Supporting Information

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SI Materials and Methods

Antibodies. The following antibodies, phalloidins, and dyes were used in this study: Diaphanous-related formin 1 (mDia1; 610848, 1 μg/mL; BD Transduction Laboratories), mDia2 (55539, 2 μg/mL; Sigma), amylase (A8273, 2 μg/mL; Sigma), Myc-tag (sc40, 2 μg/mL; Santa Cruz Biotechnology), mucin 1 (HM-1630, 1 μg/mL; Thermo Scientific), Wiskott-Aldrich syndrome-like (WASL) (HPA005750, 0.8 μg/mL; Sigma), Arp3 (A5979, 10 μg/mL; Sigma), zona occludens 1 (40–2200, 1 μg/mL; Invitrogen), Alexa Fluor goat 555 anti-mouse (A21424, 2 μg/mL; Invitrogen), Alexa Fluor goat 555 anti-rabbit (A21426, 1 μg/mL; Invitrogen), TRITC-phalloidin (P1951, 0.5 μg/mL; Sigma), FITC-phalloidin (P5282, 2 μg/mL; Sigma), and FM4-64 (F34653, 0.1 μg/mL; Invitrogen).

Construction of Recombinant Adenoviruses. cDNAs encoding mDia1FL (amino acids 1–1,255), mDia1ΔDAD, and mDia1ΔDAD-I845A (amino acids 1–1,182) were fused 5′ to a triple Myc-tag and inserted into the pShuttle (+) vector. The Lifeact-eGFP construct was inserted directly into the vector. These constructs then were introduced into adenoviruses (Capital Biosciences). Viral titer was amplified in 293A cells and monitored using the AdEasy kit (Agilent) according to manufacturer instructions.

Amylase Secretion. Fresh acini were washed twice, preincubated in resuspension medium for 30–60 min, and washed again. Resuspended acini were then aliquoted and further incubated in replicates in the absence or presence of cholecystokinin (CCK) for 30 min. All incubations were performed at 37 °C. The acinar suspension was centrifuged and the supernatant assayed for amylase activity with the use of Infinity Amylase reagent (Invitrogen). Results were expressed as percentage of total cellular amylase content at the beginning of incubation. When the effect of latrunculin A (LatA; 1 μM; Sigma) was tested, the acini were also preincubated with LatA for 15 min before stimulation. Control acini were treated with an appropriate concentration of DMSO. Results represent the average and SEM of three independent experiments. Statistical significance was determined by *t* test (two-tailed).

Quantification of Actin Bundle Properties. For the assessment of average length and diameter of the apical actin bundles, 100 bundles from eight independent live-imaging experiments were measured using Softworx software (Applied Precision). Bundle length was measured only for bundles in which both ends were visible. The average number of bundles per length was calculated by dividing the number of actin bundles by the length of the lumen perimeter, in lumens formed by at least three Lifeact-GFP–expressing cells and displaying at least two bundles. Because of the scarcity of bundles in mDia1ΔDAD-I845A–expressing cells, bundle density also was measured in lumens that depicted compound secretion but not bundles. Results represent the average and SD measured along the perimeter of 15–22 lumens, from four independent experiments. Statistical significance was determined by *t* test (two-tailed). Data of actin-coated vesicle speed and length of travel over actin bundles represent the average and SD of 40 distinct vesicle trajectories taken from seven independent experiments.

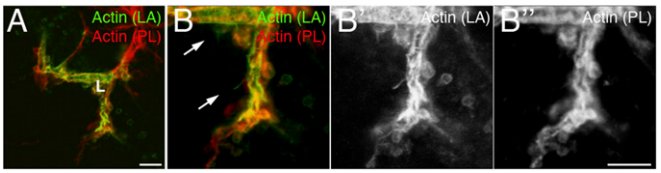


Fig. S1. Apical actin bundles can be detected by the Lifeact-GFP probe but not by phalloidin. (*A*–*B*″) Low (*A*) and high (*B*–*B*″) magnifications of subapical actin in pancreatic acinar cells expressing Lifeact-GFP (green, gray in *B*′) and stained with TRITC-phalloidin (red, gray in *B*″). Cells were incubated in the presence of Lifeact-GFP–carrying adenoviruses for 16 h and stimulated with CCK (100 pM). Cells were then fixed and stained. Arrows indicate actin bundles emanating from the apical surface. (Scale bars, 5 μm.)

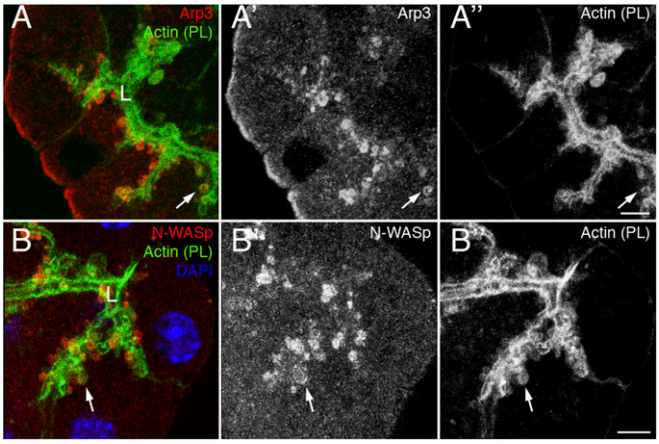


Fig. S2. Arp3 and Neural Wiskott–Aldrich syndrome protein (*N*-WASp) are enriched around actin-coated vesicles. (*A*–*A*′′) Localization of endogenous Arp3 (red, gray in *A*′) in pancreatic acinar cells. Acini were isolated and allowed to recover for 30 min before stimulation with CCK (100 pM). Cells were then fixed and stained. F-actin is labeled with phalloidin (PL; green, gray in *A*′′). Arrows indicate actin-coated vesicles positive for Arp3. (*B*–*B*′′) Localization of endogenous *N*-WASp (red, gray in *B*′) in pancreatic acinar cells. Acini were treated as in *A*. Arrows indicate actin-coated vesicles positive for *N*-WASp. F-actin is labeled with phalloidin (green, gray in *B*′′), and nuclei are labeled with DAPI (blue). (Scale bars, 5 μm.)

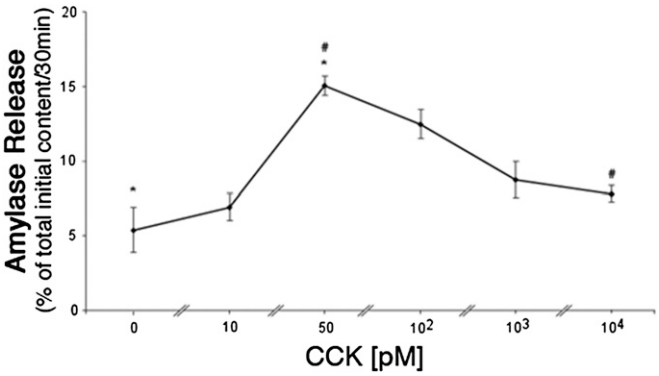
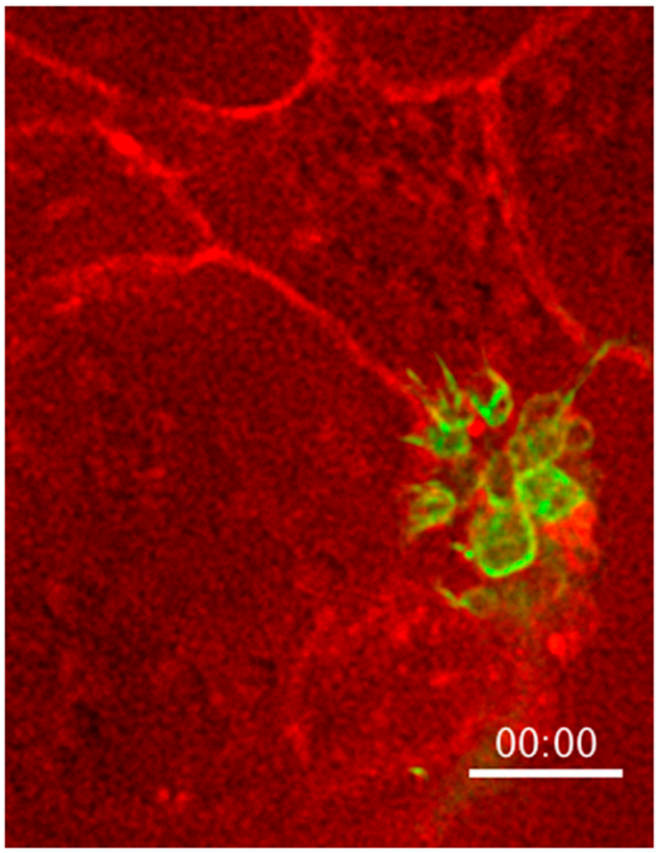
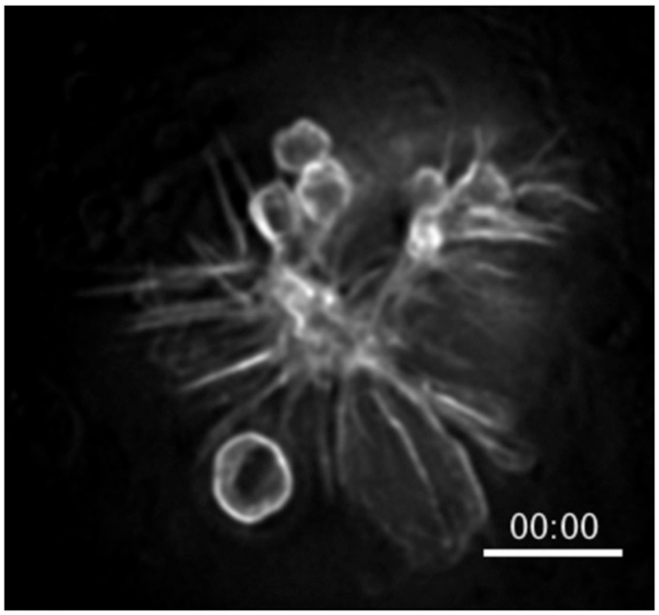


Fig. S3. Amylase release from freshly isolated acini. Acini were isolated and allowed to recover for 30 min before stimulation with the indicated concentrations of CCK. Results represent the average and SEM of three independent experiments; $^{*}P = 14.7 \times 10^{-4}$, $^{\#}P = 10.8 \times 10^{-4}$.



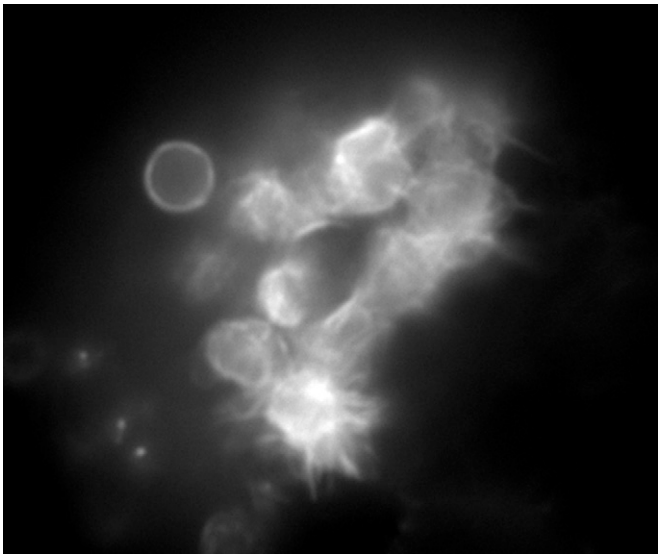
Movie S1. Time-lapse fluorescence images of acinar cells expressing Lifeact-GFP, stained with a lipophilic dye (FM4-64, red), and stimulated with 100 pM CCK, shown in Fig. 1 *B*–*C*. Cells were incubated in the presence of Lifeact-GFP–carrying adenoviruses for 16 h, washed, stained with FM4-64, and stimulated briefly before the initiation of imaging. Images were acquired at 5.5-s intervals over 4.5 min. The image sequence is displayed twice: first the merged channel, followed by the Lifeact-GFP channel. (Scale bar, 5 μm.)

Movie S1



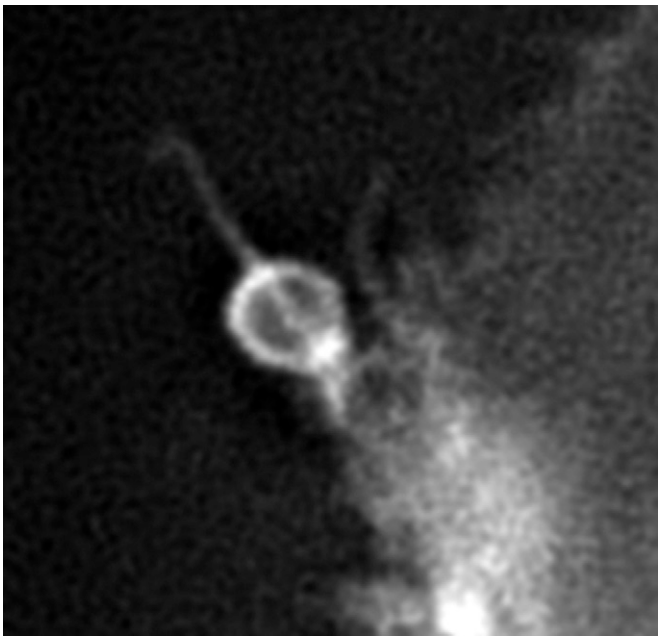
Movie S2. Time-lapse fluorescence images of acinar cells expressing Lifeact-GFP, stimulated with 100 pM CCK, shown in Fig. 1*D*. Cells were incubated in the presence of Lifeact-GFP–carrying adenoviruses for 16 h, washed, and stimulated briefly before the initiation of imaging. Images were acquired at 4-s intervals over 3.5 min. (Scale bar, 5 μm.)

Movie S2



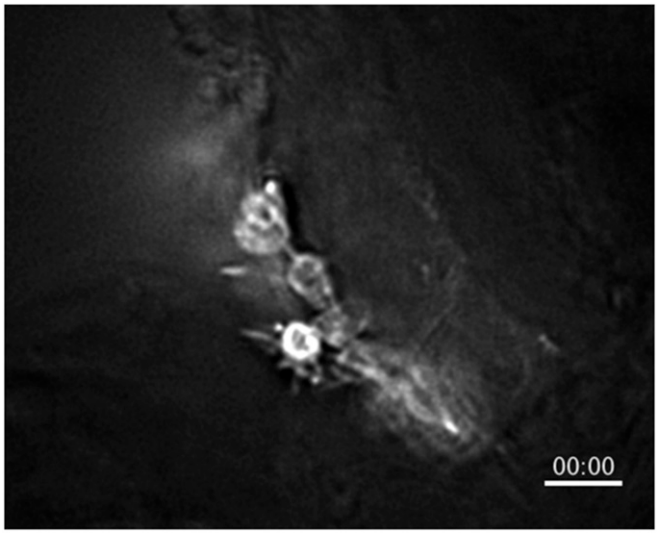
Movie S3. Time-lapse fluorescence images of acinar cells expressing Lifeact-GFP, stimulated with 100 pM CCK. Cells were incubated in the presence of Lifeact-GFP–carrying adenoviruses for 16 h, washed, and stimulated briefly before the initiation of imaging. Images were acquired at 4-s intervals over 5 min. (Scale bar, 5 μm.)

Movie S3



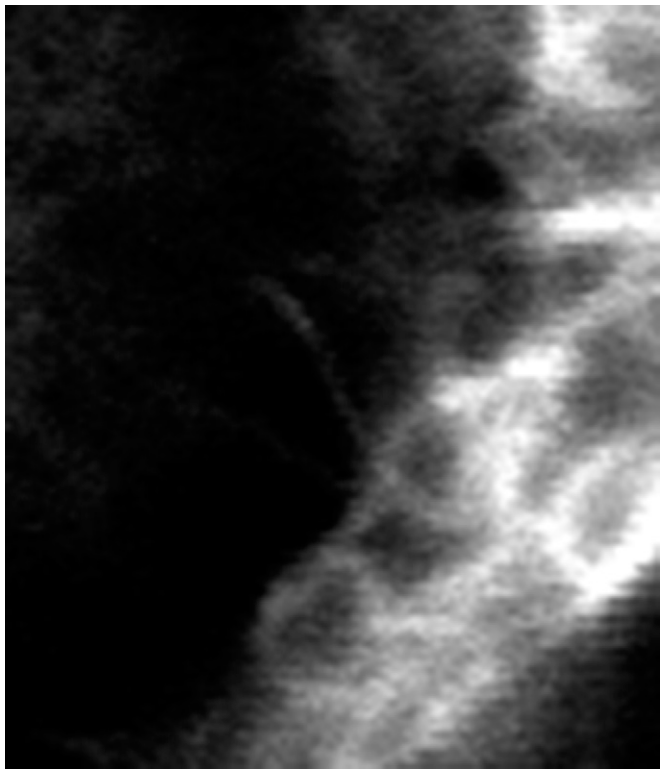
Movie S4. Time-lapse fluorescence images of acinar cells expressing Lifeact-GFP, stimulated with 100 pM CCK, shown in Fig. 1*F*. Cells were incubated in the presence of Lifeact-GFP–carrying adenoviruses for 16 h, washed, and stimulated briefly before the initiation of imaging. Images were acquired at 4-s intervals over 1.3 min. (Scale bar, 5 μm.)

Movie S4



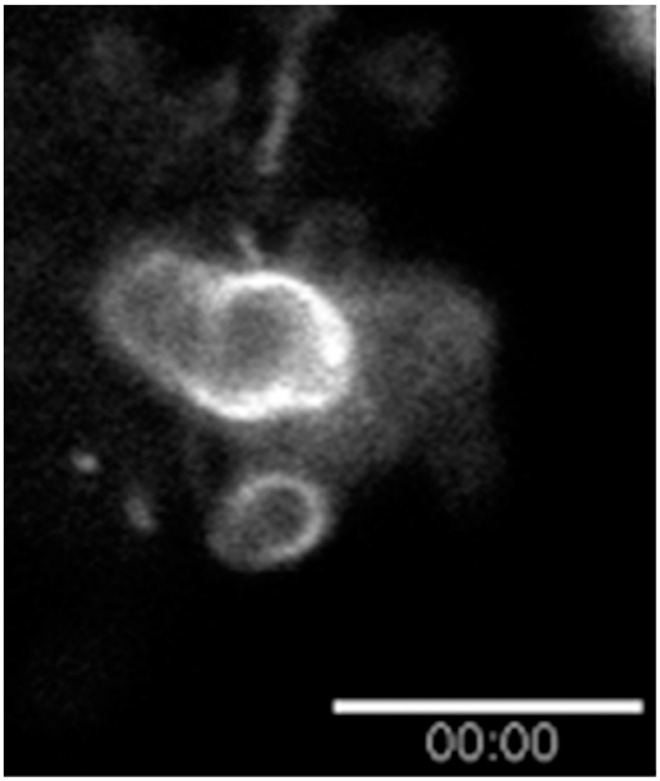
Movie S5. Time-lapse fluorescence images of acinar cells expressing Lifeact-GFP and stimulated with 100 pM CCK following exposure to LatA (1μM), shown in Fig. 1*G*. Cells were incubated in the presence of Lifeact-carrying adenoviruses for 16 h, washed, and stimulated briefly before the initiation of imaging. LatA was introduced when indicated. Images were acquired at 5-s intervals over 12.5 min. (Scale bar, 5 μm.)

Movie S5



Movie S6. Time-lapse fluorescence images of acinar cells expressing Lifeact-GFP and stimulated with 100 pM CCK, depicting de novo bundle growth, shown in Fig. 1 *H* and *I*. Cells were incubated in the presence of Lifeact-carrying adenoviruses for 16 h, washed, and stimulated briefly before the initiation of imaging. Images were acquired at 1.2-s intervals over 43 s. (Scale bar, 5 μm.)

Movie S6



Movie S7. Time-lapse fluorescence images of acinar cells coinfected with Ad-Lifeact-GFP and Ad-mDia1ΔDAD-I845A and stimulated with 100 pM CCK, depicting compound secretion events, shown in Fig. 3*C*. Cells were incubated in the presence of the adenoviruses for 16 h, washed, and stimulated briefly before the initiation of imaging. Images were acquired at 1.1-s intervals over 2.1 min. (Scale bar, 5 μm.)

Movie S7