Cell Host & Microbe, Volume *3*

Supplemental Data

Bacterial Actin Assembly Requires Toca-1 to Relieve N-WASP Autoinhibition

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Table S1. Actin tail assembly by *L. monocytogenes* in cells in which Toca-1 has been depleted.

	Transfection vector	**Bacteria with well-formed actin tails**[a]		**Bacteria associated with actin**[b]	
		% of bacteria	Mean length (um)	% of bacteria	Mean length (um)
Toca-1 depletion[c]	3 Toca-1 shRNAi[c]	37.9 ± 3.3[d]	5.5 ± 0.6	80.5 ± 2.4	2.2 ± 0.2
	Control shRNAi	43.1 ± 3.7[d]	5.5 ± 1.1	79.7 ± 4.6	2.3 ± 0.4

[a] Longer than 0.83 um (10 pixels).

[b] Associated with detectable polymerized actin.

[c] >95% depletion of Toca-1.

[d] $p = 0.1$.

Table S2. Actin tail assembly by *S. flexneri* that conditionally express the type III secretion activator VirB.

IPTG	VirB expression	Bacteria with well-formed actin tails[a]		Bacteria associated with actin[b]	
		% of bacteria	Mean length (um)	% of bacteria	Mean length (um)
absent	absent	1.8 ± 0.7[c]	3.2 ± 0.4[d]	30.1 ± 5.1[e]	0.1 ± 0.02[f]
present	present	29.6 ± 5.1[c]	3.5 ± 0.2[d]	52.7 ± 3.4[e]	1.1 ± 0.2[f]

[a] Longer than 0.83 um (10 pixels).

[b] Associated with detectable polymerized actin.

[c] p = 0.01; [d] p = 0.2 ; [e] p = 0.008 ; [f] p = 0.02.

Table S3. The effect of Toca-1 on actin tail assembly by *S. flexneri* is independent of expression of dominant negative (DN) or constitutively active (CA) derivatives of Cdc42.

Cdc42 transfection vector	Toca-1 depletion transfection vector	Bacteria with well-formed actin tails[a]		Bacteria associated with actin[b]	
		% of bacteria	Mean length (um)	% of bacteria	Mean length (um)
N17 Cdc42 (DN)	3 Toca-1 shRNAi[c]	9.9 ± 0.3	6.3 ± 1.0	66.1 ± 8.6	0.7 ± 0.1
	Control shRNAi	40.3 ± 3.4	4.5 ± 0.4	78.4 ± 2.0	2.0 ± 0.3
L61 Cdc42 (CA)	3 Toca-1 shRNAi[c]	8.5 ± 1.1	5.8 ± 0.2	71.6 ± 5.1	0.7 ± 0.2
	Control shRNAi	43.2 ± 0.7	5.2 ± 0.5	76.4 ± 6.8	2.4 ± 0.2

[a] Longer than 0.83 um (10 pixels).

[b] Associated with detectable polymerized actin.

[c] >95% depletion of Toca-1.

Figure S1. In Toca-1 depleted cells, intracellular *S. flexneri* are in the cytosol, not in vacuoles. Absence of vacuolar membranes around intracellular wild-type *S. flexneri* in Toca-1-depleted (**A-B**) and mock-depleted (**C-D**) HeLa cells. Presence of vacuolar membranes (arrowheads) around avirulent (virulence plasmid minus) *S. flexneri* that had been phagocytosed by J774 macrophage-like cells (**E-F**). Size bar (**A, C, E,** shown in **E**), 2.5 um; (**B, D, F,** shown in **F**), 1 um.

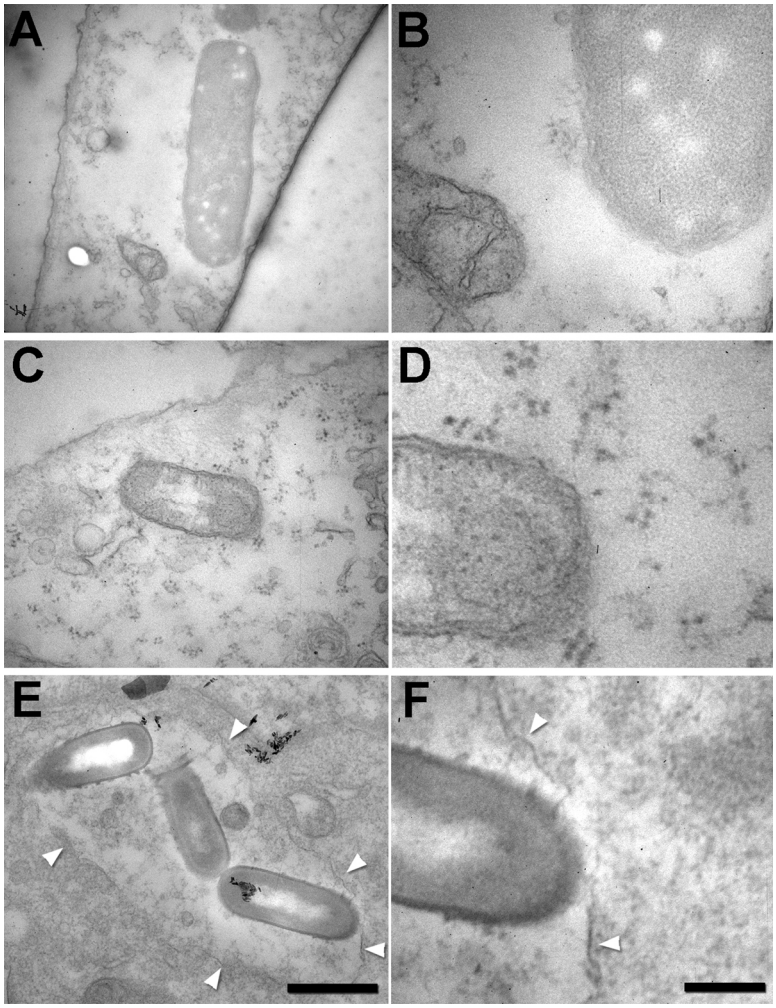

Figure S2. N-WASP expression and recruitment to intracellular *S. flexneri* is unaltered by Toca-1 depletion. (**A**) Western blot of N-WASP levels in lysates of Toca-1-depleted and mock-depleted HeLa cells. Levels of β-actin confirmed equivalent loading. (**B-C**) Recruitment of N-WASP (arrowheads, green) to intracellular bacteria was unaffected by Toca-1 depletion. *S. flexneri* infection of Toca-1-depleted (**B**) or mock-depleted (**C**) HeLa cells. Cells were co-transfected with Toca-1 shRNA (**B**) or control shRNA (**C**) vector and a vector expressing GFP-N-WASP. Bacterial and cellular DNA was labeled with DAPI (blue). Size bar (**B-C**, shown in **C**), 5 um.

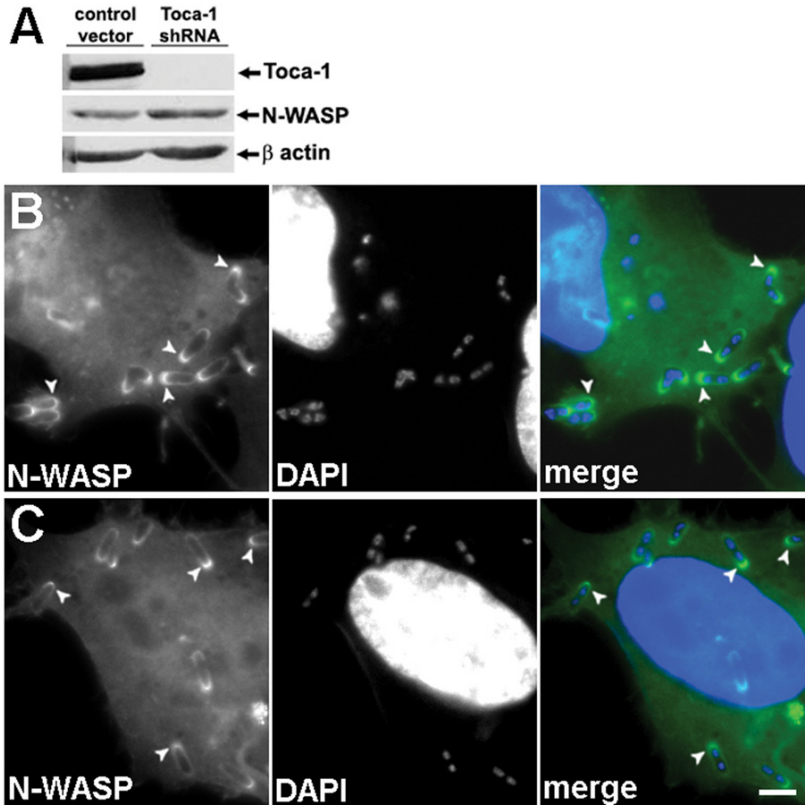

Figure S3. Levels of VirB expression by *S. flexneri* that conditionally expresses VirB. Levels of VirB in bacterial cultures of a *S. flexneri* strain that conditionally expresses *virB* under the control of a *tac* promoter (AWY3 pDSW206-P_{tac}-*virB*). Following growth in the presence of IPTG for 60 min., IPTG was removed by washing. Numbers above lanes indicate time (in min) after washout of IPTG from culture. MW marker in kilodaltons is shown at right.