Supplemental Materials

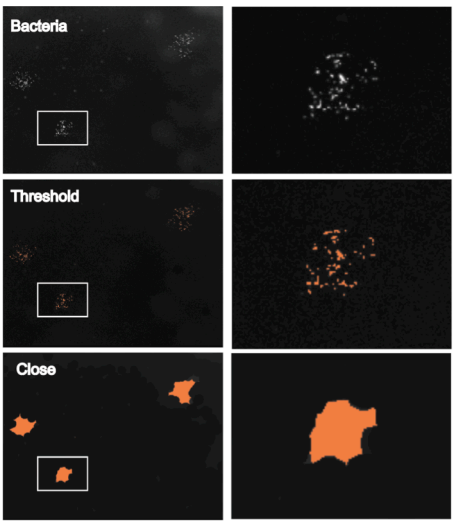

Fig. S1. Computer-assisted image analysis. Representative images of HeLa 229 cells infected with GFP-expressing *L. monocytogenes* for 8 hours (Top panels, Bacteria). The high intensity pixels corresponding to the bacteria were selected against the low intensity pixels corresponding to background by using the threshold function of the MetaMorph 7.1 imaging software (Middle panels, Threshold). Selected pixels were clustered by proximity using the "close" morphological filter in order to create single objects that represent infection foci (Bottom panels, Close). The integrated morphometry analysis (IMA) module was used to determine the size of the identified foci in a given image and their corresponding total gray intensities (total GFP signal), as shown in Figure 1B and 1C, respectively. The right panels show zoomed-in images corresponding to the boxed area of the images in the left panels.

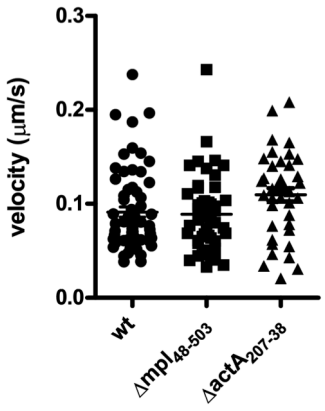

Fig. S2. Graph showing cytosolic motility recorded in cells infected with wild type, the *mpl* or the $actA_{207\text{-}238}$ mutants. Cytosolic velocity of wild type (wt, n=65), *mpl* mutant ($\Delta mpl_{48\text{-}503}$, n=49), and *actA* mutant ($\Delta actA_{207\text{-}38}$, n=40) in HeLa229 cells. Data were analyzed by one-way ANOVA using PRISM 5 (GraphPad Software). wt vs. $\Delta mpl_{48\text{-}503}$, ns; wt vs. $\Delta actA_{207\text{-}38}$, ns; $\Delta mpl_{48\text{-}503}$ vs. $\Delta actA_{207\text{-}38}$, ns.

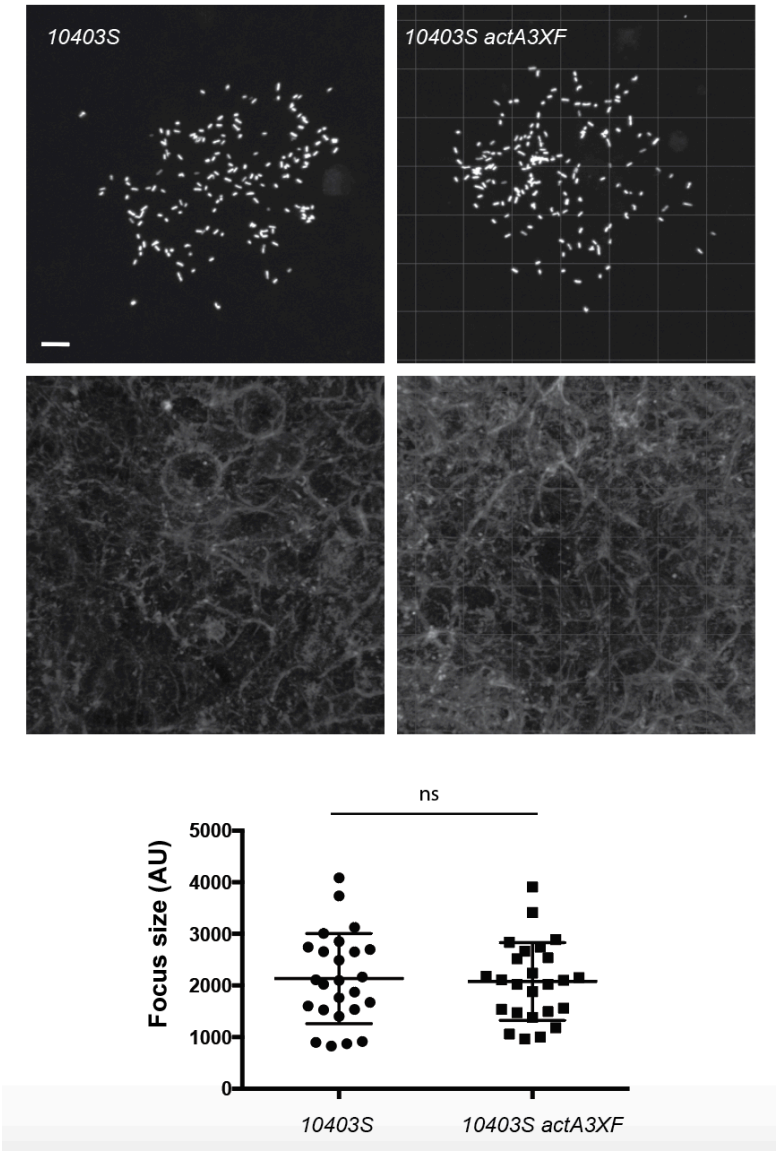

Fig. S3. Characterization of the infection foci formed in cells infected with wild type (10403S) and 3xFlag ActA (10403S *actA*3xF) bacteria. (A) Representative images showing monolayers of confluent HeLa-229 cells infected with GFP-expressing *L. monocytogenes* for 6 h (top, GFP-expressing bacteria; bottom, Phalloidin staining). Scale bar represents 10 μm. (B) Graph showing the size of the infection foci as determined by computer-assisted analysis of images as shown in (A). 10403S *actA*3xF vs. 10403S, n.s.; unpaired t-test.

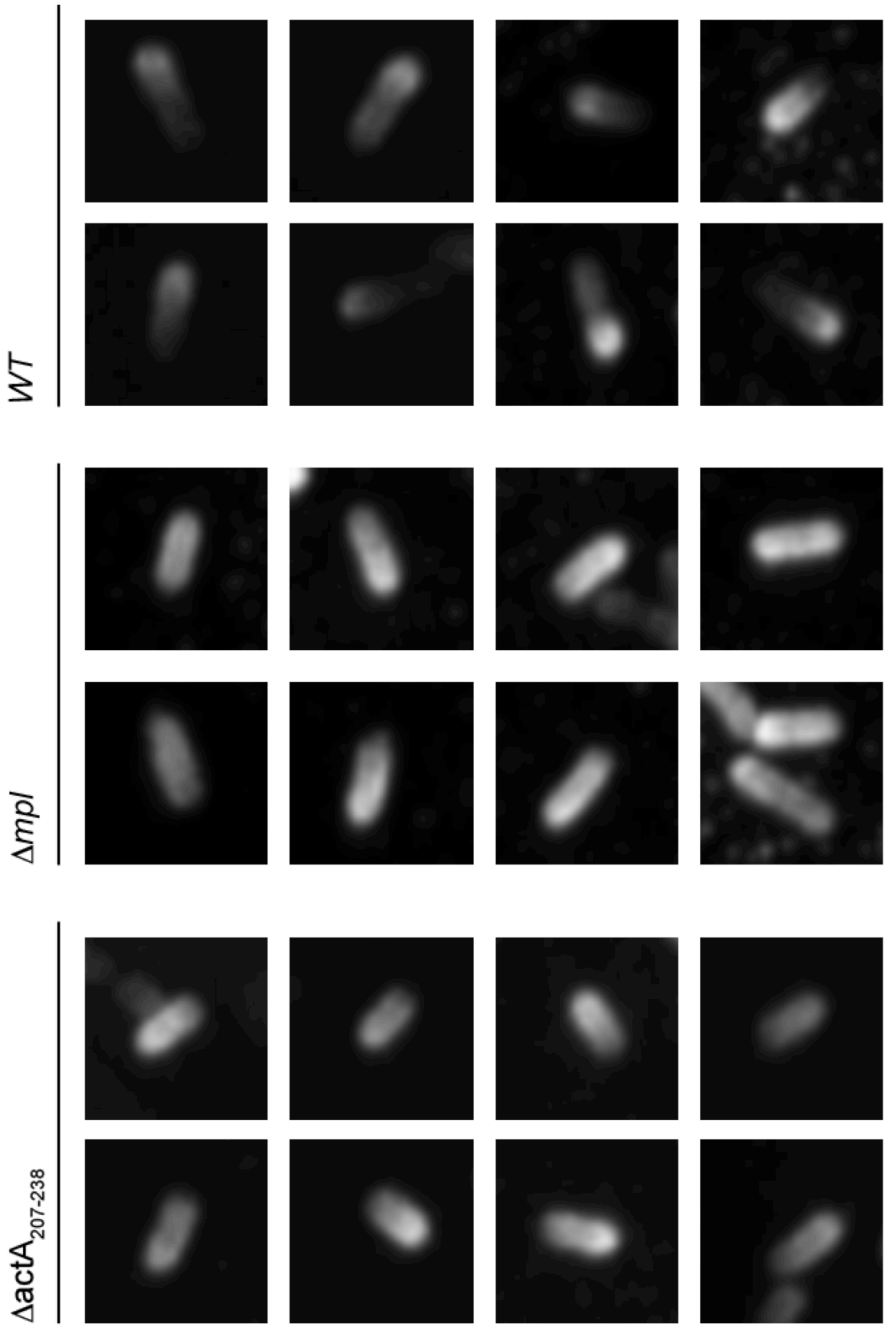

Fig. S4. Distribution of ActA on the surface of wt, *mpl* and Δ*actA*$_{207-38}$ mutant bacteria forming protrusions. Gallery of images showing the distribution of 3xFlag-ActA on the surface of bacteria in protrusions.

Table S1. Primers used in this study

Primer Name	Sequence (5'-3')
ActAEco	GAAGAATTCGAAGCAGTTGGGGTTAA
ActA3BamHI	GGAGGATCCCACTTATCAGAGCCGGCGCGC
3xFsense	ATTATAAAGATCATGACATCGATTACAAGGATGACGATAAGATCTTAAATC AAAAACCATTTTTCCC
3xFantisense	GATGTCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCCATGGCTTTTA AAGGTTGTGG
Δ207-38 sense	GACATCGATTACAAGGATGACGATGCGATTGTTGATAAAAGTGC
pMplEco	GAAGAATTCGTTTTGCACTCTTCGTAA
mpl HP BglII antisense	GAAGATCTATCCACCCGCTAACGAGTGGATAAGAATGTATTCCTCAGTTAAC CCCAAC

Table S2. Bacterial strains and relevant characteristics

Strain [a]	Relevant characteristic(s)	Reference or source
10403S	Wild type	(1)
L37	Δ$mpl_{48\text{-}503}$ [b]	This work
DP-L1935	Δ*plcB*	(2)
L35	3xFlag *actA* [c]	This work
L71	Δ$mpl_{48\text{-}503}$-3xFlag *actA* [b,c]	This work
L156	3xFlag Δ$actA_{207\text{-}238}$ [c,d]	This work
DP-L3078	Δ*actA*	(3)

[a] All strains were derived from *L. monocytogenes* 10403S
[b] In frame deletion of *mpl*
[c] 3xFlag was inserted at amino acid 200 of ActA
[d] In frame deletion of *actA*

Table S3. Plasmids and relevant characteristics

Plasmid	Relevant characteristic(s)
p304*mpl-actA-plcB*	pHT304 (4) containing *mpl-actA-plcB* full-length operon
p304*Δmpl*$_{48\text{-}503}$*-actA-plcB*	p*304mpl-actA-plcB* carrying an in frame deletion of *mpl*
p304*mpl-3xF actA*$\Delta_{207\text{-}238}$*-plcB*	p*304mpl-actA-plcB* carrying 3xFlag insertion at amino acid 200 and in frame deletion of ActA
pMAD Δ*mpl*$_{48\text{-}503}$	pMAD (5) containing in frame deletion of *mpl*
pMAD-3xFlagActA	pMAD (5) containing *actA* with 3xFlag insertion at amino acid 200
pMAD-3xFlag *actA*$\Delta_{207\text{-}238}$	pMAD (5) containing in frame deletion of *actA* with 3xFlag insertion at amino acid 200
p315hspkGFP	pHT315 (4) GFP under the control of a IPTG-inducible promoter
p304*mpl*	pHT304 (4) containing *mpl* promoter and ORF followed by *plcB* terminator
pDsRed-Monomer-Mem	N-terminal 20 amino acids of neuromodulin and monomeric RFP

References

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2. **Smith GA, Marquis H, Jones S, Johnston NC, Portnoy DA, Goldfine H.** 1995. The two distinct phospholipases C of Listeria monocytogenes have overlapping roles in escape from a vacuole and cell-to-cell spread. Infect Immun **63:**4231-4237.
3. **Skoble J, Portnoy DA, Welch MD.** 2000. Three regions within ActA promote Arp2/3 complex-mediated actin nucleation and Listeria monocytogenes motility. J Cell Biol **150:**527-538.
4. **Arantes O, Lereclus D.** 1991. Construction of cloning vectors for Bacillus thuringiensis. Gene **108:**115-119.
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