Induction of Inflammatory Macrophages in the Gut and Extra-Gut Tissues by Colitis-Mediated Escherichia coli

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HIGHLIGHTS
Increased commensal E. coli in colitis induce inflammatory macrophages
Colitic E. coli are different from other commensal and pathogenic E. coli
Gut inflammatory macrophages by E. coli need IL-18, IFN-γ, IL-12, and IL-22
PCKδ, NLRC4, caspase8, and caspase1/11 are required for E. coli-mediated activation

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Induction of Inflammatory Macrophages in the Gut and Extra-Gut Tissues by Colitis-Mediated Escherichia coli

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SUMMARY

Inflammatory macrophages play a critical role in gut and extra-gut inflammatory disorders, which may be promoted through the dysbiosis of gut microbiota. However, it is poorly understood how gut microbiota affect inflammatory macrophages. Here, we found that increased Escherichia coli (E. coli) in inflamed colon may induce inflammatory macrophages in gut and extra-gut tissues. These E. coli are different from other commensal and pathogenic E. coli in genomic components and also in ability to induce inflammatory responses. Dominant E. coli from colitic tissues induce gut inflammatory macrophages through a regulating network consisted of IL-18, IFN-γ, IL-12, and IL-22 in gut tissues. These E. coli also directly activate macrophages. Cytosolic inflammasome components PCK6, NLRC4, caspase8, and caspase1/11 are involved in E. coli-mediated activation in both gut epithelial cells and macrophages. These disclose a novel mechanism for how dysbiosis of gut microbiota in colitis cause inflammatory macrophages related to multiple diseases.

INTRODUCTION

The dysbiosis (aberrant gut microbiota composition and function) of gut microbiota may promote gut and extra-gut autoimmune and inflammatory disorders such as inflammatory bowel disease (IBD), obesity, atherosclerosis, carcinogenesis, etc (Blander et al., 2017). Although the mechanisms involved are not well understood, the inflammatory macrophages have a causal association with these diseases (Sekirov et al., 2010; Wynn et al., 2013). Thus, it is critical to understand how gut microbiota regulate these macrophages.

Tissue-resident macrophages represent a highly heterogeneous cell population able to sense and quickly adapt to environmental cues such as gut tissue macrophages, which play either protective or tolerogenic roles. In steady state conditions, the gut lamina propria (LP) macrophages display an anergic phenotype and are essential for intestinal homeostasis (De Schepper et al., 2018); but under inflammatory settings such as DSS-mediated colitis, the conditioning of murine Ly6C+ blood monocytes is impaired, and they give rise to inflammatory macrophages (Zigmond et al., 2012). These inflammatory macrophages produce large amounts of mediators such as TNFα, IL6, IL-1β, reactive oxygen intermediaries, and nitric oxide to cause diseases (MacDonald et al., 2011). Thus, the transformation of suppressive macrophages back into proinflammatory phenotype or inflammatory macrophages into anti-inflammatory cells has a major impact on the progression and resolution of the inflammation-associated diseases. It is unclear how the transformation of these macrophages is induced and maintained in these diseases. Alterations in the microbiome population and/or changes in gut permeability may promote microbial translocation into the distal tissues and/or organs. Danger signals derived from the microbiome can trigger the inflammatory cascade and activate macrophages to transform into inflammatory macrophages. However, what danger signal(s) of gut microbiota induce inflammatory macrophages remains poorly understood.

Certain members of the microbiota have been linked to inflammatory responses and intestinal pathology in mouse models such as that the members of the Enterobacteriaceae family, Klebsiella pneumoniae and Proteus mirabilis (Garrett et al., 2010). Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis (Garrett et al., 2010). E. coli, another member of Enterobacteriaceae family, is present in very less proportion in gut contents under normal physiological conditions (Schieber et al., 2015). However, a high abundance of commensal E. coli (facultative anaerobic
Figure 1. Characteristics of E. coli O160:H7 Isolated from Inflamed Colon Tissues
(A and B) 16s rDNA analyses of colon contents in DSS-treated wt (male, n = 5) and un-molested control mice (male, n = 5). The samples were clustered at phylum levels using the sample phylum count matrices and composition of colon bacteria (phylum levels) in control (A) and DSS-treated (B) mice. Mice were fed a 2.5% DSS solution in drinking water for 7 days.
(C) Fluorescent in situ hybridization (FISH) of E. coli in colon tissues of DSS-treated and un-molested mice (one representative, n = 6). Red, E. coli; Green, mucus; Blue, nuclei.
(D) Percent of E. coli O160:H7 clones in colitic tissues. The bacteria from colon tissues of DSS-treated and un-molested mice were in vitro cultured and then CFU of bacteria were sequenced through V1-V9 regions (n = 6).
(E and F) Survival rate (E), body weight, and disease activity index (F) in DSS-treated mice infused by E. coli O160:H7, heat-killed dead (killed) E. coli O160:H7, and E. coli IA139 (isolated from mice by us) (n = 12). Mice were treated using pan-antibiotics for one week before infusing E. coli. Data in F are represented as mean ± SD.
(G) Length of colon were monitored at day 7 after DSS. Data are represented as mean ± SD.
Proteobacteria in phylum and Enterobacteriaceae in genus) is commonly observed during inflammation in the colon (Winter and Baumler, 2014), including chemically induced colitis, antibiotic-treated mice, infection with enteric pathogens, and genetically induced colitis (Winter and Baumler, 2014). Microbial communities in patients with inflammatory bowel diseases also exhibit an increased prevalence of E. coli (Winter and Baumler, 2014). However, the physiological and pathological function(s) of these E. coli are poorly understood. One isolated E. coli strain from antibiotic-treated mice may cause lethal inflammasome activation (Ayres et al., 2012), whereas another strain E. coli may protect mice against muscle wasting and loss of fat during enteric Salmonella typhimurium or respiratory Burkholderia thailandensis infections (Schieber et al., 2015). Here, we found that a high abundance of commensal E. coli in inflamed colon not only indirectly induce inflammatory macrophages through gut epithelial cells but also directly activate extra-gut macrophages through cytosolic inflammasome complexes consisted of PCK8 (phosphoenolpyruvate carboxykinase 8), NLRC4 (NLR family CARD domain-containing protein 4), caspase8, and caspase1/11. These inflamed tissues derived E. coli do not cause acute disease symptoms.

RESULTS

E. coli O160:H7 Isolated from Inflamed Colon Promotes Sensitivity to DSS-mediated Colitis

To characterize inflammation-mediated E. coli, we employed chemically induced colitis (dextran sulfate sodium [DSS]-mediated colitis), in which there is a relative luminal abundance of Proteobacteria phylum (Enterobacteriaceae genus, E. coli species) (Schieber et al., 2015). Consistent with this report, the increased gut Proteobacteria phylum, Enterobacteriaceae genus, and E. coli was detected in the colonic contents and tissues of DSS-treated mice (Figures 1A and 1B and https://www.ncbi.nlm.nih.gov/sra/PRJNA512937). Using culturing techniques, serotyping, and genetic and molecular characterization, we identified a dominant E. coli strain from these inflamed colon tissues, named as E. coli O160:H7 strain (Figures S1A–S1C, 1C, and 1D, Table S1A and http://www.ncbi.nlm.nih.gov/bioproject/513139). E. coli O160:H7 strain was also present in the microbiota of unmanipulated mice but was not abundant, suggesting it is not able to compete efficiently for intestinal colonization. We next sequenced the genome of E. coli O160:H7 isolate and aligned the reads to reference E. coli genomes (Table S1B). The composition of E. coli O160:H7 gene clusters was different from other pathogenic E. coli O157:H7 and E. coli CFT073 and also unpathogenic E. coli str.k12 substr.MG1655 (Figures S1B and S1C). The fiC gene, encoding flagellin (H-antigen), was similar to that of E. coli O157:H7 isolates (Figure S1D). But, type III secretion system (T3SS) of E. coli O160:H7 was different from pathogenic E. coli O157:H7 such that T3SS of E. coli O160:H7 contained hxiB, irp1, HMWP1, paqkl, hokA, faaB, fdoG, fahH, tueB, bax, PTS-Dga, EIIc, dgaD, glmS, GFPt, ABC-2, and CPSE-A, which were not detected in E. coli O157:H7 (Table S1C). Notably, we did not find virulence-related membrane protein genes such as enterotoxin, EspB, EspA, SepZ, SepD, Hcp-like protein, protein TerZ, protein TerA, protein TerF, prohead protease, and antirepressor protein in E. coli O160:H7 isolate, which were encoded by E. coli O157:H7 (Table S1C). T3SS of E. coli O160:H7 was different from other unpathogenic E. coli str.k12 substr.MG1655 and pathogenic E. coli CFT073 (Table S1C). E. coli O160:H7 also encoded type IV secretion system (T4SS) (Table S1D) and other factors, including those for adhesion such as fim gene cluster (fimA, fimB, fimC, fimD, fimE, fimF, fimH, fimG, fimI, etc) and pil gene cluster (pilID, pilIT), papC, and papD, and internalization gene such as csg etc (Table S1D). However, other disease-associated factors such as Afa/Dr adhesins, traA (encoding pilin), and mxiX (marker for pathogenicity-associated island from strain CFT073), which were found in patients (Mansan-Almeida et al., 2013), was not detected in E. coli O160:H7 (Table S1D). E. coli O160:H7 also had multiple drug-resistant genes such as oprM, emhC, ttgC, cusC, adeK, smeF, mtrE, cmeC, gesC, acrA, mexA, adel, smeD, mtrC, and cmeA (http://www.ncbi.nlm.nih.gov/bioproject/513139). Taken together, the gene composition of genome in E. coli O160:H7 is different from other identified pathogenic and unpathogenic E. coli.

We next examine whether E. coli O160:H7 may cause pathological responses in gut tissues. Although E. coli O160:H7 were infused into wt mice, pan-antibiotic-treated wt mice and germ-free (GF) mice, no remarkable symptoms of acute gut diseases such as diarrhea, colonic bleeding, and reduced body weight were observed,
Figure 2. *E. coli* O160:H7 Induces the Accumulation of Inflammatory Macrophages in Colon Tissues

(A) Flow cytometry of F4/80⁺CD11b⁺, F4/80⁺CD11c⁺, and F4/80⁺TNFα⁺ cells in DSS-treated and unmolested mice (n = 6).

(B) QRT-PCR of TNFα, IL-6, IL-1β, INOS, arginase-1, and IL-10 in the colon tissues of DSS-treated and unmolested mice (n = 6).

(C) Flow cytometry of MHCII⁺Ly6C⁺ inflammatory macrophages (CD45⁺CX3CR1⁺CD11b⁺CD103⁻/⁻ F4/80⁺ MHCII⁺Ly6C⁺) in the colon LP of DSS-treated and unmolested mice (n = 6). % cells and total Ly6c⁺ MHCII⁺ cell number per colon were analyzed (right).

(D) Flow cytometry of inflammatory macrophages in the colon LP of mice with or without different *E. coli* infusion (n = 3). % cells and total Ly6c⁺ MHCII⁺ cell number per colon were analyzed (right).

(E) CFU of bacteria in colon tissues of mice infused different *E. coli*.

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*Figure 2. E. coli O160:H7 Induces the Accumulation of Inflammatory Macrophages in Colon Tissues*

(A) Flow cytometry of F4/80⁺CD11b⁺, F4/80⁺CD11c⁺, and F4/80⁺TNFα⁺ cells in DSS-treated and unmolested mice (n = 6).

(B) QRT-PCR of TNFα, IL-6, IL-1β, INOS, arginase-1, and IL-10 in the colon tissues of DSS-treated and unmolested mice (n = 6).

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(D) Flow cytometry of inflammatory macrophages in the colon LP of mice with or without different *E. coli* infusion (n = 3). % cells and total Ly6c⁺ MHCII⁺ cell number per colon were analyzed (right).

(E) CFU of bacteria in colon tissues of mice infused different *E. coli*.
consistent with the genome sequencing data that virulence-related genes are not detected in E. coli O160:H7. However, oral administration of E. coli O160:H7 promoted sensitivity to DSS-mediated colitis (Figures 1E–1H). This E. coli O160:H7 isolate was much more effective than un-dominant E. coli IA139 strain isolated from same mice in promoting sensitivity to DSS-mediated colitis (Figures S2A–S2E). E. coli O160:H7 was also different from other identified pathogenic E. coli. It was weaker than E. coli CFT073 but stronger than unpathogenic E. coli such as E. coli Str. k12.Substr.MG1655 and E. coli Nissle 1917 (Figures S2F–S2K). Oral administration of these E. coli resulted in high levels of colonization (Figures S2D and S2I). Notably, E. coli O160:H7 isolate and other pathogenic and unpathogenic E. coli had a similar ability in inducing TLR4-mediated NF-kB activity (Figure S2L), implying that difference of O160:H7 with other gram-negative E. coli in promoting sensitivity to DSS-mediated colitis may not depend on LPS. Taken together, E. coli O160:H7 from inflamed colon tissues promotes sensitivity to DSS-mediated colitis, but it is different from other pathogenic and unpathogenic E. coli.

**Figure 2. Continued**

(F) Flow cytometry of inflammatory macrophages in the colon LP of E. coli colonized GF mice (n = 6). GF/pseudo, pseudomonas-colonized mice; GF/E. coli, E. coli O160-colonized mice; GF/killed E. coli, killed E. coli O160-infused mice. % cells and total Ly6C+MHCII+ cell number per colon were analyzed (right). (G) Flow cytometry and immunostaining of F4/80+TNFα+ macrophages in the colon tissues of GF mice with or without E. coli infusion (n = 6). % cells and total F4/80+TNFα+ cell number per colon were analyzed (right). Scale bars = 40 μm. (H) CFU of bacteria in intestine and colon tissues of E. coli infused GF mice. (I) Flow cytometry of F4/80+TNFα+ cells in the spleen and liver of mice with or without E. coli infusion (n = 6). (J) ELISA of IL-18, IL-1, and TNFα in the sera of mice with or without E. coli infusion (n = 6). (K) CFU of bacteria in intestine and colon tissues of mice infected E. coli. Mice in A–C, untreated using pan-antibiotics. Mice in D and I–K, treated using pan-antibiotics for one week before infusing bacteria. For CFU in bacteria infected mice, 106 bacteria were orally infused and then CFU were counted after 7 days. Scale bars = 40 μm in G. ANOVA plus post-Bonferroni analysis in D and F; Two-side Student’s t-test in A–C, G, I, and J; *p<0.05, **p<0.01, and ***p<0.001; NS, no significance; R. E, relative expression. Data in A–K are a representative of two or three independent experiments. See also Figure S3A.

**E. coli O160:H7 Induces Inflammatory Macrophages in Gut and Extra-gut Tissues**

To elucidate how E. coli O160:H7 promotes sensitivity to DSS-mediated colitis, we first examined the composition of gut immune cells in DSS-treated mice. There had remarkably increased F4/80+CD11B+, F4/80+CD11C+, and F4/80+TNFα+ macrophages in the colon lamina propria (LP) (Figure 2A) and higher levels of inflammatory cytokines in the colonic tissues of mice (Figure 2B), because CD11C and TNFα generally are markers of inflammatory macrophages (Bain et al., 2013), suggesting that there may exist increased inflammatory macrophages in the gut tissues of DSS-treated mice. For gut macrophage subsets, previous multiple studies (Bain et al., 2013; Mortha et al., 2014; Shouval et al., 2014; Tamoutounour et al., 2012) suggest that CX3CR1+CD11b+CD103+Ly6C−MHCII+ cells belong to proinflammatory/inflammatory macrophages (P2 stage), whereas CX3CR1+CD11b+CD103−F4/80+Ly6C−MHCII+ cells as anti-inflammatory macrophages (P3 and P4 stage) (Figure S3A). We further investigated the gut macrophage subpopulations using this classification, which was used through this manuscript. The proportion of CD45+CX3CR1+CD11b+F4/80−MHCII−Ly6C− inflammatory macrophages remarkably increased in the DSS-treated mice (Figure 2C), suggesting that E. coli from inflamed colon may induce the inflammatory macrophages. To determine that E. coli O160:H7 may induce inflammatory macrophages, we employed E. coli-infused mice including broad-spectrum antibiotics AVNM (ampicillin, vancomycin, neomycin, and metronidazole)-treated mice, GF mice, and untreated normal mice. In E. coli-infused non-antibiotics WT mice, proinflammatory macrophages did not remarkably increase as compared with mice uninfused with E. coli (unshown). But, the colonization of E. coli O160:H7 isolate could cause significantly increased inflammatory macrophages in AVNM-treated mice and GF mice, in which there existed the dysbiosis of gut microbiota (Figures 2D–2H). There was also significantly increased inflammatory macrophages in extra-gut organs and tissues with higher levels of inflammatory cytokines in peripheral blood in E. coli-infused mice, in which E. coli were detected (Figures 2I–2K). Thus, although it does not cause remarkable bowel disease symptoms, E. coli from the inflamed colon may induce inflammatory macrophages not only in colon tissues but also in extra-gut organs and tissues under the dysbiosis of gut microbiota.

**IL-18, IFNγ, IL-12, and IL-22 Are Required in E. coli-mediated Gut Inflammatory Macrophages**

We next want to understand how E. coli O160:H7 isolate induces inflammatory macrophages in colon tissues. Intestinal mononuclear phagocytes do not or only slightly produce inflammatory responses when stimulated with TLR (toll-like receptor) ligands, commensal, or pathogenic bacteria (Franchi et al., 2012). However, IFNγ may promote the generation of inflammatory macrophages (Hu and Ivashkiv, 2009).
Remarkably increased IFNγ was detected not only in DSS-mediated colitis but also in *E. coli* O160:H7 colonized colon tissues as compared with their control mice, whereas other anti-inflammatory cytokines such as IL-4 did not significantly change (Figure 3A). There also existed a drastic expansion of interferon
IL-18 has been shown to play an important role in the induction of IFNγ production, increasing NK cell activity and T cell proliferation (Nielsen et al., 2016). There also are substantial evidences for the expression and secretion of IL-18 by the intestinal epithelium. Thus, we detected whether the accumulated IFNγ producing cells were related to IL-18 in the gut epithelial cells. Indeed, the increased IL-18 was detected in the gut epithelial cells of E. coli O160:H7-colonized mice and also DSS-induced colitic tissues (Figures 4A and 4B). More mature IL-18 was also detected in crypt supernatants after in vitro stimulation by E. coli O160:H7 (Figures 4C and 4D). The colonization of E. coli O160:H7 in IL-18−/− mice did not cause the accumulation of inflammatory macrophages in the colonic LP (Figures 3F and 3G). Thus, our results demonstrate that gut IFN-γ plays a critical role in E. coli O160:H7-mediated inflammatory macrophages in colonic tissues.

**PKCδ, NLRC4, caspase8, and caspase1/11 Are Required for E. coli O160:H7-Induced IL-18**

Next question is how E. coli O160:H7 induces the expression of IL-18 in gut epithelial cells. The inactive 24 kDa precursor pro-IL-18 is constitutively expressed by gut epithelial cells and primed for release upon inflammasome activation. Gut epithelial cells have revealed an expression of an array of inflammasome components including NAIP, NLRP (NOD-like receptor protein) 1, NLRN4, NLRP6, AIM2, caspase4/5 (human)/caspase11 (mouse), caspase8, ASC, and NLRP6/3 (von Moltke et al., 2013). Cytosolic pattern recognition receptors (PRRs) are often associated with the use of pore-forming toxins or injection of effector molecules through specialized secretion systems of gram-negative bacteria (von Moltke et al., 2013), which are encoded by E. coli O160:H7. Because inner rod protein of type three secretion systems (TTSS) and functional flagellin (Flc) of gram-negative bacteria-mediated production of mature IL-18 mainly is through NLRC4/caspase1 signal pathway (Miao et al., 2010), we investigated the effects of NLRC4 and caspase1 on E. coli O160:H7-mediated mature IL-18. We found that NLRC4 and caspase1/11 was involved in E. coli O160:H7-mediated IL-18 release (Figures 5A–5C and 5A–54C). More recent studies have revealed a requirement for caspase8 in activating caspase1 within the inflammasome complex (Man and Kanneganti, 2016). The caspase8 specific inhibitor did also affect E. coli O160:H7-mediated mature IL-18 (Figures 5D and 5E). The phosphorylation of NLRC4, which is activated by PKCδ in Salmonella infection, was necessary in macrophages (Qu et al., 2012). PKCδ inhibitor also impaired E. coli O160:H7-mediated mature IL-18 in gut epithelial cells (Figures 5D and 5E). Finally, immunoprecipitation further identified...
bioactive PCK8, caspase1, caspase8, and ASC molecules being bound by NLRC4 in colon epithelial cells (Figure 5F), which are shown in macrophages infected with S. Typhimurium (Man et al., 2014). Interestingly, NLRC4 complexes also included caspase11 in colon epithelial cells (Figure 5F). It was also found that the noncanonical inflammasome also activates caspase11 in response to many gram-negative bacteria (Kayagaki et al., 2011). Critically, the colonization of E. coli O160:H7 in NLRC4/−/− or caspase1/11−/− mice did not cause accumulated inflammatory macrophages in colonic LP (Figures 5G, 5H, and S4D). In addition, caspase11 and NLRC4 inflammasome activation in gut epithelial cells may lead to a lytic cell death, resembling pyroptosis (Rauch et al., 2017). There had increased PI+ cells (pyroptosis cells) in caspase11 and NLRC4 inflammasome activation in gut epithelial cells through PKCδ/NLRC4/Caspase8/CASPASE11/1 complexes. Scale bars = 40 μm in B. Two-side student’s t-test in A, E, and G; *p<0.05, **p<0.01, and ***p<0.001; NS, no significance; R. E, relative expression. Data in A, E, G, and H are represented as mean ± SD; data in B are represented as mean ± SEM; data in all panels are a representative of two or three independent experiments. See also Figures S4 and S5.

**E. coli O160:H7 Directly Induces IL-18 and IL-1β in Macrophages**

We also observed effect(s) of E. coli O160:H7 isolate on the macrophages. E. coli O160:H7 directly activated macrophages to induce IL-1β and IL-18 in vitro (Figures 6A–6C). Furthermore, E. coli O160:H7-mediated production of IL-18 and IL-1β was also dependent on signal pathway consisted of PCK8, NLRC4, caspase8, and caspase1/11 signal pathway in macrophages (Figures 6A–6E). Intravenous injection of E. coli O160:H7 into normal wt mice caused rapidly increased IL-18 and IL-1β in peripheral blood and accumulation of inflammatory macrophages in spleen and liver (Figures 6F and 6G). NLRC4/−/− and caspase1/11−/− and IL-18−/− impaired this ability of E. coli O160:H7 to induce the production of IL-1β and IL-18 (Figures 6F and 6G). These bacteria could effectively localize in these tissues and organs (Figure 6H). Meanwhile, we also found that E. coli O160:H7 was more effective in inducing mature IL-18 or IL-1β than other unpathogenic E. coli (Figures 6I and 6J). Thus, E. coli O160:H7 also directly induce production of IL-18 and IL-1β in macrophages through activating inflammasome complexes including PCK8, NLRC4, caspase8, and caspase1/11.

**E. coli O55: HNT from Patients Has Similar Function with E. coli O160:H7**

We also investigate a dominant E. coli O55: HNT strain from colitic tissues of patients with inflammatory bowel disease. The increased E. coli could be detected in colitic tissues of patients with inflammatory bowel disease (Figures 7A and 7B), consistent with other data (Winter and Baumler, 2014). We found that the isolated E. coli O55: HNT from colitic tissues of patients with inflammatory bowel disease (Table S1E) had a similar function with mouse E. coli O160:H7 isolate. This strain E. coli O55: HNT also promoted sensitivity to DSS-mediated colitis (Figures 7C–7F) and induced inflammatory macrophages (Figure 7G). In vivo intravenously administration also caused increased inflammatory macrophages in the colonized tissues and organs (Figures 7H and 7I).

We finally compared the effects of E. coli O55: HNT and E. coli O160:H7 strain with other identified pathogenic and unpathogenic E. coli on mortality and morbidity after oral administration and in vivo...
intravenous administration. Notably, E. coli O55: HNT was similar to E. coli O160:H7 strain but not to pathogenic E. coli such as E. coli CFT073 in mortality and morbidity. Although oral administration of E. coli CFT073 caused remarkable symptom of acute gut diseases, the mice administrated with E. coli O55: HNT did not exhibit detectable symptom (Figures S6A and S6B). Colon inflammation was observed only in E. coli CFT073 but not in E. coli O55: HNT or E. coli O160:H7 infused wt mice (Figures S6A and S6B). Although oral administration of E. coli in pan-antibiotics-treated mice or GF mice, E. coli O55: HNT and E. coli O160:H7 could cause symptom of acute gut diseases and colon inflammation but much slighter than E. coli CFT073 (Figures S6C and S6D). In vivo intravenous administration mice, E. coli O55: HNT and E. coli O160:H7 could cause disease symptoms. However, these symptoms were remarkably slighter than pathogenic bacteria E. coli CFT073 (Figures S6E and S6F) although it is significantly severe than unpathogenic bacteria E. coli MG1655 (Figures S6E and S6F). Intravenous injection also exhibited tissue colonization pattern (Figure S6G). Taken together, there are remarkable differences in mortality and morbidity between E. coli O55: HNT and E. coli O160:H7 isolated from colitic tissues and other pathogenic E. coli.

**DISCUSSION**

In this study, we found that a high abundance of commensal E. coli in inflamed colonic tissues are different from other unpathogenic commensal E. coli and also pathogenic E. coli in their genome, especially T3SS and virulent factors. These E. coli may induce inflammatory macrophages in the colon tissues and extra-gut tissues but not acute infection diseases. They stimulate gut epithelial cells to produce IL-18 through inflammasome complexes that consisted of PKCδ, NLRc4, caspase8, and caspase1/11. IL-18 derived from gut epithelial cells induces Th1- and NKP46+ IFNγ-producing cells, which are necessary for the generation of inflammatory macrophages. Meanwhile, higher levels of IL-12 and IL-22 in the colon tissues are also involved in E. coli-mediated inflammatory macrophages. The isolated E. coli not only induce gut inflammatory macrophages but also directly activate extra-gut macrophages to produce proinflammatory cytokines. There also have increased pyroptosis cells in the E. coli-colonized mice, which may potentially promote microbial translocation into the distal tissues and/or organs. These results disclose a new mechanism for how colitis associated gut microbiota to cause inflammatory macrophages in the gut and extra-gut tissues and organs. Since inflammatory macrophages are related to multiple systemic diseases such as inflammatory bowel disease (IBD), obesity, atherosclerosis, carcinogenesis, etc (Blunder et al., 2017), our results imply that a high abundance of commensal E. coli in inflamed gut may play a role in the occurrence and development of these diseases. Thus, our data suggest a possible mechanism for the occurrence and development of chronic inflammation diseases, which are related to inflammatory macrophages.

Generally, gram-negative bacteria may activate inflammasomes through LPS-caspase11/1 and/or flagellin-NLRC4-caspase1 pathway to induce the production of mature IL-18 in macrophages and epithelial cells. However, several studies have exhibited difference of gram-negative bacteria in their ability to induce production of inflammatory cytokines. Pathogenic E. coli but not commensal bacteria can elicit substantial amounts of mature IL-18 by the NLRC4 pathway (Franchi et al., 2012; Lightfield et al., 2008). E. coli Nissle 1917 and commensal E. coli K12 also differentially affect the inflammasome in intestinal epithelial cells (Becker et al., 2014). We here also found that there exists a remarkable difference between inflamed colonic tissues derived E. coli and other unpathogenic and pathogenic E. coli in inducing inflammatory macrophages. Recently, E. coli strains from antibiotic-treated mice may cause lethal inflammasome activation through NLRC4 (Ayres et al., 2012), whereas another strain E. coli, which also activate NLRC4, may protect

**Figure 6. E. coli O160:H7 Directly Stimulates Macrophage to Produce IL-18 and IL-1β**

(A and B) ELISA of IL-18 and IL-1β in the supernatants of macrophages after exposed to E. coli O160:H7 at 3 h (A) and 24 h (B).

(C) Immunoblotting of pro-IL-18, mature IL-18, pro-caspase18, and mature IL-18 in the macrophages after exposed to E. coli for 1 h.

(D) IL-18 ELISA and immunoblotting after exposed to E. coli O160:H7 for 3 h in the presence of caspase8 and PKCδ inhibitor.

(E) Immunoblotting after exposed to E. coli O160:H7 with or without caspase8 and PKCδ inhibitors for 3 h.

(F) ELISA of IL-18, IL-1β, and TNFα in the peripheral blood of wt, NLRc4−/−, and caspase1/11−/− mice after injecting E. coli O160:H7 or heat-killed E. coli in tail vein at the indicated time (n = 6).

(G) Flow cytometry of F4/80+TNFα+ macrophages in spleen and liver of wt, NLRc4−/−, and caspase1/11−/− mice after injecting E. coli or heat-killed E. coli in tail vein (n = 6).

(H) CFU of E. coli in the spleen, liver, and lung of wt, NLRc4−/−, and caspase1/11−/− mice after injecting E. coli in tail vein.

(I) ELISA of IL-18 and IL-1β in the macrophages after exposed to DH5α, E. coli 1917, or E. coli O160:H7 for 3 h.

(J) Immunoblotting of in the macrophages after exposed to DH5α, E. coli nissle 1917 (1917), or E. coli O160:H7 (O160). ANOVA plus post-Bonferroni analysis in A, B, D, and F–I. *p<0.05, **p<0.01, and ***p<0.001; NS, no significance; R. E, relative expression. Data in A, B, D, F–I are represented as mean ± SD. Data are a representative of three independent experiments.
specialized secretion systems (von Moltke et al., 2013). The activation of NLRC4 inflammasome requires
with virulent bacteria, such as the use of pore-forming toxins or injection of effector molecules through
Studies have found that cytosolic PRRs respond to patterns of pathogenesis that are often associated
discrimination between pathogenic and nonpathogenic bacteria. Differences, especially in flagellin, rode-like proteins, and T3SS secreting system. Cytosolic PRRs (pattern
the presence of an intact type III (T3SS) or IV secretion system (T4SS) (Franchi et al., 2006). In addition,
colon tissues derived from their genomic characteristics. Indeed, compared analyses of the genomes between inflamed
colon tissues derived E. coli O160:H7 and other pathogenic and unpathogenic E. coli exhibit remarkable
differences, especially in flagellin, rode-like proteins, and T3SS secreting system. Cytosolic PRRs (pattern
recognition receptors) are critical for discriminating between pathogenic and nonpathogenic bacteria. Studies have found that cytosolic PRRs respond to patterns of pathogenesis that are often associated with virulent bacteria, such as the use of pore-forming toxins or injection of effector molecules through specialized secretion systems (von Moltke et al., 2013). The activation of NLRC4 inflammasome requires the presence of an intact type III (T3SS) or IV secretion system (T4SS) (Franchi et al., 2006). In addition, the release of T3SS PrgJ-like rod proteins into the cell cytosol can activate NLRC4. Thus, although the genetic factors of flagellin, rode-like protein, T3SS and/or IV secreting system change, these gram-negative E. coli may exhibit altered ability in inducing inflammatory cytokines and inflammation-associated diseases.

mice against muscle wasting and loss of fat during infections (Schieber et al., 2015). All of these may be
derived from their genomic characteristics. Indeed, compared analyses of the genomes between inflamed
colon tissues derived E. coli O160:H7 and other pathogenic and unpathogenic E. coli exhibit remarkable
differences, especially in flagellin, rode-like proteins, and T3SS secreting system. Cytosolic PRRs (pattern
recognition receptors) are critical for discriminating between pathogenic and nonpathogenic bacteria. Studies have found that cytosolic PRRs respond to patterns of pathogenesis that are often associated with virulent bacteria, such as the use of pore-forming toxins or injection of effector molecules through specialized secretion systems (von Moltke et al., 2013). The activation of NLRC4 inflammasome requires the presence of an intact type III (T3SS) or IV secretion system (T4SS) (Franchi et al., 2006). In addition, the release of T3SS PrgJ-like rod proteins into the cell cytosol can activate NLRC4. Thus, although the genetic factors of flagellin, rode-like protein, T3SS and/or IV secreting system change, these gram-negative E. coli may exhibit altered ability in inducing inflammatory cytokines and inflammation-associated diseases.

Our results suggest that gut epithelial cells exist in similar inflammasome complexes with macrophages to
be involved in gram-negative bacteria (Qu et al., 2012). There exist multiple inflammasomes, which are broadly expressed in hematopoietic and non-hematopoietic cells, such as gut epithelial cells (Hu et al.,
2010; Sellin et al., 2014), and can trigger numerous downstream responses including production of
IL-1β, IL-18, and lytic cell death (Sellin et al., 2014). Despite the fact that the functional importance of inflam-
masomes within immune cells has been well established, the contribution of inflammasomes in non-he-
matopoietic cells remains comparatively understood. We here demonstrated that E. coli isolated from
colon tissues directly stimulate gut epithelial cells through inflammasome complexes that consisted of
PKCδ, NLRC4, caspase8, and caspase11/11. Other studies also found the role of NAIP-NLRC4 (Rauch
et al., 2017) and caspase4/11 (Hagar et al., 2013; Knodler et al., 2014) in gut epithelial cells. An inflamma-
some formed by NLRC4, ASC, and potentially caspase8 is also described in a model of enteric S.
typhimurium infection (Rauch et al., 2017).

Although we demonstrate that inflamed E. coli directly and indirectly induce inflammatory macrophages
through PKCδ, NLRC4, caspase8, and caspase11/11 complexes, the question is whether the inflamed
E. coli-mediated activation of the inflammasomes in the gut and extra gut macrophages is a sufficient signal
to trigger those chronic inflammatory diseases that remain unresolved. However, Kitamura et al. reported
that transgenic mice expressing a constitutively active NLRC4 variant (H443P) develop an auto-inflamma-
tory disease (Kitamura et al., 2014). Others also found that NAIP/NLRC4 inflammasome activation in MRP8+
cells is sufficient to cause systemic inflammatory diseases (Nichols et al., 2017).

Limitations of the Study
Although we analyzed the changes of cell population and subsets using flow cytometry, the exact changes
of cell population and subsets, especially Ly6C+ inflammatory and anti-inflammatory macrophages in col-
on tissues, need to be solved through other technique(s) such as single cell analyses.
METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

DATA AND CODE AVAILABILITY
Raw 16S rRNA gene sequence data for the feces microbiota: https://www.ncbi.nlm.nih.gov/sra/PRJNA512937; Raw genome components of E. coli O160:H7: http://www.ncbi.nlm.nih.gov/bioproject/513139.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.10.046.

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AUTHOR CONTRIBUTIONS
R.Y. designed the research and wrote the paper; H. Q., Y. L., X. S., J. W. and Yingquan. L. conducted in vivo experiments and immunoassay, participated in study design and performed the statistical analysis; Y. G conducted in vitro experiments, especially immunoblotting analyses; C. Z offered patient samples and conducted some in vivo experiments. H. Z, L. S conducted germ-free mouse experiments; Y. X. X.Y and Yanmei, X conducted bacteria typing; Y. Z. offered assistances for the animal experiments. All authors read and approve the final manuscript.

DECLARATION OF INTERESTS
The authors declare no conflict of interest.

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Supplemental Information

Induction of Inflammatory Macrophages in the Gut and Extra-Gut Tissues by Colitis-Mediated *Escherichia coli*

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Figure S1. Characteristics of *E. coli* O160: H7 genome, Related to Figure 1.

(A) Circular representation of *E. coli* O160: H7 genome. From outer to inner: 1, Genome Size; 2, Forward Strand Gene, colored according to cluster of orthologous groups (COG) classification; 3, Reverse Strand Gene, colored according to cluster of
orthologous groups (COG) classification; 4, Forward Strand ncRNA; 5, Reverse Strand ncRNA; 6, Repeat; 7, GC; 8, GC-SKEW. J, Translation, ribosomal structure and biogenesis; A, RNA processing and modification; K, Transcription; L, Replication, recombination and repair; B, Chromatin structure and dynamics; D, Cell cycle control, cell division, chromosome partitioning; Y, Nuclear structure; V, Defense mechanisms; T, Signal transduction mechanisms; M, Cell wall/membrane/envelope biogenesis; N, Cell motility; Z, Cytoskeleton; W, Extracellular structures; U, Intracellular trafficking, secretion, and vesicular transport; O, Posttranslational modification, protein turnover, chaperones; X, Mobilome: prophages, transposons; C, Energy production and conversion; G, Carbohydrate transport and metabolism; E, Amino acid transport and metabolism; F, Nucleotide transport and metabolism; H, Coenzyme transport and metabolism; I, Lipid transport and metabolism; P, Inorganic ion transport and metabolism; Q, Secondary metabolites biosynthesis, transport and catabolism; R, General function prediction only; S, Function unknown.

(B) The pan-gene Venn graph of *E. coli* O160:H7 (ENK1), *E. coli* O157:H7.str.Sakai, *E. coli*.str.K12.substr.MG1655 and *E. coli* CFT073. Each ellipse represent one strain, the number in the ellipse means the only cluster number. One cluster have the genes that more than 50 percent identity and less than 0.3 length diversity.

(C) Dispensable gene heat map in each strain. Left, dispensable gene cluster; Top, strain cluster; The similarities of gene, the middle with different color represent different coverage by heat map; Color/depth, top left pic. Below, each strain name. *E. coli* O160:H7 (ENK1).

(D) FliC phylogenetic analysis. The web based program Phylogeny.fr was used for phylogenetic analysis of the fliC gene sequence from *E. coli* O160:H7 as compared to publicly available fliC gene sequences from pathogenic and non-pathogenic *E. coli* strains. *E. coli* O160:H7 is marked.
Figure S2. *E. coli* O160:H7 is different from other *E. coli* strains in promoting sensitivity to DSS-mediated colitis, Related to Figure 1.

(A and B) Survival rate (A), body weight and disease activity index (B) were monitored in mice infused by *E. coli* O160:H7 strain and *E. coli* IAI39 (isolated from mice) under DSS (n=12).
(C) Length of colon were monitored at day 7 after the start of DSS in mice infused by *E. coli* O160:H7 strain and *E. coli* IAI39 (n=6).

(D) CFU of *E. coli* in small intestine and colon of mice infused by *E. coli* O160:H7 and *E. coli* IAI39 (n=6).

(E) H&E staining and histological scores of colon tissues in mice infused by *E. coli* O160:H7 strain or *E. coli* IAI39 after DSS-treatment (n=6).

(F and G) Survival rate (F), body weight and disease index (G) were monitored in mice infused by *E. coli* O160:H7, *E. coli* G1655, *E. coli* CFT0735 or *E. coli* 1917 under DSS (n=12).

(H) Length of colon were monitored at day 7 after the start of DSS (n=6).

(I) CFU of *E. coli* in small intestine and colon of mice infused by *E. coli* O160:H7, *E. coli* G1655 and *E. coli* CFT0735 or *E. coli* 1917.

(J and K) H&E staining (J) and histological scores (K) of colon tissues in mice infused by *E. coli* O160:H7 strain, *E. coli* G1655, *E. coli* CFT0735 or *E. coli* 1917 after DSS-treatment.

(L) NF-κB activity in TLR4 expressed 293T cells after exposed to different *E. coli* O160:H7, *E. coli* G1655 and *E. coli* CFT0735 or *E. coli* 1917. NF-κB activity was detected using NF-κB reporter analysis system.

Scale bars=40 μm; Wilcoxon’s test in A and F; Analysis of variance test in B and G; ANOVA plus post-Bonferroni analysis in C, E, K and L; *P<0.05, **P<0.01 and ***P<0.001; NS, no significance; R. E, relative expression. Data in B, C, D, G, H, I and L are represented as mean+/−SD; Data in E and K are represented as mean+/−SEM. Data are a representative of two or three independent experiments.
Figure S3. IL-12 and IL-22 affect the accumulation of inflammatory macrophages, Related to Figure 2 and 3.

(A) Gating strategy was based on Bain et al. who showed that distinct macrophages subsets can be isolated without using CX3CR1-GFP reporter mice (Bain et al., 2013) and Shouval, *Immunity*. 2014). We performed some modifications to this method: following initial gating on live CD45+ cells after eliminating double and dead cells using FCS-W, SSC-W and 7-AAD staining, we gated on F4/80+ CD103− cells after gating CD11B and CXCR1, and finally gated on Ly6C and MHCII.

(B) QRT-PCR of IL-12 and IL-22 in the colon tissues of and DSS-treated mice or *E. coli* infused mice (n=3).
(C) Flow cytometry of inflammatory macrophages in the colon LP of DSS-treated mice injected neutralizing IL-12 or IL-22 Ab (n=3). Two side Student’s t-test in B; ANOVA plus post-Bonferroni analysis in C. *P<0.05, **P<0.01 and ***P<0.001; NS, no significance; R. E, relative expression. Data in B and C are represented as mean+/−SD. Data are a representative of three independent experiments.
Figure S4. *E. coli* O160: H7 mediated inflammatory macrophages needs caspase-1 in gut epithelial cells, Related to Figure 5.

(A) Immunoblotting of pre-caspase-1, mature caspase-1, pre-casepase-8, mature caspase-8, pro-IL-18 and mature IL-18 in colon tissues, lamina propria, crypts and crypt supernatants of wt mice after exposed to *E. coli* with or without pan-caspases and caspase 1 inhibitors for one hour.
(B) ELISA of IL-18 in the supernatants of colon epithelial cells after exposed to different *E. coli* with or without pan-caspase and caspase 1 inhibitor for one hour.

(C) Immunoblotting of pro-IL-18 and mature IL-18 in colon tissues, lamina propria, crypts and crypt supernatants of caspase 1/11 KO mice after exposed to *E. coli* for one hour.

(D) Flow cytometry of inflammatory macrophages and CD4^+^IFNγ^+^ in the LP of colon tissues of caspase 1/11 (C1/11) KO mice infused *E. coli* O160 (n=3)

ANOVA plus post-Bonferroni analysis in B and D; *P<0.05, **P<0.01 and ***P<0.001; NS, no significance; R. E, relative expression. Data in B and D are represented as mean+/-SD. Data are a representative of three independent experiments.
Figure S5. *E. coli* O160: H7 may cause pyroptosis of gut epithelial cells, Related to Figure 5.

Staining of propidium and fluorescent CK19, marker of gut epithelial cells in mice infused using *E. coli* O160:H7, *E.coli* G1655, *E. coli* CFT073 and positive control Salmonella Typhimurium (ST, 1×10⁹/mouse). For propidium iodide staining, mice were injected with 100µg/mouse propidium iodide intravenously 10 minutes before sacrifice (Rauch et al, Immunity, 2017). Data are represented as mean+/-SEM. Scale bars=20 µm (upper) or 40µm (lower). ANOVA plus post-Bonferroni analysis; *P<0.05, **P<0.01 and ***P<0.001; NS, no significance;
Figure S6. *E. coli* O160:H7 only cause weaker responses as compared to pathogenic *E. coli*, Related to Figure 7.

(A) Body weight and disease activity index in *wt* mice after orally infusing different kinds of *E. coli*.
(B) H&E staining of colon tissues in wt mice after infusing different E. coli.

(C) Body weight and disease activity index of different kinds of E. coli orally infused mice, which were treated using pan-anti-biotics.

(D) H&E staining of colon tissues in pan-anti-biotics treated mice after infusing E. coli

(E) Survival rate and disease activity index of mice after in vein injecting different kinds of bacteria (1×10^8/mice) (n=12)

(F) The concentration of ASK, CPK, BUN and ALT in peripheral blood of mice after in vein injecting different kinds of bacteria for 3 days (n=8).

(G) CFU in different tissues and organs of mice after injecting different kinds of bacteria (1×10^8/mice) (n=8) for 3 days.

1655, E. coli G1655; CFT073, E.coli CFT073; O160, E. coli O160:H7; 055, E. coli 055: HNT. Scale bars=40 μm. Analysis of variance test in A, C and E (right); Wilcoxon’s test in E (left); ANOVA plus post-Bonferroni analysis in B, D and F; *P<0.05, **P<0.01 and ***P<0.001; NS, no significance; R. E, relative expression. Data in A, C, E (right), F and G are represented as mean+/−SD; Data in B and D are represented as mean+/−SEM. in Data are a representative of three independent experiments.
Table S1 Characteristics of isolated E. coli from colon tissues, Related to Figure 1.
Transparent Methods

Mice

Four-to six-week-old male or female C57BL/6 mice were obtained from Nanjing Animal Center. IL-18-/- mice was from Prof. Meng, University of Chinese Academy of Sciences, shanghai; Caspase1/caspase -11-/- and NLRC4-/- were from Prof. Shao, National Institute of Biological Sciences, Beijing; IFNγ-/- mice was offered by Prof. Lian, University of Science and Technology of China, Hefei. All experimental litters were bred and maintained under specific pathogen-free (SPF) conditions in the Animal Center of Nankai University. All experimental variables such as husbandry, parental genotypes and environmental influences were carefully controlled. Male, 6-8 weeks old mice were used in this study except for special indication.

C57BL/6 germ-free (GF) mice were generated by Institute of Laboratory Animal Science, Peking Union Medical College (PUMC). All experiments in GF mice were performed in the Institute of Laboratory Animal Science, Peking Union Medical College (PUMC).

All procedures were conducted according to the Institutional Animal Care and Use Committee of the Model Animal Research Center. Animal experiments were approved by the Institute’s Animal Ethics Committee of Nankai University.

Patients

Eighty inflammatory bowel disease (IBD) patients (10 active Crohn’s disease (CD), 20 inactive CD, 50 active ulcerative colitis (UC) ) and 50 patients with colitic cancer
who regularly visited the Tianjin people hospital (Tianjin, China) from 2017 to 2018 were recruited to the study. The diagnosis of IBD was based on standard clinical, endoscopic, radiological and histological criteria (Ouyang et al., 2006). The control group consisted of sex- and age-matched healthy subjects. Patients with IBD who met any of the following criteria were excluded: (1) use of antibiotics, probiotics or prebiotics in the 3-month period immediately preceding the sampling time point; (2) current infectious diarrhea; and (3) malignancy. UC activity was evaluated using the Mayo score (D’Haens et al., 2007); Active UC was defined as UC disease activity index >2. Activity of CD was scored by Crohn’s disease activity index (CDAI) (Geubel et al., 1976); Active CD was defined as a CDAI > 150. Written informed consent was obtained from all subjects prior to their enrollment.

The study was approved by the Ethics Committee at the Tianjin People Hospital, Tianjin, China. It was conducted in accordance with guidelines expressed in the Declaration of Helsinki.

**Bacterial strains**

_E. coli_ O160: H7 and _E. coli_ IAI139 were respectively isolated from DSS-mediated colitic tissue and colon contents of mice. _E.coli_ O55: HNT was also isolated from colitic tissues of patients with inflammatory bowel disease.

_E.colistr.K12.substr.MG1655,  E.coli Nissle. 1917 and  DH5α was from ATCC;  E. coli CFT073 were from Chinese Center for Disease Control and Prevention. Samonella typhimurium  (ATCC14028 ) was from Pro. Guo , College of Life Science, Wuhan University. These bacteria were grown in LB media
shaking at 37°C overnight and stored in 25% glycerol frozen stocks and used for experiments.

**Mouse models**

For dextran sodium sulfate (DSS) induced colitis, DSS induced colitis was performed according to our previously reported method (Cao et al., 2016) with modification. Briefly, mice received 2.5% *(wt mice)*, 2.2% *(Pan-antibiotics treated mice) *(wt/vol)* DSS (40,000 kDa; MP Biomedicals) or at the indicated dose in their drinking water for 7 days, then switched to regular drinking water. The amount of DSS water drank per animal was recorded and no differences in intake between strains were observed. For survival studies, mice were followed for 12 days post start of DSS-treatment. Mice were weighed every other day for the determination of percent weight change. This was calculated as: % weight change = (weight at day X-day 0 / weight at day 0) × 100. Animals were also monitored clinically for rectal bleeding, diarrhea, and general signs of morbidity, including hunched posture and failure to groom. For microbiota transplantation, germ-free (GF) mice were orally administered 200 µl of fecal suspension or 1×10⁹ bacteria (once/week). In wt mice, mice were first treated with pan-antibiotics (ampicillin (A, 1 g/L, Sigma), vancomycine (V, 0.5g/L, Sigma), neomycin sulfate (N, 1 g/L, Sigma), and metronidazole (M, 1g/L, Sigma) ) via the drinking water for one week (sometime longer than one week) and then orally administered 200 µl of fecal suspension or 1×10⁹ bacteria (once/week). To confirm the elimination of bacteria, stools were collected from antibiotic-treated and untreated mice and cultured in anaerobic and aerobic condition. For oral infection, *E. coli* overnight grew in LB media shaking at 37 °C. Mice were gavaged with 1×10⁹ *E. coli*
in 200 μl of sterile PBS. Mice were sacrificed at the indicated days. Representative colon tissues were embedded in paraffin for hematoxylin/eosin (H&E) staining or embedded in OCT compound (Tissue-Tek, Sakura, Torrance, CA) and frozen over liquid nitrogen for immuno-staining.

Disease activity index (DAI) and histological scores were assessed according to following methods. Disease activity index was the average of these scores: (combined score of stool consistency, bleeding and weight loss)/3 (Tang et al., 2015). Diarrhea was scored daily as follows: 0, normal; 2, loose stools; 4, watery diarrhea. Blood in stool was scored as follows: 0, normal; 2, slight bleeding; 4, gross bleeding. Weight loss was scored as follows: 0, none; 1, 1%-5%; 2, 5%-10%; 3, 10%-15%; 4, >15%. Disease activity index was the average of these scores: (combined score of stool consistency, bleeding and weight loss)/3. For histological evaluation, histology was scored as follows: epithelium (E), 0=normal morphology; 1=loss of goblet cells; 2=loss of goblet cells in large areas; 3=loss of crypts; 4=loss of crypts in large areas; and infiltration (I), 0=no infiltrate; 1=infiltrate around the crypt basis; 2=infiltrate reaching the lamina (L) muscularis mucosae; 3=extensive infiltration reaching the L muscularis mucosae and thickening of the mucosa with abundant oedema; 4=infiltration of the L submucosa. Total histological score was given as E+I (Tang et al., 2015).

For in vivo injection, E. coli overnight grew in LB media shaking at 37 °C. Mice were injected intravenously with 5×10⁸ live or dead bacteria in PBS, and then survival (time to moribund) were detected. Disease indexes (DI) were scored using a quantitative scale that integrated four cardinal signs of systemic toxicity (piloerection,
ocular discharge, lethargy, and diarrhea; each scored from zero to three) (Liu et al., 2011).

For blocking experiments, mice or *E. coli* transplanted mice were injected i.p. with the anti-IFN-γ mAb (100 μg/g body weight), anti-IL18 mAb (100 μg/g), anti-IL22 mAb (100μg/g), anti-IL12 mAB (100 ng/g) or control isotypic antibody (100 ng/g) at day 1 and day 3, and then lamina propria cells were analyzed at day 6.

**Analyses of gut microbiota**

For gut microbiota analyses, the DSS-treated mice and unmolested control littermate wt mice were reared in different cages. Gut microbiota was analyzed by Majorbio Biotechnology Company (Shanghai, China) using primers that target to V3-V4 regions of 16S rRNA. Once PCR for each sample, the amplicons were purified using the QIAquick PCR purification kit (Qiagen Valencia, CA), quantified, normalized, and then pooled in preparation for emulsion PCR followed by sequencing using Titanium chemistry (Roche, Basel Switzerland) according to the manufacturer’s protocol. Operational Taxonomic Unit (OTU) analysis was performed as follows: sequences were processed (trimmed) using the Mothur software and subsequently clustered at 97% sequence identity using cd-hit to generate OTUs. The OTU memberships of the sequences were used to construct a sample-OTU count matrix. The samples were clustered at phylum, genus and OTU levels using the sample-phylum, sample-genus and sample-OTU count matrices respectively. For each clustering, Morisita-Horn dissimilarity was used to compute a sample distance matrix from the initial count matrix, and the distance matrix was subsequently used to generate a hierarchical clustering using Ward’s minimum variance method. The
Wilcoxon Rank Sum test was used to identify OTUs that had differential abundance in the different sample groups.

For colony analysis of the gut tract and extra-gut tissues, the homogenized colon and extra-colon tissues from DSS treated, unmolested mice or *E. coli* infected wt or GF mice were harvested, and then serially diluted the homogenates and plated them on bacterial medias that support the growth of *E. coli* such as LB. We then incubated the plates aerobically at 37 °C for 24 h, after which we counted colonies, classified based on colony appearance and subjected them to 16S rDNA colony PCR and sequencing. For colony PCR, we resuspended colonies in sterile PBS, boiled for 10 min at 100 °C and then V1-V9 regions were analyzed by PCR with the universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (DeSantis et al., 2007). PCR was performed on a Bio-Rad iCycler using an annealing temperature of 51 °C and the following conditions: 95 °C (5 min), followed by 30 cycles of 95 °C (30 s), annealing (1 min), 72 °C (2 min), and a final extension at 72 °C (10 min). Reactions were then subjected to a PCR cleanup using the QIAquick PCR Purification Kit (Qiagen) and sequenced using the 27F and 1492R primers (Beijing Genomics Institute (BGI)). We classified the sequences using Microbial Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

For serotyping, previously reported methods were used in this study (Bai et al., 2016). Briefly, the O antigen was initially screened using the O-genotyping PCR method to identify and classify the *E.coli* O sero groups. The complete *E.coli* O antisera (O1-O188; Statens Serum Institut, Hillerød, Denmark) were used to confirm the PCR results. The isolates were referred as O-untypable if they did not react with
any O antisera. The entire coding sequence of fliC was amplified by PCR using the primers: F-FLIC1 (5′- ATGGCACAAGTCATTAATACCCAAC-3′) and R-FLIC2 (5′-CTAACCCCTGCAGCAGAGACA-3′). Then, the PCR products were sequenced and compared to a publicly available CGE Serotype Finder database (http://cge.cbs.dtu.dk/services/) to determine the H type of each isolate. The isolate was H-untypable if fliC was negative by PCR.

For multilocus sequence typing (MLST), previously reported methods were used (Bai et al., 2016). Briefly, defined fragments of the seven mouse keeping genes (i.e., adk, icd, fumC, recA, mdh, gyrB, and purA) were amplified and sequenced according to the E.coli MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). Sequences types (STs) for each isolate were assigned based on the allelic profile of the seven house keeping genes. A neighbor-joining tree was constructed by MEGA 6 based on the concatenated sequences of the seven house keeping genes, and used to analyze the phylogenetic relationships among strains.

**Analyses of E. coli O160: H7**

For genome sequencing and assembly of E. coli O160: H7, the mouse strain ENK1 (E. coli O160: H7) genome was sequenced using a PacBio RS II platform and Illumina HiSeq 4000 platform in the Beijing Genomics Institute (BGI, Shenzhen, China). Four SMRT cells Zero-Mode Waveguide arrays of sequencing were used by the PacBio platform to generate the subreads set. PacBio subreads (length < 1 kb) were removed. The program Pbdagcon (https://github.com/PacificBiosciences/pbdagcon) was used for self correction. Draft genomic unitigs, which are uncontested groups of fragments, were assembled using the Celera Assembler against a high quality corrected circular
consensus sequence subreads set. To improve the accuracy of the genome sequences, GATK (https://www.broadinstitute.org/gatk/) and SOAP tool packages (SOAP2, SOAPsnp, SOAPindel) were used to make single-base correction. To trace the presence of any plasmid, the filtered Illumina reads were mapped using SOAP to the bacterial plasmid database (http://www.ebi.ac.uk/genomes/plasmid.html).

For genome component prediction, gene prediction was performed on the *E.coli*O160:H7 (ENK1) genome assembly by glimmer3 (http://www.cbcb.umd.edu/software/glimmer/) with Hidden Markov models. tRNA, rRNA and sRNAs recognition made use of tRNAscan-SE, RNAmmer, and the Rfam database. The tandem repeats annotation was obtained using the Tandem Repeat Finder (http://tandem.bu.edu/trf/trf.html), and the minisatellite DNA and microsatellite DNA were selected based on the number and length of repeat units. The Genomic Island Suite of Tools (GIST) was used for genomicis lands analysis (http://www5.esu.edu/cpsc/bioinfo/software/GIST/) with IslandPath-DIOMB, SIGI-HMM, IslandPicker method. Prophage regions were predicted using the PHAge Search Tool (PHAST) web server (http://phast.wishartlab.com/) and CRISPR identification using CRISPRFinder.

For gene annotation and protein classification, the best hit abstracted using Blast alignment tool for function annotation. Seven databases which are KEGG (Kyoto Encyclopedia of Genes and Genomes), COG (Clusters of Orthologous Groups), NR(Non-Redundant Protein Database databases), Swiss-Prot,and GO (Gene Ontology), TrEMBL, EggNOG are used for general function annotation. Four databases for pathogenicity and drug resistance analysis. Virulence factors and resistance gene were identified based on the core dataset in VFDB (Virulence Factors of Pathogenic Bacteria) and ARDB (Antibiotic Resistance
Genes Database) database, other two are PHI (Pathogen Host Interactions) and (Carbohydrate-Active enZYmes Database). Type III secretion system effector proteins were detected by EffectiveT3.

For comparative genomics and phylogenetic analysis, the synteny of *E. coli* O160: H7 and other pathogenic and non-pathogenic *E. coli* strains was performed using MUMmer and BLAST Core/Pan genes of *E. coli* O160: H7 and other pathogenic and non-pathogenic *E. coli* strains were clustered by the CD-HIT rapid clustering of similar proteins software with a threshold of 50% pairwise identity and 0.7 length difference cutoff in amino acid. Gene family was constructed by the gene of *E. coli* O160: H7, other pathogenic and non-pathogenic *E. coli* strains, integrating multi software: align the protein sequence in BLAST and eliminate the redundancy by solar and carry out gene family clustering treatment for the alignment results with Hcluster_sg software. The phylogenetic tree is constructed by the TreeBeST using the method of NJ.

Reference sequences were respectively from:

*E. coli* strain Nissle 1917 https://www.ncbi.nlm.nih.gov/bioproject/447975;

*E. coli* O157:H7 strain https://www.ncbi.nlm.nih.gov/bioproject/479590;

E. coli CFT073 https://www.ncbi.nlm.nih.gov/bioproject/313;

Escherichia coli str. K-12 substr. MG1655 https://www.ncbi.nlm.nih.gov/bioproject/485867;

E. coli E24377A ETEC NC_009801 https://www.ncbi.nlm.nih.gov/bioproject/13960;

E. coli 53638 EIEC AAKB00000000 https://www.ncbi.nlm.nih.gov/bioproject/15639;

E. coli APEC O1 CP000468 https://www.ncbi.nlm.nih.gov/bioproject/16718;
coli IAI39 ExPEC  https://www.ncbi.nlm.nih.gov/bioproject/33411;
E.coli 042 EAEC FN554766  https://www.ncbi.nlm.nih.gov/bioproject/40647;
Escherichia coli O113:H21 str. CL-3
https://www.ncbi.nlm.nih.gov/bioproject/72243;
Escherichia coli strain VTH-15  https://www.ncbi.nlm.nih.gov/nuccore/GQ423574.1
Escherichia coli 042  https://www.ncbi.nlm.nih.gov/bioproject/40647
Escherichia coli ABU 83972 https://www.ncbi.nlm.nih.gov/bioproject/38725
Escherichia coli O127:H6 str. E2348/69
https://www.ncbi.nlm.nih.gov/bioproject/285331
Escherichia_coli_HS  https://www.ncbi.nlm.nih.gov/bioproject/13959
Escherichia_coli_S88  https://www.ncbi.nlm.nih.gov/bioproject/33375
Escherichia_coli_IHE3034  https://www.ncbi.nlm.nih.gov/bioproject/43693
Escherichia_coli_UTI189  https://www.ncbi.nlm.nih.gov/bioproject/16259
Escherichia coli IAI39  https://www.ncbi.nlm.nih.gov/bioproject/59381
Escherichia_coli_SMS-3-5 https://www.ncbi.nlm.nih.gov/bioproject/19469
Escherichia coli STEC_B2F1 https://www.ncbi.nlm.nih.gov/bioproject/48273
Escherichia_coli_UMN026  https://www.ncbi.nlm.nih.gov/bioproject/62981
Escherichia_coli_O111_H-__str._11128 _
https://www.ncbi.nlm.nih.gov/bioproject/32513
Escherichia coli O113:H21 str. CL-3
https://www.ncbi.nlm.nih.gov/bioproject/72243
Escherichia coli strain VTH-15  https://www.ncbi.nlm.nih.gov/nuccore/GQ423574.1
Out group: Legionella pneumophila
https://www.ncbi.nlm.nih.gov/nuccore/NC_006368.1
Cell isolation and flow cytometry

For the staining of lamina propria (LP) lymphocytes, colon or small intestine were isolated, cleaned by shaking in ice-cold PBS four times before tissue was cut into 1 cm pieces. The epithelial cells were removed by incubating the tissue in HBSS with 2 mM EDTA for 30 min with shaking. The LP cells were isolated by incubating the tissues in digestion buffer (DMEM, 5% fetal bovine serum, 1 mg/ml Collagenase IV (Sigma-Aldrich) and DNase I (Sigma-Aldrich) for 40 min at 37°C with shaking. The digested tissues were then filtered through a 40-mm filter. Cells were resuspended in 10 ml of the 40% fraction of a 40:80 Percoll gradient and overlaid on 5 ml of 80% fraction in a 15 ml Falcon tube. Percoll gradient separation was performed by centrifugation for 20 min at 1,800 rpm at room temperature. LP cells were collected at the interphase of the Percoll gradient, washed and resuspended in medium, and then stained and analyzed by flow cytometry. Single-cell suspensions of MLNs, PPs and spleen were prepared by mashing in a cell strainer (70 mm).

For analysis of different immune cell populations, the cells were washed with staining buffer containing PBS, 2% FBS, 1 mM EDTA and 0.09% NaN3 and surface staining was performed with FITC, PE, APC, PercP/cy5.5, BV421, BV605, APC/Cy7 or Alexa fluor 700-labeled anti-CD4, CD11c, MHCII, F4/80, CD11b, Ly6C, CD45, CX3CR1, CD103, TNFα, IFNγ, IL-17A, Foxp3, CD103 and Ki67 antibodies and analyzed using FACScan flow cytometry (Su et al., 2014). Gating strategy was based on Bain et al. who showed that distinct macrophages subsets can be isolated without using CX3CR1-GFP reporter mice (Bain et al., 2013; Shouval et al., 2014). We performed some modifications to this method: following initial gating on live CD45+ cells we gated on CX3CR1+CD11b+CD103−F4/80+ cells, and then analyse Ly6C and MHCII (Bain et al., 2013); Double cells or other unseparated cells were gated out.
based on SSC and FSC. Dead cells were eliminated through 7-AAD staining.

For intracellular staining, the cells were cultured and stimulated for 6 hrs with 50ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma) and 1 μg/ml ionomycin (Sigma) in the presence of GolgiStop (10 ng/ml, BD Biosciences). After incubation for 6 hrs, cells were washed in PBS, and then fixed in Cytofix/Cytoperm, permeabilized with Perm/Wash buffer (BD Biosciences), and stained with FITC-, PE-, APC or PerCP/Cy5.5-conjugated antibodies. Meanwhile, dead cells were eliminated through 7-AAD staining.

**Staining**

For hematoxylin/eosin (H&E) staining, previously reported methods were used in this experiment (Cao et al., 2016; Su et al., 2014). Briefly, the entire colon was excised to measure the length of the colon and then were fixed in 4% (w/v) paraformaldehyde buffered saline and embedded in paraffin, 5 μm sections colon sections were cut and stained with H&E.

For immunostaining, immunostaining was performed according to our previous method(Cao et al., 2016; Su et al., 2014). 5-μm-thick sections were prepared from the frozen tissue and fixed in acetone (−20°C) for 10 min. After rehydration in PBS for 5 min and further washing in PBS, tissue sections were blocked with 1% (w/v) BSA and 0.2% (w/v) milk powder in PBS (PBS-BB). The primary antibody was added in PBS-BB and incubated overnight at 4°C. After PBS washing (three times, 5 min each), tissue was detected with DAB kit or fluorescence labeled second antibody. Nuclei were stained by DAPI.

For fluorescent *in situ* hybridization (FISH), mucus immune-staining was paired with fluorescent *in situ* hybridization (FISH) in order to analyze bacteria localization
at the surface of the intestinal mucosa according to reported method (Chassaing et al., 2016; Vaishnava et al., 2011). In brief, the ileum and colonic tissues (proximal colon, second centimeters from the caecum) containing fecal material were placed in methanol-Carnoy’s fixative solution (60% methanol, 30% chloroform, 10% glacial acetic acid) for a minimum of 3 hrs at room temperature. Tissue were then washed in methanol, ethanol, ethanol/xylene (1:1) and xylene, followed by embedding in paraffin with a vertical orientation. 5-μm sections were cut and dewaxed by preheating at 60 °C for 10 min, followed by bathing in xylene at 60 °C for 10 min, xylene at room temperature for 10 min and 99.5% ethanol for 10 min. The hybridization step was performed at 50 °C overnight with an probe diluted to a final concentration of 0.01μg/mL in hybridization buffer (20mM Tris-HCl, pH7.4, 0.9M NaCl, 0.1% SDS, 20% formamide). After washing for 10 min in wash buffer (20mMTris-HCl, pH7.4, 0.9MNaCl) and 10 min in PBS and block solution (5% FBS in PBS) was added for 30 min at 50 °C. Mucin 2 primary antibody (rabbit H-300, Santa) was diluted to 1: 200 in block solution and applied overnight at 4 °C. After washing in PBS, block solution containing anti-rabbit secondary antibody diluted to 1: 200 was applied to the section for 2 hrs. Nuclei were stained using DAPI. Observations were performed with a Zeiss LSM 700 confocal microscope with software Zen 2011 version 7.1. This software was used to determine the distance between bacteria and the epithelial cell monolayer, as well as the mucus thickness.

**Ex vivo stimulation**

For *ex vivo* colon stimulation, colon from healthy mice were harvested, washed and incubated with or without $1 \times 10^9$ *E. coli* in DMEM media with ATP (2 mM) for 1hr. For analyses of caspase1, caspase11 and IL-18, the colon epithelial cells were
separated from colon tissues using 0.1% EDTA, followed by three 1 min shakings by hand, a 15-min incubation at 4 °C, and passage through 70-μm filters (BD Falcon) to collect the flow through. Fraction containing intact and isolated crypts were collected by centrifugation at 75 g for 5 min. at 4°C and washed with PBS. The lamina propria was separated from crypts to enrich for mononuclear and intestinal epithelial cells, respectively. Protein extracts were analyzed by immunoblotting for pro- and mature forms of caspase1, -11, IL-18 and IL-1β. The supernatants were collected for IL-18 ELISA. The expression of caspase1, caspase8, caspase11 and IL-18 was analyzed using immunoblotting and ELISA.

For ex vivo macrophage stimulation, macrophages were generated from abdomen cavity according to previously reported method (Lu et al., 2013). After ip injecting thioglycollate for 4-5 days, macrophages were collected, and then macrophages were exposed to different kinds of bacteria and collected at indicated time (3 hrs or 24 hrs after stimulation). The expression of caspase1, caspase8, caspase11 and IL-18 was analyzed using immunoblotting and ELISA.

For caspase inhibition experiment, pan-caspase (100 μM), caspase1(100 μM) and caspase8 inhibitor (46 μM) were respectively added into culture, and then colon epithelial cells were separated, and expression of caspase1, caspase11 and IL-18 was analyzed using immunoblotting and ELISA.

For PKCδ inhibition experiment, PKCδ inhibitors (20μM) were added into culture, and then colon epithelial cells were separated at the indicated time. The expression of caspase1, caspase11 and IL-18 was analyzed using immunoblotting and ELISA.

Immunoprecipitation and immunoblot
Immunoprecipitation and immunoblot were performed according to previous methods (Cao et al., 2016; Su et al., 2014). The cells were lysed with cell lysis buffer (Cell Signaling Technology), which was supplemented with a protease inhibitor 'cocktail' (Calbiochem). The protein concentrations of the extracts were measured using a bicinchoninic acid assay (Pierce).

For immunoprecipitation (IP), IP was performed according to our previously method (Gao et al., 2018). The gut epithelial cells were lysed in IP lysis buffer (Pierce, Rockford, IL, USA) containing 10% PMSF. Protein A/G magnetic beads (Pierce) were first added into the cell lysates for preclearing. The supernatant was collected after centrifuging at 12,000 rpm and then immunoprecipitated overnight at 4°C with the anti-NLRC4 or IgG antibodies. Protein A/G Magnetic Beads Protein A/G Magnetic Beads were added into cell lysates and incubated for additional 3 hrs. After being washed with five times, lysates were denatured and resolved by SDS-PAGE gels.

For the immunoblot, hybridizations with primary antibodies were conducted for 1 h at room temperature in blocking buffer. The protein-antibody complexes were detected using peroxidase-conjugated secondary antibodies (Proteintech) and enhanced chemiluminescence (Millipore).

RT-PCR and qRT-PCR

RT-PCR and qRT-PCR were performed according to our previous methods (Cao et al., 2016; Su et al., 2014). Total RNA was extracted from the cells, tissues and organs using TRIzol reagent (Invitrogen). First-strand cDNA was generated from total RNA using oligo-dT primers and reverse transcriptase (Invitrogen Corp). The PCR products were visualized on 1.0% (wt/vol) agarose gels. Quantitative real-time PCR (qRT-PCR)
was conducted using QuantiTect SYBR Green PCR Master Mix (Qiagen) and specific primers in an ABI Prism 7000 analyzer (Applied Biosystems). GAPDH mRNA expression was detected in each experimental sample as an endogenous control. All reactions were run in triplicate. The primers used in this study were listed in the Methods.

ELISA

For the levels of TNFα, IL-1β, IL-18, IL-12 and IL-22 in mouse peripheral sera and gut tissues, ELISAs were performed according to the manufacturer’s protocol. For tissue levels of cytokines, frozen tissues were homogenized in lysis buffer (PBS, 1% TritonX100 and protease inhibitor) using a Power Lyser 24 bench top bead-based homogenizer (Mobio). Lysates were centrifuged and supernatants used for ELISA.

QUANTIFICATION AND STATISTICAL ANALYSES

Two side Student's t-test and ONE-way ANOVA Bonferroni’s Multiple Comparison Test were used to determine significance. The statistical significance of the survival curves was estimated using Kaplan and Meier method, and the curves were compared using the generalized Wilcoxon's test. Histological scores, bacteria copy numbers and cell numbers in different groups were analyzed by a Mann-Whitney U test. A 95% confidence interval was considered significant and was defined as p < 0.05. * indicates p < 0.05, ** p < 0.01, *** p < 0.001.

Key resources table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies for immunoblotting |        |            |
and immunostaining

| Antibody                                | Manufacturer         | Cat/RRID                      |
|------------------------------------------|----------------------|------------------------------|
| Anti-Mouse β-Actin (C4) antibody         | Santa Cruz Biotechnology | sc-47778 RRID:AB_626632      |
| FITC-Goat Anti-Rat IgG(H+L)              | Proteintech          | SA00003-11                   |
| Alexa Fluor 488-Goat Anti-Mouse IgG(H+L)| Proteintech          | SA00006-1                    |
| Alexa Fluor 594-Goat Anti-Rabbit IgG(H+L)| Proteintech       | SA00006-4                    |
| Alexa Fluor 488-Goat Anti-Rabbit IgG(H+L)| Proteintech       | SA00006-2                    |
| Alexa Fluor 594-Goat Anti-Mouse IgG(H+L)| Proteintech          | SA00006-3                    |
| FITC-rabbit Anti-goat IgG(H+L)           | Proteintech          | SA00003-4                    |
| Anti-Mouse F4/80 (3H2113)                | Santa Cruz Biotechnology | sc-71088 RRID:AB_1122714    |
| Anti-Mouse TNFalpha (52B83)              | Santa Cruz Biotechnology | sc-52746 RRID:AB_630341     |
| Anti-Mouse CD4 (EPR19514)                | Abcam                | ab183685 RRID:AB_2686917     |
| Anti-Mouse IFN-gamma                     | Abcam                | ab9657 RRID:AB_2123314      |
| Anti-Mouse IL-18                         | Abcam                | ab71495 RRID:AB_1209302     |
| Anti-Mouse CK19(A-3)                     | Santa Cruz Biotechnology | sc-376126 RRID:AB_10988034|
| Anti-Mouse CD11b (1B6ε)                  | Santa Cruz Biotechnology | sc-21744 RRID:AB_626882    |
| Anti-Mouse Caspase1                      | Proteintech          | 22915-1-Ap                  |
| Anti-Mouse Caspase8                      | Proteintech          | 13423-1-Ap                  |
| Anti-Mouse Caspase11 (EPR18628)          | Abcam                | ab180673                     |
| Anti-Mouse NLRC4                         | ThermoFisher         | PA5-72908 RRID:AB_2718762   |
| Anti-Mouse PKCδ                          | Proteintech          | 19132-AP                    |
| Anti-Mouse ASC/TMS1 (D2W8U)              | Cell Signaling       | 67824                       |
| Anti-Mouse IL-1β                         | ABclonal Biotechnology | A11369                      |

**Antibodies for flow cytometry**

| Antibody                                | Manufacturer         | Cat/RRID                      |
|------------------------------------------|----------------------|------------------------------|
| PerCP/Cy5.5 anti-mouse CD45 (30-F11)     | Biolegend            | 1031232 RRID:AB_893340       |
| Brilliant Violet 421™ anti-mouse CD45    | Biolegend            | 103134 RRID:AB_2562559       |
| PE anti-mouse MHCII (M5/114.15.2)        | Biolegend            | 107608 RRID:AB_313323        |
| APC anti-mouse Ly6C (HK1.4)              | Biolegend            | 128016 RRID:AB_1732076       |
| Brilliant Violet 605™ anti-mouse F4/80 (BM8)| Biolegend       | 123133 RRID:AB_2562305       |
| APC/Cy7 anti-mouse/human CD11b (M1/70)   | Biolegend            | 101226 RRID:AB_830642        |
| Antibody                        | Manufacturer | Catalogue Number | RRID         |
|--------------------------------|--------------|------------------|--------------|
| FITC anti-mouse CD103 (2E7)    | Biolegend    | Cat:121420       | RRID:AB_10714791 |
| Alexa Fluor® 700 anti-mouse CX3CR1(SA011F11) | Biolegend    | Cat:149036       | RRID:AB_2629606 |
| FITC anti-mouse CD4 (RM4-5)    | Thermo Fisher Scientific | Cat:11-0042-85   | RRID:AB_464897 |
| PE anti-mouse IFN gamma (XGM1.2) | Thermo Fisher Scientific | Cat:25-7311-82   | RRID:AB_469680 |
| PErp/cy5.5 anti-mouse NKp46(29A1.4 ) | Biolegend    | Cat:137610       | RRID:AB_10641137 |
| APC anti-mouse Ki67 (SolA15)   | Thermo Fisher Scientific | Cat:17-5698-82   | RRID:AB_2688057 |
| APC anti-mouse IL17 (eBio17B7) | Thermo Fisher Scientific | Cat:11-7177-81   | RRID:AB_763581 |
| PE anti-mouse Foxp3 (NRRF-30)  | Thermo Fisher Scientific | Cat:12-4771-82   | RRID:AB_529580 |
| FITC anti-mouse F4/80 (BM8)    | Biolegend    | Cat:123108       | RRID:AB_893502 |
| APC anti-mouse CD11c (N418)    | Biolegend    | Cat:117310       | RRID:AB_313779 |
| FITC anti-mouse CD11b(M1/70)   | Thermo Fisher Scientific | Cat:11-0112-82   | RRID:AB_464935 |
| APC anti-mouse TNFα (MP6-XT22) | Thermo Fisher Scientific | Cat:17-7321-82   | RRID:AB_469508 |
| PE anti-mouse Ly6G (1A8) mouse | BD Bioscience | Cat:551461       | RRID:AB_394208 |
| FITC-Ly6C (AL-21) mouse       | BD Bioscience | Cat:553104       | RRID:AB_394628 |

**Neutralizing antibody**

| Antibody                        | Manufacturer | Catalogue Number | RRID         |
|--------------------------------|--------------|------------------|--------------|
| Mouse IL-12 Ab antibody        | RD Systems   | Cat: AF-419-NA   | RRID:AB_354485 |
| Mouse IL-22 Ab antibody        | RD Systems   | Cat: AF582       | RRID:AB_355457 |
| Mouse IL-18 (93-10C) Ab antibody | RD Systems   | Cat: D048-3      | RRID:AB_2123796 |
| Mouse IFN gamma (37895) Ab antibody | RD Systems   | Cat: MAB485      | RRID:AB_2123047 |

**Primers for qPCR**

| Primer                          | Manufacturer | Sequence                     |
|--------------------------------|--------------|------------------------------|
| Murine GAPDH-Fs                 | BGI          | 5' -ACTCAGCACCGGCTTCA-3'     |
| Murine GAPDH-Rs                 | BGI          | 5'-AACGCTACACACTGCATTTGG-3'  |
| Murine IFNγ-Fs                  | BGI          | 5' -GACCTAAAGAGTCTGAGG-3'    |
| Murine TNFα-Fs                  | BGI          | 5' -GGTGCAAGGCCCATAGAACTG-3'|
| Murine TNFα-Rs                  | BGI          | 5' -CAGGCTTTTTCCTCAACAG-3'  |
| Murine IL-4-Fs                  | BGI          | 5' -ATTCACGCAATTTGAAACAGG-3'|
| Murine IL-4-Rs                  | BGI          | 5' -TGCACGGTCCATGAGAACACT-3'|
| Murine IL-6-Fs                  | BGI          | 5' -TCTGAAAGAATCTGTGTT-3'   |
| Murine IL-6-Rs                  | BGI          | 5' -GATGGATCTACCAACTGGA-3'  |
| Murine IL-1β-Fs                 | BGI          | 5' -GTGTTTTCCCTGAGACCT-3'   |
| Murine IL-1β-Rs                 | BGI          | 5' -AATGGGACGTCACACACA-3'   |
| Gene                        | Primer 1 (5’-3’)                  | Primer 2 (5’-3’)                  |
|-----------------------------|-----------------------------------|-----------------------------------|
| Murine NOS-Fs               | BGI 5’-TGCCCCAAGGTATCCAAGTT-3’    |                                   |
| Murine NOS-Rs               | BGI 5’-CCTCCGTCAGTCTCCCACA-3’     |                                   |
| Murine Arginase1-Fs         | BGI 5’-CTGACCTATGTGTCATTTGGG-3’   |                                   |
| Murine Arginase1-Rs         | BGI 5’-TCAGGGAAGGACACAGGTT-3’     |                                   |
| Murine IL-10-Fs             | BGI 5’-AGCCTTATCGGAATGACTCCAGT-3’ |                                   |
| Murine IL-10-Rs             | BGI 5’-GCCTTGTAGACACCTTGGT-3’     |                                   |
| Murine IL-12-Fs             | BGI 5’-TGTTTGCCATCGTTTTGCTG-3’    |                                   |
| Murine IL-12-Rs             | BGI 5’-ACAGGTAAGTCACTGTTCCT-3’    |                                   |
| Murine IL-22-Fs             | BGI 5’-GCTCAGCTCCTTGACATCA-3’     |                                   |
| Murine IL-22-Rs             | BGI 5’-CAGAGGAAGCATTTCCTCAG-3’    |                                   |

**Perimeters for detection of bacteria**

| Perimeter          | Primer 1 (5’-3’)                  | Primer 2 (5’-3’)                  |
|--------------------|-----------------------------------|-----------------------------------|
| 16s 27F            | BGI 5’-AGAGTTTGATCCTGGCTCAG-3’    |                                   |
| 16s 1492R          | BGI 5’-GGTTACCTTGTTACGACTT-3’     |                                   |
| Eubacteria-Fs      | BGI 5’-ACTCCTACGGAGGCAAGCAGT-3’   |                                   |
| Eubacteria-Rs      | BGI 5’-ATTACCGCGGCTGCTGGC-3’      |                                   |
| E. coli-Fs         | BGI 5’-TGGGATCTCATTGTCAGA-3’      |                                   |
| E. coli-Rs         | BGI 5’-CACTGGGTGGGGCATAATTCC-3’   |                                   |

**Probe**

| Gene                  | Primer 1 (5’-3’)                  | Primer 2 (5’-3’)                  |
|-----------------------|-----------------------------------|-----------------------------------|
| E.coli-Colin situ     | BGI 5’-GAG ACT CAA GAT TGC CAG TAT CAG | cy3-GAG ACT CAA GAT TGC CAG TAT CAG |

**Critical Commercial Assays**

| Assay                | Cat.     |
|----------------------|----------|
| Mouse IL-18 ELISA KIT| E-EL-M0730c |
| Mouse IL-1β ELISA KIT| E-EL-M0037c |
| Mouse TNFa ELISA KIT | E-EL-M0049c |
| Mouse CPK ELISA KIT  | EK-M21262 |
| Mouse BUN ELISA KIT  | EK-M21223 |
| Mouse ASK ELISA KIT  | EK-M20900 |
| Mouse ALTELISA KIT   | EK-M20426 |
| QIAquick PCR Purification Kit | 28104 |
| QuantiTect SYBR Green PCR Master Mix | 208052 |
| Foxp3 / Transcription Factor Fixation/Permeabilization Concentrate and Diluent | 00-5521-00 |
| Chemicals, inhibitors                                      | Supplier        | Cat. No. |
|-----------------------------------------------------------|-----------------|----------|
| Cell stimulation cocktail                                 | ebioscience     | 00-4975-03 |
| Permeabilization Buffer                                   | Thermo Fisher   | 00-8333-56 |
| Dual-Luciferase Reporter Assay System                    | Promega         | E1910    |
| ECL chemiluminescence                                     | Absin           | abs920   |
| Protease Inhibitor Cocktail                              | Sigma-Aldrich   | P8340    |
| Dextran sulfate sodium salt (DSS)                         | Mpbio           | 160110   |
| pan caspase inhibitor (Z-VAD-FMK)                         | ApexBio Technology | A1902  |
| Caspase1 inhibitor (Z-YVAD-FMK)                           | ApexBio Technology | A8955  |
| Caspase8 inhibitor(Z-IETD-FMK)                            | ApexBio Technology | B3232  |
| PKCδ inhibitor (Rottlerin)                                | MedChemExpress  | HY-18980 |
| Ampicillin                                                | Sigma-Aldrich   | BP021    |
| Vancomycine                                               | Sigma-Aldrich   | V2002    |
| Neomycin sulfate                                          | Sigma-Aldrich   | N6386    |
| Metronidazole                                             | Sigma-Aldrich   | M3761    |
| Trizol                                                    | Life Technologies | 15596026 |
| FBS                                                       | Gibco           | 10099141 |
| Collagenase IV                                            | Sigma-Aldrich   | C5138    |
| Dnase I                                                   | Solarbio        | D8071    |
| DMEM                                                      | Gibco           | 11965118 |
| HBSS                                                      | Gibco           | 14170161 |
| Pecoll                                                    | Solarbio        | P8370    |
| PMA                                                       | Sigma-Aldrich   | 79346    |
| GolgiStop                                                 | BD Biosciences  | 554724   |
| LPS                                                       | Sigma-Aldrich   | L2630    |
| 7-AAD                                                     | Thermo Fisher   | A1310    |
| ATP                                                       | Sigma-Aldrich   | FLAAS    |
| EDTA                                                      | Sigma-Aldrich   | 798681   |
| Thioglycollate                                            | Millipore       | 70157    |
| Pierce™ Protein A/G magnetic beads                        | Thermo Fisher   | 88803    |
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