Bastos et al., http://www.jcb.org/cgi/content/full/jcb.201204107/DC1

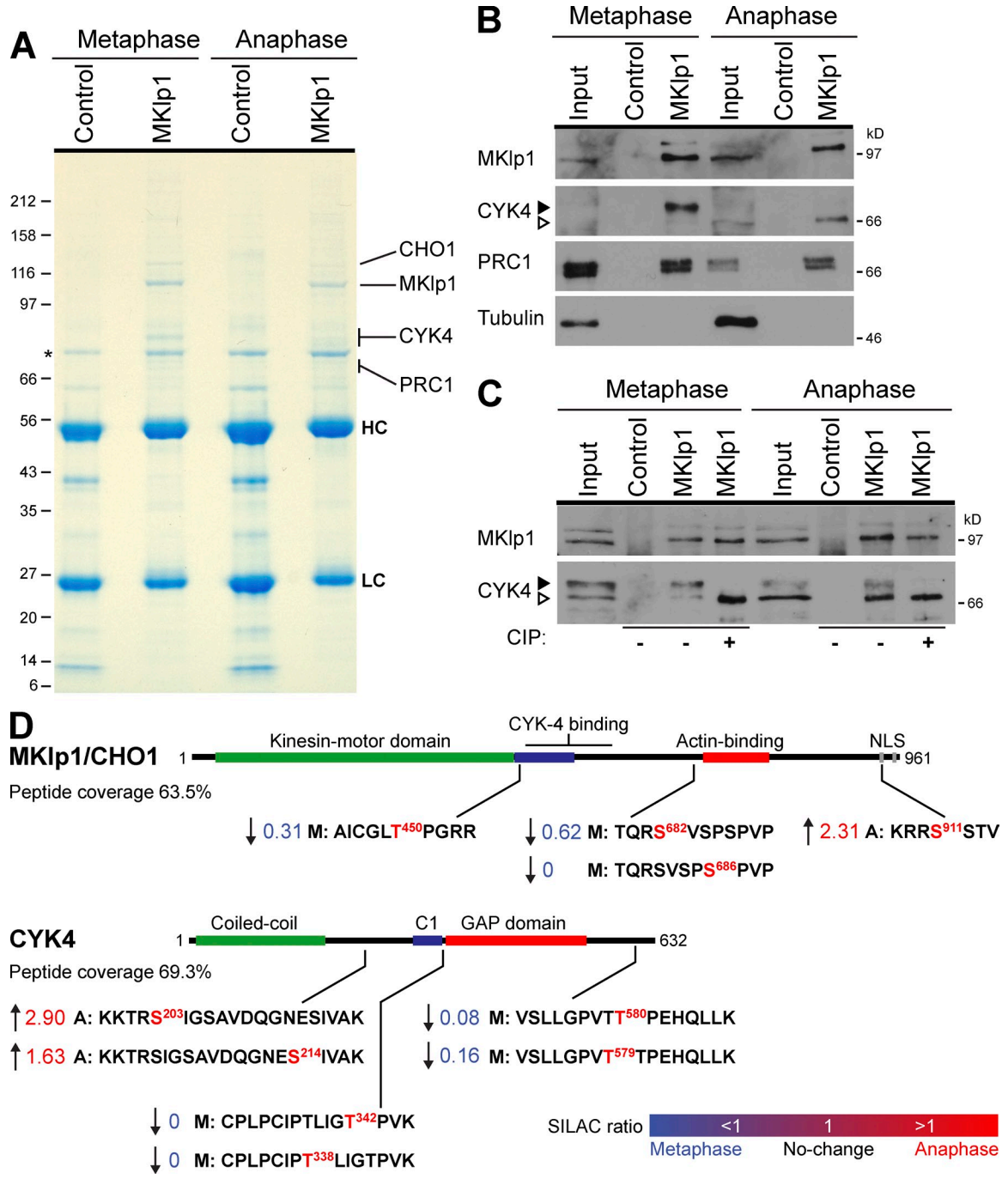


Figure S1. **Analysis of centralspindlin phosphorylation in metaphase and anaphase.** (A) Endogenous MKlp1–CYK4 centralspindlin complexes were immune precipitated using MKlp1 antibodies from synchronized populations of HeLa cells in interphase, metaphase, or anaphase states. Control isolations were performed using GFP antibodies. The asterisk marks a nonspecific contaminant, HC stands for IgG heavy chain, and LC stands for IgG light chain. (B) These complexes were analyzed by Western blotting with MKlp1, CYK4, PRC1, and tubulin antibodies. The filled and open arrowheads mark the metaphase and anaphase forms of CYK4, respectively. (C) Metaphase and anaphase MKlp1–CYK4 centralspindlin complexes were incubated with calf intestinal phosphatase (CIP) and then analyzed by Western blotting with MKlp1 and CYK4 antibodies. The filled and open arrowheads mark the metaphase and anaphase forms of CYK4, respectively. Note the calf intestinal phosphatase–treated metaphase CYK4 has the same mobility as anaphase CYK4. Molecular mass standards are indicated in kilodaltons in A–C. (D) HeLa cells were grown in medium containing "light" or "heavy" arginine and lysine as described in the Materials and methods. These light- and heavy-labeled cell populations were synchronized and arrested in metaphase using nocodazole or released into anaphase for 45–60 min by washing out the nocodazole, respectively. Equal numbers of cells were then combined and lysed, and the cell lysates were used for MKlp1 immune precipitations. The MKlp1 centralspindlin complexes were then analyzed by SDS-PAGE page and MS. MaxQuant v1.0.13.13 was used for the SILAC analysis. The SILAC ratios for the phosphorylation sites identified are shown on the schematics of MKlp1 and CYK4. High coverage was obtained for both proteins in this analysis. M, metaphase; A, anaphase.

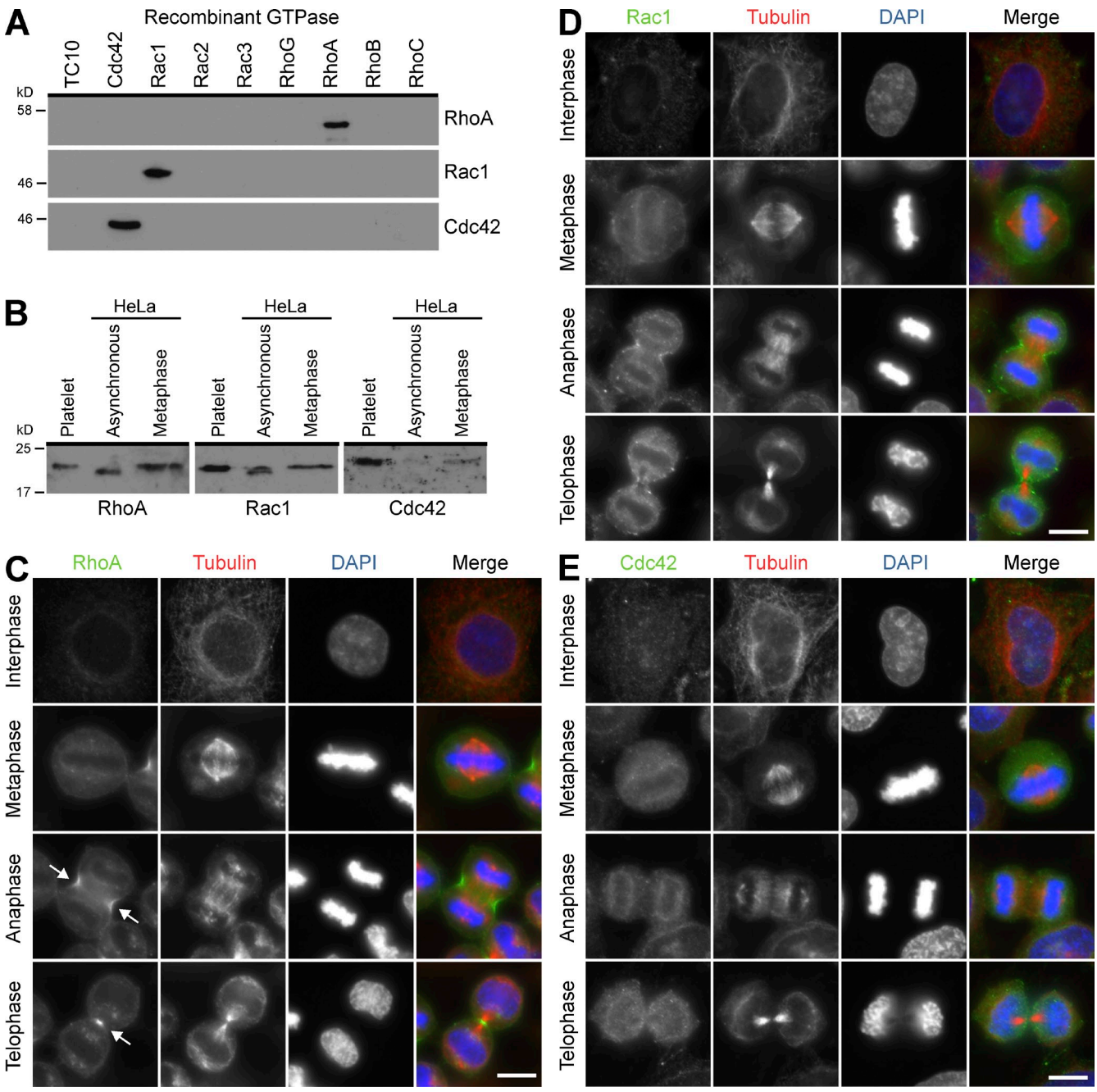


Figure S2. **Rho family GTPase in HeLa cells.** (A) The specificity of RhoA, Rac1, and Cdc42 antibodies was tested against a panel of Rho family GTPases. A 100-ng aliquot of recombinant GST-tagged GTPase was used for Western blotting as indicated in the figure. (B) Western blotting of 10 µg platelet, asynchronous, or mitotic HeLa cell extracts using Rac1-, RhoA-, or Cdc42-specific antibodies. (C–E) HeLa cells were fixed with TCA and then stained for RhoA (C), Rac1 (D), or Cdc42 and tubulin (E). DAPI was used to detect DNA. Cells at different stages of mitosis and cytokinesis are shown. Arrows mark RhoA staining in the cleavage furrow region in anaphase and telophase cells. Bar, 10 µm.

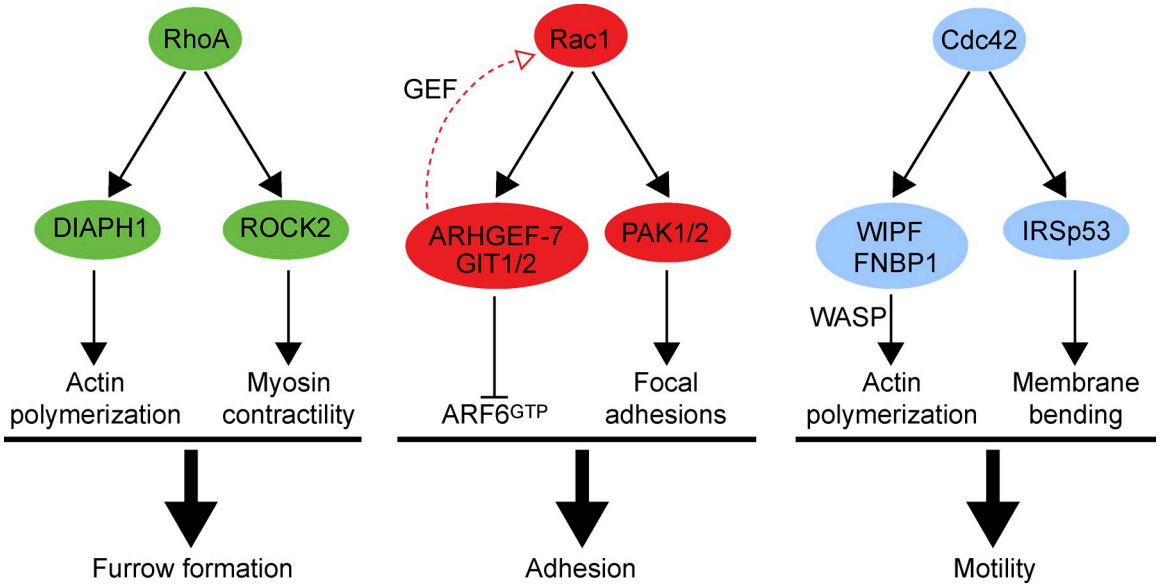


Figure S3. **Schematic depicting the RhoA, Rac1, and Cdc42 effector pathways identified.** The different effectors identified for RhoA, Rac1, and Cdc42 are depicted in the figure. Molecular functions associated with the different effectors are indicated, together with the cellular outcome (contraction, adhesion, or motility). ARHGEF7 may act as a Rac1 GEF and could therefore feed back and further activate Rac1. WASP, Wiskott–Aldrich syndrome protein.

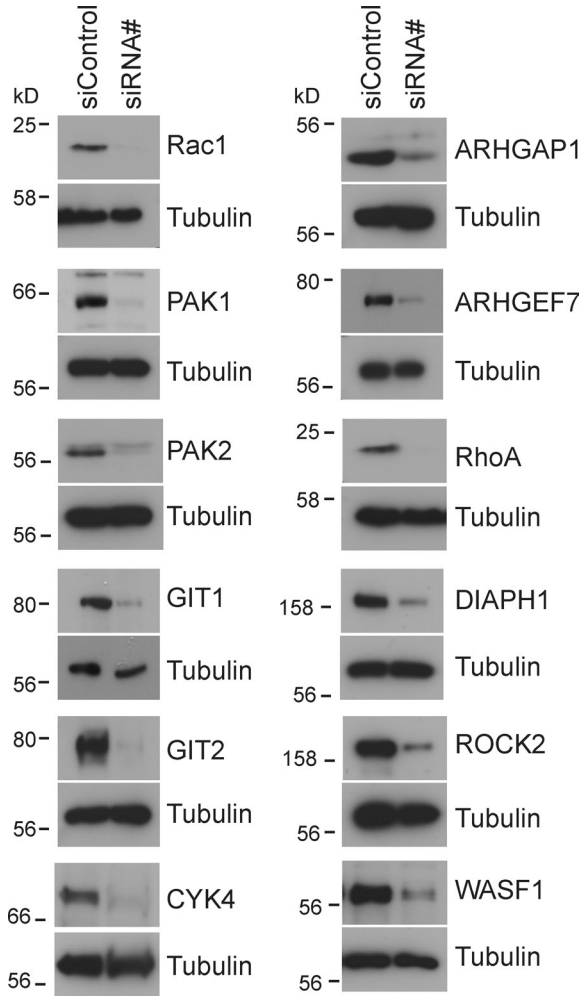


Figure S4. **Western blot analysis of RhoA and Rac1 effector proteins.** HeLa cells were treated for 48 h with siRNA duplexes targeting the various RhoA and Rac1 effector proteins shown in the figure. Total cell extracts were then Western blotted with specific antibodies to test for depletion efficiency and tubulin antibodies to confirm equal sample loading. siControl, nonsilencing control.

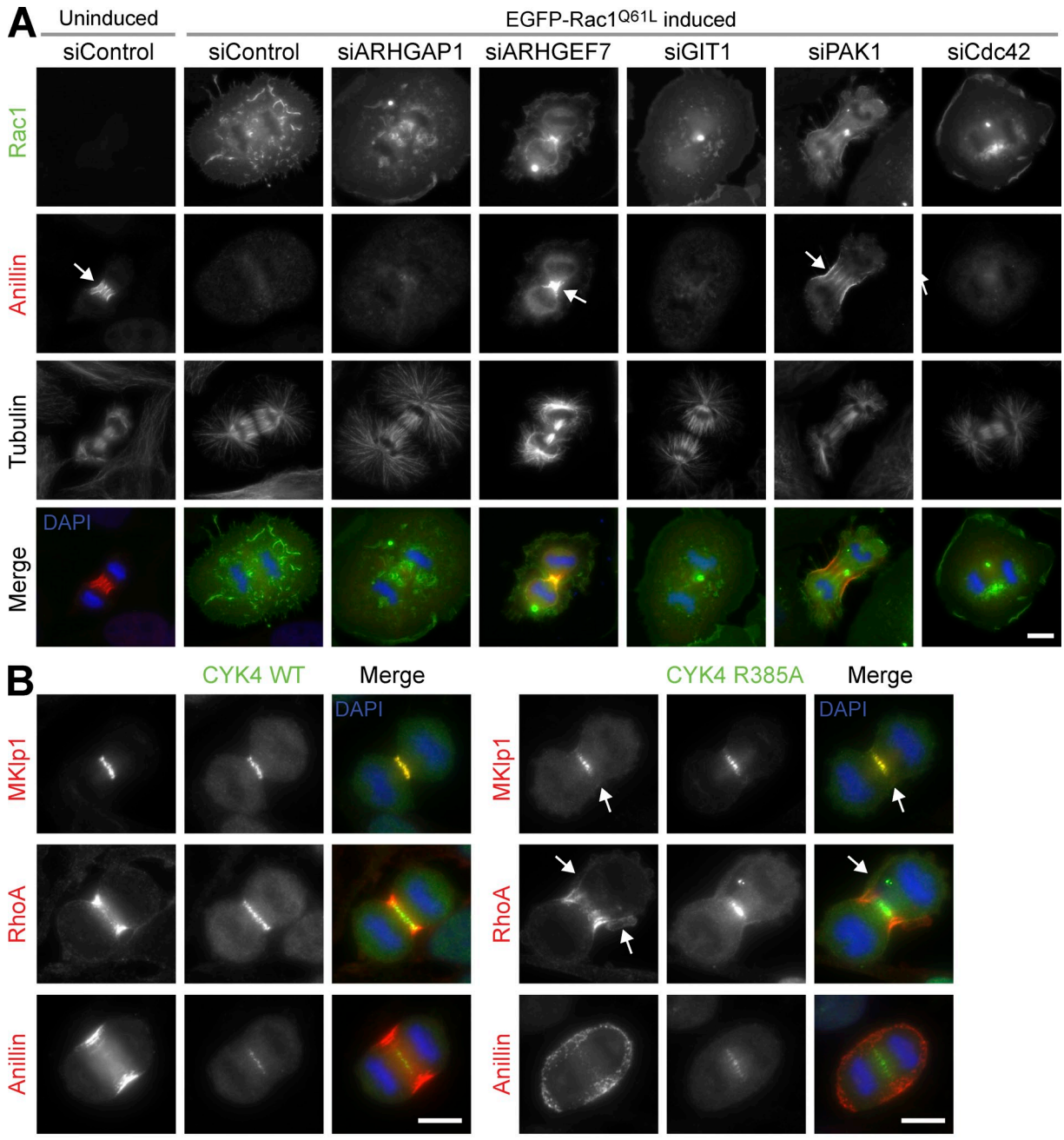
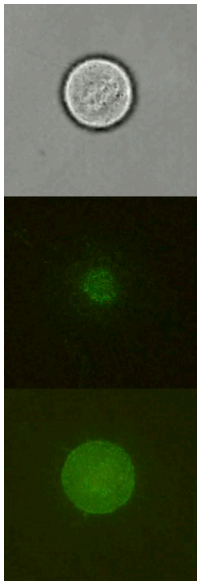
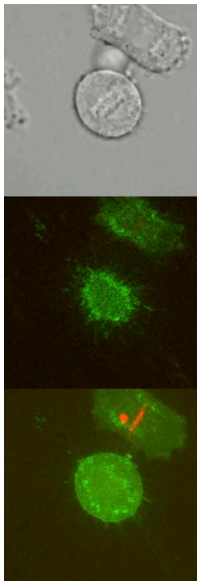


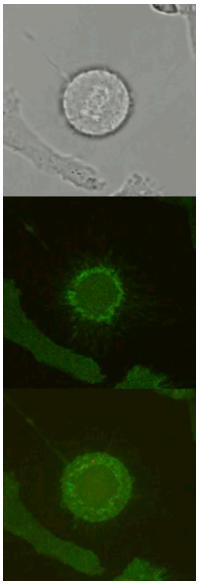
Figure S5. **Depletion of Rac1 effectors rescues anillin localization and furrow formation in cells expressing hydrolysis-defective Rac1.** (A) HeLa cells expressing inducible EGFP-Rac1^{Q61L} were transfected with siRNA duplexes targeting Rac1-specific effectors and Cdc42. Rac1^{Q61L} expression was induced for 48 h, and then, the cells were fixed and stained with antibodies to anillin and tubulin or DAPI to detect DNA. Rac1 was directly visualized by EGFP fluorescence. Arrows indicate anillin staining in the cleavage furrow. Bars, 10 µm. (B) HeLa cells expressing inducible EGFP-CYK4 or GAP activity–defective CYK4^{R385A} were transfected with control or CYK4 3′-UTR siRNA duplexes. CYK4 expression was induced for 60 h, and then, the cells were fixed and stained with antibodies to MKlp1, RhoA, or anillin and with DAPI to detect DNA. CYK4 was visualized using antibodies to EGFP. Arrows indicate membrane protrusions observed in CYK4^{R385A}-expressing cells. siControl, nonsilencing control; WT, wild type.



Video 1. **Localization of activated Rac1 in anaphase cells expressing wild-type CYK4.** HeLa cells expressing EGFP-PAK1 CRIB domain and inducible mCherry-CYK4 were transfected with CYK4 3′-UTR siRNA duplexes then used for time-lapse imaging. Images corresponding to 25 planes spaced by 0.7 μm through the cell volume were collected every minute using a spinning-disk confocal microscope and are shown for 120 min. A bright-field image (top) is shown with the EGFP-PAK1 (green) and mCherry-CYK4 (red) signals at the bottom section where the cells touch the glass growth surface (middle), and a maximum intensity projection is shown along the z axis of all sections (bottom).



Video 2. **Localization of activated Rac1 and early cytokinesis failure in cells expressing GAP mutant CYK4.** HeLa cells expressing EGFP-PAK1 CRIB domain and inducible mCherry-CYK4 R385A were transfected with CYK4 3′-UTR siRNA duplexes then used for time-lapse imaging. Images corresponding to 25 planes spaced by 0.7 μm through the cell volume were collected every minute using a spinning-disk confocal microscope and are shown for 120 min. A bright-field image (top) is shown with the EGFP-PAK1 (green) and mCherry-CYK4 (red) signals at the bottom section where the cells touch the glass growth surface (middle), and a maximum intensity projection is shown along the z axis of all sections (bottom). The cell shown fails cytokinesis within 60 min.



Video 3. **Localization of activated Rac1 and late cytokinesis failure in cells expressing GAP mutant CYK4.** HeLa cells expressing EGFP-PAK1 CRIB domain and inducible mCherry-CYK4 R385A were transfected with CYK4 3′-UTR siRNA duplexes then used for time-lapse imaging. Images corresponding to 25 planes spaced by 0.7 µm through the cell volume were collected every minute using a spinning-disk confocal microscope, and are shown for 120 min. A bright-field image (top) is shown with the EGFP-PAK1 (green) and mCherry-CYK4 (red) signals at the bottom section where the cells touch the glass growth surface (middle panel), and a maximum intensity projection is shown along the z axis of all sections (bottom). The cell shown fails cytokinesis at a late stage.

Table S1 shows a list of siRNA duplexes used in the study and is provided in an Excel file.