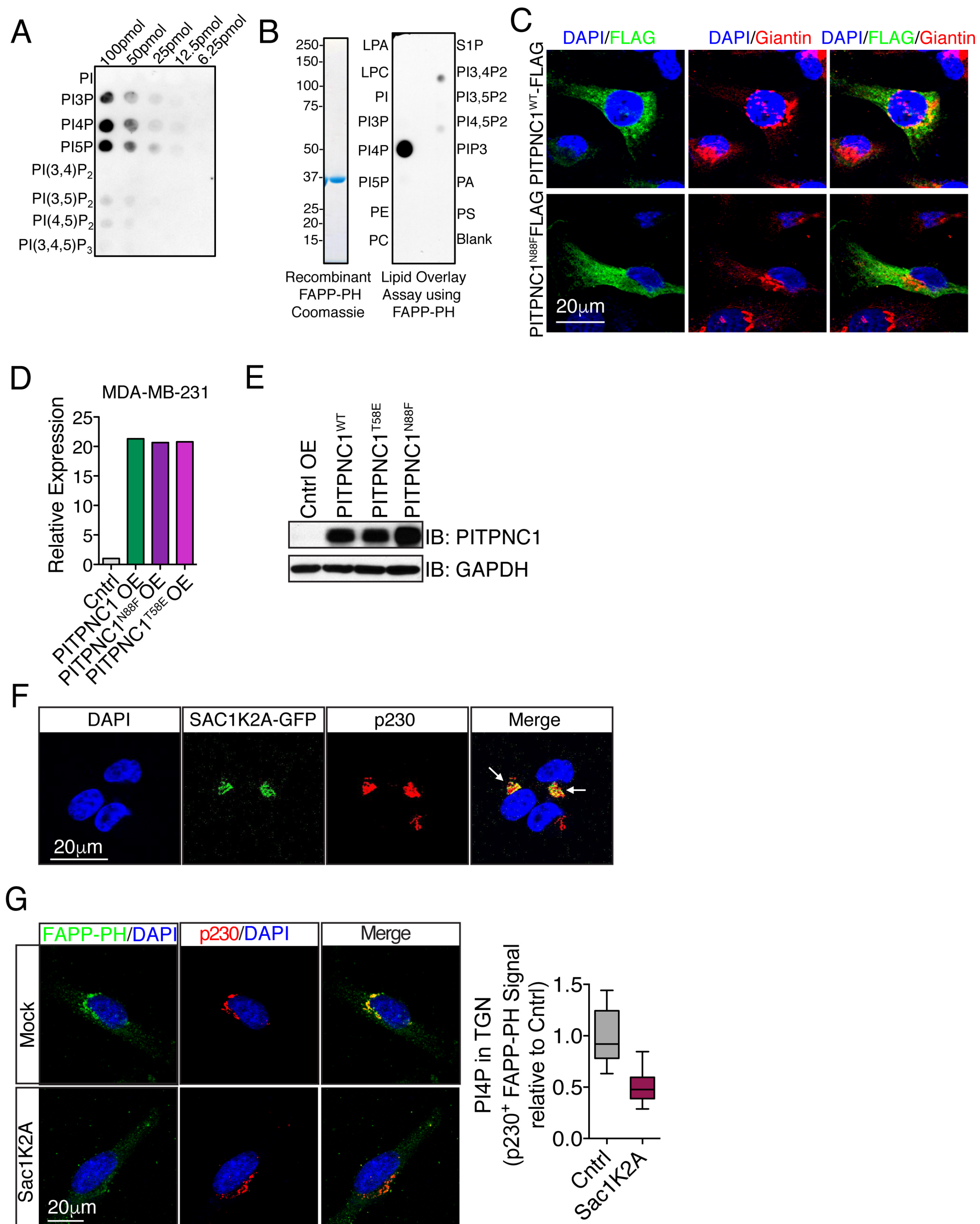


Figure S2 | Identification of PI4P as the lipid substrate of PITPNC1, Related to Figure 2

(A) Lipid overlay assay used to determine interactions between phosphorylated forms of PI and PITPNC1. Lipid bound recombinant PITPNC1-GST was detected with anti-GST antibody.

(B) Left, gel electrophoresis of purified recombinant GST-tagged FAPP-PH domain stained with colloidal blue. Right, lipid-binding specificity of FAPP-PH domain using the lipid overlay assay. Bound GST-FAPP-PH was detected with anti-GST antibody.

(C) Representative images for Figure 2F. MDA-MB-231 cells expressing either Flag-tagged wild-type or the N88F lipid mutant form of PITPNC1 were subjected to immunofluorescence analysis for the Flag epitope and the Golgi marker Giantin.

(D,E) MDA-MB-231 cells were transduced with retrovirus expressing PITPNC1, PITPNC1^{N88F}, PITPNC1^{T58E}, or control vector. PITPNC1 expression levels were determined by qRT-PCR (D) and western blot analysis (E).

(F) MDA-MB-231 were transfected with GFP-SAC1K2A and subjected to immunofluorescence analysis for GFP, p230 and DAPI 48 hours after. Arrows in the merge indicate signal overlap between GFP and the *trans*-Golgi marker p230.

(G) MDA-MB-231 were transfected with mock or GFP-SAC1K2A and subjected to immunofluorescence analysis for PI4P using the FAPP-PH domain, p230, and DAPI 48 hours after. Right, quantification of the FAPP-PH signal intensity in p230-positive areas. N=20/group.

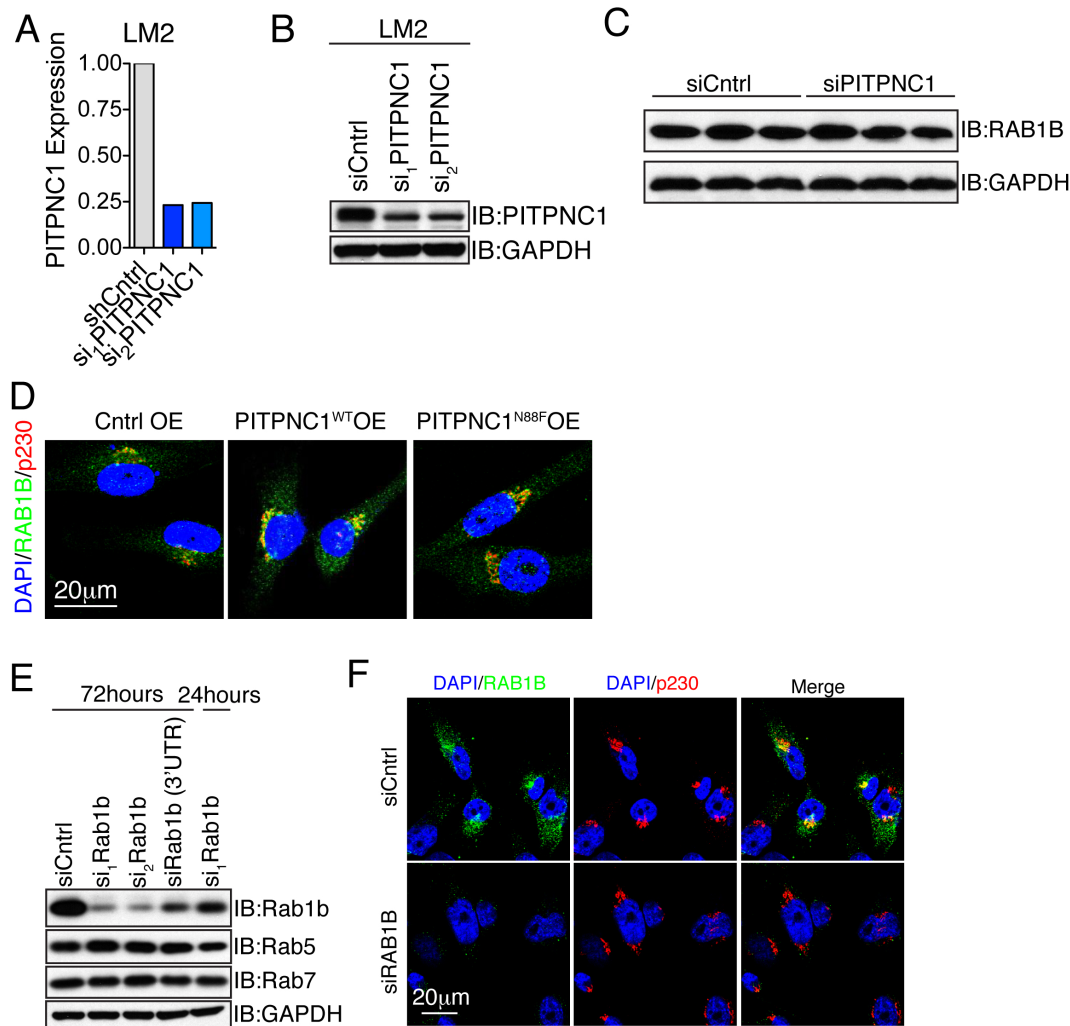


Figure S3 | PITPNC1 forms a protein complex with 14-3-3 and RAB1B, Related to Figure 3

(A,B) LM2 cells were transfected with siRNAs targeting PITPNC1 or a control siRNA. 72 hours after transfection, PITPNC1 expression levels were determined by qRT-PCR (A) and by western blotting (B).

(C) Western blot analysis of RAB1B and GAPDH in the lysates of LM2 transfected with either control siRNA or siRNA targeting PITPNC1.

(D) Representative images for Figure 3H showing immunostaining of RAB1B (green), p230 (red) and DAPI (blue) in MDA-MB-231 cells expressing either a control vector, wild type PITPNC1 or the lipid mutant N88F version of PITPNC1.

(E) Depletion of RAB1B following siRNA treatment was confirmed using western blotting analysis of endogenous RAB1B levels after 24 and 72 hours of transfection. Analysis of RAB5 and RAB7 was included to determine the antibody specificity for RAB1B.

(F) Depletion of RAB1B following siRNA treatment was confirmed using immunostaining. LM2 breast cancer cells were transfected with siRNA targeting RAB1B or a control and stained with anti-RAB1B 72 hours after.

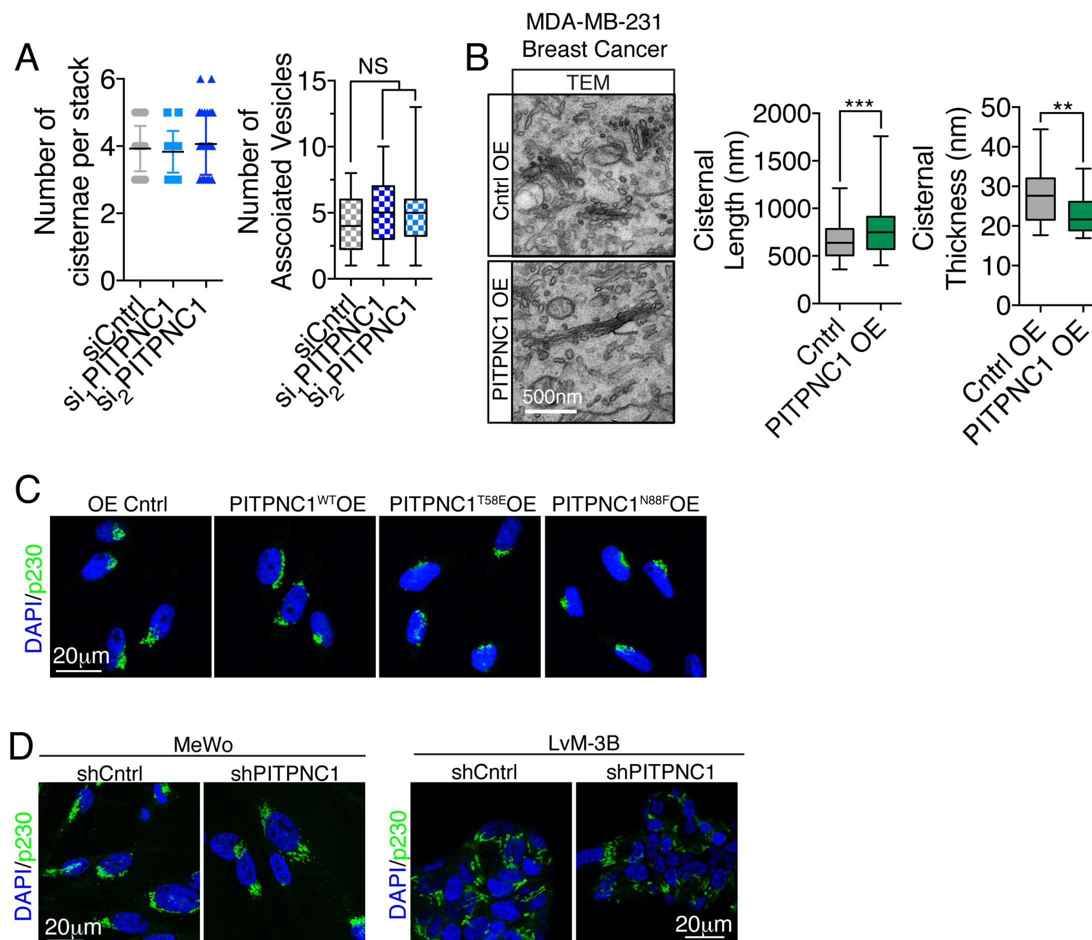


Figure S4 | PITPNC1 promotes Golgi extension, a phenotype associated with metastasis, Related to Figure 4

(A) LM2 cells were transfected with either control siRNA or siRNAs targeting PITPNC1 and analyzed by TEM 72 hours after. The number of stacks per Golgi (left) and the number of associated vesicles (right) were quantified.

(B) MDA-MB-231 cells transduced with PITPNC1 over-expression or a control vector were analyzed by TEM for cisternae length and thickness of the Golgi. N=22/group.

(C) Representative images for Figure 4F. MDA-MB-231cells over-expressing wild-type PITPNC1, the lipid mutants T58E and N88F or a control vector were stained for p230 (green), and DAPI (blue).

(D) Representative images for Figure 4G. Metastatic MeWo melanoma or LvM-3B colorectal cancer cells were transduced with lentiviral containing shRNA targeting PITPNC1 or a control shRNA. The cells were immunostained with an antibody to p230 (green) and DAPI (blue).

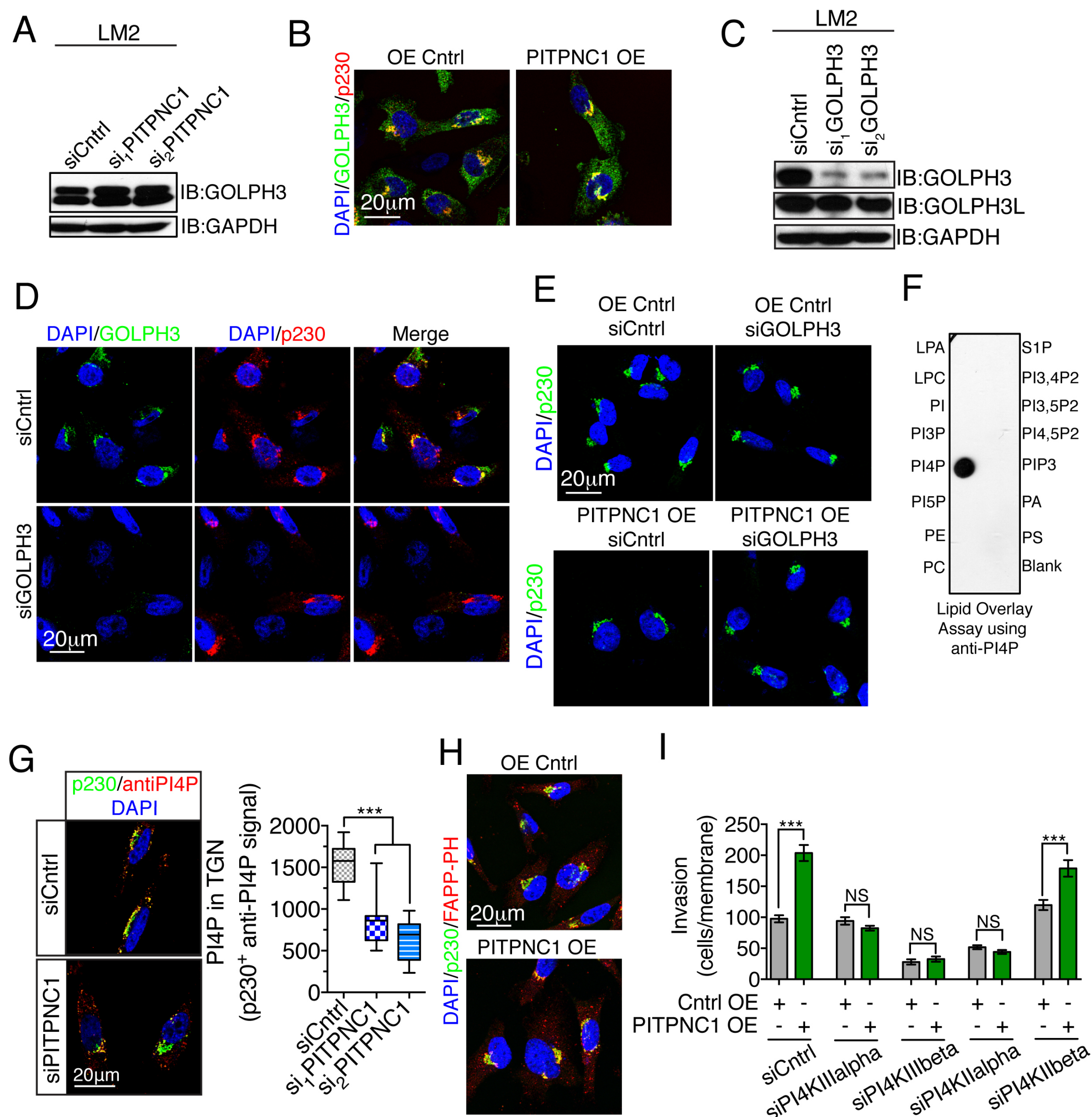


Figure S5 | PITPNC1 facilitates Golgi extension through the recruitment of GOLPH3, Related to Figure 5

(A) Western blot analysis of LM2 cells transfected with a control siRNA or siRNAs targeting PITPNC1 using anti-GOLPH3 and anti-GAPDH.

(B) Representative images for Figure 5C. MDA-MB-231 cells transduced with PITPNC1 over-expression or control vectors were stained for GOLPH3 (red), DAPI (blue) and p230 (green).

(C) Depletion of GOLPH3 following siRNA treatment was confirmed using western blotting. LM2 breast cancer were transfected with siRNA targeting GOLPH3 or a control siRNA and analyzed by western blotting by using anti-GOLPH3 and anti-GOLPHL3 as measure for the specificity of the GOLPH3 antibody.

(D) Depletion of GOLPH3 following siRNA treatment was confirmed using immunofluorescence. LM2 breast cancer were transfected with siRNA targeting GOLPH3 or a control siRNA and analyzed using anti-GOLPH3 (green), anti-p230 (red) and DAPI (blue).

(E) Representative images from Figure 5D. MDA-MD-231 cells retrovirally transduced with a vector over-expressing PITPNC1 or a control vector were subsequently transfected with siRNA targeting GOLPH3 or a control siRNA. After 72 hours the cells were stained with anti-p230 (green) and DAPI (blue).

(F) Left, lipid-binding specificity of anti-PI4P using the lipid overlay assay.

(G) Quantification of the PI4P Golgi abundance in LM2 cells transfected with either a control siRNA or siRNAs targeting PITPNC1 as determined by the mean anti-PI4P signal intensity in p230 positive areas. N=20/group.

(H) Representative images for Figure 5H. MDA-MB-231 cells transduced with PITPNC1 over-expression or control vectors were stained for PI4P using the FAPP-PH domain (red), DAPI (blue) and p230 (green).

(I) Matrigel invasion by MDA-MB-231 cells retrovirally transduced with PITPNC1 over-expression or control vectors combined with transfection with either control or siRNA targeting PI4KIIIalpha, PI4KIIIbeta, PI4KIIbeta and PI4KIIalpha. N=5/group.

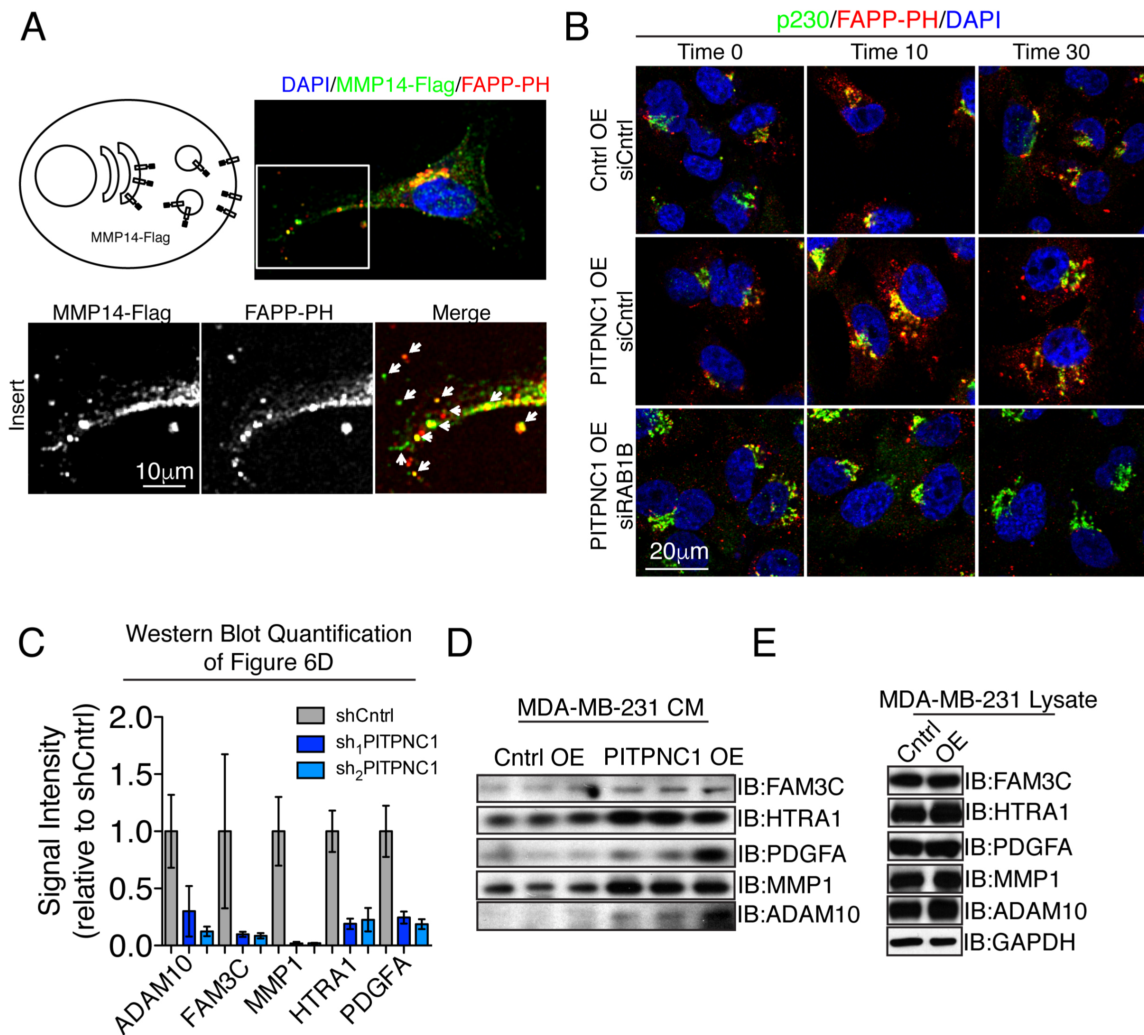


Figure S6 | PITPNC1 facilitates malignant secretion, Related to Figure 6 (A-E).

(A) MDA-MB-231 cells were transduced with a C-terminal Flag-tagged version of MMP14 (a plasma membrane bound form of the MMP family). The cells were then immunostained for the flag epitope and PI4P (FAPP-PH). The arrows in the inserts highlight the overlap between the marker of post-Golgi carriers (flag-tagged MMP14) and PI4P.

(B) Representative images for Figure 6B. MDA-MB-231 cells retrovirally transduced with a vector over-expressing PITPNC1 or a control vector was subsequently transfected with siRNA targeting RAB1B or a control siRNA. 72 hours after siRNA transfection, the cells were subjected

to the Golgi exit assay and stained for PI4P by the FAPP-PH domain (red), p230 (green) and DAPI (blue) at the indicated time points.

(C) Optical density quantification of the western blot shown in Figure 6D

(D) Western blotting analysis of FAM3C, HTRA1, PDGFA, MMP1, and ADAM10 in the conditioned media collected from MDA-MB-231 overexpressing a control vector or a vector expressing PITPNC1.

(E) Western blotting analysis of endogenous levels of ADAM10, FAM3C, HTRA1, MMP1, and PDGFA in MDA-MB-231 overexpressing a control vector or a vector expressing PITPNC1.

Error bars represent S.E.M.

A

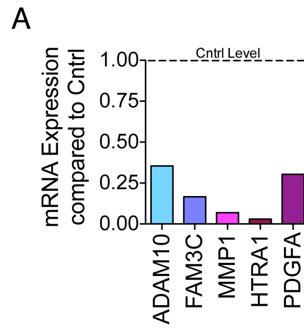


Figure S7 | PITPNC1 promotes metastasis by enhancing secretion of pro-invasive and pro-angiogenic proteins, Related to Figure 7

(A) MDA-MB-231cells were retrovirally transduced with a vector over-expressing PITPNC1. Cells were further transduced with shRNAs targeting ADAM10, FAM3C, HTRA1, PDGFA, siRNA targeting MMP1, or a control hairpin. Expression levels of each gene were determined by qRT-PCR.

EXTENDED EXPERIMENTAL PROCEDURES

Cell lines

MDA-MB-231, LM2, 4T1, CN34par, CN34Lm1a1, MeWo-LM2 and LS174T-LvM3 were all propagated in DMEM supplemented with 10% Fetal Bovine Serum. BT549 and HCC1806 were propagated in RPMI supplemented with 10% Fetal Bovine Serum. Human epidermal primary melanocytes, darkly pigmented (HEMn-DP, Life Technologies) and FHC colon epithelial cells (ATCC) were propagated according to the manufacturers' protocols.

Generation of lentivirus and retrovirus for knockdown and over-expression cell lines

Performed as previously described (Minn et al., 2005; Tavazoie et al., 2008). Primers used to clone over-expression constructs can be found in Supplementary Table 1. shRNA and siRNA sequences can be found in Supplementary Tables 2 and 3.

Mutagenesis

Mutagenesis was performed using the QuikChange Multi Site-Directed Mutagenesis Kit (Agilent Technologies). Mutagenesis primer sequences are listed in Supplementary Table 4.

Quantitative PCR

Performed as previously described (Tavazoie et al., 2008). Primer sequences are listed in Supplementary Table 5.

Transient transfection

siRNAs were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. 48 hours after transfection, cells were used for invasion and endothelial recruitment assays. 72 hours after transfection, cells were used for immunofluorescence

analysis, except for the experiments described in Figure 4A and 5C where the cells were analyzed 24 hours after transfection with siRAB1B or a control siRNA.

Sac1K2A were transfected into cells using Lipofectamine 2000 and used for invasion and endothelial recruitment assays or immunofluorescence analysis 16 hours post-transfection.

Proliferation Assay

20,000 MDA-MB-231 cells were seeded in 6-well plates and counted after 1, 3, and 5 days.

Migration assay

Cancer cells were conditioned in 0.2% FBS DMEM-based media for 16 hr. Cells were seeded at 50,000 cells per well in starvation medium into 3.0µm HTS Fluoroblock trans-well migration insert (BD Falcon) and incubated at 37°C for 6hr. The inserts were processed and analyzed as described for the matrigel invasion assay.

Genomic amplification

Analysis was performed using the data from Tumorscape (Beroukhim et al., 2010) (http://www.broadinstitute.org/tumorscape/pages/portalHome.jsf) and the cBioPortal (hhtp://www.cbioportal.org).

Western blot analysis

Conditioned media was collected from cells incubated in serum-free media for 16 hours. Collected media was 0.2µM-filtered to remove dead cells and concentrated 20-fold using spin-filter columns (Millipore). Cellular protein extract were prepared by lysing cells in RIPA buffer containing protease and phosphatase inhibitors (Roche). Secreted and cellular proteins were separated using SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane. The following antibodies were used for protein detection: anti-PITPNC1 (Sigma), anti-RAB5

(Cell Signaling Technologies), anti-RAB7 (Cell Signaling Technologies), anti-FAM3C (Abcam), anti-MMP1 (Acris Antibodies), anti-HTRA1 (Sigma), anti-PDGFA (Acris Antibodies), anti-ADAM10 (Millipore), anti-RAB1B (Proteintech), anti-GOLPH3 (Abcam), anti-GAPDH (Sigma), followed by the appropriate HRP-conjugated secondary antibodies (Invitrogen). Quantification was performed using Photoshop (Adobe).

Flag immunoprecipitation

MDA-MB-231 cells retrovirally transduced to express c-terminal flag-tagged PITPNC1 were lysed in TNET buffer (50mM Tris, 150mM NaCl, 1 mM EDTA, 1% Triton X-100, pH 7.4). Following centrifugation at 15000 rpm for 15 min the supernatant was mixed with magnetic anti-flag beads (Sigma) and left to rotate end over end at 4C for 5 hours. Immunoprecipitated material was washed twice in 2xPBS and once in 1xPBS before eluting the bound protein complexes by addition of 3xFlag peptide (Sigma). Immunoprecipiated material was analyzed by mass spectrometry and western blotting.

Lipid overlay assay

Nitrocellulose membranes were spotted with the indicated lipids (Echelon Biosciences). The membranes were blocked in 3% FFA-free BSA (Sigma-Aldrich) and incubated with 1μg recombinant PITPNC1 overnight at 4°C. PITPNC1 was detected using anti-GST antibody (Cell Signaling Technologies) and HRP-conjugated anti-rabbit antibody (Invitrogen).

Liposome pull-down assay

Performed as previously described (He et al., 2009). Briefly, solutions of phosphatidylcholine, phosphatidylethanolamine and phosphoinositides (65/24/4) were dried under argon. The lipids were resuspended in lipid transfer buffer (10mM HEPES pH 7.4, 150mM NaCl) for a total 4mM total lipid and incubated at 65°C for 1 hour. The lipids were frozen in liquid nitrogen and thawed

at 37°C three times. Resulting liposomes were pelleted by centrifugation at 25,000 x *g* for 20min at 22°C. Pelleted liposomes were resuspended in lipid transfer buffer and mixed with 4ug recombinant protein. After an incubation of 30 min at room temperature the liposomes were collected by centrifugation (25,000 x *g*, 20 min, 22°C) and levels of PITPNC1 in the supernatant and pellet were detected by SDS-PAGE followed by SimplyBlue staining (Invitrogen). The liposome bound fraction was determined as PITPNC1 protein levels in the pellet compared to the supernatant.

Protein production

cDNA of human PITPNC1 and the FAPP-PH domain was cloned into the pGEX-6P-1 vector (GE Healthcare Biosciences) and transformed into BL21(DE3) cells (Agilent Technologies). Cells were grown in LB medium supplemented with 100µg/mL ampicillin to OD_{600} = 0.6 at 37°C. Isopropyl-B-D-thiogalactaside (IPTG, Sigma) was added to 0.2mM and incubation was continued at 18°C overnight. Cells were collected by centrifugation and resuspended in 50mM Tris-HCl, 150mM NaCl, 2mM DTT, pH 7.5 plus protease inhibitors (Roche). The lysate was sonicated and spun down at 10,000 RPM for 20 minutes at 4°C. Protein was further purified from the supernatant using the Pierce GST Spin Purification kit (Thermo Scientific) according to the manufacturer’s instructions.

Metastasis-free survival analysis

Published microarray data from GSE17536 was used to obtain probe-level expression values for PITPNC1 in colorectal cancer patients. Each sample was classified as PITPNC1 positive if the signal was above the median signal for the population.

Kaplan–Meier metastasis-free survival curves were generated using Graphpad Prism 5 software (GraphPad Software).

Immunofluorescence

Immunocytochemical detection of cellular proteins as well as inositol lipids was performed as previously described (Hammond et al., 2009). Briefly, cells were fixed in 4% paraformaldehyde/PBS for 15 minutes at room temperature, washed, and permeabilized for 5 minutes with 20μM digitonin. Blocking and all subsequent staining steps were carried out in 5% goat serum/PBS. The following primary antibodies where used: anti-p230 (BD Biosciences), FAPP-PH-GST (recombinant protein), anti-GM130 (BD Biosciences), anti-GOLPH3 (Abcam), anti-RAB1B (Proteintech), anti-TGN46 (AbD Serotec), anti-Flag (Sigma), anti-PI4P (Echelon Bioscience), anti-Giantin (Abcam) and anti-GST (Cell Signaling). Primary antibodies were detected using the appropriate Alexa Fluor-labeled secondary antibodies (Invitrogen). Cells were counter-stained with DAPI and mounted in ProLong Gold (Invitrogen). Fluorescence images were acquired on an inverted TC5 SP5 laser scanning confocal microscope. Image analysis was performed using ImageJ software. To measure lipid/protein contents in the *trans*-Golgi compartments, the Golgi markers (p230, TGN46, Giantin) were thresholded and the magic wand tool (ImageJ) was used to demarcate the *trans*-Golgi compartments. Mean signal intensity of the lipid/protein was then measured in the demarcated compartment. Golgi extent was calculated as the length of p230 covered nucleus relative to the nuclear circumference multiplied by 100. For quantification of released PI4P containing vesicles in the Golgi exit assay, *trans*-Golgi PI4P abundance was subtracted from the whole cellular PI4P abundance and divided by the cell area. The data was then normalized to the time 0 value.

All imaging experiments were repeated minimum 2 times. Results are displayed as box and whiskers plot with minimum and maximum data points represented as upper and lower error bars, respectively.

Histology

Lungs were prepared by intravenous perfusion of saline followed by 4% paraformaldehyde as well as infusion of paraformaldehyde in the trachea. Removed lungs were paraffin embedded, sectioned and stained with hematoxilin and eosin.

Transmission electron microscopy

Cells were fixed in 4% paraformaldehyde and 2.5% glutaraldehyde in 0.075M sodium cacodylate buffer pH 7.4 for 30min. Subsequently, cells were washed in the buffer, post-fixed with 1% osmium tetra-oxide for 1h, stained *en bloc* with 2% Uranyl acetate for 30 min, dehydrated by a graded series of ethanol, depletion of ethanol with propylene oxide, infiltrated with a resin (Electron Microscope Sciences) and embedded with the resin. After polymerization at 60°C for 48h, ultra-thin sections were cut, post-stained with 2% uranyl acetate and 1% lead citrate and visualized by electron microscopy (100CX JEOL) with the digital imaging system XR41-C, Advantage Microscopy Technology (AMT Corp.). Quantification of the Golgi structures (cisternae length and thickness, number of Golgi stacks and number of associated vesicles) were performed using ImageJ.

Stable Isotope Labeling of Amino acids in Culture (SILAC) LC-MS/MS experiment

Cells were grown in DMEM-Flex media contained in the SILAC Protein ID & Quantitiation Kit (Invitrogen) for 7 days according to the manufacturer's instructions. Media was changed to serum-free DMEM-Flex media 16 hours prior to collection of conditioned media. Conditioned media was 0.2µM-filtered to remove dead cells, and concentrated 100x using Amicon Ultra-15 centrifugal filter units (Millipore). Reduced and alkylated proteins were separated by 1D gel electrophoresis, stained by SimplyBlue (Invitrogen), and protein bands were excised and trypsinized (Promega), following published protocol (Shevchenko et al., 1996). Extracted peptides were desalted on a trap column following separation using a 12cm/75µm reversed phase C_{18} column (Nikkyo Technos Co., Ltd.). The resolving gradient, increasing from 10% B to

45% B in 45 minutes (A: 0.1% Formic Acid, B: Acetonitrile/0.1% Formic Acid), was delivered at 300 nL/min. The liquid chromatography setup (Dionex) was connected to an Orbitrap XL (Thermo Scientific) operated in CID *top-7-mode*. Acquired data was searched against a forward and a reversed (Elias et al., 2005) human data base (ipi.HUMAN v3.87) appended with common contaminants (Bunkenborg et al., 2010) and quantified using MaxQuant version 1.2.2.5 (Cox et al., 2011). Tandem MS data were queried using full tryptic constraint allowing for a maximum of 3 missed cleavages. Oxidation of methiones and N-terminal acetylation of protein was allowed. Cysteines were treated as being fully carbamidomethylated. Matched peptides fulfilling a False Discovery Rate of 1% or lower were accepted as valid matches.

Label Free Quantification LC-MS/MS experiment

Co-immunoprecipitated and 3xFLAG eluted proteins were trypsinized in-solution overnight. Peptides were desalted using home-made Empore C18 columns prior to being analyzed by LC-MS/MS (Dionex 3000 HPLC coupled to Orbitrap XL, Thermo Scientific). The mass spectrometer was operated in a high/low mode. Peptides were separated at 300nL/min using a gradient increasing from 10% B to 45% B in 120 minutes (A: 0.1% Formic Acid, B: Acetonitrile/0.1% Formic Acid). Generated LC-MS/MS data were queried against Uniprots complete Human Proteome (July 2014) and quntified using MaxQuant 1.5.0.30. In short, Peptide Spectrum Match false discovery rate was set to 1% while protein false discovery rate was set to 1%. A total of 563 proteins were matched. Match between runs were used for the label free quantification. Generated label free quantification values were analyzed using Perseus 1.5.0.9. All LFQ values were log2 transformed and filtered, requiring that a given protein was matched in two of the three replicates. An excellent correlation was measured between replicate samples (r^2 of 0.98 for each condition). Missing LFQ values were imputed (width: 0.3, down shift: 1.8). Differences between the two conditions were assessed by a 2-sample t-test using a permutation based FDR cut-off of $p<0.05$. In addition to PITPNC1, several 14-3-3 protein isoforms and RAB1B were

found to be significantly different between the two samples. RAB1B was matched with 6, 8, and 9 peptide spectrum matches (PSMs) in bait samples versus 2, 1 and 2 PSMs in control.

Tables S1-S4

Table S1: Cloning primers

	Forward
PITPNC1 (isoform a)	CCGGCCTACGTAATGCTGCTGAAAGAGTACCG
PITPNC1-GFP (isoform a)	CCGGCCAAGCTTATGCTGCTGAAAGAGTACCGG
PITPNC1-GST (isoform a)	CCGGCCGAATTCCTGCTGAAAGAGTACCGGATC
PITPNC1-Flag (isoform a)	CCGGCCTACGTAATGCTGCTGAAAGAGTACCG
FAPP1-PH	CCGGCCGAATTCATGGAGGGGGTGTTGTACAA
MMP14-Flag	CCGGCCGAATTCATGTCTCCCGCCCCAAGACC
	Reverse
PITPNC1 (isoform a)	CCGGCCGAATTCTTACTCAGATTTGGGCCGACA
PITPNC1-GFP (isoform a)	CCGGCCCTCGAGCTCAGATTTGGGCCGACA
PITPNC1-GST (isoform a)	CCGGCCCTCGAGTTACTCAGATTTGGGCCGACA
PITPNC1-Flag (isoform a)	CCGGCCGAATTCTTACTTGTCGTCATCGTCTTTGTA GTCCTCAGATTTGGGCCGACATG
FAPP1-PH	CCGGCCCTCGAG TCACCTTGTATCAGTCAAACATG
MMP14-Flag	CCGGCCGTCGACTCACTTATCGTCGTCATCCTTGTAATC GACCTTGTCCAGCAGGGAAC

Table S2: shRNA sequences

Cntrl	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT

PITPNC1#1	CCGGCGGGTGTATCTCAACAGCAAACTCGAGTTTGCTGTTGAGATACACCCGTTTTTG
PITPNC1#2	CCGGCAATGGATGAAGTCCGAGAATCTCGAGATTCTCGGACTTCATCCATTGTTTTTG
ADAM10	CCGGGCAGGTTCTATCTGTGAGAAACTCGAGTTTCTCACAGATAGAACCTGCTTTTT
FAM3C	CCGGGATGCAAGTTTAGGAAATCTACTCGAGTAGATTTCCTAAACTTGCATCTTTTTG
HTRA	CCGGCGGTGAAGTGATTGGAATTAACTCGAGTTAATTCCAATCACTTCACCGTTTTTG
PDGFA	CCGGGAATCCGGATTATCGGGAAGACTCGAGTCTTCCCGATAATCCGGATTCTTTTTTG

Table S3: siRNA sequences

PITPNC1 #1	CCACAGACGCACCCGAAUU
PITPNC1 #2	CGAUGAAAUUCCAGAGCGC
GOLPH3	GCUUGUGGAAUGAGACGUA
MMP1	GGAGGUAUGAUGAAUAUAA
RAB1B	GCCAGCGAGAACGUCAAU

Table S4: Mutagenesis Primers

	Forward
T58E	CCCTCACCATGGCAATGGGCAGTTCGAAGAGAAGCGGGTGTATCTCAACAGCA
N88F	ATTTTATGTGACAGAGAAGGCTTGGTTCTATTATCCCTACACAATTACAGAAT
S274A	TCTTCCGTCCGCAGTGCGCCTTCTGCCGCTCCATCCACCCCTCTCTCCACA
S299A	CCCAAAGATCGGCCCCGGAAAAAGGCCGCCCCAGAAACTCTCACACTTCCA

PITPNC1 Silent	TGATGAGTGGTATGACATGACTATGGACGAGGTACGTGAGTTTGAACGAGCCACTCAGGAA
	Reverse
T58E	TGCTGTTGAGATACACCCGCTTCTCTTCGAACTGCCCATTGCCATGGTGAGGG
N88F	ATTCTGTAATTGTGTAGGGATAATAGAACCAAGCCTTCTCTGTCACATAAAAT
S274A	TGTGGAGAGAGGGGTGGATGGAGCGGCAGAAGGCGCACTGCGGACGGAAGA
S299A	TGGAAGTGTGAGAGTTTCTGGGGCGGCCTTTTTCCGGGGCCGATCTTTGGG
PITPNC1 Silent	TTCCTGAGTGGCTCGTTCAAACTCACGTACCTCGTCCATAGTCATGTCATACCACTCATCA

Table S5: Quantitative PCR primers

PITPNC1	For: GCGCTACTACAAAGAATCTGAGG
	Rev: GAGCACATGATAGGCTGATGAC
ADAM10	For: AGCAACATCTGGGGACAAAC
	Rev: CCCAGGTTTCAGTTTGCATT
FAM3C	For: ATCTCAAAAGCTTGCCCTGA
	Rev: AAATGGTGCCACATCTCCTC
MMP1	For: AGGTCTCTGAGGGTCAAGCA
	Rev: AGTTCATGAGCTGCAACACG
HTRA1	For: TGGAATCTCCTTTGCAATCC
	Rev: CCTTCAGCTCTTTGGCTTTG

PDGFA	For: ACACGAGCAGTGTCAAGTGC
	Rev: ACCTCACATCCGTGTCCTCT

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