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An Inducible, Large-Intestine-Specific Transgenic Mouse Model for Colitis and Colitis-Induced Colon Cancer Research

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Abstract

Background—Animal models are an important tool to understand intestinal biology. Our lab previously generated C57BL/6-Tg(*Carl-cre*)5Flt transgenic mice (CAC) with large intestine specific Cre recombinase (Cre) expression as a model to study colon health.

Aims—To expand the utility of the CAC mouse model by determining the impact of chemically induced colitis on CAC transgene expression.

Methods—CAC mice were crossed to Rosa reporter mice (*Rosa26R^{flox/flox}*) with a lox-STOP-lox signal controlling β -galactosidase (β gal) expression and then further crossed with *Apc^{CKO/CKO}* mice in some experiments to delete *Apc* alleles (*Apc⁵⁸⁰*) Initially, 8-wk-old CAC^{Tg/WT}; *Rosa26R^{flox/WT}*; *Apc^{580/WT}* mice were treated with dextran sulfate sodium (DSS) in drinking water (5 d, 0, 0.65%, 1.35%, or 2.0%). Colon tissue damage and β gal labelling were analyzed 10-d after stopping DSS. Next, 8-wk-old CAC^{Tg/WT}; *Rosa26R^{flox/flox}* mice were treated with 0 or 1.35% DSS, and colonic β -gal labeling was assessed at 30-d post DSS treatment. Finally,

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Ms. Fa Wang and Dr. DeSmet have no conflicts to report.

Dr. Johnson is currently an employee of Eli Lilly and Company.

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Ethical approval: All procedures performed in studies involving animals were in accordance with the ethical standards of Purdue University Animal Care and Use Committee at which the studies were conducted.

This article does not contain any studies with human participants performed by any of the authors.

Authors contributions: Conception and design: JCF; Development of methodology: FW and RLJ; Acquisition of data: FW, RLJ and MLD; Analysis and interpretation of data: FW, RLJ and JCF; Administrative, technical or material support: JCF and PWS; Study supervision: JCF.

10-wk-old $CAC^{Tg/WT};Apc^{580/WT}$ mice were treated with DSS (0 or 2%) for 5 d and colonic tumors were analyzed at 20-wk.

Results— $CAC^{Tg/WT};Rosa26R^{floxed/WT};Apc^{580/WT}$ mice had a DSS dose-dependent increase in colon epithelial damage that correlated with increased epithelial β -gal labeling at 10-d ($r^2 = 0.9$, $\beta = 0.75$). The β -gal labeling in $CAC^{Tg/WT};Rosa26R^{floxed/floxed}$ mice colon remained high at 30-d, especially in the crypts of the healed ulcer. DSS also increased colon tumor incidence and multiplicity in $CAC^{Tg/WT};Apc^{580/WT}$ mice.

Conclusions—DSS-mediated epithelial damage induces a persistent, Cre-mediated recombination of floxed alleles in CAC mice. This enables the examination of gene function in colon epithelium during experimental colitis and colitis-induced colon cancer.

Keywords

Colorectal cancer; Transgenic animal model; Cre recombinase; Colitis

Introduction

Colorectal cancer is a multi-factorial disease and its development can be influenced by genetics, diet, inflammation, environment and other factors [1-7]. Unfortunately, the etiology of colorectal cancer remains unclear, thus barriers exist to develop approaches to prevent and treat the disease [8-10]. Controlled animal studies are an important approach to understand colorectal cancer biology and to test promising strategies for disease prevention and treatment. As such, an optimal animal model of colorectal cancer should capture the pathological changes, molecular mechanisms, and clinical symptoms presented in the human disease. In addition, the cancer effect should be limited to colon to limit any confounding response from other tissues.

Many animal models have been developed for colorectal cancer research, and they have been reviewed in several recent comprehensive reviews [11-13]. Our lab previously generated a transgenic mouse model for colorectal cancer research with large intestine-specific Cre recombinase (Cre) transgene expression, i.e. the C57BL/6-Tg(Car1-cre)5Flt or CAC mouse [14]. In colon epithelial cells of CAC mice, Cre is expressed in approximately 10% of the epithelium. Here we report that Cre expression in colon epithelial cells of CAC mice can be induced during dextran sulfate sodium (DSS) induced colitis. In addition, our data suggest that these recombination events induce a persistent effect that can be used to study genes involved in the epithelial response to colitis or in colitis-associated colon cancer.

Materials and Methods

Animals

C57BL/6-Tg(Car1-cre)5Flt mice were created by our group [14] and maintained as heterozygotes ($CAC^{Tg/WT}$). The *Rosa26R* strain, B6.129S4-*Gt(ROSA)26Sortm1Sor/J*, was obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained as homozygotes ($ROSA^{floxed/floxed}$). *Rosa26R* mice contain a STOP sequence that is flanked by LoxP sites upstream from the *E. coli* β -galactosidase (β gal) gene within a gene trap of the *Rosa26* allele

[15]. The *Apc*^{CKO} mouse carrying a floxed exon 14 of the *Apc* gene (*Apc*^{CKO/CKO}) was obtained from the Mouse Models of Human Cancer Consortium (National Cancer Institute, Frederick, MD). In cells expressing Cre, exon 14 is removed resulting in a truncated 580 amino acid long protein produced from one (*Apc*^{580/WT}) or both (*Apc*^{580/580}) *Apc* alleles. Genotypes were determined by standard PCR methods as previously described for the CAC [14], *Rosa26R* [15], and floxed *Apc* alleles [14]. Each strain of mouse was maintained on a C57BL/6J background. Mice were bred to create experimental animals with specific combinations of genotypes. All mouse breeding colonies were housed individually, given a standard chow diet and water *ad libitum*, and exposed to a 12 hours light/12 hours dark cycle. The experiments were conducted with approval from the Purdue University Animal Care and Use Committee.

Experimental Design

Experiment 1—CAC^{Tg/WT}; *Rosa26R*^{flox/flox} mice were crossed to *Apc*^{CKO/CKO} mice to generate CAC^{Tg/WT}; *Rosa26R*^{flox/WT}; *Apc*^{580/WT} mice. Experimental mice were weaned at 3 weeks of age and fed the AIN93G diet [16]. Mice were randomly assigned into one of four dextran sulfate sodium (DSS) treatment groups (0%, 0.65%, 1.35% and 2%, n=16 per group, 8 male and 8 female). DSS (MW = 36,000-50,000 d, MP Biomedicals, LLC, Solon, OH) was diluted in deionized water (w/v) to the appropriate concentrations. At 8 weeks of age, mice received the DSS solution in place of water *ad libitum* for 5 consecutive days. Afterwards, DSS was replaced with deionized water and this was provided until the day of sacrifice. Half of the mice in each group were sacrificed 2 days after ending the DSS treatment and half were sacrificed 10 days after ending DSS treatment. All mice were fasted overnight before harvest. At necropsy, the proximal and distal colon were prepared as Swiss rolls and examined for β-galactosidase levels by immunohistochemistry.

Experiment 2—24 CAC^{Tg/WT}; *Rosa26R*^{flox/flox} mice (12 female and 12 male) were weaned at 3 weeks of age and fed AIN93G diet. At 8 weeks of age, 16 mice (8 male and 8 female) were treated with 1.35% DSS for 5 days and the other 8 mice (4 male and 4 female) were used as vehicle controls (0% DSS). Half of the DSS treated mice and all the vehicle control mice were sacrificed 10 days after completing the DSS treatment. The other mice in the DSS treatment group were sacrificed 30 days after completing the DSS treatment. All mice were fasted overnight before harvest. At necropsy, the proximal and distal colon were prepared as Swiss rolls and examined for β-galactosidase levels by immunohistochemistry. Small intestine, kidney, spleen, liver and lung were also collected for detection of β-galactosidase enzymatic activity.

Experiment 3—13 CAC^{Tg/WT}; *Rosa26R*^{flox/WT}; *Apc*^{580/580} mice with two recombined *Apc* alleles were generated by crossing CAC^{Tg/WT}; *Rosa26R*^{flox/WT}; *Apc*^{580/WT} mice with *Apc*^{CKO/CKO} mice. Mice were sacrificed at 4 weeks of age after an overnight fast. At necropsy, the proximal and distal colon were prepared as Swiss rolls and examined for β-galactosidase levels by immunohistochemistry.

Experiment 4—81 CAC^{Tg/WT}; *Apc*^{580/WT} mice were generated by crossing CAC^{Tg/WT} mice with *Apc*^{CKO/CKO} mice. CAC^{Tg/WT}; *Apc*^{580/WT} pups were weaned at 3 weeks of age

and fed an AIN93G diet. $CAC^{Tg/WT};Apc^{580/WT}$ mice were treated with either 2% DSS (n=38) or deionized water (n=43) at 10 weeks of age for 5 days, then the DSS was replaced with water. Mice were sacrificed at 20 weeks of age after an overnight fast. The colon was removed, opened longitudinally, and cleaned with PBS. Digital images were collected and analyzed to determine the number, location, and size of each tumor.

Detection of β -galactosidase Activity in Organs

Tissues were dissected from the mice, rinsed briefly in cold PBS, and fixed for 1-2 hours in ice cold 4% paraformaldehyde. After rinsing the tissue for 15 minutes in PBS, tissues were incubated for 4 hours in 1 mg/mL 5-bromo-4-chloro-3-indoyl- β -D-galactopyranoside (X-gal; Invitrogen; B-1690) solution (pH 7.6) at 37°C as previously described [14]. In tissues expressing β gal (indicative of Cre-mediated recombination of the *Rosa26R* locus), the enzyme converted the X-gal substrate into a dark blue crystalline precipitate that is visible upon gross examination.

Tissue Preparation and Immunohistochemical Detection of β -galactosidase

The colon was opened longitudinally, rinsed with cold PBS, and transected at its midpoint to separate the proximal and distal colon. Each section was split longitudinally and Swiss-rolled [17], resulting in 2 rolls of proximal colon and 2 rolls of distal colon per mouse. Tissues were immersion fixed for 48-72 hours in 10% neutral buffered formalin at 4°C before being transferred to 70% ethanol. Tissues were processed and embedded in paraffin within 7 days of collection at the Purdue Histology and Phenotyping Laboratory.

Four micron tissue sections were cut, de-paraffinized, and rehydrated through xylene, ethanol, and water by standard methods. For antigen retrieval, slides were submerged in 0.01 mol/L sodium citrate (pH 6.0) and heated to 96°C for 20 minutes in a laboratory microwave (PELCO, Edmond, OK). Immunohistochemistry was performed on a Dako Autostainer (Dako, Denmark). Slides were incubated with 0.03% hydrogen peroxide (5 minutes) and serum-free protein block (30 minutes; Dako; Denmark), followed by a 120 minute incubation with rabbit polyclonal anti- β -galactosidase (β gal) antibody (1:400 dilution; Novus; NB100-65209). Antigen binding was detected with a horseradish peroxidase-labeled anti-rabbit IgG polymer (Vector laboratories; MP-7410) and 3, 3'-diaminobenzidine (DAB; Vector laboratories; SK-4100) as the chromogen. Slides were counterstained with Harris hematoxylin (EK Industries, Juliet, IL). The digital images were collected with an Aperio ScanScope slide scanner (Leica Technologies, Buffalo Grove, IL) at 20X magnification.

Image Analysis

Mucosal transgene expression was determined by quantifying the percent β gal positive pixels after immunohistochemistry. The colonic mucosa was differentiated from the tunica muscularis using the Genie histology pattern recognition software (Aperio, Leica Biosystem) trained at 5X magnification and 1000 iterations to a mean training accuracy of 96%. A positive pixel count algorithm was then used to analyze DAB labeling within the mucosa. This analysis was done by a board certified veterinary pathologist (RLJ) and confirmed independently (by FW).

The area of β gal positive crypts was determined by manually measuring the length of the mucosal surface containing β gal labeled crypts. β gal positive crypts were included only if the entire crypt axis, from base to lumen, was within the plane of section or if the crypt base could be seen immediately adjacent to the muscularis mucosa. The percentage of area represented by β gal positive crypts was calculated by dividing the length of the β gal positive area by the total length of the mucosa surface.

The damaged tissue surface area included the areas of ulceration and areas undergoing active healing. Ulceration was defined as sections of the colon mucosa devoid of crypts. This phenotype is obvious at day 2 after DSS termination and increased with DSS dose. Tissue healing included restitution (a single layer of epithelial cells covering an ulcer bed), crypt fission, and crypt regeneration - two events that repopulate the organized crypt structure within an ulcer. These phenotypes were observed at day 10 after DSS termination and continued through day 30 after DSS termination. The percentage of damaged surface was defined as the length of damaged mucosa divided by the total length of the mucosal surface.

Statistical Analysis

Statistical analysis was performed with SAS Enterprise 5.1 software (SAS Institute, Cary, NC). All data were reported as the mean \pm standard error of the mean (SEM). Normality of the data was determined by evaluation of its histogram and a Shapiro-Wilk normality test ($p > 0.05$). All of the data were normally distributed. Statistical comparisons were made by two-tailed student's t-tests, one-way ANOVA or Chi-square test as appropriate. Following one-way ANOVA, the Bonferroni multiple comparison test or planned orthogonal contrasts were used to compare groups. Differences were considered significant at $p = 0.05$ for all the statistical comparisons.

Results

Cre-mediated recombination is increased by DSS treatment in $CAC^{Tg/WT}; Rosa26R^{flox/WT}; Apc^{580/WT}$ mice

Our group has previously reported that CAC mice have recombination of the Rosa allele that is restricted to the large intestine epithelial cells [14]. In our experiments, β -gal labeling is used as an indicator of Cre-mediated transgene recombination. In experiment 1, we examined the proximal and distal colon of $CAC^{Tg/WT}; Rosa26R^{flox/WT}; Apc^{580/WT}$ mice without DSS treatment (Fig 1A-D). The proximal colon under the basal, non-DSS condition had significantly more β -gal positive cells than the distal colon (Fig. 1E, $p = 0.0023$). However, β -gal labeling in proximal colon was mostly superficial, while entire crypts were labeled in distal colon (Fig. 1B, D). As a result, 5-fold more β -gal positive crypts were observed in the distal colon than the proximal colon, (Fig. 1F, $p = 0.0011$).

Ten days after DSS treatment β -gal labeling was significantly increased at all three DSS doses (Fig. 2A, vehicle vs DSS, Proximal colon: $p < 0.0001$; Distal colon: $p = 0.0007$). In both the proximal and distal colon, a significant increase in β -gal labeling was observed as more DSS was used, although the labeling plateaued in the 2% DSS treatment group (Fig. 2A). In addition, the increased β -gal labeling was present from the surface to the crypt base in both

colonic segments (Fig. 3). The impact of DSS treatment on β -gal activity was also examined in the small intestine, liver, spleen, lung and kidney. As reported previously [14], β -gal activity was not observed in the small intestine, even after DSS induction (data not shown). Both liver and lung had low level baseline transgene recombination but this was not increased by DSS treatment. Kidney had non-specific β -gal activity in non-transgenic mice but this did not increase in either the CAC mouse or the CAC mouse treated with DSS. In the spleen there was no transgene expression either at baseline or after DSS treatment (Supplemental Fig. S1).

As expected, the percentage of damaged epithelial surface was higher with increasing DSS dose in both proximal and distal colon (Fig. 2B). In addition, there was a direct, positive correlation between epithelial damage and β -gal expression in both colonic segments ($r^2 > 0.9$, slope = 0.75, Fig. 2C). Also, regardless of the DSS dose, approximately 60% of the abnormal crypts in the areas of epithelial damage were β -gal positive (data not shown). This indicates that Cre-mediated recombination of the LacZ locus has occurred in the epithelium that expanded to heal the DSS induced ulcers.

Increased β -gal expression resulting from DSS induced colon damage is sustained after tissue recovery

In normal colon mucosa, the crypts are well organized and separated by very little lamina propria (Fig 4A,B). 10 days after DSS treatment, the ulcerated mucosa was undergoing active healing, characterized by epithelial restitution, crypt fission, and crypt regeneration (Fig 4C, D). By 30 days after DSS treatment, the ulcerated mucosa was filled with normal-appearing crypts that were separated by slightly increased amounts of lamina propria (Fig 4E, F). As we observed in the first study, the total percentage of β -gal positive crypts was significantly higher in the DSS treatment groups than the vehicle controls at 10-day post DSS (proximal colon in Fig. 5A, $p < 0.0001$, distal colon in Fig. 5D, $p = 0.0024$) and greater than 60% of the crypts in the healing ulcer were β -gal positive (Fig. 5B, E). This increased β -gal staining remained 30 days after termination of DSS treatment (Fig. 4, Supplemental Fig. S2, and Fig. 5). In fact, the percentage of β -gal positive crypts in the healing/healed ulcer was higher at 30-day post DSS than 10-day post DSS (e.g. $75.2 \pm 4.4\%$ vs $57.9 \pm 6.3\%$ in proximal colon). The DSS treatment group also had significantly more β -gal positive crypts in areas of normal-appearing crypts that were adjacent to healing mucosa (Fig. 5C, F). This indicated that the transgene expression induced by DSS was sustained in the tissue after recovery of the damaged epithelium.

DSS induced transgene recombination increases tumor formation

We first examined whether β -gal staining occurs coincident with adenomatous phenotypes that result from deletion of two floxed Apc alleles. In $CAC^{Tg/WT}; Rosa26R^{lox/WT}; Apc^{580/580}$ mice, 30% of the distal colon exhibited an adenomatous phenotype consistent with recombination of both Apc alleles (i.e. increased cytoplasmic basophilia, increased nuclear to cytoplasmic ratio, loss of nuclear basal polarity, disruption of crypt architecture). While only 2.4% of crypts in normal mucosa were β -gal positive (Fig. 6A), within the adenomatous regions 88.8 \pm 1.5% of the epithelial cells were positive for β -gal (Fig. 6B). The co-occurrence of β -gal labeling (indicative of

recombination at the ROSA26R locus) and the adenomatous phenotype (indicative of recombination of both floxed *Apc* alleles) suggests that Cre mediated recombination of floxed alleles is very efficient.

Since DSS treatment induces Cre-mediated recombination in crypts, we hypothesized that DSS would increase tumor incidence in CAC^{Tg/WT}; *Apc*^{580/WT} mice by expanding the colon-specific inactivation of a single *Apc* allele, coupled with the well-established tumor-promoting effects of inflammation [18]. In the vehicle treated group, 18.6% of the mice had a colonic tumor and the tumors were all in the distal colon (Table 1, 1.25 per mouse). In contrast, DSS treatment significantly increased the percentage of mice with colonic tumors to 78.9% (DSS vs Vehicle, $p < 0.0001$) and this included the appearance of tumors in the proximal colon in 34% of mice. The number of colonic tumors developed per mouse was also significantly higher in the DSS treated mice compared to the vehicle controls (4.20 vs 1.25 per mouse, $p < 0.0001$). The DSS treatment resulted in twice as many mice with tumors in the distal colon compared to the proximal colon (Table 1, 74% vs 34%, respectively) and twice as many tumors in the distal versus the proximal colon (Table 1, 3.71 vs 1.47 per mouse, respectively). Thus the CAC mediated recombination of *Apc* alleles induced by DSS along with the pro-inflammatory environment within DSS-induced ulcers was a potent promoter of colon cancer.

Discussion

Our results expand the utility of the CAC mouse model by demonstrating that recombination of floxed alleles driven by the Cre recombinase transgene is increased during DSS-colitis. This effect was restricted to large intestinal epithelial cells, was tightly correlated with DSS induced epithelial damage, was visible during the healing of ulcers, and persisted in crypts within the healed epithelium. However, since DSS causes colon epithelial damage that is accompanied by local inflammation, it is not clear whether induction of transgene activity is a result of the epithelial damage, healing, inflammation, or the factors combined. While the mechanism for this effect is not known, two lines of evidence suggest that the colonic stem cell has been affected. First, recombination is evident in epithelial cells from the luminal surface to the crypt base where colon stem cells reside, and second, the effect is persistent in crypts and lasts after the damaged epithelium has healed.

In addition to the CAC mouse we developed and used in these experiments [14], several other mouse models exist for colon research and each have advantages and disadvantages [12]. A 12.4 kb region of the mouse villin promoter drives Cre recombinase expression uniformly throughout the epithelial cells of the intestine [19]. However, villin-Cre transgene expression extends into the small intestine [19] and this can confound interpretation of events occurring in the colon. An improvement on the villin-Cre mouse was accomplished by using a 9 kb villin promoter to drive a CreER^{T2} transgene that is tamoxifen inducible and which can cause gene deletions that are spatially and temporally controlled. However, 60 days after treating villin-CreER^{T2} mice with tamoxifen, the persistence of recombination in small intestine was seen in 80% of the epithelium while in the colon it was just 40% [20]. Moreover, villin promoter driven Cre or Cre-ER^{T2} transgene expression level is lower in the

crypt base where stem cells are found [19,20] and this suggests that Cre activity in the colon stem cell compartment may not be sufficient for optimal recombination.

Deletion of floxed alleles directly in intestinal stem cells can be accomplished using the *Lgr5* promoter-CreER^{T2} mouse [21] and the *Lrig1* promoter-CreER^{T2} mouse [22]. Unfortunately, inducible Cre-ER^{T2} transgene activity is very low in the large intestine of *Lgr5* promoter-CreER^{T2} mice [23] and so, like the villin promoter models, the *Lgr5* promoter-CreER^{T2} mouse is best for inducing small intestinal cancer. In contrast, the *Lrig1* promoter-CreER^{T2} mouse forms multiple adenomas in the distal colon when it is used to delete an *Apc* allele; however, many tumors are also seen in the small intestine [22].

An improvement on these models is the CDX2 promoter-Cre mouse whose transgene expression is seen only in cecum, colon and distal ileum of adult mice. When it is used to delete a floxed *Apc* allele, tumors form in the ileum and distal colon [24]. Unfortunately, transient expression of the CDX2-Cre transgene during embryonic development causes recombination of floxed alleles throughout the distal half of the body. This could reduce the utility of the CDX2-Cre model for many genes whose functions are present outside the colon. A tamoxifen-inducible version of this model, CDX2-CreER^{T2}, avoids the problem of embryonic transgene recombination [23]. However, the induced transgene activity is much higher in proximal colon than the distal colon so its utility may be limited.

Our original CAC model [14] has a major advantage over the other models just described - intestinal transgene expression is specific to the large intestine. This makes the model useful to study the role of specific genes in basic colonic biology and in cancer. A weakness of the CAC model is that CAC transgene expression affects just a small portion of the colonic epithelium and extends to the crypt base mostly in the distal colon. Here we report a major advance for the CAC mouse – that Cre-mediated recombination is induced during DSS-mediated epithelial damage in the colon. The effect of DSS-induced colitis on CAC transgene activity occurs to a comparable degree in both the proximal and distal colon. Thus, the DSS/CAC model will allow researchers to evaluate the role of epithelial cell specific genes in the development and recovery of colitis as well as in colitis-associated colon cancer. The DSS/CAC model also has the advantage that the increase in transgene activation is directly related to the dose of DSS used but that increase does not involve the entire mucosa. For example, we found that only 40-50% of the epithelial surface in the distal colon became transgene positive after treating mice with 2% DSS for 5 days. Thus, in this model both normal tissue (without transgene expression) and tissue with recombined alleles will be adjacent to one another. This will reduce the biological variation caused by intra-animal differences by providing an internal control for paired analysis of treatment effects.

As a preliminary test of the DSS/CAC model, we used it to examine the impact of *Apc* allele deletion on the development of colon cancer. We found that in CAC mice with one floxed *Apc* allele, DSS increased colonic tumor incidence 4-fold (to 78.9%) and tumor number per mouse 3-fold (to 4.5 colonic tumors per mouse). However, despite the fact that DSS-induces recombination of floxed alleles equally in both proximal and distal colon, tumors formed predominantly in the distal colon. This suggests that the different environmental influences

interact with inflammation and Apc allele loss to affect cellular transformation and tumor development in the proximal and distal colon.

In summary, we observed a novel phenotype for the CAC model - that the Cre transgene mediated recombination is induced following DSS treatment. This opens the opportunity to study gene function at the time of colitis development as well as after the recovery of colitis. We believe the DSS/CAC model is an efficient, reliable model for research on the epithelial cell biology of colitis and inflammation-induced colon cancer. This will make the CAC/DSS model a powerful tool to study the mechanisms by which inflammation promotes transformation as well as to assess interventions to minimize the impact of inflammation on carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

APC	adenomatosis polyposis coli
CA1	carbonic anhydrase I
CDX2	caudal type homeobox 2
DSS	dextran sulfate sodium
WT	wild-type

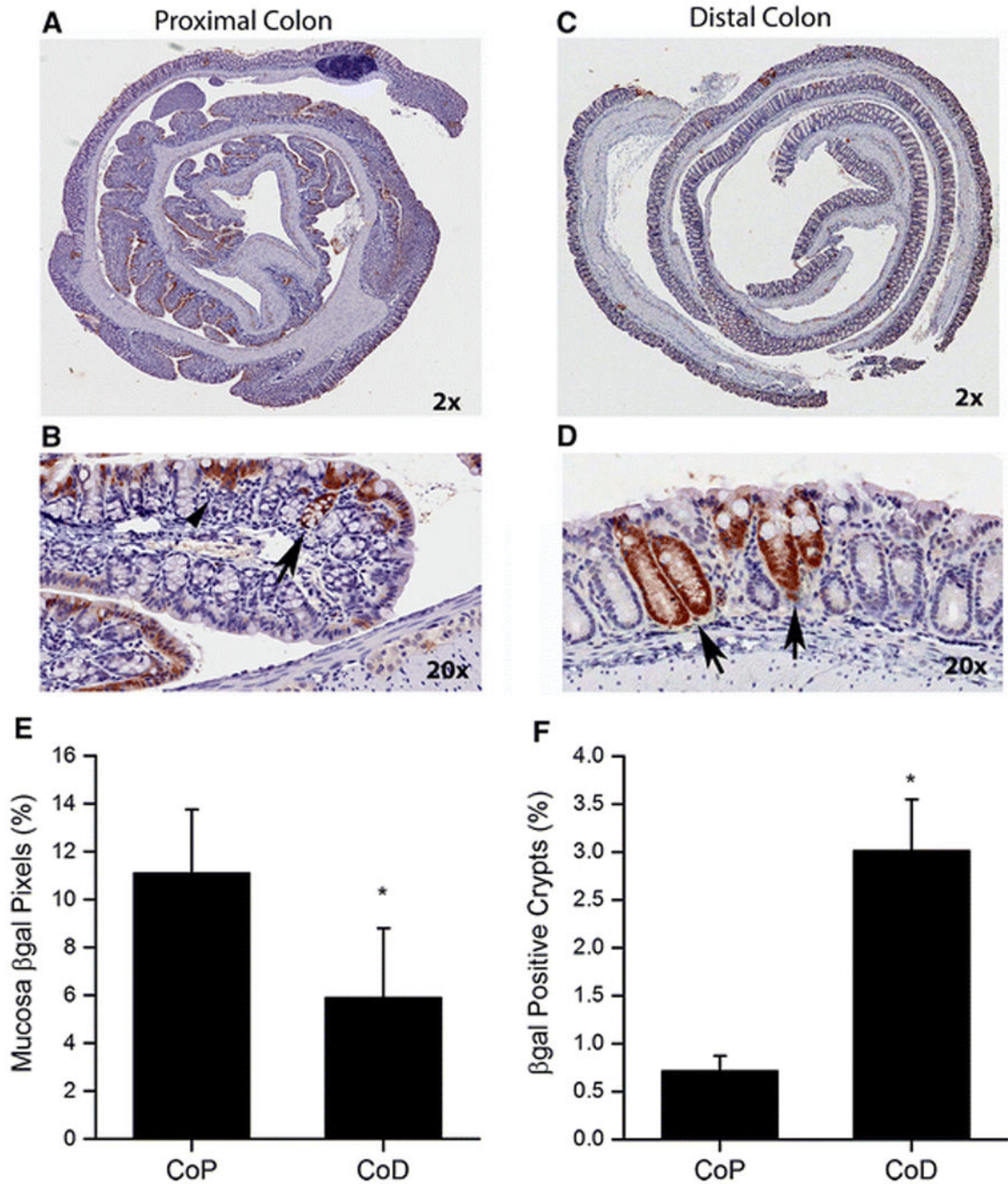


Figure 1. Baseline β gal expression patterns are different between proximal and distal colon in $CAC^{Tg/WT};Rosa26R^{flox/WT};Apc^{580/WT}$ mice

Colons from 10 weeks old mice were prepared as Swiss rolls, processed, and stained for β -gal expression by immunohistochemistry. Representative images were generated using an Aperio ScanScope digital slide scanner and are presented for proximal colon (A, B) and distal colon (C, D). The top pictures are at 2X magnification and the bottom pictures are at 20X magnification. (B) β -gal labeling in proximal colon Swiss rolls was predominantly superficial (arrow head) with occasional labeling to the crypt base (arrow). (D) β -gal labeling in distal colon Swiss rolls was seen from the crypt base to the luminal surface

(arrow). (E) Total mucosal β -gal positive pixels and (F) β -gal positive crypts were quantified. The bars represent the means \pm SEM of 8 animals for data from the proximal colon (CoP) and distal colon (CoD). * $p < 0.05$ vs CoP.

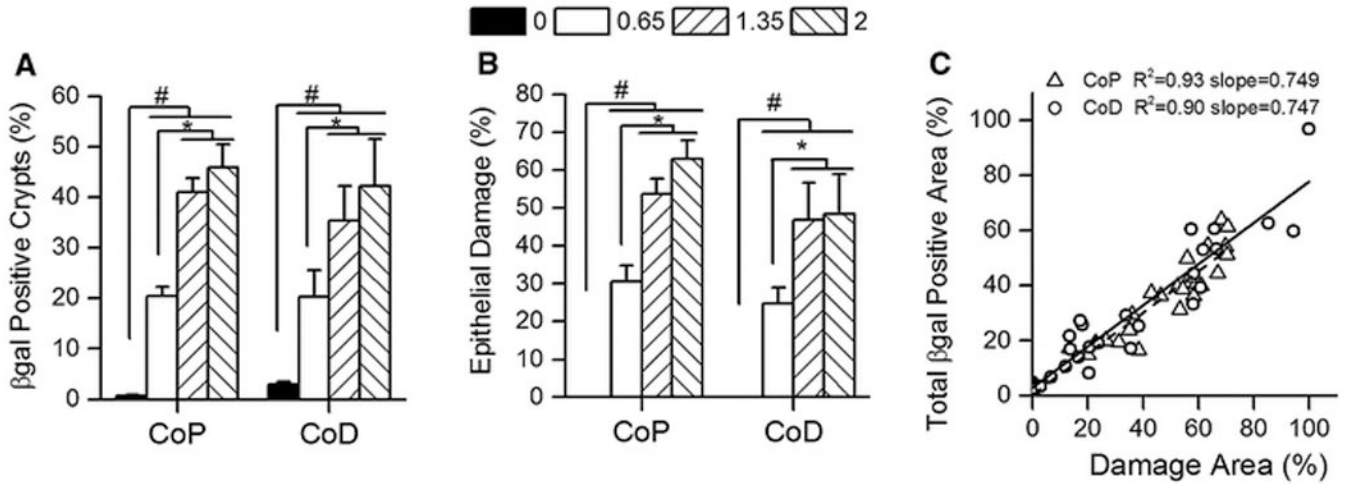


Figure 2. Colon β -gal expression is positively correlated with epithelial damage in $CAC^{1g/WT}; Rosa26R^{lox/WT}; Apc^{380/WT}$ mice following DSS treatment

Mice were given increasing amounts of DSS for 5 days then examined histologically 10 days later. (A) The percentage of β -gal positive crypts in the proximal (CoP) or distal (CoD) colon. (B) The percentage of damaged epithelium (includes colon epithelial ulceration, restitution, crypt fission and other abnormal crypt structures observed during the healing process). Bars represent the mean \pm SEM of 8 animals per group. Planned orthogonal comparisons were performed between no DSS vs the DSS treatment groups (# $p < 0.05$), the low DSS vs high DSS groups (* $p < 0.05$), and the two high DSS groups (not significant). (C) The relationship between the percentage epithelial damage and β -gal positive area in colon. The regression line for the proximal colon is shown as a dashed line. The regression line for the distal colon is shown as a solid line.

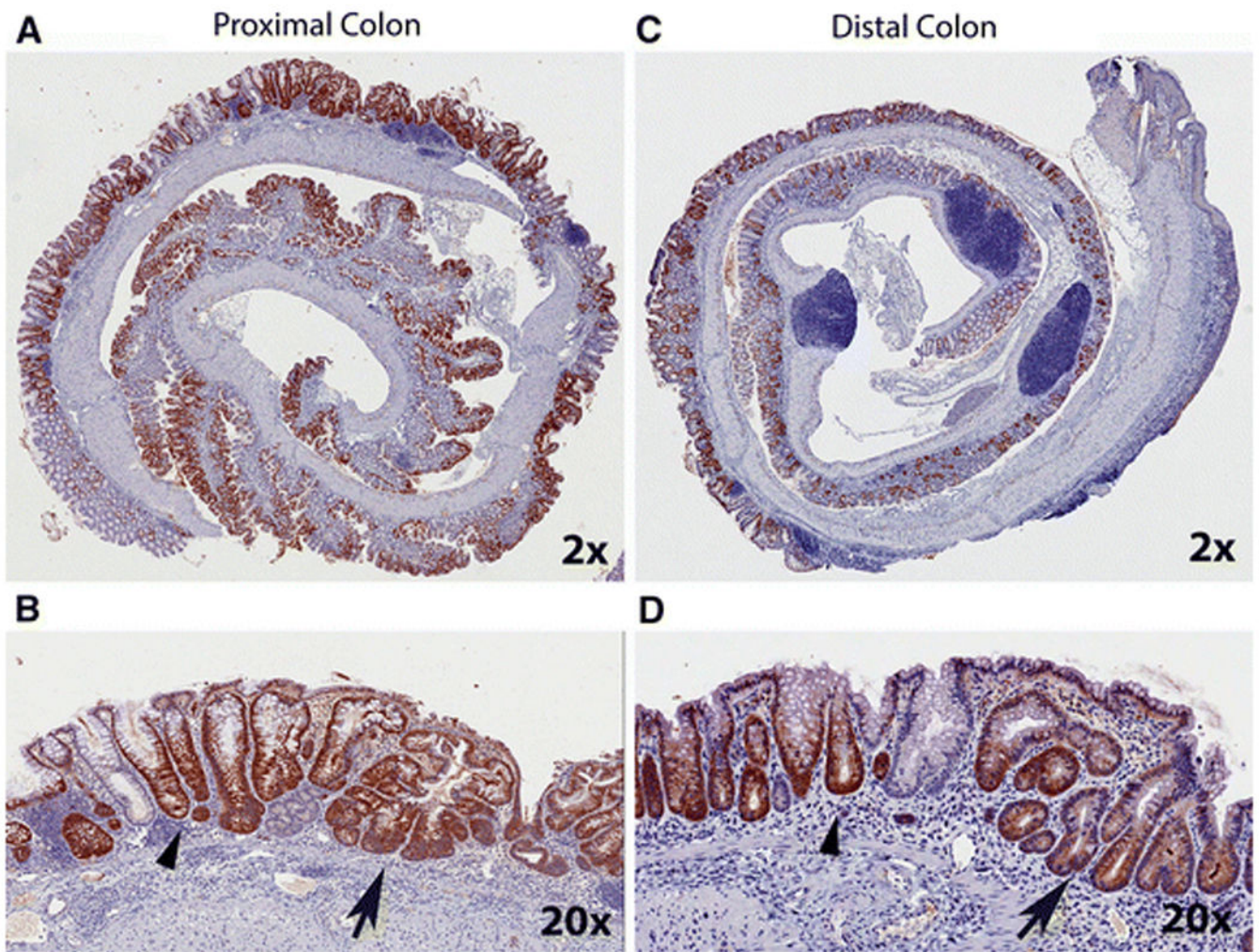


Figure 3. Immunohistochemical labeling for β gal in proximal and distal colon of DSS-treated $CAC^{Tg/WT}; Rosa26R^{lox/WT}; Apc^{580/WT}$ mice
 Animals were treated with 2% DSS for 5 days, and the tissues were collected at 10 days after stopping DSS treatment. Representative images were generated using an Aperio ScanScope digital slide scanner and are presented for proximal colon (A, B) and distal colon (C, D). The top pictures are at 2X magnification and the bottom pictures are at 20X magnification. Arrow head = normal crypt structure adjacent to a healing area. Arrow = crypt fission phenotype within a healing area.

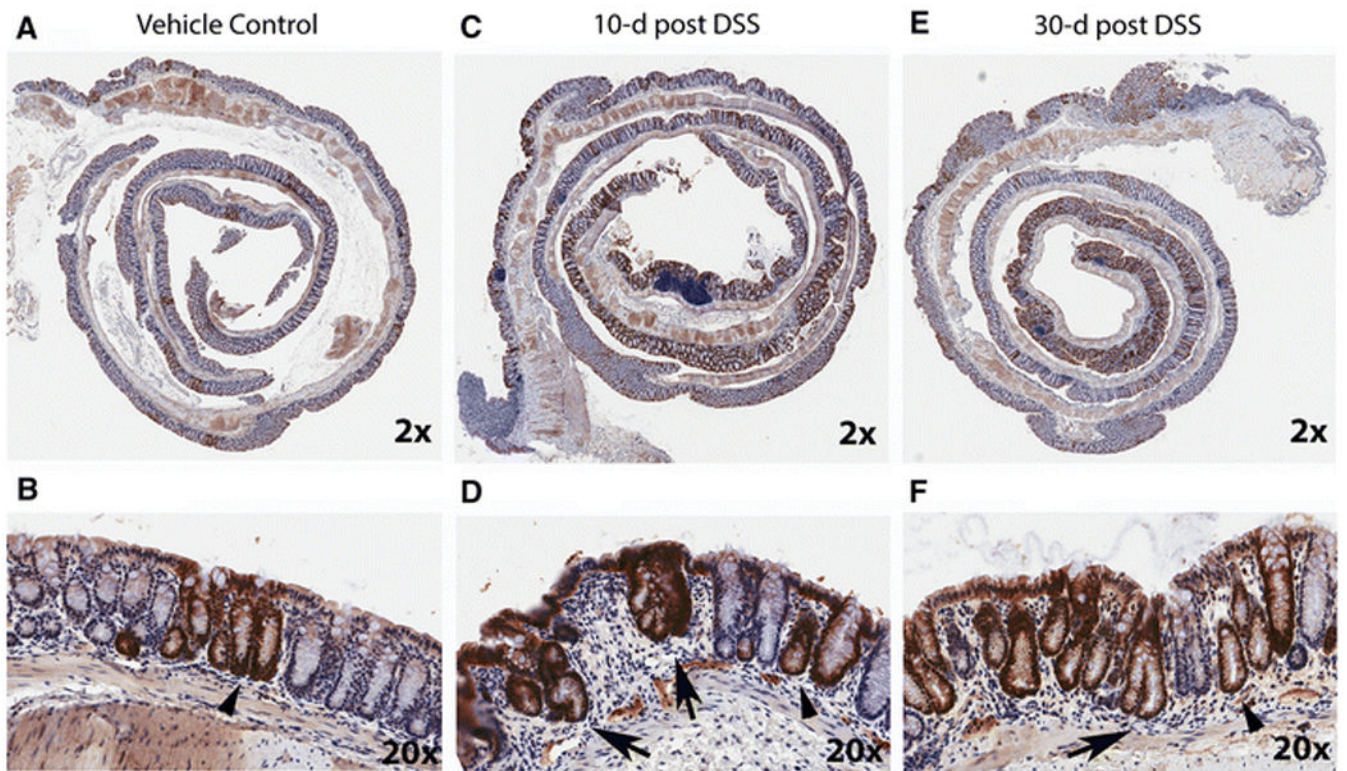


Figure 4. After DSS treatment transgene expression is increased in regenerating crypts and is sustained after healing of ulcers in distal colon

(A, B) Representative images of β gal expression level in the distal colon of a $CAC^{Tg/WT};Rosa26R^{flox/flox}$ mouse treated with vehicle under 2X (A) and 20X (B) magnification. Crypt base labeling is highlighted with arrow head. (C, D) Images of β gal labeling in a $CAC^{Tg/WT};Rosa26R^{flox/flox}$ mouse 10 days after the end of treatment with 1.35% DSS under 2X (C) and 20X (D) magnification. (E, F) Images of β gal labeling in a $CAC^{Tg/WT};Rosa26R^{flox/flox}$ mouse 30 days after the end of treatment with 1.35% DSS under 2X (E) and 20X (F) magnification. In (C,D, E, F), Arrow = β gal positive crypts undergoing regeneration. Arrow head = β -gal positive crypts with normal phenotype adjacent to a healing area. All the images were generated using an Aperio ScanScope digital slide scanner.

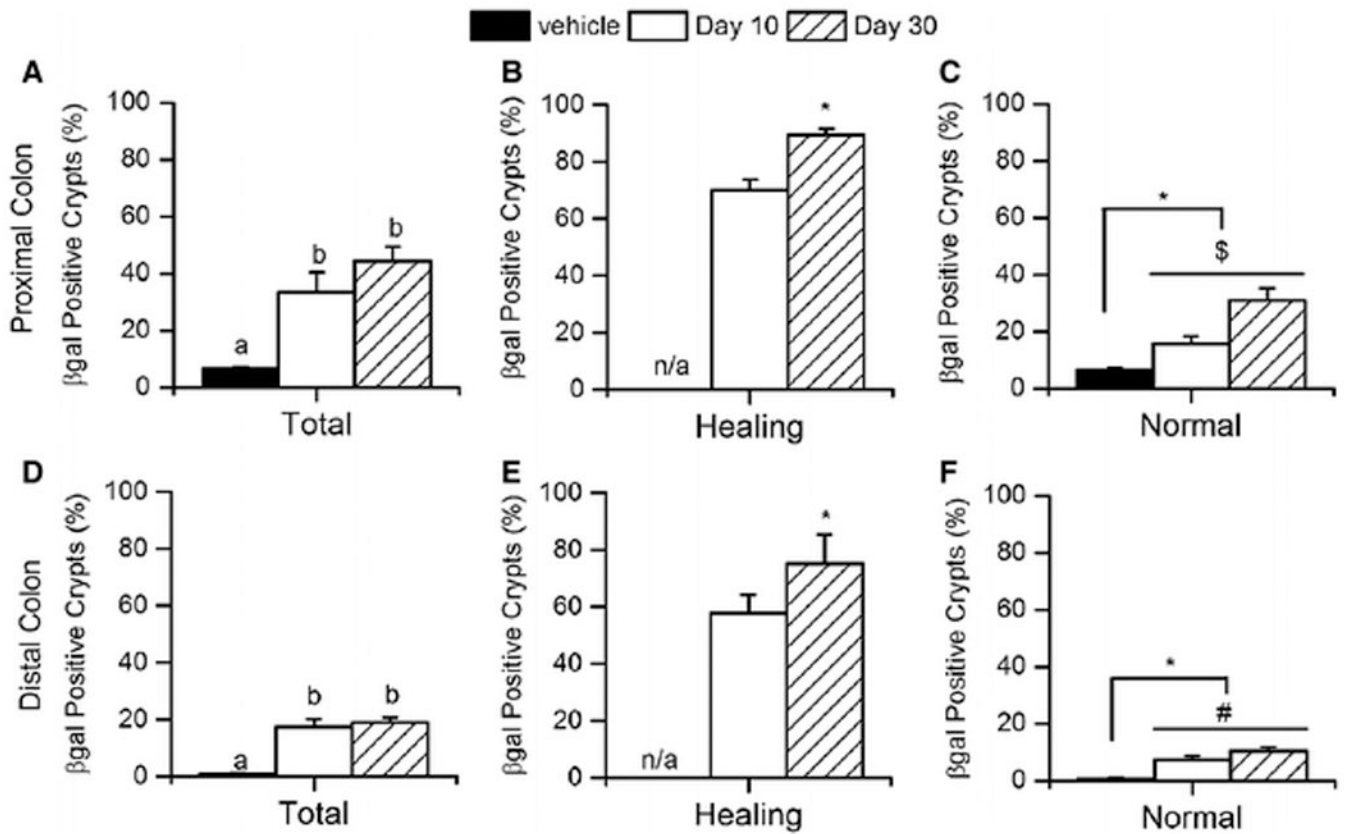


Figure 5. The percentage of β -gal positive crypts increases after DSS treatment and is sustained after regeneration in the colon of $CAC^{Tg/WT};Rosa26R^{lox/lox}$ mice

(A, D) The percentage of β -gal positive crypts was measured in proximal (A) and distal (D) colon at 10 or 30 days after ending DSS treatment. Means with different letters are significantly different (Bonferroni, $p < 0.05$). (B, E) The percentage of β -gal positive crypts in mucosa that exhibits a healing phenotype in the proximal (B) and distal (E) colon. Student t-tests were used to compare values between day 30 and day 10 ($* p < 0.05$). (C, F) The percentage of β -gal positive crypts in normal mucosa was measured in proximal (C) and distal (F) colon. Planned orthogonal comparisons were performed ($* p < 0.05$, day 0 vs post-treatment values; $\$ p < 0.05$, day 10 vs day 30; $\# p < 0.10$, day 10 vs day 30). Bars represent the mean \pm SEM of 8 animals per group.

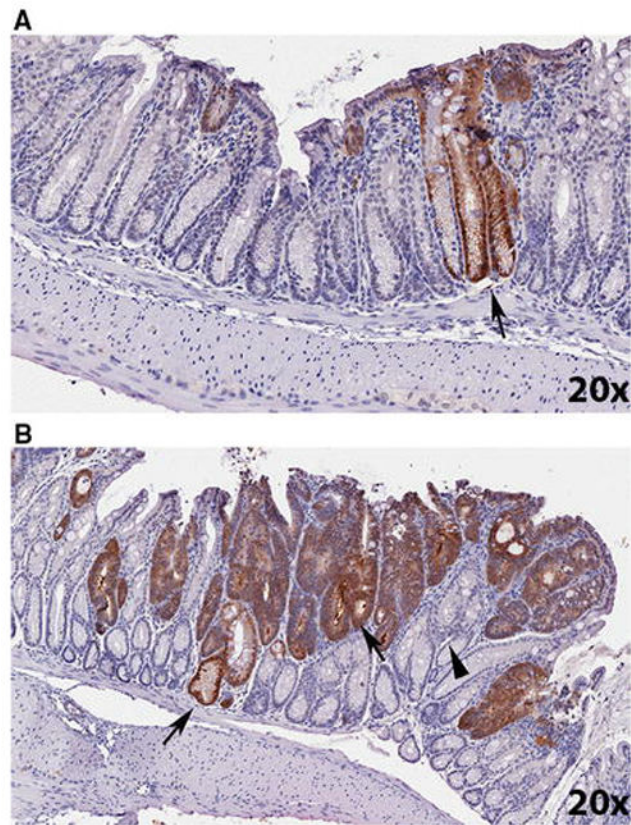


Figure 6. Colon tissue with an adenomatous phenotype show high βgal expression level in 4-week-old $CAC^{Tg/WT}; Rosa26R^{flox/WT}; Apc^{580/580}$ mice

(A) βgal expression in a normal area of the distal colon. Arrow = βgal labeling in a normal crypt. (B) βgal expression in distal colon adenomatous phenotype. Arrow = adenomatous phenotype with βgal positive labeling. Arrow head = adenomatous phenotype with βgal negative labeling. Images were generated using an Aperio ScanScope digital slide scanner. Magnification: 20X.

Table 1

DSS Increases Tumor Incidence in CAC^{Tg/WT};Apc^{580/WT} Mice

	Vehicle				DSS			
	n	# Tumors	Incidence (%)	# per mouse ^a	n	# Tumors	Incidence (%)	# per mouse ^a
Any Tumor	9	12	20.9	1.33±0.24	37	186	97.4 [*]	5.03±0.56 [#]
Cecum	2	2	4.7	1.00±0.00	30	60	81.6 [*]	2.00±0.32 [#]
Proximal colon	0	0	0.0	0.00	15	22	34.2 [*]	1.47±0.29 [#]
Distal colon	8	10	18.6	1.25±0.25	28	104	73.7 [*]	3.71±0.35 [#]
Proximal + Distal	8	10	18.6	1.25±0.25	30	126	78.9 [*]	4.20±0.40 [#]
No Tumor	34	0	79.1		1	0	2.6	
Total Mice	43				38			

Notes: Significantly different from the vehicle group

^{*} Chi-square test, p<0.05;

[#] Student's T-tests, p<0.05.

^a Average number of tumors per tumor-bearing mouse