Supporting Information

Hemrajani et al. 10.1073/pnas.0911609106

SI Materials and Methods

Bacterial Strains and Growth Conditions. The strains and primers used in this study are listed in Tables S1 and S2. The lambda red system (1) was used to delete *nleH1* and *nleH2* from WT EPEC strain E2348/69 (2), generating strain ICC303. *nleH1*-specific primer pair 1–2 was used to amplify the kanamycin cassette from pSB315 (3). *nleH2*-specific primer pair 3–4 was used to amplify the chloramphenicol cassette from pKD3 (1). Deletion of *nleH1* and *nleH2* was confirmed using primer pairs 19–20 (*nleH* external), 21–22 (kanamycin cassette–specific), 23–24 (*nleH2* external), and 25–26 (chloramphenicol cassette–specific). The *C. rodentium* ICC180 Δ*nleH* mutant (ICC285) was prepared as described previously (4).

Bacteria were cultured in LB broth at 37 °C for 18 h with appropriate antibiotics (100 μg mL^{-1} of ampicillin, 25 μg mL^{-1} of chloramphenicol, or 50 μg mL^{-1} of kanamycin). Overnight LB cultures were primed for infection by 1:100 dilution in DMEM containing 1,000 mg/L of glucose without phenol red, followed by a further incubation for 3 h at 37 °C without agitation; 1 mM isopropyl-β-D-thiogalactopyranoside was added during the final 30 min when needed (for expression from pSA10-derived plasmids). HeLa cells were infected with primed bacteria normalized to a multiplicity of infection (MOI) of 1:50 or 1:100 for the specified time points.

Plasmids and *nleH* Gene Cloning. The plasmids used in this study are listed in Table S3. For purification of His-tagged NleH, *nleH1* was amplified using primers 29 and 30, and then cloned into pET28a (generating pICC451). pICC451 and pICC449 were used as a template for site-directed mutagenesis (K159A) using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) and the primer pair 31–32 (generating pICC452 and pICC450, respectively). For cloning into the bacterial expression vector pSA10 (5), *neH1* and *nleH2* were amplified using primer pairs 5–6 and 7–8, respectively (generating pICC443 and pICC444, respectively). For Y2H screening, *neH1, nleH2*, and *ospG* were cloned into pGBT9 (Clontech) to generate pICC447, pICC448, and pICC456, respectively, using primer pairs 9–10, 11–12, and 35–36, respectively. Deletion of the 100 first amino acids of NleH was done by inverse PCR on pGBT9(*nleh1*) using the primer pair 33–34, generating pGBT9($nleh1_{\Delta100}$) (pICC455). For transfection experiments, *nleH1 and* $nleH1_{K159A}$ were cloned into the transfection vector pHM6 (Roche) using primers 13–14 and E2348/69 DNA or pICC452 as templates, respectively, generating pHM6(*nleH1*) (pICC449) and pHM6($nleH1_{K159A}$) (pICC450). The pEGFP-N1 (Clontech) vector was used as a control in all transfection experiments. For transfection experiments, *bi-1* was cloned into the transfection vector pRK5-Myc using primers 37–38 and pICC458 as a template.

Protein Purification. NleH1-His and NleH1(K159A)-His were purified using a Ni^{2+} agarose His-Bind Resin Column (Novagen). Purified NleH1-His and NleH1(K159A)-His were dialyzed against EB50 buffer [50 mM Tris (pH 8), 2 mM DTT, 2 mM EDTA, 10% glycerol, and 50 mM NaCl]. Dialyzed proteins were then concentrated using 10 kDa of Amicon Centricons (Millipore) and frozen in 50-μL aliquots at −80 °C. NaCl concentration was adjusted, and 0.1% Triton X-100 was added equally to all samples, to prevent precipitation during storage.

Kinase Assay. NleH1-His and NleH1(K159A)-His were added to the kinase assay reagent mix [50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, and 50 μM ATP] with 5 μCi of [γ-33P]ATP (370 MBq/mL, 3,000 Ci/mmol; MP Radiochemicals) with or without 10 μg of the general substrate MBP (Sigma-Aldrich). The positive control was 100 units of Abl tyrosine kinase (New England Biolabs). The mixture was incubated for 30 min at 37 °C and subjected to SDS/PAGE, followed by Coomassie blue staining and autoradiography.

Tissue Culture, Transfection, and Pharmacology. HeLa cells were grown and infected as described previously (6). Cells were transfected with the different expression vectors using Lipofectamine 2000 (Invitrogen) or with 20 μM BI-1 or control siRNA using Hyperfect (Qiagen) according to the manufacturers' recommendations. After 72 h, knockdown of BI-1 expression was tested by RNA isolation using the Qiagen RNAeasy Kit according to the manufacturer's instructions, and by semiquantitative RT-PCR using BI-1 (h)-PR (sc-37298-PR; Santa Cruz Biotechnology) or GADPH (primers 15 and 16) primers.

Apoptosis was induced by the addition of 1 μM of STS (Calbiochem) for 4 h during EPEC infection and for 6 h during transfection. ER stress was induced by the addition of 10 μg/mL of BFA (Sigma-Aldrich) or 5 μg/mL of TUN (Sigma-Aldrich), followed by an 18-h incubation. The global caspases inhibitor z-VAD-fmk (Calbiochem) was used at a final concentration of 66 μM (added together with bacteria or with STS).

Epifluorescence Microscopy and Scanning Electron Microscopy. For analysis of nuclear condensation, cleaved caspase-3 staining, and membrane blebbing, HeLa cells were infected with a MOI of 1:50 for 1 h as described previously (6). Following five washes with HBSS, 250 μg/mL of gentamicin (Invitrogen) was added to kill the bacteria (to avoid cytotoxic effects of bacterial multiplication) for a further 2 h before processing for DNA staining with Hoescht 33342 (Molecular Probes), cleaved caspase-3 staining, or SEM.

Membrane blebbing was analyzed by phase contrast and was distinguished from attached bacteria stained with Hoescht 33342. Membrane blebbing was confirmed by SEM. For SEM, cells were fixed with formaldehyde, washed in phosphate buffer (0.1 M, pH 7.3), fixed overnight in 3% glutaraldehyde and postfixed in 1% osmium tetroxide for 30 min. Samples were processed using standard dehydration and critical point drying methods, sputter coated using a gold sputter (gold-palladium target). Samples were examined using a JEOL JSM-6390 scanning electron microscope.

For cleaved caspase-3 staining, transfected cells were fixed in prechilled methanol for 10 min at −20 °C, permeabilized, washed and quenched for 30 min with 50 mM NH_4Cl, and stained as described previously (6). Cleaved caspase-3 (active) was detected with rabbit anti-cleaved caspase-3 (Cell Signaling Technology), incubated overnight at 4 °C, and HA-NleH1 was stained with mouse anti-HA (Covance), diluted at 1:1,000 and 1:500, respectively, in 5% BSA. Donkey anti-rabbit IgG conjugated to rhodamine (RRX) and donkey anti-mouse IgG conjugated to Cy2 (Jackson Laboratories) were used at concentrations recommended by the manufacturer. For myc-BI-1/HA-NleH1 colocalization, transfected cells were fixed with paraformaldehyde, permeabilized with Triton X-100, washed with PBS, and quenched for 30 min with 50 mM NH_4Cl. HA-NleH1 was detected with mouse anti-HA (Covance) diluted at 1:500 and then with donkey anti-mouse IgG conjugated to RRX. Myc-BI-1 was next detected with mouse anti-myc conjugated to FITC (Sigma-Aldrich) diluted at 1:240 in 5% BSA.

Before epifluorescence observation, the coverslips were mounted on slides using ProLong Gold antifade reagent (Invitrogen) and visualized with a Zeiss Axioimager immunofluorescence microscope. All images were analyzed using Zeiss Axiovision Rel 4.5 software.

Cell Viability Assay. HeLa cells grown in 24-well plates were infected for 1 h with an MOI of 1:100 and then treated with 250 μg/mL gentamicin for 4 h. Cells were washed three times with PBS and then trypsinized; trypsin was inactivated with culture media. Then 0.05% Trypan blue in PBS was mixed with HeLa cells at a 1:1 ratio just before counting on a Neubauer hemocytometer (Sigma-Aldrich). All counts were compared with the level of uninfected untreated cells and plotted as a percentage of adherent cells.

Detection of Procaspase-3 by Western Blot Analysis. HeLa cells, grown in six-well plates, were infected for 1 h with an MOI of 1:100. After five washes in HBSS, 250 μg/mL of gentamicin and STS were added, and the cells were incubated at 37 °C for 3 h. After washing, the cells were lyzed in 1% Triton X-100 containing a protease inhibitor mixture (Sigma-Aldrich). Cells were scraped on ice, and the lysates were sonicated. Whole-cell lysates were concentrated using Biomax protein-concentrating columns (Millipore), run on 12% SDS/PAGE gels, and transferred to PVDF membrane. Membranes were stained overnight at 4 °C with anti-procaspase-3 (Cell Signaling Technology), followed by incubation with anti-rabbit IgG-HRP conjugate (Dako), diluted 1:3,000, for 45 min at room temperature. Membranes were incubated with ECL reagents (GE Healthcare), and luminescence was detected using a Fuji LAS 3000 imager. The same membrane was then washed and incubated with the primary mouse anti-tubulin antibody (Developmental Studies Hybridoma Bank) and secondary anti-mouse IgG-HRP conjugate (Dako).

Measurement of Cytosolic Ca^{2+} Levels. Cytosolic Ca^{2+} levels were measured using the commercially available fluorescent indicator Fluo-4 Direct (Invitrogen) according to the manufacturer's instructions. HeLa cells were grown in 96-well microplates, incubated with Fluo-4 Direct for 1 h at 37 °C, and then infected for 3.5 h using primed bacteria (MOI 1:100). Fluorescent intensities were determined using a fluorometer set for excitation at 494 nm and emission at 516 nm.

Yeast Two-Hybrid System. A pretransformed Matchmaker HeLa cell cDNA library (Clontech) was screened against the NleH1 bait according to the manufacturer's protocol. Plasmid from positive colonies was purified using the yeast plasmid purification protocol described in Clontech's Yeast Protocols Handbook (PT3024-1); transformed into HB101; selected on M9 minimal media supplemented with proline, thiamine, streptomycin (100 μg/mL), and ampicillin (100 μg/mL); and sequenced. Protein interactions were confirmed by cotransforming the yeast AH109 with pGBT9 (*nleH1*), pGBT9($nleH1_{\Delta 100}$), pGBT9(*nleH2*) or pGBT9(*ospG*), and pGAD(*bi-1*) or pGAD($bi\text{-}1_{1\text{–}40}$), and plating on selective media (-Ade, His, Trp, Leu).

Murine Models. All animal experiments were performed in accordance with the Animals Act 1986 and were approved by the local Ethical Review Committee. Specific-pathogen-free female WT inbred C57BL/6 6- to 8-week-old mice were used in the study. All animals were housed in individually HEPA-filtered cages with sterile bedding. Independent experiments were performed using at least four mice per group.

Oral Inoculation and Harvesting. Mice were orally inoculated using a gavage needle with 200 μL of an overnight LB-grown bacterial suspension of *C. rodentium* in PBS (~5×10^9 CFU). The number of viable bacteria used as the inoculum was determined by retrospective plating onto LB agar–containing antibiotics. Stool samples were recovered aseptically at various time points after inoculation, and the number of viable bacteria per gram of stool was determined after homogenization at 0.1 g mL^{-1} in PBS and plating onto LB agar containing the appropriate antibiotics.

Tissue Collection and IFA Staining. Segments of the cecum and the terminal colon of each mouse were collected at 9 days postinoculation, rinsed of their contents, and fixed in 10% buffered formalin for microscopic examination. Formalin-fixed tissues were then processed, embedded in paraffin, and sectioned at 5 μm. Sections were unwaxed in Histoclear with two 10-min washes and then rehydrated in 100%, 95%, and 80% ethanol before immersion in PBS 0.1% Tween 20 0.1% saponin (PBS-TS). Sections were then placed in 10 mM sodium citrate and heated to 100 °C for 10 min for heat-induced antigen unmasking. Blocking was conducted in PBS-TS with 10% normal donkey serum before labeling. Caspase-3 cleavage was analyzed by indirect immunofluorescence assay (IFA) using chicken anti-intimin antibody to label bacteria and rabbit anti-cleaved caspase-3 (Cell Signaling) overnight at 4 °C and Hoechst at room temperature for 1 h in a humidified chamber for DNA labeling. Multiple separate sections of each mouse were examined, and at least four sites of adherent bacteria were analyzed per mouse.

Sections were examined with an Zeiss Axio Imager M1 microscope. Images were acquired using a Zeiss AxioCam MRm monochrome camera and computer-processed using Zeiss AxioVision, Adobe Photoshop 5.0, and Adobe Illustrator 8.0 software.

Statistics. All statistical tests were performed using commercially available GraphPad InStat version 3.06 software on experiments tested in triplicate and repeated a minimum of three times.

1. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 97:6640–6645.
2. Levine MM, et al. (1978) *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are noninvasive. *Lancet* 1:1119–1122.
3. Dahan S, et al. (2005) EspJ is a prophage-carried type III effector protein of attaching and effacing pathogens that modulates infection dynamics. *Infect Immun* 73:679–686.
4. Hemrajani C, et al. (2008) Role of NleH, a type III secreted effector from attaching and effacing pathogens, in colonization of the bovine, ovine, and murine gut. *Infect Immun* 76:4804–4813.
5. Schlosser-Silverman E, Elgrably-Weiss M, Rosenshine I, Kohen R, Altuvia S (2000) Characterization of *Escherichia coli* DNA lesions generated within J774 macrophages. *J Bacteriol* 182:5225–5230.
6. Whale AD, et al. (2007) TccP2-mediated subversion of actin dynamics by EPEC 2: A distinct evolutionary lineage of enteropathogenic *Escherichia coli*. *Microbiology* 153:1743–1755.

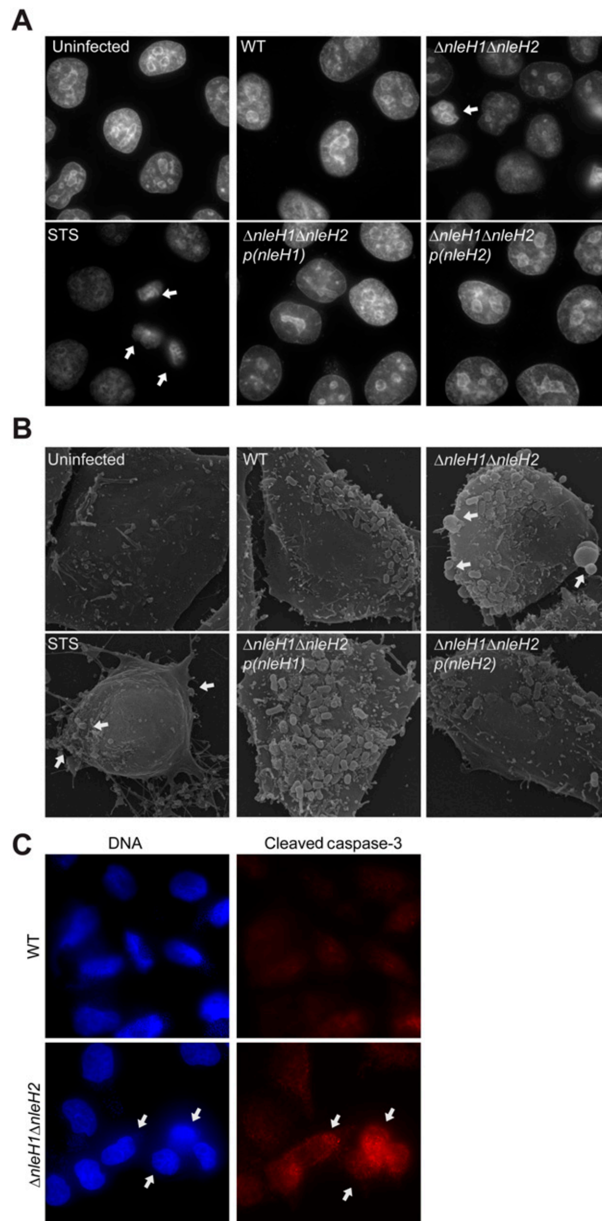

Fig. S1. HeLa cells infected with EPEC Δ*nleH* undergo apoptosis. Cells infected for 3 h with WT EPEC, Δ*nleH1*Δ*nleH2*, and complemented strains p(*nleH1*) or p (*nleH2*) were stained with Hoechst for evaluation of nuclear condensation and fragmentation (*A*, arrows), processed for SEM to visualize membrane blebbing (*B*, arrows), or stained with cleaved caspase-3 antibody (*C*, arrows). Uninfected and STS-treated cells were used as controls.

Fig. S2. Autoradiograph (*A*) and Coomassie blue staining (*B*) of NleH1 kinase assay using [γ-33P] ATP. Kinase assays were conducted using purified proteins in the presence or absence of the general substrate MBP. NleH1 phosphorylated MBP (lane 1) and autophosphorylated itself (lane 2). A K159A substitution resulted in loss of kinase activity (lanes 3 and 4). Abl kinase was used as a positive control (lanes 5 and 6). The MBP substrate used in the kinase assays is shown in lane 7. (*C*) Quantification of live adherent HeLa cells following infection with WT EPEC, Δ*nleH1*Δ*nleH2* mutant, and p(*nleH1*)- or p(*nleH1 K159A*)-complemented strains. Complementing the double-*nleH* mutant with a plasmid encoding $NleH1_{K159A}$ significantly increased the number of adherent cells, although not to a WT level. The nonparametric Mann-Whitney test was used to determine significance, because the data did not follow Gaussian distribution. $*P < 0.05$.

A

```
              1        10        20        30        40        50        60        70        80
NleH1         MLSPSSVNLGCSWNSLTRNLTSPDSRILSSVRDAAASSDNGAQVKVGNRTYRVVVTDNKFCVTRESHSGCFTNLLHRLGWPKGEISRKI
NleH2         MLSPSSINLGCSWNSLTRNLTSPDNRVLSSVRDAAVHSDSGTQVTVGNRTYRVVVTDNKFCVTRESHSGCFTNLLHRLGWPKGEISRKI
OspG          .........................................................................................
consensus>50  mlspssvnlgcswnsltrnltspd.rilssvrdaa..sd.g.qv.vgnrtyrvvvtdnkfcvtreshsgcftnllhrlgwpkgeisrki

              90        100       110       120                 130       140       150       160
NleH1         EVMLNSSPVNRAMERGAVHSNRPDLPPVDYAPPELPSVDY........NSLPVPGYVIGKGGNAVVYEDAEDATKVLKMFTT.....
NleH2         EAMLNTSPVSTTIERGSVHSNRPDLPPVDYAQPELPPADYTQSELPRVSNNKSPVPGNVIGKGGNAVVYEDMEDTTKVLKMFTI.....
OspG          ........................MKITSTIIQTPFPFENNNSHAGIVTEPILGKLIGQGSTAEIFEDVNDSSALYKKYDLIGNQY
consensus>50  e.mln.spv....erg.vhsnrpdlppvdya.pelp..dy.q.e......n..P!pG.vIGkGgnAv!%ED.#D.tkvlKm%t......

                  170       180       190       200       210         220       230       240       250
NleH1         SQSNEEVTNEVRCFNQYYGAGSAEKIYGDNGDIIGIRMDKINGESLLNISS..LPAQAEHAIYDMFDRLEQKGILFIDTTETNVLYDRT
NleH2         SQSHEEVTSEVRCFNQYYGSGSAEKIYNDNGNVIGIRMNKINGESLLDIPS..LPAQAEQAIYDMFDRLEKKGILFVDTTETNVLYDRM
OspG          NEILEMAWQESELFNAFYGDEASVVI..QYGGDVYLRMLRVPGTPLSDIDTADIPDNIESLYLQLICKLNELSIIHYDLNTGNMLYDKE
consensus>50  s#s.EevtnEvrcFNq%YG.gsaekIy.#nGdi!giRMdk!nGesLl#I.s..lPa#aE.aiy#$fdrL#qkgIlfiDttetNvLYDr.

                   260       270          280       290
NleH1         RNEFNPIDISSYNISERSWSENQ...IMQSYHGGKQDLISVVLSKI.
NleH2         RNEFNPIDISSYNVSDISWSEHQ...VMQSYHGGKLDLISVVLSKI.
OspG          SESLFPIDFRNIYAEYYAATKKDKEIIDRRLQMRTNDFYSLLNRKYL
consensus>50  r#efnPIDissynise.swse.#...!mqsyhggkqDliSvvlsKi.
```

B

% cells with cleaved caspase 3

50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 0

- | - | OspG | GFP | Transfection

- | STS

C

I, II, III, IV, V, VI

Fig. S3. (*A*) Sequence alignment of NleH1, NleH2 of EPEC strain E2348/69, and OspG of *Shigella flexneri* 2A strain 320. ! and $ correspond to hydrophobic residues (I or V and L or M), % corresponds to aromatic residues (F or Y), # corresponds to charged residues (N, Q, D, and E). (*B*) Ectopic expression of OspG did not prevent cleavage of procaspase-3 following treatment with STS, similar to mock- or GFP-transfected cells. (*C*) NleH/OspG–BI-1 protein interaction. Yeast cells co-transformed with pGBT9-NleH1/pGAD-BI-1 [III] and pGBT9-NleH1Δ100/pGAD-BI-1 [VI] grew on selective medium, whereas yeast co-transformed with pGBT9-NleH1Δ100/pGAD-BI-1 [I], pGBT9-NleH2/pGAD [II], pGBT9-OspG/pGAD-BI-1 [IV] and pGBT9-OspG/pGAD [V] did not, demonstrating specific interaction of NleH2 or NleH1Δ100 with BI-1.

Table S1. Strains used in this study

Strains	Characteristics	Reference
E2348/69	Wild-type EPEC O127:H6	(1)
ICC303	EPEC E2348/69 Δ*nleH1*Δ*nleH2*, *nleH1*::*Kan*, *nleH2*::*Cm* (Kan^r Cm^r)	This study
AH109	*S. cerevisiae MATa* mating type with *HIS3*, *ADE2*, *lacZ*, and *MEL1* reporters for interaction, and *TRP1* and *LEU2* transformation markers	Clontech
Y187	*S. cerevisiae MAT*α mating type with *lacZ*, and *MEL1* reporters for interaction, and *TRP1* and *LEU2* transformation markers	Clontech
ICC180	Bioluminescent *C. rodentium* harboring the *Photorhabdus luxCDABE* operon (Kan^r)	(2)
ICC285	ICC180 Δ*nleH* (Kan^r Cm^r)	(3)

Kan^r, kanamycin; Cm^r, chloramphenicol.

1. Levine MM, et al. (1978) *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are noninvasive. *Lancet* 1:1119–1122.
2. Wiles S, et al. (2004) Organ specificity, colonization and clearance dynamics in vivo following oral challenges with the murine pathogen *Citrobacter rodentium*. *Cell Microbiol* 6: 963–972.
3. Hemrajani C et al. (2008) Role of NleH, a type III secreted effector from attaching and effacing pathogens, in colonization of the bovine, ovine, and murine gut. *Infect Immun* 76: 4804–4813.

Table S2. Plasmids used in this study

Plasmids	Characteristics	Reference
pSA10	pKK177-3 expression vector containing *lacI* gene	(1)
pICC443	p(*nleH1*), Derivative of pSA10, expressing NleH1	This study
pICC444	p(*nleH2*), Derivative of pSA10, expressing NleH2	This study
pGBT9	Y2H expression vector with GAL4 DNA binding domain (GAL4-BD)	Clontech
pGAD-T7 Rec	Y2H vector containing library inserts fused to GAL4 DNA activation domain (GAL4-AD)	Clontech
pGAD424	Y2H expression vector with GAL4 DNA activation domain (GAL4-AD)	Clontech
pICC447	pGBT9(*nleH1*), Derivative of pGBT9, expressing NleH1 fused to GAL4-BD	This study
pICC448	pGBT9(*nleH2*), Derivative of pGBT9, expressing NleH2 fused to GAL4-BD	This study
pICC455	pGBT9($nleH1_{\Delta 100}$), Derivative of pGBT9 expressing NleH1 deleted from its first 100 amino acids fused to GAL4-BD	This study
pICC456	pGBT9 (*OspG*), Derivative of pGBT9 expressing OspG fused to GAL4-BD	This study
pICC457	pGAD(*bi-1*), Derivative of pGAD424 expressing BI-1 fused to GAL4-AD	Clontech
pHM6	Mammalian expression vector with N-term HA tag	Roche
pICC449	pHM6(*nleH1*), Derivative of pHM6, expressing HA-NleH1	This study
pICC450	pHM6($nleH1_{K159A}$), Derivative of pHM6, expressing HA-$NleH1_{K159A}$	This study
pET28a	Vector for expression of His-tagged proteins	Novagen
pICC451	pET28a expressing NleH1 His tagged	This study
pICC452	pET28a expressing $NleH1_{K159A}$ His tagged	This study
pSB315	Source of *aphT* cassette	(2)
pKD4	*oriRg*, *blaM*, *KanR* cassette flanked by FRT sites	(2)
pKD46	*ori101*, *repA 101* (ts), *araBp-gam-bet-exo*, *blaM*	(2)
pRK5-myc	Mammalian expression vector with N-term Myc tag	(3)
pICC458	pRK5-myc transfection vector expressing BI-1	This study
pRK5-myc-OspG	pRK5-myc transfection vector expressing OspG	(4)

1. Schlosser-Silverman E, Elgrably-Weiss M, Rosenshine I, Kohen R, Altuvia S (2000) Characterization of *Escherichia coli* DNA lesions generated within J774 macrophages. *J Bacteriol* 182: 5225–5230.
2. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 97:6640–6645.
3. Berger CN, Crepin VF, Jepson MA, Arbeloa A, Frankel G (2009) The mechanisms used by enteropathogenic *Escherichia coli* to control filopodia dynamics. *Cell Microbiol* 11:309–322.
4. Kim DW, et al. (2005) The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. *Proc Natl Acad Sci USA* 102: 14046–14051.

Table S3. Primers used in this study

	Sequence 5′–3′
1	ATGCTATCACCATCTTCTGTAAATTTGGGGTGTTCATGGAATTCTTTAACCACGTTGTGTCTCAAAATCTC
2	AATTTTACTTAATACCACACTAATAAGATCTTGCTTTCCTCCATGATAAGGAATTCCCCGGATCCGTCGAC
3	ATGTTATCGCCCTCTTCTATAAATTTGGGATGTTCATGGAATTCTTTAACGTGTAGGCTGGAGCTGCTTC
4	TATCTTACTTAATACTACACTAATAAGATCCAGCTTTCCTCCGTGATAAGCATAGAATATCCTCCTTA
5	CCGCGGGTCGACATGCTATCACCATCTTCTG
6	AAAACTGCAGTCATCAAATTTTACTTAATACCACACTAAT
7	CCGCGGGTCGACATGTTATCGCCCTCTTCTA
8	AAAACTGCAGTCATCATATCTTACTTAATACTACACTAAT
9	CGGGATCCTGCTATCACCATCTTCTG
10	CCAATGCATTGGTTCTGCAGCTAAATTTTACTTAATACC
11	CGGGATCCTGTTATCGCCCTCTTCTA
12	CCAATGCATTGGTTCTGCAGTTATATCTTACTTAATACT
13	CCCGGAAGCTTGATGCTATCACCATCTTCT
14	ATAGTTAGCGGCCGAATTTTACTTAATACCACACTAAT
15	AAGGTCGGAGTCAACGGATTTGGT
16	AGTGATGGCATGGACTGTGGTCAT
17	CTATTCGATGATGAAGATACCCCACCAAACCCAAAAAAAGAG
18	TTAGCATCTATGACTTTTTGGGGCGTTCAAGTG
19	GTCATGGTGATGTTTGTTAAG
20	TACCAACTCCATCCATGCAAC
21	CAGGATCTTGCCATCCTATGGA
22	GCTTGATGGTCGGAAGAGCATA
23	GCAAATCCTGCGTGCTGACGG
24	CGGATCCTCATCCACATTGTAAAGATCC
25	TTATACGCAAGGCGACAAGG
26	GATCTTCCGTCACAGGTAGG
27	AAAACTGCAGTCATCATACCACACTAATAAGATCTTGCTT
28	AAAACTGCAGTCATCATACTACACTAATAAGATCCAGCTT
29	GGAATTCCATATGCTATCACCATCTTCTG
30	GGATCCAGAACCCTAAATTTTACTTAATACC
31	GAT GCA ACA AAA GTC CTG GCG ATG TTT ACT ACA TCT CAA AGC
32	GCT TTG AGA TGT AGT AAA CAT CGC CAG GAC TTT TGT TGC ATC
33	GCTGTTCATTCGAATAGACCTG
34	CAGGATCCCCGGGAATTCCG
35	GCGAATTCAAAATTACCAGCACCATTATTCA
36	CGGGATCCTCACAGATATTTGCGGTTCAGCA
37	CCGGAATTCCAACATATTTGATCGAAA
38	CCCAAGCTTTCATTTCTTCTCTTTCTTCTTA

Table S4. Percentage of cells infected and with cleaved caspase-3 staining

	Percent cells with cleaved caspase-3
Uninfected	0.33 ± 0.50
Wild type	1.18 ± 1.40
Δ*nleH1*Δ*nleh2*	43.00 ± 5.54
Δ*nleH1*Δ*nleh2* p(*nleh1*)	23.78 ± 5.30
Δ*nleH1*Δ*nleh2* p($nleh1_{K159A}$)	34.30 ± 8.81