

Supplementary Information

Figure S1 PAO treatment does not affect β -COP localization at the Golgi complex.

HeLa cells were treated for 5 min with 0.1% DMSO or 10 μ M PAO and then visualized by immunofluorescence microscopy with anti-arfaptin1 and anti- β -COP antibodies. Scale bars, 10 μ m.

Figure S2 Protein-lipid overlay assay with recombinant arfaptin1 and2.

Membranes spotted with 100 pmol of the indicated phosphoinositides and modified lipids were incubated with recombinant non-tagged arfaptin1 or arfaptin2. Binding was detected by incubating the strips with the goat anti-arfaptin1 or the mouse anti-arfaptin2 antibodies followed by HRP-conjugated secondary antibody.

Figure S3 Expression of the deletion mutants of arfaptin1 in HeLa cells.

HeLa cells were transfected with constructs coding for either the wild type full-length arfaptin1 or the indicated deletion mutants of arfaptin1 fused to GFP. After 24 h, cells were lysed and the lysates were western blotted with anti-GFP antibody.

Figure S4 PKD phosphorylates the long isoform of arfaptin1 inhibiting its localization at the TGN.

The coding sequence of the 373-aa form of human arfaptin1 (GenBank accession no. NP_001020766.1) was amplified by RT-PCR from HeLa cells and cloned into the pGEX-6P1 plasmid for expression and purification of recombinant GST fusion

proteins, and into the pMePy-GST plasmid for expression of GST-tagged versions in HeLa cells.

(A) Schematic representation of the 341- and 373-aa isoforms of arfaptin1 and the 341-aa form of arfaptin2. The longer splicing isoform of arfaptin1 (named here as arfaptin1b) contains an alternative exon which precedes the 40-aa region required for the localization of the arfaptin1 BAR domain at the TGN. Numbers indicate amino acid position. The triangle marks the position of the serine phosphorylated by PKD which is numbered as Ser100 for the short isoform or as Ser132 for the longer isoform. (B) In vitro kinase assay using the constitutively active form of PKD2 and the wild type form or the indicated alanine mutants of arfaptin1, arfaptin1b and arfaptin2. (C) HeLa cells expressing wild type, S132A and S132E versions of arfaptin1b tagged with GST were fixed and stained with anti-GST and anti-arfaptin2 antibodies. Scale bars, 10 μ m.

Figure S5 Analysis of the effect of arfaptin1 or 2 knockdown on PAUF secretion.

Normal HeLa cells were transfected with either non-targeting (siControl), arfaptin1 (siArfaptin1 #2), or arfaptin2 (siArfaptin2 #4) siRNA oligonucleotides. After 48 h, cells were transfected with a plasmid encoding PAUF with a His tag. 24 h later, cells were incubated at 20°C for 2 h in Opti-MEM and then shifted to 32°C for 1 h. The levels of PAUF in the medium and in the cell lysates were measured by western blotting with anti-His antibody.

Figure S6 *Drosophila* arfaptin localizes at the Golgi membranes in S2 cells.

Drosophila S2 cells stably expressing Mannosidase II-GFP were transfected with a plasmid coding for Flag-tagged *Drosophila* arfaptin. Expression of the Flag-tagged

arfaptin was induced by incubating the cells with Cu^{2+} for 12 h. Then, the cells were plated onto a concanavalin A-coated glass coverslip and, after 2 h, processed for immunofluorescence with an anti-Flag antibody. Scale bars, 10 μm .

Figure S1

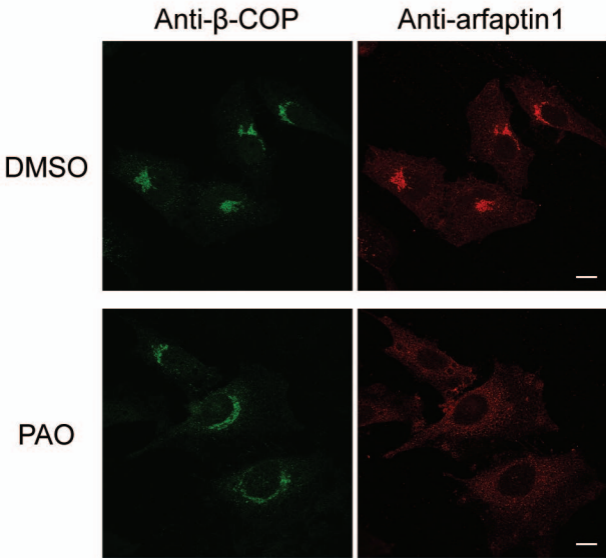
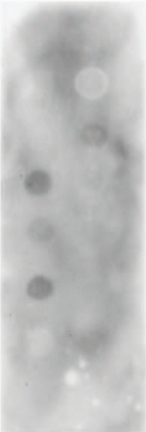


Figure S2

Arfaptin1

LPA		S1P
LPC		PI(3,4)P ₂
PI		PI(3,5)P ₂
PI(3)P		PI(4,5)P ₂
PI(4)P		PI(3,4,5)P ₃
PI(5)P		PA
PE		PS
PC		Blank



Arfaptin2

LPA		S1P
LPC		PI(3,4)P ₂
PI		PI(3,5)P ₂
PI(3)P		PI(4,5)P ₂
PI(4)P		PI(3,4,5)P ₃
PI(5)P		PA
PE		PS
PC		Blank




Figure S3

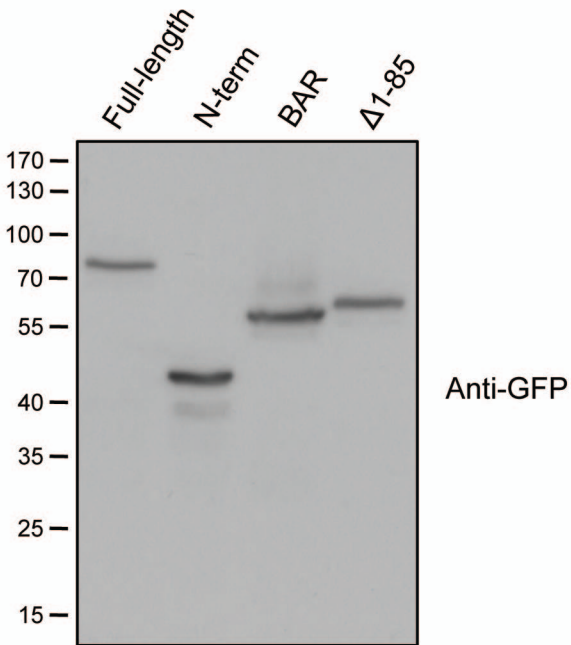
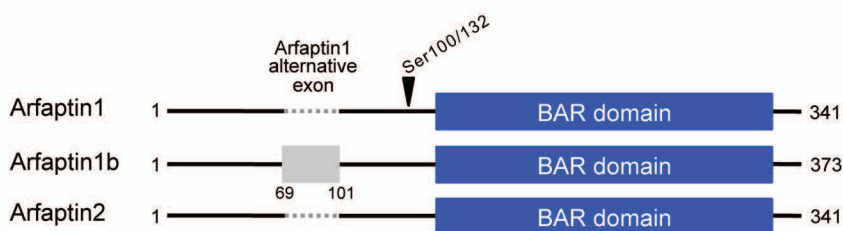
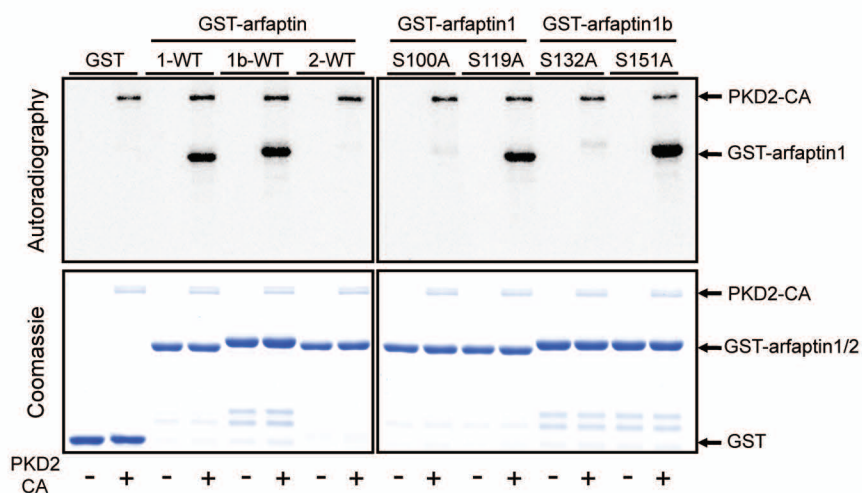


Figure S4

A



B



C

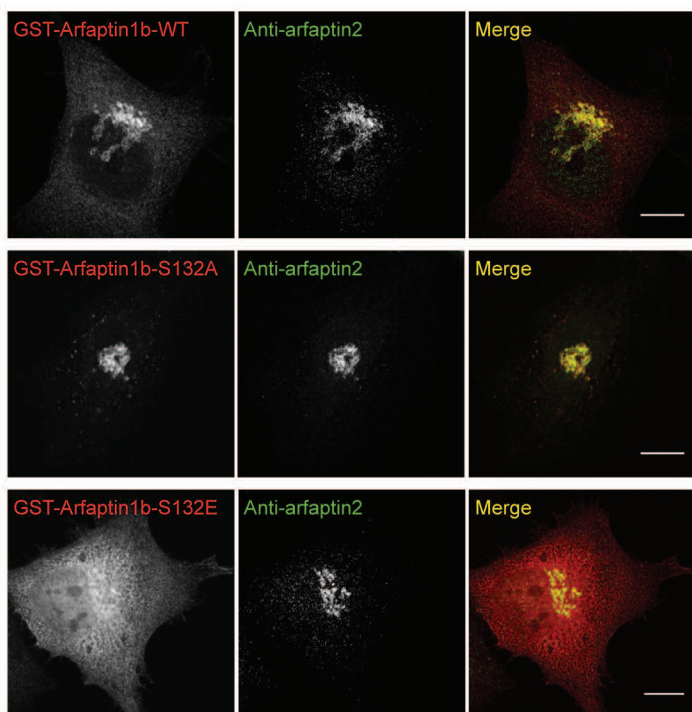


Figure S5

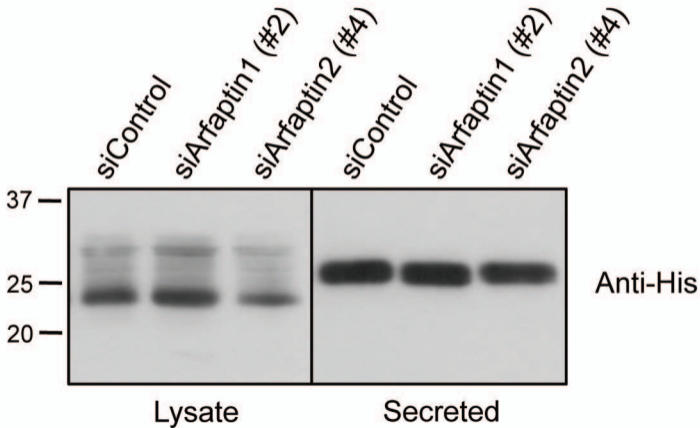
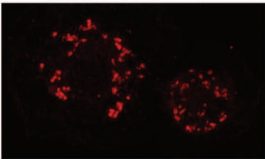
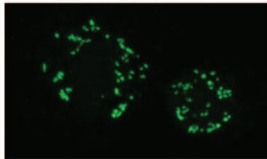


Figure S6

Drosophila arfaptin



Mannosidase II-GFP



Merged

