Supplementary Information

SUPPLEMENTARY METHODS

Animals, antibodies, cytokines, growth factors, inhibitors, and reagents.

Animals

EGFR^{wa2} and EGFR^{wa5} mice on a C57BL/6 background were obtained from Dr. David Threadgill (University of North Carolina, Chapel Hill). PCR primers specific for the EGFR sequence containing the relevant point mutation were used for genotyping (sequences available upon request).

Antibodies

Rabbit polyclonal active caspase-3, Akt, Bax, Bcl-2, phospho-Ser 473 Akt, phospho-Tyr1068 EGFR antibodies, horseradish peroxidase (HRP) or FITC-conjugated anti-rabbit antibodies, and Cy3-conjugated anti-mouse IgG antibodies were from Cell Signaling Technology, Beverly, MA. Rabbit polyclonal anti-EGFR antibody was from Upstate USA Inc., Charlottesville, VA. Rabbit polyclonal ZO-1 antibody was from Invitrogen Corporation, Carlsbad, CA. Mouse monoclonal β-actin antibody was from Sigma-Aldrich, St. Louis, MO. Mouse monoclonal E-cadherin antibody was from BD Biosciences, San Jose, CA.

Mouse polyclonal antibodies used for flow cytometry, anti-Gr-1 conjugated with phycoerythrin (PE), anti-F4/80 conjugated with allophycocyanin (APC), anti-CD-3 conjugated with FITC, anti-CD-4 conjugated with PE- cyanine (Cy)5.5, anti-TNF conjugated with PE-Cy7, were from BD Biosciences. Anti-IL-13 conjugated with PE was from eBioscience Inc., San Diedo, CA.

Cytokines and growth factors

Murine EGF was a gift from Stanley Cohen (Vanderbilt University, Nashville, TN). Murine TNF, IL-1α, IFN-γ were from Pepro Tech, Inc., Rocky Hill, NJ. Insulin, transferrin, and selenous acid were from BD Biosciences, San Jose, CA.

Inhibitors

EGFR kinase inhibitor, AG1478, and PI3K inhibitor ,Wortmannin, were from Calbiochem, San Diego, CA. Golgi inhibitor was from BD Biosciences.

Reagents and kits

Mouse EGFR SMARTpool siRNA were from Dharmacon, Inc, Lafayette, CO. pcDNA3.1/Zeo vector and Lipofectamine 2000 were from Invitrogen, Carlsbad, CA.

MicroAmp Optical 96-Well Reaction Plates, High Capacity cDNA Reverse Transcription kit , Taqman Gene Expression Master Mix were from Applied Biosystems, Foster City, CA.

EK/LIC Cloning Kit and Ni-MAC purification kit were from Novagen, La Jolla, CA. RNA isolation kit and QIAquick Gel Extraction Kit were from QIAGEN Company, Valencia, CA. Wizard® Genomic DNA Purification Kit was from Promega Corporation, Madison, WI.

Annexin V-FITC and propidium iodide were from Calbiochem/EMD Biosciences, Darmstadt, Germany. ApopTag™ *In Situ* Oligo Ligation Kit (ISOL) was from Intergen Company, Purchase, NY. Sulforhodamine multi-caspase activity kit was from Biomol International, LP, Plymouth Meeting, PA. Alexa Fluor® 488 Protein Labeling Kit was from Invitrogen Corporation, Carlsbad, CA.

Accutase was from Innovative Cell Technologies, Inc. San Diego, CA. CellLytic™ IB Inclusion Body Solubilization Reagent, FITC-conjugated dextran (4000 mol wt), protease and phosphatase 1 and 2 inhibitor cocktails, pectin from Citrus fruit, and zein from Maize were from Sigma-Aldrich. DC protein assay was from Bio-Rad Laboratories, Hercules, CA. DSS (molecular weight 36-50 kDa) was from MP Biomedicals, LLC, Solon, OH. 2,6,4-trinitrobenzenesulfonic acid (TNBS), Oxazolone, and CelLytic™ MT mammalian tissue lysis/extraction reagent were from Sigma-Aldrich.

Vectashield™ Mounting Medium and Antigen Unmasking Solution were from Vector laboratories, Inc. Burlingame, CA. Netwell™ insert (mesh size of 500 μM) was from Corning Incorporated Life Sciences, Acton, MA.

Generation of His-tagged p40 recombinant protein. To generate the p40-expressing plasmid, a ligation-independent directional cloning method without the need for restriction enzyme digestion or ligation reactions (EK/LIC Cloning Kit) was used following the manufacturer's instructions. Briefly, LGG genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit, and this DNA was used as a template for PCR. Primer sequences were 5'-GAGGAGAAGCCCGGTTTAAACGTAGCTGC and 5'-GACGACGACAAGATGAAATTC. PCR products were excised from agarose gels and purified using a QIAquick Gel Extraction Kit. PCR products were cloned into EK/LIC vector. The recombinant plasmid was cloned into NovaBlue GigaSingles™ competent cells. Then the cloned plasmid was transformed into BL21 (DE3)pLysS competent expression cells. Protein expression was induced by IPTG. Inclusion Body Solubilization Reagent was used to solubilize the His-p40 protein from inclusion bodies. p40 recombinant protein was purified using the Ni-MAC Purification Kit. Protein bound to the column was eluted by imidazole and dialyzed to permit refolding. His-p40 was labeled with FITC using regents provided in the Alexa Fluor® 488 Protein Labeling Kit, following the manufacturer's instruction.

The p40 recombinant proteins migrated with an apparent molecular mass of 50 kDa in SDS-PAGE gels. Western blot analysis with anti-p40 and anti-His antibodies was performed to detect this protein (Supplementary Figure 1).

Cell culture

YAMC and EGFR$^{-/-}$MCE cells were maintained in RPMI 1640 media supplemented with 5% fetal bovine serum (FBS), 5 U/ml of murine IFN-γ, 100 U/ml penicillin and streptomycin, 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenous acid on collagen-coated plates at 33°C (permissive condition) with 5% CO_2. Prior to all experiments, cells were serum-starved for 16-18 h in RPMI 1640 containing 0.5% FBS and 100 U/ml penicillin and streptomycin (no IFN-γ) at 37°C (nonpermissive conditions).

The HT29 human colonic epithelial carcinoma cells were grown in DMEM media supplemented with 10% FBS and 100 U/ml penicillin and streptomycin at 37°C. HT29 cells were serum-starved (0.5%) for 16-18 h before experiments.

Preparation of cellular lysates and Western Blot analysis.

Crypts isolated from the colon and cell monolayers were washed twice with ice-cold PBS and then scraped into cell lysis buffer (10mM Tris-HCl (pH 7.4), 150mM NaCl, 1%Triton X-100, 1 mM EDTA, and 1 mM EGTA) with protease and phosphatase 1 and 2 inhibitor cocktails. The scraped suspensions were centrifuged (14,000 x g, 10 min) at 4^0C and protein content was determined using DC protein assay. Cellular lysates were mixed with Laemmli sample buffer and proteins were separated by SDS-PAGE for Western blot analysis with anti-phospho-Ser 473 (P)-Akt, anti-Akt, anti-phospho-Tyr1068 EGFR, anti-EGFR, anti-Bax, anti-Bcl-2, anti-active Caspase-3, and anti-Actin antibodies.

TNBS and p40 treatment. To evaluate the role of p40 in TNBS-induced colitis, mice were treated with 100 μl of 70 mM TNBS in 50% ethanol intrarectally. Control mice received 100 μl of 50% ethanol intrarectally. Mice were sacrificed 4 days after TNBS treatment. Mice were gavaged with p40 (10 μg/day/mouse), beginning on the same day of intrarectal TNBS treatment, until sacrificed.

Analysis of colon inflammation. Paraffin-embedded colon tissue sections were stained with hematoxylin and eosin for light microscopic examination to assess colon injury and inflammation. Samples from the entire colon were examined by a pathologist blinded to treatment conditions. A modified combined scoring system(1) including degree of inflammation (scale of 0-3) and crypt damage (0-4), percentage of area involved by inflammation (0-4) and crypt damage (0-4), and depth of inflammation (0-3) was applied for colitis induced by DSS. The scoring system used to assess oxazolone or TNBS-induced colitis was modified from a previous scoring system (2, 3); lamina propria mononuclear cell and polymorphonuclear cell infiltration,

enterocyte loss, crypt inflammation, and epithelial hyperplasia were scored from 0 to 3, yielding an additive score between 0 (no colitis) and 15 (maximal colitis).

Immunohistochemistry. To unmask antigens, colon sections were boiled for 15 min in Antigen Unmasking Solution. For ZO-1 staining, sections were blocked using 10% goat serum or 1 h and stained with an anti-ZO-1 antibody overnight at 4°C and FITC-labeled secondary antibody for 1 h at room temperature. EGFR-Tyr1068 and E-cadherin double staining was performed by blocking sections using 10% goat serum for 1 h, then incubating with an rabbit polyclonal anti-EGFR-Tyr1068 antibody overnight followed by a mouse monoclonal anti-E-cadherin antibody for 1 hour at 4°C. Then, sections were incubated sequentially with FITC-labeled anti-rabbit (1 h) followed by Cy3-labeled anti-mouse antibodies (1 h) at room temperature. Sections were then mounted using Mounting Medium containing DAPI for nuclear counter-staining and observed by fluorescence microscopy. FITC and DAPI images were taken from the same field.

Mouse colon organ culture. Colon explants obtained from 6-8 week old wt C57BL/6 and $EGFR^{wa5}$ (EGFR dominant negative)(4) mice on a C57BL/6 background were used for organ culture, as described(5). Briefly, the colon was opened and washed with sterile PBS and DMEM media, and then cut into 4 x 4 mm pieces. The colon explants were cultured on Netwell™ inserts in DMEM containing 0.5% FBS at 37 ^{0}C with 5% CO_2 for 1 h before treatment. At the end of the experiment, colon tissue was lysed in tissue homogenization buffer(6) or fixed in 4% paraformaldehyde at 4^{0}C overnight before preparing paraffin-embedded tissue sections.

Colonic cell isolation for immunophenotyping. The colon was weighed, cut into small pieces, and digested in DMEM containing 1 mg/ml dispase, 0.25 mg/ml collagenase A, and 25 U/ml DNase at 37° for 20 minutes with shaking. The suspension was passed through a 70-μm cell strainer. Cells were harvested by centrifugation and washed with DMEM. Viable cells were counted with trypan blue. Cells were incubated with Golgi inhibitor in DMEM containing 10% FBS at 37° for 4 hours to block protein transport (secretion).

To label cell surface markers for testing cell types, cells were labeled with fluorescent-tagged antibody mixtures containing antibodies specific against respective cell markers for 30 min at room temperature. We used the following antibodies for detection of indicated cell types: anti Gr-1-PE (neutrophil, dilution 1:100); anti F4/80-APC (macrophage, dilution 1: 200); anti CD-3-FITC (dilution 1: 100); and anti CD-4-PE-Cy5.5 (dilution 1: 250).

The cells were then fixed in 0.1% paraformaldyhyde/PBS at 4° overnight and then permibilized for labeling intracellular cytokine proteins. Cells were labeled with anti TNF-Cy7 (dilution: 1:200) or IL-13-PE (dilution: 1:200) 30 minutes at 4°C.

Cells were analyzed by multi color flow cytometry to determine the percentage of positive cells using a BD LSRII system (BD Biosciences). The results were calculated as: % of cells x total immune cells / colon weight (gram) = number of cells/gram of colon weight.

Real-Time PCR analysis. Total RNA was isolated from homogenized whole mouse colon tissue using the Qiagen RNA isolation kit and was treated with RNase-free DNase. Reverse transcription was performed using the High Capacity cDNA Reverse Transcription kit. For Real-Time-PCR reactions, 25 µl reactions were set up by addition of 1.25 µl of primer mix (containing 5 µM of reverse and forward primers), 5 µl of diluted cDNA template, and 12.5 µl of Taqman Gene Expression Master Mix. Real-Time PCR was performed using the 7300 Real Time PCR System (Applied Biosystems). The data were analyzed using the Sequence Detection System V1.4.0 software. The relative abundance of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used to normalize levels of the mRNAs of interest. All cDNA samples were analyzed in triplicate. Sequences of primer and probe sets (Applied Biosystems) used in this study, including TNF, IFN-γ, KC, IL-1β, IL-6, Il-10, IL-13, and IL-17, will be provided upon requested.

SUPPLEMENTARY RESULTS

p40 prevents TNBS-induced colitis in mice.

TNBS-induced colitis is mediated by IL-12 driven Th1 immune responses, including increased TNF and IFN-γ production, and has been shown to resemble the pathogenesis of Crohn's disease(7). We sought to determine the role of p40 in prevention of colitis in this model. Wt mice on the C57Bl/6 background were gavaged with p40 beginning on the same day of intrarectal TNBS administration, until mice were sacrificed after 4 days of TNBS treatment. p40 treatment prevented TNBS-induced body weight loss (Supplementary Figure 12A) and shortening of the colon length (Supplementary Figure 13B), compared to the p40 untreated group. In addition, TNBS-induced histological changes, including the disruption of the epithelial monolayer and inflammatory cell infiltration were relieved by p40 treatment (Supplementary Figure 12C-D). We did not find significant changes in FITC-dextran levels in the serum and distribution of ZO-1 protein in the colon epithelium in C56Bl/6 mice treated with TNBS for 4 days, compared to enthanl treated mice. However, TNBS increased colon epithelial cell apoptosis, which was inhibited by p40 treatment in mice (Supplementary Figure 12E). We also fould that p40 treatment decreased levels of TNF and IFN-γ in the colon mucosa in this model (data not shown). Thus, these data suggest that p40 exerts beneficial effects on prevention of TNBS-induced colitis.

p40 regulates immune responses in colitis

In addition to regulation of intestinal epithelial homeostasis, probiotics have been reported to regulate immune responses in several diseases, including colitis(8-10). Therefore, we tested the effects of p40 on inflammatory cytokine production in DSS or oxazolone-induced colitis models. We used real-time PCR analysis of RNA isolated from the colon mucosa to detect cytokine message levels and flow cytometry to detect intracellular cytokine protein levels in macrophages and lymphocytes.

First, we found that p40 treatment significant decreased infiltration macrophage (Supplementary Figure 9A) and neutrophil (Supplementary Figure 9C) into the colon in DSS-induced colitis. However, either DSS treatment only or DSS with p40 co-treatment affected CD3

(Supplementary Figure 9D) or CD4 (Supplementary Figure 9E) cell infiltration into the colon.

We further found that DSS treatment increased TNF, IL-6, KC, IFN-γ, IL-17, IL-10 and IL-1β levels, but not IL-13 level, in wt mice (Supplemental Figures 10). p40 treatment reduced TNF, IL-6, KC, IFN-γ and MCP-1 production, but not IL-1β, IL-10, and IL-17 in wt mice treated with DSS (Supplementary Figure 10). We further found that p40 inhibited TNF-positively stained macrophages in DSS-treated wt mice using flow cytometry (Supplementary Figure 9B). p40 regulation of cytokine production in DSS model has also been confirmed in our preliminary data using Luminex assay (data not shown).

Based on the cytokine profiles that are regulated by p40, it is possible that p40 plays a role in regulation of innate immunity and a Th1 immune response in DSS-induced colitis, such as regulation of TNF, IL-6 and KC production by macrophages and IFN-γ production by Th1 cells.

Since IL-13-mediated Th2 response is the driving inflammatory stimulus for oxazolone-induced colitis (11), we detected the effects of p40 on IL-13 production in this model. We found that p40 treatment did not affect IL-13 production detected using flow cytometry or mRNA levels by real-time PCR in oxazolone treated mice (Supplementary Figure 11). These data indicate that p40 may not have effects on the Th2-driven immune responses. Since we found that p40 failed to prevent oxazolone-induced colitis in $EGFR^{f/f}$-Cre mice with EGFR specifically deleted in the intestinal epithelium (Figure 9D-F), we suggest that activation of EGF receptor in the intestinal epithelial cells play a role in ameliorating colitis in this model.

FIGURE LEGENDS

Supplementary Figure 1. Generation of a recombinant His-tagged p40 fusion protein. Proteins present in the soluble fraction and inclusion bodies of competent cells and eluted fractions from a Ni-MAC column were separated by SDS-PAGE and stained with Colloidal Blue Staining kit

(A). Anti-p40 and anti-His antibodies were used in Western blot analysis (B). His-p40 was used to treat YAMC cells to detect Akt activation by Western blot analysis as described in Figure 1 (C). Arrows represent the His-p40 band. Cont: bacterial culture with IPTG induction. S: soluble fraction, I: inclusion bodies, p40 in (B): the purified fraction of p40, Y: YAMC cellular lysate.

Supplementary Figure 2. Kinetic comparison of EGFR and Akt activation by p40 to EGF. YAMC cells were treated with 2.5 μM p40 or EGF for the indicated times. EGFR and Akt phosphorylation was detected by Western blot analysis of cellular lysates as described in Figure 1. Anti-actin antibody was used as a loading control. The relative densities of protein bands on Western blots were determined by comparing densities of the phospho-Akt bands to the actin bands. The relative density of band from the control was set as 100%, and the relative densities of bands from p40 or EGF-treated samples were compared to those in the control group. Data in this Figure are representative of three separate experiments. * $p<0.001$ and # $p<0.05$, compared to the un-treated control group.

Supplementary Figure 3. Delivery of p40 to the colon using pectin/zein hydrogel beads. Pectin/zein beads containing p40 or FITC-labeled p40 (10 μg) were administered to wt C57BL/6 mice by gavage. Mice were sacrificed 4 h after gavage. FITC-labeled p40 in paraffin-embedded colon sections was visualized using fluorescence microcopy (green staining). Nuclei were stained using DAPI (blue staining). Three parts of the colon, proximal, middle, and distal colon are shown.

Supplementary Figure 4. p40 reduces colon epithelial cell apoptosis in an EGFR-dependent manner during the treatment of DSS-induced colitis. Mice were treated with DSS for 4 days to induce colitis and then p40 was administered for the following 3 days as described in Figure 6. Paraffin-embedded tissue sections were prepared for ISOL staining to detect apoptosis.

Apoptotic nuclei labeled with peroxidase were visualized using DIC microcopy (A). Arrows indicate ISOL-labeled apoptotic nuclei (brown in B). The number of apoptotic nuclei per 100 crypts is shown (B). ISOL: In situ oligo ligation kit. In Figure 4B, * $p<0.01$ compared to water groups in wt or EGFR^{wa2} mice, and # $p<0.05$ compared to wt mice treated with DSS.

Supplementary Figure 5. EGFR kinase activity is required for p40 to restore intestinal barrier function during the treatment of DSS-induced colitis. Mice were treated as described for Supplementary Figure 4. Paraffin-embedded colon tissues were stained with anti-ZO-1 antibody (green staining) and DAPI (blue staining), as described in Figure 7.

Supplementary Figure 6. Specific deletion of EGFR in the intestinal epithelium in EGFR$^{f/f}$-Cre mice. Paraffin-embedded colon sections were prepared for immunohistochemistry to detect EGFR expression using a rabbit anti-EGFR antibody and FITC-conjugated secondary antibody (Green staining) and nuclei using DAPI staining (blue staining) (A). Colon epithelial cells and stroma were isolated for Western blot analysis to detect EGFR and E-cadherin (an epithelial cell marker) expression using a rabbit anti-EGFR and a mouse anti-E-cadherin antibody, respectively. Anti-actin antibody was used as a loading control. Green arrow in (A) indicates EGFR expression.

Supplementary Figure 7. p40 fails to prevent DSS-induced colitis in EGFR$^{f/f}$-Cre mice. Mice were treated with 3% DSS in drinking water for 7 days (7D), and were gavaged with pectin/zein control beads or beads containing p40 at 10 μg/mouse/day, beginning on the same day of DSS treatment until the end of the experiment, as described in Figure 4. Control mice received water alone. Body-weight changes are shown (A). Paraffin-embedded colon sections were stained with H&E for light microscopic assessment of epithelial damage. Colon injury scores are shown (B). The length of colon was measured (C). In A, * $p<0.05$ compared to wt mice at the same day

of DSS treatment. In B and C, * $p<0.05$ compared to water groups in $EGFR^{f/f}$ or $EGFR^{f/f}$-Cre mice, and # $p<0.05$ compared to DSS-treated $EGFR^{f/f}$ mice.

Supplementary Figure 8. The N-terminal portion of p40, but not the C-terminal portion, mimics the activity of full-length p40. Recombinant His-tagged p40 N-terminal 1-180 aa (p40-N) and C-terminal 181-412 aa (p40-C) peptides were expressed and purified, using the same method as for full-length p40. Purified peptides were analyzed by Western blot analysis, using anti-p40 and anti-His antibodies (A). YAMC cells were treated with recombinant full-length (FL) p40, p40-N, and p40-C peptides for 1 hour. EGFR and Akt activation were detected by Western blot analysis of total cellular lysates using antibodies against EGFR-phospho (P)-Tyr1068 and Akt-phospho (P) Ser473, respectively. Actin blot was used as protein loading control (B). HT29 cells were treated with the "cytokine cocktail" containing TNF (100 ng/ml), IL-1α (10 ng/ml) and γ-IFN (100 ng/ml) for 16 hours in the presence or absence of 1-hour pretreatment with p40-FL, p40-N and p40-C (10 ng/ml). HT29 cells were dissociated and stained with Annexin V-FITC and propidium iodide, and analyzed by flow cytometry. Percentage of apoptotic cell populations is shown (C). C57BL/6 mice were treated with 3% DSS in drinking water for 4 days in the presence or absence of gavage with pectin/zein beads contain p40-N or p40-C peptides. Paraffin-embedded colon sections were stained with H&E for light microscopic assessment of epithelial injury and inflammation. Colon injury scores are shown (D). In (C), * $p<0.01$ compared to control, and # $p<0.05$ compared to cytokine treatment only or cytokine with p40-C 181-412 aa. In (D), * $p<0.01$ compared the water group, and # $p<0.05$ compared to DSS treatment only or DSS with p40-C.

Supplementary Figure 9. p40 blocks DSS-induced neutrophil and macrophage infiltration into the colon and TNF production in colon macrophages. Mice were treated as described in Figure 4. The colon tissues were weighted and the total number of colonic immune cells were isolated and recorded for flow cytometric staining of macrophage marker F4/80 (A), TNF (B), neutrophil

marker, Gr-1 (C), CD3 (D) and CD4 (E). For TNF staining, cells were incubated with a Golgi inhibitor to block TNF secretion.

Supplementary Figure 10. p40 suppresses DSS-induced proinflammatory cytokine gene expression in the mouse colon. Mice were treated as described in Figure 4. mRNA was isolated from the colon tissues for real-time PCR analysis of indicated cytokine mRNA expression levels. The cytokine mRNA expression level in control mice (water only) was set as 100%, mRNA expression levels in treated mice were compared to the control group.

Supplementary Figure 11. P40 does not affect IL-13 production in oxaxolone treated mice. Mice were treated with oxazolone (Oxa) as described in Figure 8. The colon tissues were weighted and the total number of colonic immune cells were isolated and recorded. Cells were incubated with a Golgi inhibitor to block IL-13 secretion and stained using an anti-IL-13-PE antibody. IL-13 production was analyzed by flow cytometry.

Supplementary Figure 12. P40 prevents TNBS-induced colitis in mice. TNBS was administered rectally in the presence or absence of gavage with p40 containing-beads at 10 μg/mouse/day, beginning on the day of rectal TNBS administration until the end of the experiment. Mice were sacrificed 4 days after intrarectal challenge of TNBS. Control mice received ethanol only. Body-weight changes after intrarectal challenge of ethanol or TNBS are shown (A). Colon length was recorded (B). Paraffin-embedded colon sections were stained with H&E for light microscopic assessment of epithelial damage (C). Colon injury scores are shown (D). Paraffin-embedded tissue sections were analyzed using ISOL staining to detect apoptosis, as described in Figure 6. The number of apoptotic nuclei per 100 crypts is shown (E).

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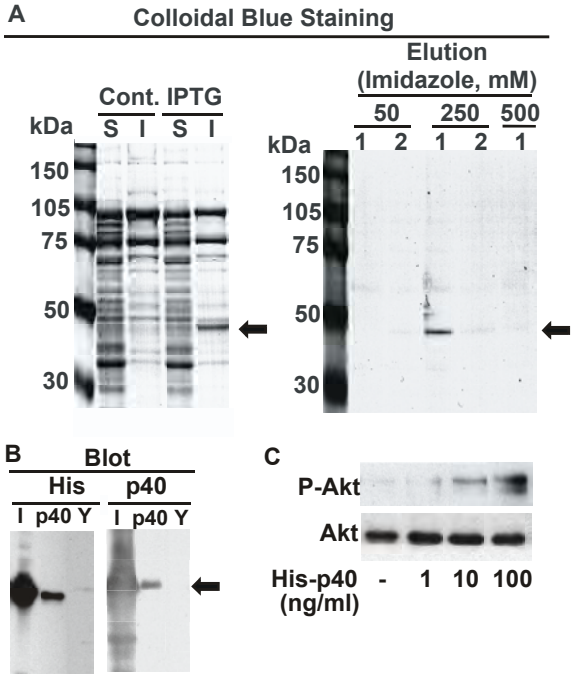
Supplementary Figure 1
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism



Supplementary Figure 2
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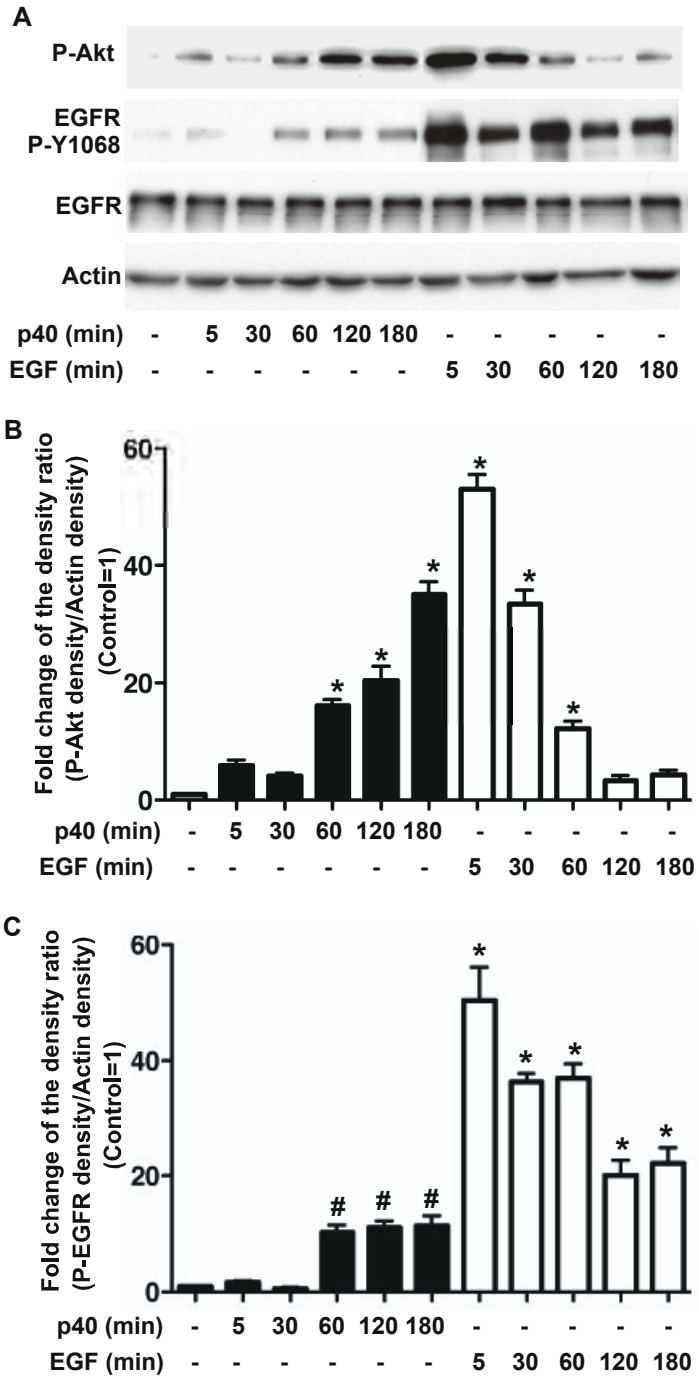

Supplementary Figure 3
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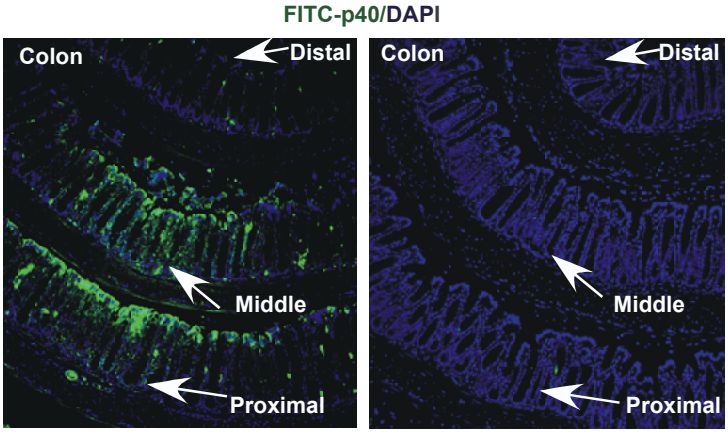
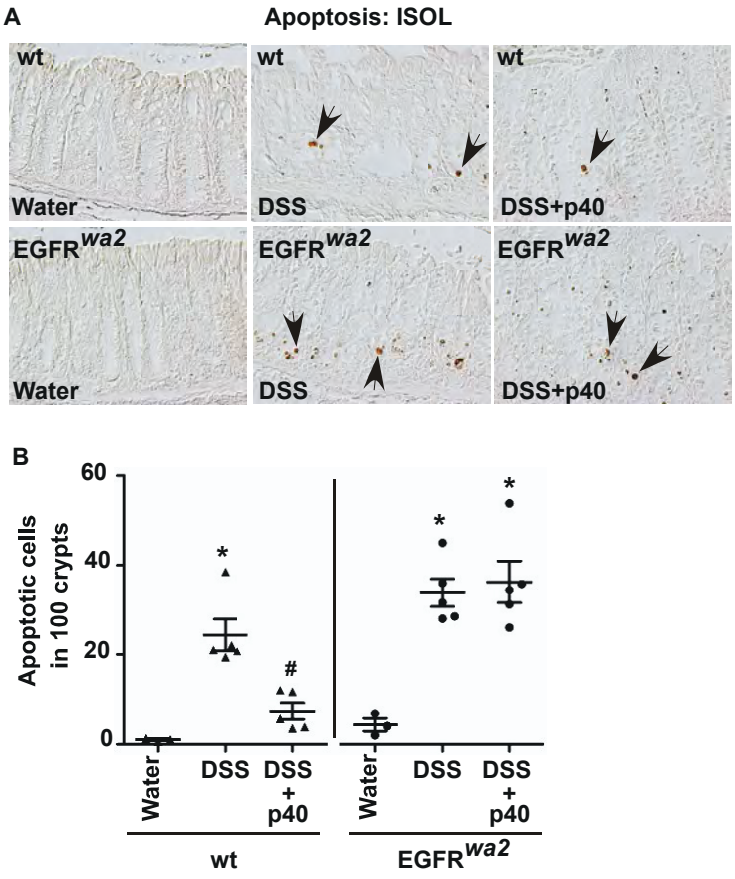
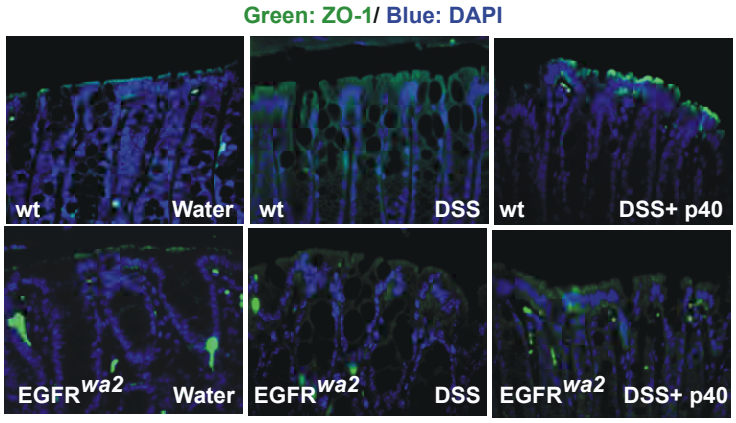


Supplementary Figure 4
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism



Supplementary Figure 5
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism



Supplementary Figure 6
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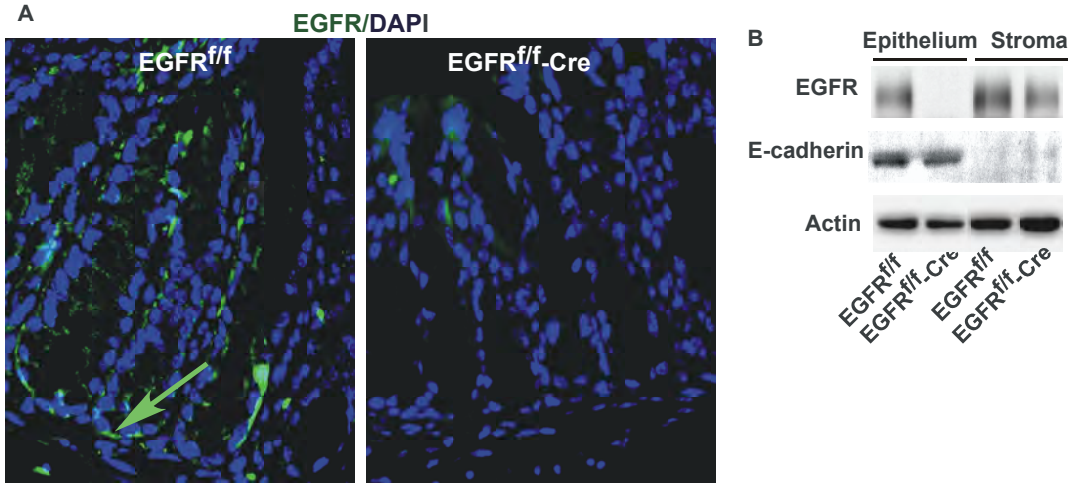
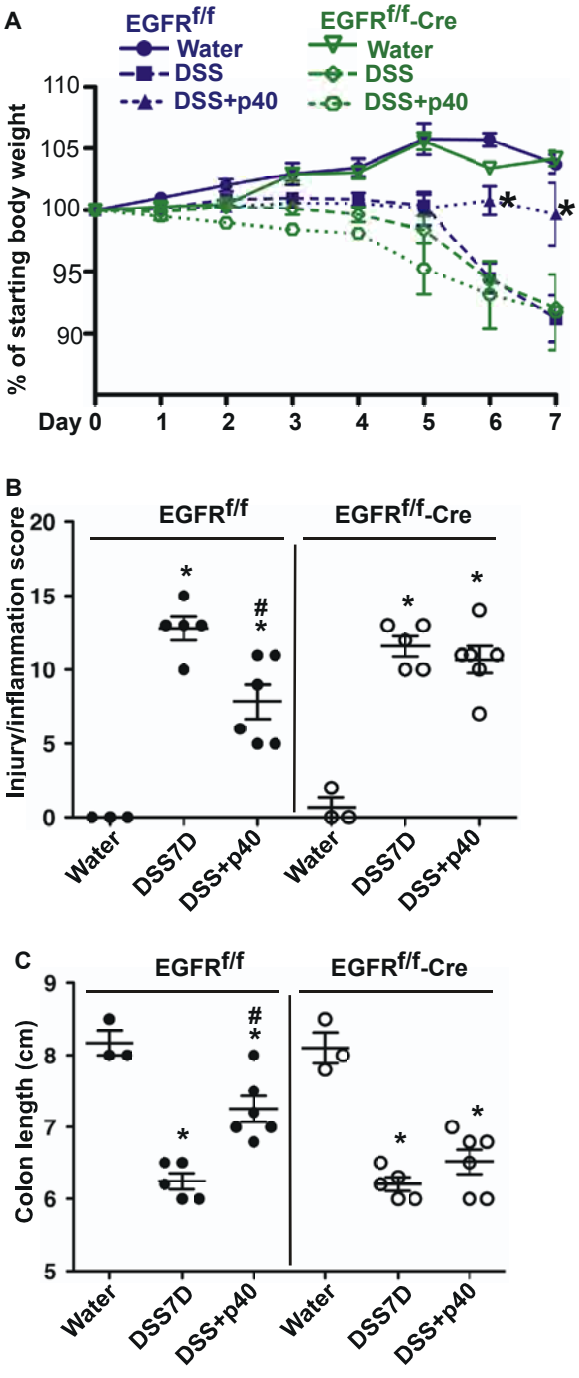
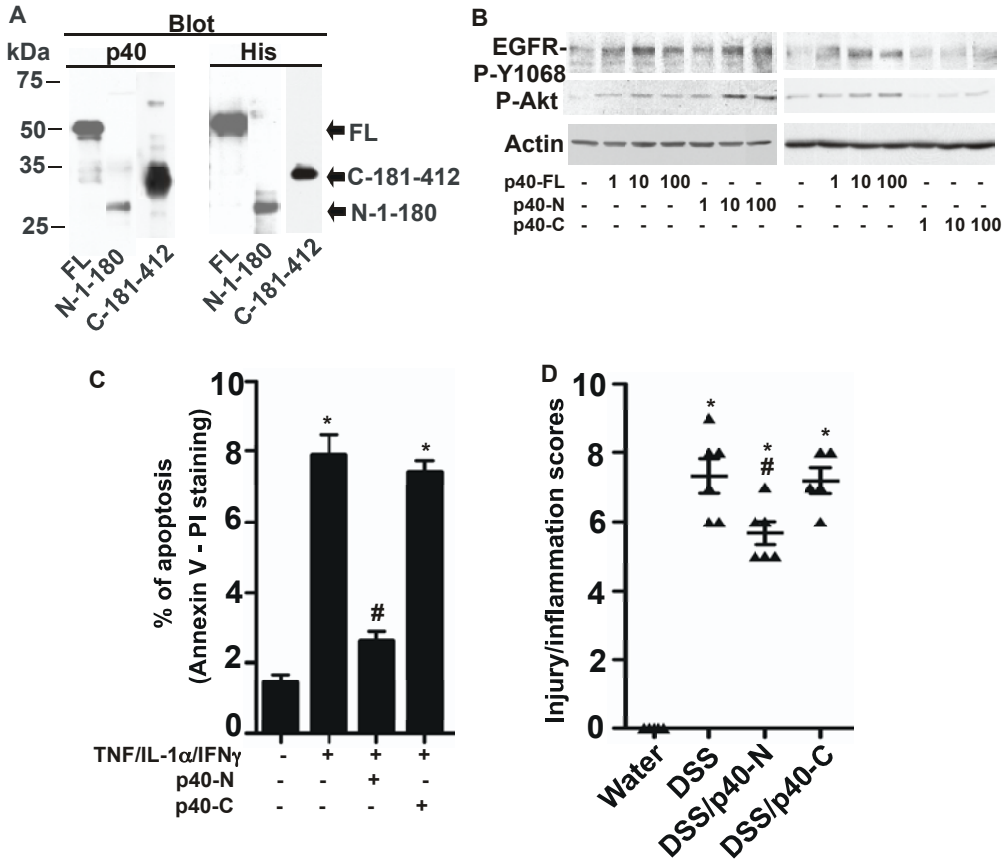


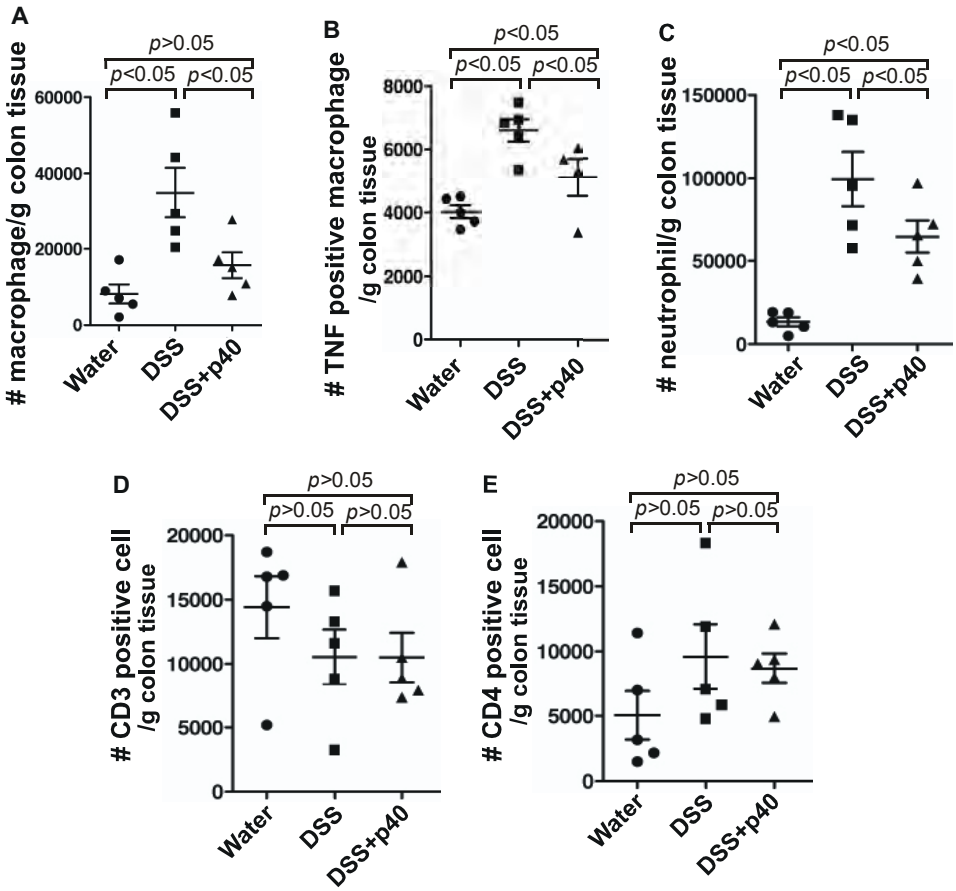
Supplemental Figure 7
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism



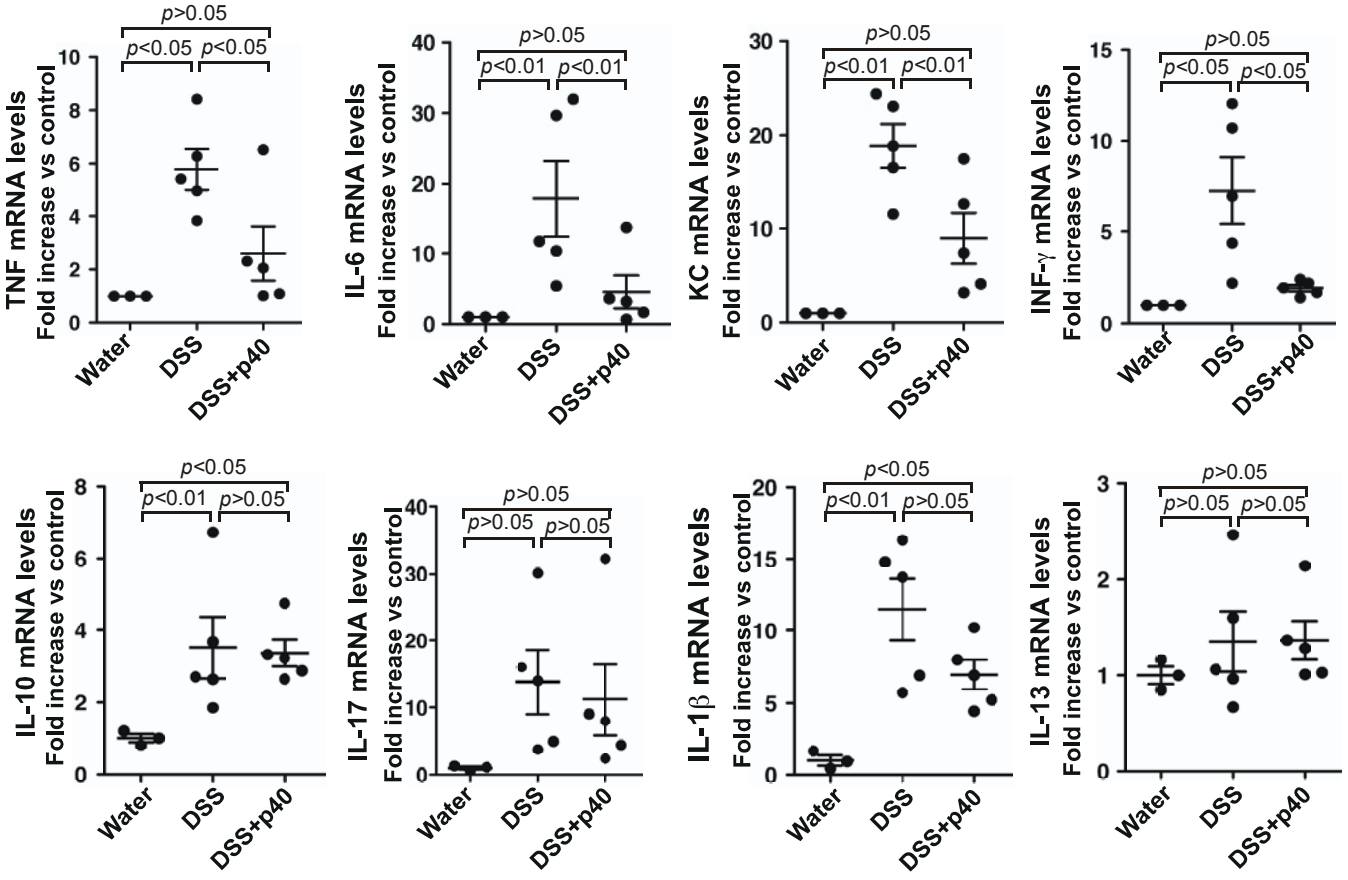
Supplementary Figure 8
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism



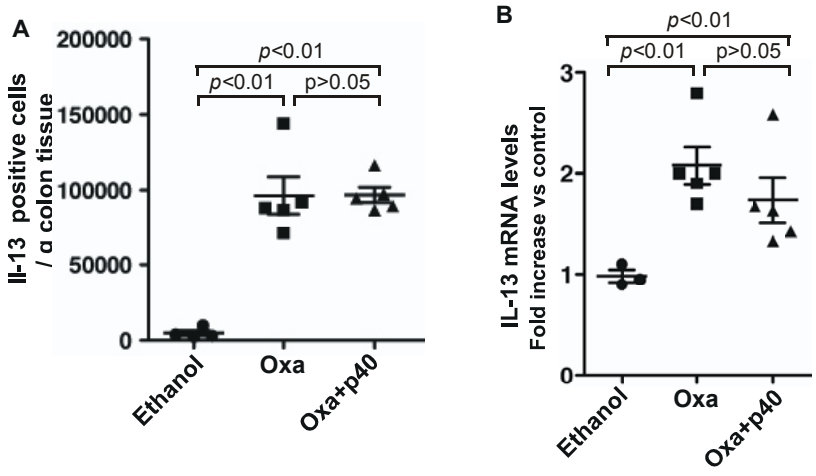
Supplementary Figure 9
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism



Supplementary Figure 10
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism



Supplementary Figure 11
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism



Supplementary Figure 12
Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGF receptor-dependent mechanism