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# Cathelicidin Signaling via the Toll-Like Receptor Protects Against Colitis in Mice

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## Abstract

**Background & Aims—**Cathelicidin (encoded by *Camp*) is an anti-microbial peptide in the innate immune system. We examined whether macrophages express cathelicidin in colons of mice with experimental colitis and patients with inflammatory bowel disease; we investigated its signaling mechanisms.

**Methods—**Quantitative, real-time, reverse transcription PCR, bacterial 16S PCR, immunofluorescence, and small interfering (si)RNA analyses were performed. Colitis was induced in mice using sodium dextran sulfate (DSS); levels of cathelicidin were measured in human primary monocytes.

**Results—**Expression of cathelicidin increased in the inflamed colonic mucosa of mice with DSS-induced colitis, compared with controls. Cathelicidin expression localized to mucosal macrophages in inflamed colon tissues of patients and mice. Exposure of human primary monocytes to *E coli* DNA induced expression of *Camp* mRNA, which required signaling by ERK;



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Hon Wai Koon---acquisition, analysis and interpretation of data; drafting of the manuscript; David Shih, Jeremy Chen, Tressia Hing, Ivy Law, Samantha Ho & Ryan Ichikawa--- acquisition of data; Kyriaki Bakirtzi, Dezheng Zhao, Hua Xu, Richard Gallo, Paul Dempsey, Genhong Cheng and Stephan Targan---provision of materials and services;
Charalabos Pothoulakis---critical revision of manuscript and study supervision.

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expression was reduced by siRNAs against toll-like receptor (TLR)9 and MyD88. Intracolonic administration of bacterial DNA to wild-type mice induced expression of cathelicidin in colons of control mice and mice with DSS-induced colitis. Colon expression of cathelicidin was significantly reduced in *TLR9* −/− mice with DSS-induced colitis. Compared with wild-type mice, *Camp* −/− mice developed a more severe form of DSS-induced colitis, particularly after intracolonic administration of *E coli* DNA. Expression of cathelicidin from bone marrow-derived immune cells regulated DSS induction of colitis in transplantation studies in mice.

**Conclusions—**Cathelicidin protects against colitis induction in mice. Increased expression of cathelicidin in monocytes and experimental models of colitis involves activation of TLR9–ERK signaling by bacterial DNA. This pathway might be involved in pathogenesis of ulcerative colitis.

### Keywords

Cramp; LL-37; IBD; mouse models; endogenous inhibitors; immune regulation

## Introduction

Cathelicidin belongs to a peptide family with established antimicrobial functions in innate immune responses to protect the host against infection[1]. Cathelicidin possesses distinct anti-bacterial, anti-viral, and anti-fungal functions [2–4]. Different species have one or more forms of cathelicidin. The human form of cathelicidin is LL-37 while mCRAMP represents the mouse form [1, 5, 6]. LL-37 is largely secreted from surfaces exposed to the exterior environment such as cornea [3, 7], airway [8], skin [9] and gut [10]. In the colonic milieu with abundant presence of microbes, colonization of colonic bacteria is controlled in part by anti-microbial peptides, including members of the cathelicidin and defensin family of proteins.

Toll-like receptors (TLRs) are sensors of bacteria acting as an interface between the exterior environment and colonic cellular responses. TLRs recognize different pathogen-associated molecular patterns (PAMPs) such as Gram-negative bacterial lipopolysaccharide (TLR4), lipoprotein from Gram-positive bacteria (TLR1, 2, 6), double-stranded RNA (TLR3), flagellin (TLR5) or bacterial hypomethylated DNA (TLR9). Binding of PAMPs to TLRs trigger innate and adaptive immune responses mediating gastrointestinal homeostasis. TLR2, 4 and 9 mediate LL-37 secretion in monocyte-derived macrophages [11]. Moreover, bacterial DNA from *Mycobacterium tuberculosis* binds to TLR9 and mediates LL-37 expression in macrophages [11].

Inflammatory bowel diseases (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) represent complex immune disorders associated with abnormal responses to bacteria [12]. Interestingly, LL-37 mRNA expression is increased in colon biopsies from UC, but not CD patients [13]. Moreover, intracolonic administration of mCRAMP attenuates dextran sulfate (DSS)-induced colitis in mice [14]. However, studies to characterize the particular cells in the colonic mucosa expressing cathelicidin during experimental colitis have not been done and the mechanism(s) involved in cathelicidin upregulation has not been fully elucidated.

We used bacterial DNA, human primary monocytes and mCRAMP deficient (KO) and wild type (WT) mice to examine the *in vivo* cellular mechanisms involved in increased cathelicidin expression during colonic inflammation. Since TLR9 specifically recognizes bacterial DNA, we also studied a possible link between cathelicidin expression and TLR9 in monocytes and TLR9 deficient (KO) mice. Here we show that expression of cathelicidin is highly up-regulated in colon of mice with DSS colitis, both in macrophages and the epithelium and present direct evidence that increased cathelicidin expression is intimately correlated with TLR9-dependent signaling. Our results with cathelicidin KO mice also

demonstrate that endogenous cathelicidin play an important functional role in the modulation of colitis.

## Materials and Methods

### Bacteria DNA detection

(Qualitative method) Bacterial DNA was detected by a 16S ribosomal DNA (rDNA) PCR kit (#4370653, Applied Biosystems) followed by electrophoresis (2% agarose gel in TBE buffer). (Spectrophotometry method) *E. coli* cells were cultured overnight in LB broth and diluted to ($10^1$ – $10^8$ CFU/ml), bacterial DNA was extracted, and detected by spectrophotometry at 260 nm with a standard curve made by 0–1000 ng/ml of standard *E. coli* DNA (D4889, Sigma). (Absolute PCR method) Colonic tissue DNA was extracted and the bacterial 16S rDNA was detected by absolute 16S PCR with custom 16S primer sets and known *E. coli derived* bacterial DNA standard (0–1000 ng/ml) from different CFU/ml ($10^1$ – $10^8$ CFU/ml) as previously described [15]. Bacteria in colon tissues were detected by Gram Staining (HT90T, Sigma, St Louis, MO).

### Mouse colitis model

Male 8–10 week old c57/BL6 mice (n=6 per group) were used. A breeding colony of TLR9 and mCRAMP deficient mice was established and maintained at the University of California, Los Angeles (UCLA) animal facility under standard conditions. Mice received standard pelleted chow and tap water *ad libitum*, except the colitis group, which received water containing 5% (w/v) dextran sodium sulfate (DSS) for 5 days, as previously described [16]. To induce cathelicidin expression, 2.5 mg/kg *E. coli* genomic DNA (50 μg/mouse) was administered intracolonically under transient isoflurane anesthesia at day 0, 2 and 4 of the DSS colitis experiments. After 5 days, mice were sacrificed by carbon dioxide euthanasia. Colonic tissues were excised, homogenized in RIPA buffer, and equal amounts of protein (40 μg/lane) were subjected to Western blotting and ELISA. Some colonic tissues were also used for H&E staining and TUNEL assays as previously described [16]. Animal studies were approved by the institutional animal research committee of UCLA.

### Human primary monocyte cell culture

Blood was obtained after informed consent in accordance with procedures established by the Cedars-Sinai Institutional Review Board IRB#3358. PBMC were isolated as previously described [17]. Monocyte preparations were >90% pure as determined by esterase stain (Sigma-Aldrich, St. Louis, MO). Human primary monocytes were isolated and cultured in RPMI1640 medium (Invitrogen) containing 10% fetal calf serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen) as previously described [17].

## Results

### Up-regulation of cathelicidin in monocytes/macrophages in the colons of DSS-induced mouse colitis

Schauber *et al* showed that cathelicidin LL-37 expression is increased in the colon of UC but not in CD patients [13]. Here we characterized colonic expression of mouse cathelicidin (mCRAMP) protein by ELISA, Western blotting and immunofluorescence staining. We found that colonic mCRAMP protein expression level is increased by ~ 3–4 fold ($p<0.001$) in DSS-treated (5 days) mice by both ELISA and Western blot analysis (Figure 1A, 1B & 1C). Cathelicidin expression in the mucosal epithelium was also increased in mice with DSS colitis and UC patients (Figure 1D and 1E), in agreement with a previous report [13]. Since monocytes/macrophages may be a source of cathelicidin [11], we examined whether mucosal macrophages express cathelicidin during colitis by immunofluorescence staining using a

specific antibody against a mouse macrophage marker. Our results show increased expression of cathelicidin (red signal) in colonic macrophages (green signal) of DSS-exposed mice (Figure 1D) and UC patients (Figure 1E), compared to controls.

### Bacterial invasion into colonic tissues during DSS induced colitis

Bacterial DNA with CpG motifs, but not human host-DNA, is a natural ligand for TLR9 [18]. Moreover, detection of prokaryotic-specific 16S ribosomal DNA (rDNA) is a well established approach for detection of bacteria in mucosal samples of IBD patients [1920]. Based on these considerations, we hypothesized that DSS-induced colitis would also lead to bacterial invasion into colonic tissues. We detected bacterial 16S rDNA from mouse colons by PCR with agarose gel electrophoresis. While in DNA samples from normal colon 16S rDNA is below detectable levels, a 16S rDNA band is clearly evident in DNA samples from DSS-exposed mouse colons (Figure 2C). Similarly, we used quantitative PCR to determine 16S rDNA and correlated the bacterial count [measured as colony forming unit (CFU/ml)] with the amount of bacterial 16S DNA (by 16S PCR) and total bacterial genomic DNA (by spectrophotometry) (Figure 2A and 2B). DSS-exposed colons had significantly higher 16S rDNA expression than normal colons, indicating increased bacterial penetration (Figure 2D). We also observed more Gram positive bacteria (purple color) and Gram negative bacteria (slightly darker orange) present in colon tissues of DSS-treated mice compared to controls (Figure 2E). Thus, DSS-induced colitis is associated with increased bacterial invasion.

### Bacterial genomic DNA induces LL-37 gene expression in human primary monocytes

To determine whether bacterial genomic DNA induces cathelicidin gene expression in human, LL-37 levels were determined in primary human monocytes after *E. coli* genomic DNA exposure for up to 24 hours. *E. coli* genomic DNA increased LL-37 level in human primary monocytes over time (Figure 3A). We did not observe any increase in secreted LL-37 in the conditioned media from monocytes exposed to bacterial DNA (Figure 3A). Real-time RT-PCR experiments showed that the LL-37 mRNA expression peaked around 2 hours after exposure to bacterial genomic DNA and then gradually returned to normal levels (Figure 3B). Similarly, *E. coli* genomic DNA stimulated increased LL-37 mRNA in monocytes in a concentration-dependent fashion (Figure 3C).

### Bacterial DNA- induced LL-37 gene expression in monocytes is ERK1/2- and TLR9-dependent

To further explore the mechanism of LL-37 expression in monocytes, we treated human monocytes with *E. coli* DNA and found a time-dependent increase in ERK1/2 phosphorylation (Figure 3D). We also pretreated human monocytes with inhibitors of various signaling pathways, then exposed cells to *E. coli* DNA and measured LL-37 expression in the cell lysates by ELISA. Pretreatment with the selective ERK1/2 inhibitor PD98059 almost completely abolished LL-37 expression induced by *E. coli* DNA (Figure 3E), consistent with a previous report with sodium butyrate in HT-29 cells [21].

*E. coli* DNA is an established TLR9 ligand [22]. Pretreatment with the TLR9 inhibitor ODN-TTAGGG diminished LL-37 expression induced by *E. coli* DNA (Figure 3E), suggesting that *E. coli* DNA-mediated LL-37 expression involves TLR9. In contrast, inhibition of NF-κB by caffeic acid phenethyl ester (CAPE) did not alter LL-37 expression in response to *E. coli* DNA (Figure 3E). Moreover, pretreatment with the protein translation inhibitor Cycloheximide or the RNA transcription inhibitor Actinomycin D also significantly inhibited LL-37 expression induced by *E. coli* DNA (Figure 3F), indicating the involvement of *de novo* RNA transcription and protein synthesis in this response.

TLRs may modulate LL-37 expression in human alveolar macrophages during *Mycobacterium tuberculosis* infection [11]. TLR ligand binding also activates the downstream signaling molecule MyD88 that mediates various cellular responses, including cytokine secretion [23]. To determine the roles of TLR9 and MyD88 in LL-37 secretion, TLR9 and MyD88 genes were silenced in human monocytes with specific siRNAs and LL-37 levels were then evaluated. Basal LL-37 levels were not affected by either siRNA (Figure 4A). In contrast, RNA interference of TLR9 and MyD88 significantly reduced LL-37 expression induced by *E. coli* DNA (Figure 4A). The knock-down efficiency of TLR9 and MyD88 RNA interference is shown in Figure 4B.

### Bacterial DNA induces colonic cathelicidin secretion in vivo

To confirm the significance of bacterial DNA in the induction of cathelicidin expression during colitis, we injected *E. coli* DNA intracolonically and measured the levels of mCRAMP in the colon of normal or DSS-exposed mice as illustrated in Figure 4C. Colonic administration of *E. coli* DNA significantly increased colonic mCRAMP protein (Figure 4D) and mRNA (Figure 4E) in mice treated with DSS or water (control). Exposure of mice to *E. coli* DNA and DSS resulted in decreased colonic TLR9 mRNA expression (Figure 4F).

### Reduced colonic mCRAMP levels in TLR9 KO mice with colitis

To directly demonstrate the role of TLR9 in cathelicidin expression during colitis, we treated wild type (WT) and TLR9 KO mice with either water alone, or water containing 5% DSS for 5 days, followed by determination of colonic mCRAMP levels. As expected from a previous study [24], TLR9 KO mice showed worsened colitis than WT mice after DSS (Figure 5C). Moreover, no histological differences were evident between TLR9 KO and WT mice exposed to water alone (Figure 5C). Compared to WT, however, DSS-exposed TLR9 KO mice had significantly lower mCRAMP protein and mRNA levels (Figure 5A and 5B). Basal mCRAMP levels were statistically indistinguishable between WT and TLR9 KO mice (Figure 5A and 5B). These results directly confirm a major role for TLR9 in colonic cathelicidin expression during colitis.

### Cathelicidin deficiency and bacterial DNA administration aggravates colitis

To directly assess the role of endogenous cathelicidin in the development of colitis, WT and mCRAMP KO mice were provided with 5% DSS to induce colitis. Some mice groups were administered with *E. coli* DNA intracolonically. Our findings showed that mCRAMP KO mice develop more severe colitis (Figure 6C) with larger body weight drop (Figure 6A), indicated by higher colitis (Figure 6D) and histological scores (Figure 6E) compared to WT. Intracolonic bacterial DNA administration did not affect the severity of colitis in WT mice, but worsened colitis in mCRAMP KO mice, as indicated by colitis and histological scores (Figure 6D and 6E) with a further body weight drop (Figure 6A). Bacterial DNA treatment did not significantly affect body weight (Figure 6B) and histology score (Figure 6E) in water- treated control mice of either genotype. Colonic tissue apoptosis and apoptosis index during DSS-induced colitis were significantly augmented in mCRAMP KO mice and further increased by *E. coli* DNA administration (Figure 6F). These results directly demonstrate an important role for bacterial DNA-driven endogenous cathelicidin in the development of colitis.

### Bone marrow derived cathelicidin plays more important role in modulation of colitis

Since endogenous cathelicidin is produced by both colonic epithelial and immune cells, we next assessed the role of these two cell populations in the development of colitis. By bone marrow transplantation (Figure 7A), the genotypes of bone marrow derived cells can be exchanged (i.e. WT to KO or KO to WT). The efficiency of bone transplantation was

verified by flow cytometry of bone marrow and blood cells (Supplementary Figure 1). When the transplanted mice were exposed to DSS, KO to KO transplanted mice had worst colitis as expected, when compared to WT to WT transplanted mice (Figure 7A and 7C). Transplantation of WT bone marrow to KO recipient mice significantly improved colitis score (Figure 7A), and reduced body weight drop (Figure 7B), apoptosis (Figure 7C), histology score (Figure 7D), and colonic TNFα mRNA levels (Figure 7F). This indicates that cathelicidin-expressing WT bone marrow cells are involved in reduced colonic inflammation in KO recipient mice.

On the other hand, transplantation of KO bone marrow to WT recipient significantly increased colitis score (Figure 7A), body weight drop (Figure 7B), apoptosis (Figure 7C), histology score (Figure 7D), and colonic TNFα mRNA levels (Figure 7F), suggesting that cathelicidin deficient bone marrow cells worsen colonic inflammation in WT recipient mice. As control, the histology score (Figure 7D), body weight change (Figure 7B) and TNFα mRNA levels (Figure 7F) were not affected by bone marrow transplantation among all water-treated normal groups. However, the mCRAMP protein levels of cross-over mice (WT to KO or KO to WT) were lower than those of WT to WT mice and higher than those of KO to KO mice (Figure 7E). These results suggest that bone marrow cathelicidin may play an important role in the development of colitis.

## Discussion

Initiation and progression of intestinal inflammation, including IBD, requires the presence of bacterial pathogens [2526]. To maintain normal homeostasis, the host evolved several anti-bacterial defense systems, including anti-microbial peptides, such as cathelicidin, to defend against microorganisms [27]. An important advance in the cathelicidin field has been evidence indicating that cathelicidin may also play a role in innate immunity. Schauber *et al* showed increased colonic LL-37 mRNA expression in UC, but not in CD patients [13]. In line with these findings we present evidence for increased expression of cathelicidin in the colon of DSS-exposed mice, a model of UC. Using bacterial *E. coli* DNA (a natural TLR9 ligand) and human monocytes, we identified an ERK1/2 dependent signaling pathway involved in increased transcription of the LL-37 gene (Figure 3). Thus, interactions of bacterial DNA and TLR9 may contribute to the increase of endogenous cathelicidin during colitis.

Several pieces of evidence point to the importance of TLR9 in increased expression of cathelicidin during intestinal inflammation: a) Elevated colonic mouse cathelicidin levels in DSS-induced colitis are associated with increased expression of TLR9 in both DSS colitis (Figure 4F) and UC colons [28]. b) Bacterial DNA stimulates LL-37 expression in monocytes that can be inhibited by TLR9 and MyD88 gene silencing (Figure 4A, B). c) Monocytes express TLR9 that mediates cytokine secretion induced by bacterial DNA [29]. d) Notably, TLR9 deficient mice have reduced colonic expression of mouse cathelicidin during experimental colitis (Figure 5).

During DSS-induced colitis, bacteria invade into the mucosal tissue in close contact with local immune cells (Figure 2). Similar bacterial invasion had been observed in colons of UC but not in normal patients [30]. In human monocytes, bacterial 16S DNA activates TLR9 signaling which in turn increases ERK1/2 dependent LL-37 gene transcription. Although the exact molecular mechanism mediating this response was not examined in our study, Kida *et al* showed that ERK1/2 as well as other MAP kinases stimulate activation of AP-1 that binds to its specific consensus sequence on the LL-37 promoter to initiate LL-37 transcription [31]. Sodium butyrate-activated MAPK and AP-1 pathways lead to LL-37 gene transcription in human lung epithelial cells [31] and induces LL-37 expression via AP-1 in intestinal epithelial cells [32]. NF-κB antagonism does not alter *E. coli* DNA-induced LL-37 expression (Figure

3E). Consistent with our data, TLR9 activation by bacterial *E. coli* DNA activates ERK1/2 and AP-1 but not NF-κB-dependent interleukin-8 (IL-8) expression in human colonic epithelial T84, HT29, and Caco-2 cells [33].

Our results with mCRAMP KO mice directly demonstrate that endogenous cathelicidin modulates the development of colitis (Figure 6), consistent with the report by Tai *et al* [14] showing that exogenous cathelicidin administration reduces the severity of DSS-induced colitis. Another potential mechanism involved in the exacerbation of DSS-associated colitis in mCRAMP KO mice may be related to the anti-microbial effect of cathelicidin [34], since cathelicidin KO mice have aggravated infection in a wide variety of bacterial models of infection [35, 3637383940].

Moreover, in mCRAMP KO, but not WT mice, *E. coli* DNA exacerbates DSS-mediated colitis (Figure 6). Even in water-treated groups, mCRAMP KO mice developed very mild inflammation that was only observed by detailed histological scoring system (Figure 6E) and was not detected by gross clinical colitis scoring (Figure 6D). Intracolonic administration of *E. coli* DNA moderately promoted mild colonic inflammation in both water treated WT and mCRAMP KO mice, but the difference was not statistically significant (Figure 6E). Since the TLR9 ligand (*E. coli* DNA) increases expression of the pro-inflammatory chemokine IL-8 [33], this finding suggests that endogenous cathelicidin also stabilizes the pro-inflammatory effects of the TLR9 ligand in the colon.

In conclusion, increased expression of cathelicidin is observed in the DSS mouse model of colitis. Bacterial DNA that triggers TLR9-ERK1/2 mediated mechanism leads to elevated levels of cathelicidin expression. Bone marrow derived endogenous cathelicidin also plays an important role in modulating the development of colitis. These previously unrecognized pathways further signify the importance of the interactions of microbial recognition patterns with the mucosal defense system in the pathophysiology of intestinal inflammation and IBD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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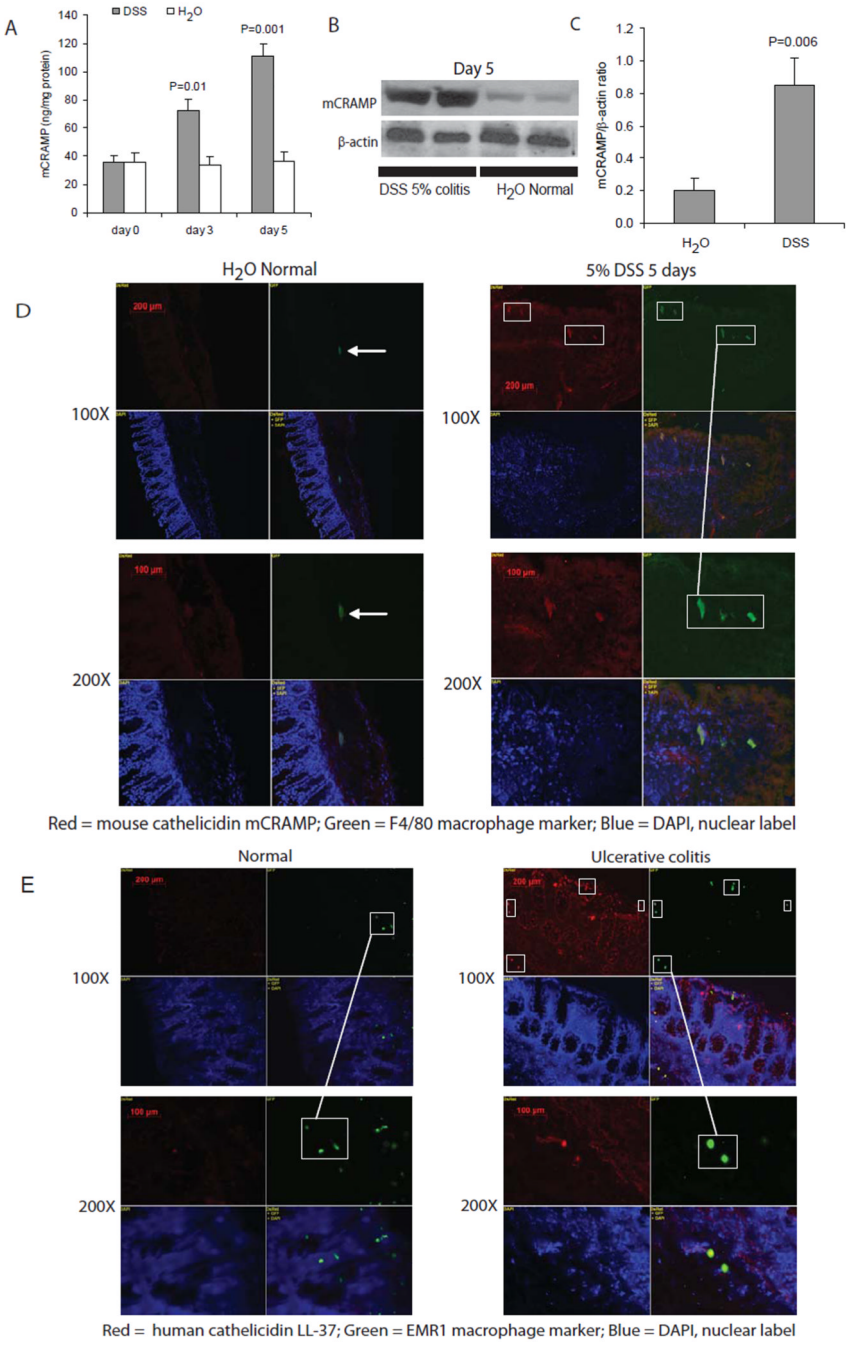


**Figure 1. Increased mCRAMP expression in colonic macrophages of mice with DSS-induced colitis**

Mice were provided with either water alone or water containing 5% of DSS. Animals were sacrificed and colonic tissues were removed on days 0, 3 and 5. (A) Colonic mCRAMP protein was measured by ELISA. The mCRAMP level was significantly increased in DSS treated mice when compared to day 0. (B) mCRAMP protein in colons was detected by Western blot analyses. (C) Densitometry of mCRAMP Western blot analyses. (D) Immunofluorescence staining of mCRAMP (red) and F4/80+ macrophages (green), counterstained by nuclear DAPI (blue) label. Overlapping expression of mCRAMP and F4/80 in merged images is indicated by yellowish color at 100–200X magnification. Results

are representative of 4 mice per group. (E) Immunofluorescence staining of LL-37 (red) and EMR1+ macrophages (green), counterstained by nuclear DAPI (blue) label. Overlapping expression of LL-37 and EMR1 in merged images was displayed in yellowish color at 100–200X magnification. Results are representative of 4 UC and 4 normal patients. Increased colonic cathelicidin protein expression is localized at macrophages and epithelium during colitis.

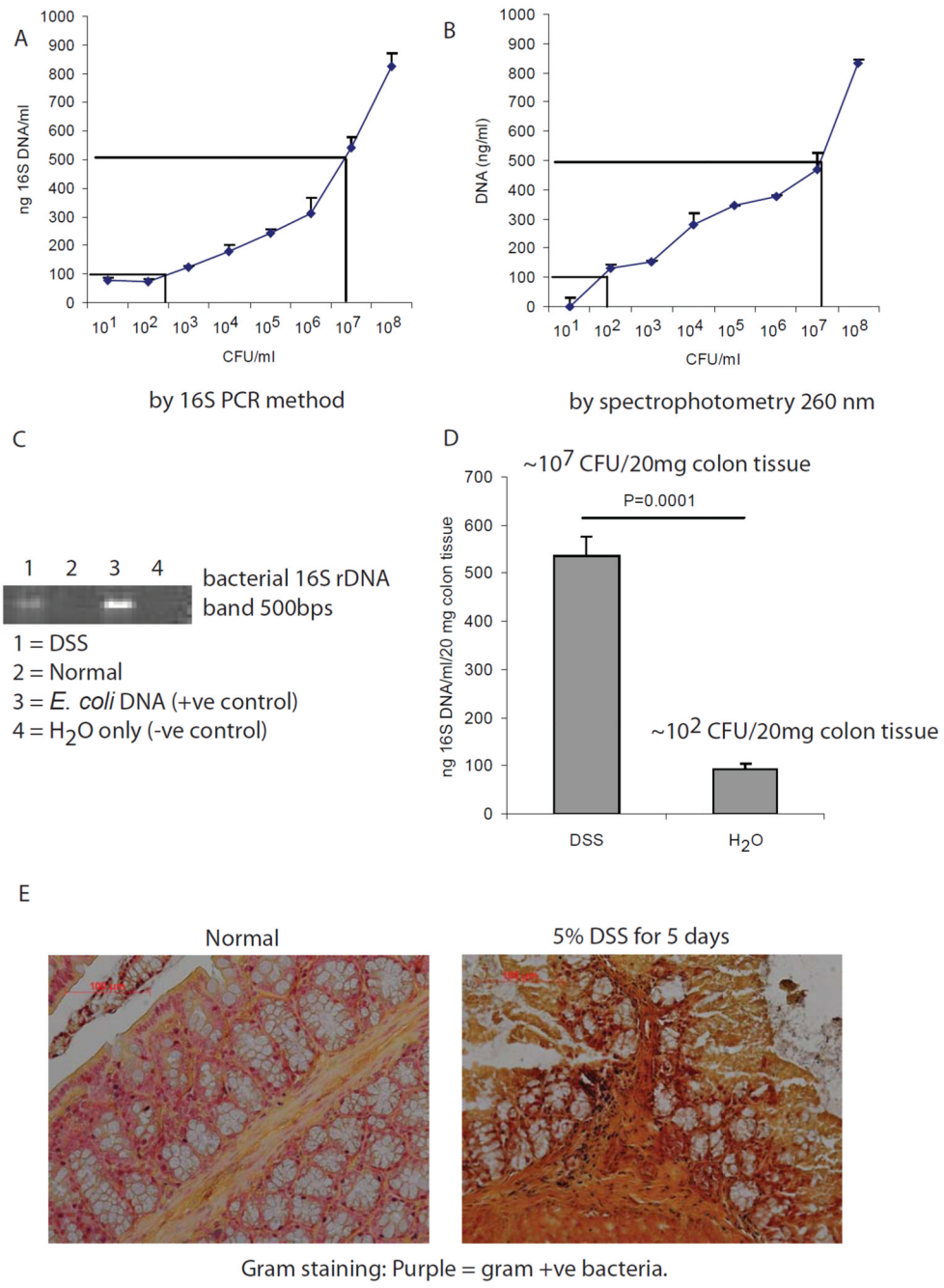


**Figure 2. Increased bacterial invasion into inflamed colons of DSS-exposed mice**

(A) *E. Coli* bacterial cells were cultured and diluted to different CFU/ml. The bacterial DNA was extracted and detected by (A) absolute bacterial specific 16S rDNA real-time PCR and (B) by spectrophotometry. Both methods used in (A) and (B) show similar correlation between CFU/ml and bacterial DNA concentration. (C) PCR band at 500 base pairs showing the presence of bacterial 16S rDNA in colonic DNA samples with positive and negative control groups. (D) Quantitative real-time PCR of 16S rDNA. Bacterial 16S DNA level was significantly increased in DSS group when compared to normal group. Using the standard curves in (A) and (B), the approximate CFU per 20mg colon tissue was calculated. (E) Gram staining for gram positive bacteria (purple) and gram negative bacteria (orange) in the colon

tissues of normal and DSS exposed mice at 200X magnification. Results are representative of 4 mice per group. Bacterial invasion into colon tissues is evident in DSS induced colitis.

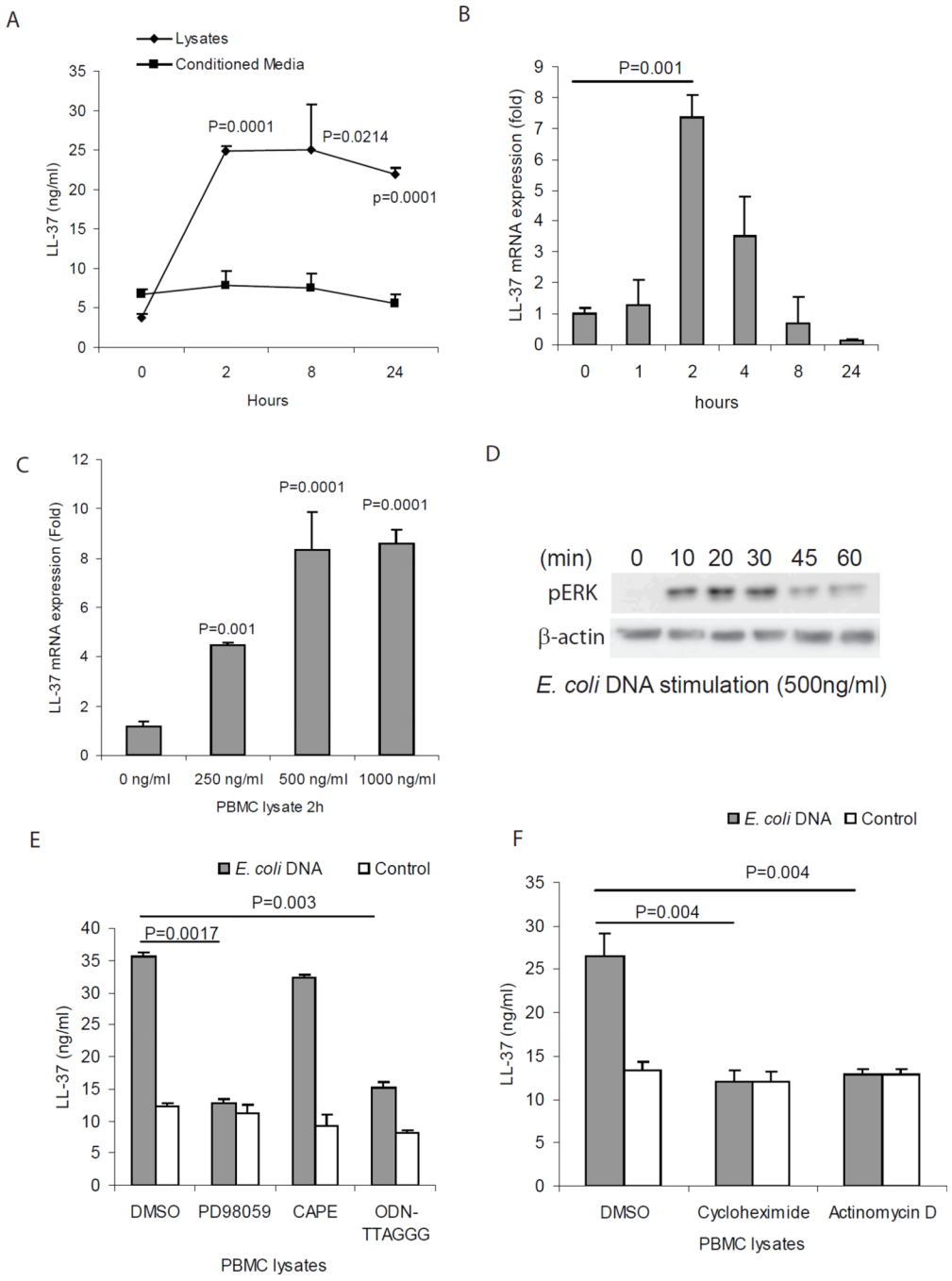


**Figure 3. Bacterial DNA stimulates LL-37 expression in human monocytes**

(A) Human primary monocytes were incubated with 500 ng/ml of *E. coli* genomic DNA for 0–24 hours. LL-37 in conditioned media and cell lysates were measured by ELISA. LL-37 levels in conditioned media were significantly increased 2–24 hours when compared to respective 0 hour. (B) LL-37 mRNA was determined by real-time RT-PCR. LL-37 mRNA expression was significantly increased at 2 hour when compared to respective 0 hour. (C) Human primary monocytes were treated with 0–1000 ng/ml of *E. coli* DNA and LL-37 mRNA expression was measured by real-time RT-PCR. LL-37 mRNA expression was significantly increased when compared to control. (D) Human primary monocytes were treated with 500 ng/ml of *E. coli* DNA for 0–60 mins. Phosphorylated ERK1/2 and β-actin

were determined by Western blot analyses. (E & F) Human primary monocytes were pretreated with the DMSO (control vehicle), ERK1/2 inhibitor PD98059 (10 μM), the NF-κB inhibitor CAPE (1 μM), and TLR9 receptor antagonist ODN-TTAGGG (25 μM), the protein synthesis inhibitor Cycloheximide (CHX, 1 μM), and the RNA transcription inhibitor Actinomycin D (ActD, 1 μM) for 30 min before incubation with *E. coli* DNA (500 ng/ml) for 4 hours. LL-37 levels in cell lysates were measured by ELISA. The decrease was statistically significant when compared to DMSO and *E. coli* DNA treated group. Results are representative of 3 separate experiments. *E. coli* DNA induces LL-37 *de novo* mRNA and protein synthesis in monocytes that are ERK and TLR9 dependent.

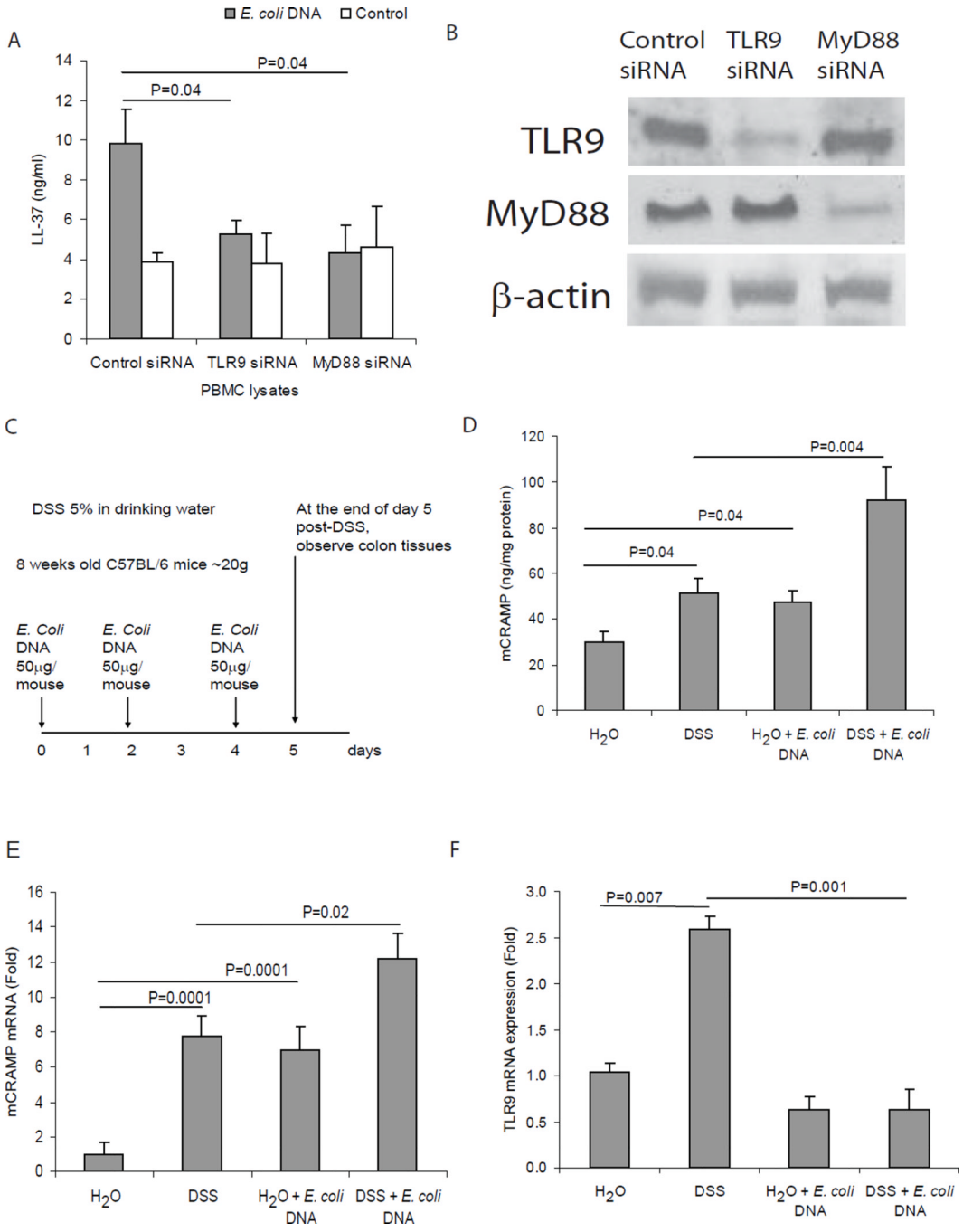


**Figure 4. *E. coli* DNA induces TLR9-dependent LL-37 expression in human monocytes**

(A) Monocytes were co-transfected with control, TLR9 and MyD88 siRNAs. Transfected cells were treated with *E. coli* DNA (500 ng/ml) for 4 hours. Cell lysates were used for LL-37 ELISA. The decrease was statistically significant when compared to control siRNA transfected *E. coli* DNA treated group. (B) Western blot showing the successful knockdown of TLR9 and MyD88 by respective siRNA. (C) Experimental plan of multiple intracolonic *E. coli* DNA administrations to normal and DSS exposed mice. Wild-type or mCRAMP deficient mice (~20 g) were given 5% DSS in their drinking water or water alone and injected intracolonically with 2.5 mg/kg *E. coli* DNA in 50 μl. (D & E) Colonic levels of mCRAMP (D) protein and (E) mRNA on day 5 were measured by ELISA and real-time RT-

PCR. Colonic mCRAMP expression is increased after DSS treatment and is further augmented by *E. coli* DNA administration. (F) Colonic levels of TLR9 mRNA. TLR9 mRNA expression was significantly increased after DSS treatment but was reduced by intracolonic *E. coli* DNA administration. Each group includes 6 mice.

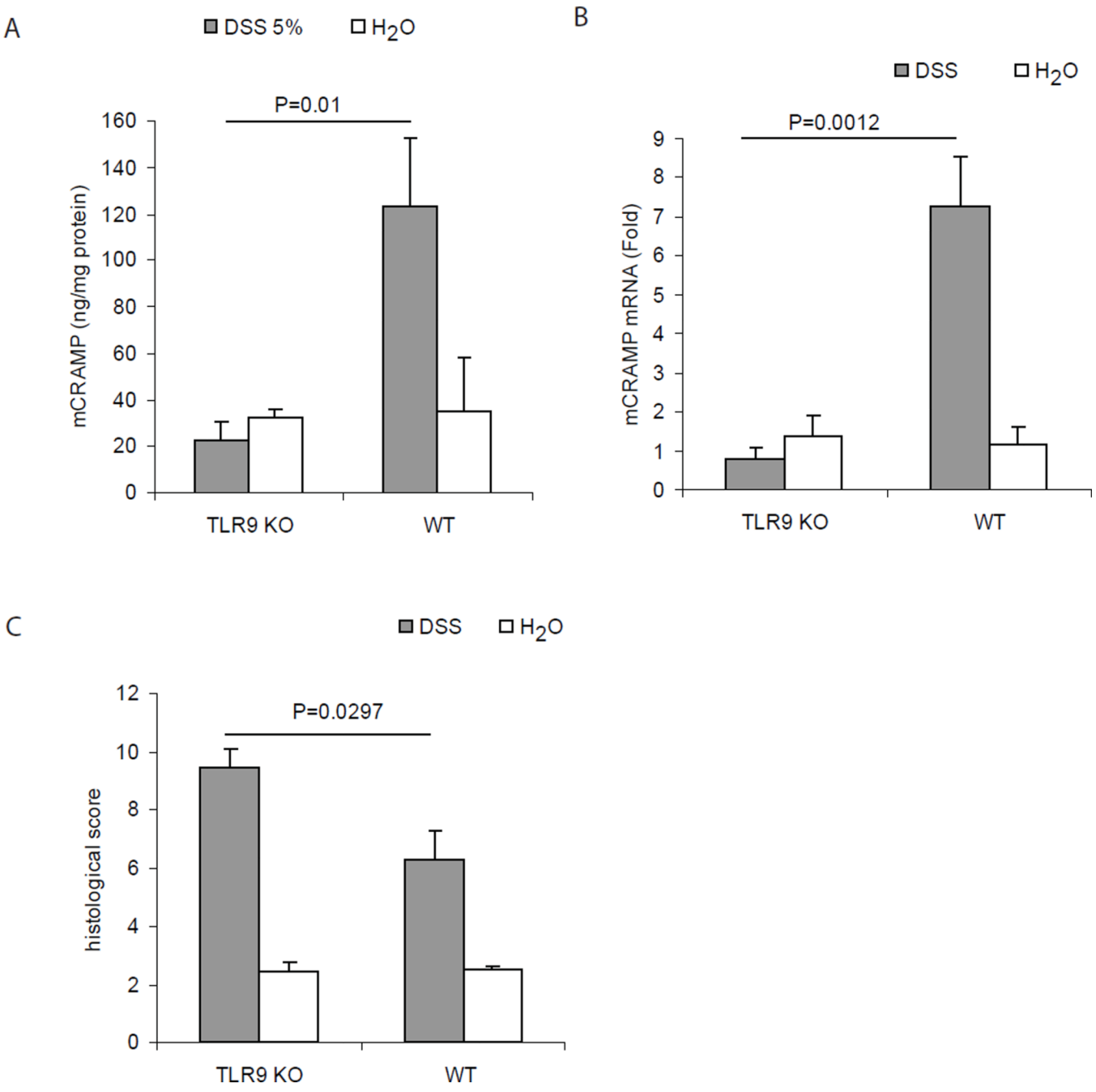


**Figure 5. TLR9 mediates colonic cathelicidin expression *in vivo***

(A & B) TLR9 deficient mice and wild-type mice were given 5% DSS in their drinking water or water alone for 5 days. Colonic levels of mCRAMP peptide (A) and mRNA (B) were measured by ELISA and real-time RT-PCR. The decrease was statistically significant when compared to DSS 5% treated wild-type mice. Each group includes 4 mice. After exposure to DSS, colonic mCRAMP levels are lower in TLR9 KO than those of wild-type mice. (C) Histological score of TLR9 KO and wild-type mice. TLR9 KO mice developed significantly more severe histological damage than wild-type mice after DSS exposure.

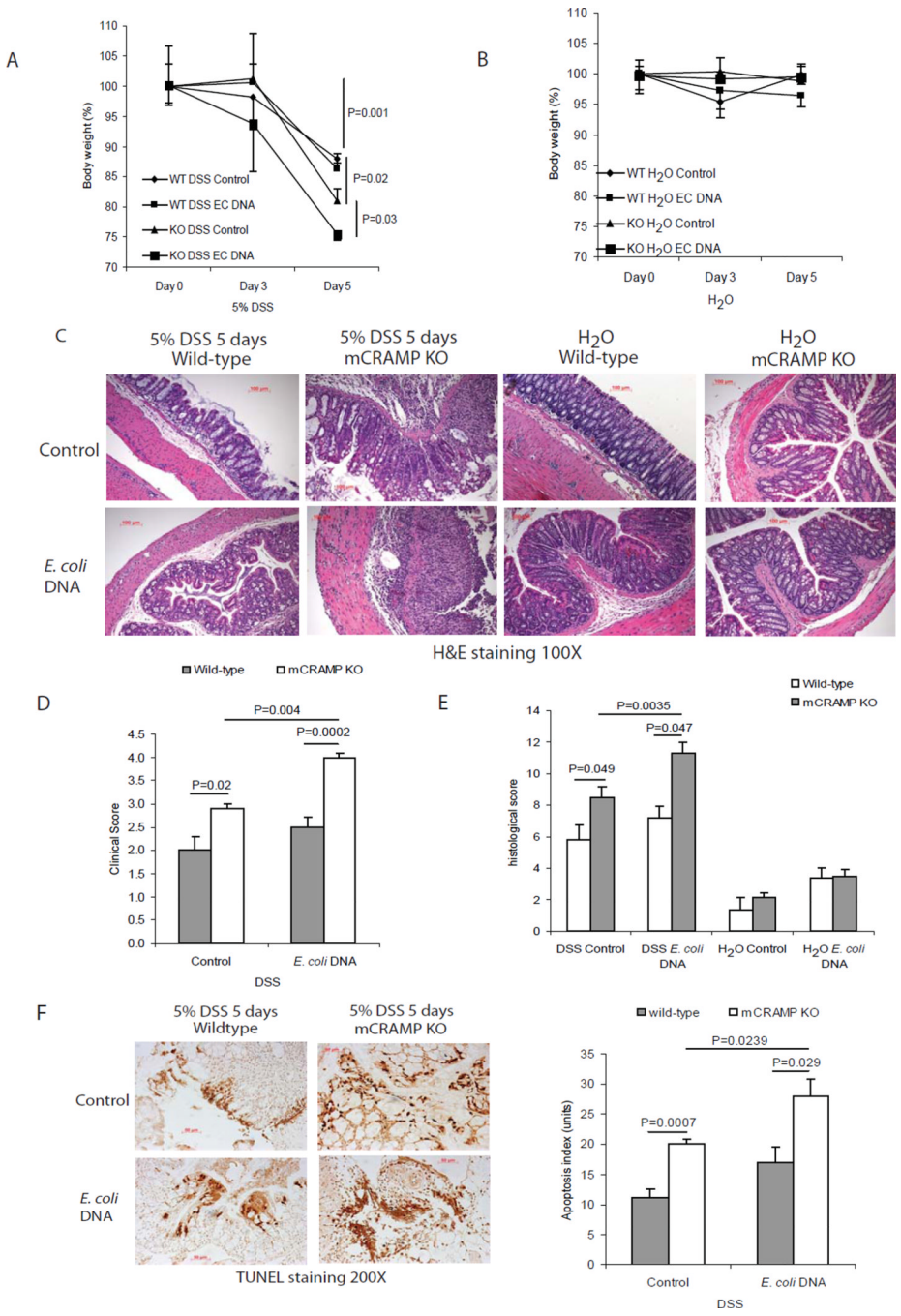


**Figure 6. Endogenous mCRAMP modulates course of DSS colitis**

Wild-type and mCRAMP KO mice (~20 g) were administered with 2.5 mg/kg *E. coli* DNA intracolonically in 50 μl and provided with water containing a 5% DSS solution for 5 days. (A) and (B) Body weight change of mice. (A) DSS exposure led to significant decrease of body weight which was augmented in mCRAMP KO mice and further worsened by intracolonic E. coli DNA administration. (B) No significant change of body weight of all water treated groups. (C) H&E staining of colons of mice. (D) Clinical score and (E) histological score of DSS colitis of various groups of wild-type and mCRAMP KO mice. The difference between control wild-type mice and mCRAMP KO mice was statistically significant. Also the increase of clinical score and histological score in *E. coli* DNA treated

mCRAMP KO group was significant when compared to control mCRAMP KO group. (F) TUNEL staining of colons of DSS exposed mice with apoptosis index. Apoptotic cells were visualized as brown spots. mCRAMP KO mice generally develop more serious colonic apoptosis than wild-type mice after exposure to DSS. Intracolonic administration of *E. coli* DNA exacerbates apoptosis in mCRAMP KO mice but not in wild-type mice. Results are representative of 4 mice per group.

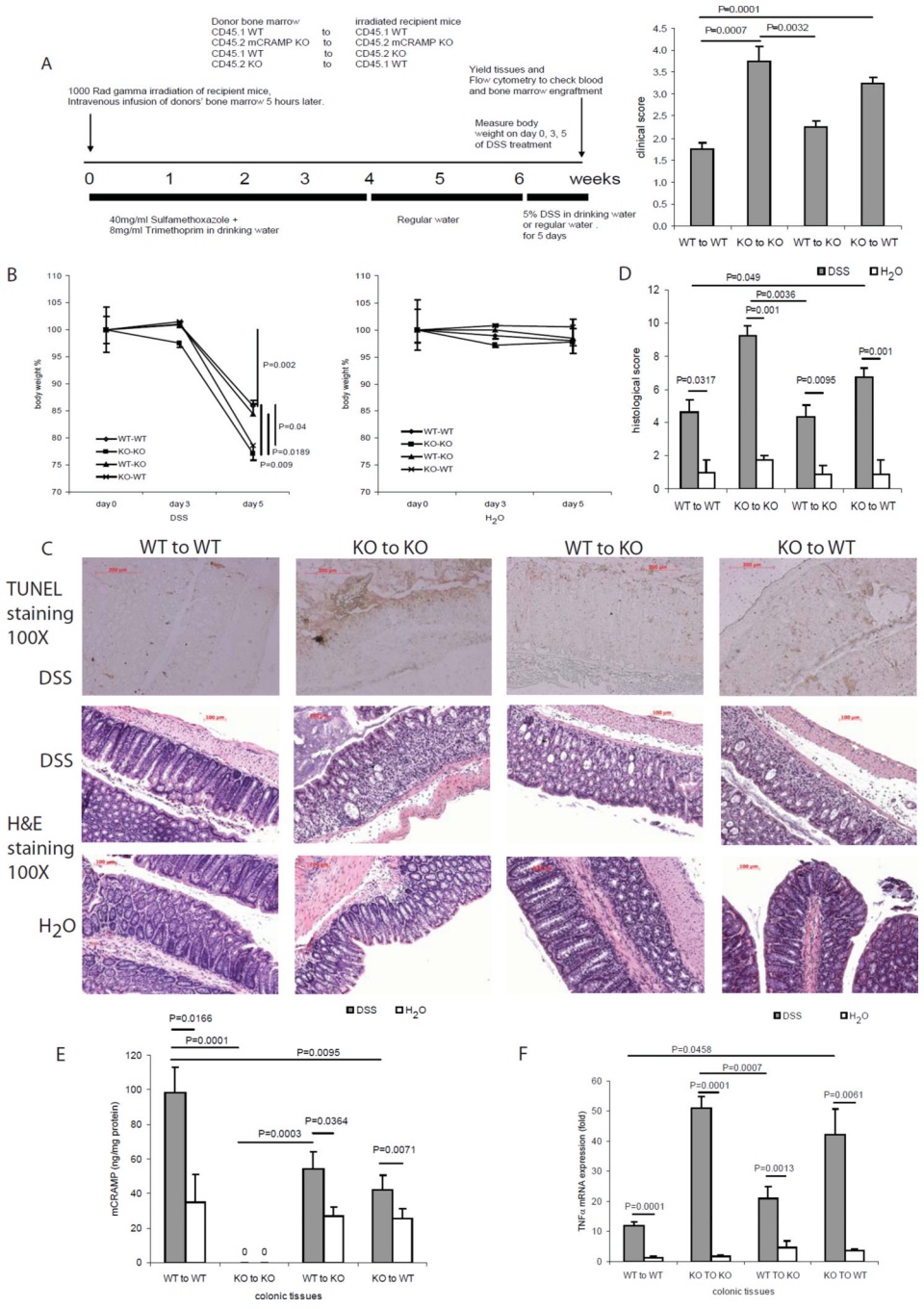


**Figure 7. Bone marrow derived cathelicidin modulates colitis**

(A) Plan of bone marrow transplantation experiment with clinical score of DSS colitis groups. $H_2O$ treated normal groups had all zero score. (B) Body weight change of transplanted mice. (C) H&E and TUNEL staining of colonic tissue 100X. (D) Histological score of transplanted mice. (E) Colonic mCRAMP protein levels of transplanted mice. (F) Colonic TNFα mRNA levels of transplanted mice. WT bone marrow transplantation to mCRAMP KO mice increases colonic mCRAMP mRNA levels and ameliorates colitis. KO bone marrow transplantation to WT mice decreases colonic mCRAMP mRNA levels and worsens colitis. Results are representative of 6 mice per group.