Reversi et al., http://www.jcb.org/cgi/content/full/jcb.201309079/DC1

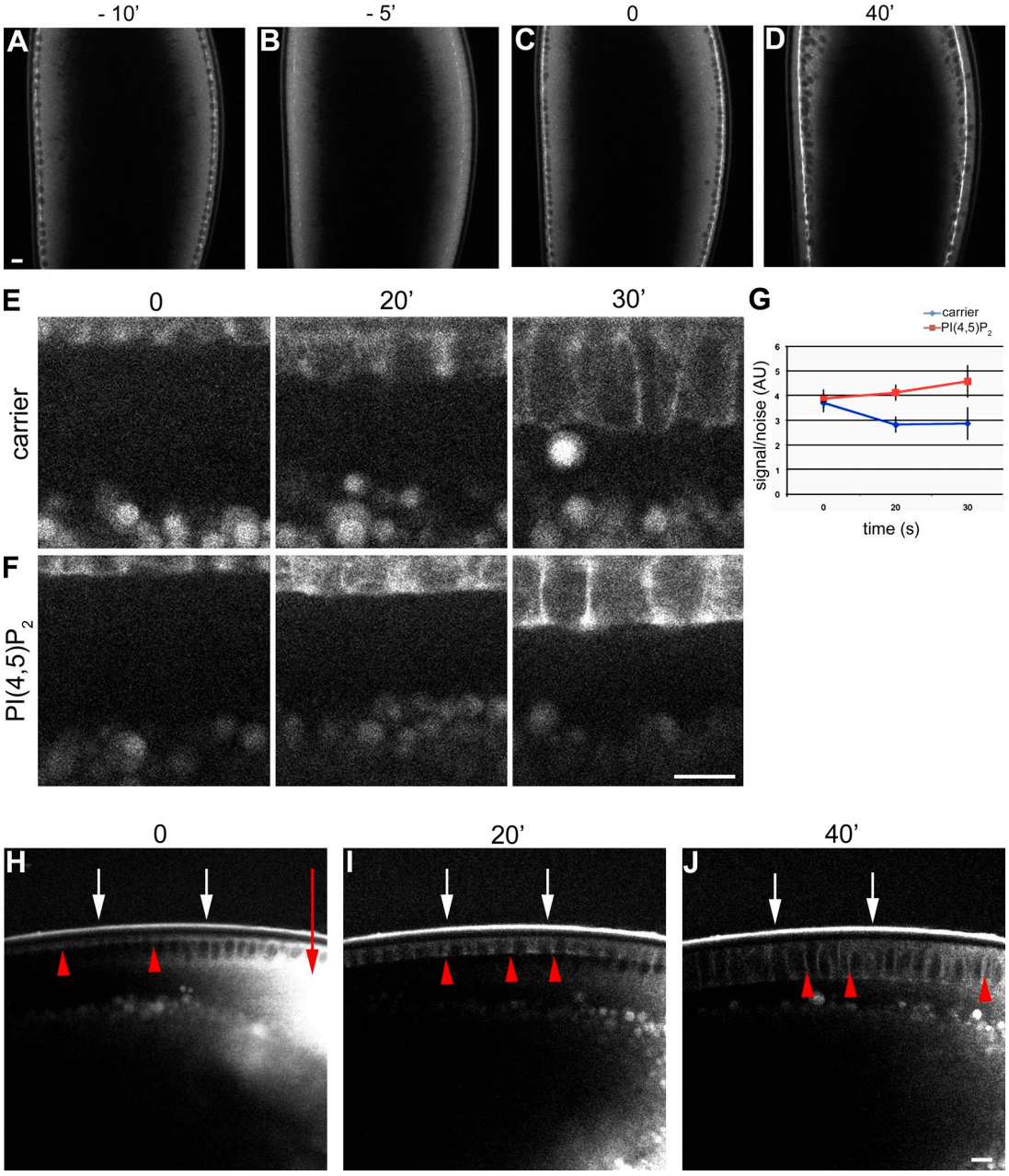


Figure S1. **PI(4,5)P_2-induced phenotype is manifested only after the onset of cellularization.** (A–D) Still frames from a time-lapse two-photon movie of a cellularizing embryo expressing Sqh::GFP injected before cycle 13 with PI(4,5)P_2/AM. Four different time points are shown: 10 min before the onset of cellularization (A); 5 min before the onset of cellularization (B); 0 min (C, onset of cellularization); and 40 min after the beginning of cellularization (D). Injection of PI(4,5)P_2/AM before cycle 13 did not alter earlier stages of development, and metaphase furrows formed normally (A) and progressed for ~5 µm before completely regressing (B). The hyper-constricted phenotype became manifested only when embryos entered cycle 14. In addition, some nuclei detached from the cortex, leading to a partial nuclear fallout phenotype (C and D). Bar, 10 µm. (E and F) Still frames from a time-lapse two-photon movie of a cellularizing embryo expressing the PI(4,5)P_2 sensor, PLCδ1-PH::GFP injected with carrier (E, DMSO) or PI(4,5)P_2/AM (F) before the beginning of cellularization. Three different time points are shown: 0, 20, and 30 min. PI(4,5)P_2/AM injection leads to increased recruitment of PLCδ1-PH-GFP to the plasma membrane (F) compared with the control (E). Bar, 10 µm. (G) Quantification of PLCδ1-PH::GFP fluorescence signal/noise ratio in embryos injected either with carrier (DMSO) or with PI(4,5)P_2/AM. For each time point, the signal was calculated by measuring fluorescence intensity in the cell-containing region of the embryo and the noise in an area, of equal size, immediately below. PI(4,5)P_2/AM injection causes a significant increase in the signal/noise ratio, indicating increased plasma membrane recruitment (Student's *t* test, P = 0.043). Error bars represent mean ± SD (n = 3). (H–J) Still frames from a time-lapse two-photon movie of a cellularizing embryo injected with fluorescently labeled PI(4,5)P_2 showing that ectopic PI(4,5)P_2 is correctly incorporated into the plasma membrane (red arrowheads). Three different time points are shown: 0, 20, and 40 min. Red arrow in H indicates the site of injection. White arrows point to the autofluorescence of the vitelline membrane. Bar, 10 µm.

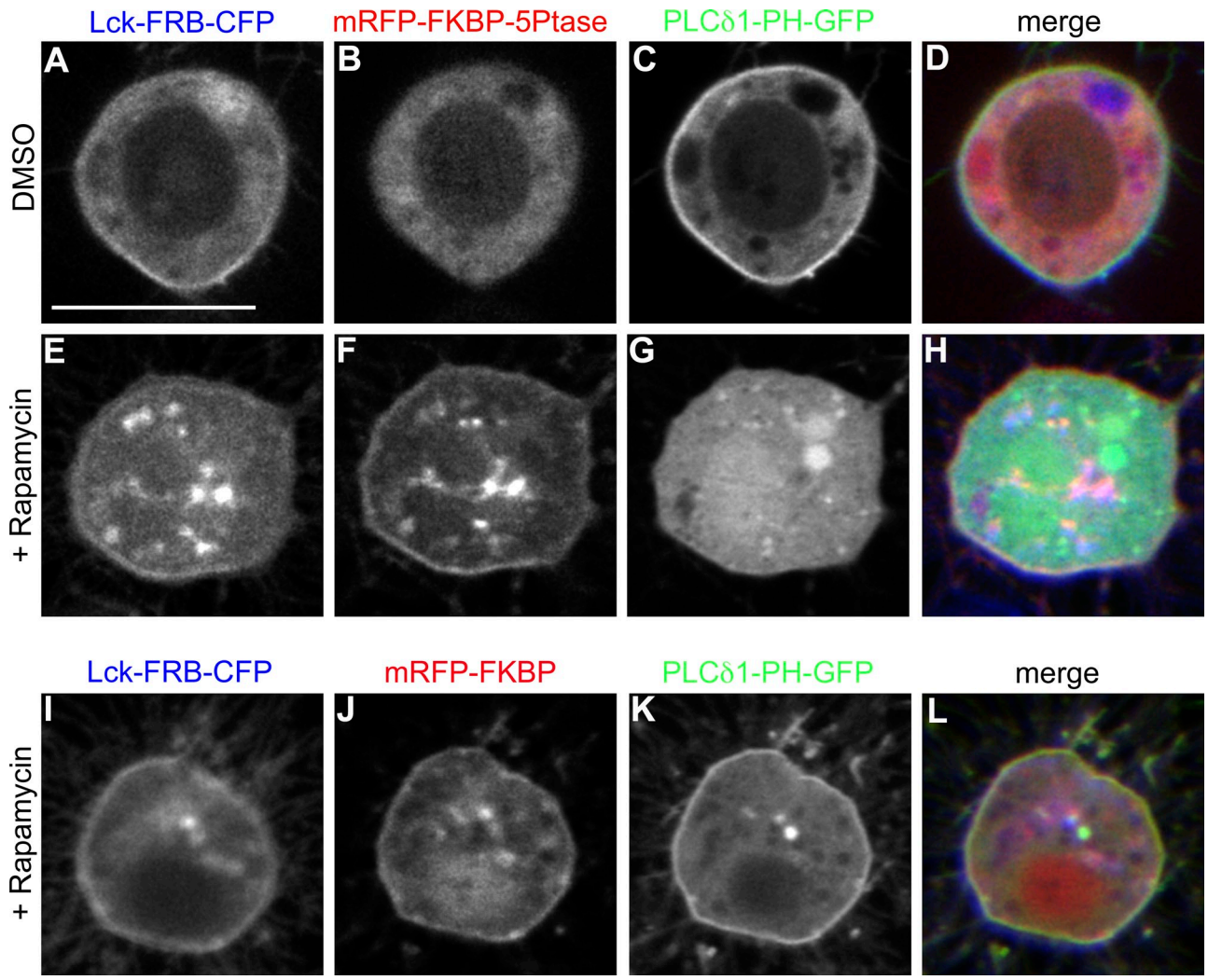


Figure S2. **Depletion of PI(4,5)P_2 causes PLCδ1-PH-GFP dissociation from the plasma membrane.** (A–H) Representative confocal images of S2 cells coexpressing the plasma membrane anchor Lck-FRB-CFP (A and E), mRFP-FKBP-5Ptase (B and F), and PLCδ1-PH-GFP (C, G, and K). (A–C) In control cells the 5Ptase remains cytosolic (B) and PLCδ1-PH-GFP is localized at the plasma membrane (C). Addition of rapamycin induces translocation of the 5Ptase to the plasma membrane (F), resulting in a complete loss of PLCδ1-PH-GFP plasma membrane localization (G). D and H correspond to the merge of A–C and E–G, respectively. Addition of rapamycin also results in clustering of Lck-FRB-CFP and mRFP-FKBP-5Ptase in cytoplasmic blobs, presumably indicating that a pool of the Lck-FRB-CFP anchor is also cytosolic in S2 cells. (I–L) Addition of rapamycin in control cells expressing the plasma membrane anchor Lck-FRB-CFP (I) and the FKBP construct lacking the 5Ptase catalytic domain (J) shows no changes in PLCδ1-PH-GFP localization (K). Bar, 10 µm. L shows the merge of I–K.

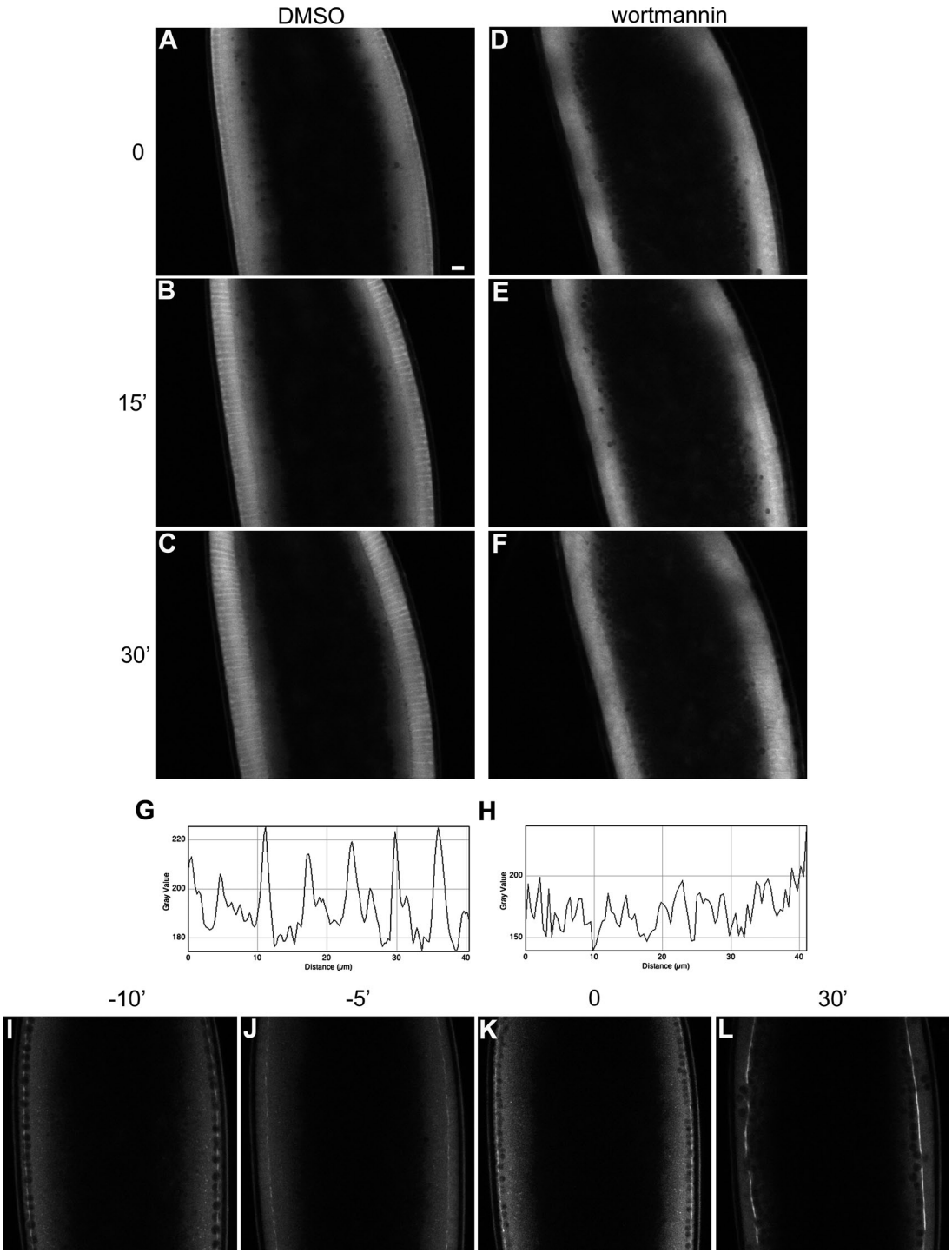


Figure S3. **PI3-kinase inhibition results in reduced PI(3,4,5)P_3 levels at the plasma membrane during cellularization.** (A–F) Still frame from time-lapse two-photon movies of cellularizing embryos expressing GFP::Akt-PH injected at the onset of cellularization with either DMSO (A–C) or wortmannin (D–F). (A–C) In control-injected embryos, GFP::Akt-PH localizes to the plasma membrane. (D–F) Upon wortmannin injection, the signal is partially lost from the plasma membrane and redistributed to the cytoplasm. Three different time points are shown: 0 min (A and D), 15 min (B and E), and 30 min (C and F). Bar, 10 μm. (G and H) Profile plots illustrating the decrease in GFP::Akt-PH signal at the plasma membrane upon wortmannin injection. Fluorescence intensity was calculated by drawing a line (40 μm long) perpendicular to the plasma membrane at ~15 μm from the vitelline membrane. In controls (G), fluorescence intensity peaked every ~5 μm in correspondence with the plasma membrane (peaks mean ± SD = 217 ± 7.17). The mean fluorescence intensity in between peaks, corresponding to the cytosolic pool, is 186.5 (SD ± 8.2). This difference is significant; Student's *t* test, $P = 2.5 \times 10^{-5}$ ($n = 7$). No significant difference in fluorescence intensity was observed in wortmannin-injected embryos (H; peaks mean ± SD = 182 ± 16.88; cytosolic mean ± SD = 172 ± 10.62; Student's *t* test P = 0.206, $n = 7$). (I–L) Still frames from a time-lapse two-photon movie of a cellularizing embryo expressing Sqh::GFP injected before cycle 13 with wortmannin. Four different time points are shown: 10 min before the onset of cellularization (I), 5 min before the onset of cellularization (J), 0 min (K; onset of cellularization), and 30 min after the onset of cellularization (L). Injection of wortmannin before cycle 13 did not alter earlier stages of development, and metaphase furrows formed normally (I) and progressed for ~5 μm before completely regressing (J and K). The hyper-constricted phenotype became manifested only when embryos entered cycle 14 (L). Bar, 10 μm.

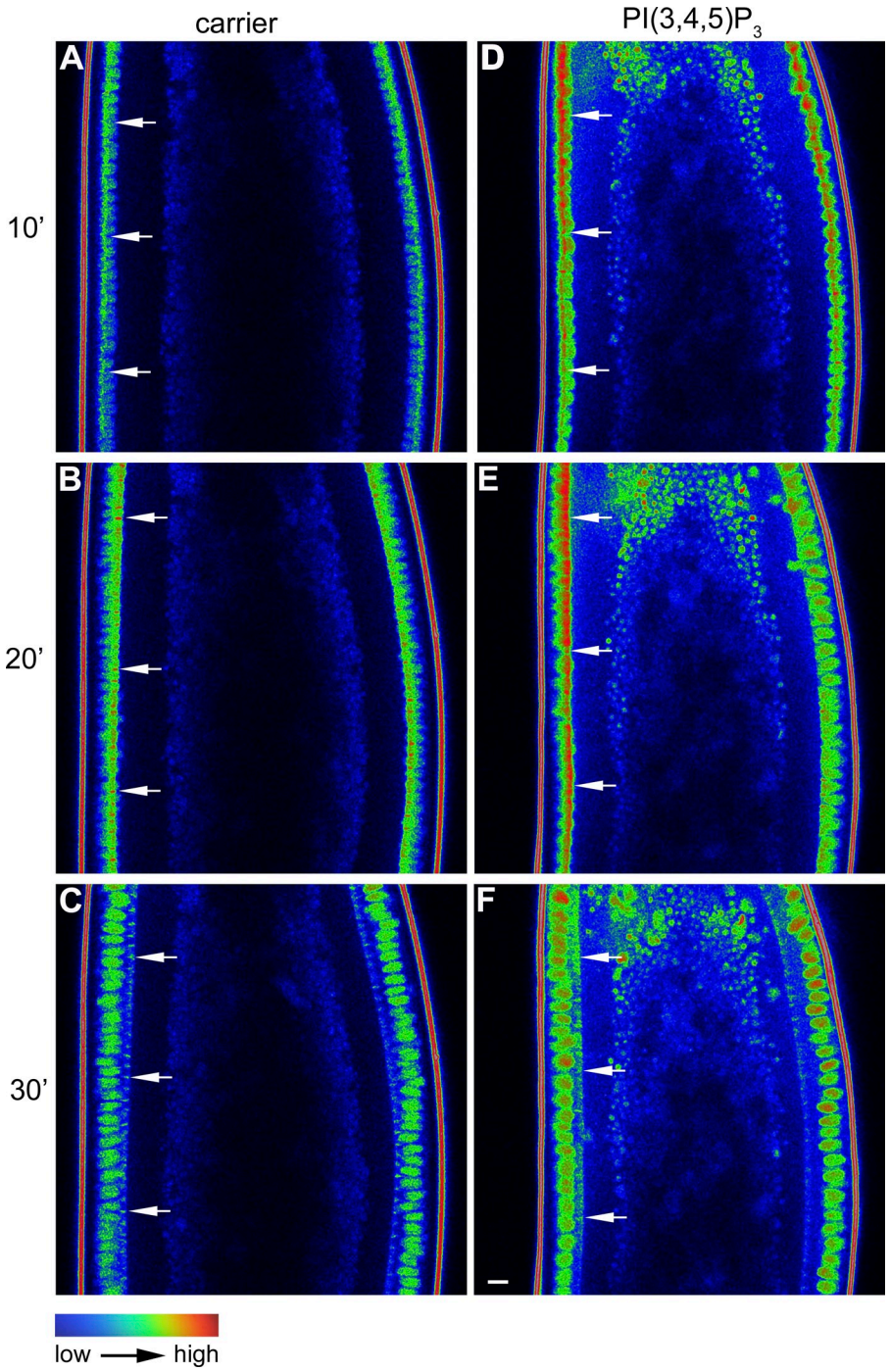


Figure S4. **PI(3,4,5)P_3/AM injection causes increased Bottleneck recruitment.** (A) Still frames from time-lapse two-photon movies of embryos expressing YFP::Bnk, driven by its endogenous promoter, injected either with carrier (DMSO; A–C) or PI(3,4,5)P_3/AM (D–F) at the onset of cellularization. Grayscale 8-bit still images were pseudo-colored with the rainbow LookUp Table (LUT; ImageJ software) to produce false-color images. Pixels with a value of 0 are black and pixels with a value of 255 are red. Three different time points are shown: 10 min (A and D), 20 min (B and E), and 30 min (C and F). YFP::Bnk signal increases at the furrows (arrows) upon PI(3,4,5)P_3/AM injection, during the slow phase (D and E). Loss of Bnk from the furrows did not appear to be affected upon PI(3,4,5)P_3/AM injection (C and F). Bar, 10 µm.

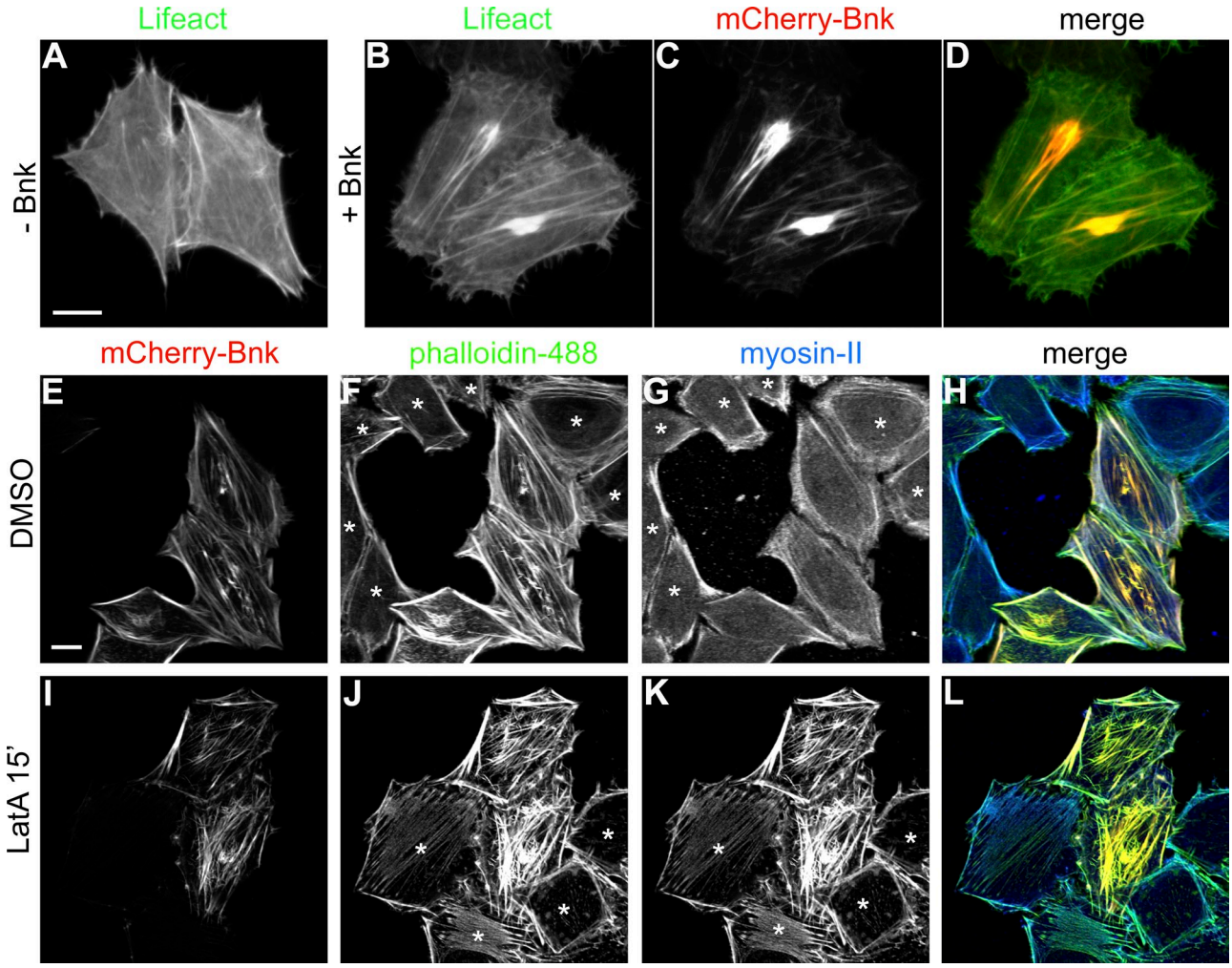
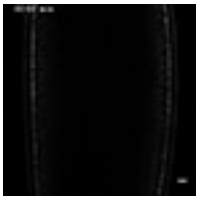
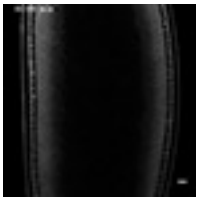


Figure S5. **Bottleneck acts as a stabilizer of actin filaments.** (A–D) Confocal images of HeLa cells expressing Lifeact alone (A) or together with mCherry::Bnk (B and C) showing the thickening of actin filaments induced upon Bnk expression and the colocalization between mCherry::Bnk and Lifeact in yellow (D). Bar, 10 µm. (E–L) Confocal images of HeLa cells treated with either DMSO (E–H) or latrunculin A (I–L) for 15 min, fixed, and stained with phalloidin 488 (F and J) and myosin-II antibody (G and K). In Bnk-transfected cells, stress fibers do not depolymerize. DMSO-treated cells are shown as a control. H and L correspond to the merge of E–G and I–K, respectively. Asterisks in E–L indicate nontransfected cells. Bar, 10 µm.

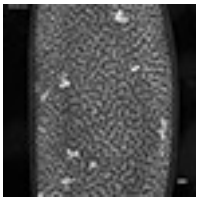
Video 1. **Sqh::GFP dynamics in a control-injected embryo.** Control-injected embryo expressing Sqh::GFP (white) imaged in an optical cross section over the course of cellularization by two-photon microscopy (LSM 780 NLO; Carl Zeiss). Frames were acquired every 2 s for 1 h. Bar, 10 µm.



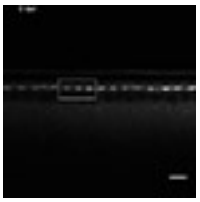
Video 2. **Sqh::GFP dynamics in a PI(4,5)P_2-injected embryo.** PI(4,5)P_2/AM-injected embryo expressing Sqh::GFP (white) imaged in an optical cross section over the course of cellularization by two-photon microscopy (LSM 780 NLO; Carl Zeiss). Frames were acquired every 2 s for 1 h. Bar, 10 µm.



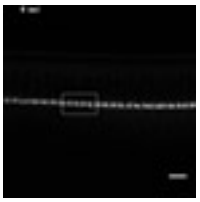
Video 3. **Sqh::GFP dynamics in a PI(3,4,5)P_3-injected embryo.** PI(3,4,5)P_3/AM-injected embryo expressing Sqh::GFP (white) imaged in an optical cross section over the course of cellularization by two-photon microscopy (LSM 780 NLO; Carl Zeiss). Frames were acquired every second for 1 h. Bar, 10 µm.



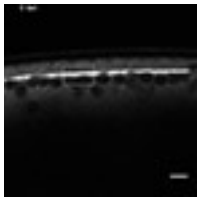
Video 4. **4D imaging of Sqh::GFP.** Z-stack projection (z = 1 µm for a total of 70 µm) of Sqh::GFP (white)-expressing embryo over the course of cellularization imaged by two-photon microscopy (LSM 780 NLO; Carl Zeiss). Frames were acquired every 3.3 s/focal plane for 1 h. Bar, 10 µm.



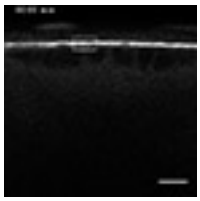
Video 5. **Fluorescence recovery after photobleaching of a control-injected embryo expressing Sqh::GFP during the slow phase of cellularization.** FRAP of a control-injected embryo during early stage of cellularization imaged by two-photon microscopy (LSM 780 NLO; Carl Zeiss). White box indicates the bleached region; Sqh::GFP (white). Frames were acquired every 300 ms for 3 min. Bar, 10 µm.



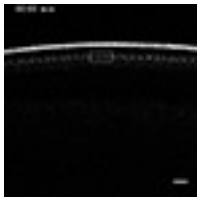
Video 6. **Fluorescence recovery after photobleaching of a control-injected embryo expressing Sqh::GFP during the fast phase of cellularization.** FRAP of a control-injected embryo during late stage of cellularization imaged by two-photon microscopy (LSM 780 NLO; Carl Zeiss). White box indicates the bleached region of interest; Sqh::GFP (white). Frames were acquired every 300 ms for 3 min. Bar, 10 µm.



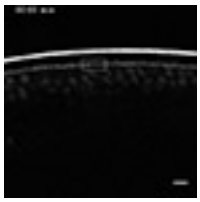
Video 7. **Fluorescence recovery after photobleaching of a PI(4,5)P_2/AM-injected embryo expressing Sqh::GFP during the slow phase of cellularization.** FRAP of a PI(4,5)P_2/AM-injected embryo during early stage of cellularization imaged by two-photon microscopy (LSM 780 NLO; Carl Zeiss). White box indicates the bleached region of interest; Sqh::GFP (white). Frames were acquired every 300 ms for 3 min. Bar, 10 µm.



Video 8. **Fluorescence recovery after photobleaching of a *bottleneck* mutant embryo expressing Sqh::GFP during the slow phase of cellularization.** FRAP of a *bottleneck* mutant embryo during the slow phase of cellularization imaged by two-photon microscopy (LSM 780 NLO; Carl Zeiss). White box indicates the bleached region of interest; Sqh::GFP (white). Frames were acquired every 200 ms for 3 min. Bar, 10 µm.



Video 9. **Fluorescence recovery after photobleaching of a control-injected embryo expressing YFP::Bnk.** FRAP of a control-injected embryo during the slow phase of cellularization imaged by two-photon microscopy (LSM 780 NLO; Carl Zeiss). White box indicates the bleached region; YFP::Bnk (white). Frames were acquired every 400 ms for 2 min. Bar, 10 µm.



Video 10. **Fluorescence recovery after photobleaching of a wortmannin-injected embryo expressing YFP::Bnk.** FRAP of a wortmannin injected embryo during the slow phase of cellularization imaged by two-photon microscopy (LSM 780 NLO; Carl Zeiss). White box indicates the bleached region; YFP::Bnk (white). Frames were acquired every 400 ms for 2 min. Bar, 10 µm.