Supplemental materials

Wong et al., http://www.jcb.org/cgi/content/full/jcb.200706167/DC1

Role of microtubules and PIP3

We asked in dHL60 cells whether PIP3 and microtubule regulate the spatial distribution of PRG by treating cells with a PI3K inhibitor, PIK-90 (Knight et al., 2006; Van Keymeulen et al., 2006), or a drug that disrupts microtubules, nocodazole (Fracek and Margulis, 1979). Cells lacking PIP3 make multiple pseudopods after stimulation with fMLP (16/37 cells). The distribution of PRG-YFP in these cells (not depicted) is similar to that seen in blebbistatin-treated cells, in agreement with

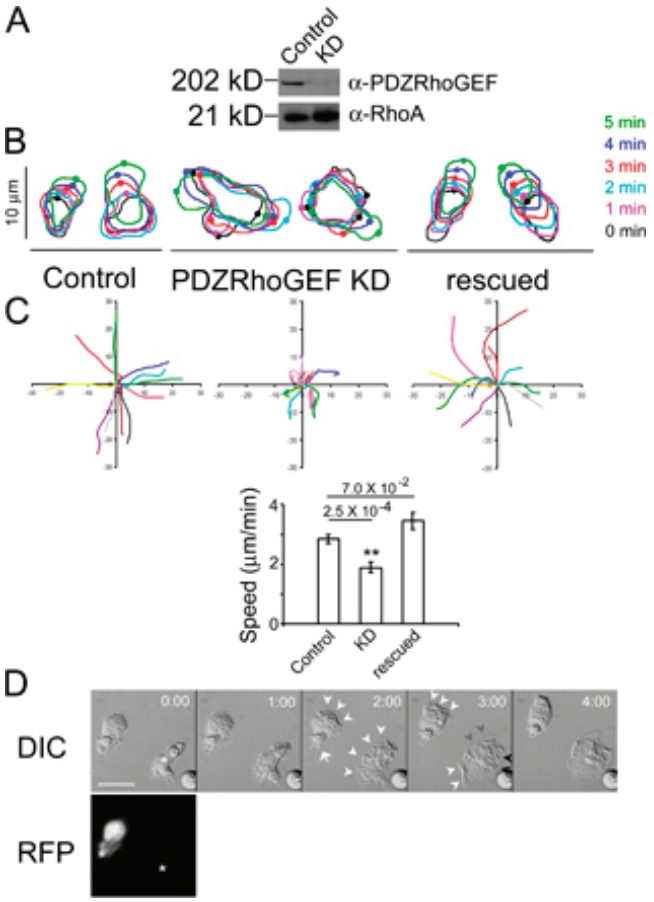

Figure S1. **Protein expression of endogenous PRG in control or PRG KD cells.** Whole cell lysates were prepared from these groups and protein level revealed by Western blotting using antibody specific to PDZRhoGEF. RhoA was used as a loading control. (B) Outlines of representative control, KD, and KD expressing Myc-rPRG (rescued) cells at the indicated time (color coded) during the initial 5 min of stimulation with uniform concentration of 100 nM fMLP. Small circle depicts center of a protruding pseudopod at the corresponding time (second panel). (C) Trajectories of migrating control (11), KD (13), and rescued (13) cells during the same time period. Different colors depict different cells (third panel). Plot of average migration speed of control (n = 11), KD (n = 20), and rescued (n = 13) cells during 0 to 5 min after uniform stimulation with 100 nM fMLP (bottom panel). P values are as indicated. ** marks statistical significance based on t test ($P < 0.001$). Error bars represent ± 1 SEM. (D) Expression of Myc-rPRG rescues multiple-front phenotype in PRG knock-down cells. Cells stably expressing PRG shRNA were transiently cotransfected with Myc-rPRG and RFP (to mark transfected cells). Time-lapse images of DIC and fluorescent images of representative cell expressing and not expressing (asterisk) Myc-rPRG are shown. Time indicates the duration after addition of 100 nM fMLP. Different color of arrowheads marks different pseudopod in each cell. Arrow depicts back of the cell. Bar = 10 μm.

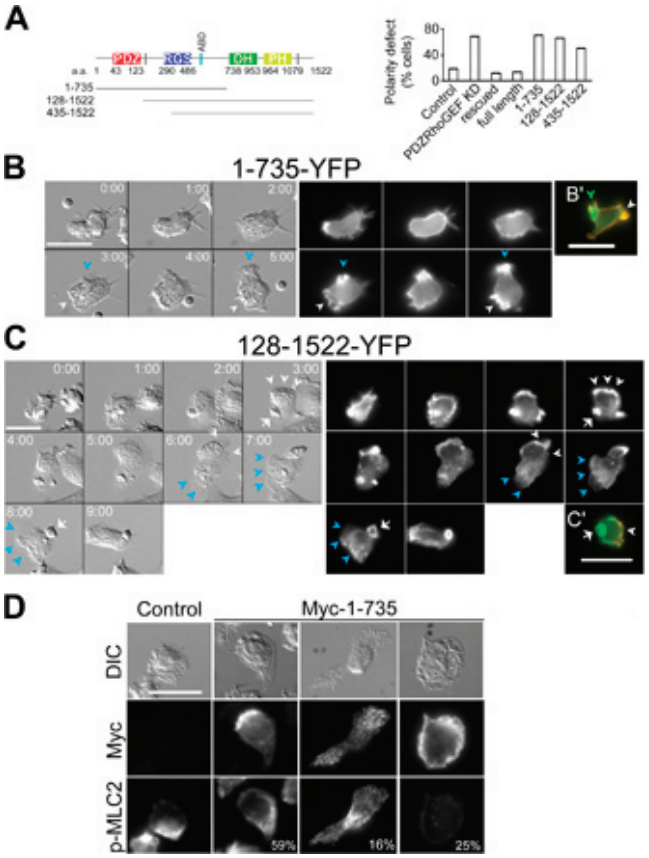

Figure S2. **Expression of truncated PRG mutants disrupts fMLP-induced polarity.** (A) Left, schematic domain structures of full-length PRG and truncation mutants. PDZ, PSD95/DlgA/ZO-1 domain; RSG, regulator of G protein signaling domain; DH, Dbl homology domain; PH, pleckstrin homology domain; ABD, actin-binding domain; gray bars, proline rich domains. Numbers depict the amino acid positions in the full-length protein. The truncation mutants contain sequences indicated by the lines associated with them. Right, graph summarizing percent of cells that showed polarity defect after 3 min uniform stimulation with 100 nM fMLP in control (n = 110), cells expressing shRNA targeting PRG without (n = 213) or with exogenous rat PRG (rescued; n = 48), and cells expressing C-terminal YFP-tagged full-length (n = 58), 1–735 (n = 70), 128–1522 (n = 28), or 435–1522 (n = 24) PRG. (B and C) DIC and fluorescent time-lapse images of dHL60 cells expressing YFP-tagged (B) 1–735 or (C) N-terminal truncated (128–1522-YFP) PRG mutants. Fluorescent overlays of (B') 1–735-YFP or (C') 128–1522-YFP (green) expressing cells and actin (red) after fMLP stimulation, fixed and processed as in Fig. 1 C. Time indicates elapsed time after uniform stimulation with 100 nM fMLP. Different arrowhead colors indicate separate pseudopod. Arrow points to the back. (D) Localization of p-MLC2 in dHL60 cells expressing Myc-1–735 PRG. Cells were processed as above and stained for myc and p-MLC2. DIC and fluorescent images of representative cells are shown. Percentages in the lower right hand corner represent relative frequencies of each phenotype in 60 cells expressing the mutant construct. Bars = 10 μm.

our previous observation (Van Keymeulen et al., 2006) that fMLP-dependent activation of RhoA depends on PIP3.

We also explored possible roles of microtubules in regulating localization of PRG. Unlike normal cells treated with nocodazole, most nocodazole-treated PRG-YFP-expressing cells (34 of 47 cells examined) fail to polarize after fMLP stimulation. Instead of pseudopods, they form multiple pointed protrusions at various portions of the cell periphery; in these cells, PRG-YFP distributes all over the cell periphery (not depicted). A smaller proportion (13 of 47 examined) of nocodazole-treated cells show ability to polarize and to accumulate PRG-YFP to the back (unpublished data). Because the nocodazole treatment does completely disrupt dHL60 cell microtubules, the latter result suggests that microtubules are not required to exclude PRG from the front, although they may regulate PRG in other ways.

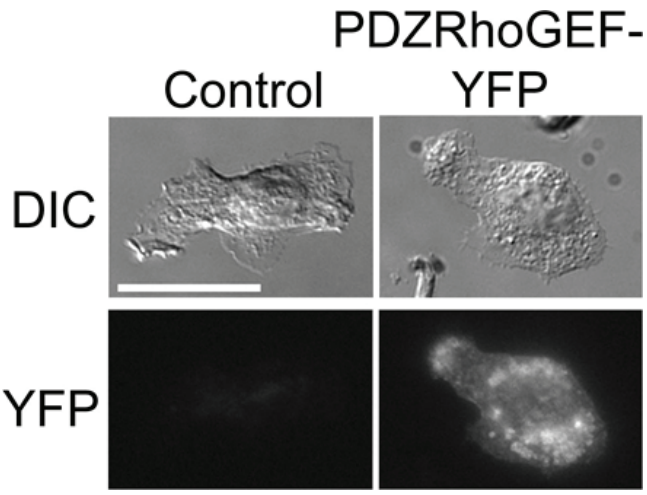

Figure S3. **Background fluorescence of blebbistatin.** dHL60 cells expressing or nonexpressing PRG-YFP were treated with 100 uM blebbistatin for 45 min, stimulated with 100 nM fMLP for 3 min, fixed, and imaged under the exact same conditions. Bar = 10 μm.

References

Fracek, S., and L. Margulis. 1979. Colchicine, nocodazole and trifluralin: different effects of microtubule polymerization inhibitors on the uptake and migration of endosymbiotic algae in Hydra viridis. *Cytobios.* 25:7–16.

Knight, Z.A., B. Gonzalez, M.E. Feldman, E.R. Zunder, D.D. Goldenberg, O. Williams, R. Loewith, D. Stokoe, A. Balla, B. Toth, et al. 2006. A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. *Cell.* 125:733–747.

Van Keymeulen, A., K. Wong, Z.A. Knight, C. Govaerts, K.M. Hahn, K.M. Shokat, and H.R. Bourne. 2006. To stabilize neutrophil polarity, PIP3 and Cdc42 augment RhoA activity at the back as well as signals at the front. *J. Cell Biol.* 174:437–445.