Severity of DSS-induced colitis is reduced in Ido1-deficient mice with down-regulation of TLR-MyD88-NF-kB transcriptional networks

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Indoleamine 2,3-dioxygenase 1 (IDO1) catalyzes L-tryptophan to kynurenine in the first and rate-limiting step of tryptophan metabolism. IDO1 is expressed widely throughout the body, with especially high expression in colonic intestinal tissues. To examine the role of IDO1 in the colon, transcriptome analysis was performed in both Ido1−/− and Ido1+/+ mice. Gene set enrichment analysis identified the Inflammatory Response as the most significant category modulated by the absence of IDO1. This observation prompted us to further investigate the function of IDO1 in the development of tissue inflammation. By using DSS-induced experimental colitis mice models, we found that the disease in Ido1−/− mice was less severe than in Ido1+/+ mice. Pharmacological inhibition of IDO1 by L-1MT attenuated the severity of DSS-colitis as well. Transcriptome analyses revealed that pathways involving TLR and NF-kB signaling were significantly down-regulated by the absence of IDO1. Furthermore, dramatic changes in TLR and NF-kB signaling resulted in substantial changes in the expression of many inflammatory cytokines and chemokines. Numbers of inflammatory cells in colon and peripheral blood were reduced in IDO1 deficiency. These findings suggest that IDO1 plays important roles in producing inflammatory responses and modulating transcriptional networks during the development of colitis.
metabolites, which promotes cell-cycle arrest of and apoptosis of T cells and induce the differentiation of T regulatory cells (Treg)\(^{16}\).

Despite knowledge of the immune-modulatory role of IDO1, the mechanism by which IDO1 mediates inflammatory reactions is a field of active investigation and remains controversial. A contradictory effect of IDO1 on inflammatory responses during the development of colitis was recently described. Mechanistic studies using trinitrobenzene sulfonic acid (TNBS)-induced colitis suggested that inhibition of IDO1 leads to increased severity of colitis due to down-regulation of Treg cell responses within the intestinal tract\(^{17,18}\). Conversely, another study reported that local increases in IDO1 production during active inflammatory responses resulted in more severe colitis promoted by key mediators of pro-inflammatory signaling\(^{19}\). Most previous results were drawn from studies of T cell-associated functions of IDO1. For example, TNBS is a chemical that induces colitis in a T cell-dependent manner\(^{17}\) as a result of delayed-type hypersensitivity reaction to haptenized proteins; however, because other studies raised the points that IDO1 may play inflammatory roles in a T cell-independent manner, different approaches are needed to elucidate the role of the enzyme in the development of colitis. One of the potential approaches is to study the roles of IDO1 using dextran sulfate sodium (DSS) which induces colitis by disrupting epithelial barrier function of colon tissues.

In this study, we performed for the first time an array-based transcriptome analysis to identify differentially-expressed genes targeted by IDO1 using Id01 knock-out (Id01\(^{-/-}\)) mice. We identified new molecular targets of IDO1 and described functionally distinct molecular mechanisms regulated by IDO1. In addition, we further examined the pathophysiological roles played by IDO1 in colitis development in studies using DSS-induced colitis model. We determined the effects of Id01 expression on colitis-related clinical parameters and histopathological damage. We also assessed changes in inflammatory cell recruitment using flow cytometry, and performed gene expression profiling analyses. To our knowledge, this is the first report of a comprehensive gene expression profile analysis of Id01\(^{-/-}\) mice in either non-stimulated or DSS-stimulated conditions. This large-scale profiling may enhance our understandings of the contributions of IDO1 to colitis development, and provide novel target genes regulated by IDO1.

**Results**

**Identification of differentially expressed genes in Id01 knock-out mice.** As a first attempt to identify the targets of IDO1, which catalyzes the first and rate-limiting step of tryptophan degradation, transcriptome analysis was performed using Id01\(^{-/-}\) and Id01\(^{+/+}\) mice. Total RNAs from the colon tissue of each mouse was respectively applied to Illumina Mouse WG-6 v.2 BeadChip arrays containing a total of 45,281 transcripts. Principal component analyses (PCA) confirmed that the gene expression profiles of Id01\(^{-/-}\) mice were readily distinguishable from those of Id01\(^{+/+}\) mice as shown in Fig. 1a. Differentially expressed genes in Id01\(^{-/-}\) and Id01\(^{+/+}\) mice were identified by unpaired t-test with thresholds of 1% false discovery rate [FDR] and 2-fold change restriction. A total of 102 significant genes were identified of 45,281 transcripts. Principal component analyses (PCA) confirmed that the gene expression profiles of Id01\(^{-/-}\) mice in either non-stimulated or DSS-stimulated conditions. This large-scale profiling may enhance our understandings of the contributions of IDO1 to colitis development, and provide novel target genes regulated by IDO1.

**Contribution of IDO1 to development of DSS-induced colitis.** Based on the array analyses showing that the inflammatory response was the most significant biological process affected by the absence of IDO1 in untreated mice, we next sought to study the effects of IDO1 deficiency on colitis development. We used an established murine model of colitis induced by oral administration of DSS, a reagent that disrupts the barrier function of mucosal epithelial cells\(^{20}\). Id01\(^{-/-}\) and Id01\(^{+/+}\) mice were given either 1% or 2% DSS in their drinking water for 7 days and daily water intakes were measured. There was no difference in DSS-containing water consumption among all groups (see Supplementary Fig. S1). While treatment with 2% DSS resulted in a marked inflammatory reaction, 1% DSS treatment did not produce significant inflammatory reaction compared to the 0% control in Id01\(^{+/+}\) mice. In treatment with 2% DSS, progressive weight loss, increased disease activity index (DAI) including severe diarrhea and intestinal bleeding, and greater shortening of colon length were detected in Id01\(^{+/+}\) compared to Id01\(^{-/-}\) mice (Fig. 2a–c). These results suggest that IDO1 may play roles in promoting the inflammatory response in
Figure 1. Differential gene-expression profiles of Ido1−/− mice compared with Ido1+/+ mice detected by microarray analysis. (a) 3-D view of PCA scores plot of Ido1−/− group (n = 3) versus Ido1+/+ group (n = 3). Groups are shown by different colors and dots represent individual strains. Blue spots represent Ido1+/+ group and the red spots represent Ido1−/− group. On a 3D-PCA plot of Ido1−/− group can be clearly distinguished from Ido1+/+ group. (b) Hierarchical clustering and heat map of up- or down-regulated genes that are differentially expressed (>2-fold, 1% [FDR]) in the absence of IDO1. Red indicates high relative expression and green indicates low expression of genes as shown in the scale bar. Cluster analysis functionally categorized by IPA; Canonical pathway significantly detected in Ido1−/− mice compared to Ido1+/+ mice. Statistical significance of pathway modulation was calculated via a right-tailed Fisher’s exact test in Ingenuity Pathway. (c,d) Gene-pathway networks using IPA. (c) Gene Expression and (d) Inflammatory Response pathway are displayed as networks. Green and red symbols denote down-regulated and up-regulated genes in Ido1−/− compared with Ido1+/+ mice, respectively. Arrows with unbroken lines indicate a direct interaction between two molecules, with the mode of action in the direction of the arrow; arrows with broken lines denote an indirect interaction. (e) We performed gene-set-enrichment analysis (GSEA) to determine whether the filtered gene list from Ido1−/− mice versus Ido1+/+ mice showed specific enrichment in the inflammatory response in the rank-based analysis. Rank of 102 genes in our data sets ordered by expression level with enrichment plots for the up-regulated and down-regulated genes. ES (Enrichment Score) is a value that represents how well the gene set is enriched within the selected gene list. The FDR q value <0.01 for specific enrichment of the gene set is as indicated. The leading edge analysis of our microarray data identified that the gene is highly correlated with Inflammatory Response.
DSS-challenged mice. In addition, although treatment with 1% DSS did not induce significant differences in body weight and DAIs between \( \text{Ido1}^{-/} \) and \( \text{Ido1}^{+/+} \) mice, it caused histological changes showing that the extent of tissue damage in \( \text{Ido1}^{+/+} \) mice was much greater than those for \( \text{Ido1}^{-/} \) mice (Fig. 2d). Inflammatory cell infiltration and crypt damage was more apparent in these mice as evidenced on H&E staining.

Figure 2. Phenotypic comparisons of DSS-induced colitis outcomes in gene deletion (\( \text{Ido1}^{-/-} \) vs. \( \text{Ido1}^{+/+} \) mice) and pharmacologic inhibition (placebo vs. L-1MT). (a–c) All data presented as the mean ± S.E.M of each genotype (n = 4–8 per group). Unpaired student’s t test was used to determine the significant difference between 2% DSS treatment groups. *(asterisk) indicates the significant difference at \( p < 0.05 \). (a) Body weight curves of \( \text{Ido1}^{-/-} \) and \( \text{Ido1}^{+/+} \) mice in an acute model of DSS-induced colitis for 7 days. (b) Stool consistency, fecal bleeding and weight loss were observed on daily basis and DAI (Disease activity index) was scored for each mouse in \( \text{Ido1}^{-/-} \) and \( \text{Ido1}^{+/+} \). DAI score was graded on a scale of 0–4 as describe in the Methods. (c) Representative image of the DSS-induced colitis in \( \text{Ido1}^{-/-} \) and \( \text{Ido1}^{+/+} \) mice. Colon length of DSS-induced mice was measured on the 7th day after the start of DSS treatment. (d) Representative H&E staining of colon tissue sections of each genotype treated with 1% DSS. Scale bars shows magnification 100\( \mu \)m for upper panels and 200\( \mu \)m for lower panels. Histological score is calculated by the sum of severity of inflammation (0–3), damage (0–4), and extension (0–4). Data are presented as mean ± S.E.M (n = 3–5 per group) and \( p \) value was estimated by unpaired t test. Bar with *indicates the significant difference at \( p < 0.05 \). (e–f) All data are presented as mean ± S.E.M of each group (n = 3 per group). Unpaired student’s t test was used to determine the significant difference between 2% DSS treatment groups. *(asterisk) indicates the significant difference at \( p < 0.05 \). (e) Stool consistency, fecal bleeding and weight loss were observed on daily basis and DAI was scored for each mouse in placebo (\( \text{Ido1}^{+/+} \)) and L-1MT (\( \text{Ido1}^{+/+} \) + L-1MT). (f) Representative image of the DSS-induced colitis in placebo and L-1MT mice. Colon length of DSS-induced mice was measured on the 8th day after the start of DSS treatment.
Furthermore, to explore whether pharmacological inhibition of IDO1 reproduces the results observed following Idol gene deletion, we administered Idol+/− mice with L-1MT, a specific IDO1 inhibitor, then followed by colitis induction with 2% DSS treatment. IDO1 blockade by L-1MT ameliorated severe diarrhea and intestinal bleeding, resulting in significant reduction in the DAI. Shortening of colon length was notably attenuated in mice administered with L-1MT compared to the placebo (Fig. 2e,f). Taken together, our data indicate that the severity of DSS-induced colitis development was significantly reduced in Idol−/− mice and L-1MT administered mice, suggesting that IDO1 deficiency might protect against pro-inflammatory signals. We next carried out transcriptome analysis to elucidate the underlying molecular mechanisms of this effect.

**Gene expression profiling analysis of inflamed colon tissues in Idol−/− mice.** We analyzed the gene expression profiles of three to five Idol−/− or Idol+/− mice from the controls and from cohorts treated with 1% or 2% DSS (Fig. 3a). As expected, the transcription levels of Idol were significantly increased in the inflamed colon tissues of Idol+/− mice, suggesting that the inflammatory response induced Idol transcription (data not shown). PCA analysis showed that Idol−/− mice had a distinct pattern compared with Idol+/− mice only in the 2% DSS treatment group (Fig. 3a). We performed a one-way ANOVA analysis to identify differentially-expressed genes with statistical thresholds of 5% FDR and 2-fold change restriction among the six groups. A total of 6,421 genes were identified as significant and were classified further based on their biological functions. As expected, the Inflammatory Response category was the most significant key function (p = 1.23E-35 to 3.89E-08) (Fig. 3b). In comparisons of Idol−/− and Idol+/− mice, much higher numbers of genes were differentially expressed under DSS-stimulated conditions than in the basal non-stimulated state. Cytokines including interleukins and interferons are known small molecule that has been observed experimentally to affect gene expression directly or indirectly. We next carried out transcriptome analysis to elucidate the underlying molecular mechanisms of this effect.

**IDO1 deficiency results in down regulation of TLR and NF-kB signaling pathways.** We next conducted an upstream regulator analysis to identify major upstream molecules of the differentially expressed genes identified in our data set. As a point of interest, the greatest overlap with the regulators was in the pathways related to the Toll-like receptor and NF-kB signaling. Given these findings, we focused on genes belonging to those pathways in more detail. The most striking result was that expression of the majority of genes involved in TLR signaling was down-regulated in DSS-treated Idol−/− mice, suggesting that TLR signaling is essential for the contributions of IDO1 to the development of DSS-induced colitis (Fig. 5b). In the basal state...
(non-stimulated), expressions of Tlr2, Tlr6, and Myd88 remained low and the levels were similar in Ido1−/− and Ido1+/+ mice. However, treatment with DSS resulted in dramatic increases in the expressions of those genes in Ido1+/+ mice in a dose-dependent manner. The expressions of these genes were...
not significantly induced by DSS treatment in IDO1-deficient mice (Fig. 5d). Importantly, MyD88 is a key adaptor protein in the signal transduction cascades shared by most TLRs and MyD88-dependent TLR signaling pathways were found to be down-regulated in our study (Fig. 5b). This suggests that the differences in colitis development between \( \text{Ido1}^{-/-} \) and \( \text{Ido1}^{+/+} \) mice result from dysregulation of the TLR-MyD88 signaling pathway. We also ascertained that TLR-triggered cascades downstream of NF-kB signaling molecules like NF-kB (\( p = 1.54 \times 10^{-4} \)), JNKs (JUN N-terminal kinases, \( p = 6.62 \times 10^{-3} \)), and IRF5 (interferon regulatory factor 5, \( p = 1.20 \times 10^{-3} \)), which were all down-regulated in DSS-treated \( \text{Ido1}^{-/-} \) mice (Fig. 5c). These results implied that in mice with DSS-induced colitis, TLR signaling was inhibited by IDO1-deficiency, which suppressed pro-inflammatory cytokine and chemokine production through the regulation of a multitude of transcription factors such as NF-kB.

**Discussion**

IDO1-mediated tryptophan metabolism in various tissues is linked to numerous biological and physiological functions. Heightened expression of IDO1 in colonic intestinal tissues led to the hypothesis that...
IDO1 plays critical roles in gut homeostasis. Various microorganisms and food antigens exist in the lumen and challenge the intestinal immune system. As the luminal surface of the gastrointestinal tract continually interacts with foreign antigens such as pathogenic bacteria, the mucosal immune system maintains immune tolerance to limit inflammatory responses elicited by these antigens. In the present study, we showed that in the basal state in which there was no chemical induction of inflammation, IDO1 deficiency did not lead to pathophysiologic changes in the gut. However, gene expression...
profiling analysis revealed that a transcriptional network of inflammatory responses was significantly down-regulate in the absence of IDO1.

When the mucosal immune system fails to maintain tolerance, pathogenic microbes are able to infect the host. Impaired immune tolerance in the intestine can lead to inflammatory bowel diseases such as Crohn's disease and ulcerative colitis\(^{26-30}\). Treatment with DSS treatment mimics the condition of impaired immune tolerance because it disrupts the mucosal barrier of epithelial cells on the luminal surface. Studies of the DSS-induced colitis model system enabled us to characterize the role of IDO1 in driving inflammatory response, which is not apparent in the non-stimulated state. We showed that DSS-treated IDO1-deficient mice did not develop colitis of the same severity as normal control mice by assessing the loss in body weight, intestinal bleeding, diarrhea, shortening of colon length and histological lesions. It should be noted that there are conflicting results regarding the role of IDO1 in different mouse models of colitis. In studies that utilized TNBS to induce colitis, inhibition of IDO1 was found to exacerbate colitis\(^{37-31}\). This discrepancy can probably be explained, at least in part, by the fact that TNBS induces a T cell-dependent disease while the DSS induction is in a different manner. Although both DSS and TNBS colitis are used for inflammatory bowel disease animal model, DSS colitis has been distinguished from TNBS models by many others in the several points\(^{20,32}\). For instance, while TNBS-induced colitis develops as a result of delayed-type hypersensitivity reaction to haptenized proteins, DSS-induced colitis is the result of a change in epithelial barrier function\(^{32}\). Additionally, it is known that while TNBS induces Crohn's disease-like colitis, DSS induces ulcerative colitis\(^{39}\). Therefore, disease exacerbation in IDO1-deficient TNBS-treated mice was attributed to the inhibition of T\(_{reg}\) activity\(^{37-31}\), which is a distinct mechanism from what we found in IDO1-deficient DSS-treated mice.

It is well established that TLRs and NF-κB signaling pathways make important contributions to inflammatory responses. Strikingly, our transcriptome analyses showed that the expression of members of the TLR-MyD88-NF-kB signaling pathways was significantly decreased in the absence of IDO1. Consequently, down-regulation of those signaling pathways resulted in dramatic changes in the expression of cytokines and chemokines. We showed that in IDO1 deficient mice, the frequencies of circulating inflammatory cells in peripheral blood and in the gut were decreased as well. These findings suggest that IDO1 may require TLR-MyD88-NF-kB signaling to promote the development of colitis. It is notable that compared to normal mice, IDO1-deficient mice had higher expression of Muc1, which encodes mucin protein, the first line of host defense against invading bacteria (Fig. 5d, \(p = 3.20E-3\)). Reduction in the expression of Muc1 severely affects epithelial barrier function\(^{33,34}\). Several studies reported that mucin may be a negative regulator of TLR signaling\(^{35,36}\). This suggests that the absence of IDO1 may lead to increased mucin expression and subsequently down-regulate TLR-MyD88-NF-kB signaling.

IDO has become an emerging target for the treatment of cancer, infection, autoimmunity, and other diseases associated with inflammatory responses and immunosuppression\(^{37-40}\). The present report utilizing the Ido1\(^{−/−}\) mouse model provides the first comprehensive analysis of IDO1 targets at the transcriptome level and broadens our understanding of the diverse functions of IDO1 during inflammatory responses. This study also suggest that IDO1 is likely to be a promising target of therapeutic intervention in colitic diseases.

**Methods**

**Mice.** Ido1\(^{−/−}\) mice of the C57BL/6 (B6) genetic background were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Ido1\(^{−/−}\) mice were crossed with C57BL/6 wild-type (The Jackson Laboratory) to generate the Ido1\(^{−/−}\) and Ido1\(^{+/+}\) offspring used in this study. Genotypes of knockout mice were verified via PCR typing. The mice used were 10–12 weeks old and weighed 18–23 g. Age- and weight-matched female littersmates were used as controls. C57BL/6 mice and Ido1\(^{−/−}\) mice were maintained under specific pathogen free (SPF) condition at the Center of Animal Resource Development, Seoul National University College of Medicine. The mice were maintained based on the guidelines of Seoul National University Animal Experiment Ethics Committee. All animal experimental protocols were approved by the Committee on the Ethics of animal experiments of Seoul National University (Institutional Animal Care and Use Committee permit number: SNU-150119-5). All experiments were carried out in accordance with the guidelines and regulations.

**Induction of colitis and evaluation of colitis severity.** To generate an acute colitis experimental model, dextran sulfate sodium (DSS) (molecular mass 36–50 kDa; MP Biomedicals, Illkirch, France) was added to the drinking water at concentrations of 1% or 2% (w/v) given ad libitum for 7 days. Control mice received drinking water without DSS. The subsequent course of colitis development was evaluated by monitoring daily weight changes. Colitis severity also was scored by evaluating clinical disease activity through daily observation of the following parameters: weight loss (0 points = No weight loss or weight gain, 1 point = 5–10% weight loss, 2 points = 11–15% weight loss, 3 points = 16–20% weight loss, 4 points = > 21% weight loss); stool consistency (0 points = normal and well formed, 2 points = very soft and unformed, 4 points = watery stool); and bleeding stool score (0 points = normal color stool, 2 points = reddish color stool, 4 points = bloody stool). The disease activity index (DAI) was calculated based on the combined scores of weight loss, stool consistency, and bleeding ranging from 0 to 12. All parameters were scored from day 0 to day 7. At the 7th day after DSS-colitis induction, mice were sacrificed and the entire colon was quickly removed. After colon length was determined as a marker of

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inflammation, the entire colon was cut open lengthwise and gently flushed with sterile phosphate-buffered saline (PBS) to remove any traces of feces. Colon segments were immediately frozen in liquid nitrogen and stored at −80 °C for subsequent extraction of total RNA. For histological analysis, colon segments were fixed in 10% neutral buffered formalin phosphate and stored at room temperature until study for evidence of inflammation.

**Administration of L-1MT.** 9–11 weeks old Ido1+/+ mice were administered L-1MT. To prepare L-1MT for oral gavage, 1g of L-1MT (purchased from Sigma-Aldrich) was added to a 15ml conical tube with 10ml Methocel/Tween [0.5% Tween 80/0.5% Methylcellulose (v/v in water; both from Sigma-Aldrich)]. The mixture was bead milled overnight by adding 2–3 mm glass beads and mixing inversion. The next day, the L-1MT concentration was adjusted to 80 mg/ml by adding an additional 2.5 ml Methocel/Tween and mixing again. The L-1MT slurry was administered by oral gavage at 400 mg/kg/dose (100 μl of total volume) using a curved feeding needle (20-guage 1 1/2 in; Fisher) as previously described. For twice a day dosing, L-1MT was administered once in the morning and once in the evening. On day 5 of the experiment, all mice received 2% DSS treatment and mice were sacrificed on day 13.

**Histological analysis of colitis.** Routinely processed, 4–6μm paraffin-embedded sections of colon samples were prepared and stained with hematoxylin and eosin (H&E) for histological grading. Histological scores, including severity of colitis, were evaluated in a blinded manner as previously described by Laroui et al. Grades were evaluated from 0–4 for the following three criteria: severity of inflammation (0, rare inflammatory cell in the lamina propria; 1, increased inflammatory cells in the lamina propria; 2, confluent inflammatory cells extending into the submucosa; and 3, transmural extension of the inflammatory cell infiltrate); damage (0, none; 1, loss of the basal 1/3 of the crypt; 2, loss of the basal 2/3 of the crypt; 3, loss of the entire crypt but intact epithelial cells; and 4, loss of the entire crypt and of the surface epithelial cells); extension (0, none; 1, focal; 2, lesion involving 1/3 of the intestine; 3, lesion involving 2/3 of the intestine; and 4, lesion involving the entire intestine). Scores for each criterion were added to give an overall inflammation score for each sample with a range of 0–11. The histological grades were determined for each section, and the sum of the grades was reported as the histological score for each mouse. The level of colitis was blindly assessed by two histopathologists.

**Flow cytometry (FACS) analyses.** Fresh peripheral blood lymphocytes (PBLs) were prepared by incubating blood with ACK (ammonium-chloride-potassium) buffer to lyse red blood cells at room temperature for 3–5 minutes, and stained using FACS buffer (1X phosphate-buffered saline [PBS] with 0.1% bovine calf serum and 0.05% sodium azide). Colonic cells in the lamina propria of mice were isolated according to the previously described protocol. Briefly, colon pieces (1 cm pieces) were treated with 5ml of predigestion solution (1× HBSS containing 2mM EDTA and 1mM DTT) for 20 min at 37°C. After incubation, cells epithelial cells were decanted and again incubated the pieces for 20 min at 37°C. Intestinal pieces were washed with 1× PBS to remove remaining EDTA. And collected tissues were incubated with digestion solution (1.5 mg/ml of collagenase D [Roche], 0.1 mg/ml of DNase I [Sigma-Aldrich] and 5% of fetal bovine serum in 100 ml of 1× PBS) for 20 min at 37°C and repeated. Cells were harvested and centrifuge for 10 min at 1,500 rpm, and resuspended with 5 ml of 40% percoll solution and overlayed to 80% percoll solution. Cells were centrifuged for 20 min at 1,000g and resuspended with FACS buffer. And cells were stained FITC- and eFluor® 450-conjugated anti-Ly6G (Gr-1; RB6-8C5, eBioscience, San Diego, CA, USA), PE- and PE-Cy7-conjugated anti-CD11b (M1/70, eBioscine), APC-conjugated anti-Ly6C (HK1.4, eBioscience), PE-Cy5-conjugated anti-F4/80 (BM8, eBioscience), APC-Cy7-conjugated anti-CD11c (N418, Biologend, San Diego, CA, USA) antibodies at 4 °C for 30 min. After washing with FACS buffer, the cells were analyzed by a FACS Calibur (BD Bioscience, Franklin Lakes, NJ, USA) and FACS LSRII (BD Bioscience) and Flowjo software (Tree star, Ashland, OR, USA).

**Microarray hybridization.** The gene expression profile was determined using the MouseWG-6 v.2 Expression BeadChips (Illumina®). For microarray hybridization, total RNA was isolated by homogenizing colon tissue samples and was purified using a DNA-free RNA isolation kit (RNAqueous-4PCR kit; Ambion, Austin, TX, USA) in accordance with the manufacturer’s instructions. Total RNA integrity and quantity were assessed with a Nanodrop-2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Only total RNA with an OD 260/280 ratio >2.0 was used for microarray hybridization. RNA samples were first amplified for array analyses using the Illumina Total Prep RNA Amplification Kit (Ambion, Austin, TX, USA) according to the manufacturer’s instructions. Briefly, 500ng of total RNA, isolated from colon tissue, was used to prepare labelled cRNA with overnight incubation according to the manufacturer’s protocol. The quality and quantity of the labelled cRNA were monitored using a Nanodrop-2000 Spectrophotometer. Amplified cRNA (1.5μg) was hybridized on MouseWG-6 Expression BeadChip arrays, containing more than 45,281 well-annotated Ref transcripts, according to the manufacturer’s standard protocol. The arrays were then scanned on a BeadArray Reader (BeadStation 500G Instrument, Illumina Inc.), and Spot images identification and quantification were obtained by the Genome Studio software v1.0.2. (Illumina Inc.).
Identification of significant genes. The raw data were pre-processed through three steps: background correction was performed, the data were then log-transformed to log 2 scale, and normalized by quantile normalization method implemented in the Genome Studio software (Illumina Inc.). Significant difference between two genotypes in each dose (0% DSS [baseline], 1% DSS treatment, 2% DSS treatment), differences between dose response effect in each genotype, and difference between genotype x dose interaction were identified using ANOVA test (p < 0.05) on log 2-transformed normalized intensities using by Partek® Genomics Suite software v6.3 (Partek, St Louis, MI) (http://www.partek.com/partekgs). Transcripts with more than 2-fold differential expression and a false discovery rate (FDR) < 0.01 were selected for each specific comparison analyzed.

Functional enrichment and clustering analysis. Functional categorization analysis was performed based upon gene ontology consortium (GO). Broad Gene Set Enrichment Analysis (GSEA) was done to examine the significance of each functional category classified by GO44 (http://www.broadinstitute.org/gsea/index.jsp). Hierarchical clustering analysis was carried out with Genesis software v1.7.535 using the Pearson correlation distance matrix with average linkage algorithm.

Statistical Analysis. Data were expressed as the mean ±S.E.M. Statistical significance (p value < 0.05) was evaluated either by unpaired student's t test between two groups or one-way analysis of variance (ANOVA) to compare multiple groups using dose and genotype as factors. Statistical analyses were performed using Graph Pad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and SAS Enterprise Guide 6.1 (SAS Institute Inc., Cary, NC, USA). For gene analyses, significances for functional enrichment of specific genes were determined by a right-tailed Fisher's exact test as the negative log of the probability that the number of focus genes is not due to random chance.

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**Author Contributions**

D.S. and E.C designed the study. W.S., Y.L. and J.S. conducted the experiments. W.S., Y.L., E.C. and D.S. analyzed data and interpreted the results. W.S. and D.S. wrote the manuscript. All authors reviewed the manuscript.

**Additional Information**

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