Chao et al., http://www.jcb.org/cgi/content/full/jcb.200911110/DC1

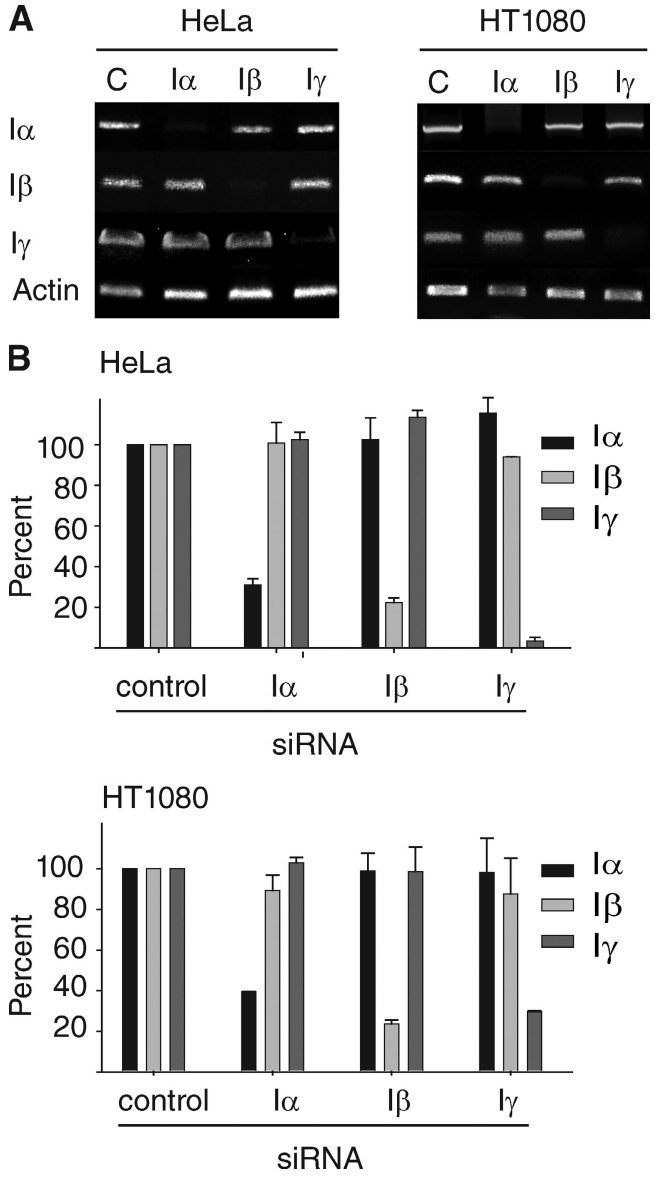

Figure S1. **Efficiency and specificity of mRNA and PIPKI-α protein knockdown.** (A) Quantitative RT-PCR was performed using gene-specific PCR primers to determine PIPK I-α, -β, and -γ (γ661 and γ635) mRNA levels in HeLa or HT1080 cells that were transfected with the indicated PIPKI-specific siRNAs or control (C) siRNAs. mRNA was isolated 48 h after siRNA treatment and subjected to RT-PCR analysis. Data are representative samples from three experiments. (B) Extent of PIPKI-α, -β, or -γ protein knockdown is shown. Cells were transfected with the indicated siRNA pools, and cell lysates were generated 48 h later and subjected to SDS-PAGE and Western blot analysis using isoform-specific antibodies. Quantitations of the Western blot analysis are shown. The data shown are representative of three independent experiments. Error bars indicate SEM.

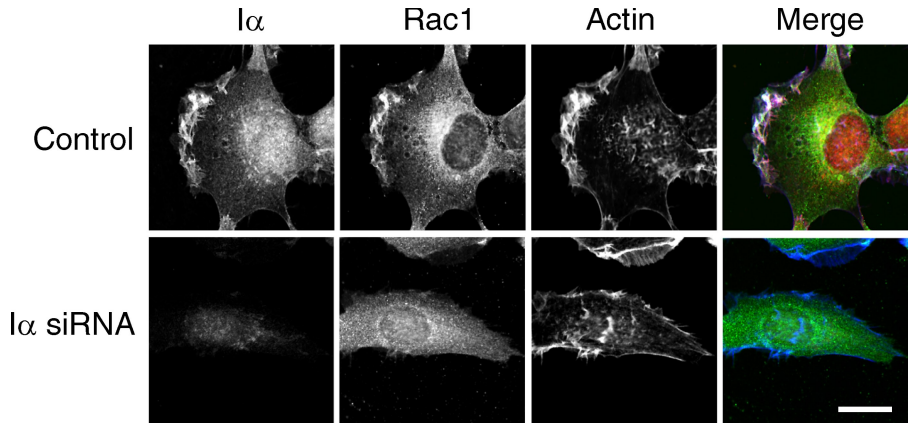

Figure S2. **PIPKI-α and Rac1 colocalize at the plasma membrane.** HT1080 cells were transfected with control siRNA or siRNAs targeting PIPKI-α. After 36 h, cells were fixed and stained with specific antibodies directed at PIPKI-α and Rac1, respectively. F-actin was visualized with Alexa Fluor 594–conjugated phalloidin. Endogenous PIPKI-α and Rac1 colocalize at membrane ruffles at the cell front. Upon PIPKI-α knockdown, Rac1 targeting to the plasma membrane is attenuated. Bar, 10 μm.

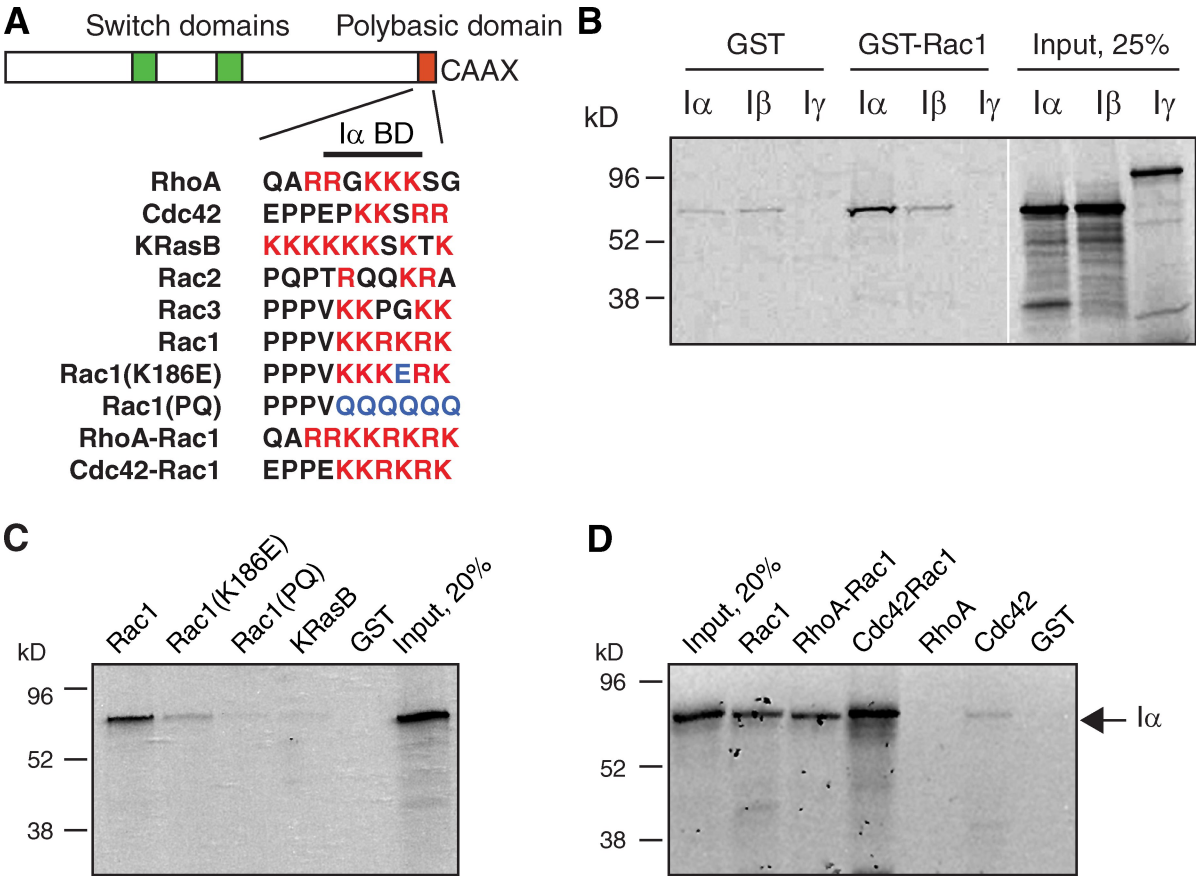

Figure S3. **PIPKI-α specifically and directly binds Rac1 via the polybasic tail of Rac1.** (A) Amino acid sequences of the C-terminal regions of Rac1 and related GTPases are shown. The polybasic domain is indicated by a black bar, basic residues are indicated in red, and amino acid substitutions in Rac1 mutants are indicated in blue. (B) ^{35}S-labeled PIPK Iα, -β, and -γ generated by in vitro transcription and translation were used in pull-down assays with purified recombinant GST, GST-Rac1, GST-RhoA, GST-CDC42, and GST-Arf6. Bound proteins were analyzed by SDS-PAGE and autoradiography. The white line indicates that intervening lanes have been spliced out. (C) ^{35}S-labeled PIPKI-α generated by in vitro transcription and translation was used in pull-down assays with purified recombinant GST or with GST fusions to wild-type Rac1 or the mutant Rac1 variants Rac1(K186E) and Rac1(PQ) as well as K-RasB. Bound proteins were analyzed as above. (D) ^{35}S-labeled PIPKI-α was used in pull-down assays with GST fusions to wild-type Rac1, Cdc42, RhoA, and the Cdc42-Rac1 and RhoA-Rac1 chimeras containing the Rac1 polybasic domain. Bound proteins were analyzed as above.

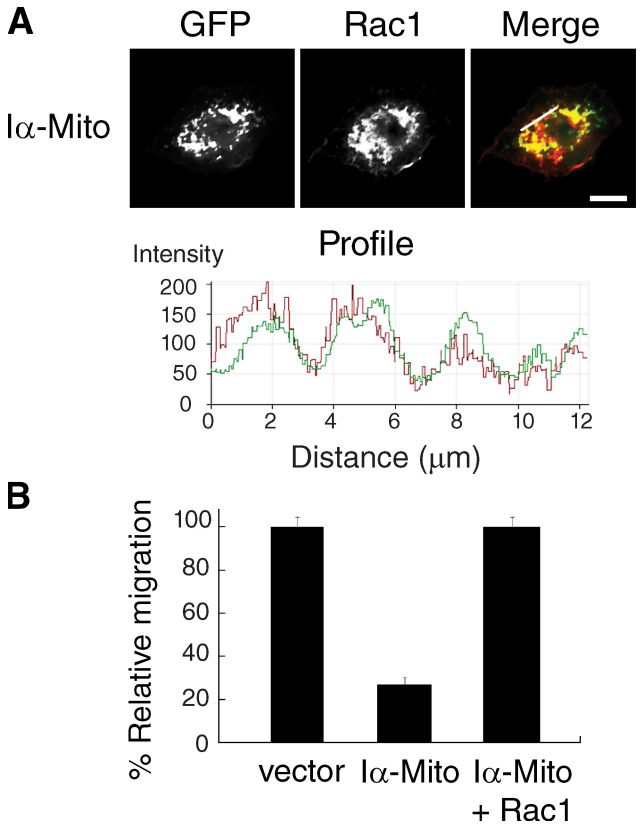

Figure S4. **Mitochondrial targeting of PIPKI-α affects Rac1 subcellular localization and chemotaxis.** (A) HeLa cells transiently transfected with EGFP–Iα-Mito were fixed and stained with mouse anti-Rac1 antibodies followed by Cy3-conjugated goat anti–mouse antibodies to visualize endogenous Rac1 protein (middle). GFP fluorescence (left) was recorded directly. (right) Fluorescence intensity profiles were determined along the line drawn. Colors indicate Rac1 (red) and EGFP–Iα-Mito (green) fluorescence. Bar, 10 μm. (B) HeLa cells transiently transfected with EGFP-tagged Iα-Mito, empty EGFP-OMP25 vector, or the combination of EGFP–Iα-Mito and Flag-Rac1 were serum starved, loaded into the top well of a Transwell chamber, and allowed to migrate for 2 h at 37°C in response to serum (10% FBS) present in the lower chamber. The number of cells passing through the membrane was determined. Results are shown as a relative percentage of migrated cells. Error bars indicate SEM (n = 3).

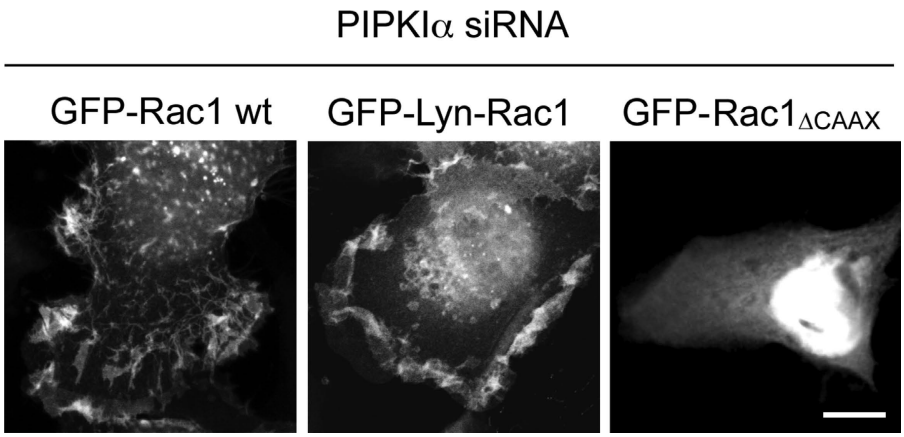

Figure S5. **Targeting of GFP-tagged Rac1 variants in PIPKI-α–depleted cells.** HT1080 cells treated with PIPKI-α–specific siRNAs were transiently transfected with EGFP-tagged wild-type (wt) Rac1, Lyn-Rac1, or a Rac1 mutant lacking the CAAX motif (ΔCAAX). Cells were fixed, and GFP fluorescence was recorded. Bar, 10 μm.