Supplementary Materials and Methods

Cellular phosphoinositide and liposome overlay assay

The extracted acidic lipids and PC/PE/phosphoinositides liposomes were produced as previously described (19). The extracted acidic lipids or liposomes were spotted onto nitrocellulose membranes. The membranes were incubated in a blocking buffer (2% skim milk, 1.5% BSA, and 0.05% Tween 20 in phosphate-buffered saline) and then probed with 10 μg/ml e

GST-tagged protein in blocking buffer. After three washes with TBS-0.05% Tween 20, the membranes were incubated with anti-GST antibody.
RT-PCR

Total RNA was isolated from cells using TRIzol reagent (Life Technologies) and used as a template for cDNA synthesis with the PrimeScript II 1st-strand cDNA Synthesis Kit (TAKARA). RT-PCR was performed using the following primers:

(5’-TCCTGGTTTGCACAGGGGCCAGTTC-3’ and 5’-CTACCACCATGAAAAAGGGCTTC) for PI4KIIα;

(5’-CTGACAAAAGGATTGGACAAAGCCAC-3’ and 5’-CTACCAGGAGGAAAAATGGCTTC-3’) for PI4KIIβ;

(5’-GTCTCCCTGGTCACTCTCATGTTGG-3’ and 5’-TCAGTAGGGGATTTGGTCGTT-3’) for PI4KIIIα;

(5’-GAGTCAACGGATTTGGTCGT-3’ and 5’-TGTGGTCATGAGTCCTTCCA-3’) for PI4KIIIβ;

for GAPDH.

Supplementary Figure Legends
**Supplementary Figure 1. Knockdown of SAC1 in T47D cells.**

(A) The indicated siRNA-transfected T47D cells were fixed and stained with anti-E-cadherin antibody and phalloidin. Scale bar = 20 μm.

(B) Control- or SAC1-siRNA-transfected T47D cells were lysed and immunoblotted with the indicated antibodies.

(C) The indicated siRNA-transfected T47D cells were analyzed in a migration assay. The data represent the mean (SEM) of three independent experiments. *, P < 0.05. Scale bar = 100 μm.

**Supplementary Figure 2. Quantification of phosphoinositides.**

(A) Lipids were extracted from the indicated siRNA-transfected MCF7 cells, and the amount of phosphoinositides present was quantified by liposome overlay assay.

(B) Quantification of total PI(4)P levels in SAC1-attenuated MCF7 cells. The amount of PI(4)P was normalized to that of PI(3)P. The data represent the mean (SEM) of three independent experiments. N.S., not significant.
(C) HeLa cells were fixed, permeabilized with 0.5% saponin or 20 M digitonin, and stained with anti-OSBP antibody and Alexa Fluor 647-labeled Fapp1 2 × PH domain preincubated with or without PC/PE or PC/PE/PI(4)P liposomes. Scale bar = 20 μm.

**Supplementary Figure 3. Knockdown of SAC1 and PI4KI1β in MCF7 cells.**

(A) Lower phase image of figure 2B. The indicated siRNA-transfected MCF7 cells were fixed and stained with phalloidin. Scale bar = 20 μm.

(B) The indicated siRNA-transfected MCF7 cells were lysed and immunoblotted with SAC1 and PI4KI1β antibodies.

(C) MCF7 cells were transfected with SAC1 siRNA in combination with PI4KI1β siRNA and then fixed and stained with phalloidin. Scale bar = 20 μm.

(D) Control- or PI4KI1β-siRNA-transfected MCF7 cells were lysed and immunoblotted with a PI4KI1β antibody.

(E) MCF7 cells were transfected with the indicated siRNAs and then fixed and stained with anti-GM130 antibody together with Alexa Fluor 647-labeled Fapp1 2 × PH
domain. Scale bar = 20 μm.

(F) Quantification of Golgi PI(4)P levels in MCF7 cells transfected with PI4KIIIβ siRNA. The data shown are the mean (SEM) from more than 100 cells. *, P < 0.001.

Supplementary Figure 4. Knockdown of PI4K variants.

(A) Depletion of PI4K isoforms by the indicated siRNAs in MDA-MB-231 cells. The results of the RT-PCR analysis are shown.

(B) The indicated siRNA-transfected MDA-MB-231 cells were fixed and stained with phalloidin. Scale bar = 20 μm.

(C) Control- or PI4KIIIβ-siRNA-transfected MDA-MB-231 cells were lysed and immunoblotted with the indicated antibodies.

(D) MDA-MB-231 cells were transfected with SAC1 siRNA in combination with PI4KIIIβ siRNA and then fixed and stained with phalloidin.

Supplementary Figure 5. Knockdown of PI4KIIIβ in Hs578t cells.
(A) Control- or PI4KIIIβ-siRNA-transfected Hs578t cells were lysed and
immunoblotted with the indicated antibodies.

(B) The indicated siRNA-transfected H578t cells were fixed and stained with
anti-N-cadherin antibody and phalloidin. Scale bar = 20 μm.

(C) The indicated siRNA-transfected H578t cells were fixed and stained with
anti-cadherin-11 and anti-β-catenin antibodies in addition to phalloidin. Scale bar =
20 μm.

Supplementary Figure 6. Knockdown of SAC1, PI4KIIIβ and GOLPH3.

(A) Control- or PI4KIIIβ-siRNA-transfected MDA-MB-231 cells were lysed and
immunoblotted with the indicated antibodies.

(B) Control- or SAC1-siRNA-transfected MCF7 cells were lysed and immunoblotted
with the indicated antibodies.

(C) The indicated siRNA-transfected MCF7 cells were fixed and stained with
anti-GOLPH3 and anti-GM130 antibodies and phalloidin. Scale bar = 20 μm.

(D) Fluorescence intensity of GOLPH3 was quantified in control- or
SAC1-siRNA-transfected MCF7 cells. The data shown are the mean (SEM) from more than 150 cells measured. *, P < 0.0001.

(E) The indicated siRNA-transfected MCF7 cells were fixed and stained with anti-E-cadherin antibody in addition to phalloidin. Scale bar = 20 μm.

(F) Nitrocellulose membranes spotted with PC/PE/phosphoinositide liposomes were incubated with GST, GST-FAPP1 2 × PH, GST-GOLPH3 (wt), or GST-GOLPH3 (R90L). The bound protein was detected using an anti-GST antibody.

Supplementary Table 1. SAC1 expression in cancer.

Supplementary Table 2. PI4KIIIβ expression in cancer.