Veltman et al., http://www.jcb.org/cgi/content/full/jcb.201309081/DC1

THE JOURNAL OF CELL BIOLOGY

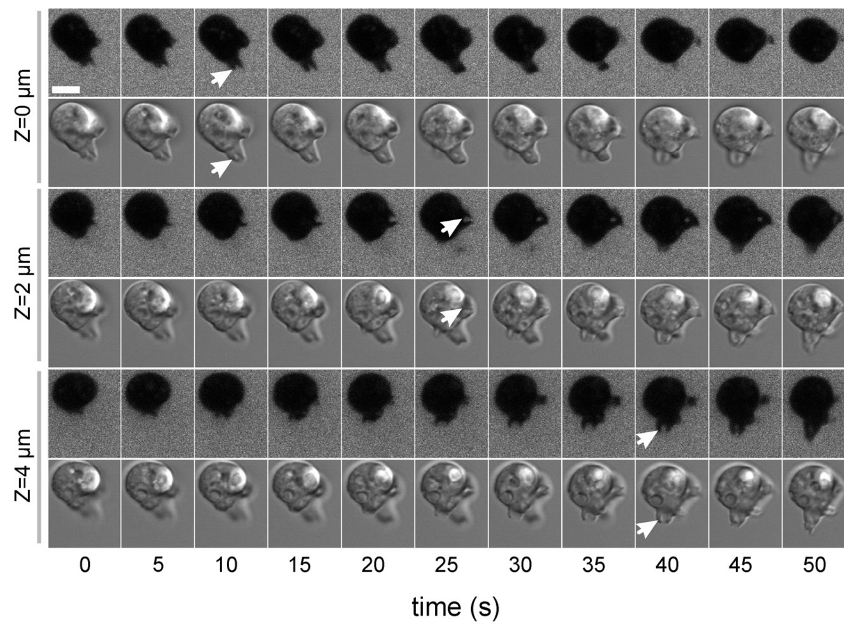


Figure S1. **Determination of the rate of macropinocytosis.** The rate of macropinocytosis was determined via direct visualization of uptake events of fluorescent dextran. Cells (shown is AX2) were allowed to settle on a glass-bottomed dish under medium supplemented with FITC-dextran. The lowest confocal plane (z = 0) was taken just above the glass substratum. Individual uptake events are marked with the arrows. Bar, 10 µm.

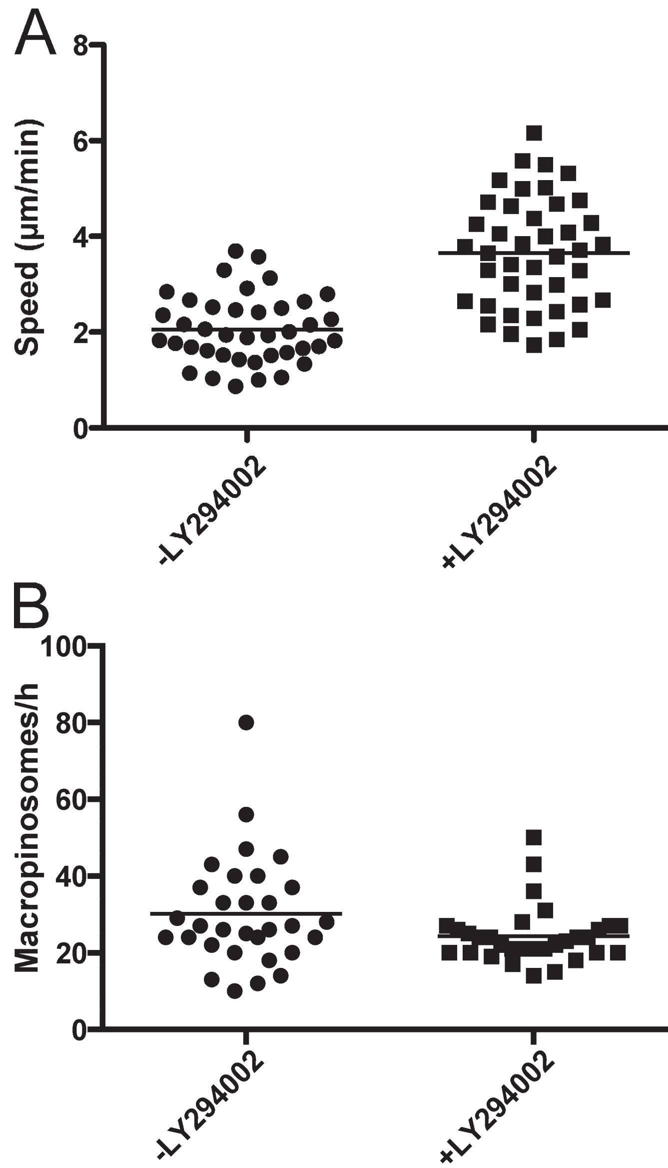
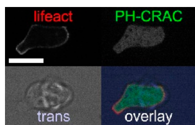
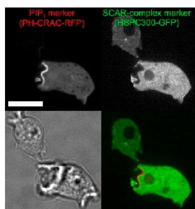


Figure S2. **LY294002 inhibits macropinocytosis and increases migration speed.** (A) Speeds of randomly moving Lifeact-mRFP–transfected AX2 cells were measured as described in Materials and methods, in the absence or presence of 10 μM LY294002. Treated cells are significantly faster (two-tailed *t* test, $n = 40$, $P < 0.0001$). (B) Macropinosomes in Lifeact-mRFP–transfected AX2 cells were measured by counting closed, circular actin structures as described in Materials and methods, in the absence or presence of 10 μM LY294002. Treated cells make significantly fewer macropinosomes (two-tailed *t* test, $n = 31$, $P < 0.05$)



Video 1. **PIP_3 induces negative curvature at the leading edge.** AX2 cells were transfected with a vector that expresses the F-actin marker Lifeact-mRFP and the PIP_3 marker PH-CRAC-GFP. Cells were grown axenically in HL5 medium and incubated overnight in LoFlo medium to reduce autofluorescence. Cells were transferred to an acid-washed glass-bottom dish (MatTek Corporation) and allowed to migrate randomly without stimulation. Images were acquired sequentially on a spinning disk microscope in the following order: mRFP, GFP, trans. Exposure time per channel was 90 ms, and one set of images was collected every 2 s.



Video 2. **The SCAR complex is recruited to the edge of PIP_3 patches.** AX2 cells were transfected with a vector that expresses the PIP_3 marker PH-CRAC-RFP and the SCAR–WAVE complex marker HSPC300-GFP. Cells were cultured axenically in HL5 medium and incubated for 3 h in SorMC buffer before imaging to reduce autofluorescence. Cells were transferred to an acid-washed glass-bottom dish (MatTek Corporation) and allowed to migrate randomly. Images were acquired sequentially on a spinning disk microscope in the following order: RFP, GFP, trans. Exposure time per channel was 90 ms, and one set of images was collected per second. The RFP channel was corrected for bleaching using standard methods.