TITLES AND LEGENDS TO SUPPLEMENTARY MATERIAL

Table S1: Summary of assays and effects from RPPA analyses (see also detailed results in raw data files 1 – 8)

Table S2: List of differentially expressed transcripts
pval = uncorrected p-value; qval = FDR-adjusted p-value

Table S3: Results of Pathway Enrichment Analysis

Movie 1: Protease protection assay
S2-007 cells were co-transfected with Plac8-CFP (left panels/green pseudocolor in overlay) and YFP (middle panels/red pseudocolor in overlay). At the 60 s time point, cells were treated with digitonin, and at the 400 s time point trypsin was added.
Scale bars: 10 µm

Movie 2: Plac8-PrP-vesicle
3D rendering of stacked images of a Plac8- and PrP-positive intracellular vesicle.

Movie 3: FRAP assay
In a Plac8-YFP-transfected S2-007 cell, a region of interest (ROI) covering a Plac8-positive membrane area (white rectangle) was photobleached using high-power laser intensities. Recovery of fluorescence in the ROI was recorded and quantified over time. Fluorescence recovery was complete within 100 s.
Scale bar: 10 µm

Movie 4: FLIP assay
S2-007 cells were transfected with Plac8-CFP (left panel) or YFP (right panel). ROIs (white outlines) in individual cells were repeatedly photobleached using high-power laser intensities and fluorescent signals recorded in regular intervals for 20 min of total duration.

Movie 5: TIRF assay

S2-007 cells were transfected with Plac8-YFP and TIRF images of an individual cell recorded over a timeframe of 9 min.

Figure S1: Quantification of Plac8 signal intensities

(A) Representative image showing the method of Plac8 signal intensity quantification. Thin confocal slices of S2-007 cells transfected with YFP-Plac8 were analyzed by defining regions of interest representing background (blue square), autofluorescence of untransfected cells (white square), cytosolic localization (green squares), and membrane localization (red rectangles).

(B) Background-substracted signal intensities (arbitrary units; A.U.) were measured and plotted as mean fluorescence intensity +/- SD (n=12 independent measurements).

Figure S2: Plac8/PrP immunofluorescence

(A) S2-007 cells were co-transfected with Plac8-CFP (left panel/red pseudocolor in overlay) and PrP-YFP (middle panel/green pseudocolor in overlay). Plac8- and PrP-positive vesicles showed Plac8 signals at the perimeter and PrP signals in the lumen.

(B) Intensity plots of CFP (red line) and YFP (green line) fluorescence across 2 individual intracellular vesicles. The cross-sections that were analyzed are indicated by white lines in the merged image in (A).

Figure S3: Nocodazole treatment and flow cytometry

48h after transfection with siRNAs, cells (A: S2-007; B: PaTu8988T) were either left untreated or incubated with 0.1 µg/ml nocodazole. Cells were fixed after 3h, 6h and 9h of incubation and cell cycle distribution of treated and control cells analyzed by flow cytometry. While both, G1/S and S/G2 transition, were attenuated in cells treated with siRNAs 2 and 3, cells were completely arrested in G1 after transfection with siRNA1.

si1-si3=Plac8-specific siRNAs; siC=non-silencing control siRNA; UT=untreated cells.

Figure S4: Plac8 knockdown does not affect Akt/ phospho-Akt and c-Myc levels

A) Akt and phospho-Akt levels were not uniformly influenced upon loss of Plac8 in the pancreatic cancer cell lines as shown by Western blot analyses (representative blots for PaTu-8988T cells).

B) c-Myc mRNA expression in tumor tissue samples of different mice as analyzed by quantitative RealTime reverse transcription PCR (qRT-PCR). Expression was normalized to ribosomal protein, large, P0 (RPLP0) mRNA levels. n= 4 for KC, Plac8$^{-/-}$KC, KPC mice, and n= 3 for Plac8$^{-/-}$KPC mice.

C) Plac8 levels were not systematically influenced by c-Myc expression levels in the pancreatic cancer cell lines as shown by Western blot analyses (representative blots for PaTu-8988T cells). While c-Myc levels were already reduced in control-transfected cells, the two c-Myc-specific siRNAs used showed different efficiencies of c-Myc downregulation. Plac8 levels did not vary significantly in response to any changes in c-Myc expression.

Figure S5: D*ifferentialy expressed transcripts*

Heat maps of transcripts downregulated (left panel, A) and upregulated (right panel, B) in response to Plac8 knockdown in S2-007 cells. For generation of heat maps, mean values from 3 independent experiments were used and expression values mean centered across the four experimental groups. Red denotes high expression, green low expression and black intermediate expression levels.