Gastroenterology 2019;156:1098–1111

ATF4 Deficiency Promotes Intestinal Inflammation in Mice by Reducing Uptake of Glutamine and Expression of Antimicrobial Peptides

Xiaoming Hu,[1] Jiali Deng,[1] Tianming Yu,[2] Shanghai Chen,[1] Yadong Ge,[2] Ziheng Zhou,[1] Yajie Guo,[1] Hao Ying,[1] Qiwei Zhai,[1] Yan Chen,[1] Feixiang Yuan,[1] Yuguo Niu,[1] Weigang Shu,[2] Huimin Chen,[2] Caiyun Ma,[2] Zhanju Liu,[2] and Feifan Guo[1]

[1]*Key Laboratory of Nutrition, Metabolism and Food Safety, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; and* [2]*Department of Gastroenterology, The Shanghai Tenth People's Hospital, Tongji University, Shanghai, China*

BACKGROUND & AIMS: Activating transcription factor 4 (ATF4) regulates genes involved in the inflammatory response, amino acid metabolism, autophagy, and endoplasmic reticulum stress. We investigated whether its activity is altered in patients with inflammatory bowel diseases (IBDs) and mice with enterocolitis. **METHODS:** We obtained biopsy samples during endoscopy from inflamed and/or uninflamed regions of the colon from 21 patients with active Crohn's disease (CD), 22 patients with active ulcerative colitis (UC), and 38 control individuals without IBD and of the ileum from 19 patients with active CD and 8 individuals without IBD in China. Mice with disruption of *Atf4* specifically in intestinal epithelial cells (*Atf4*ΔIEC mice) and *Atf4*-floxed mice (controls) were given dextran sodium sulfate (DSS) to induce colitis. Some mice were given injections of recombinant defensin α1 (DEFA1) and supplementation of L-alanyl-glutamine or glutamine in drinking water. Human and mouse ileal and colon tissues were analyzed by quantitative real-time polymerase chain reaction, immunoblots, and immunohistochemistry. Serum and intestinal epithelial cell (IEC) amino acids were measured by high-performance liquid chromatography–tandem mass spectrometry. Levels of ATF4 were knocked down in IEC-18 cells with small interfering RNAs. Microbiomes were analyzed in ileal feces from mice by using 16S ribosomal DNA sequencing. **RESULTS:** Levels of ATF4 were significantly decreased in inflamed intestinal mucosa from patients with active CD or active UC compared with those from uninflamed regions or intestinal mucosa from control individuals. ATF4 was also decreased in colonic epithelia from mice with colitis vs mice without colitis. *Atf4*ΔIEC mice developed spontaneous enterocolitis and colitis of greater severity than control mice after administration of DSS. *Atf4*ΔIEC mice had decreased serum levels of glutamine and reduced levels of antimicrobial peptides, such as *Defa1*, *Defa4*, *Defa5*, *Camp*, and *Lyz1*, in ileal Paneth cells. *Atf4*ΔIEC mice had alterations in ileal microbiomes compared with control mice; these changes were reversed by administration of glutamine. Injections of DEFA1 reduced the severity of spontaneous enteritis and DSS-induced colitis in *Atf4*ΔIEC mice. We found that expression of solute carrier family 1 member 5 (SLC1A5), a glutamine transporter, was directly regulated by ATF4 in cell lines. Overexpression of SLC1A5 in IEC-18 or primary IEC cells increased glutamine uptake and expression of antimicrobial peptides. Knockdown of ATF4 in IEC-18 cells increased expression of inflammatory cytokines, whereas overexpression of SLC1A5 in the knockdown cells reduced cytokine expression. Levels of *SLC1A5* were decreased in inflamed intestinal mucosa of patients with CD and UC and correlated with levels of *ATF4*. **CONCLUSIONS:** Levels of ATF4 are decreased in inflamed intestinal mucosa from patients with active CD or UC. In mice, ATF4 deficiency reduces glutamine uptake by intestinal epithelial cells and expression of antimicrobial peptides by decreasing transcription of *Slc1a5*. ATF4 might therefore be a target for the treatment of IBD.

Keywords: Gene regulation; immune response; mouse model; intestine.

BASIC AND TRANSLATIONAL AT

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), constitutes one of the most prevalent gastrointestinal diseases worldwide.[1] CD develops along the gastrointestinal tract and affects all layers of the intestinal wall, whereas UC is restricted to the colon and affects only the mucosa.[2] Many factors have been found to contribute to the pathogenesis of IBD, including the inflammatory response,[3] endoplasmic reticulum (ER) stress,[4] innate and adaptive immunity,[5] antimicrobial defence,[6] autophagy,[7] and gut mircobiota.[8] However, the molecular mechanisms underlying the pathogenesis of IBD remain poorly understood.

Abbreviations used in this paper: **ATF4, activating transcription factor 4; ATG16L1, autophagy related 16 like 1; CAMP, cathelicidin; CD, Crohn's disease; DEFA, defensin α; DSS, dextran sulfate sodium; EIF2A, eukaryotic translation initiation factor 2a; ER, endoplasmic reticulum; Gln, glutamine; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; IHC, immunohistochemistry; IL, interleukin; LPS, lipopolysaccharide; LYZ1, lysozyme; mRNA, messenger RNA; NOD2, nucleotide binding oligomerization domain containing 2; OTUs, operational taxonomic units; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; SEM, standard error of the mean; SLC1A5, solute carrier family 1 member 5; Th, T helper; UC, ulcerative colitis; WT, wild type.**

Most current article

0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2018.11.033

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Activating transcription factor 4 (ATF4) regulates genes involved in the inflammatory response, amino acid metabolism, autophagy, and endoplasmic reticulum stress.

NEW FINDINGS

Levels of ATF4 were decreased in inflamed intestinal mucosa from patients with active IBD, compared to individuals without IBD. In mice, ATF4 deficiency reduced glutamine uptake by intestinal epithelial cells and expression of antimicrobial peptides by decreasing transcription of *Slc1a5*.

LIMITATIONS

This study did not determine the role of SLC1A5 in animal models of IBD.

IMPACT

ATF4 might be a target for the treatment of IBD.

Intestinal epithelial cells (IECs), principally enterocytes, Paneth cells, goblet cells, neuroendocrine cells, and intestinal stem cells, form the epithelial barrier that is disrupted during the inflammatory process in IBD.[9] Paneth cells are specialized epithelial cells located at the base of small intestinal crypts that act as important effectors of innate immunity through their secretion of antimicrobial peptides such as α-defensins.[10] Numerous studies have indicated that disruption of genes associated with Paneth cell functions, including the expression and secretion of antimicrobial peptides, increases susceptibility to IBD. Such genes include x-box binding protein 1 (*Xbp1*), autophagy-related 16–like 1 (*Atg16l1*), nucleotide binding oligomerization domain-containing 2 (*Nod2*), and transcription factor 4 (*Tcf4*).[4,11–13] Moreover, CD patients often exhibit a reduced number of healthy Paneth cells and decreased expression of α-defensins in areas of acute mucosal inflammation.[14] Therefore, understanding the signaling cascades that regulate Paneth cell function is critical for the design of new therapeutic approaches for these diseases.

Amino acids are the building blocks of proteins, and they function as key metabolic regulators in many processes.[15] Recent work has indicated an important role for amino acids in antimicrobial peptide expression and gut health maintenance.[16–23] For example, dietary tryptophan regulates the expression of intestinal antimicrobial peptides and gut microbiota composition, and disorders of tryptophan metabolism cause intestinal inflammation.[16–18] Glutamine (Gln), the main respiratory substrate of IECs, plays an important role in intestinal physiology, and decreased Gln levels have been shown to be associated with immune dysfunction.[19] Moreover, Gln has also been found to increase antimicrobial peptide expression and reduce gut inflammation in animal models of IBD.[20–23] Hence, the dysfunction of genes involved in intestinal amino acid homeostasis might be expected to increase susceptibility to IBD.

Activating transcription factor 4 (ATF4) is a basic leucine zipper transcription factor expressed in many tissues, including the intestine. It is involved in the regulation of various physiological processes, including the inflammatory response,[24] amino acid and lipid metabolism,[25,26] ER stress,[27] autophagy,[28] and energy homeostasis.[29,30] Dysregulation of ATF4 expression induces a variety of disorders, such as liver steatosis,[31] Parkinson's disease[32] and Alzheimer's disease.[33] The potential roles of ATF4 in the pathogenesis of IBD, however, have not been studied yet. Given the importance of the inflammatory response, ER stress, autophagy, and amino acid metabolism in IBD development, we speculated that ATF4 might also be involved in the pathogenesis of this condition. The aim of our current study was to investigate this possibility and elucidate the underlying mechanisms.

Methods

Participants

All patients with IBD were recruited from the Department of Gastroenterology, the Shanghai Tenth People's Hospital of Tongji University (Shanghai, China) from October 2016 through May 2018. Biopsy samples were taken from inflamed and/or uninflamed regions of the colon from patients with active colonic CD (n = 21; mean age, 32.0 years; range, 15–56 years), patients with active UC (n = 22; mean age, 40.3 years; range, 19–59 years), and control individuals without IBD (n = 38; mean age, 47.4 years; range, 17–64 years) and from inflamed and/or uninflamed regions of the ileum from patients with active ileal CD (n = 19; mean age, 34.7 years; range, 17–57 years) and control individuals (n = 8, mean age, 45.5 years; range, 29–50 years) who had endoscopy. The diagnosis of CD or UC was based on clinical, radiologic, and endoscopic examination and histologic findings, as described previously.[34,35] The baseline characteristics are described in Supplementary Table 1. The disease severity was assessed according to international standard criteria: the CD Activity Index for CD patients and Mayo scores for UC patients. The study was approved by the Institutional Review Board for Clinical Research of the Shanghai Tenth People's Hospital of Tongji University. Written informed consent was also obtained from all participants before the study protocol began.

Mice

All mice were of the C57BL/6J background. *Atf4*-floxed mice (control mice) [31] were intercrossed with *Villin*-Cre mice (obtained from the Shanghai Biomodel Organism Science and Technology Development Company, Shanghai, China) to generate IEC-specific *Atf4*-deletion mice (*Atf4*ΔIEC mice). Control and *Atf4*ΔIEC mice were kept separately in different cages after weaning at 4 weeks old. Most of the experiments were conducted with male mice; female mice were used only for characterizing IBD phenotype. Male C57BL/6J wild-type (WT) mice were purchased from the Shanghai Laboratory Animal Company (Shanghai, China). Mice were housed in laboratory cages at 23°C ± 3°C at 35% ± 5% humidity under a 12-hour dark/light cycle. With free access to a regular chow diet (Shanghai Laboratory Animal Company), all mice were maintained under specific pathogen-free conditions. All protocols of animal experiments were approved by the Institutional Animal Care and Use Committee at the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism, version 6.0 (GraftPad Software, San Diego, CA). All values are presented as mean ± standard error of the mean (SEM). Statistical comparisons were performed using an unpaired 2-tailed Student *t* test, paired Student *t* test, or 1-way analysis of variance, and the nonparametric Mann–Whitney *U* test was used for analysis of microbial data. Pearson correlation was performed to analyze the correlation of solute carrier family 1 member 5 (*SLC1A5*) mRNA levels and *ATF4* expression in intestinal mucosa from IBD patients. Statistical significance was set at $P < .05$. See the supplementary material for additional information.

Results

ATF4 Expression Is Decreased in the Inflamed Intestinal Mucosa of Patients With Active IBD and the Colonic Epithelia of Mice With Dextran Sulfate Sodium–Induced Colitis

To explore the potential role of ATF4 in the pathogenesis of IBD, we measured its expression in intestinal mucosa from patients with active IBD and control individuals. ATF4 expression was markedly decreased in inflamed colon mucosa from patients with active colonic CD or UC compared with uninflamed mucosa from control individuals, as evaluated by quantitative reverse-transcription polymerase chain reaction (qRT-PCR), Western blot analysis, and immunohistochemical (IHC) staining, respectively (Figure 1*A*–*C*). ATF4 expression was also lower in inflamed areas of colon mucosa than in adjacent normal mucosa from the same IBD patients (Figure 1*D*). Similar results were obtained in ileum mucosa from ileal CD patients (Supplementary Figure 1*A* and *B*). Consistent with these findings, ATF4 expression was also found to be decreased in colonic epithelia of dextran sulfate sodium (DSS)–treated WT mice compared with controls (Figure 1*E* and *F*). These data suggest that intestinal epithelial ATF4 may play an important role in the pathogenesis of IBD.

Atf4 *Deletion in IECs Causes Spontaneous Enterocolitis and Susceptibility to DSS-Induced Colitis*

To further study the involvement of ATF4 in the development of IBD, we generated *Atf4*ΔIEC mice. Expression levels of ATF4 and its target, TRB3, were decreased in jejunal, ileal, and colonic epithelia but not in the liver of these mice, indicating the efficacy of the deletion (Supplementary Figure 2*A*–*C*).

*Atf4*ΔIEC mice appeared normal at birth but spontaneously developed a disease phenotype after 3 weeks of age that resulted in an increase of mortality (about 20% in *Atf4*ΔIEC mice but only 2.7% in control mice) (Supplementary Figure 3*A*). We observed that the mice that died showed serious intestinal damage and extensive lymphocyte infiltration in both the ileum and colon (Supplementary Figure 3*B* and *C*). The body weight of surviving *Atf4*ΔIEC mice (19.66 ± 0.61g) was significantly lower than that of control mice (24.61 ± 0.54g) at 8 weeks of age, and spleen weight was significantly increased (Supplementary Figure 3*D*–*F*), although no difference in food intake and drinking water was observed between those mice (Supplementary Figure 3*G* and *H*).

Furthermore, we found that surviving *Atf4*ΔIEC mice had a 10.6% frequency of diarrhea and spontaneous rectal prolapse (Figure 2*A* and *B*) and significantly decreased colon length (Figure 2*C*), indicating that they suffered from spontaneous intestinal disease. Histologic analysis showed that *Atf4*ΔIEC mice developed serious spontaneous enterocolitis, characterized by altered intestinal architecture, including villous damage, crypt loss, and extensive lymphocyte infiltration in the ileum (Figure 2*D* and *E*), as well as modified intestinal architecture and increased lymphocyte infiltration of the colon compared with control mice (Figure 2*G* and *H*). Consistent with these inflammatory changes, messenger RNA (mRNA) expression levels of inflammatory cytokines, including interleukin (IL) 1b (*Il1b*), *Il6*, and tumor necrosis factor α (*Tnfa*), were significantly higher in the ileal and colonic tissues of *Atf4*-ΔIEC mice than that of controls (Figure 2*F* and *I*). Inflammatory response, such as lymphocyte infiltration, is implicated in the pathogenesis of IBD.[36] Consistently, the numbers of neutrophils and $CD4^+$ T cells were also markedly increased in the lamina propria of the ileums and colons of *Atf4*ΔIEC mice compared with controls (Supplementary Figure 4*A* and *B*).

We next investigated the influence of ATF4 on the induction of mucosal inflammation using a DSS-induced colitis model. The clinical signs of colitis, including weight loss, rectal bleeding, and colon shortening, were more severe in *Atf4*ΔIEC mice than controls after DSS challenging (Supplementary Figure 5*A*–*C*). The epithelial damage, including mucosal erosion, crypt loss, and lymphocyte infiltration, and the mRNA expression of inflammatory cytokines (e.g., *Il1b*, *Il6*, and *Tnfa*) were also increased in the colons of *Atf4*ΔIEC mice (Supplementary Figure 5*D*–*F*). Additionally, female *Atf4*ΔIEC mice exhibited a similar inflammatory phenotype (Supplementary Figures 6*A*–*F* and 7*A*–*F*).

Atf4 Deletion in IECs Compromises Ileal Paneth Cell Function

Paneth cells and goblet cells, which produce antimicrobial peptides and mucins, respectively, play key roles in intestinal innate immunity.[10,37] Reduced numbers of goblet cells, as determined by periodic acid–Schiff staining, and diminished mRNA expression of the goblet cell marker *Muc2* were also observed in the ileal epithelia of *Atf4*ΔIEC mice compared with controls at 8 weeks of age (Supplementary Figure 8*A* and *C*), but electron microscopy showed no obvious differences in the secretory granules from goblet cells (Supplementary Figure 8*B*). Moreover, no differences in goblet cell number and *Muc2* mRNA expression were seen in the colonic epithelia between *Atf4*ΔIEC mice and controls

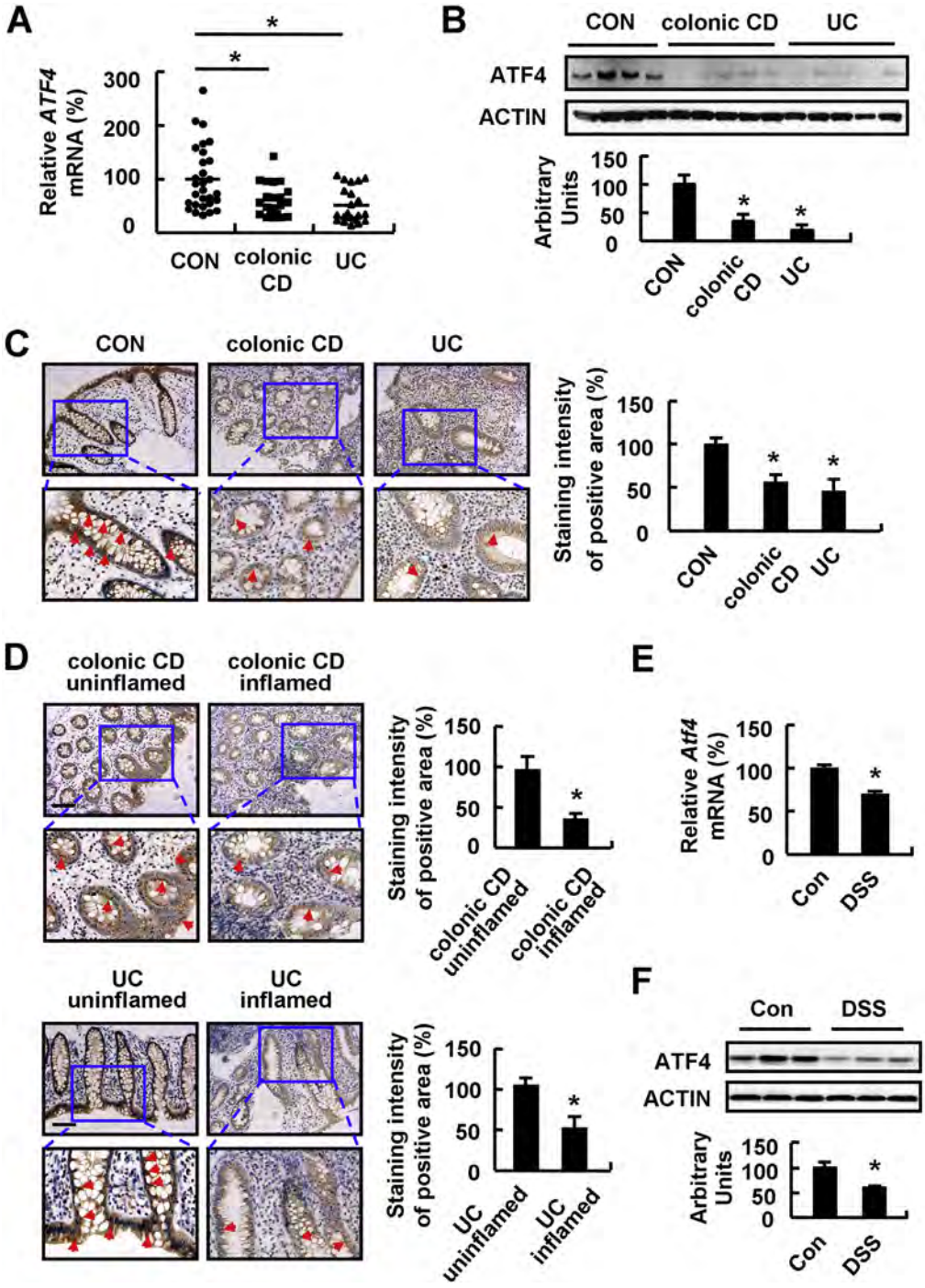

Figure 1. ATF4 is down-regulated in the inflamed colon mucosa of active IBD patients and the colonic epithelia of mice with DSS-induced colitis. (*A*) qRT-PCR analysis of *ATF4* mRNA in colonic biopsy samples from control individuals (CON) (n = 31) and patients with inflamed mucosa of active colonic CD (n = 21) and active UC (n = 22). *$P < .05$ vs CON. (*B*) Western blot analysis of ATF4 protein in colon biopsy samples from control individuals (n = 4) and patients with colonic CD (n = 5) and UC (n = 5). Quantification of band intensity is shown in the lower graphs. *$P < .05$ vs CON. (*C*) Representative intestinal sections were prepared from the colon mucosa of control individuals and the inflamed mucosa of active colonic CD and UC patients and were stained with anti-ATF4 monoclonal antibody by IHC. (*D*) ATF4 protein expression in uninflamed and inflamed colon mucosa from the same colonic CD and UC patients by IHC. The boxed areas are shown enlarged in the lower panels. The red arrows indicate ATF4-positive cells. Scale bar, 50 μm. The staining intensity of the positive area was quantified by ImageJ software (National Institutes of Health, Bethesda, MD) and illustrated with bar charts. n = 3 patients per group. (*E*, *F*) WT mice were administered normal water as control (Con) or 2.5% DSS in drinking water for 7 days to induce acute colitis. qRT-PCR and Western blot analyses were performed of *Atf4* mRNA and protein in colonic epithelia, respectively. Values are expressed as mean ± SEM (n = 6 mice per group). *$P < .05$.

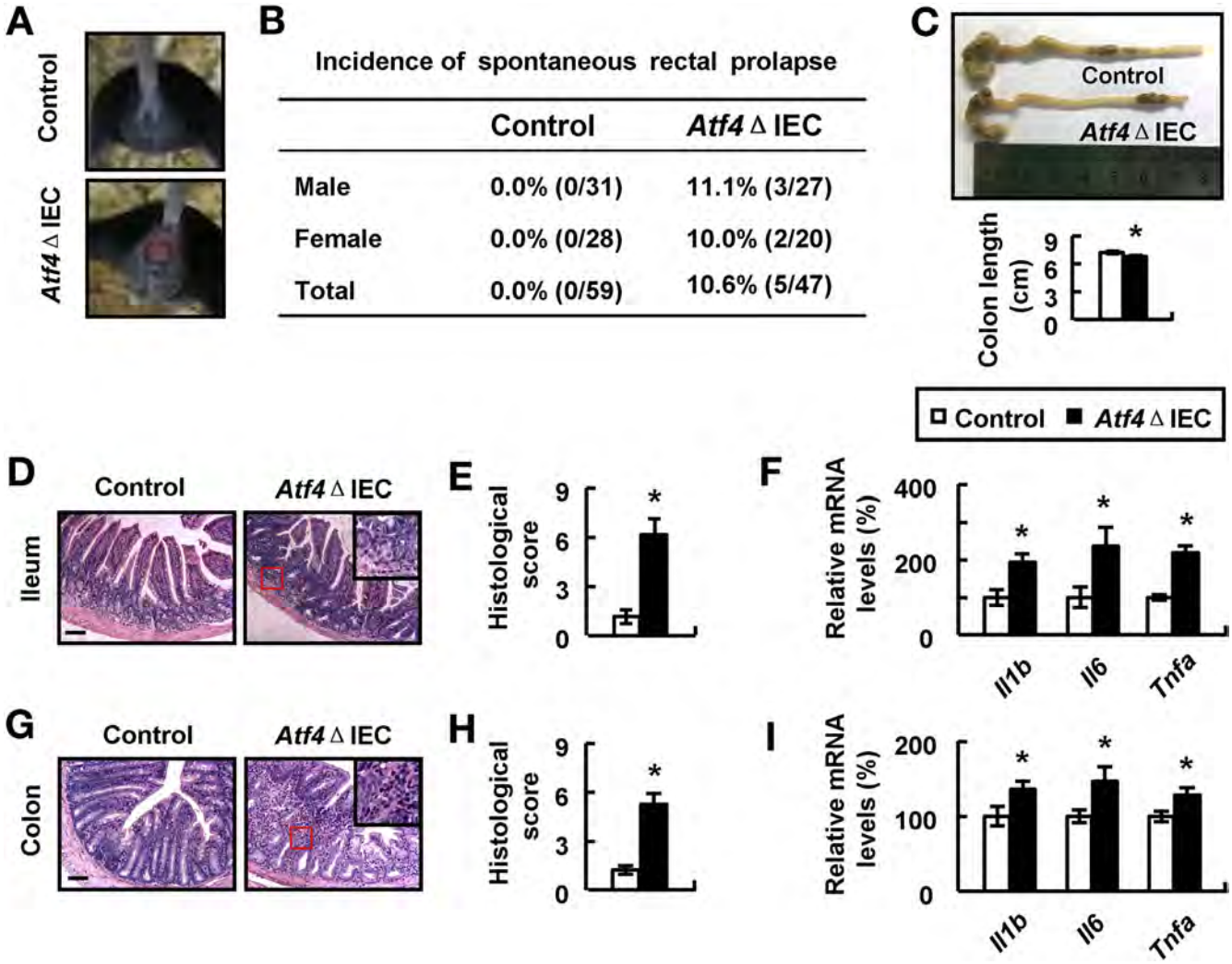

Figure 2. *Atf4* deletion in IECs causes spontaneous enterocolitis. (*A*) Visible rectal prolapse in male *Atf4*ΔIEC mice (12 weeks old). (*B*) The incidence of spontaneous rectal prolapse was determined in male and female mice (8–16 weeks). (*C*) Colon length. (*D*) H&E-stained ileal sections from 8-week-old *Atf4*ΔIEC and control mice. Scale bar, 50 μm. (*E*) Histologic scores. (*F*) qRT-PCR analysis of mRNA expression of inflammatory cytokines (*Il1b*, *Il6*, and *Tnfa*) in distal ileal tissues. (*G*) H&E-stained of colonic sections from 8-week-old *Atf4*ΔIEC and control mice. (*H*) Histologic scores. (*I*) qRT-PCR analysis of mRNA expression of inflammatory cytokines in distal colonic tissues. Values are expressed as mean ± SEM (n = 6 mice per group). *$P < .05$. Data are representative of 3 independent experiments.

(Supplementary Figure 8*D* and *E*). Notably, numbers of Paneth cell secretory granules were markedly reduced in the crypts of *Atf4*ΔIEC mice, as indicated by the decreased cryptdins observed by H&E staining (*arrows* in Figure 3*A* and Supplementary Figure 9*A*) and rudimentary electron-dense granules visualized by electron microscopy (*arrows* in Figure 3*B*). Consistent with a reduction in Paneth cell granule numbers, the expression of transcripts encoding antimicrobial peptides, including cryptdin (*Defa1* [defensin α]), *Defa4*, *Defa5*, cathelicidin (*Camp*), and lysozyme (*Lyz1*), was substantially diminished in the ileal epithelia of *Atf4*-ΔIEC mice compared with controls (Figure 3*C* and Supplementary Figure 9*B*).

Subsequently, we conducted a time course experiment to explore whether the Paneth or goblet cell alterations were present before the emergence of intestinal inflammation. To this end, we examined the intestinal histology of the ileum and colon and the expression of Paneth and goblet cell markers in ileal epithelia from 1-, 3-, and 5-week-old *Atf4*ΔIEC and control mice. The results showed that expression of ileal Paneth cell markers, such as *Defa1* and *Defa5*, was significantly increased in 5-week-old control mice vs 3-week-old control mice and was significantly compromised in *Atf4*ΔIEC mice (Figure 3*H*). However, the expression of goblet cell *Muc2* had no significant difference between *Atf4*ΔIEC and control mice at 5 weeks old (Figure 3*I*). Moreover, we observed that development of serious ileal and colonic inflammation in *Atf4*ΔIEC mice occurred at 5 weeks old (Figure 3–*G*). Taken together, these results suggest that impairment of ileal Paneth cell function in *Atf4*ΔIEC mice may play a key role in promoting intestinal inflammation.

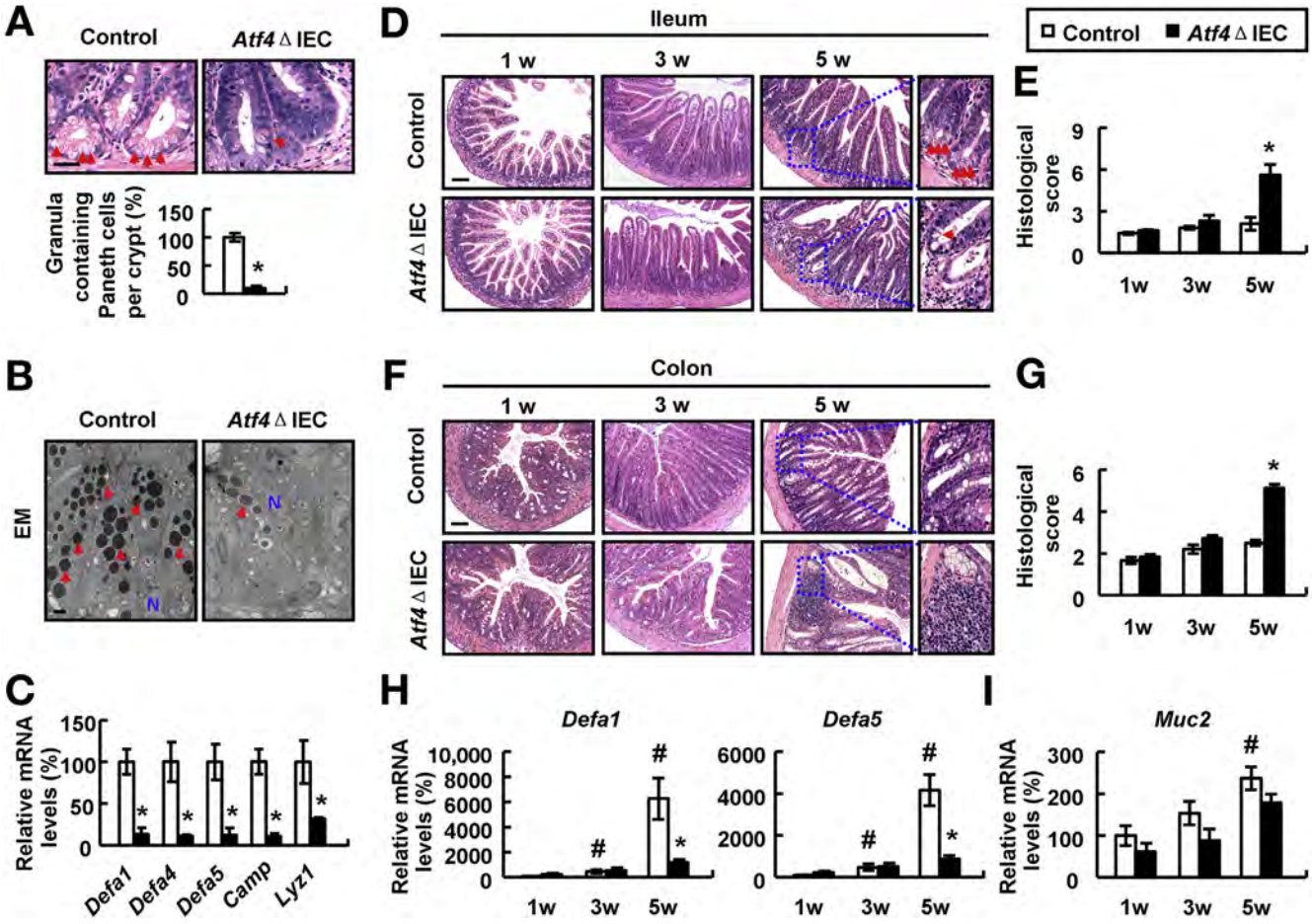

Figure 3. *Atf4* deletion in IECs compromises ileal Paneth cell function. (*A*) Paneth cells with typical eosinophilic granules (*red arrows*) on H&E-stained sections at the base of crypts of 8-week-old control mice but not *Atf4*ΔIEC mice. Scale bar, 20 μm. Quantification of granula containing Paneth cells per crypt is shown in the lower graphs. (*B*) Electron microscopy analysis of Paneth cells. Red arrows indicate rudimentary electron-dense granules. Scale bar, 2 μm. (*C*) qRT-PCR analysis of mRNA expression of antimicrobial peptides cryptdin (*Defa1*), *Defa4*, *Defa5*, cathelicidin (*Camp*), and lysozyme (*Lyz1*) in ileal epithelia. Values are expressed as mean ± SEM (n = 6 mice per group). $^{*}P < .05$. (*D*, *F*) H&E-stained ileal and colonic sections from 1-, 3-, 5-week-old *Atf4*ΔIEC and control mice, respectively. The boxed areas are shown enlarged in the right panels. Red arrows indicate secretory granules by Paneth cells. Scale bar, 50 μm. (*E*, *G*) Histologic scores. (*H*, *I*) qRT-PCR analysis of mRNA expression of (*H*) Paneth cell markers *Defa1* and *Defa5* and (*I*) goblet cell marker *Muc2* in ileal epithelia, respectively. Values were expressed as mean ± SEM (n = 5–6 mice per group). $^{*}P < .05$ vs control mice, $^{\#}P < .05$ vs 1-week-old mice. EM, electron microscopy; w, week.

Treatment of Recombinant DEFA1 Alleviates Spontaneous Enteritis and DSS-Induced Colitis

Emerging evidence has indicated that Paneth cells are a site of origin for intestinal inflammation.[38] To test whether the compromise of antimicrobial peptide production by Paneth cells was underlying the phenotype present in these animals, we studied the effect of a recombinant form of DEFA1, a major α-defensin secreted by Paneth cells in mice,[39,40] on gut inflammation in *Atf4*ΔIEC mice. *Escherichia coli* was significantly decreased and *Lactobacillus* species were increased in the ileum of *Atf4*ΔIEC mice by DEFA1 treatment (Supplementary Figure 10*A* and *B*). Furthermore, signs of spontaneous enteritis, but not colitis, were markedly ameliorated, as evidenced by decreased epithelial damage and lymphocyte infiltration, as well as mRNA expression of inflammatory cytokines (Supplementary Figure 10*C*–*H*). Moreover, DSS-induced colitis in *Atf4*ΔIEC mice was also obviously improved by recombinant DEFA1 treatment (Supplementary Figure 11*A*–*F*). These data show that gut inflammation caused by IEC-specific *Atf4* deletion may be ascribable to the compromised antimicrobial peptide production.

Gln Supplementation Rescues IEC-Specific Atf4 Deletion-Induced Ileal Paneth Cell Antimicrobial Peptide Expression and Intestinal Inflammation

The small intestinal Paneth cells arise from pluripotent intestinal stem cells.[10] We found that proliferation marker Ki-67 staining and the genes involved in intestinal stem cell differentiation in the small intestine were unaffected in *Atf4*ΔIEC mice, suggesting that the decrease of antimicrobial

peptide production was not due to impairment of Paneth cell proliferation and differentiation (Supplementary Figure 12*A* and *B*).

Because amino acids play a key role in regulating antimicrobial peptide production and ATF4 is involved in amino acid metabolism,[16,20,25] we sought to investigate the potential role of amino acids in mucosal inflammation in *Atf4*ΔIEC mice, presumably via regulating antimicrobial peptide expression. For this purpose, we measured serum amino acids in *Atf4*ΔIEC and control mice, showing a decrease of serum Gln, tryptophan, and histidine but an increase of methionine in *Atf4*ΔIEC mice compared with controls. No differences in other amino acids tested (e.g., glycine, alanine, serine, and leucine) were evident (Supplementary Figure 13*A*).

The decreased levels of serum Gln in *Atf4*ΔIEC mice were of particular interest to us, because Gln has been known to reduce gut inflammation in animal models of IBD.[21–23] We found that *Defa1* expression was stimulated by Gln addition and inhibited by Gln deprivation in confluent IEC-18 cells and primary IECs (Supplementary Figure 13*B* and *C*). Control mice fed with a Gln-free diet also resulted in a significant down-regulation of antimicrobial peptides, including *Defa1*, *Defa4*, *Defa5*, *Camp*, and *Lyz1* (Supplementary Figure 13*D*). To test whether decreased levels of Gln contributed to the development of mucosal inflammation in *Atf4*ΔIEC mice, we provided Ala-Gln dipeptide or Gln in drinking water or control vehicle in such mice. Supplementation of both Ala-Gln and Gln restored Gln levels in sera and ileal epithelia (Figure 4*A* and

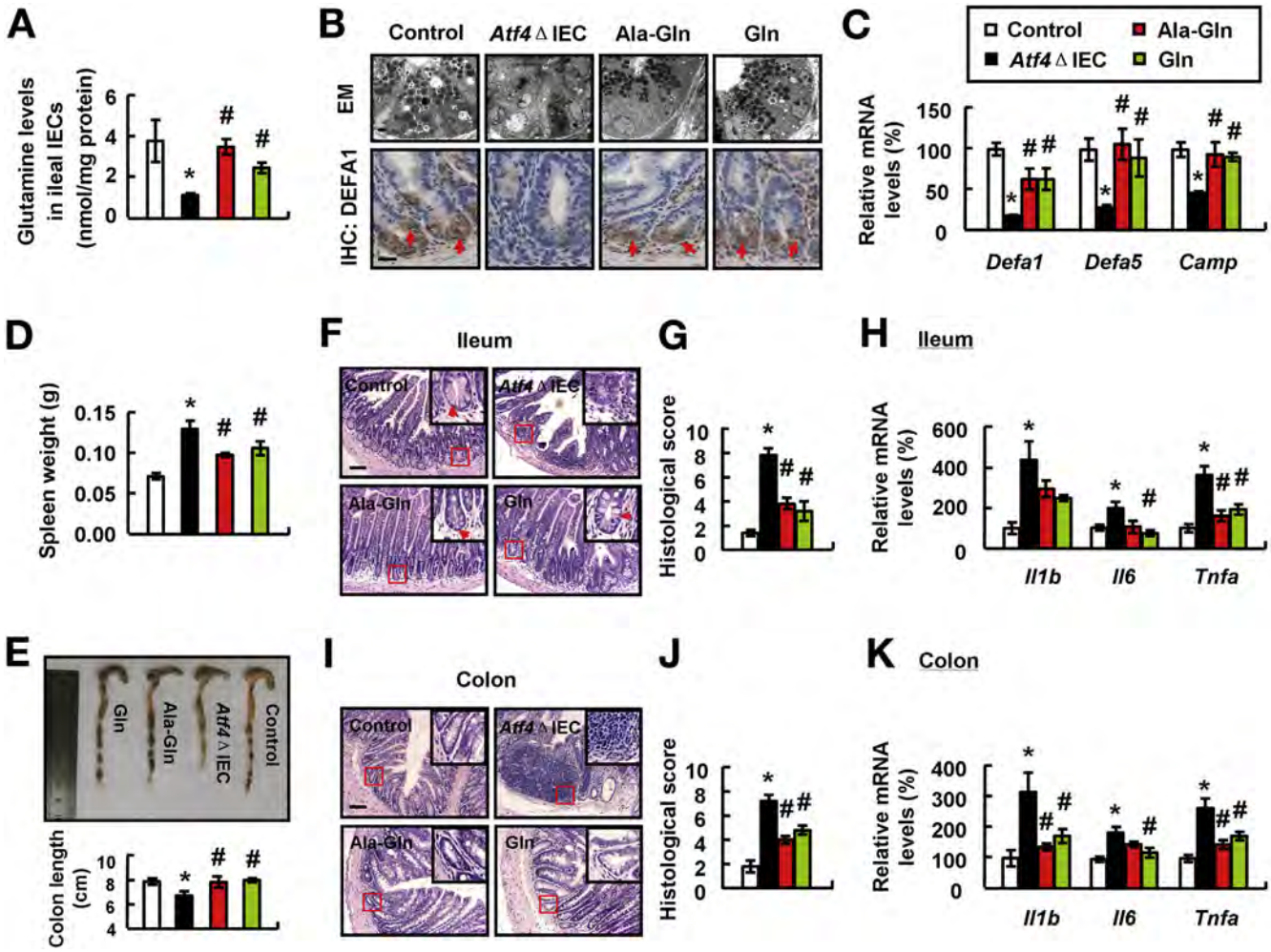

Figure 4. Gln supplementation rescues IEC-specific *Atf4* deletion-induced ileal Paneth cell antimicrobial peptide expression and intestinal inflammation. Control mice received water (Control), and *Atf4*ΔIEC mice received water (*Atf4*ΔIEC) or 2% Ala-Gln dipeptide (Ala-Gln) or 2% Gln in drinking water for 30 days. (*A*) Gln levels of ileal epithelial cells. (*B*) Electron microscopy analysis of Paneth cell granules (scale bar, 2 μm) and IHC analysis of DEFA1 expression (scale bar, 50 μm). Red arrows indicate DEFA1-positive cells. (*C*) qRT-PCR analysis of mRNA expression of Paneth cell antimicrobial peptides in ileal epithelia. (*D*) Spleen weight. (*E*) Colon length. (*F*) H&E-stained of ileal sections. The boxed areas are shown enlarged. Red arrows indicate secretory granules by Paneth cells. Scale bar, 50 μm. (*G*) Histologic scores. (*H*) qRT-PCR analysis of mRNA expression of inflammatory cytokines in distal ileal tissues. (*I*) H&E-stained of colonic sections. The boxed areas are shown enlarged. Scale bar, 50 μm. (*J*) Histologic scores. (K) qRT-PCR analysis of mRNA expression of inflammatory cytokines in distal colonic tissues. Values are expressed as mean ± SEM (n = 5–6 mice per group). $^{*}P < .05$ vs control mice, $^{\#}P < .05$ vs the *Atf4*ΔIEC group. Data are representative of 3 independent experiments. EM, electron microscopy.

Supplementary Figure 14*A*), rescued Paneth cell antimicrobial peptide expression (Figure 4*B* and *C*), and ameliorated the spontaneous enterocolitis (Figure 4*D*–*K*). Furthermore, Ala-Gln supplementation increased *Defa1* expression and secretion in primary IECs isolated from the ileum of *Atf4*ΔIEC mice (Supplementary Figure 14*B* and *C*). To explore whether the rescue effect of Gln on intestinal inflammation in *Atf4*ΔIEC mice was dependent on modulating antimicrobial peptide expression, we injected *Atf4*-ΔIEC mice with or without DEFA1 antibody in the presence of Gln and found that DEFA1 antibody significantly blocked the rescue effect of Gln on the expression of inflammatory cytokines in ileal tissues of *Atf4*ΔIEC mice (Supplementary Figure 14*D*). These results suggest that changes in Paneth cell function certainly contribute to the reversal effect of Gln on IBD in *Atf4*ΔIEC mice.

Because inflammatory responses, including mononuclear infiltration and T helper (Th) type 17 cell activation, are involved in the pathogenesis of IBD,[3,36] we next examined relevant changing patterns and found that Ala-Gln or Gln administration also inhibited mononuclear cell infiltration and Th17 cell differentiation (Supplementary Figure 15*A*–*D*). We also investigated the effect of Gln on the inflammatory responses in vitro. As expected, overexpression of ATF4 blocked lipopolysaccharide (LPS)-induced up-regulation of inflammatory cytokines, including *Il1b* and *Il6*, compared with controls (Supplementary Figure 16*A* and *B*). On the contrary, Gln deprivation reversed the inhibitory effects of ATF4 overexpression on LPS-induced increases in inflammatory cytokine expression (Supplementary Figure 16*C*).

Gln Supplementation Rescues the Composition of Gut Microbiota

Accumulating evidence has shown that antimicrobial peptides are able to shape the gut microbiota, prevent them from direct contact with the epithelium, and induce intestinal inflammation.[10] We used 16S ribosomal DNA sequencing and clarified the ileal microbial diversity and composition of *Atf4*ΔIEC mice, Gln-treated *Atf4*ΔIEC mice, and control littermates. Rarefaction and Shannon index analysis showed that the sequencing depth covered rare new phylotypes and most of the diversity (Supplementary Figure 17*A*). Nonmetric multidimensional scaling analysis on the basis of microbial composition showed major differences between *Atf4*ΔIEC and control mice (Figure 5*A*), although there was no significant difference in the diversity of the gut microbiota in these groups (Supplementary Figure 17*B*). Operational taxonomic unit (OTU) profiling and unweighted UniFrac distance-based principal coordinate analysis showed a statistically significant difference in microbiota between control and *Atf4*ΔIEC mice, whereas the gut microbiota of *Atf4*ΔIEC mice receiving Gln treatment was similar to that of control mice (Figure 5*B* and Supplementary Figure 17*C*). Detailed analysis of the top 40 bacterial taxa showed that Gln treatment significantly increased the abundance of *Lactobacillus* and *Faecalibaculum* species but markedly decreased the pathogenic bacteria *Escherichia/Shigella* and *Enterococcus* species in *Atf4*ΔIEC mice (Figure 5*C* and *D*). Collectively, these results strongly suggest that the deletion of *Atf4* in IECs has a profound influence on gut microbiota composition and that Gln treatment could reverse this alteration.

Atf4 Deletion in IECs Decreases Gln Uptake by the Gut Epithelia by Directly Suppressing Slc1a5 Expression

Several transporters, including SLC1A5, SLC6A19, SLC6A14, SLC3A2, and SLC38A2, are known to transport Gln in gut epithelia.[41] However, we found that only SLC1A5 was markedly reduced in the ileal epithelia of *Atf4*ΔIEC mice compared with controls, with no changes in other Gln transporters (Figure 6*A*). Together with the observation that knockdown and overexpression of ATF4 suppressed and promoted *Slc1a5* expression, respectively, in IEC-18 cells (Figure 6*B*–*E*), these findings indicate an essential role of ATF4 in maintaining basal levels of intestinal *Slc1a5* expression.

Previous studies have shown that *SLC1A5* expression is regulated by ATF4 in tumor cells,[42,43] and our analysis of the chromatin immunoprecipitation sequencing data set (Gene Expression Omnibus: GSE28332) also showed that ATF4 binds to the promoter of murine *Slc1a5*.[44] To examine whether ATF4 directly regulates *Slc1a5* transcription and clarify the region responsible for transactivation by ATF4 through promoter activity experiments, we generated a series of constructs containing fragments of the human *SLC1A5* promoter, each of which was then co-transfected into 293T cells with ATF4 overexpression plasmids. As shown in Figure 6*F*, overexpression of ATF4 significantly enhanced P3 and P4 promoter activity in reporter assays, whereas no activity was observed when promoters P1 and P2 were used. Our bioinformatics analysis further identified a conserved amino acid response element at the ATF4 binding site in the human, mouse, and rat *SLC1A5* promoters (Supplementary Figure 18*A* and *B*). When this amino acid response element was mutated or deleted in promoter P3, transactivation of promoter activity by ATF4 was entirely absent (Figure 6*G*). Thus, these results indicate that ATF4 plays a critical role in *SLC1A5* transactivation.

We subsequently investigated the regulatory role of SLC1A5 in antimicrobial peptide expression and inflammatory response in vitro. As shown in Figure 6*H*, uptake of Gln into IEC-18 cells was decreased by *Atf4* knockdown but increased by overexpression of SLC1A5. Moreover, overexpression of SLC1A5 could restore antimicrobial peptide expression in primary *Atf4*-knockout IECs and suppress *Atf4* knockdown-induced up-regulation of inflammatory cytokines such as *Il6* and *Tnfa* (Figure 6*I* and Supplementary Figure 18*C*).

SLC1A5 Expression Is Decreased in Inflamed Intestinal Mucosa From Patients With Active IBD

We next assessed SLC1A5 expression in intestinal mucosa from IBD patients and control individuals. Consistent with decreased ATF4 expression, as described, *SLC1A5*

expression was found to be lower in the inflamed colon mucosa of patients with active colonic CD or UC than in normal mucosa from control individuals (Figure 7*A*). IHC staining further confirmed that the number of SLC1A5-positive cells was significantly decreased in inflamed colon mucosa from active colonic CD and UC patients compared with control individuals (Figure 7*B*). SLC1A5 expression was also found to be lower in inflamed than in uninflamed colon mucosa from the same IBD patients (Figure 7*C*). Similar results were also obtained in the ileum mucosa from ileal CD patients (Supplementary Figure 19*A* and *B*). Moreover, both ATF4 and SLC1A5 protein expression levels were significantly decreased in inflamed ileum mucosa compared with those in uninflamed ileum mucosa from the same active ileal CD patients (Figure 7*D*). We observed that the levels of IEC-derived *SLC1A5* positively correlated with *ATF4* expression among IBD patients (Figure 7*E*).

Discussion

Genome-wide association studies by genetics consortia have identified numerous variants associated with IBD.[45–47]

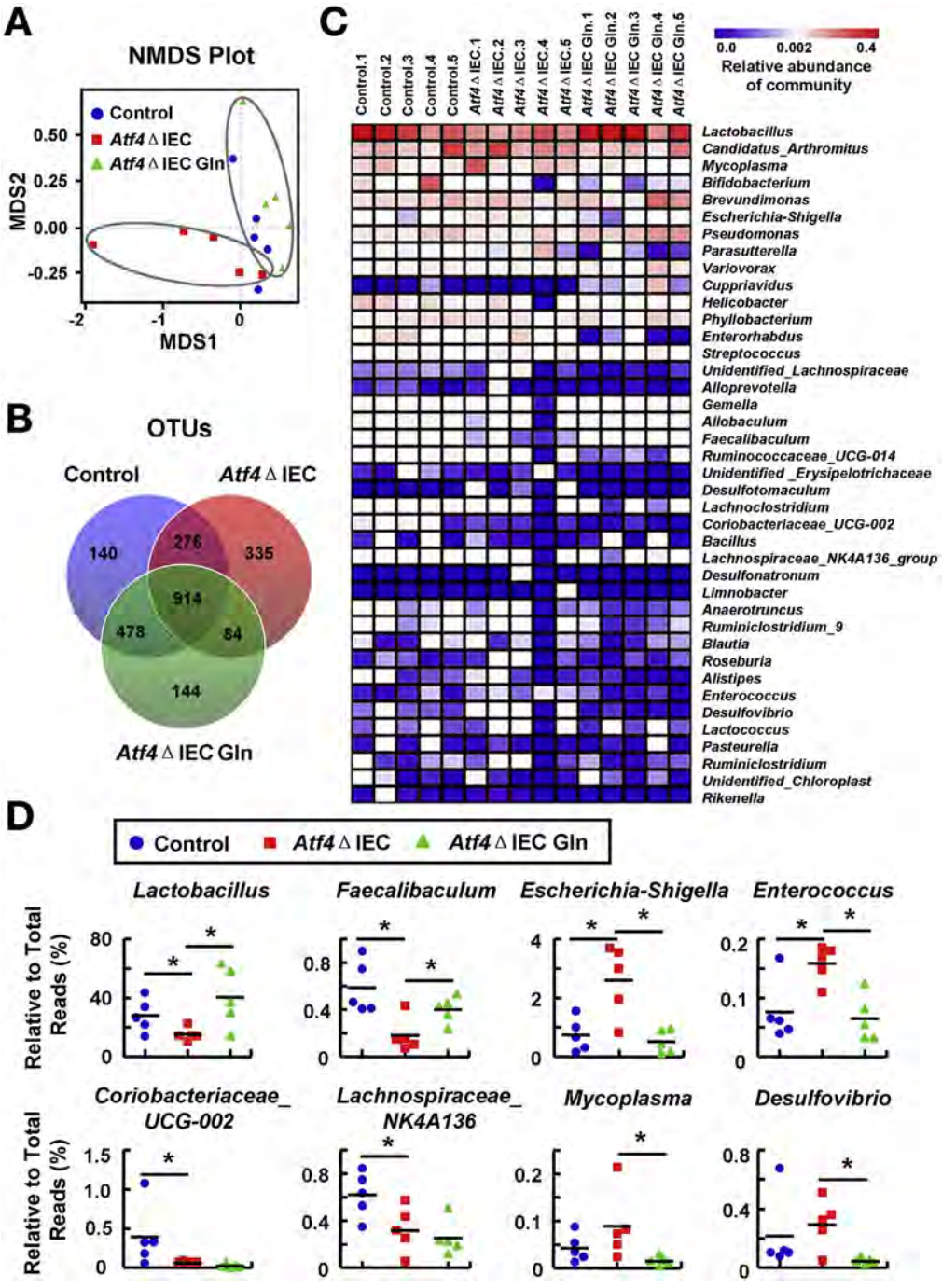

Figure 5. Gln supplementation rescues the composition of gut microbiota. (*A*) Nonmetric multidimensional scaling analysis on OTUs (n = 5 mice per group). Each symbol represents a single sample (from 1 mouse) and is colored according to group. (*B*) The Venn diagram shows that OTUs were overlapped in each group. (*C*) The heat map shows an abundance of the top 40 bacterial taxa. (*D*) The bacterial genera with significant changes in each group are shown. **P* < .05, by nonparametric Mann–Whitney *U* test. n = 5 mice per group. NMDS, nonmetric multidimensional scaling.

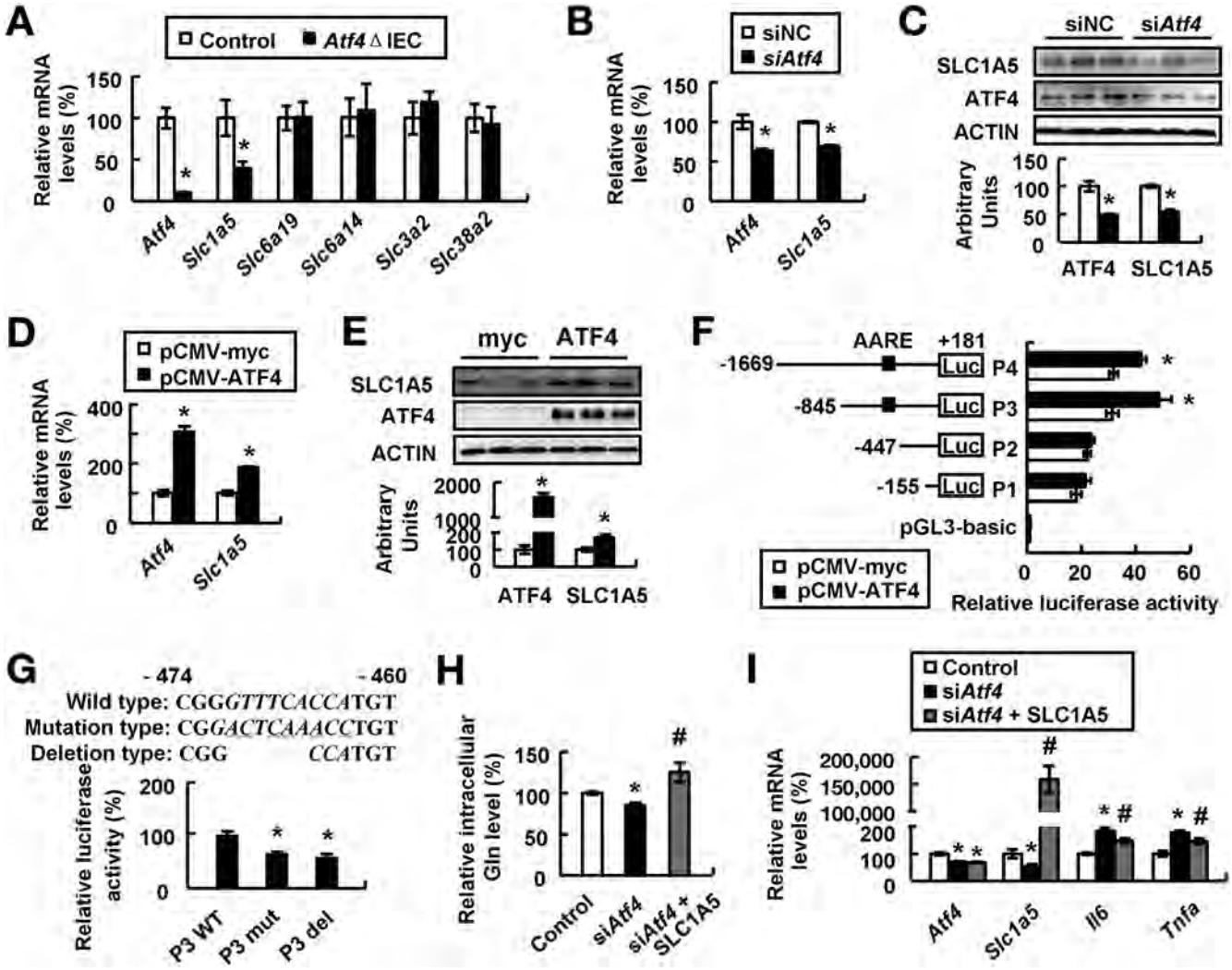

Figure 6. *Atf4* deletion in IECs decreases the Gln uptake by the gut epithelia through directly suppressing *Slc1a5* expression. (*A*) Ileal epithelia from *Atf4*ΔIEC and control mice were analyzed for mRNA expression of *Slc1a5*, *Slc6a19*, *Slc6a4*, *Slc3a2*, and *Slc38a2*, respectively. Values are expressed as mean ± SEM (n = 6 mice per group). **P* < .05. (*B–E*) Knockdown of ATF4 in IEC-18 cells represses *Slc1a5* mRNA and protein expression, and overexpression of ATF4 enhances *Slc1a5* mRNA and protein expression by qRT-PCR and Western blot analysis, respectively. (*F*) The truncated *SLC1A5* promoter reporters (P1–P4) and pRL-*Renilla* as a transfection control were co-transfected with pCMV-ATF4 or empty vector (pCMV-myc) into 293T cells, and the promoter activity was determined as the ratio of firefly/*Renilla* luciferase activities. Results are expressed relative to the activity of cells transfected with the empty pGL3-basic vector. (*G*) Relative promoter activity of the WT of P3 (P3 WT) promoter and the mutated (P3 mut) and deleted (P3 del) constructs. The dual-luciferase activity was measured after 48 hours of transfection. Values are expressed as mean ± SEM (n = 3). **P* < .05 vs P3 WT group. (*H*, *I*) The relative intracellular Gln levels and mRNA expression of inflammatory cytokines were determined in IEC-18 cells after transfection with control siRNA or *Atf4* siRNA or co-transfected with *Atf4* siRNA and *Slc1a5* expression vectors, respectively. Values are expressed as mean ± SEM (n = 3). **P* < .05. Data are representative of 3 independent experiments. Luc, luciferase; myc, c-myc gene product; pCMV, Cytomegalovirus promoter; si, small interfering.

To date, more than 200 IBD risk loci have been identified, implicating the innate immune response, adaptive immunity, autophagy, ER stress, intestinal epithelial barrier function, and antimicrobial defense pathways in this condition.[47] The detailed etiology of IBD, however, remains to be described. In the present study, we showed that ATF4 expression was markedly decreased in inflamed intestinal mucosa from patients with active IBD and that IEC-specific deletion of *Atf4* caused spontaneous enterocolitis and increased susceptibility to DSS-induced colitis in mice. Our findings suggest that ATF4 exerts critical effects on maintaining the intestinal epithelial homeostasis.

We found that Paneth cell function, especially antimicrobial peptide expression, was strikingly impaired in the *Atf4*ΔIEC mice. Based on the recombinant DEFA1 reverse experiments, we speculated that the role of ATF4 in regulating antimicrobial peptide expression might underlie the observed phenotype. We found that secretory granules from Paneth cells and antimicrobial peptide expression were increased in *Atf4*ΔIEC mice receiving Ala-Gln or Gln

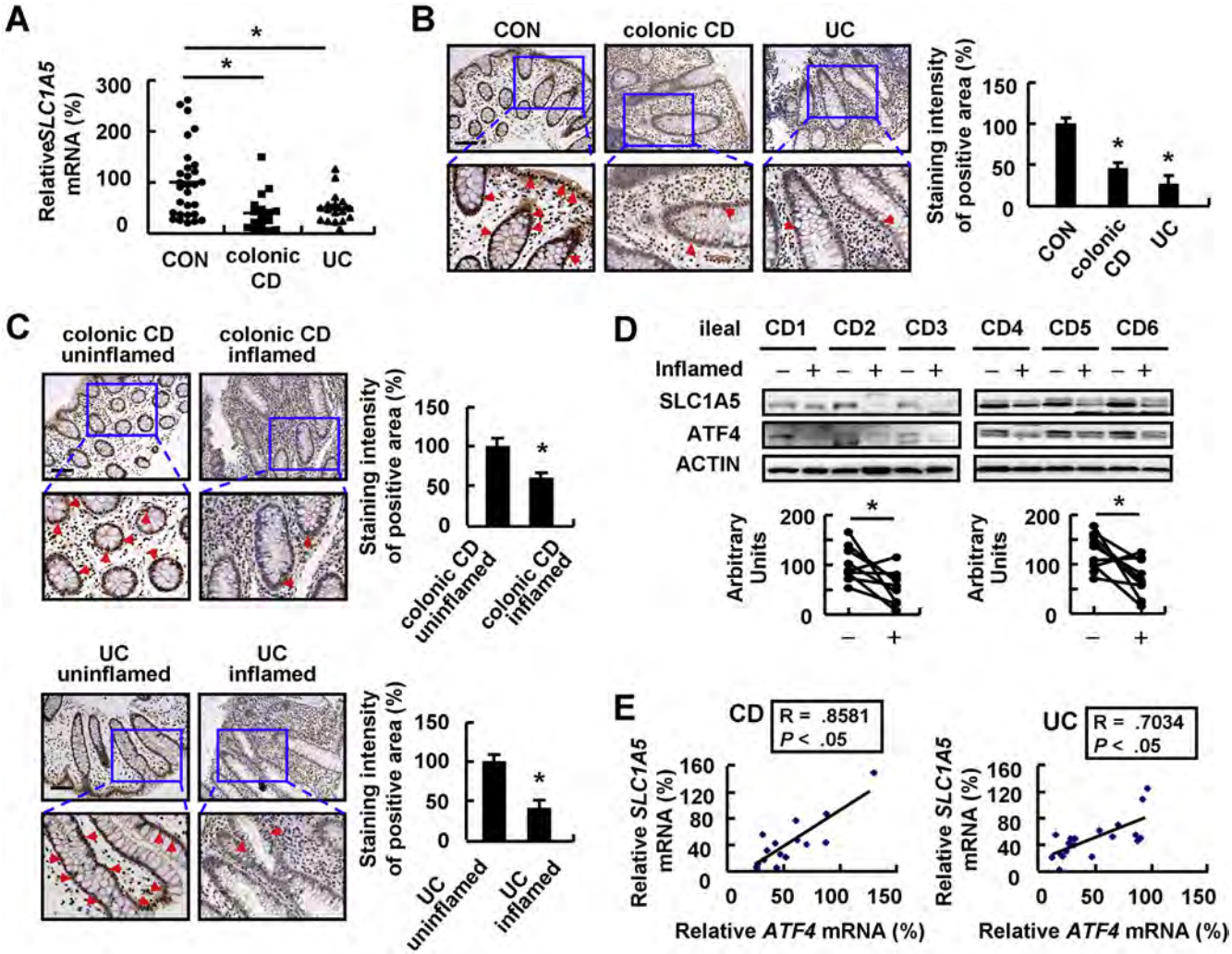

Figure 7. SLC1A5 expression is decreased in inflamed intestinal mucosa from active IBD patients. (*A*) Colonic biopsy samples, as described in Figure 1, and *SLC1A5* expression were analyzed by qRT-PCR. *$P < .05$ vs CON. (*B*) Representative intestinal sections were prepared from the colon mucosa of control individuals and the inflamed mucosa of active colonic CD and UC patients and were stained with anti-SLC1A5 monoclonal antibody by IHC. (*C*) SLC1A5 protein expression in uninflamed and inflamed intestinal mucosa from the same active colonic CD and UC patients by IHC. The boxed areas are shown enlarged in the lower panels. The red arrows indicate SLC1A5-positive cells. Scale bar, 50 μm. Staining intensity of the positive area was quantified by ImageJ software (National Institutes of Health) and are illustrated as bar charts (n = 3 patients per group). *$P <$ .05. (*D*) Western blot analysis of ATF4 and SLC1A5 expression levels in uninflamed and inflamed ileal mucosa from the same active ileal CD patients. –, uninflamed; +, inflamed. n = 9 patients per group. *$P < .05$. (*E*) A correlation analysis was performed between the relative levels of *SLC1A5* and *ATF4* mRNA in intestinal mucosal biopsy samples of active CD patients (n = 17) and active UC patients (n = 21) (Spearman rank correlation coefficient: A-CD, $R = 0.8664$, $P < .05$; A-UC: $R = 0.8543$, $P < .05$). CON, control.

supplementation and that in *Atf4*ΔIEC mice, Gln treatment rescued the alteration of gut microbiota, suggesting that epithelial Paneth cell dysfunction and intestinal inflammation may ensue from IEC-specific *Atf4* deletion through decreased intestinal Gln levels.

Previous studies have shown that SLC1A5 acts as a high-affinity, sodium-dependent Gln transporter[48] and that its inhibition attenuates cell growth and mTOR (mammalian target of rapamycin) signaling.[49] Moreover, SLC1A5 is important in the growth of cancer cells, including those of lung, prostate, and breast malignancies.[50–52] However, its role in regulating epithelial cell function remains unclear. In the current study, SLC1A5 was observed to be associated with reduced Gln uptake in *Atf4*ΔIEC mice. SLC1A5 expression was positively regulated by ATF4 in vivo and in vitro, and ATF4 directly regulated transcription of *Slc1a5* by binding to its promoter. Additionally, we also found that overexpression of SLC1A5 restored antimicrobial peptide expression and inhibited *Atf4* knockdown-induced up-regulation of inflammatory cytokines in vitro. SLC1A5 expression was also decreased in inflamed mucosa from patients with active CD or UC compared with normal mucosa from control individuals. IEC-derived *SLC1A5* was found to positively correlate with *ATF4* expression in patients with active IBD. Therefore, *SLC1A5* may be directly targeted by ATF4 to regulate Gln uptake and inflammation in the intestine.

Previous studies have shown that epithelial autophagy,[53,54] unfolded protein response pathway defects, and ER stress[4,55] may contribute to the pathogenesis of IBD and that ATF4 is critical in the response to autophagy[28] and unfolded protein response pathway/ER stress.[44] In this study, we found that expression levels of most autophagy-related genes and proteins were not significantly changed in the ileal and colonic epithelia of *Atf4*ΔIEC vs control mice at any age examined (Supplementary Figure 20*A*–*D*). By contrast, ER stress markers p-EIF2A and CHOP (C/EBP homologous protein) were increased in ileal and colonic epithelia of *Atf4*ΔIEC mice at 5 weeks of age (Supplementary Figure 21*A*–*D*), and ER stress inhibitor tauroursodeoxycholic acid treatment led to moderate recovery of the spontaneous enterocolitis compared with vehicle treatment (Supplementary Figure 22*A*–*G*). These results suggest that ER stress, but not autophagy, may contribute to the development of IBD in these mice, which needs to be investigated in the future.

A previous study reported that activation of the EIF2AK4-EIF2A-ATF4 pathway serves as a host defense mechanism to protect against intestinal inflammation.[28] Using a human colonic epithelial cell line, our studies also showed that overexpressing ATF4 protected against LPS-induced inflammatory response (Supplementary Figure 16*B*). Furthermore, many studies report that Gln plays a role in attenuating enterocyte apoptosis and protecting colonic barrier function.[56,57] Our studies indicate that ATF4 and Gln might also have an important role in modulating the functions of absorptive enterocytes and colonocytes. Moreover, we found that Ala-Gln or Gln supplementation significantly inhibited mononuclear infiltration and Th17 cell differentiation, suggesting that Gln may improve the enterocolitis of *Atf4*ΔIEC mice via regulating immune homeostasis. These Paneth cell-independent regulatory mechanisms cannot be ruled out.

Genetic mutation of some of genes, such as *ATG16L1* or *NOD2*, is implicated in human IBD,[58,59] whereas mice with these mutations do not develop spontaneous enterocolitis.[12,53] By contrast, IEC-specific *Atf4* deletion induces spontaneous enterocolitis, and we speculate that genetic variation of *ATF4* may be a risk factor for human IBD. Although the correlation between ATF4 expression and the pathogenesis of IBD was observed in Asian patients, its relevance to other ethnic groups must be verified in the future, because IBD-related genetic variants of some genes are not always applicable to all ethnicities.[46,60] Up-regulation of colonic α-defensins has been reported in both CD and UC patients.[61,62] In our study, deletion of *Atf4* reduced ileal but not colonic α-defensin expression in mice without DSS treatment, suggesting that disruption of *Atf4* expression most likely is linked to the ileal form of CD, although *Defa5* expression is relatively suppressed in the colonic epithelia of *Atf4*ΔIEC mice after DSS treatment (Supplementary Figure 23*A* and *B*).

In summary, we discovered that ATF4 expression was decreased in inflamed intestinal mucosa from patients with active IBD and that IEC-specific *Atf4* deletion induces spontaneous enterocolitis in a mouse model. ATF4 deficiency in intestinal epithelia decreases Gln uptake and antimicrobial peptide expression by diminishing *SLC1A5* transcription. Thus, ATF4 plays a critical role in maintaining intestinal homeostasis, and therapy targeting this transcription factor may provide a novel strategy for the treatment of IBD.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, https://doi.org/10.1053/j.gastro.2018.11.033.

References

1. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012;142:46–54.
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427–434.
3. **He C**, **Shi Y**, **Wu R**, et al. miR-301a promotes intestinal mucosal inflammation through induction of IL-17A and TNF-α in IBD. Gut 2016;65:1938–1950.
4. **Kaser A**, **Lee AH**, Franke A, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell 2008;134:743–756.
5. Cader MZ, Kaser A. Recent advances in inflammatory bowel disease: mucosal immune cells in intestinal inflammation. Gut 2013;62:1653–1664.
6. Stappenbeck TS, McGovern DPB. Paneth cell alterations in the development and phenotype of Crohn's disease. Gastroenterology 2017;152:322–326.
7. **Cabrera S**, **Fernández AF**, **Mariño G**, et al. ATG4B/autophagin-1 regulates intestinal homeostasis and protects mice from experimental colitis. Autophagy 2013;9:1188–1200.
8. **Imhann F**, **Vila A**, Bonder M, et al. Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. Gut 2017;67:108–119.
9. Zhang K, Hornef MW, Dupont A. The intestinal epithelium as guardian of gut barrier integrity. Cell Microbiol 2015;17:1561–1569.
10. Bevins CL, Salzman NH. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat Rev Microbiol 2011;9:356–368.
11. **Cadwell K**, **Stappenbeck TS**, **Virgin HW**, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature 2008;456:259–263.
12. **Kobayashi KS**, **Nuñez G**, **Flavell RA**, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005;307:731–734.
13. Wehkamp J, Wang G, Kübler I, et al. The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. J Immunol 2007;179:3109–3118.
14. Wehkamp J, Salzman NH, Porter E, et al. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci USA 2005;102:18129–18134.

15. Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids 2009;37:1–17.
16. **Hashimoto T**, **Perlot T**, Rehman A, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature 2012;487:477–481.
17. Lamas B, Richard ML, Leducq V, et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. Nat Med 2016;22:598–605.
18. **Nikolaus S**, **Schulte B**, Al-Massad N, et al. Increased tryptophan metabolism is associated with activity of inflammatory bowel diseases. Gastroenterology 2017;153:1504–1516.
19. Newsholme P, Curi R, Pithon Curi TC, et al. Glutamine metabolism by lymphocytes, macrophages, and neutrophils: its importance in health and disease. J Nutr Biochem 1999;10:316–324.
20. **Ren W**, **Duan J**, Yin J, et al. Dietary L-glutamine supplementation modulates microbial community and activates innate immunity in the mouse intestine. Amino Acids 2014;46:2403–2413.
21. Vicario M, Amat C, Rivero M, et al. Dietary glutamine affects mucosal functions in rats with mild DSS-induced colitis. J Nutr 2007;137:1931–1937.
22. Hsiung YC, Liu JJ, Hou YC, et al. Effects of dietary glutamine on the homeostasis of $CD4^+$ T cells in mice with dextran sulfate sodium-induced acute colitis. PLoS One 2014;9:e84410.
23. **Jeong SY**, **Im YN**, Youm JY, et al. L-glutamine attenuates DSS-induced colitis via induction of MAPK phosphatase-1. Nutrients 2018;10:e288.
24. Iwasaki Y, Suganami T, Hachiya R, et al. Activating transcription factor 4 links metabolic stress to interleukin-6 expression in macrophages. Diabetes 2014;63:152–161.
25. **Zhao E**, **Ding J**, Xia Y, et al. KDM4C and ATF4 cooperate in transcriptional control of amino acid metabolism. Cell Rep 2016;14:506–519.
26. Wang C, Huang Z, Du Y, et al. ATF4 regulates lipid metabolism and thermogenesis. Cell Res 2010;20:174–184.
27. Bouman L, Schlierf A, Lutz AK, et al. Parkin is transcriptionally regulated by ATF4: evidence for an interconnection between mitochondrial stress and ER stress. Cell Death Differ 2011;18:769–782.
28. **Bretin A**, **Carrière J**, Dalmasso G, et al. Activation of the EIF2AK4-EIF2A/eIF2α-ATF4 pathway triggers autophagy response to Crohn disease-associated adherent-invasive Escherichia coli infection. Autophagy 2016;12:770–783.
29. **Deng J**, **Yuan F**, Guo Y, et al. Deletion of ATF4 in AgRP neurons promotes fat loss mainly via increasing energy expenditure. Diabetes 2017;66:640–650.
30. Xiao Y, Deng Y, Yuan F, et al. ATF4/ATG5 signaling in hypothalamic proopiomelanocortin neurons regulates fat mass via affecting energy expenditure. Diabetes 2017;66:1146–1158.
31. **Li K**, **Xiao Y**, Yu J, et al. Liver-specific gene inactivation of the transcription factor ATF4 alleviates alcoholic liver steatosis in mice. J Biol Chem 2016;291:18536–18546.
32. Sun X, Liu J, Crary JF, et al. ATF4 protects against neuronal death in cellular Parkinson's disease models by maintaining levels of parkin. J Neurosci 2013;33:2398–2407.
33. Baleriola J, Walker CA, Jean YY, et al. Axonally synthesized ATF4 transmits a neurodegenerative signal across brain regions. Cell 2014;158:1159–1172.
34. Mojtahed A, Khanna R, Sandborn WJ, et al. Assessment of histologic disease activity in Crohn's disease: a systematic review. Inflamm Bowel Dis 2014;20:2092–2103.
35. Mosli MH, Feagan BG, Zou G, et al. Development and validation of a histological index for UC. Gut 2017;66:50–58.
36. Habtezion A, Nguyen LP, Hadeiba H, et al. Leukocyte trafficking to the small intestine and colon. Gastroenterology 2016;150:340–354.
37. McGuckin MA, Lindén SK, Sutton P, et al. Mucin dynamics and enteric pathogens. Nat Rev Microbiol 2011;9:265–278.
38. **Adolph TE**, **Tomczak MF**, **Niederreiter L**, et al. Paneth cells as a site of origin for intestinal inflammation. Nature 2013;503:272–276.
39. Ouellette AJ. Paneth cells and innate immunity in the crypt microenvironment. Gastroenterology 1997;113:1779–1784.
40. Eisenhauer PB, Harwig SS, Lehrer RI. Cryptdins: antimicrobial defensins of the murine small intestine. Infect Immun 1992;60:3556–3565.
41. Scalise M, Pochini L, Galluccio M, et al. Glutamine transport. From energy supply to sensing and beyond. Biochim Biophys Acta 2016;1857:1147–1157.
42. Ren P, Yue M, Xiao D, et al. ATF4 and N-Myc coordinate glutamine metabolism in MYCN-amplified neuroblastoma cells through ASCT2 activation. J Pathol 2015;235:90–100.
43. Timosenko E, Ghadbane H, Silk JD, et al. Nutritional stress induced by tryptophan-degrading enzymes results in ATF4-dependent reprogramming of the amino acid transporter profile in tumor cells. Cancer Res 2016;76:6193–6204.
44. **Han J**, **Back SH**, Hur J, et al. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nat Cell Biol 2013;15:481–490.
45. **Anderson CA**, **Massey DC**, Barrett JC, et al. Investigation of Crohn's disease risk loci in ulcerative colitis further defines their molecular relationship. Gastroenterology 2009;136:523–529.
46. **Brant SR**, **Okou DT**, **Simpson CL**, et al. Genome-wide association study identifies African-specific susceptibility loci in African Americans with inflammatory bowel disease. Gastroenterology 2017;152:206–217.
47. **Huang H**, **Fang M**, **Jostins L**, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature 2017;547:173–178.
48. Broer S. Amino acid transport across mammalian intestinal and renal epithelia. Physiol Rev 2008;88:249–286.
49. Nakaya M, Xiao Y, Zhou X, et al. Inflammatory T cell responses rely on amino acid transporter ASCT2

facilitation of glutamine uptake and mTORC1 kinase activation. Immunity 2014;40:692–705.
50. Hassanein M, Hoeksema MD, Shiota M, et al. Slc1a5 mediates glutamine transport required for lung cancer cell growth and survival. Clin Cancer Res 2013;19:560–570.
51. Wang Q, Hardie RA, Hoy AJ, et al. Targeting ASCT2-mediated glutamine uptake blocks prostate cancer growth and tumour development. J Pathol 2015; 236:278–289.
52. van Geldermalsen M, Wang Q, Nagarajah R, et al. ASCT2/SLC1A5 controls glutamine uptake and tumour growth in triple-negative basal-like breast cancer. Oncogene 2016;35:3201–3208.
53. **Conway KL**, **Kuballa P**, Song JH, et al. Atg16l1 is required for autophagy in intestinal epithelial cells and protection of mice from *Salmonella* infection. Gastroenterology 2013;145:1347–1357.
54. **Tschurtschenthaler M**, **Adolph TE**, **Ashcroft JW**, et al. Defective ATG16L1-mediated removal of IRE1α drives Crohn's disease–like ileitis. J Exp Med 2017;214: 401–422.
55. Cao SS, Zimmermann EM, Chuang BM, et al. The unfolded protein response and chemical chaperones reduce protein misfolding and colitis in mice. Gastroenterology 2013;144:989–1000.
56. Liu N, Ma X, Luo X, et al. L-glutamine attenuates apoptosis in porcine enterocytes by regulating glutathione-related redox homeostasis. J Nutr 2018; 148:526–534.
57. Akagi R, Ohno M, Matsubara K, et al. Glutamine protects intestinal barrier function of colon epithelial cells from ethanol by modulating Hsp70 expression. Pharmacology 2013;91(1-2):104–111.
58. **Wehkamp J**, **Harder J**, Weichenthal M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. Gut 2004;53:1658–1664.
59. Salem M, Ammitzboell M, Nys K, et al. ATG16L1: a multifunctional susceptibility factor in Crohn disease. Autophagy 2015;11:585–594.
60. Feki S, Bouzid D, Abida O, et al. Genetic association and phenotypic correlation of TLR4 but not NOD2 variants with Tunisian inflammatory bowel disease. J Dig Dis 2017;18:625–633.
61. Wehkamp J, Schwind B, Herrlinger KR, et al. Innate immunity and colonic inflammation: enhanced expression of epithelial alpha-defensins. Dig Dis Sci 2002;47:1349–1355.
62. Fahlgren A, Hammarström S, Danielsson A, et al. Increased expression of antimicrobial peptides and lysozyme in colonic epithelial cells of patients with ulcerative colitis. Clin Exp Immunol 2003;131: 90–101.

Author names in bold designate shared co-first authorship.

Received March 22, 2018. Accepted November 9, 2018.

Reprint requests
Address requests for reprints to: Feifan Guo, PhD, Key Laboratory of Nutrition, Metabolism, and Food Safety, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China. e-mail: ffguo@sibs.ac.cn; fax: +86-21-54920291. Zhanju Liu, MD, PhD, Department of Gastroenterology, The Shanghai Tenth People's Hospital, Tongji University, Shanghai 200072, China. e-mail: liuzhanju88@126.com; fax: +86-21-66303983.

Acknowledgments
Author contributions: Feifan Guo and Zhanju Liu planned and supervised the experimental work and data analysis; Xiaoming Hu, Jiali Deng, Tianming Yu, Shanghai Chen, Yadong Ge, Ziheng Zhou, Yajie Guo, Feixiang Yuan, and Yuguo Niu performed all the experiments; Xiaoming Hu, Hao Ying, Qiwei Zhai, Yan Chen, and Feifan Guo analyzed the data; Tianming Yu, Yadong Ge, Weigang Shu, Huimin Chen, Caiyun Ma, and Zhanju Liu diagnosed the patients and provided clinical information and samples; and Xiaoming Hu, Zhanju Liu, and Feifan Guo wrote the manuscript. All authors discussed and revised the manuscript.

Conflicts of interest
The authors disclose no conflicts.

Funding
This work was supported by grants from National Natural Science Foundation (81325005, 81390350, 81470822, 81500622, 81600623, 81630017, 81700750, and 31830044), Basic Research Project of Shanghai Science and Technology Commission (16JC1404900 and 17XD1404200), and CAS Interdisciplinary Innovation Team. Feifan Guo was also supported by the One Hundred Talents Program of the Chinese Academy of Sciences. Xiaoming Hu was supported by Youth Innovation Promotion Association CAS.