Supplementary text

LEGENDS TO SUPPLEMETARY FIGURES

fig. S1. Tyrosine phosphorylation at Y474 of Tir is required for efficient actin polymerization and the recruitment of ZO-1, but not for disruption of the epithelial barrier.

(A) Production of actin-rich pedestals by EPEC is dependent on the tyrosine phosphorylation of Tir at Y474. HeLa cells were infected by *Δtir* containing p99-tir (*Δtir*/Tir) or p99-tirY474F. (*Δtir*/TirY474F), as described in the Materials and Methods. The cells were fixed and stained for phosphotyrosine with a monoclonal antibody 4G10 (green), for F-actin with rhodamine-phalloidin (red), and for EPEC with anti-intimin antiserum (blue). When the cells were infected with *Δtir*/Tir expressing a wild-type Tir, anti-phosphotyrosine antibody detected phosphorylated Tir at the interface between the bacteria and pedestals (upper row), whereas no phosphorylation of Tir was detected with *Δtir*/TirY474F expressing a mutant Tir with a substitution of Y474 by F474 (lower row). Scale bar, 5 mm.

(B) Tyrosine phosphorylation at Y474 is required for ZO-1's recruitment. HeLa cells were infected with a wild-type EPEC (WT), *Δtir,* or *Δtir*/Tir or *Δtir*/TirY474F, as described in the Materials and Methods. The cells were fixed and stained for ZO-1 (green) and F-actin (red), and for bacteria with anti-intimin antiserum (blue). It is notable that no recruitment of ZO-1 was observed in the absence of Tir, and even in its presence, the substitution at Y474 of Tir abolished the recruitment. Scale bar, 5 μm.

(C) EPEC expressing a mutant TirY474F causes a drop of TER, as observed with wild-type EPEPC. The measurement of TER across Caco/B7 monolayers was performed as described previously (1). Briefly, Caco/B7 monolayers cultured on filter supports (Transwell COL) for 5 days were infected by either wild-type EPEC or the mutants expressing Tir from plasmids at a MOI of 1 to 3. The TERs across the monolayers were measured with Millicell-ERS (Millipore) every two hours. Closed square, wild-type EPEC; open square, *Δtir*; open triangle, *Δtir* expressing a wild-type Tir from p99-tir; open circle, *Δtir* expressing a mutant Tir (Y474F) from p99-tirY474F. Data represent the means (± SD) from three independent experiments.

fig. S2. Recruitment of ZO-1 to the actin tails induced by *S. flexneri* and *L. monocytogenes* is mediated through the proline-rich region of the ZO-1 molecule.

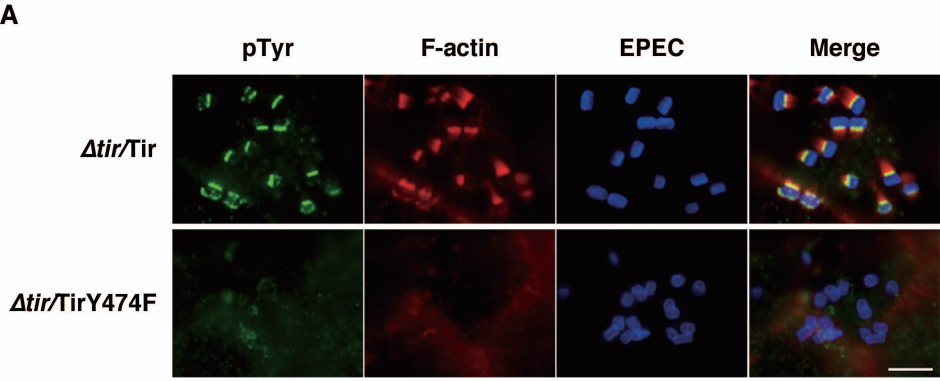
HeLa cells expressing NZO-1 (upper row), control mEGFP (middle row), or mEGFP-PRR (lower row) were infected by *S. flexneri* (panel A) or *L. monocytogenes* (panel B) as described in the Materials and Methods. F-actin and bacteria were visualized with rhodamine-phalloidin (red) and DAPI (blue), respectively. NZO-1 and PRR were detected with anti-VSVG tag and with the fluorescence of mEGFP, respectively. Scale bars, 5 μm.

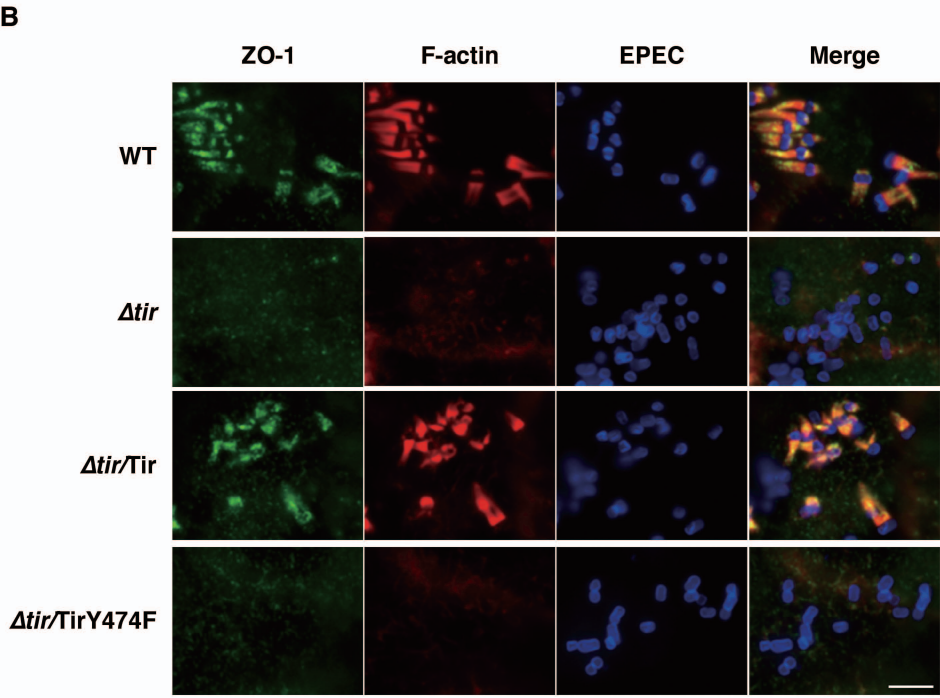
fig. S3. Clustering of the membrane-targeted Nck SH3 domains induces the recruitment of ZO-1 and formation of actin-tails.

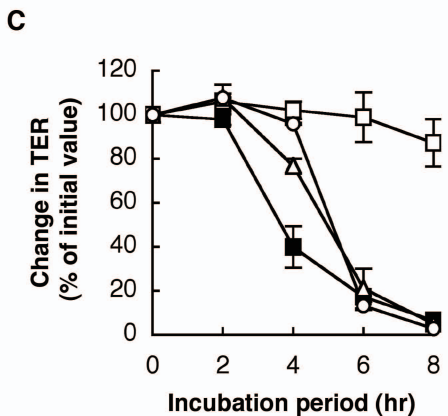
NIH-3T3 cells expressing either Nck SH3(3) (upper row) or Nck SH3(1+2+3) (lower row) were treated with anti-CD16 and Alexa546-conjugated anti-rat IgG antibodies. After fixation, the clustered fusion proteins were observed under a fluorescence microscope (CD16, red). F-actin (panel A) and ZO-1 (panel B) were visualized with Alexa488-phalloidin, and T8-754 and Alexa488-anti-mouse IgG antibodies, respectively (green). Insets are a higher magnification of the regions outlined by white dotted lines. Scale bar, 10 μm.

REFERENCE

1. **Miyake, M., M. Hanajima, T. Matsuzawa, C. Kobayashi, M. Minami, A. Abe, and Y. Horiguchi.** 2005. Binding of intimin with Tir on the bacterial surface is prerequisite for the barrier disruption induced by enteropathogenic *Escherichia coli*. Biochem. Biophys. Res. Commun. **337:**922-927.


A
pTyr
F-actin
EPEC
Merge
Δtir/Tir
Δtir/TirY474F


B
ZO-1
F-actin
EPEC
Merge
WT
Δtir
Δtir/Tir
Δtir/TirY474F


C
Change in TER
(% of initial value)
120
100
80
60
40
20
0
0
2
4
6
8
Incubation period (hr)

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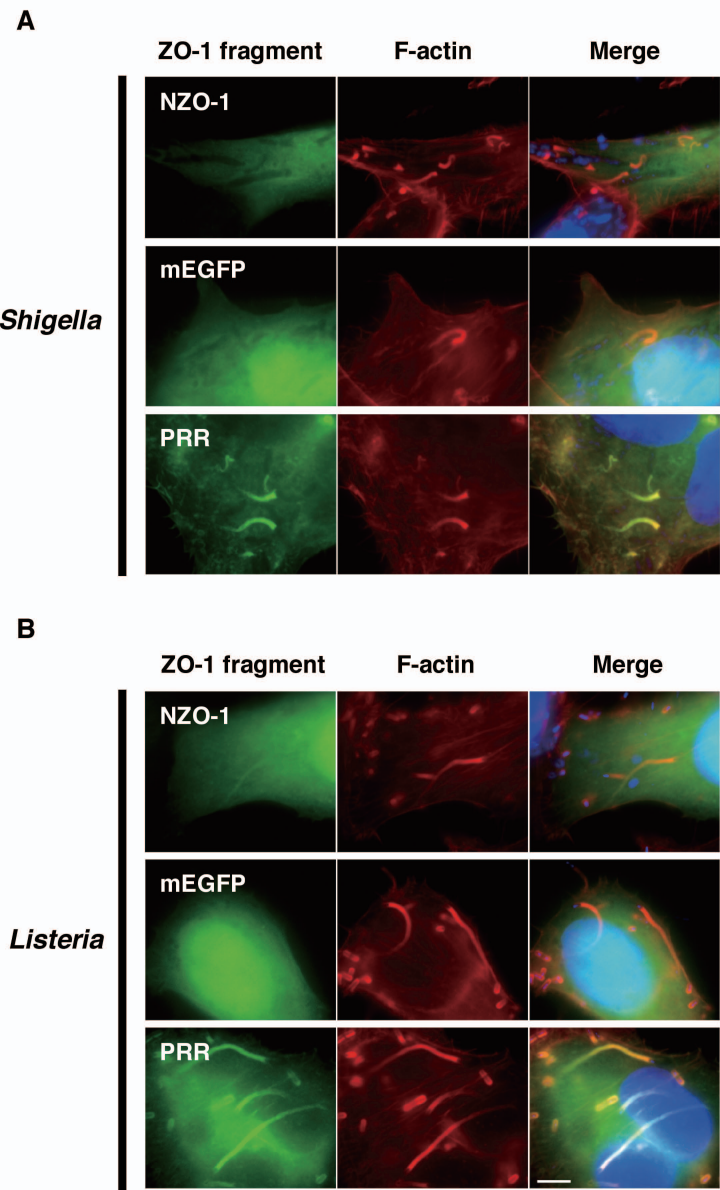

fig.S3 Hanajima, M. et al.