Veltman et al., http://www.jcb.org/cgi/content/full/jcb.201205058/DC1

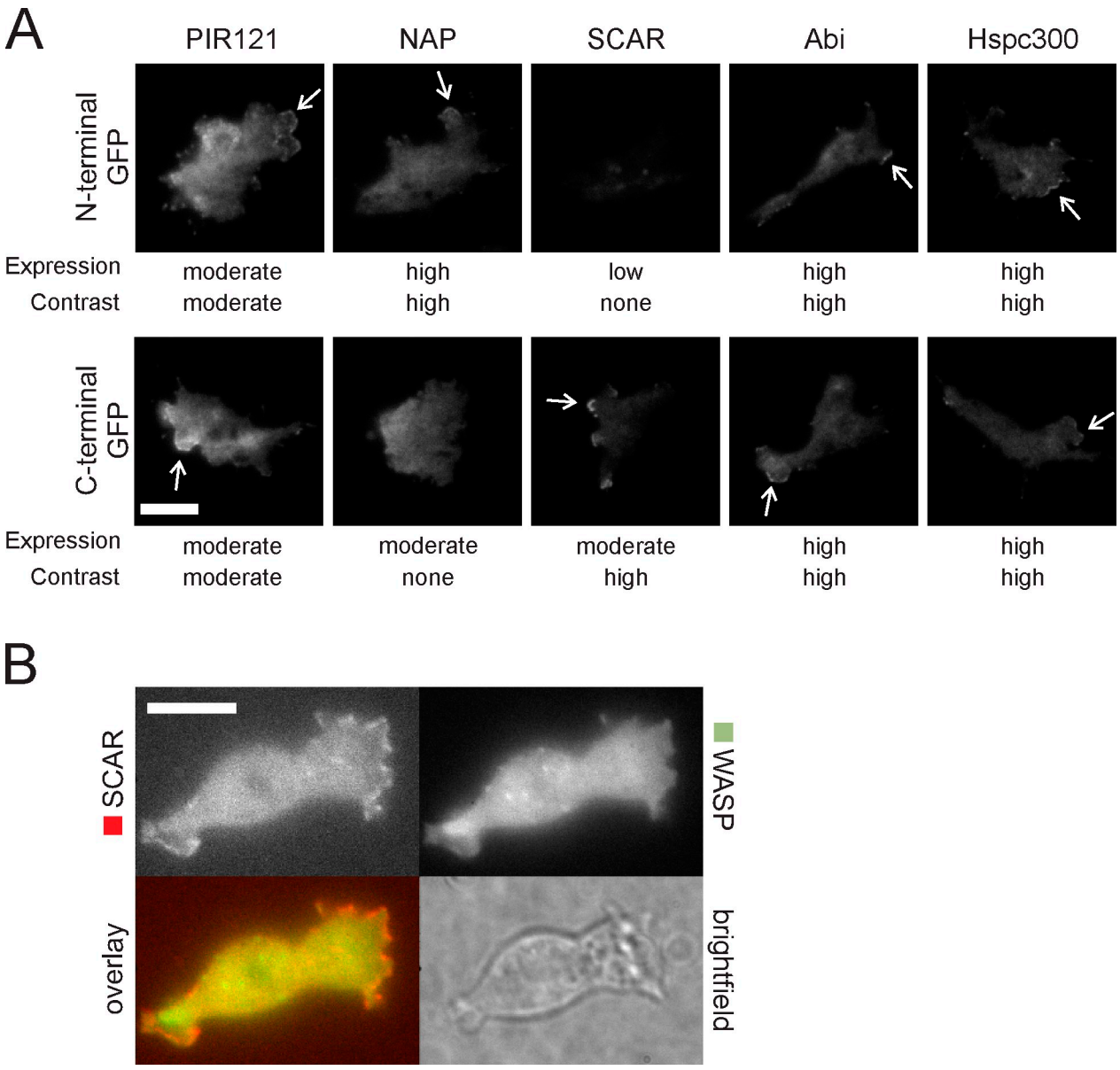


Figure S1. **Localization of SCAR complex and WASP in wild-type cells.** (A) Each different subunit of the SCAR complex was tagged with GFP at either the N terminus or C terminus. Constructs were transfected to wild-type cells and imaged on a TIRF microscope. Expression levels and the contrast of the signal at the leading edge compared with the cytosolic background were scored for each cell strain. Arrows mark active protrusions. (B) Visualization of the SCAR complex (SCAR C-terminally tagged with RFP) and WASP (N-terminally tagged with GFP) using wide-field epi-illumination. Bars, 5 µm.

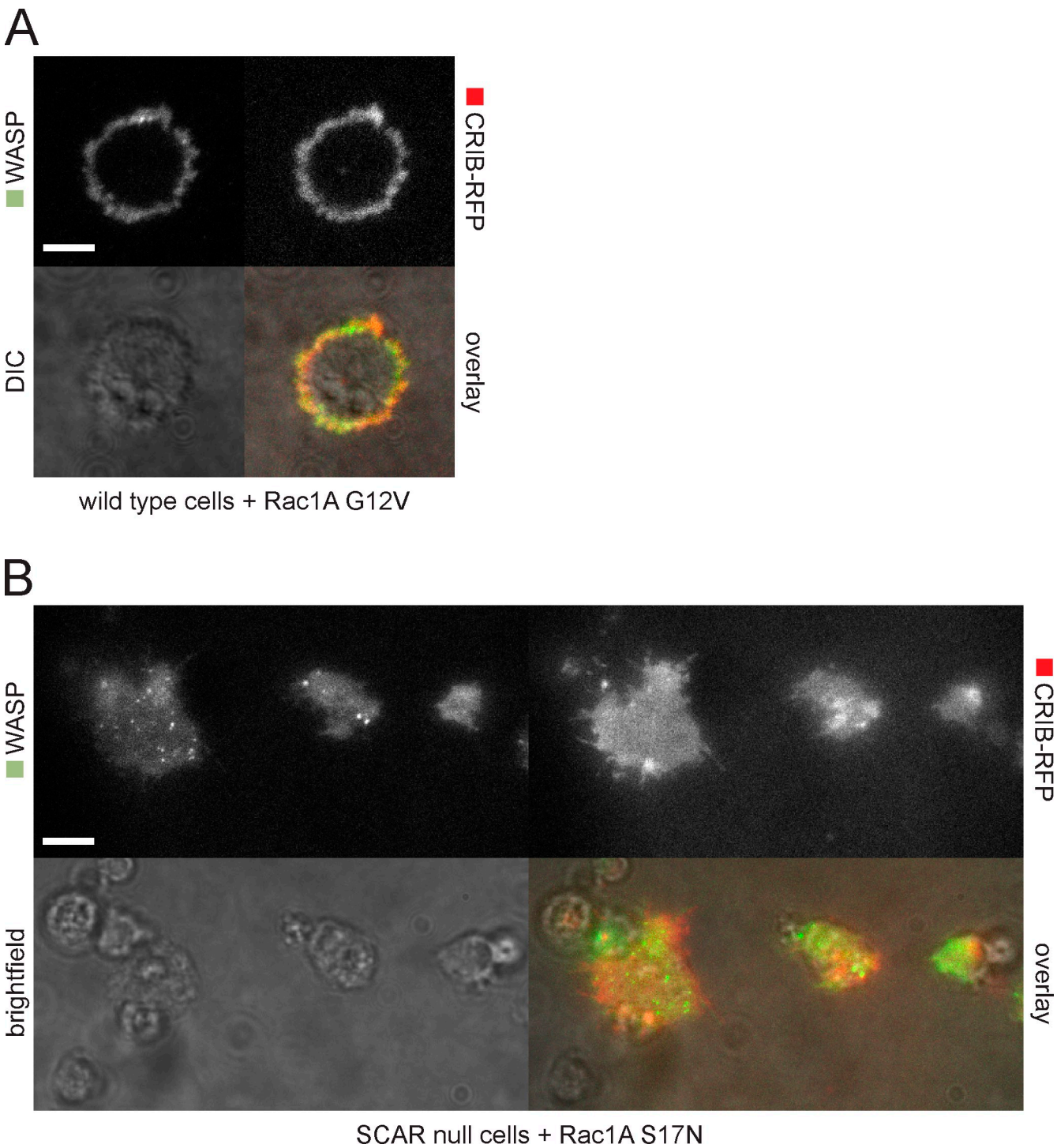
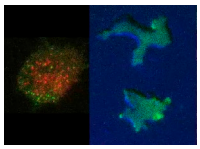
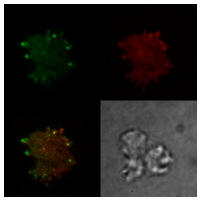


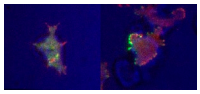
Figure S2. **Effects of dominant Rac mutants on WASP localization.** (A) GFP-WASP and CRIB-RFP were stably integrated into the genome of wild-type cells. An extrachromosomal vector with doxycycline-inducible constitutively active Rac1A G12V was transfected to these cells. Expression of Rac1A G12V was induced by adding 10 μg/ml doxycycline, and cells were imaged 4 h after induction. (B) GFP-WASP and CRIB-RFP were stably integrated into the genome of SCAR-null cells. An extrachromosomal vector with doxycycline-inducible dominant-negative Rac1A S17N was transfected to these cells. Expression of Rac1A S17N was induced by adding 10 μg/ml doxycycline, and cells were imaged 4 h after induction. DIC, differential interference contrast. Bars, 5 μm.



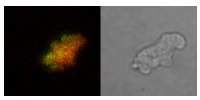
Video 1. **WASP and SCAR complex distribution in wild-type cells.** (left) Wild-type AX3 cells were transfected with GFP-WASP and clathrin light chain–mRFP. Cells were seeded onto a glass-bottom dish and incubated overnight in low fluorescent medium to reduce autofluorescence. Medium was aspirated, and cells were overlaid with 1% agarose to ensure consistent contact with the glass substratum. Time-lapse images were captured at two frames per second (f/s) on a microscope (Eclipse TE2000-U) that was fitted with a custom TIRF condenser. (right) Wild-type AX3 cells were transfected with the SCAR complex marker HSPC300-GFP. Cells were developed on Petri dishes under phosphate buffer until the onset of cell streaming. Cells were harvested, transferred to a glass-bottom dish, and overlaid with a thin layer of 0.4% agarose to ensure consistent contact with the glass substratum. Time-lapse images were collected at one frame per second on a microscope (Eclipse TE2000-U) that was fitted with a custom TIRF condenser. A differential interference contrast (DIC) image of the cell is overlaid in blue.



Video 2. **WASP and Arp2/3 complex in SCAR-null cells.** SCAR-null cells were transfected with GFP-WASP (green) and the Arp2/3 complex marker mRFP-ArpC4 (red). Cells were seeded on a glass-bottom dish and incubated overnight in low fluorescent medium to reduce autofluorescence. Time-lapse images were collected at one frame per 2 s on a microscope (Eclipse TE2000-U) that was fitted with a custom TIRF condenser. Red and green channels are combined in the bottom left image, and a bright-field image of the cell is displayed on the bottom right.



Video 3. **Visualization of WASP and Arp2/3 in cell spreading.** Wild-type AX3 cells and SCAR-null cells were transfected with both GFP-WASP (green) and the Arp2/3 complex marker mRFP-ArpC4 (red). Cells were incubated overnight in low fluorescent medium to reduce autofluorescence. Cells were then resuspended and transferred onto a fresh glass-bottom dish. Time-lapse images were captured while the cells were still dropping out of suspension and adhering and spreading on the glass substratum at one frame per 2 s (f/s) on a microscope (Eclipse TE2000-U) that was fitted with a custom TIRF condenser. A bright-field image of the cell is overlaid in blue.



Video 4. **Self-propagating waves of WASP in SCAR-null cells.** SCAR-null cells were transfected with GFP-WASP (green) and the Arp2/3 complex marker mRFP-ArpC4 (red). Cells were seeded on a glass-bottom dish and incubated overnight in low fluorescent medium to reduce autofluorescence. Time-lapse images were collected at one frame per 2 s on a microscope (Eclipse TE2000-U) that was fitted with a custom TIRF condenser. A bright-field image of the cell is shown on the right.