SUPPLEMENTARY MATERIALS AND METHODS:

Protein extraction and Western blot

Colons collected from mice were flushed with PBS. Each colon was then cut open longitudinally and placed on a clean glass slide with luminal surface facing up. Epithelial cells from the luminal side were scraped off, collected and immediately placed in a tube containing acid washed glass beads (425-600 µm) (Sigma) and lyses buffer containing 50 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS) and 20% glycerol, and vortexed for 3-4 min for homogenization. Insoluble material was removed by centrifugation at 12,000 r.p.m., and the supernatant was collected for protein quantification. Following quantification, β-mercapto ethanol and bromophenol blue were added to a final concentration of 5% (v/v) and 0.1% (w/v), respectively. Samples were then used for SDS gel electrophoresis. Following protein transfer, the membrane was immunoblotted with the following primary antibodies: rabbit anti-IκBα (1:500; Santa Cruz), rabbit anti-Klf4 (1:500; H180, Santa Cruz) and mouse monoclonal anti-β actin (1:2,000; Sigma), overnight at 4˚C. The membrane was incubated for 1h at RT with appropriate secondary antibodies, and the signal detected by chemiluminescence.

RNA extraction and cDNA synthesis

Colons collected from mice were flushed with PBS/RNAlater (Life Technologies, Carlsbad, CA) (1:1). Each colon was then cut open longitudinally and placed on a clean glass slide with luminal surface facing up. Epithelial cells from the luminal side were scraped off, collected and immediately placed in a tube containing acid washed glass beads (425-600 µm) (Sigma) and RLT tissue lyses buffer (RNeasy, Qiagen) containing 0.1% β-mercapto ethanol, and vortexed for 1-2 min for tissue disruption. RNA isolation and purification then proceeded according to manufacturer's recommendations (Qiagen). This was followed by a further purification step using lithium chloride in order to purify the isolated RNA from all polysaccharides including DSS [63]. In brief, the isolated RNA was incubated with 0.1 volume of 8

M LiCl diluted in RNase-free water on ice for 2 h and then centrifuged at 14,000 g for 30 min at 4°C. The supernatant was discarded and the pellets of RNA resuspended in 200 µl of RNase-free water. The 2-hour incubation with lithium chloride, the centrifugation and the pellet suspensions were repeated once more. The RNA was then precipitated at −20°C for 30 min, in 0.1 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of 100% absolute ethanol. The RNA was then centrifuged at 14,000 g for 30 min at 4°C. The supernatants were discarded and the pellets were washed with 100 µl of 70% ethanol and centrifuged at 14,000 g for 10 min at 4°C. The supernatants were removed and the RNA pellet was dissolved in 20–50 µl of RNase-free water. Equal amounts of purified RNA from mice per group were then pooled for cDNA synthesis. First strand cDNA was synthesized using SuperScript VILO cDNA Synthesis Kit (Life Technologies).

Histology, Immunohistochemistry (IHC) and immunofluorescence (IF)

Following stool analysis, isolated colons were flushed with modified Bouin's fixative (50% ethanol, 5% acetic acid), and cut-open longitudinally for gross examination. The colons were then Swiss-rolled, fixed, and embedded in paraffin and 5-mm sections were cut and stained with hematoxylin and eosin for histological examination.

For IHC, sections were deparaffinized in xylene, incubated in 3% hydrogen peroxide in methanol for 30 minutes, rehydrated in ethanol gradient, and then treated with 10 mM Na citrate buffer, pH 6.0, at 120°C for 10 minutes in a pressure cooker. The histological sections were incubated with a blocking buffer (3% bovine serum albumin and 0.01% Tween 20 in 1X Tris-buffered PBS (TTBS)) for 1 hour at RT. Sections were then stained using goat anti-Klf4 (1:300 dilution; R&D Systems, Minneapolis, MN), mouse anti-BrdU (1:50 dilution; BD Pharmingen, San Jose, CA), rabbit anti-Ki67 (1:200 dilution; Leica Microsystems, Buffalo Grove, IL), mouse anti-NFκB (p65) (1:200 dilution; sc-190x, Santa Cruz) and mouse anti-γH2AX (1:500 dilution; 05-636, EMD Millipore, Billerica, MA). Washes were done using 1X TTBS and detection of primary antibodies for IF was carried out using appropriate AlexaFluor labeled secondary antibodies

(Molecular Probes, Carlsbad, CA) at 1:500 dilutions in 3% BSA in TTBS for 30 minutes at 37°C, counterstained with Hoechst 33258 (2 μg/ml), mounted with Prolong gold (Molecular Probes), and cover-slipped. For Klf4 detection by IF, secondary unconjugated rabbit anti-goat antibody (Jackson Immuno Research, West Grove, PA) was added at 1:300 dilution in 3% BSA in TTBS for 30 minutes at 37°C. After washing, goat ant-rabbit AlexaFluor labeled tertiary antibody (Molecular Probes) was then added at 1:500 dilutions in 3% BSA in TTBS for 30 minutes at 37°C, followed by counterstaining and mounting as described above. For inflammation markers, antigen retrieval was carried out in 10 mM Na citrate buffer, pH 6.0, at 120°C for 5 minutes in a pressure cooker, except for F4/80 where antigen retrieval was done by 1% sodium dodecyl sulfate at room temperature for 5 minutes. The following antibodies: AlexaFluor 488 conjugated rat anti-mouse F4/80 (clone BM8, rat IgG2a, Molecular Probes), FITC conjugated hamster anti-mouse CD11c (clone HL3, IgG1 λ2, BD Biosciences), FITC conjugated rat anti-mouse Ly-6G (clone RB6-8C5, IgG2bκ, BD Biosciences), PE conjugated rat anti-mouse IL-6 (clone MP5-20F3, IgG1, BD Biosciences), were used at 1:100 dilution in 3% BSA in TTBS overnight at 4°C. Slides were washed, counterstained and mounted as mentioned above. Detection of primary antibodies for IHC was carried out using appropriate HRP-conjugated secondary antibodies at 1:500 dilutions for 30 minutes at 37°C, and color development was performed using Betazoid DAB Chromogen Kit (BiocareMedical, Concord, CA). Sections were then counterstained with hematoxylin, dehydrated, and cover-slipped. Images were acquired using a Nikon *eclipse* 90i microscope (Nikon Instruments Inc.) equipped with DS- Qi1Mc and DS-Fi1, CCD cameras (Nikon Instruments Inc., Melville, NY). Confocal microscopy using Zeiss LSM510 Meta confocal microscope was employed to visualize γH2AX foci (Zeiss, Thornwood, NY).

Heterotypic cell adhesion assay:

Confluent Caco2-BBE cells were transfected with SC-siRNA, Klf4 siRNA (Ambion/Life Tech, Carlsbad, CA), pEGFP vector expressing GFP, pKlf4-EGFP vector expressing Klf4-GFP

fusion protein, empty vector or with construct encoding Klf4, using Lipofectamine 2000 (LifeTech) according to the manufacturer's instructions in 6 well plates, The following day, Jurkat cells were labeled with 5 uM 2',7' bis-(2-carboxyethyl)-5 (and-6) carboxyfluorescein acetoxymethyl ester (BCECF-AM, Molecular Probes), in culture media for 30 min at 37 °C, then washed 2x PBS and resuspended in RPMI medium. Caco2-BBE medium was then removed and the labeled Jurkat cells were added ($10x10^6$ cells in 2.5 ml of RPMI medium per well) to confluent Caco2-BBE cells and incubated for 30 min at 37 °C. After incubation of Jurkat cells with Caco2-BBE monolayers, the media containing nonattached Jurkat cells were removed, and cells remaining in the wells were washed twice in PBS and lysed in a Triton lysis buffer (10 mM Tris, 150 mM, NaCl, 3 mM, EDTA 1%TritonX-100). The fluorescence was then measured using SpectraMax M3 fluorescence spectrophotometer (Molecular Devices; excitation wavelength, 492 nm; emission wave-length, 520 nm.

SUPPLEMENTARY FIGURE LEGENDS:

Supplementary figure 1. Differences in mRNA levels of Klf4 and inflammatory cytokines and in inflammatory cell infiltrates in the colon between *Klf4*$^{fl/fl}$ and *Klf4*$^{\Delta IS}$ mice following DSS treatment.

(A-D) RT-PCR analysis for relative mRNA levels of Klf4, TNFα, IL-1β and IL-6. (A) Increased Klf4 mRNA level in DSS treated *Klf4*$^{fl/fl}$ mice compared to DSS treated *Klf4*$^{\Delta IS}$ mice. (B-D) compared to DSS treated *Klf4*$^{\Delta IS}$ mice, DSS treated *Klf4*$^{fl/fl}$ mice had elevated mRNA levels of inflammatory cytokines TNFα, IL-1β and IL-6, respectively. (E) Increased inflammatory cell infiltrates: macrophages (F4/80), lymphocytes (CD11c), granulocytes and monocytes (Ly-6G) and for IL-6 positive cells, in *Klf4*$^{fl/fl}$ mice following DSS treatment as compared to DSS treated *Klf4*$^{\Delta IS}$ mice

Supplementary figure 2. Klf4 modulates lymphocytes adhesion to colonic epithelial cells *in vitro* and DSS treatment evokes increase in proliferation in *Klf4*$^{\Delta IS}$ mice.

(A) Heterotypic cell adhesion assay showing significant reduction and increase in lymphocytes (Jurkat cells) adhesion to Caco2-BBE colonic epithelial cells following suppression or overexpression of Klf4 in Caco2-BBE cells, respectively. (B1-3) Ki67 and BrdU IF staining in DSS treated *Klf4*$^{fl/fl}$ mice. (B4-5) Ki67 and BrdU IF staining in DSS treated *Klf4*$^{\Delta IS}$ mice.

Supplementary figure 3. DSS treatment induces DNA damage in the colonic epithelial cells.

IF staining for γH2AX foci marking DNA breaks in the nucleus of colonic epithelial cell of *Klf4*$^{fl/fl}$ mice (A-C), DSS treated *Klf4*$^{fl/fl}$ mice (D-F), *Klf4*$^{\Delta IS}$ mice (G-I) and DSS treated *Klf4*$^{\Delta IS}$ mice (J-L).

Supplementary figure 4. Suppression of Klf4 expression *in vitro* and minimal colonic epithelium loss and inflammation in WT mice given DSS and NP/Klf4 siRNA.

(A) Western blot analysis of WT MEFs transfected or not with Klf4 siRNA. Klf4 expression level was reduced in WT MEFs transfected with Klf4 siRNA. (B) H&E staining of WT mice colon given DSS and NP/SC siRNA (1) or DSS and NP/Klf4 siRNA (2) showing minimal loss of colonic epithelium and ulceration regions in mice given DSS and NP/Klf4 siRNA.

Supplementary figure 5. WT mice given DSS and NP/Klf4 siRNA show reduced Klf4 expression and have sustained proliferation in the colonic epithelium.

(A) Western blot analysis of colonic protein extracts from WT mice (4 individual mice per group) treated or not with DSS and given or not NP/Klf4 siRNA shows reduced Klf4 expression level in colon of WT mice given DSS and NP/Klf4 siRNA. (B) IHC staining for Ki67 colon of WT mice given (1) NP/SC siRNA, (2) NP/Klf4 siRNA, (3) DSS and NP/SC siRNA, (4) DSS and NP/Klf4 siRNA.