Supplementary materials

Supplementary figures

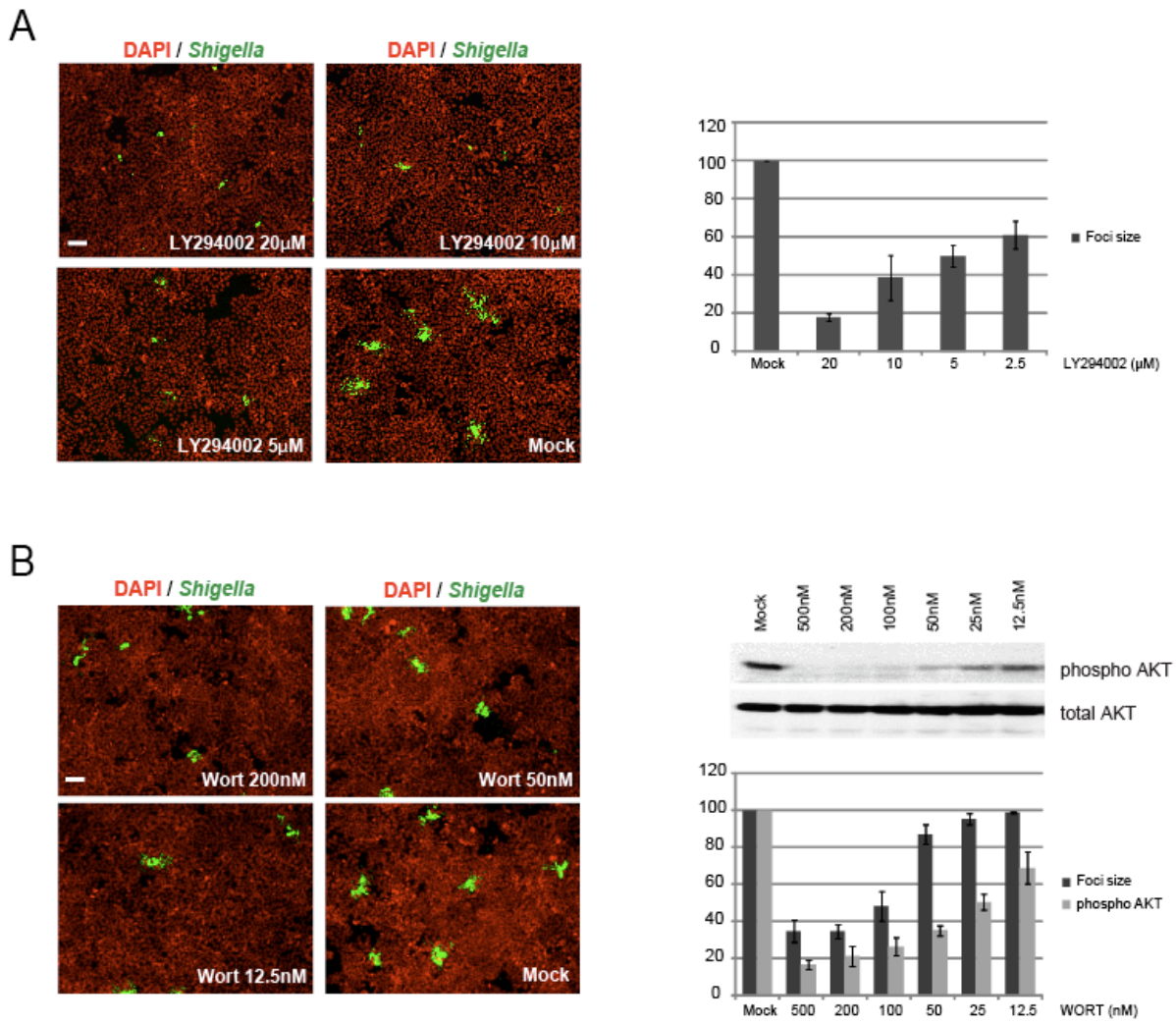

Figure S1. Effect of PIK3 inhibitors on *S. flexneri* dissemination in HT-29 cells

(A) Representative images of HT-29 cells pretreated for 1h with various concentrations of Wortmannin and infected with GFP-expressing *S. flexneri* for 8h. (red, nuclei; green, *Shigella*). Graph showing the analysis of focus size in Wortmannin-treated HT-29 cells relative to the size recorded in mock-treated cells set at 100%. Values represent the mean +/- SD of three

independent experiments. (B) Upper panel: western blot analysis of Akt phosphorylation in mock-treated and Wortmannin-treated cells. Lower panel: Akt phosphorylation levels were quantified in mock-treated and Wortmannin-treated cells using ImageJ software analysis and normalized to the total Akt levels. Graph showing Akt phosphorylation level in Wortmannin-treated cells calculated as percentile level of mock-treated cells (phospho Akt). Statistical analysis: Mock vs. Wort500 $p< 0.0001$, Mock vs. Wort200 $p< 0.0001$, Mock vs. Wort100 $p<0.0005$, Mock vs. Wort50 $p< 0.05$, Mock vs. Wort25 NS, Mock vs. Wort12.5 NS. The experiment shows the inhibitory effect of Wortmannin on Akt phosphorylation in the 50-12.5 nM concentration range, with little to no effect on *Shigella* dissemination.

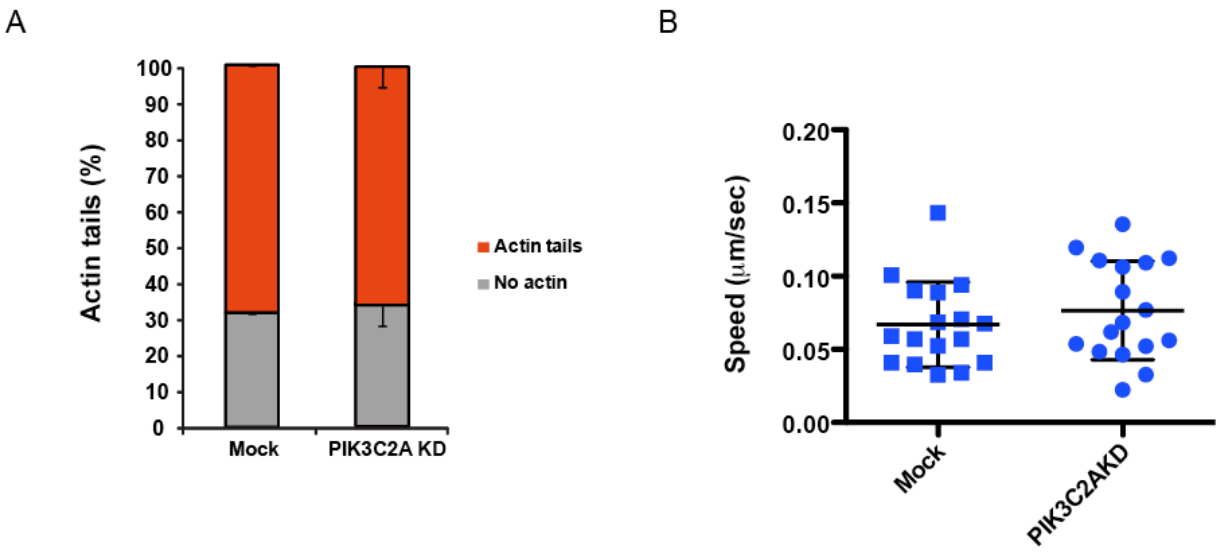

Figure S2. PIK3C2A is not required for cytosolic motility

(A) Graph showing counts of actin tails in mock-treated and PIK3C2A-depleted cells. Values represent the mean +/- SD of three independent experiments. (B) Graph showing measurements of *S. flexneri* velocity in the cytosol of mock-treated and PIK3C2A-depleted HT-29 cells. The

tracking module of the Volocity software was used to score motile bacteria and determine their velocity (μm/s).

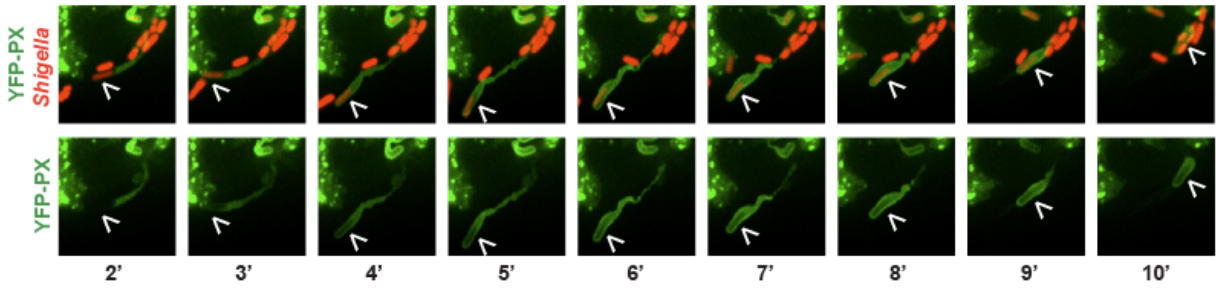

Figure S3. Localization of YFP-PX in *S. flexneri* protrusions

Representative time-lapse images of YFP-PX-expressing HT-29 cells (green) infected with RFP-PIK3C2A-expressing *S. flexneri* (red). Note the progressive YFP-PX enrichment in the plasma membrane surrounding protrusions overtime (arrowhead).

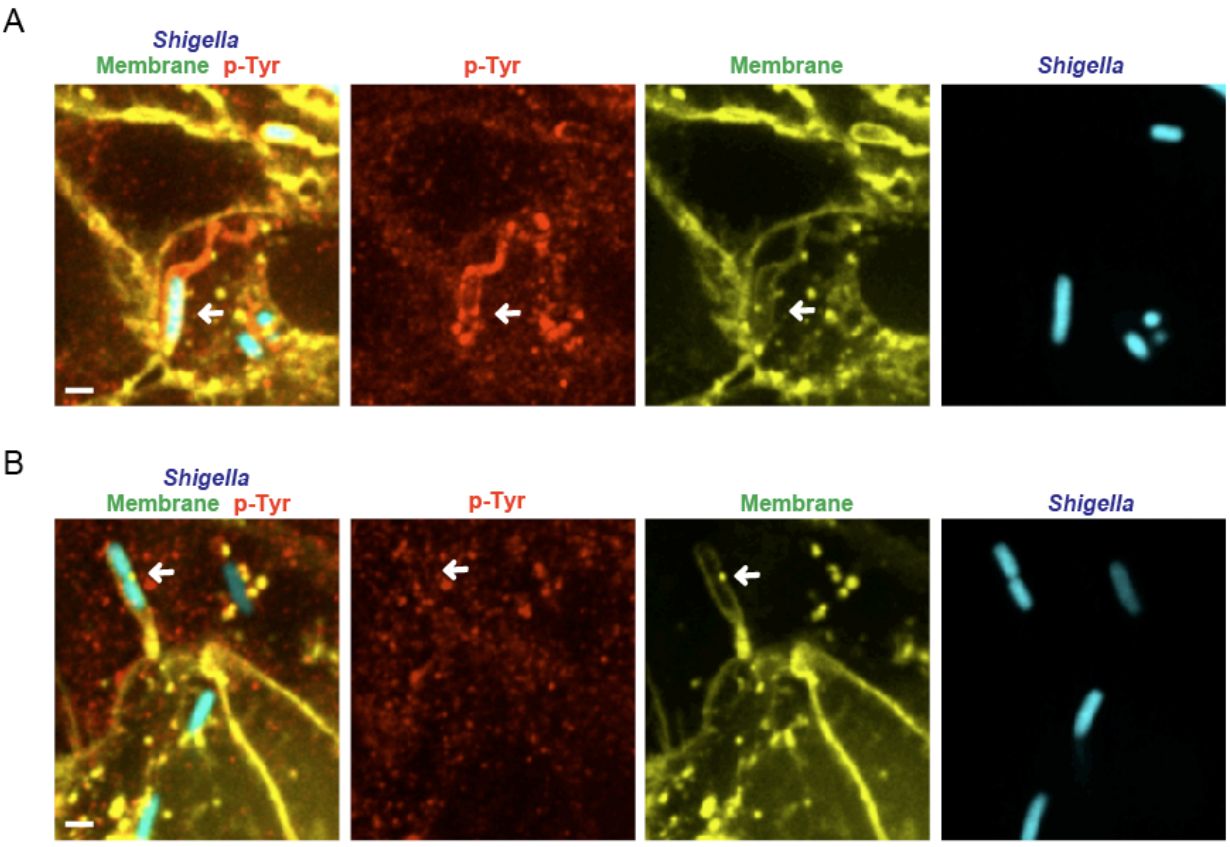

Figure S4. Phospho-tyrosine residues in *S. flexneri* protrusions

Representative images of HT-29 cells infected with CFP-expressing *S. flexneri* (blue) displaying protrusions (arrows, membrane-YFP, yellow) that scored (A) phospho-tyrosine positive (red) or (B) phospho-tyrosine negative (red), as counted in Figure 7C.

Supplementary movies

Movie S1. Time lapse imaging of protrusion resolution into vacuole through VLP formation

HT-29 cell expressing membrane GFP (yellow) were infected with RFP-expressing *Shigella flexneri* and imaged every five minutes. Scale bar: 5 μm. The movie shows the formation of a protrusion that successfully transitions into a Vacuole-Like-Protrusion (VLP) and resolve into a vacuole.

Movie S2. Time lapse imaging of unsuccessful protrusions resolution into vacuole

HT-29 cell expressing membrane GFP (yellow) were infected with RFP-expressing *Shigella flexneri* and imaged every five minutes. Scale bar: 5 μm. The movie shows the formation of a protrusion that fails to transitions into a Vacuole-Like-Protrusion (VLP) and retracts bringing the pathogen back to the primary infected cell.