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# The Unfolded Protein Response and Chemical Chaperones Reduce Protein Misfolding and Colitis in Mice

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## Abstract

**BACKGROUND & AIMS—**Endoplasmic reticulum (ER) stress has been associated with development of inflammatory bowel disease. We examined the effects of ER stress–induced chaperone response and the orally active chemical chaperones tauroursodeoxycholate (TUDCA) and 4-phenylbutyrate (PBA), which facilitate protein folding and reduce ER stress, in mice with colitis.

**METHODS—**We used dextran sulfate sodium (DSS) to induce colitis in mice that do not express the transcription factor ATF6$\alpha$ or the protein chaperone P58$^{IPK}$. We examined the effects of TUDCA and PBA in cultured intestinal epithelial cells (IECs); in wild-type, *P58*$^{IPK-/-}$, and *Atf6a*$^{-/-}$ mice with colitis; and in *Il10*$^{-/-}$ mice.

**RESULTS—***P58*$^{IPK-/-}$ and *Atf6a*$^{-/-}$ mice developed more severe colitis following administration of DSS than wild-type mice. IECs from *P58*$^{IPK-/-}$ mice had excessive ER stress, and apoptotic signaling was activated in IECs from *Atf6a*$^{-/-}$ mice. Inflammatory stimuli induced ER stress signals in cultured IECs, which were reduced by incubation with TUDCA or PBA. Oral administration of either PBA or TUDCA reduced features of DSS-induced acute and chronic colitis in wild-type mice, the colitis that develops in *Il10*$^{-/-}$ mice, and DSS-induced colitis in *P58*$^{IPK-/-}$ and *Atf6a*$^{-/-}$ mice. Reduced signs of colonic inflammation in these mice were associated with significantly decreased ER stress in colonic epithelial cells.



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**CONCLUSIONS—**The unfolded protein response induces expression of genes that encode chaperones involved in ER protein folding; these factors prevent induction of colitis in mice. Chemical chaperones such as TUDCA and PBA alleviate different forms of colitis in mice and might be developed for treatment of inflammatory bowel diseases.

**Keywords**

IBD; Mouse Model; Ulcerative Colitis; Therapeutic Agent

The processes of protein folding, modification, and maturation in the endoplasmic reticulum (ER) are sensitive to environmental changes and multiple cellular disturbances, including ER $Ca^{2+}$ depletion, defective glycosylation, metabolic stimuli, altered redox status, energy deprivation, inflammatory stimuli, and increased protein secretion. When ER protein folding is perturbed or when cells are stimulated to secrete large amounts of protein, unfolded/misfolded proteins accumulate in the ER lumen, a condition called ER stress.[1] To restore ER function and improve protein-folding homeostasis (proteostasis), eukaryotes evolved the unfolded protein response (UPR). In mammalian cells, the UPR is initiated by 3 ER-localized transmembrane protein sensors: activating transcription factor 6$\alpha$ (ATF6$\alpha$), inositol-requiring kinase 1$\alpha$ (IRE1$\alpha$), and PKR-like ER kinase.[2–4] UPR signaling can lead to either adaptation or apoptosis. In the adaptive UPR, ER protein folding is remodeled through transactivation of genes encoding ER chaperones, ER trafficking machinery and ER-associated protein degradation, and eIF2$\alpha$ phosphorylation-mediated global translation attenuation.[5–8] Alternatively, prolonged and/or severe ER stress leads to the activation of the proapoptotic UPR, including the transcription factor CHOP and the IRE1$\alpha$-activated c-Jun-N-terminal kinase (JNK) pathway.[9] Moreover, chronic ER stress impairs cellular homeostasis through energy depletion, leakage of ER $Ca^{2+}$, mitochondrial damage, oxidative stress, and activation of caspases.[10] Therefore, persistent protein misfolding in response to chronic environmental stress and/or ineffective adaptive UPR signaling can compromise cell function and homeostasis and induce apoptosis.[11]

Recent studies link ER stress to the pathogenesis of inflammatory bowel disease (IBD). For example, patients with active Crohn's disease and ulcerative colitis exhibit signs of ER stress in their ileal and/or colonic epithelium.[12–15] In addition, human genetic studies of IBD have identified primary genetic abnormalities in several genes, including *XBP1, AGR2*, and *ORMDL3*, that encode proteins associated with ER stress.[15–19] Previous studies have indicated that cells with a high load of protein folding and secretion are sensitive to altered ER homeostasis and this can induce inflammatory response gene expression.[5,20,21] Intestinal microbiota and their molecules stimulate intestinal epithelial cells (IECs) to increase secretion of mucins and antimicrobial peptides that can overwhelm their protein secretory capacity. On the other hand, exposure to inflammatory stimuli can cause ER stress, although the precise mechanism is not well understood.[21] On exposure to high levels of exogenous antigens and inflammatory cytokines in the intestinal lumen, IECs may require efficient UPR-mediated ER chaperone induction to survive the heavy burden of protein folding and secretion.

In this study, we show that protein misfolding in the ER caused by deletion of the ER cochaperone gene *P58$^{IPK}$/Dnajc3* exacerbates experimental colitis in mice. ATF6$\alpha$ is a potent transcriptional activator for a number of ER chaperone genes, including *BiP, Grp94*, and *P58$^{IPK}$* in many cell types.[2–4] Although whole body deletion of *Atf6a* does not generate an obvious phenotype under normal conditions, it is required for cells to survive chemical-induced ER stress.[8] We found that in the absence of $P58^{IPK}$ or ATF6$\alpha$, mice are sensitive to colitis and exhibit reduced induction of ER chaperone genes and hyperactivation of proapoptotic UPR signaling in colonic IECs. The chemical chaperones

tauroursodeoxycholate (TUDCA) and 4-phenylbutyrate (PBA) are Food and Drug Administration–approved bioactive small molecules that function to facilitate protein folding and reduce ER stress both in vitro and in vivo by stabilizing protein-folding intermediates and preventing protein aggregation.[22–28] In this study, we show that oral delivery of either TUDCA or PBA dramatically decreases the clinical, histologic, and biochemical signs of inflammation in both innate immunity– and T cell–dependent colitis through reducing ER stress signaling in colonic IECs.

## Materials and Methods

### Mice

*Atf6α*$^{-/-}$ and *P58*$^{IPK-/-}$ mice (C57BL/6J background) were described previously.[8,29] Wild-type C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal care and procedures were conducted according to the protocols and guidelines approved by the University of Michigan University Committee on Use and Care of Animals and the Sanford-Burnham Medical Research Institute Institutional Animal Care and Use Committee.

### Cell Culture

IEC-6 cells (passages 8–10) were kindly provided by Dr Linda Samuelson (Department of Physiology, University of Michigan Medical Center, Ann Arbor, MI). The cells were maintained in Dulbecco's modified Eagle medium containing 4.5 mg/mL glucose and supplemented with 2 mmol/L L-glutamine, 10 mmol/L HEPES, 100 μg/mL streptomycin, and 100 U/mL penicillin. Cells were seeded $5 \times 10^5$ cells/well in 12-well plates, and all experiments were performed 1 day after cultures reached confluence. Cells were treated with 100 ng/mL rat tumor necrosis factor (TNF)-α (R&D Systems, Minneapolis, MN), 100 ng/mL rat MCP-1 (R&D Systems), 25 ng/mL rat interleukin (IL)-1β (R&D Systems), and 5 mmol/L PBA (Scandinavian Formulas, Inc, Sellersville, PA) or TUDCA (EMD Chemicals, Billerica, MA).

## Results

### Dextran Sodium Sulfate–Induced Colitis Induces ER Stress in Colonic Epithelium

To characterize the role of ER stress and the UPR in IECs during intestinal inflammation, we used the dextran sodium sulfate (DSS)-induced colitis murine model. C57BL/6J mice at 8 weeks of age were fed 3% DSS in their drinking water for 3 or 5 days to develop colitis in the large intestine. The purity of isolated colonic epithelial cells was greater than 95%, measured by flow cytometry using an antibody against murine epithelial cell adhesion molecule (Supplementary Figure 1*A*). Analysis of UPR markers, including the ER chaperone BiP, phosphorylation of the ER stress sensor PKR-like ER kinase, and the UPR transcription factors ATF4, CHOP, and spliced XBP1 showed a time-dependent induction in colonic epithelium that coincided with progression of DSS-induced colitis. A similar messenger RNA induction pattern of UPR genes was observed by quantitative reverse-transcription polymerase chain reaction analysis and immunohistochemistry (IHC) staining (Supplementary Figure 1*C* and *D*). The ER stress induction in the DSS-induced colitis model is similar to that observed in intestinal tissues from patients with active IBD,[12–15] suggesting that this experimental colitis model is valid for studies of ER stress and the UPR in IECs on intestinal inflammation.

### *The ER Cochaperone* P58$^{IPK}$ *Protects From DSS-Induced Colitis*

To determine the requirement for proper protein folding in the ER for IEC function, we analyzed mice with deletion in *P58*$^{IPK}$. P58$^{IPK}$ is a heat-shock 40-kilodalton protein that

belongs to the DNAJ chaperone family and resides in the ER lumen in association with the ER chaperone BiP and promotes proper protein folding.[30–32] The UPR induces transcription of *P58$^{IPK}$*, while cells and mice deleted in *P58$^{IPK}$* display slight protein misfolding and are sensitive to ER stress.[29,30] The role of P58$^{IPK}$ in colonic epithelia was studied using bone marrow chimeras of *P58$^{IPK+/-}$* and *P58$^{IPK-/-}$* mice to exclude the effect of *P58$^{IPK}$* deletion in hematopoietic cells. On challenge with DSS, *P58$^{IPK-/-}$* mice displayed severe body weight loss, rectal bleeding, and shortening of the large intestine (Figure 1*A* and *B* and Supplementary Figure 2*A*). Consistently, the *P58$^{IPK-/-}$* mice showed significantly more severe mucosal damage, loss of goblet cells, and inflammatory cell infiltration in the colon compared with their heterozygous littermates (Figure 1*C* and Supplementary Figure 2*B*). After DSS challenge, immunoblots showed up-regulation of ER stress markers BiP and phosphor-IRE1*α* in isolated colonic IECs from *P58$^{IPK-/-}$* mice compared with both *P58$^{IPK+/+}$* and *P58$^{IPK+/-}$* mice. The proapoptotic transcription factor CHOP was also induced in *P58$^{IPK-/-}$* colonic IECs before and after treatment with DSS (Figure 1*E*). Consistently, IHC indicated increased expression of CHOP in *P58$^{IPK-/-}$* colonic epithelium with DSS-induced colitis (Figure 1*F*). Bim, a BH3-only member of the Bcl-2 family that is transactivated by CHOP, plays a critical role in ER stress–induced apoptosis.[33] On DSS challenge, Bim and cleaved caspase-3 were highly induced in the colonic epithelium of *P58$^{IPK-/-}$* mice compared with both *P58$^{IPK+/+}$* and *P58$^{IPK+/-}$* mice (Figure 1*E*). These data suggest that the elevated susceptibility of *P58$^{IPK-/-}$* mice to DSS-induced colitis is due to a hyperactivated ER stress response and proapoptotic UPR signaling, including CHOP and Bim, in colonic IECs during inflammation.

## ATF6α-Mediated ER Chaperone Induction Protects Against DSS-Induced Colitis

To further elucidate the role of ER chaperones induced during intestinal inflammation, we studied mice with a deletion in *Atf6α*, the master regulator of ER chaperone gene expression. The colon of *Atf6α$^{-/-}$* mice is indistinguishable from wild-type, as indicated by H&E and periodic acid–Schiff staining (Supplementary Figure 3*A*). Age- and sex-matched *Atf6α$^{+/+}$*, *Atf6α$^{+/-}$*, and *Atf6α$^{-/-}$* littermate mice were reconstituted with wild-type bone marrow cells. The genetic ablation of *Atf6α* exacerbated symptoms of DSS-induced colitis, including severe body weight loss, rectal bleeding (Figure 2*A* and *B*), and significantly greater mucosal damage, goblet cell loss, and macrophage infiltration in the colon (Figure 2*C* and *D* and Supplementary Figure 3*B*). *Atf6α$^{-/-}$* mice displayed reduced expression of ER chaperone genes, including *BiP, Grp94*, and *P58$^{IPK}$*, in both protein and messenger RNA levels (Figure 2*E* and Supplementary Figure 3*C*), indicating that the adaptive UPR signaling in colonic IECs of *Atf6α$^{-/-}$* mice is compromised in response to intestinal inflammation. In contrast, the proapoptotic IRE1*α*-JNK pathway was induced in inflamed colonic epithelium of *Atf6α$^{-/-}$* mice (Figure 2*E*). After induction of colitis, the apoptotic markers cleaved caspase-3 and DNA fragmentation (by terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling [TUNEL] staining) were also increased in colonic epithelium of *Atf6α$^{-/-}$* mice compared with *Atf6α$^{+/+}$* and *Atf6α$^{+/-}$* mice (Figure 2*E* and Supplementary Figure 3*D* and *E*). Consistently, IHC showed reduced expression of the ER chaperone BiP and increased expression of the proapoptotic transcription factor CHOP in *Atf6α$^{-/-}$* colonic epithelium (Figure 2*F*). These data are consistent with the notion that ATF6*α* is an important transactivator of ER chaperone genes in colonic IECs during colitis. The impaired ER chaperone induction in *Atf6α$^{-/-}$* mice leads to unresolved ER stress, which induces proapoptotic UPR signaling in colonic IECs and exacerbates DSS-induced colitis.

## TUDCA and PBA Alleviate Inflammation-Induced ER Stress in an IEC Line

Given the protective role of the ER chaperone response in IECs against intestinal inflammation, we tested whether the chemical chaperones TUDCA and PBA can reduce ER

stress in IECs and alleviate colitis in mice. We first analyzed the effect of PBA and TUDCA in the nontransformed rat IEC line IEC-6 that was treated with physiologically relevant stimuli to cause ER stress. The inflammatory cytokines TNF-$\alpha$, MCP-1, and IL-1$\beta$ are highly up-regulated in animal models of enterocolitis and patients with IBD. We found that a combined cocktail of TNF-$\alpha$, MCP-1, and IL-1$\beta$ induces ER stress in IEC-6 cells, as monitored by the up-regulation of ER stress markers BiP and CHOP (Figure 3*A*). Prior treatment and cotreatment of IEC-6 cells with either PBA or TUDCA reduced CHOP and BiP expression in response to the inflammatory stimuli (Figure 3*A*). These data indicate that proinflammatory cytokines induce ER stress in IECs in vitro, and this cellular stress can be mitigated by treatment with either PBA or TUDCA.

## TUDCA and PBA Alleviate Signs of DSS-Induced Colitis by Reducing ER Stress Signaling in Colonic Epithelial Cells

The therapeutic potential of chemical chaperones was tested in a prevention paradigm in the DSS-induced colitis murine model. C57BL/6J mice were fed 2.5% DSS in their drinking water for 8 days. TUDCA was administrated orally by gavage at 500 mg/kg body wt per day (single dose) throughout the whole period. Compared with mice subjected to DSS challenge only, feeding of TUDCA significantly ameliorated the symptoms of DSS-induced colitis, as indicated by the lower clinical scores (Figure 3*B* and *C* and Supplementary Figure 4). TUDCA dramatically reduced the histologic manifestations of DSS-induced colitis (Figure 3*D* and *E*). In parallel, the expression of proinflammatory cytokines IL-1$\beta$ and TNF-$\alpha$ in the colon was significantly reduced by feeding of TUDCA (Figure 3*F*). Additionally, the induction of oxidative/nitrosative stress and apoptotic signaling was inhibited by treatment with TUDCA, as indicated by diminished expression of genes encoding iNOS, NOX2, and Bim (Figure 3*F*). As expected, the induction of ER stress markers BiP, $P58^{IPK}$, CHOP, GADD34, and ERO1$\alpha$ in colonic IECs was considerably reduced in mice fed with TUDCA during the induction of DSS colitis (Figure 3*F*), which is consistent with the observations in IEC-6 cells treated with inflammatory signals and TUDCA. Similarly, feeding of PBA dramatically ameliorated the symptoms of DSS-induced colitis (Figure 4*A*–*E*). Induction of ER stress in colonic epithelium was significantly reduced on administration of PBA during the induction of DSS colitis (Figure 4*F*).

## TUDCA and PBA Reverse DSS-Induced Chronic Colitis

We then examined whether the chemical chaperones can reverse the symptoms of chronic colitis. The mice were fed with 3 cycles of 2% DSS in drinking water and then received 300 mg/kg body wt TUDCA daily (double dose, 150 mg/kg body wt per dose) by oral administration for 10 days. Dramatically, the histologic scores of the large intestine, including damaged area involved, ulceration, mucodepletion of glands, and inflammatory cell infiltration, were significantly reduced after the administration of TUDCA compared with the controls (Figure 5*A* and *B*). For delivery of PBA, C57BL/6J mice with chronic colitis were fed 500 mg/kg body wt PBA daily (double dose, 250 mg/kg body wt per dose) for 10 days. PBA-treated mice also showed a similar recovery from chronic colitis (Figure 5*C* and *D*).

## TUDCA and PBA Complement the Requirement for $P58^{IPK}$ and ATF6α in Preventing DSS-Induced Colitis

To further explore the molecular mechanisms of how chemical chaperones function in alleviating intestinal inflammation, we tested the 2 compounds on DSS-induced colitis in the $P58^{IPK}$ or *Atf6α* mice. Where $P58^{IPK-/-}$ and $Atf6\alpha^{-/-}$ mice were more susceptible to DSS-induced colitis, as shown in Figures 1 and 2, feeding of TUDCA or PBA reduced the clinical and histologic scores of $P58^{IPK-/-}$ and $Atf6\alpha^{-/-}$ mice to levels similar to those of their littermate controls (Figure 6*A*–*F*). These data indicate that the chemical chaperones can

complement the requirement for molecular chaperones, correct the protein folding defects in animals with an impaired ER chaperone response, and protect mice against intestinal inflammation.

### *TUDCA and PBA Dramatically Mitigate Colitis in* Il10$^{-/-}$ *Mice*

Because TUDCA and PBA can alleviate both acute and chronic colitis induced by DSS, we then examined whether the 2 chemical chaperones can ameliorate nonsteroidal anti-inflammatory drug–induced colitis in *Il10*$^{-/-}$ mice, a T cell–dependent IBD model. *Il10*$^{-/-}$ mice with established colitis received either TUDCA or PBA in the drinking water at a concentration of 2 mg/mL (5.2 mg/mouse per day) for 3 weeks. Dramatically, the histologic scores of the large intestine were dramatically reduced after the administration of either TUDCA or PBA (Figure 7*A* [*upper and middle panels*], *B*, and *C*). Furthermore, trichrome staining indicated that fibrosis in the large intestine was significantly reduced by the feeding of TUDCA or PBA (Figure 7*A* [*lower panel*] and *D*). In the colonic epithelia of *Il10*$^{-/-}$ mice with colitis, the induction of ER stress markers including BiP, phospho-eIF2$\alpha$, and CHOP, mitochondrial UPR marker HSP60, as well as apoptotic markers including cleaved caspase-3/12 were considerably reduced after treatment with TUDCA or PBA (Figure 7*E*). These data show that the chemical chaperones have potent anti-inflammatory and antifibrotic effects in the *Il10*$^{-/-}$ colitis model through the suppression of ER stress and cell death in colonic epithelium.

## Discussion

Recent studies indicate that inflammatory conditions in the gastrointestinal tract can activate the UPR in IECs.[12–15] However, it is unknown whether these pathway(s) function to disrupt cellular homeostasis and induce apoptosis or to restore ER function and prevent cell death. Previous studies showed that P58$^{IPK}$ binds to newly synthesized secretory proteins in the ER and promotes protein folding/maturation in cells.[30–32] Consistent with a role for P58$^{IPK}$ in reducing protein misfolding in the cell, we showed that P58$^{IPK}$ prevents dysfunction of IECs and progression of DSS-induced colitis. During the development of colitis, *P58*$^{IPK-/-}$ mice display increased expression of the proapoptotic factor CHOP and reduced expression of the prosurvival protein Bcl2 in IECs due to an unresolved/prolonged ER stress. CHOP is a major cell death–inducing factor during the UPR[9,15] and has been shown to exacerbate colitis in mice.[34] Previous studies have shown that a hypomorphic mutation in the gene encoding S1P in mice enhances the sensitivity to DSS-induced colitis.[35] However, given that S1P targets several ER stress–induced bZIP transcription factors, including Luman, OASIS, and CREBH, as well as the SREBPs,[36] it was not clear whether the increased susceptibility is attributed to reduced activation of ATF6 or other transcription factors. In this study, we showed that colonic IECs from *Atf6*$\alpha^{-/-}$ mice have reduced adaptive ER chaperone expression and increased proapoptotic UPR signaling, including the IRE1$\alpha$-JNK pathway.[9,15] In mice with an IEC-specific deletion of *Xbp1*, hyperphosphorylated IRE1$\alpha$ activates JNK and induces spontaneous inflammation in the ileum.[15,37] Therefore, compromised ER chaperone expression, by loss of either an ER chaperone itself or the upstream transactivator, leads to unresolved ER stress and activation of proapoptotic signaling and therefore impairs cell function and exacerbates inflammation. In these experiments, we used bone marrow chimeras to exclude the effect of *P58*$^{IPK}$ or *Atf6*$\alpha$ deletion in hematopoietic cells, including macrophages, neutrophils, T cells, and B cells, during intestinal inflammation. However, bone marrow reconstitution is not able to replace lamina propria fibroblasts and smooth muscle cells in the gut, which may still contribute to colitis. Villin-*Cre*–directed conditional deletion models, if available, would be ideal for this study.

Consistent with the protective role of ER chaperones in IECs for intestinal homeostasis, we showed that the chemical chaperones TUDCA and PBA, which promote ER homeostasis and increase ER folding capacity, reduce ER stress signaling in IECs and alleviate colitis in mice. Furthermore, we showed that feeding of TUDCA or PBA corrected the defects in $P58^{IPK-/-}$ and $Atf6\alpha^{-/-}$ mice with impaired ER chaperone induction during DSS-induced colitis, suggesting that TUDCA and PBA resolve intestinal inflammation due to their function in promoting protein folding and alleviating ER stress.

Recent studies showed that TUDCA inhibits the expression of UPR genes in an intestinal epithelial cell line induced by the widely used ER stressor tunicamycin.[38] In our study, we showed that either TUDCA or PBA reduces ER stress gene expression in IECs induced by inflammatory stimuli that are physiologically relevant to IBD. More strikingly, the feeding of either TUDCA or PBA dramatically protected the intestinal mucosa in both a DSS-induced model and a T cell–dependent genetic model of colitis. These data suggest that TUDCA and PBA exert an epithelial-protective effect during the intestinal inflammation that is predominated by either the innate or adaptive immune response in the mucosa.

The chemical chaperones TUDCA and PBA are Food and Drug Administration–approved drugs that have outstanding safety profiles in humans. TUDCA is safely used as a hepatoprotective drug for the treatment of primary biliary cirrhosis.[39] PBA is approved for clinical use in urea cycle disorders.[40] Both compounds are in clinical trials for the treatment of a number of diseases that are associated with protein misfolding in the ER, including cystic fibrosis, amyotrophic lateral sclerosis, spinal muscular atrophy, Huntington's disease, and type 2 diabetes (http://clinicaltrialsfeeds.org/clinical-trials/results/term=TUDCA; http://clinicaltrialsfeeds.org/clinical-trials/results/intr=4-phenylbutyric+acid).[2,41–44] Current medications used to treat IBD, including corticosteroids, immunosuppressants, and biologics, have significant risks and adverse effects.[45–47] If efficacy can be shown in patients with IBD, given the safety profile and potential for oral delivery of TUDCA and PBA, this therapy could fill an important gap in our current therapeutic armamentarium. Ursodeoxycholate, the unconjugated bile salt of TUDCA, is a promising drug for chemoprevention of colorectal cancer.[48] Although TUDCA can inhibit inflammation-induced ER stress in nontransformed IEC-6 cells, it exacerbates ER stress in some colon cancer cell lines (unpublished results May, 2012.) It would be worthwhile to determine whether TUDCA is able to suppress the growth of carcinogenic colonocytes while protecting normal colonic epithelial cells against ER stress in patients with ulcerative colitis. Based on our findings in multiple murine models of colitis, the chemical chaperones TUDCA and PBA may warrant clinical investigation as a novel treatment for IBD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations used in this paper

| | |
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| **ATF6$\alpha$** | activating transcription factor 6$\alpha$ |
| **DSS** | dextran sulfate sodium |
| **ER** | endoplasmic reticulum |
| **IBD** | inflammatory bowel disease |
| **IEC** | intestinal epithelial cell |
| **IHC** | immunohistochemistry |
| **IL** | interleukin |
| **IRE1$\alpha$** | inositol-requiring kinase 1$\alpha$ |
| **JNK** | c-Jun-N-terminal kinase |
| **PBA** | 4-phenylbutyrate |
| **PBS** | phosphate-buffered saline |
| **TNF** | tumor necrosis factor |
| **TUDCA** | tauroursode-oxycholate |
| **UPR** | unfolded protein response |

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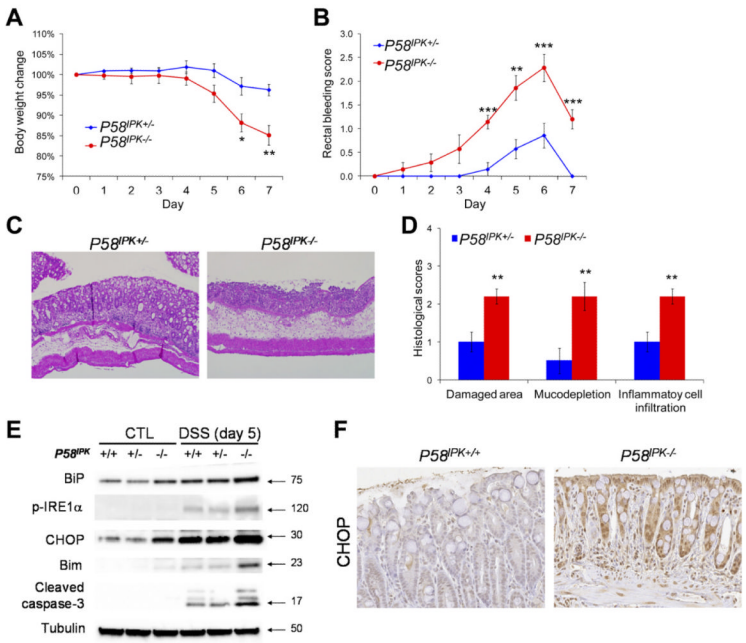


**Figure 1.**

Loss of P58$^{IPK}$ exacerbates DSS-induced colitis in mice due to a hyperactivated proapoptotic UPR. *P58*$^{IPK+/-}$ and *P58*$^{IPK-/-}$ littermates with wild-type bone marrow cells were fed 2.5% DSS in drinking water for 5 days, followed by 2 days of fresh water. (*A*) Body weight and (*B*) rectal bleeding were measured over 7 days. (*C*) After administration of DSS, the colons were isolated and fixed for H&E staining. Representative images are shown (original magnification 100×). (*D*) Histologic scores were measured in mice with DSS-induced colitis. (*E*) The proapoptotic transcription factor CHOP was induced whereas antiapoptotic Bcl2 was reduced in *P58*$^{IPK-/-}$ IECs. (*F*) IHC shows CHOP is induced in *P58*$^{IPK-/-}$ colonic epithelium with DSS-induced colitis. n = 7 for each group. **P* < .05, ***P* < .01, ****P* < .001.

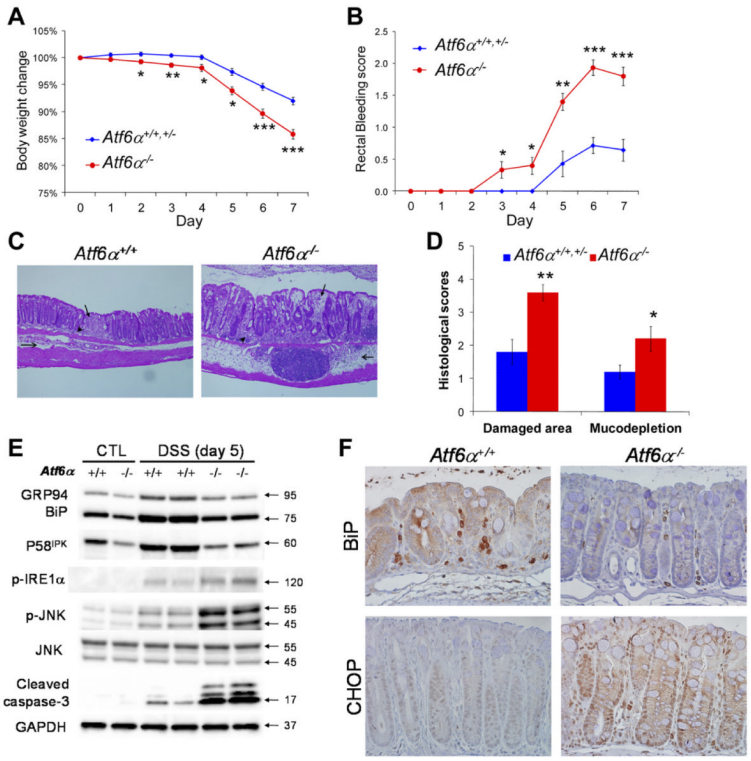


**Figure 2.**

Loss of ATF6$\alpha$ exacerbates DSS-induced colitis in mice due to defective ER chaperone induction and a hyperactivated proapoptotic UPR. *Atf6a*$^{+/+}$, *Atf6a*$^{+/-}$, and *Atf6a*$^{-/-}$ littermates with wild-type bone marrow cells were fed 3% DSS in drinking water for 5 days, followed by 2 days of fresh water. (*A*) Body weight and (*B*) rectal bleeding were measured over 7 days. After administration of DSS, the colons were isolated and fixed for H&E staining. Representative images are shown (*C*; original magnification 100×). (*D*) Histologic scores were measured in mice with DSS-induced colitis. (*E*) The expression of ER chaperones BiP, GRP94, and P58$^{IPK}$ is reduced whereas the proapoptotic IRE1$\alpha$-JNK pathway and caspase-3 are activated in *Atf6a*$^{-/-}$ IECs with DSS-induced colitis. (*F*) IHC shows that expression of the ER chaperone BiP is impaired whereas the proapoptotic transcription factor CHOP is increased in *Atf6a*$^{-/-}$ colonic epithelium with DSS-induced colitis. n = 14 or 15. **P* < .05, ***P* < .01, ****P* < .001.

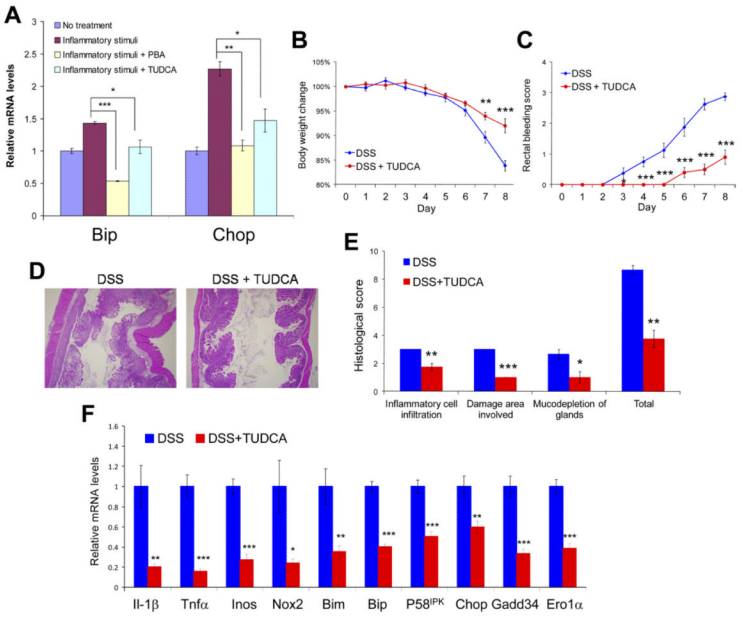


**Figure 3.**

TUDCA and PBA alleviate inflammatory stimuli-induced ER stress in IECs in vitro; TUDCA ameliorates DSS-induced colitis by reducing ER stress in colonic epithelium in vivo. (*A*) IEC-6 cells were treated with a combination of inflammatory cytokines (TNF-$\alpha$, MCP-1, and IL-1$\beta$) for 8 hours or pretreated with 5 mmol/L TUDCA or PBA for 4 hours, followed by treatment with the same inflammatory signals with 5 mmol/L TUDCA or PBA for 8 hours. The cells were then collected for RNA extraction and quantitative reverse-transcription polymerase chain reaction. The messenger RNA levels were normalized to the expression of 18S ribosomal RNA. Wild-type mice were fed 2.5% DSS in drinking water and received 500 mg/kg body wt TUDCA or the same amount of phosphate-buffered saline (PBS) without TUDCA daily by gavage (n = 8 or 10 per group). (*B*) Body weight and (*C*) rectal bleeding were measured over 8 days. (*D*) After administration of DSS, the colons were isolated and fixed for H&E staining. Representative images are shown (original magnification 40×). (*E*) Histologic scores are shown from TUDCA-treated and control mice with DSS-induced colitis. (*F*) Expression of genes associated with inflammation, oxidative stress, and apoptosis in colonic mucosa as well as ER stress markers in colonic IECs is shown (normalized to the expression of *Gapdh*). n = 8 for each group; $*P < .05$, $**P < .01$, $***P < .001$.

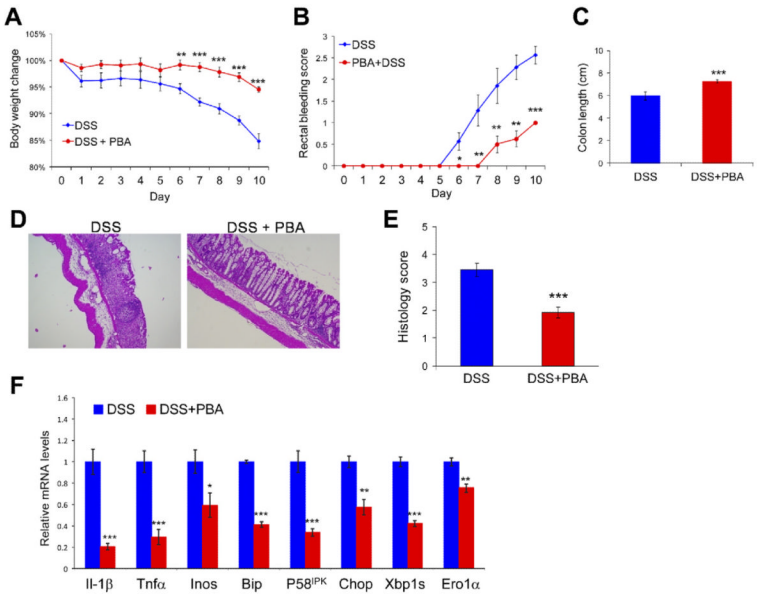


**Figure 4.**

PBA alleviates DSS-induced colitis by reducing ER stress in colonic epithelium. Wild-type mice were fed 2% DSS in drinking water and received 500 mg/kg body wt PBA or the same amount of PBS without PBA daily by gavage. (*A*) Body weight and (*B*) rectal bleeding were measured over 10 days. (*C*) Colon lengths were measured after induction of DSS colitis. (*D*) After administration of DSS, the colons were isolated and fixed for H&E staining. Representative images are shown (original magnification 40×). (*E*) Histologic scores are shown from control and PBA-treated mice with DSS-induced colitis. (*F*) Expression of genes associated with inflammation, oxidative stress, and ER stress in colonic mucosa as well as ER stress markers in colonic IECs is shown (normalized to the expression of *Gapdh*). n = 8 for each group; **P* < .05, ***P* < .01, ****P* < .001.

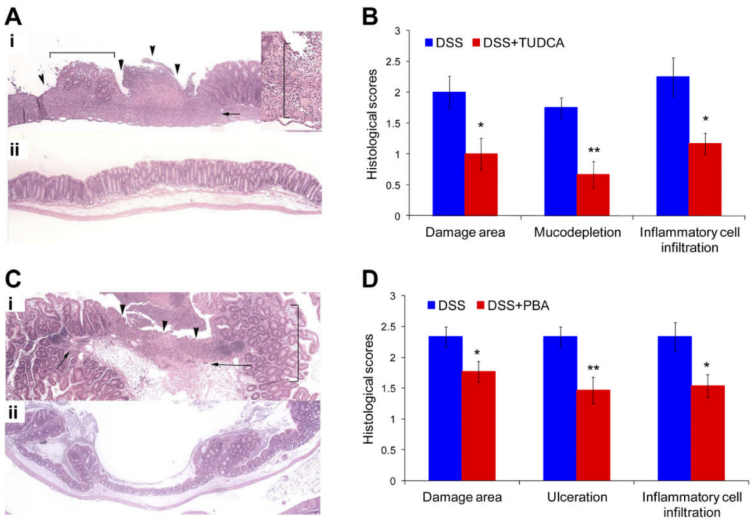


**Figure 5.**

Either TUDCA or PBA mitigates inflammation in mice with chronic colitis. (*A*) Wild-type mice with established chronic DSS-induced colitis received 300 mg/kg body wt TUDCA or the same amount of PBS without TUDCA (control) daily by gavage for 10 days. (*a*) Colon from a mouse with chronic colitis fed with PBS alone (control), showing severe epithelial ulceration (*arrowheads*), loss of goblet cell morphology (mucodepletion; *bracket*), and transmural inflammatory infiltrate (*arrow*). *Inset* shows transmural inflammatory infiltrate at the edge of an ulcer (*bracket*). (*b*) Mice with chronic colitis treated with TUDCA display reduced mucosal damage and inflammation (original magnification 40×; 100× for *inset*). (*B*) Histologic scores, including damaged area involved, mucodepletion of glands, and inflammatory cell infiltration, are shown from TUDCA-treated and control mice with chronic DSS-induced colitis. n = 6 or 8 for each group. (*C*) Wild-type mice with established chronic DSS-induced colitis received 500 mg/kg body wt PBA or the same amount of PBS without PBA (control) daily by gavage for 10 days. (*a*) Cecum from a mouse with chronic colitis fed with PBS alone (control) showing severe ulceration (*arrowheads*), inflammatory infiltrates (*arrows*), and loss of goblet cell morphology (*bracket*). (*b*) Cecum from a mouse with chronic colitis treated with PBA showing reduced mucosal damage and inflammation (original magnification 40×). (*D*) Histologic scores are shown from PBA-treated and control mice with chronic DSS-induced colitis. n = 9 or 13 for each group. **P* < .05, ***P* < .01.

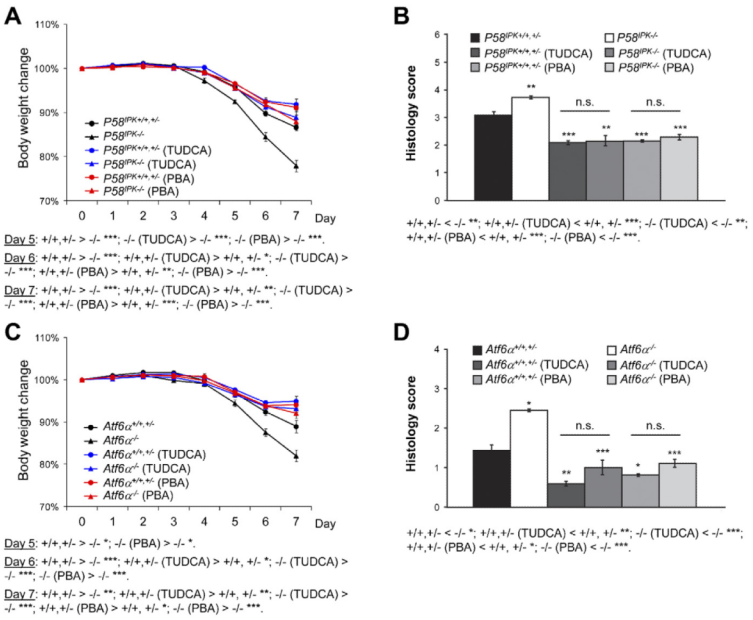


**Figure 6.**

Feeding of TUDCA or PBA corrects the defects of $P58^{IPK-/-}$ and $Atf6\alpha^{-/-}$ mice in response to DSS-induced colitis. $P58^{IPK+/+}$, $P58^{IPK+/-}$, and $P58^{IPK-/-}$ mice and $Atf6\alpha^{+/+}$, $Atf6\alpha^{+/-}$, and $Atf6\alpha^{-/-}$ mice were reconstituted with wild-type bone marrow cells and fed 3% DSS in drinking water for 5 days, followed by 2 days of fresh water. During the same period, the animals received 500 mg/kg body wt TUDCA, PBA, or the same amount of PBS without TUDCA/PBA daily by gavage. (*A* and *C*) Body weight was measured over the 7-day period. (*B* and *D*) After treatment, the colons were isolated and fixed for H&E staining and histologic scoring. n = 7–14 for each group; $*P < .05$, $**P < .01$, $***P < .001$.

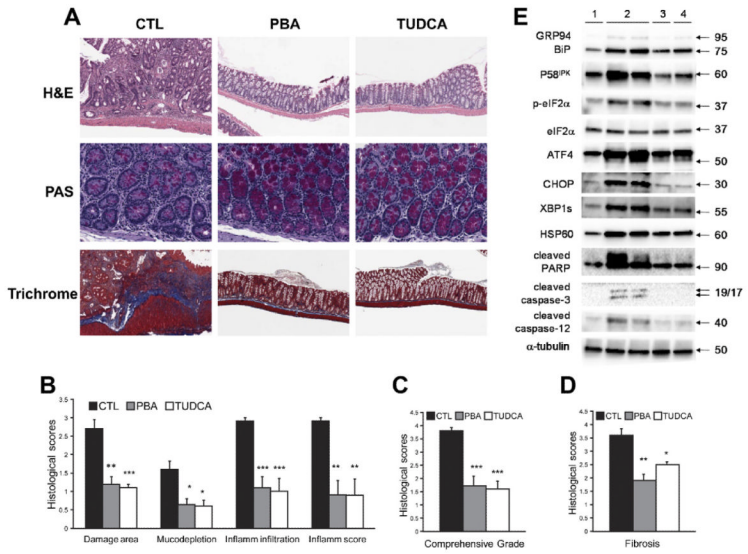


**Figure 7.**

Either TUDCA or PBA alleviates chronic colitis in *Il10*$^{-/-}$ mice. *Il10*$^{-/-}$ mice with piroxicam-induced colitis received 2 mg/mL PBA or TUDCA in the drinking water for 3 weeks. (*A*) Feeding of TUDCA or PBA reduces signs of chronic colitis in *Il10*$^{-/-}$ mice. Periodic acid–Schiff (PAS) staining shows mucin in goblet cells, and trichrome staining indicates collagen deposition in the colon. (*B*) Histologic scores are shown using the standard of Otuska et al.[49] (*C*) Histologic scores are shown using the standard of Berg et al.[50] (*D*) Histologic scores are shown for colonic fibrosis. n = 10 –11 for each group. (*E*) The mice were killed after the experiment, and the colonic IECs were isolated for protein extraction and Western blotting. Representative immunoblots are shown. 1, no piroxicam-induced colitis; 2, colitis; 3, colitis → TUDCA; 4, colitis → PBA. **P* < .01, ***P* < .001, ****P* < .0001.