Supplemental Materials

Molecular Biology of the Cell

Isozaki et al.

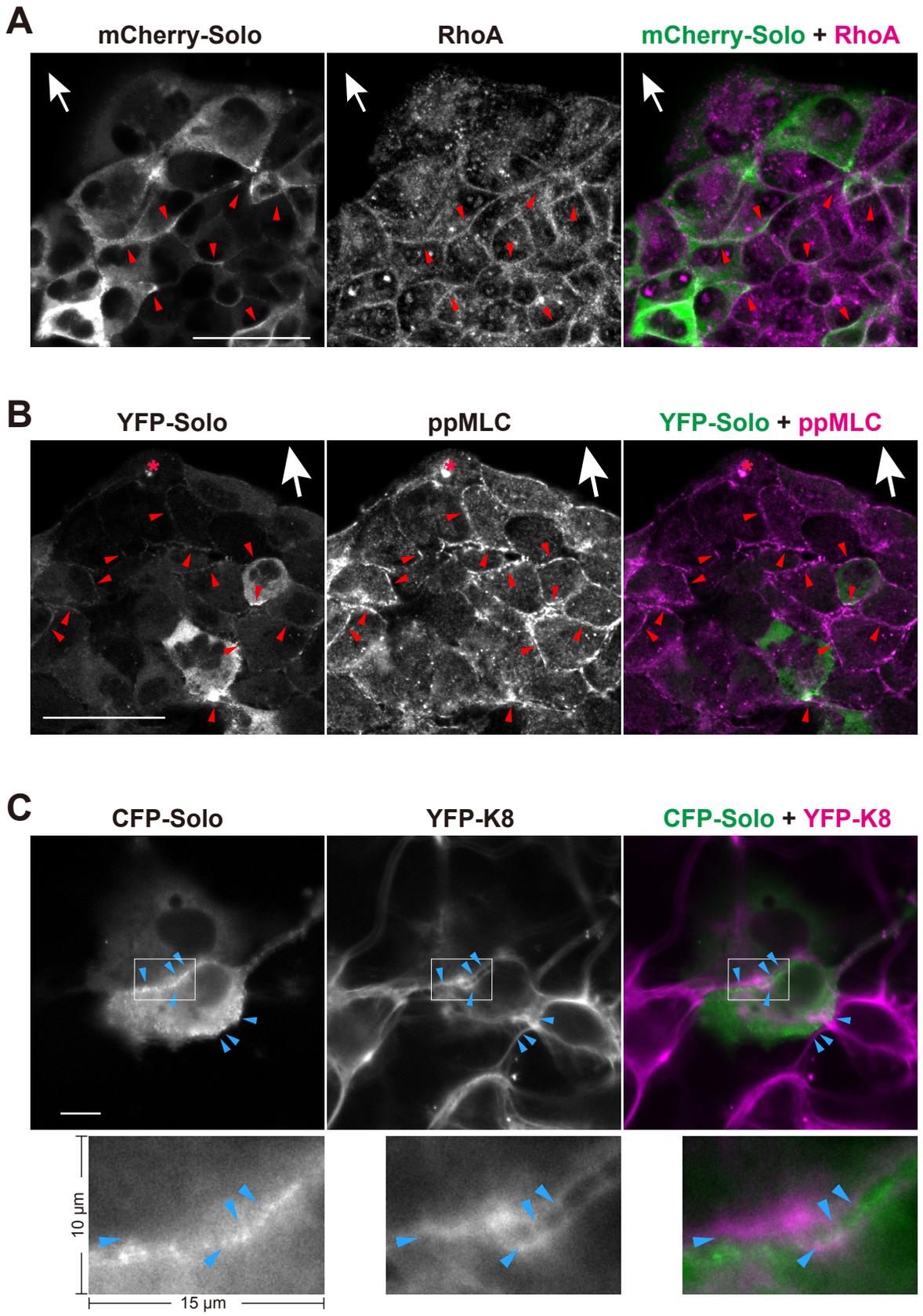
A
mCherry-Solo
RhoA
mCherry-Solo + RhoA
B
YFP-Solo
ppMLC
YFP-Solo + ppMLC
C
CFP-Solo
YFP-K8
CFP-Solo + YFP-K8
10 µm
15 µm

Figure S1: Localization of RhoA, ppMLC and K8 with Solo in collectively migrating cells. (A) Confocal immunofluorescence images of RhoA and mCherry-Solo at the cell-cell contact sites in finger-like protrusions. The collectively migrating MDCK cells, which transiently express mCherry-Solo, were fixed and stained with anti-RhoA antibody and Alexa-488 anti-mouse IgG secondary antibodies. The localization of RhoA and mCherry-Solo at the cell-cell contact sites were visualized with the Alexa-488 and mCherry fluorescence, respectively, in a single plane of cross-sectional images. White arrows indicate the putative migration direction. Red arrowheads indicate colocalization of Solo and RhoA at the cell-cell contact sites. Scale bars = 50 μm. (B) Confocal immunofluorescence images of YFP-Solo and dual-phosphorylated myosin right chain (ppMLC) at the cell-cell contact sites in finger-like protrusions. Collectively migrating YFP-Solo-expressing MDCK cells were fixed and stained with anti-ppMLC antibodies and Alexa-568 anti-mouse IgG secondary antibodies. The localization of ppMLC and Solo were visualized with the Alexa-568 and YFP fluorescence, respectively, in a single plane of cross-sectional images. White arrows indicate the putative migration direction. Red arrowheads indicate coaccumulation of Solo and ppMLC at the cell-cell contact sites. Scale bars = 50 μm. (C) Fluorescence images of CFP-Solo and YFP-K18 at the cell-cell contact sites. The collectively migrating YFP-K8-expressing MDCK cells, which were transiently transfected with CFP-Solo, were fixed. The localization of Solo and K8 filaments were visualized with CFP and YFP fluorescence, respectively. Images in lower panel represent magnified images of the white boxes in the images. Blue arrowheads indicate the relative localization of Solo and K8 bundles at the cell-cell contact sites.

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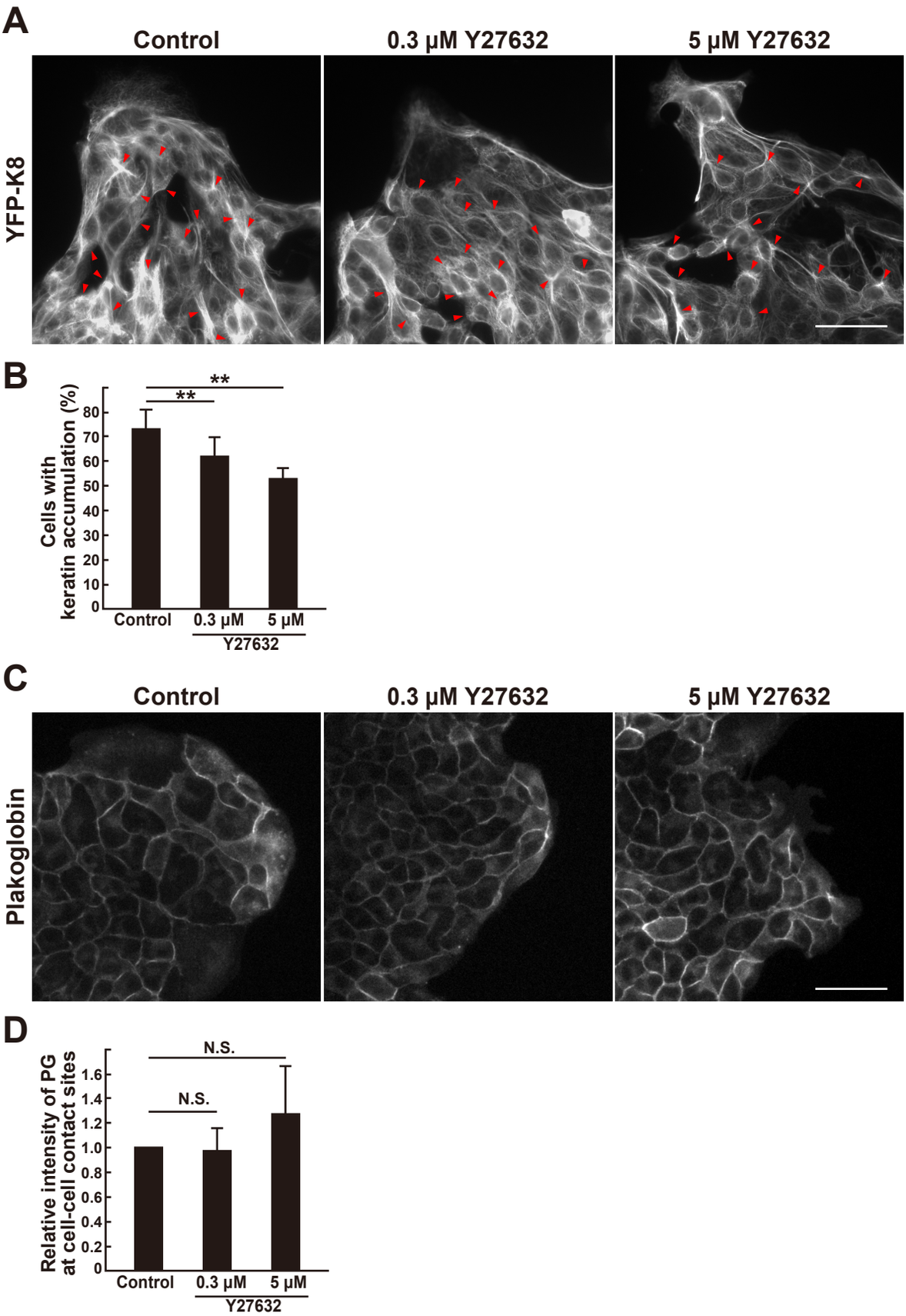

Figure S2: Effects of Y-27632 on K8 networks and plakoglobin in collectively migrating MDCK cells. (A) Fluorescence images of YFP-K8 in MDCK cells. Collectively migrating YFP-K8-expressing MDCK cells were exposed to Y-27632 at the indicated concentrations for 5 h. The cells were fixed and K8 networks were visualized with YFP fluorescence. Red arrowheads indicate the accumulation of K8 filaments in the interior cells. Scale bars = 50 μm. (B) Quantification of the percentage of interior cells with K8 accumulation in control and Y-27632-exposing finger-like protrusions. Data are mean ± SD of four independent experiments (30 cells/experiment) (C) Immunofluorescence images of PG at the cell-cell contact sites following treatment with the indicated concentrations of Y-27632 for 5 h. Collectively migrating MDCK cells were treated, as described in (A) and the cells were fixed and stained with anti-PG antibody. (D) Quantification of the relative fluorescence intensities of PG at the cell-cell contact sites in control and Y-27632-exposing cells. Scale bars = 50 μm. Data are mean ± SD of three independent experiments (100 cells/experiment). N.S., not significant (one-way ANOVA followed by Dunnett's test).

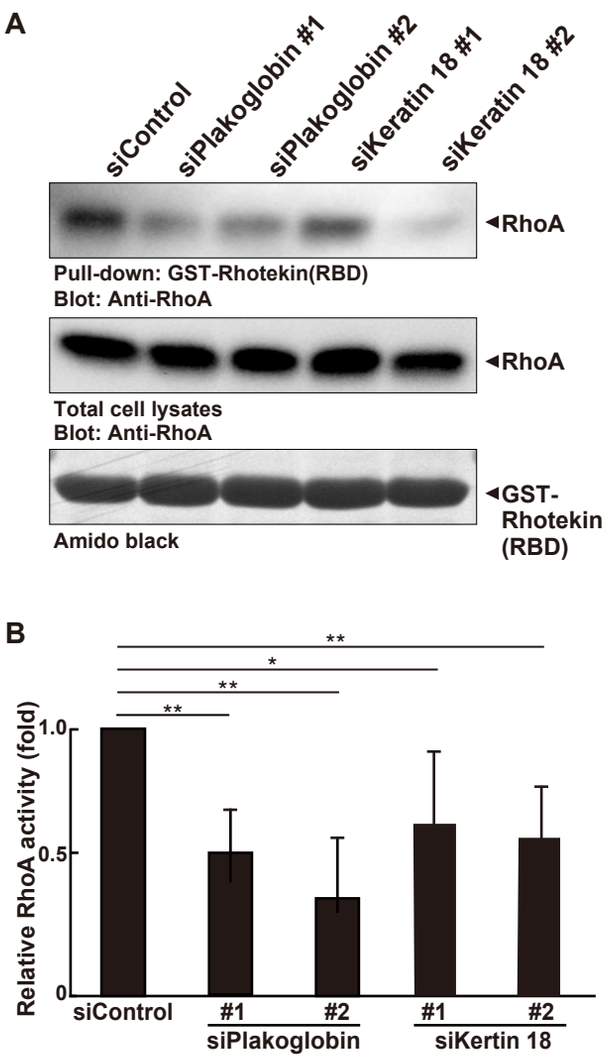

Figure S3: Effects of K18 or plakoglobin knockdown on the RhoA activity in MDCK cells. (A) Control, PG or K18 siRNA-transfected MDCK cells were treated under the conditions described in Fig. 6. Active RhoA was analyzed by the GST-RBD pulldown assays as in Fig. 6. (B) Quantification of the relative RhoA activities. The value of the control cells is set to 1.0. Each value is the mean ± SD of five independent experiments. *P < 0.05, and **P < 0.01 (one-way ANOVA followed by Dunnett's test).

Supplementary Information

Supplementary Movie 1

Time-lapse observation of finger-like protrusions comprising control or Solo siRNA-transfected MDCK cells on collagen gel. Images were obtained every 10 min. Trajectories of individual cells were overlaid. This movie corresponds to Fig. 1B. showing finger-like protrusions comprising control- or Solo siRNA-transfected MDCK cells.

Supplementary Movie 2

Time-lapse observation of control or Solo siRNA-transfected solitary cultured cells on collagen gel. Images were obtained every 10 min. Trajectories of the cells were overlaid. This movie corresponds to Fig. 2A.

Supplementary Movie 3

Time-lapse observation of finger-like protrusions comprising MDCK cells treated with the indicated dose of Y-27632. Images were obtained every 5 min. Trajectories of the cells were overlaid. This movie corresponds to Fig. 5A.

Supplementary Movie 4

Time-lapse observation of finger-like protrusions comprising the control, keratin-18- or plakoglobin-siRNA-transfected MDCK cells on collagen gel. Images were obtained every 5 min. Trajectories of individual cells were overlaid. This movie corresponds to Figs. 8C and 8D.