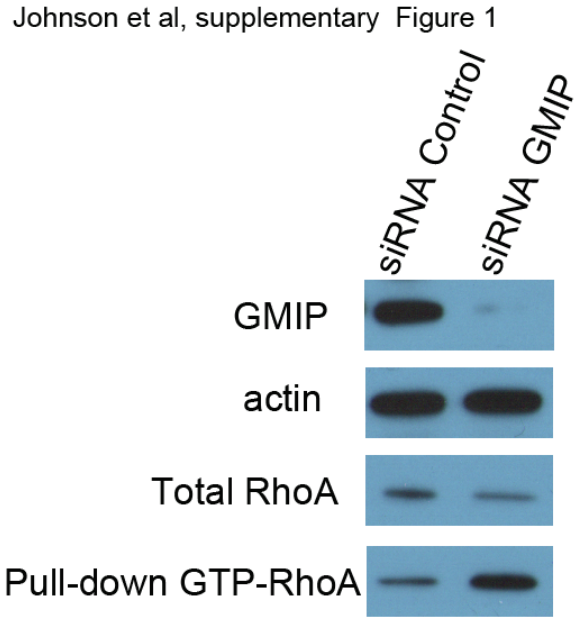
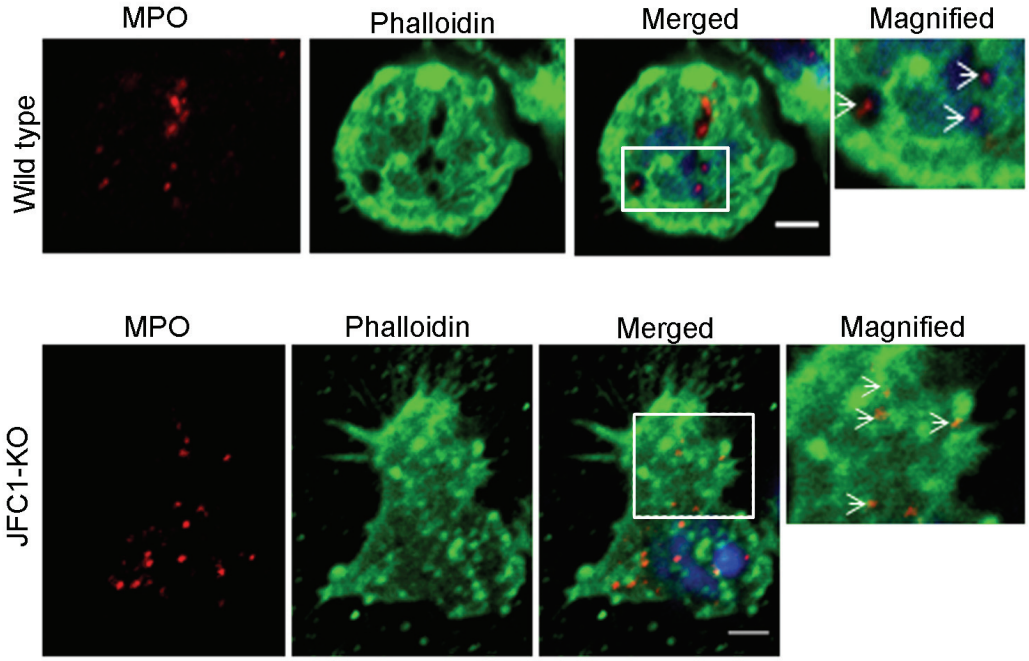
SUPPLEMENTAL MATERIALS



Supplementary Figure S1
RhoA pull-down was performed using GST-RBD of Rhotekin (50 μg) to pull-down GTP-RhoA from lysates of GMIP-downregulated or control 293T cells. The level of expression of GMIP, actin and RhoA (total RhoA) in the lysates was evaluated by Western blotting. GTP-RhoA in the pull-downs was detected using mouse monoclonal anti-RhoA (26C4) from Santa Cruz biotechnology.

Johnson el al, Supplementay Figure S2



Supplementary Figure S2
Confocal microscopy analysis of the distribution of azurophilic granules related to cortical actin. JFC1-KO or control neutrophils were stimulated with fMLF for 10 min, fixed and analyzed by immunofluorescence confocal microscopy after staining for endogenous MPO or actin (Phalloidin). The arrows point at azurophilic granules that either have a halo of depolymerized actin around them (wild type) or are trapped in cortical actin (JFC1-KO).

Supplementary movie 1
Dynamics of EGFP-LAMP3 granules in a control cells
Cells were transfected with non-silencing siRNA and with a vector for the expression of the azurophilic granule marker EGFP-LAMP3. Images were obtained by TIRFM at 1 second intervals and recorded for 2 minutes. The video is shown at 6 fps.

Supplementary Movie 2
Dynamics of EGFP-LAMP3 granules in a GMIP-downregulated cell
Cells were transfected with GMIP-silencing siRNA and with a vector for the expression of the azurophilic granule marker EGFP-LAMP3. Images were obtained by TIRFM at 1 second intervals and recorded for 2 minutes. The video is shown at 6 fps.

Supplementary Movie 3
Actin remodeling and vesicular dynamics in an untreated HL-60 cell
Cells were transfected with YFP-actin and DsRED-JFC1 and analyzed by TIRFM. Images were obtained by TIRFM at 1 second intervals and recorded for 1 minute. The video is shown at 10 frames per second (fps).

Supplementary Movie 4
Actin remodeling and vesicular dynamics in a cytochalasin D-treated HL-60 cell
Cells were transfected with YFP-actin and DsRED-JFC1, treated with cytochalasin D for 30 min and analyzed by TIRFM. Images were obtained at 1 second intervals and recorded for 1 minute. The video is shown at 10 fps.