

SUPPORTING INFORMATION

Supporting dataset S1, Supporting videos S1-S4, Supporting figures S1-S6

Supporting Dataset S1

Complete results from RNAi and invasion screen in *Drosophila* S2R+ cells, including (A) common and *Drosophila*-specific name of genes targeted by RNAi, (B) FlyBase gene annotation symbol, (C) protein function, and (D-G) percent internalization after knockdown.

Supporting Video S1

Actin recruitment around a group of invading *Rickettsia. R. parkeri* GFP-3 (red) is shown in a Cos7 cell expressing LifeAct-mCherry (green). Images were captured every 10 s starting approximately 23 min post-infection and are displayed at 10 frames per second. Bar, 5 μm .

Supporting Video S2

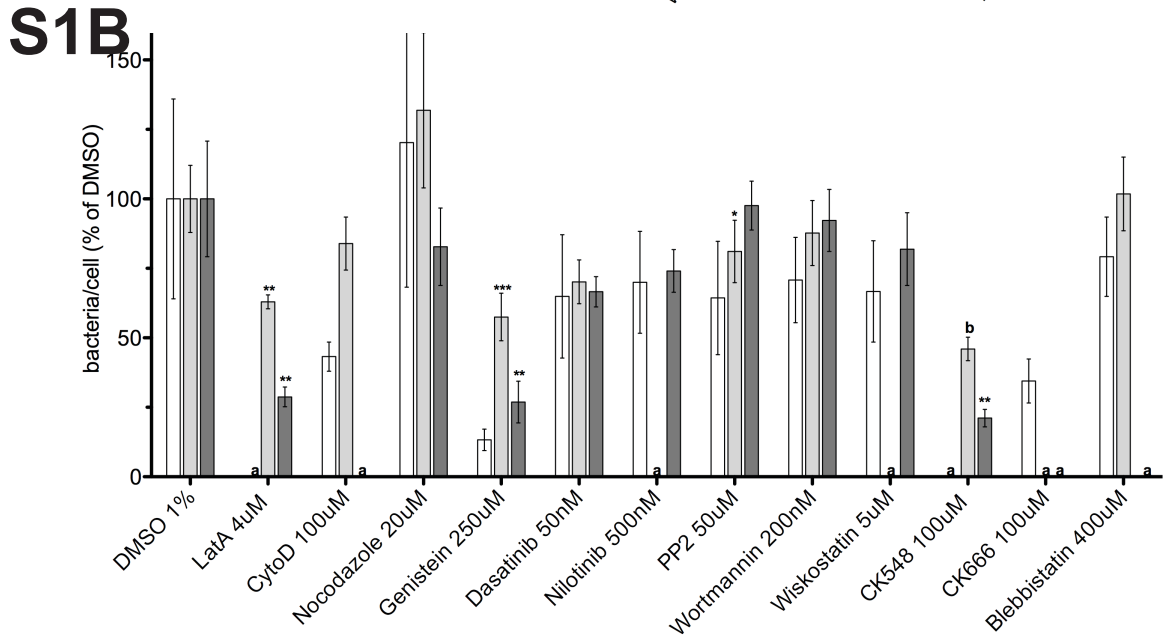
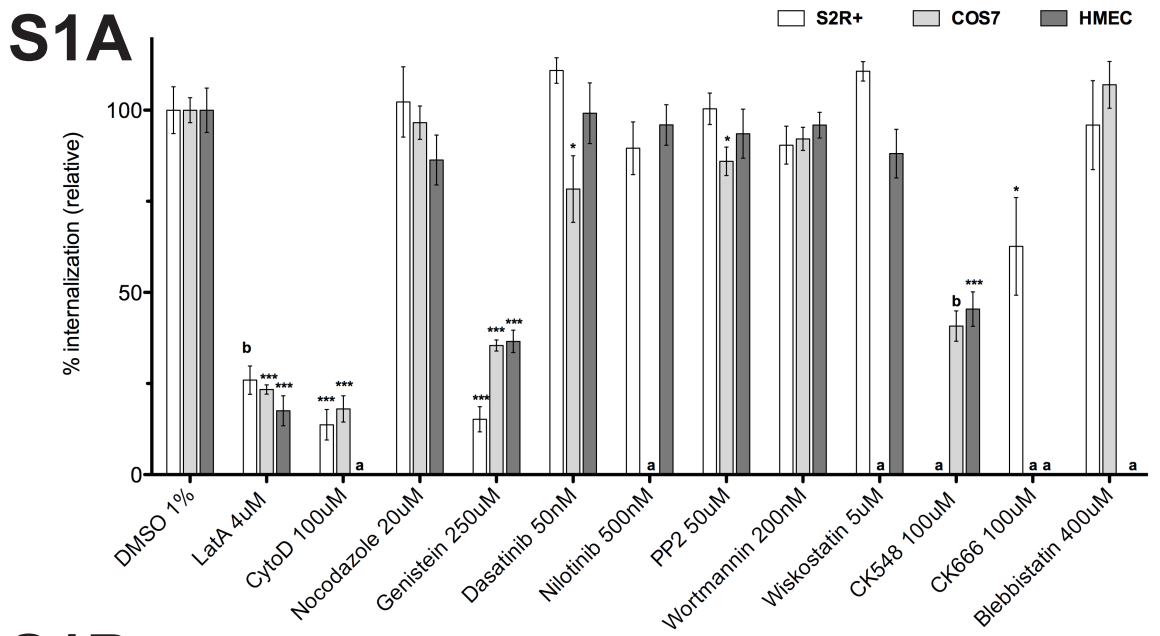
Two internalizing bacteria associated with actin. *R. parkeri* GFP-3 (red) is shown in a Cos7 cell expressing LifeAct-mCherry (green). Images were captured every 10 s starting approximately 30 min post-infection and are displayed at 10 frames per second. Bar, 5 μm .

Supporting Video S3

Internalization of a bacterium surrounded by mCherry-PAK-PBD. *R. parkeri* GFP-3 (red) is shown in a Cos7 cell expressing mCherry-PAK-PBD (green). Images were captured every 10 s starting approximately 11 min post-infection and are displayed at 10 frames per second. Bar, 5 μm .

Supporting Video S4

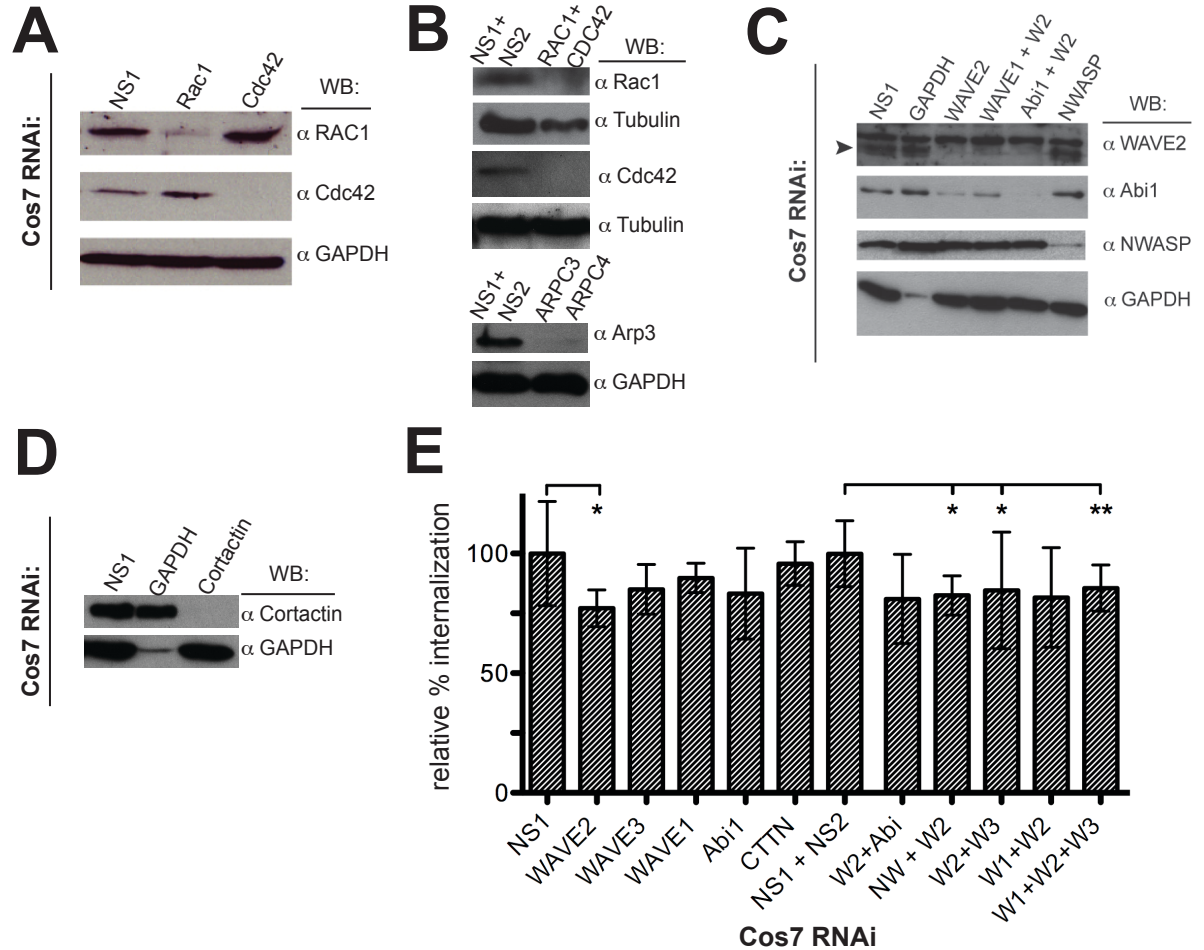
Internalization of two individual bacteria associated with mCherry-PAK-PBD. *R. parkeri* GFP-3 (red) is shown in a Cos7 cell expressing LifeAct-mCherry (green). Images were captured every 10 s starting approximately 30 min post-infection and are displayed at 10 frames per second. Bar, 5 μm .



***R. parkeri* adherence to and invasion of host cells does not require microtubules, Myosin II, N-WASP or a single tyrosine kinase family.**

(A) Percent internalization and (B) average bacteria per host cell after 15 mins of infection by *R. parkeri* in S2R+ (white bars) COS-7 (light grey bars) and HMEC-1 cells (dark grey bars); cells were either pretreated with 1% DMSO alone, or treated 30 min before infection with drugs diluted to the indicated concentrations in 1% DMSO. Data are the mean of two (S2R+) or three (COS-7 and HMEC) independent experiments and were normalized with the average of DMSO-treated control cells set as 100%. (a) indicates a treatment not performed for a particular cell type, (b) indicates that data were normalized to control values not shown in histogram. Abbreviations: LatA, latrunculin A; CytoD, cytochalasin D, Error bars, SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, raw data compared versus matched DMSO controls by unpaired t-test.

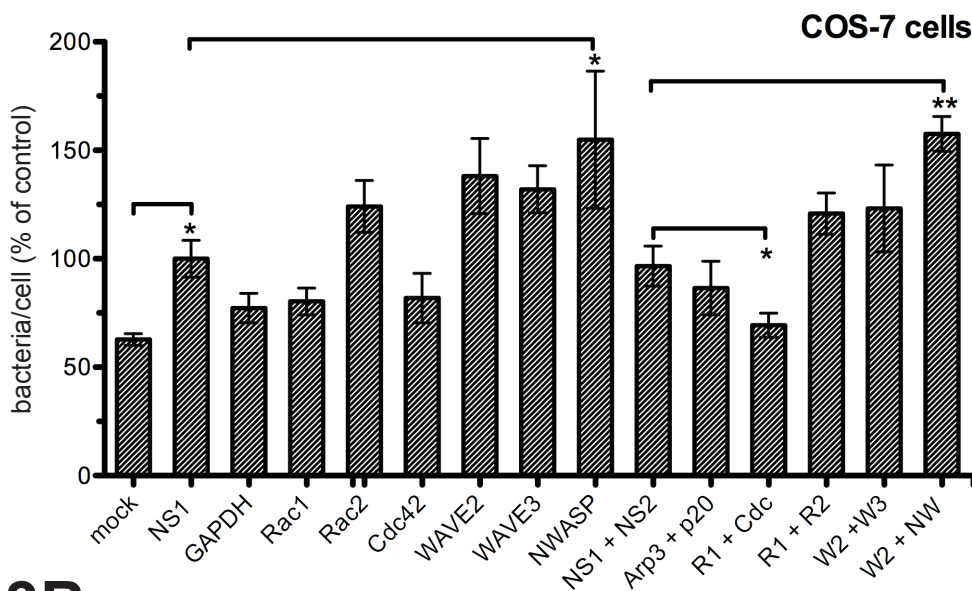
S2



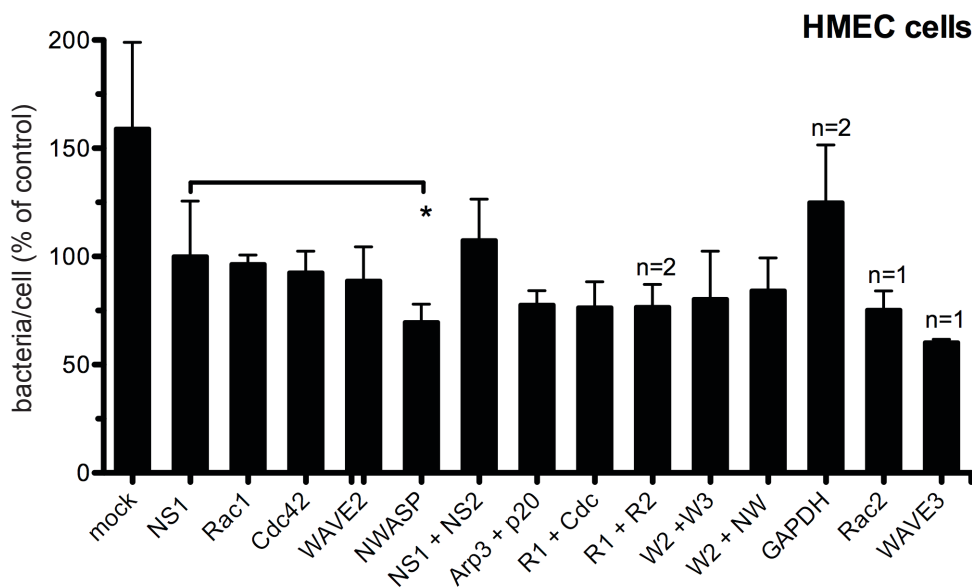
Invasion of COS-7 cells does not require Cortactin or Abi.

(A) Western blots of lysates from cells treated with the indicated siRNAs and collected in parallel during one experiment represented in Figure 3E. (B) Western blots of lysates from cells treated with the indicated siRNAs and collected in parallel during one experiment represented in Figure 3F (C) Western blots of lysates from cells treated with the indicated siRNAs and collected in parallel during one experiment represented in Figure 4B and in panel (E) of this figure. (D) Western blots of lysates from cells treated with the indicated siRNAs and collected in parallel during one experiment represented in panel (E) of this figure. (E) Relative percentage internalization at 15 min post infection with *R. parkeri* in COS-7 cells. Data represent the average of at least three independent and were normalized with nonspecific-RNA transfected COS-7 cells set as 100%. Abbreviations: NS1, nonspecific control RNA 1; NS2, nonspecific control RNA 2; W1, W2, W3, WAVE1, WAVE2, WAVE3 respectively; NW, N-WASP; CTTN, Cortactin; *= $p < 0.05$, **= $p < 0.01$ indicate values significantly different from indicated controls by unpaired t-test.

S3A



S3B

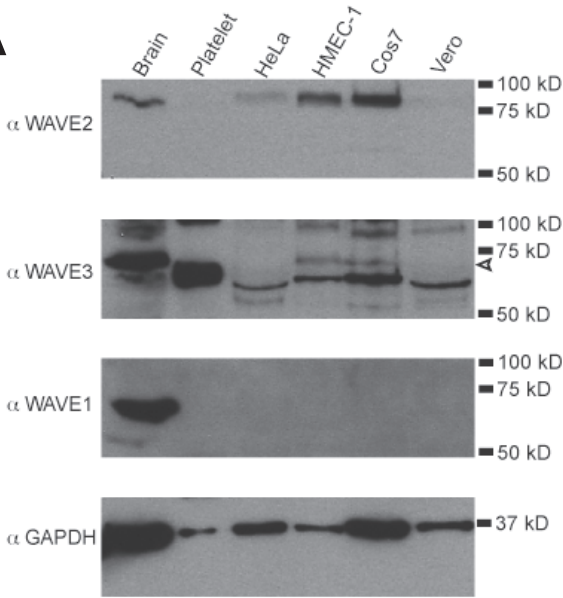


Relative changes in *R. parkeri* adherence to host cells following siRNA treatment.

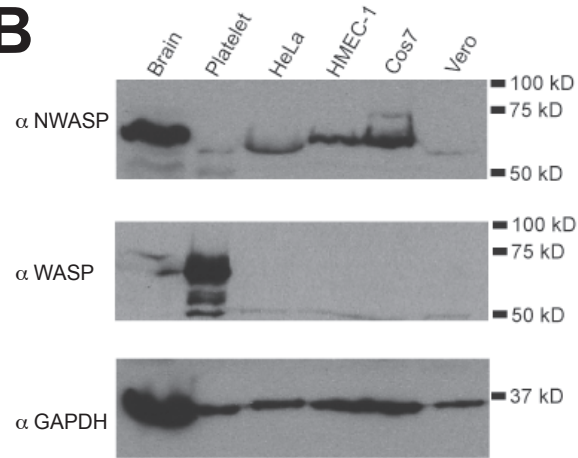
Percentage of bacteria associated with each host cell, relative to NS1 RNAi treatment, in (A) COS-7 cells, hashed bars or (B) HMEC-1 cells, black bars. Data are the mean of three independent experiments unless otherwise indicated and were normalized with the average of NS1-treated control cells set as 100%. Error bars, SEM, * $p < 0.05$, ** $p < 0.01$, raw data compared versus indicated control by unpaired t-test.

S4

A

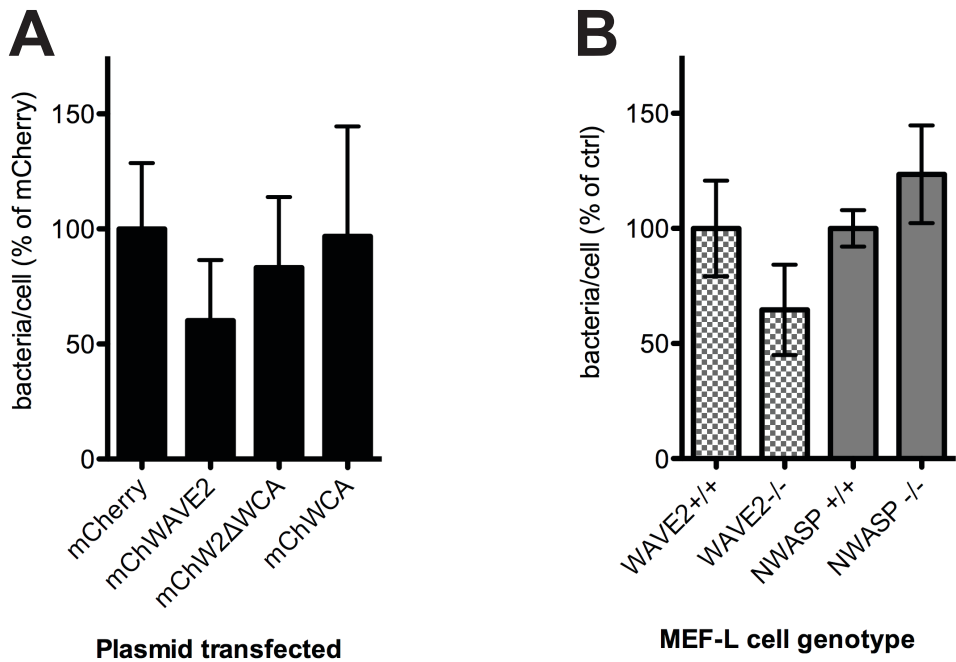


B



WAVE and WASP family protein expression in various mammalian cell lines.

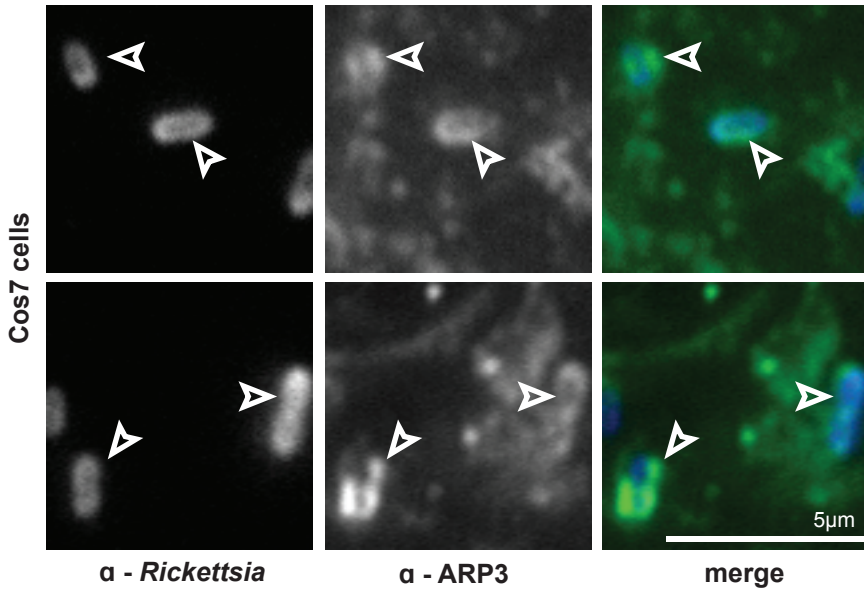
Western blots of cell lysates from pig brain extract, human platelet extract, HeLa, HMEC-1, COS-7, and Vero cells. **(A)** Expression of WAVE1, WAVE2, and WAVE3 in various cell lines. Arrowhead indicates WAVE3-specific band. WAVE1 is expressed only in brain extract. **(B)** Expression of N-WASP and WASP in various cell lines. WASP is expressed only in platelets.

S5

Binding of *Rickettsia parkeri* to host cells is not affected by absence or overexpression of WAVE2.

(A) Percentage of bacteria associated with each host cell, relative to mCherry control, in HMEC cells transfected with plasmids expressing the indicated proteins. (B) Percentage of bacteria associated with each host cell, relative to sibling control cells, in MEF-L cells genetically lacking WAVE2 or NWASP. Data are the mean of three independent experiments and were normalized with the average of control cells set as 100%. Error bars, SEM.

S6



Arp3 is recruited surrounding bacteria invading COS-7 cells. COS-7 cells infected for 7 min with *R. parkeri*, fixed and stained with anti-Rickettsia antibody to visualize bacteria (left, blue in merge), and anti-Arp3 antibody (center, green in merge). Open arrowheads indicate bacteria associated with Arp3 protein.