Supplementary Methods

Generation of Bone Marrow Chimeras

Littermate mice of *Atf6α*$^{+/+}$, *Atf6α*$^{+/-}$, *Atf6α*$^{-/-}$ and *P58*$^{IPK+/+}$, *P58*$^{IPK+/-}$, and *P58*$^{IPK-/-}$ were lethally irradiated with 950 rad ionizing irradiation. After two hours, 5×10^6 bone marrow cells isolated from the tibias and femurs of wild-type mice were injected into the lethally irradiated recipient mice through the tail vein. After transplantation, mice were treated with 4 weeks of antibiotics in their drinking water and allowed to recover for another 4 weeks prior to treatment with DSS to induce colitis.

DSS-induced Colitis, Piroxicam-induced Colitis in Il10$^{-/-}$ *Mice, and Drug Administration*

For acute colitis, mice received 2–3% (w/v) DSS (MW 36,000–50,000; MP Biomedicals, Solon, OH) in drinking water for indicated days. To develop long-lasting chronic DSS colitis, 8-week old C57BL/6J mice received 3 cycles of 2% (w/v) DSS (7 days DSS, 14 days water) as previously described.[1]

Six-month old *Il10*$^{-/-}$ mice (C57BL/6J background) were fed 200 ppm piroxicam, a non-steroidal anti-inflammatory drug (NSAID), in the diet for two weeks to induce colitis as previously described.[2] The mice were subject to PBA/TUDCA treatment for three weeks after feeding of prixicam-diet.

For administration of TUDCA (EMD Chemicals USA, Gibbstown, NJ) and PBA (Scandinavian Formulas, Inc., Sellersville, PA), the sodium salt was dissolved in sterile PBS (Invitrogen, Carlsbad, CA) in a volume of 200 μL and given to mice by gavage, or dissolved in drinking water at a concentration of 2 mg/mL. Water intake measurements showed that *Il10*$^{-/-}$ mice with colitis consumed an average of 2.6 mL of water with or without drug every day. Therefore, each *Il10*$^{-/-}$ mouse received an average of 5.2 mg of PBA or TUDCA per day.

Isolation of Colonic Epithelial Cells

Colons were cut open longitudinally and feces were removed by washing with ice-cold PBS. Colons were then cut into 2–3 mm pieces and incubated in Ca^{2+}, Mg^{2+} - free PBS buffer containing 10 mM EDTA in a 50 mL conical tube at 4°C for 1hr with gentle rotation. Then the tubes were rigorously shaken to elute the epithelium from colon sections. The supernatant was removed and sieved through a cell strainer (500 um; Fisher HealthCare, Houston, TX). The flow-through was centrifuged; the resulting cell pellet was washed twice in ice-cold PBS.[3] The purity of isolated colonocytes was determined by flow cytometry using an antibody against murine epithelial cell adhesion molecule from BioLegend (San Diego, CA) following the manufacturer's protocol. Trypan blue staining confirmed the presence of $>$ 85% viable epithelial cells after the 2-hr isolation procedure. Isolated cells were snap-frozen in liquid nitrogen for protein and RNA extraction.

Quantitative Real-Time PCR

RNAs from isolated murine colonic epithelial cells and IEC-6 cells were extracted by using RNeasy kit (Qiagen, Valencia, CA); RNA from 5 mm distal colons was isolated by using TRIzol reagent (Invitrogen, Carlsbad, CA). Q-RT-PCR was performed as previously described.[4,5] Q-RT-PCR results were normalized to the level of 18S rRNA or mRNA encoding glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Primer sequences are listed in Supplemental Table 1.

Western Blotting

The isolated colonic epithelial cells were lysed in RIPA buffer for protein extraction. Protein content was measured by Protein Assay (Bio-Rad, Hercules, CA), and protein samples were analyzed by 10–20% reducing SDS/PAGE (Tris-HCl precast gels, Bio-Rad) and were detected with the following antibodies: anti-GRP94/BiP (Enzo Life Sciences International, INC, Plymouth Meeting, PA), anti-phosphor-PERK (Cell Signaling Technology, Danvers, MA), anti-ATF4 (Santa Cruz Biotechnology, Santa Cruz CA), anti-CHOP (Santa Cruz Biotechnology), anti-XBP1 (Santa Cruz Biotechnology), anti-Bim (Cell Signaling Technology), anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Millipore Corporation, Billerica, MA), and anti-tubulin (Cell Signaling Technology). After overnight incubation of primary antibodies at 4°C, the membranes were washed and then incubated with secondary HRP-coupled antibodies (GE Healthcare, Piscataway, NJ) and developed with chemiluminescent detection system (GE Healthcare).

Immunohistochemical Staining

Colonic tissues were fixed overnight in 10% formalin, embedded in paraffin, and cut into 5 μm sections. Paraffin-embedded tissue sections were rehydrated, heat-induced antigen retrieval was performed either in 10 mM sodium citrate, 0.05% Tween-20, pH 6.0, or in 10 mM Tris, 1 mM EDTA, pH 8.0, for 10 minutes. Primary antibodies: anti-BiP antibody (Santa Cruz Biotechnology) at a dilution of 1:200 at room temperature; anti-CHOP antibody (Santa Cruz Biotechnology) at a dilution of 1:50 at room temperature; anti-XBP1 antibody (BioLegend) at a dilution of 1:50 overnight at 4° temperature. Hematoxylin was performed for counterstaining.

Histological Scoring

Hematoxylin and eosin (H&E) stained colon sections were scored in a blinded manner by board certified veterinary pathologists as previously described by Berg et al,[2] Otuska et al,[6] and Lee et al.[7]

References

1. Wirtz S, Neufert C, Weigmann B, et al. Chemically induced mouse models of intestinal inflammation. Nat Protoc 2007;2:541–546.
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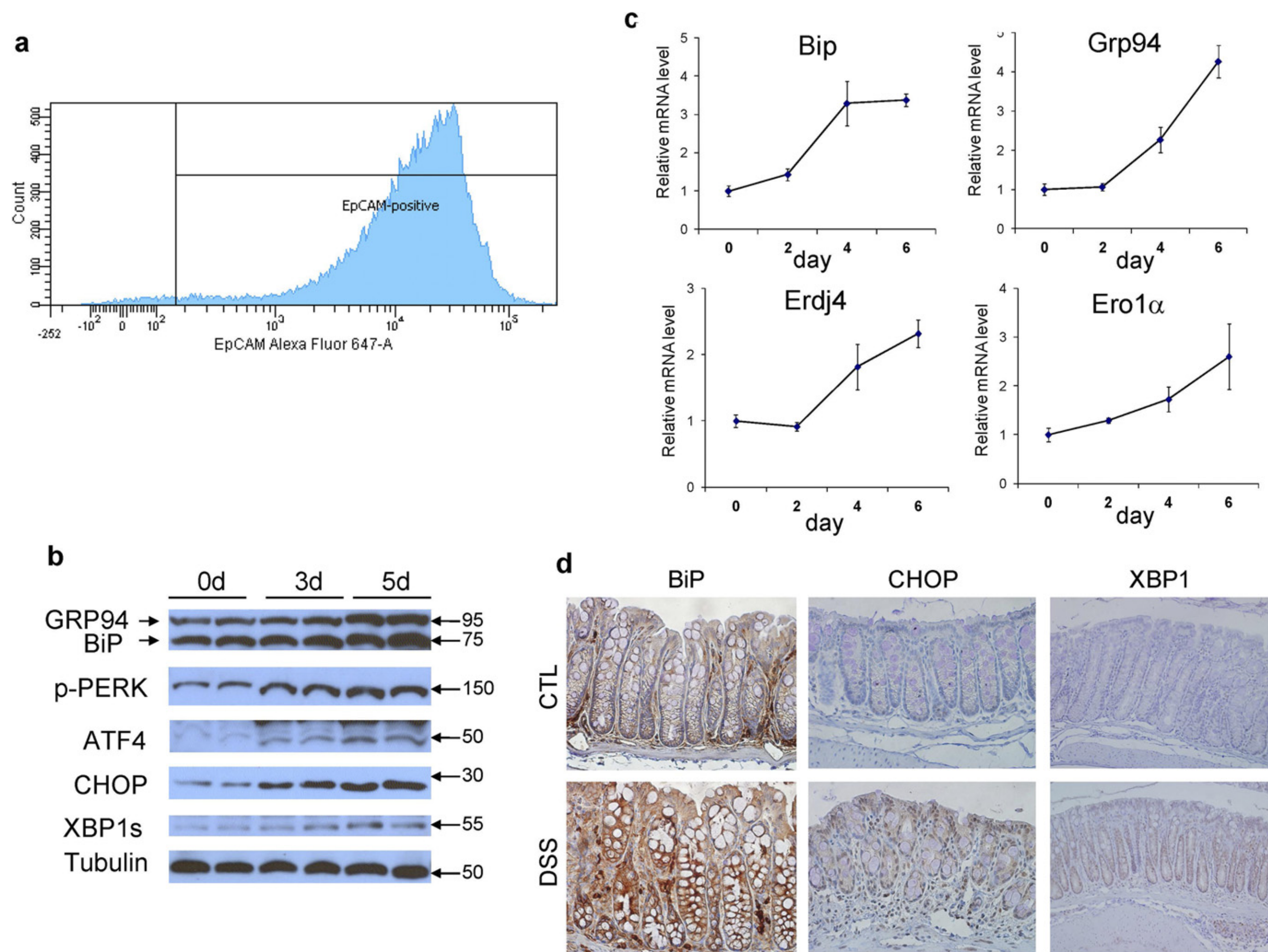
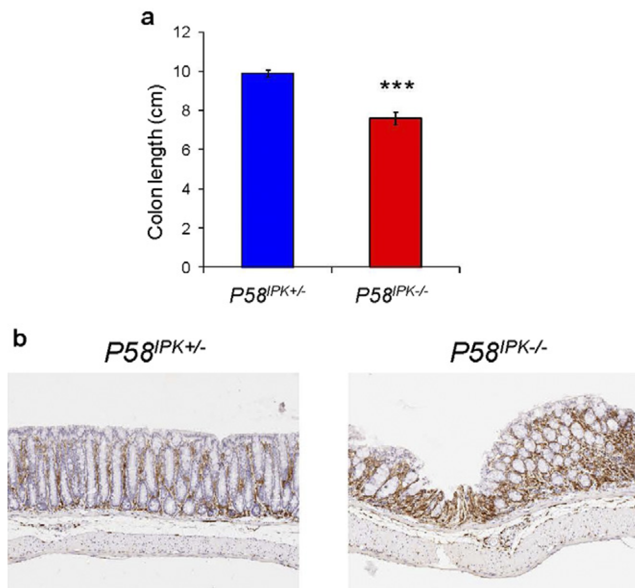
Author names in bold designate shared co-first authorship.



Supplementary Figure 1. DSS colitis induces ER stress in colonic epithelial cells. (**a**) The purity of isolated colonic epithelial cells was determined by flow cytometry using an antibody against murine EpCAM. (**b**) Wild-type mice were fed 3% DSS in the drinking water for 0, 3 and 5 days. After DSS administration for the indicated days, the mice were euthanized and the colonic IECs were isolated for protein extraction and western blotting. Representative immunoblots of colon tissues from mice are shown. (**c**) Wild-type mice were fed 3% DSS in the drinking water for 0, 2, 4 and 6 days. After DSS administration for the indicated days, the mice were euthanized and the colonic IECs were isolated for RNA extraction and Q-RT-PCR. The mRNA levels were normalized to the expression of *Gapdh.* (**d**) Wild-type mice were fed 3% DSS in the drinking water for 6 days, then the mice were euthanized and the colons were removed, fixed and paraffin embedded for immunohistochemical staining of BiP, CHOP and XBP1. Representative immunohistochemical images are shown; each group contained a minimum of 6 individual mice.



Supplementary Figure 2. *P58*$^{IPK-/-}$ mice exhibit shorter colon lengths (**a**) and higher levels of macrophage marker F4/80 in colonic mucosa (**b**) upon DSS colitis.

a
Atf6α+/+ Atf6α-/-
H&E
PAS

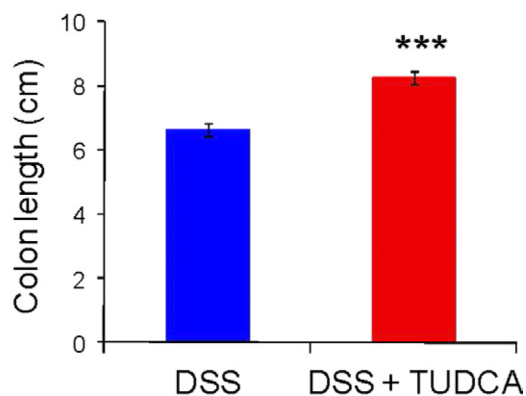
b
Atf6α+/+ Atf6α-/-
With DSS colitis (IHC: F4/80)

c
Relative mRNA levels
Atf6α+/+ Atf6α-/-
1.6 1.2 0.8 0.4 0
Bip Grp94 P58IPK Pdi
With DSS colitis

d
Atf6α+/+ Atf6α-/-
With DSS colitis (TUNEL)

e
Apoptotic index
60 50 40 30 20 10 0
*
Atf6α+/+,+/- Atf6α-/-

Supplementary Figure 3. (**a**) *Atf6*$\alpha^{-/-}$ mice display normal colon morphology under normal conditions. Colons were isolated from two-month old *Atf6*$\alpha^{+/+}$ and *Atf6*$\alpha^{-/-}$ littermates mice for H&E and PAS staining. (**b**) *Atf6*$\alpha^{-/-}$ mice showed higher levels of macrophage marker F4/80 in colonic mucosa upon DSS colitis. (**c**) *Atf6*$\alpha^{-/-}$ mice exhibit an impaired induction of ER chaperone genes including *Bip, Grp94, P58*^{IPK} and *Pdi* in colonic IECs upon DSS colitis. (**d**) Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)-staining of apoptotic epithelial cells in the colon sections from *Atf6*$\alpha^{+/+}$ and *Atf6*$\alpha^{-/-}$ mice with DSS colitis. (**e**) The apoptotic indices were calculated as the number of TUNEL-positive epithelial cells per 100 randomly selected crypts in the colon sections. 4 mice per group; * $p < 0.05$.



Supplementary Figure 4. Shortening of colon lengths upon DSS colitis was significantly mitigated by the feeding of TUDCA.

Supplementary Table 1. Sequence of primers used in this study

Primer name	Oligo sequence (5′ to 3′)
rat CHOP (f)	AGAGTGGTCAGTGCGCAGC
rat CHOP (r)	CTCATTCTCCTGCTCCTTCTCC
rat BiP (f)	TGGGTACATTTGATCTGACTGGA
rat BiP (r)	CTCAAAGGTGACTTCAATCTGGG
rat 18S (f)	AGTCCCTGCCCTTTGTACACA
rat 18S (r)	GATCCGAGGGCCTCACTAAAC
mouse IL-1beta (f)	CAACCAACAAGTGATATTCTCCATG
mouse IL-1 beta (r)	GATCCACACTCTCCAGCTGCA
mouse TNF-alpha (f)	CCCTCACACTCAGATCATCTTCT
mouse TNF-alpha (r)	GCTACGACGTGGGCTACAG
mouse iNOS (f)	CAGCTGGGCTGTACAAACCTT
mouse iNOS (r)	CATTGGAAGTGAAGCGTTTCG
mouse BiP (f)	TCATCGGACGCACTTGGA
mouse BiP (r)	CAACCACCTTGAATGGCAAGA
mouse GRP94 (f)	AATAGAAAGAATGCTTCGCC
mouse GRP94 (r)	TCTTCAGGCTCTTCTTCTGG
mouse Chop (f)	GTCCCTAGCTTGGCTGACAGA
mouse Chop (r)	TGGAGAGCGAGGGCTTTG
mouse ERdj4 (f)	CCCCAGTGTCAAACTGTACCAG
mouse ERdj4 (r)	AGCGTTTCCAATTTTCCATAAATT
mouse P58IPK (f)	TCCTGGTGGACCTGCAGTACG
mouse P58IPK (r)	CTGCGAGTAATTTCTTCCCC
mouse Ero1a (f)	GCATTGAAGAAGGTGAGCAA
mouse Ero1a (r)	ATCATGCTTGGTCCACTGAA
mouse Gadd34 (f)	CCCGAGATTCCTCTAAAAGC
mouse Gadd34 (r)	CCAGACAGCAAGGAAATGG
mouse Nox2 (f)	CCCTTTGGTACAGCCAGTGAAG
mouse Nox2 (r)	CAATCCCAGCTCCCACTAACAT
mouse Bim (f)	GGAGATACGGATTGCACAGGAG
mouse Bim (r)	CCTTCTCCATACCAGACGGAAG
mouse GAPDH (f)	TTCAACGGCACAGTCAAGG
mouse GAPDH (r)	CATGGACTGTGGTCATGAG