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## Crohn's disease-associated *Escherichia coli* survive in macrophages by suppressing NF $\kappa$ B signaling

Khalidur Rahman, PhD<sup>1,3</sup>, Maiko Sasaki, MS<sup>1,3</sup>, Asma Nusrat, MD<sup>2</sup>, and Jan-Michael A. Klapproth, MD<sup>1,3,\*</sup>

<sup>1</sup>Division of Digestive Diseases; Emory University, Atlanta, GA

<sup>2</sup>Epithelial Pathobiology and Mucosal Inflammation Research Unit, Department of Pathology and Laboratory Medicine; Emory University, Atlanta, GA

<sup>3</sup>Atlanta VA Medical Center, Decatur, GA

### Abstract

**Background**—Epidemiological and genetic studies suggest a role for enteric flora in the pathogenesis of Crohn's disease (CD). CD-associated *Escherichia coli* (CDEC) are characterized by their ability to invade epithelial cells, and survive and induce high concentration of TNF- $\alpha$  from infected macrophages. However, the molecular mechanisms of CDEC survival in infected macrophages are not completely understood.

**Methods**—Intracellular survival of CDEC strain LF82 isolated from inflamed ileum tissue, 13I isolated from inflamed colonic tissue, and control *E. coli* strains were tested in the murine macrophage cell line, J774A.1 by Gentamicin protection assay. Modulation of intracellular cell signaling pathways by the *E. coli* strains were assessed by western blot analysis and confocal microscopy.

**Results**—13I demonstrated increased survival in macrophages with 2.6-fold higher intracellular bacteria compared to LF82, yet both strains induced comparable levels of TNF- $\alpha$ . LF82 and 13I differentially modulated key Mitogen-activated protein kinase (MAPK) pathways during the acute phase of infection; LF82 activated all three MAPK pathways, whereas 13I activated ERK1/2 pathway but not p38 and JNK pathways. Both 13I and LF82 suppressed nuclear translocation of NF $\kappa$ B compared to non-invasive *E. coli* strains during the acute phase of infection. However, unlike non-invasive *E. coli* strains, 13I and LF82 infection resulted in chronic activation of NF $\kappa$ B during the later phase of infection.

**Conclusions**—Our results showed that CDEC survive in macrophages by initially suppressing NF $\kappa$ B activation. However, persistence of bacterial within macrophages induces chronic activation of NF $\kappa$ B, which correlates with increased TNF- $\alpha$  secretion from infected macrophages.

### Keywords

Crohn's disease; CD-associated *Escherichia coli*; Macrophages; NF $\kappa$ B

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\* Contact (corresponding author): Division of Digestive Diseases, Suite 201, 615 Michael Street, Atlanta, GA 30322, U.S.A, Tel: (404) 727-5638, Fax: (404) 727-5767, jklappr@emory.edu.

## INTRODUCTION

Crohn's disease (CD) is a form of inflammatory bowel disease characterized by patchy, transmural, granulomatous inflammation commonly involving the terminal ileum<sup>1</sup>. As a result, patients with CD suffer from abdominal cramps and pain, diarrhea, tenesmus, and weight loss. The etiology of CD is multifactorial, and includes environmental and bacterial triggers in a genetically susceptible host. Genome-wide association studies have suggested that mutations in genes involved in intracellular bacterial detection and clearance predispose to CD<sup>2, 3</sup>. Furthermore, presence of intramucosal bacteria in CD lesions, and the impaired ability of CD patients to clear intracellular bacteria due to defective innate immune responses implicate bacteria in the initiation and pathogenesis of CD<sup>4-6</sup>. These studies suggest that defective processing of intracellular bacteria plays an important role in CD pathogenesis<sup>7-9</sup>.

The concept that *Escherichia coli* are involved in the pathogenesis of CD is supported by multiple studies demonstrating intramucosal or mucosa-associated *E. coli* in CD patients<sup>7</sup>. This hypothesis is further strengthened by culture-independent metagenomic analyses of gut microbiota showing increased abundance of *E. coli* in IBD patients<sup>10-12</sup>. One of the best characterized CD-associated *E. coli* (CDEC) strain, LF82 can adhere to and invade intestinal epithelial cells, infect and replicate in macrophages, and induce high amounts of TNF- $\alpha$  from infected macrophages<sup>13</sup>. Further studies showed that LF82 can also invade Peyer's patches, translocate across M cells, and infect and replicate in neutrophils<sup>13-15</sup>.

CDEC strain LF82 survive and replicate in the acidic environment of active phagolysosomes in macrophages without causing cell death and surprisingly, induce secretion of TNF- $\alpha$ <sup>9, 16, 17</sup>. In contrast to the survival strategy of LF82 in macrophages, LF82 blocks autophagosome maturation and triggers death in neutrophils through NETosis<sup>15</sup>. Survival of LF82 in macrophages and neutrophils, first line of innate immune defense against invading bacteria, suggest that LF82 has the ability to modulate pro-inflammatory signaling pathways to avoid clearance by the professional phagocytes. The molecular mechanism of LF82 survival in macrophages and neutrophils are not fully elucidated, however, in human epithelial cells, LF82 is shown to suppress inflammatory responses by preventing IFN- $\gamma$  mediated STAT1 activation<sup>18</sup>. Another CDEC strain, 13I, isolated in our laboratory, suppresses IL-8 expression in infected epithelial cells through yet an unknown mechanism<sup>19</sup>. Similarly to LF82, 13I survives in macrophages and induces high concentrations of TNF- $\alpha$ <sup>19</sup>. Given the inherent ability of CDEC strains to survive in professional phagocytes, it will be of paramount interest to study the molecular mechanisms of LF82 and 13I mediated effects on pro-inflammatory signaling in professional phagocytes of the innate immune system.

Therefore, to understand the molecular mechanism of CDEC survival in macrophages, we investigated the ability of CDEC strains LF82 and 13I to modulate cell signaling pathways in a previously established murine macrophage cell model<sup>16, 19</sup>. Here we show that the CDEC strains initially suppress NF $\kappa$ B activation, but induce chronic activation of NF $\kappa$ B in the later phase of infection, which correlates with increased TNF- $\alpha$  secretion from infected macrophages. We speculate that these data have implications for the pathophysiology of

CD, allowing invading bacteria to gain a foothold in the host to induce chronic inflammation in the susceptible host.

## MATERIALS AND METHODES

### Bacterial strains

The CD-associated *E. coli* strain 13I was isolated from biopsy samples taken from macroscopically inflamed colonic tissue of a CD patient. The UC associated *E. coli* strain 30A was isolated from normal appearing UC tissue. The isolation of *E. coli* from the biopsy tissue was described previously<sup>19</sup>. The CD-associated *E. coli* strain LF82, isolated from inflamed ileum tissue, was a gift from Arlette Darfeuille–Michaud, Institut Universitaire de Technologie, Genie Biologique, Aubiere, France. The non-pathogenic *E. coli* strain EFC1, isolated from a healthy female volunteer was a gift from M.S. Donnenberg, University of Maryland, Baltimore, MD.

### Cell Culture

The murine macrophage cell line, J774A.1 and the human epithelial colorectal adenocarcinoma cell line, Caco-2 (ATCC, Manassas, VA) were maintained at 37°C in a humidified 5% CO<sub>2</sub> atmosphere in DMEM (Invitrogen, Carlsbad, CA) supplemented with 50 µg/ml Gentamicin (Invitrogen) and 10% FCS (Gemini, Calabasas, CA). Macrophages and Caco-2 cells were used between passages 4–12 and 21–30, respectively.

### Bacterial Invasion Assay

Bacterial invasion of macrophages and epithelial cells was measured as described previously<sup>19</sup>. The macrophages and Caco-2 cells were infected with overnight cultures of aerobically grown bacteria at a multiplicity of infection (MOI) of 10 bacteria per macrophage or Caco2 cell. After 3 h incubation at 37°C, infected macrophages and Caco-2 cells were washed three times in PBS and fresh DMEM supplemented with 100 µg/ml Gentamicin was added to kill extracellular bacteria. Cells were incubated for an additional hour, washed three times with PBS, lysed in 1% Triton-X-100/PBS and serial dilutions were plated on LB plates. The initial inoculum (I/O) was determined by plating serial dilutions of bacterial cultures used for infection on LB agar plates and counting the number of colonies the next day. Invasion was considered significant if a minimum of 1% of I/O could be recovered from the infected cells. Data are reported as mean ± SD.

### Cytokine Analysis

For cytokine analysis, J774A.1 macrophages were cultured on a 96 well plate and infected in quadruplicate at MOI 10:1 of an overnight culture of different *E. coli* strains for 3 h. Infected macrophages were washed three times with PBS, and cultured overnight in DMEM supplemented with 50 µg/ml Gentamicin and 10% FCS. Culture supernatants were harvested after 24 h and were analyzed by ELISA (eBioscience, San Diego, CA) in quadruplicate for TNF-α, IL-6, and IL-10 according to manufacturer's specifications. Data were presented as mean ± SD per  $1 \times 10^5$  cells.

## Immunofluorescence Confocal Microscopy

J774A.1 macrophages cultured on coverslips were infected at MOI 10:1 for 30 min and 1 h, and cells were fixed with 3.7% paraformaldehyde in PBS, permeabilized with 0.2% Triton X100 (Sigma), and blocked in 3% bovine serum albumin in PBS. Cells were probed with NF $\kappa$ B p65 (Santa Cruz, CA) at 1:1000 dilution and Alexa Fluor 546 conjugated secondary goat antibody diluted at 1:1000 was used for detection. F-actin was labeled with phalloidin conjugated Alexa Fluor 488 (1:1000; Invitrogen) and nucleus was labeled with Topro-3 (Life Technologies, Grand Island, NY). Stained cells were mounted in Prolong Gold (Invitrogen) and visualized with an Axioskope 2 plus scope (Zeiss, Jena, Germany). For the 6 h infection experiments, macrophages cultured on coverslips were infected at MOI 10:1 for 3 h. Infected macrophages were washed three times with PBS and treated with 100  $\mu$ g/mL Gentamicin for one hour to kill extracellular bacteria. Macrophages were then washed three times with PBS and cultured for an additional 2 h in DMEM supplemented with 50  $\mu$ g/mL Gentamicin and 10% FCS.

**Western blot analysis and densitometry**—For western blot analysis J774A.1 macrophages were infected with various *E. coli* strains as described above. After infection, total protein extract were subjected to western blots analysis. Blots were quantified by densitometry using Image J software<sup>20</sup>. Experiments were replicated three times, and representative blots are shown.

**Statistical Analysis**—All data were analyzed by Student's *t* test with the significance level set at 0.05.

**Ethical Considerations**—No human or animal subjects were used for this study.

## RESULTS

### CD-associated *Escherichia coli* invade and replicate in macrophages and epithelial cells without inducing apoptosis

We have previously demonstrated that 13I induces high concentrations of TNF- $\alpha$  in infected macrophage cultures<sup>19</sup>. Since the ability of CDEC to invade macrophages correlates positively with TNF- $\alpha$  secretion<sup>9</sup>, we sought to determine if 13I invades macrophages by Gentamicin protection assay. Invasion of macrophage cultures by 13I was determined in comparison to CDEC reference strain LF82, non-pathogenic *E. coli* isolate EFC-1, and UC-associated *E. coli* strain 30A. As shown in Fig. 1A, at three hours post-infection, significantly higher percentage of initial inoculum (I/O) was recovered from the 13I infected macrophages ( $8\% \pm 0.5$ ; mean  $\pm$  SD) compared to LF82 infected macrophages ( $2.5\% \pm 0.4$ ;  $p > 0.05$ ). In contrast, no intracellular bacteria were isolated from macrophages infected with control strains EFC-1 and 30A.

In parallel experiments, we determined if 13I invades epithelial cells. For this analysis we used a model intestinal epithelial cell line, Caco2. Infection of differentiated Caco-2 epithelial cells with 13I and LF82 resulted in the recovery of 7% ( $\pm 2$ ) and 6% ( $\pm 0.6$ ) of I/O, respectively, whereas no intracellular EFC-1 and 30A were recovered from infected

Caco-2 cells (Fig. 1B). Taken together, these results show that CDEC strains 13I and LF82 effectively invade macrophages and epithelial cells *in vitro*.

### CD associated *E. coli* induce high concentrations of TNF- $\alpha$ and IL-6 in macrophage cultures

We and others have previously reported that CD associated *E. coli* regulate cytokine expression; specifically increase concentration of TNF- $\alpha$  in infected macrophage cultures<sup>9, 19</sup>. TNF- $\alpha$  is central to the pathogenesis of CD and neutralization with anti-TNF- $\alpha$  antibodies has been successfully used to treat active disease<sup>21-24</sup>. Our goal was to expand on previous observations and better define key cytokine expression in response to infection with CDEC. In agreement with our previous results<sup>19</sup>, both LF82 and 13I induced a significant 2.5-fold increase in TNF- $\alpha$  secretion from infected macrophages compared to non-pathogenic EFC-1 and 30A isolated from UC patient (Fig. 2A). In contrast to TNF- $\alpha$  expression, IL-6 concentrations were increased above background by EFC-1, LF82, 13I, and 30A, which were not significantly different among all four strains tested (Fig. 2B). Next, we sought to determine the effect of CDEC on anti-inflammatory cytokine IL-10. Interestingly, commensal strain EFC-1 induced significantly higher concentrations of IL-10 in macrophage cultures compared to LF82, 13I, and UC strain 30A (Fig. 2C). These findings indicate that CD-associated *E. coli* induce an imbalance of pro- and anti-inflammatory cytokine expression in infected macrophage cultures.

### 13I activates ERK1/2, but not p38 and JNK pathways

Mitogen activated protein kinases (MAPK) mediate pro-inflammatory signaling in response to bacterial effector proteins to aid in cellular survival<sup>25</sup>. To investigate whether CD strain 13I modulates the MAPK pathway, activation of Extracellular Signal-Regulated protein kinases 1 and 2 (ERK1/2), p38 MAPK (p38), and c-Jun NH(2)-terminal kinase (JNK) were determined during the early phase of infection. Infection of macrophage cultures with all *E. coli* strains resulted in a significant increase in ERK1/2 phosphorylation at 30 min and at 60 min post infection (Fig. 3A/B). Highest level of ERK1/2 activation was observed for 13I infected macrophages at 30 min post infection; a significant 2-fold increase in ERK1/2 phosphorylation compared to non-pathogenic *E. coli* strain EFC-1 and a significant 4-fold increase compared to UC strain 30A and LPS. Interestingly, level of ERK1/2 phosphorylation in LF82 infected macrophages was not significantly different from EFC-1 infected cells. By 60 min post infection, ERK1/2 activation increased in all macrophage cultures regardless of *E. coli* strain.

Next, we sought to determine activation status of MAPK p38 in response to infection of macrophage cultures with EFC-1, LF82, 13I, and 30A (Fig. 3 C/D). In comparison to control cultures, phosphorylation of p38 was significantly increased in all *E. coli* strains and LPS at both, 30 min and 60 min post infection. LF82 infection resulted in significant 2-fold increase in p38 activation compared to 13I, 30A, and LPS at 30 min, but no significant differences in p38 phosphorylation were observed at 60 min post infection. Although 13I infection resulted in significant 7-fold increase in p38 activation compared to negative control, activation of p38 in 13I infected macrophages was significantly lower than EFC-1, LF82, and 30A at both time points investigated.

As for JNK, all *E. coli* strains tested, in addition to LPS, increased phosphorylation of JNK compared to negative control at 30 min and 60 min post infection (Fig.3 E/F). At 30 min post infection, both EFC-1 (15-fold) and LF82 (25-fold) infection of macrophage cultures resulted in a significant increase in JNK phosphorylation in comparison to 13I (4-fold) and 30A (6-fold). Interestingly, JNK activation in 13I infected macrophages was even lower than LPS (6-fold) treatment. At 60 min post infection, JNK phosphorylation increased further in 30A (23-fold) infected and LPS (19-fold) treated macrophages. Lack of p38 and JNK activation in 13I infected macrophages suggest that 13I may regulate cytokine expression through activation of ERK1/2. In contrast, LF82 elicits a stimulatory effect on MAPK's activation predominantly through p38 and JNK.

### **13I suppresses activation and nuclear translocation of NF $\kappa$ B p65 during the initial phase of infection**

To determine how CD-associated strain 13I induces increased concentrations of TNF- $\alpha$  in macrophage culture supernatants, but not apoptosis (data not shown), we examined activation of NF $\kappa$ B; a master regulator of transcriptional program governing proliferation and inflammation<sup>25</sup>. We used an antibody against phosphorylated serine<sup>536</sup> (S<sup>536</sup>) to measure activation of NF $\kappa$ B p65 by immunoblotting. As seen in Fig 4A, at 30 min post infection, with the exception of 13I (5-fold), *E. coli* strains EFC-1(21-fold), LF82 (28-fold), and 30A (19-fold) significantly increased phosphorylation of NF $\kappa$ B p65 at serine 536. To further visualize the effect of infection with Gram negative bacteria on macrophage cultures, cells were stained with anti-NF $\kappa$ B p65 at 30 min post infection. As shown in Fig 4B, infection with both, EFC-1 and 30A, caused increased translocation of NF $\kappa$ B p65 into the nucleus compared to negative control and LPS treated cells. In contrast, LF82 and 13I infection resulted in a moderate increase in NF $\kappa$ B p65 translocation. However, the lack of NF $\kappa$ B S<sup>536</sup> phosphorylation in 13I and LF82 infected macrophages was transient and at 60 min post infection, none of the *E. coli* infected or LPS treated macrophages showed differences in NF $\kappa$ B S<sup>536</sup> phosphorylation. Interestingly, nuclear translocation of NF $\kappa$ B p65 was still higher in EFC-1 and 30A infected macrophages compared to cells infected with LF82 and 13I. The decreased nuclear translocation of NF $\kappa$ B p65 in LF82 and 13I infected macrophages compared to EFC-1 and 30A at 30 min suggests either a lack of activation or active suppression of NF $\kappa$ B p65.

### **CD associated *E. coli* strains persistently activate NF $\kappa$ B p65 during the later phase of infection**

To further assess whether the lack of nuclear translocation of NF $\kappa$ B p65 in LF82 and 13I infected macrophages is sustained during the later phase of infection, nuclear translocation of NF $\kappa$ B was determined at 6 hrs post infection. In the absence of extracellular bacteria, at 6 hrs post infection, nuclear translocation of NF $\kappa$ B p65 was terminated in EFC-1 and 30A infected macrophages. Surprisingly, nuclear translocation of NF $\kappa$ B was still apparent in CD strains LF82 and 13I infected cells suggesting that ongoing intracellular replication of LF82 and 13I, and/or persistent action of bacterial effector proteins results in persistent activation of NF $\kappa$ B in the infected macrophages.

## DISCUSSION

*Escherichia coli* strains associated with CD are characterized by their ability to invade immune and non-immune cells and induce high concentrations of TNF- $\alpha$ <sup>9, 17, 19</sup>. We recently reported a CDEC strain, 13I, that exhibits the characteristics of CDEC reference strain LF82 in its ability to adhere and infect epithelial cells and macrophages, and to induce high amounts of TNF- $\alpha$  from infected macrophages<sup>19</sup>. Further analysis of the strain 13I showed that 13I is significantly more invasive than reference strain LF82, but nevertheless induces similar level of TNF- $\alpha$  from infected macrophages. Here, we used a previously validated mouse macrophage cell line and the *E. coli* strains LF82 and 13I to show that these strains differentially modulate MAPK pathways but similarly suppress NF $\kappa$ B pathway during the acute phase of infection<sup>16, 19</sup>. However, persistence of CD-associated *E. coli* strains in macrophages induces chronic activation of NF $\kappa$ B, which may contribute to survival of the infected macrophages.

Recognition of bacteria or bacterial products by the pattern recognition receptors leads to activation of MAPK pathways that regulate both pro-survival and pro-inflammatory response pathways<sup>25</sup>. The differential modulation of MAPK pathways by LF82 and 13I points to the diversity in the pathogenicity of CDEC strains. It is also worth noting that LF82 was isolated from inflamed ileum of a CD patient whereas 13I was isolated from inflamed colonic tissue of a CD patient. The anatomical location of these two strains at the time of isolation might explain the differences in their ability to modulate MAPK pathways and is due to aberrant host mucosal recognition of bacteria<sup>26</sup>, tissue tropism<sup>27</sup>, or local dysbiosis<sup>12</sup>. During the early phase of infection, while LF82 activated all three MAPK pathways, 13I infection activated ERK1/2 pathway but not p38 and JNK pathways. Given the role of ERK1/2 as a pro-survival pathway that contributes to the regulation of cell proliferation and differentiation<sup>28</sup>, activation of ERK1/2 pathway by the CDEC strains fits well with the lack of apoptosis in the LF82 and 13I infected macrophages. Unlike ERK1/2 pathway, the p38 and JNK pathways regulate apoptotic cell death, and therefore the lack of p38 and JNK activation during the acute phase of infection may result in increased survival of 13I in macrophages<sup>29, 30</sup>. Furthermore, p38 MAPK pathway negatively regulates the fusion of phagosomes with lysosomes, and *Mycobacterium tuberculosis* exploits this pathway to inhibit fusion of phagosomes with lysosomes to ensure survival of bacillus in the phagosomes<sup>31</sup>. In contrast to *M. tuberculosis*, AIEC reference strain LF82 survives in phagolysosomes and the acidic environment of the phagolysosome is favorable for its replication<sup>17</sup>. It is possible that 13I may suppress p38 pathway to increase phagosome maturation to create a favorable environment for its replication. Further experiments are needed to identify the effector proteins used by the CDEC strains to modulate MAPK pathways.

Manipulation of NF $\kappa$ B pathway is a common strategy employed by many pathogenic bacteria to suppress host immune responses for their survival<sup>25, 32</sup>. The lack of NF $\kappa$ B activation during the acute phase of LF82 and 13I infection points to the possibility that CDEC strains initially modulate NF $\kappa$ B pathway to suppress acute phase pro-inflammatory responses. However, during the later phase of infection, survival of CDEC in infected macrophages results in persistent activation of NF $\kappa$ B, which may account for the high level

of TNF- $\alpha$  induced by CDEC infected macrophages. Alternatively, high level of TNF- $\alpha$  secreted by infected macrophages may in turn result in persistent activation of NF $\kappa$ B<sup>33, 34</sup>. TNF- $\alpha$  is a pro-inflammatory cytokine that can either induce apoptosis by activating caspase-8 and -10<sup>35, 36</sup>, or inhibit apoptosis by activating NF $\kappa$ B<sup>37-40</sup>. The survival of infected macrophages in the presence of high amounts of TNF- $\alpha$  in LF82 and 13I infected macrophages suggest that LF82 and 13I strains may exploit the anti-apoptotic property of TNF- $\alpha$  to survive in infected macrophages. It been shown that the intra-macrophagic replication of the AIEC strain LF82 is dependent on the concentration of TNF- $\alpha$  as neutralization of this cytokine decreases while addition of exogenous TNF- $\alpha$  increases intra-macrophagic replication of LF82<sup>9</sup>. It is possible that CDEC strains suppress initial burst of pro-inflammatory responses by suppressing NF $\kappa$ B activation, but later allow for activation of NF $\kappa$ B through TNF- $\alpha$  to induce anti-apoptotic pathways. Although a number of different bacterial effector proteins that can either inhibit or activate NF $\kappa$ B signaling have been characterized, regulation of NF $\kappa$ B signaling through TNF- $\alpha$  would be the first report of a mechanism where a pathogenic bacteria hijacks a host pro-inflammatory cytokine for its survival. More work is needed to understand the mechanisms underlying this pathway in order to design therapies to decrease bacterial persistence, and resulting chronic inflammation in CD patients.

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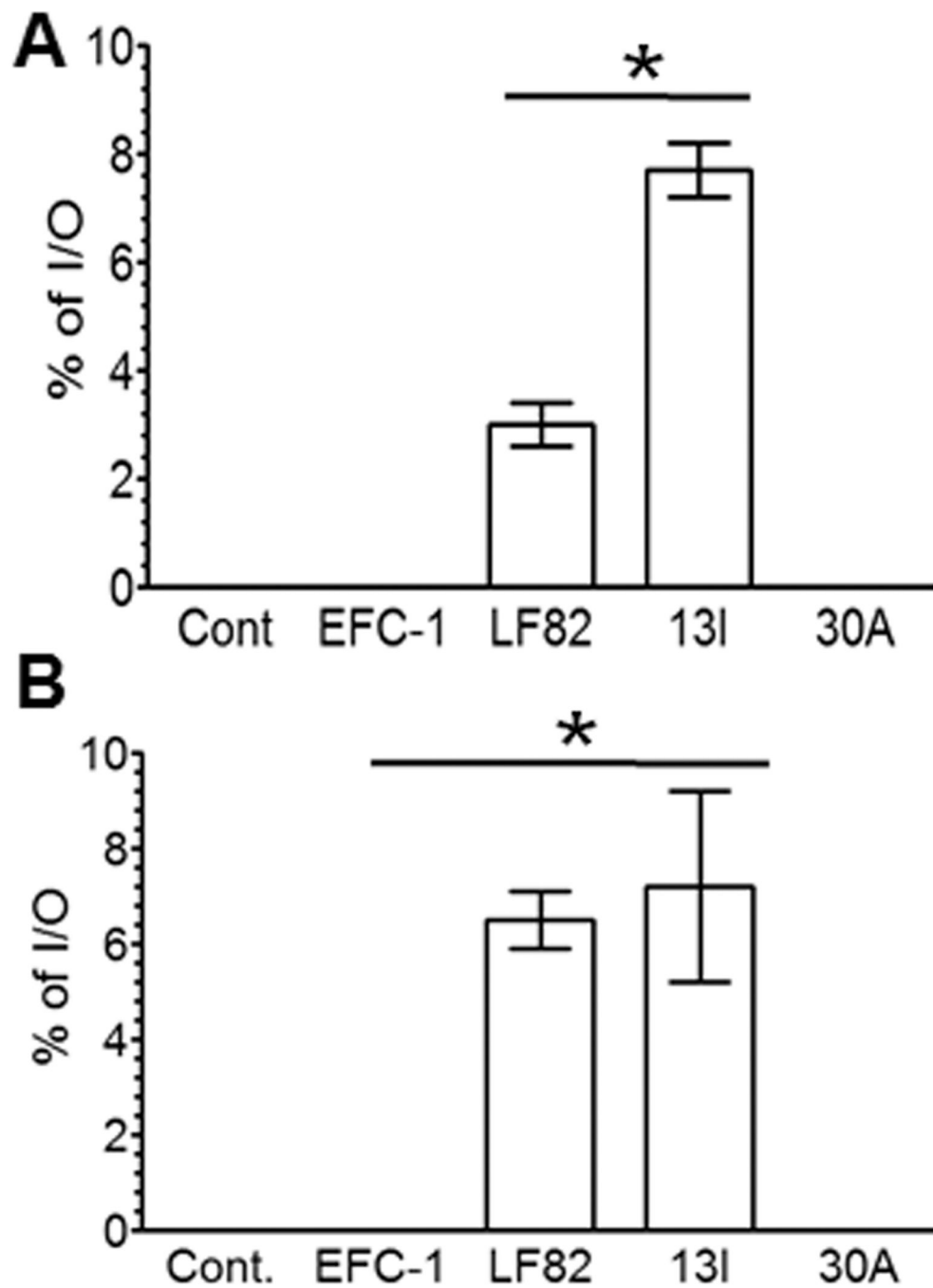
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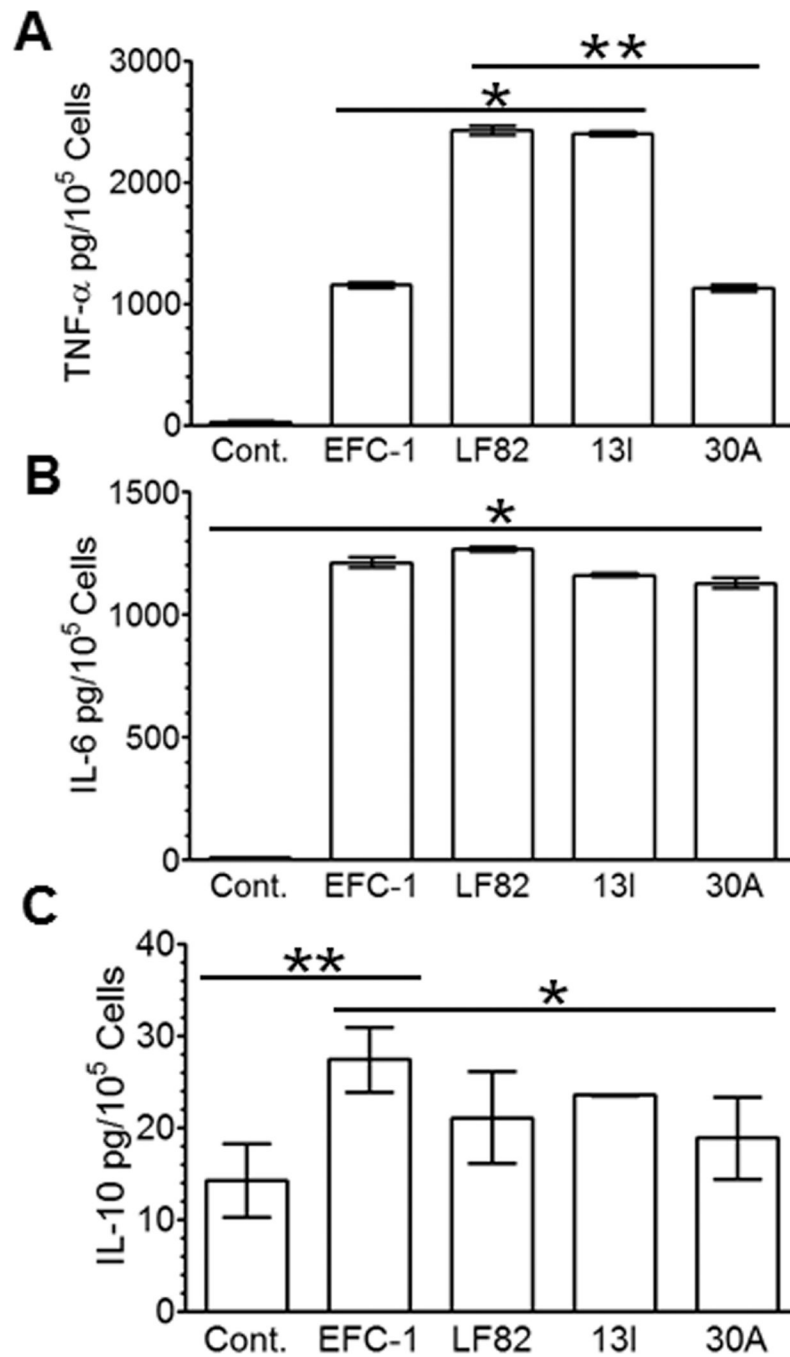
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**Fig. 1. Crohn's disease-associated *Escherichia coli* (CDEC) strain, 13I invades and replicates in macrophages and epithelial cells**

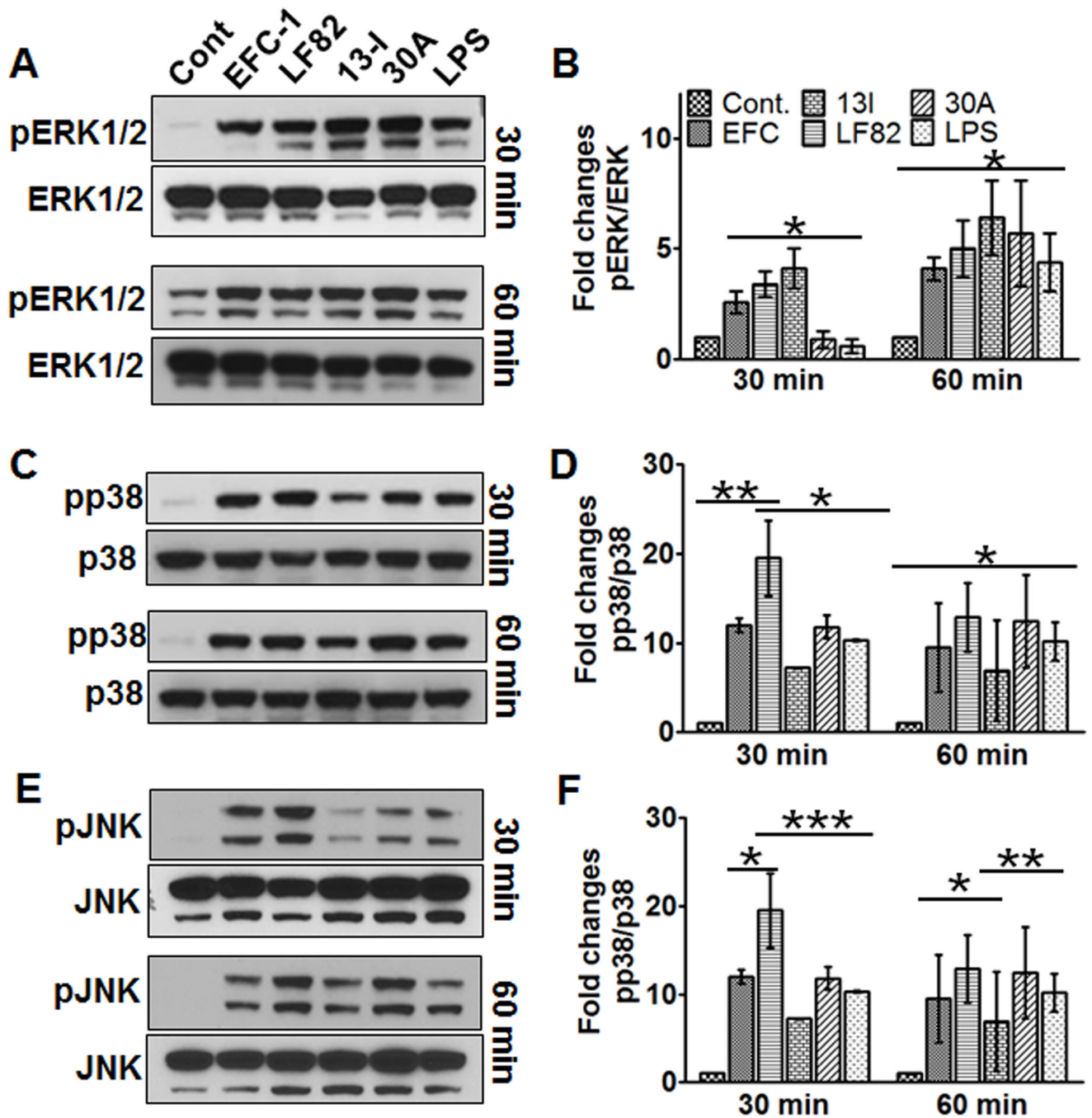
(A) J774.A1 macrophages and (B) Caco2 cells were infected for 3 hrs with CD-associated *E. coli* strains LF82 and 13I, and intracellular CFU was measured by Gentamicin protection assay, and expressed as percentage of initial inoculum (I/O). Non-pathogenic *E. coli* strain, EFC-1 and UC-associated *E. coli* strain, 30A were used as controls. Data are mean  $\pm$  S.D. Statistical significance was determined by student's *t* test.



**Fig. 2. Crohn's disease-associated *Escherichia coli* (CDEC) infected macrophages secrete high level of TNF $\alpha$**

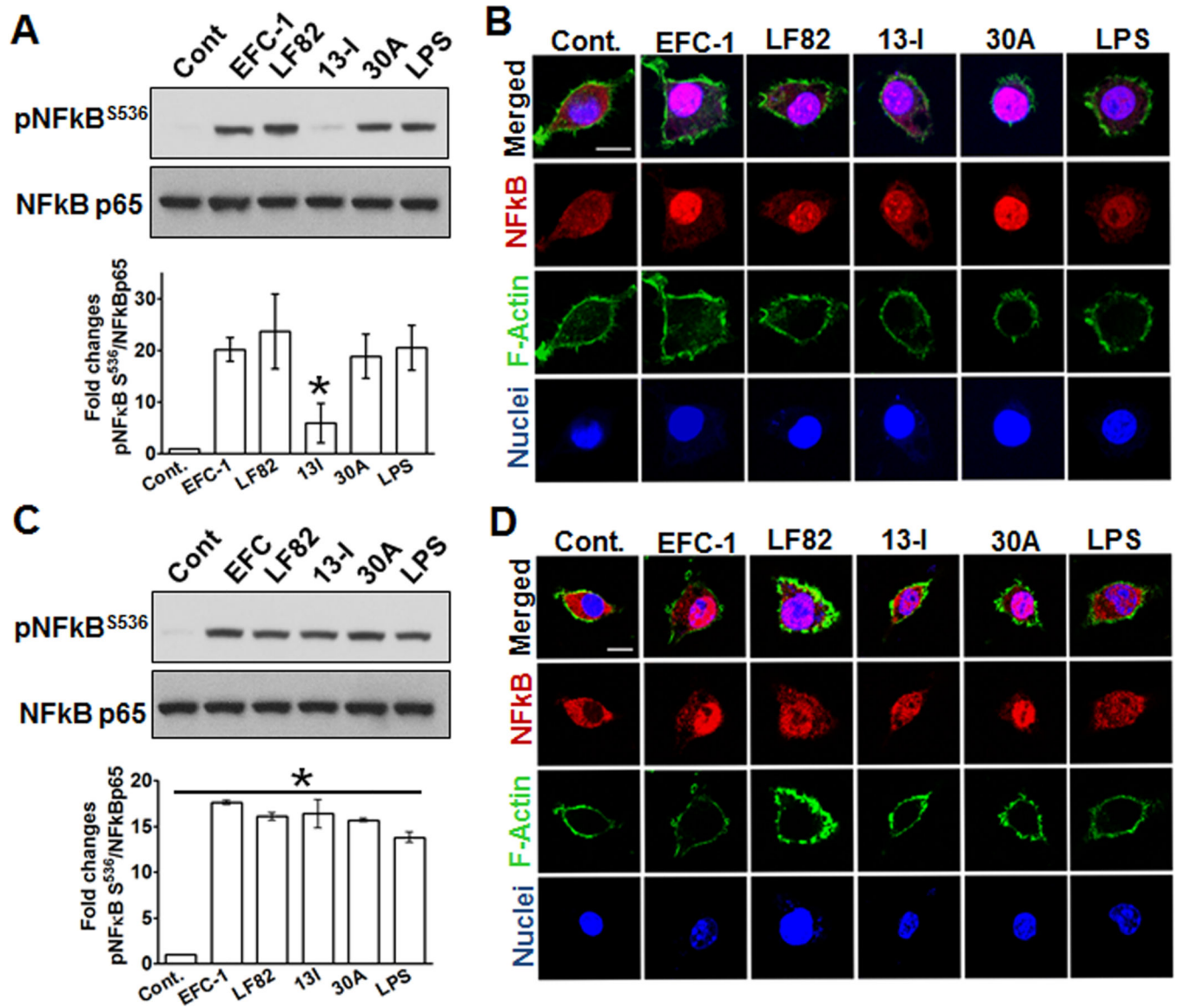
Cytokines secreted by J774.A1 macrophages infected with *E. coli* strains were measured by ELISA at 24 hrs post infection. (A) TNF $\alpha$  (B) IL-6 (C) IL-10. Data are mean  $\pm$  S.D.

Statistical significance was determined by student's *t* test.



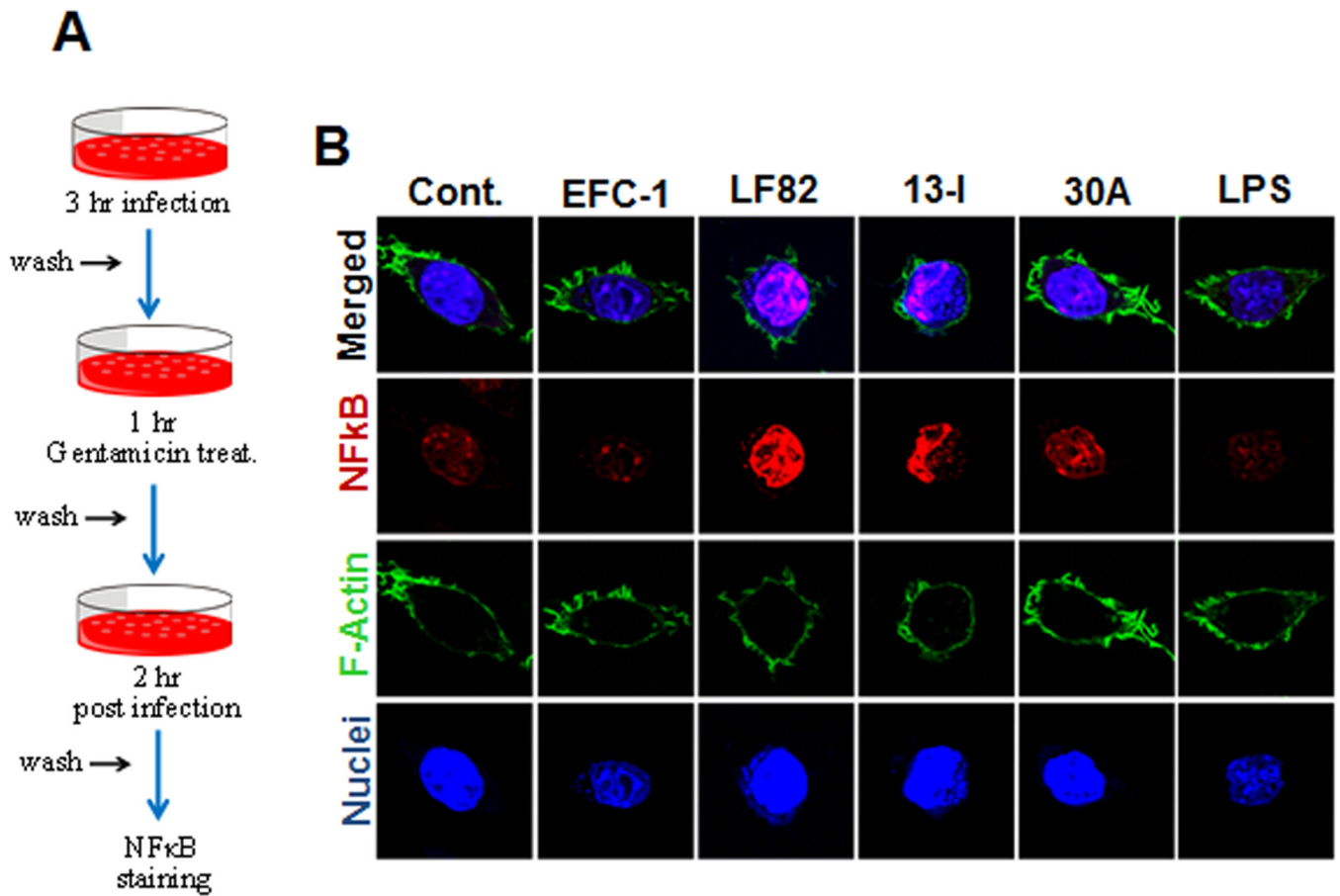
**Fig. 3. Crohn's disease-associated *Escherichia coli* (CDEC) strain, 13I activates ERK1/2 but not p38 and JNK pathways**

(A, C, E) Representative western blots of cell lysates from J774.A1 macrophages infected with *E. coli* strains for 30 min or 1 hr and (B, D, F) densitometric analyses of the western blots of three independent experiments. Data are mean  $\pm$  S.D. Statistical significance was determined by student's *t* test.



**Fig. 4. Crohn's disease-associated *Escherichia coli* (CDEC) suppress activation and nuclear translocation of NFκB during the initial phase of infection**

(A, C) J774.A1 macrophages were infected with *E. coli* strains for (A) 30 min or (C) 1 hr and cell lysates were analyzed by western blotting. The western blots are representative of three independent experiments and the histograms represent densitometric analyses of western blots from two independent experiments. (B, D) Macrophages grown on coverslips were infected with *E. coli* strains for (B) 30 min or (D) 1 hr and were subjected to confocal imaging. Scale 10 μM.



**Fig. 5. Intracellular replication of Crohn's disease-associated *Escherichia coli* (CDEC) results in activation of NFκB during the chronic phase of infection**

(A) J774.A1 macrophages grown on coverslips were infected with *E. coli* strains for 3 hr. After 3 hrs, cells were washed and again incubated for 3 hrs in complete medium supplemented with 10% serum and 50 ug/mL Gentamicin. Scale 10 μM.

**Table 1**

## Strains used in this study

<b>Name</b>	<b>Characteristics</b>	<b>References</b>
EFC-1	<i>E. coli</i> from healthy female volunteer	41
LF82	Isolated from inflamed small bowel tissue of a CD patient	13
13I	Isolated from inflamed colon tissue of a CD patient	19
30A	Isolated from normal colonic tissue of a UC patient	19