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Genetic Deletion of Klf4 in the Mouse Intestinal Epithelium Ameliorates Dextran Sodium Sulfate–induced Colitis by Modulating the NF-κB Pathway Inflammatory Response

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Abstract

Background—Krüppel-like factor 4 (KLF4) is a zinc finger transcription factor expressed in the differentiated epithelial cells lining of the intestine. Under physiological conditions, KLF4 inhibits cell proliferation. Conversely, KLF4 mediates proinflammatory signaling in macrophages and its overexpression in the esophageal epithelium activates cytokines, leading to inflammation-mediated esophageal squamous cell cancer formation in mice. Here, we tested whether KLF4 has a proinflammatory activity in experimental colitis in mice.

Methods—Villin-Cre;$Klf4^{fl/fl}$ mice with intestine-specific *Klf4* deletion ($Klf4^{\Delta IS}$) and control mice with floxed *Klf4* gene ($Klf4^{fl/fl}$) were treated or not with 3% dextran sodium sulfate (DSS) for 7 days to induce colitis. Additionally, WT mice were administered or not, nanoparticles loaded with scrambled or Klf4-siRNA, and concomitantly given DSS.

Results—Compared with DSS-treated $Klf4^{fl/fl}$ mice, DSS-treated $Klf4^{\Delta IS}$ mice were significantly less sensitive to DSS-induced colitis. DSS treatment of $Klf4^{fl/fl}$ mice induced Klf4 expression in the crypt zone of the colonic epithelium. DSS-treated $Klf4^{\Delta IS}$ mice had increased proliferation relative to DSS-treated control mice. DSS treatment induced NF-κB signaling pathway in $Klf4^{fl/fl}$ mice colon but not $Klf4^{\Delta IS}$ mice. Additionally, WT mice given DSS and nanoparticle/Klf4-siRNA were less sensitive to colitis and had reduced Klf4 expression and while maintaining the proliferative response in the colonic epithelium.

Conclusions—Our results indicate that Klf4 is an important mediator of DSS-induced colonic inflammation by modulating NF-κB signaling pathway and could be involved in the pathogenesis and/or propagation of inflammatory bowel disease. Thus, Klf4 may represent a novel therapeutic target in inflammatory bowel disease.

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Keywords

Klf4; DSS; NF-κB

Inflammatory bowel disease (IBD) is a complex multifactorial disease[1–3] and is characterized by severe inflammation of the small and/or large intestine leading to recurrent diarrhea and abdominal pain.[4] Crohn's disease and ulcerative colitis are the 2 chronic inflammatory conditions of the gastrointestinal tract that collectively identify IBD.[5]

Dextran sodium sulfate (DSS)–induced colitis is a well-established animal model of mucosal inflammation that has been used in the study of ulcerative colitis pathogenesis and preclinical studies.[6,7] DSS is known to be directly cytotoxic to the cells at multiple levels resulting in the induction of colonic epithelium breakdown.[8–13] The intestinal epithelium is a physical and immunological barrier that prevents direct contact of the intestinal mucosa with the luminal microbiota. The breakdown of the epithelium leads to enhanced uptake of toxic antigens from the gut lumen inducing mucosal and systemic inflammatory processes that then promotes colitis.[14] The exposure to the gut flora leads to a significant increase in the expression of several proinflammatory cytokines, chemokines, nitric oxide, and inducible nitric oxide synthase.[15–19] However, the susceptibility to DSS-induced colitis model is dependent on several factors, individually or combined: (1) the colonic epithelium and its ability to tolerate damage from DSS itself and/or from inflammatory response, (2) the ability of the intestinal mucosa to limit the inflammatory response, and (3) the response of the mucosal immune cells.[14,20]

The zinc finger transcription factor, Krüppel-like factor 4 (KLF4), is normally expressed in the differentiated epithelial cells of the intestine, and it may function in the switch from proliferation to differentiation.[21,22] In vitro, KLF4 inhibits cell proliferation by functioning as a cell cycle checkpoint protein.[21,23] In vivo, KLF4 exhibits a tumor suppressive effect on intestinal tumorigenesis[24] and was found to be downregulated in a variety of human cancers.[25–29] In contrast, KLF4 can also promote tumorigenesis in a context-dependent manner, such as, in the absence of $p21^{CIP1}$.[30,31] Additionally, Klf4 have been shown to be a proinflammatory factor because it activates epithelial cytokines in the esophageal squamous epithelium[32] and mediates proinflammatory signaling in macrophages.[32,33]

Studying the physiological function of KLF4 in the intestinal epithelium has been impeded by the early lethality of mice with whole body deletion of *Klf4*.[34,35] Mice with targeted deletion of *Klf4* from the intestine have been previously described.[36] They have altered differentiation, proliferation, migration, and positioning of intestinal epithelial cells, demonstrating an essential role for KLF4 in maintaining normal intestinal epithelial homeostasis.[36] In this study, we provide the first evidence that Klf4 in the colonic epithelium plays a crucial role in promoting DSS-induced colitis by modulating NF-κB pathway inflammatory response.

MATERIALS AND METHODS

Generation of Mice with Intestine-specific Deletion of the Klf4 Gene

C57BL/6 mice carrying floxed *Klf4* gene ($Klf4^{fl/fl}$) were previously described.[35] C57BL/6 mice carrying *Cre* recombinase gene under the regulation of *villin* promoter (*Vil/Cre*) were purchased from The Jackson Laboratory in Bar Harbor, ME.[37] Mice lacking *Klf4* in their intestinal epithelium were generated by mating $Klf4^{fl/fl}$ mice with *Vil/Cre* mice followed by backcrossing to produce mice with intestinal specific deletion of *Klf4* ($Klf4^{\Delta IS}$). All protocols involving mouse work has been approved by the Institutional Animal Care and Use Committee of Stony Brook University and of Georgia State University, and executed according to the criteria outlined by the Guide for the Care and Use of Laboratory Animals.

DSS Treatment and Clinical Scoring

For acute colitis induction, mice were given or not 3% DSS in water ad libitium for 6 days. Mice were weighed daily and were euthanized when there was a weight loss of > 10% (around day 7). For clinical scoring and histological and immunohistochemical characterization, the large intestines were removed from age-matched littermates of *Klf4* mutant mice ($Klf4^{\Delta IS}$) and control ($Klf4^{fl/fl}$) mice on the last day of the experiment. The colon length of each mouse was measured. Stool content was examined for consistency and for occult blood using Hematocult SENSA kit (Beckman Coulter, Pasadena, CA). Clinical scoring was given after scoring system as described by Cooper et al.[38]

In Vivo Anti-inflammatory Effect of Encapsulated Klf4-siRNA-loaded Nanoparticles

Scrambled small interfering RNA (siRNA) (SC-siRNA) or Klf4-siRNA (Santa Cruz, Dallas, TX) were loaded into biomaterial nanoparticles (NPs) prepared as detailed before.[39] In brief, 0.5 ng of siRNA in 1 mg of polylactic acid NPs that was encapsulated in hydrogel composed of alginate and chitosan, and 100 μL of the hydrogel were administered by gavage per mice a day. Mice were given or not 3% DSS in water ad libitium for 7 days and were gavage fed daily during the 7 days with the encapsulated Klf4-siRNA or SC-siRNA. Mice were weighed daily; and on the last day of treatment, the mice were euthanized and the large intestines were removed. The colon length of each mouse was measured, and clinical scoring was done as explained above.

RNA Extraction and Real-time PCR

Total RNA was extracted from colons collected from mice, RNeasy kit (Qiagen, Valencia, CA) followed by a further purification step using lithium chloride to purify the isolated RNA from all polysaccharides including DSS[40] (see Text, Supplemental Digital Content 1, http://links.lww.com/IBD/A441). Real-time PCR was carried out using primers for the following: Klf4 (catalog: QT00104174; Qiagen), Il-1β,[41] Il-6,[41] and TNFα.[41] Samples were run in triplicates on real-time thermal cycler Mastercycler ep realplex machine (Eppendorf, Hauppauge, NY).

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Protein Extraction and Western Blot

Epithelial cells from the luminal side of collected colons were scraped off and collected for protein extraction and Western blot analysis. Antibodies against IκBα, Klf4, and β actin for Western blot protein detection (see Text, Supplemental Digital Content 1, http://links.lww.com/IBD/A441).

Histology, Immunohistochemistry, and Immunofluorescence

Following stool analysis, isolated colons were fixed and paraffin embedded and sectioned. Some sections were used for standard H&E staining. For immunohistochemistry (IHC), sections were stained for Klf4, BrdU, Ki67, NF-κB (p65), γH2AX, F4/80, CD11c, Ly-6G and IL-6 (see Text, Supplemental Digital Content 1, http://links.lww.com/IBD/A441).

Heterotypic Cell Adhesion Assay

In vitro assay of Jurkat E6.1 T-cell line as a model of T lymphocytes adhesion to Caco2-BBE monolayers as a model of colonic epithelium was carried out as described before[42] (see Text, Supplemental Digital Content 1, http://links.lww.com/IBD/A441). Results are expressed as percent of fluorescence change compared with controls.

5-Bromo-2-deoxyuridine Labeling

Mice were injected intraperitoneally (IP) with Bromo-2-deoxyuridine (BrdU) (Sigma, St. Louis, MO) at 50 μg/g of body weight then euthanized at 4-hour postinjection.

Statistical Analysis

Statistical analysis for significance between treatments was performed by *t* test and one-way analysis of variance.

RESULTS

Intestine-specific Deletion of Klf4 Renders Mice Less Susceptible to DSS-induced Colitis

To determine the role of deleting Klf4 in DSS-induced colitis, mice with or without intestine-specific Klf4 deletion, $Klf4^{\Delta IS}$ or $Klf4^{fl/fl}$, respectively, were given regular water (control) or 3% of DSS in drinking water for 7 days. Control $Klf4^{fl/fl}$ and $Klf4^{\Delta IS}$ mice had no significant weight change over the experimental period (Fig. 1A). $Klf4^{fl/fl}$ given DSS showed significant weight loss compared with control $Klf4^{fl/fl}$ mice; whereas on the other hand, $Klf4^{\Delta IS}$ mice showed significantly less weight loss compared with DSS-treated $Klf4^{fl/fl}$ (Fig. 1A). Compared with DSS-treated $Klf4^{fl/fl}$ mice, $Klf4^{\Delta IS}$ mice had overall significantly lower clinical score and MPO activity (Fig. 1B–E). The protection of $Klf4^{\Delta IS}$ mice from DSS-induced colitis was further confirmed by examining H&E-stained colon sections form DSS-treated $Klf4^{fl/fl}$ and $Klf4^{\Delta IS}$ mice. As shown in Fig. 2, $Klf4^{fl/fl}$ mice had increased loss of colonic epithelium (Fig. 2A, B), whereas $Klf4^{\Delta IS}$ mice had minimal colonic epithelium loss and inflammation(s).

Colonic NF-κb Signaling Pathway Is Suppressed After DSS Treatment of Mice with Intestine-specific Deletion of Klf4 (*Klf4*$^{\Delta IS}$)

To determine the effects of DSS treatment on Klf4 expression in the colonic epithelial cells and on the inflammatory response in the presence and absence of Klf4, colonic epithelium was harvested every day for 6 days from *Klf4*$^{fl/fl}$ and *Klf4*$^{\Delta IS}$ mice given DSS or not. As shown in Fig. 3A, western blot analysis of Klf4 protein level in *Klf4*$^{fl/fl}$ mice was increased in response to DSS treatment, and, as expected, *Klf4*$^{\Delta IS}$ mice had no or very low levels of Klf4, even after DSS treatment. Relative Klf4 mRNA levels mirrored the change in Klf4 expression level shown in Fig. 3A (see Fig. A, Supplemental Digital Content 2, http://links.lww.com/IBD/A442). NF-κB has been shown to be activated by DSS treatment[43] and to play an important role in intestinal inflammation.[44–46] Additionally, Klf4 has been shown to mediate NF-κB signaling pathway. [32,33] Consistent with the previous findings, *Klf4*$^{fl/fl}$ mice had low-to-moderate increase of IκB (a suppressor of NF-κB) after DSS treatment, whereas *Klf4*$^{\Delta IS}$ mice had relatively higher levels of IκB after DSS treatment, as compared with DSS-treated *Klf4*$^{fl/fl}$ mice (Fig. 3A). Staining for NF-κB (p65 subunit) showed basal nuclear localization and comparable staining level of NF-κB in the colonic epithelium in both *Klf4*$^{fl/fl}$ and *Klf4*$^{\Delta IS}$ mice (Fig. 3B, 1 and 2, respectively). However, after DSS treatment, *Klf4*$^{fl/fl}$ mice had increased cytoplasmic and nuclear staining of NF-κB (Fig. 3B, 3), as compared with untreated *Klf4*$^{fl/fl}$ mice. Interestingly, *Klf4*$^{\Delta IS}$ mice showed reduction both in overall staining and in the nuclear localization of NF-κB after DSS treatment (Fig. 3B, 4), as compared with both untreated *Klf4*$^{fl/fl}$ and *Klf4*$^{\Delta IS}$ mice. On analyzing the mRNA levels of inflammatory cytokines Il-1β, Il-6, and TNFα after DSS treatment, *Klf4*$^{\Delta IS}$ mice had significantly lower levels of these cytokines when compared with *Klf4*$^{fl/fl}$ mice (see Fig. B–D, Supplemental Digital Content 2, http://links.lww.com/IBD/A442). Additionally, quantitation of inflammatory cell infiltrates: macrophages (F4/80), lymphocytes (CD11c), granulocytes and monocytes (Ly-6G), and for IL-6 positive cells by immunofluorescence staining showed a significant increase of inflammatory cell infiltrate in *Klf4*$^{fl/fl}$ mice after DSS treatment as compared with DSS-treated *Klf4*$^{\Delta IS}$ mice (see Fig. E, Supplemental Digital Content 2, http://links.lww.com/IBD/A442). Our in vitro data did not indicate whether there is direct or indirect interaction between Klf4 and NF-κB; however in a heterotypic cell adhesion assay, there was significant reduction and increase in lymphocytes (Jurkat cells) adhesion to Caco2-BBE colonic epithelial cells after suppression or overexpression of Klf4 in Caco2-BBE cells, respectively (see Fig. A, Supplemental Digital Content 3, http://links.lww.com/IBD/A443).

DSS Treatment in WT Mice Alters the Pattern of Distribution of Klf4 Expression in the Colonic Crypts and Suppresses Proliferation

Next, we examined the effects of DSS treatment on Klf4 expression pattern in the colonic crypts. Klf4 is normally expressed in the upper one-half of the colonic epithelium, sparing the proliferating crypt cells in the lower half (Fig. 4A, B). On treatment of *Klf4*$^{fl/fl}$ mice with DSS, Klf4 expression extended to the proliferating zone of the colonic crypts (Fig. 4C, D). *Klf4*$^{\Delta IS}$ mice showed complete absence of Klf4 in the colonic epithelial cells (Fig. 4E, F). Because Klf4 has been previously shown to suppress proliferation, we examined the effects of DSS treatment on proliferation in the colonic epithelium crypts by staining for both BrdU

and the proliferation marker, Ki67. *Klf4*$^{fl/fl}$ mice had reduced staining for both BrdU and Ki67 (see Fig. B1–B3, Supplemental Digital Content 3, http://links.lww.com/IBD/A443), whereas *Klf4*$^{\Delta IS}$ mice had more proliferation as evidenced by increased number of cells staining positive for both BrdU and Ki67 (see Fig. B4–B6, Supplemental Digital Content 3, http://links.lww.com/IBD/A443). Several stress factors are known to induce Klf4 expression such as DNA damage[47,48] and oxidative stress.[49,50] DSS is known to cause inflammation and oxidative stress to colonic epithelial cells,[9–11,51] and the resulting oxidative stress can induce DNA damage.[12,52] To confirm the presence of DNA damage in the colonic epithelium after DSS treatment, we stained for γH2AX (a marker for DNA double-strand breaks[53]) in *Klf4*$^{fl/fl}$ and *Klf4*$^{\Delta IS}$ mice given DSS or not. Untreated *Klf4*$^{fl/fl}$ and *Klf4*$^{\Delta IS}$ mice had low or no staining (see Fig. A–C and D–F, Supplemental Digital Content 4, http://links.lww.com/IBD/A444, respectively), whereas DSS-treated mice of both genotypes showed strong nuclear staining for γH2AX (see Fig. G–I and J–L, Supplemental Digital Content 4, http://links.lww.com/IBD/A444, respectively).

WT Mice Treated with NP/Klf4-siRNA Are Protected from Symptoms of DSS-induced Colitis

To investigate whether temporary inhibition of Klf4 expression in the colonic epithelium during DSS treatment will render the mice resistant to DSS-induced colitis, WT mice were administered, or not, biodegradable nontoxic NPs loaded with SC-siRNA (NP/SC-siRNA) or Klf4-siRNA (NP/Klf4-siRNA). First, we tested the effectiveness of Klf4-siRNA in suppressing Klf4 expression in WT mouse embryonic fibroblasts. As shown in Figure A, Supplemental Digital Content 5, http://links.lww.com/IBD/A445, Klf4 protein level was efficiently suppressed as determined by Western blot. Next, WT mice were administered DSS, or not, together with NP/SC-siRNA or NP/Klf4-siRNA. Control mice administered water plus NP/SC-siRNA or NP/Klf4-siRNA had no significant weight change over the experimental period (Fig. 5A). Mice given DSS plus NP/SC-siRNA showed significant weight loss compared with control mice given water plus NP/SC-siRNA. However, mice given DSS plus NP/Klf4-siRNA showed significant protection from weight loss compared with mice given DSS plus NP/SC-siRNA (Fig. 5A). Compared with mice treated with DSS plus NP/SC-siRNA, mice given DSS plus NP/Klf4-siRNA had significantly reduced clinical score (as indicated by longer colon length) and MPO activity (Fig. 5B, C). The protection of mice given DSS plus NP/Klf4-siRNA from DSS-induced colitis was further confirmed by examining H&Estained colon sections. Mice given DSS plus NP/SC-siRNA had increased loss of colonic epithelium and infiltration of inflammatory cells (see Fig. B(1), Supplemental Digital Content 5, http://links.lww.com/IBD/A445), whereas mice given DSS plus NP/Klf4-siRNA had minimal colonic epithelium loss and minimal inflammation (see Fig. B(2), Supplemental Digital Content 5, http://links.lww.com/IBD/A445).

Administration of NP/Klf4-siRNA to WT Mice During DSS Treatment Results in Suppression of Klf4 Expression in the Crypt Proliferative Zone and in Sustained Proliferation

To determine the extent of Klf4 suppression in the colonic epithelium in mice given NP/Klf4-siRNA, colons from mice were administered DSS or not together with NP/SC-siRNA or NP/Klf4-siRNA were stained for Klf4. NP/SC-siRNA had no effect on Klf4 expression (Fig. 6A). In mice given NP/Klf4-siRNA, there was no visible difference in the level or the

extent of Klf4 expression as compared with the mice given NP/SC-siRNA (Fig. 6B). In mice given both DSS and NP/SC-siRNA, the expression of Klf4 was increased and extended to the proliferative zone of the colonic crypt (Fig. 6C). However, in mice given both DSS and NP/Klf4-siRNA, there was an overall decrease in Klf4 expression; and importantly, the expression of Klf4 did not extend to the proliferative zone of the colonic crypt (Fig. 6D). Additionally, Western blot analysis for Klf4 from colonic protein extracts confirmed the reduction of Klf4 level in mice given both DSS and NP/Klf4-siRNA compared with mice given NP/Klf4-siRNA alone or to those given both DSS and NP/SC-siRNA (see Fig. A, Supplemental Digital Content 6, http://links.lww.com/IBD/A446). The effect of treating mice with NP/Klf4-siRNA on proliferation in the colonic crypts was also examined. Mice given water and either NP/SC-siRNA or NP/Klf4-siRNA had no differences in proliferation as indicated by Ki67 staining (see Fig. B(1) and B(2), Supplemental Digital Content, respectively). As expected, proliferation was reduced in mice given DSS and NP/SC-siRNA (see Fig. B(3), Supplemental Digital Content 6, http://links.lww.com/IBD/A446). However, proliferation was sustained in mice given DSS and NP/Klf4-siRNA (see Fig. B(4), Supplemental Digital Content 6, http://links.lww.com/IBD/A446).

DISCUSSION

Under physiologic conditions, KLF4 is mainly expressed in the postmitotic, differentiated epithelial cells in the small intestine and colon.[21,54] We have previously demonstrated that Klf4 has a crucial role in regulating intestinal epithelial cell homeostasis in vivo.[36]

Here, we tested the role of Klf4 in the colonic epithelium during DSS-induced colitis. Deletion of Klf4 from the colonic epithelium rendered the mice resistant to DSS-induced colitis (Figs. 1 and 2). The reduced inflammatory response obsereved in $Klf4^{\Delta IS}$ mice (Fig. 1) indicates that Klf4 plays a role in mediating the proinflammatory signaling in response to DSS treatment. Several lines of work suggest that NF-κB activation actively contributes to the development and maintenance of intestinal inflammation. NF-κB was found to be activated in mucosal cells of patients with inflammatory bowel disease[44] and to play an important role in intestinal inflammation.[44–46] It has been previously shown that NF-κB is activated by DSS treatment and plays a role in mediating DSS-induced colitis,[43] and the inhibition of NF-κB activity ameliorated intestinal inflammation in mouse models of colitis.[45,55,56] These studies also suggest that NF-κB inhibition can have therapeutic effects. However in these studies, it was not clear whether the pathogenic effect of NF-κB was due to NF-κB activation in epithelial or in mucosal immune cells.[20] Additionally, Klf4 has been shown to be a crucial mediator of NF-κB signaling pathway.[32,33] Our results indicate that under normal conditions, Klf4 might not play a role in the NF-κB signaling pathway since both the level of IκB protein and the level and localization of NF-κB p65 were similar between untreated $Klf4^{fl/fl}$ and $Klf4^{\Delta IS}$ mice groups (Fig. 3). However, compared with DSS-treated $Klf4^{fl/fl}$ mice, the increase in IκB level and the reduction in NF-κB p65 staining in DSS-treated $Klf4^{\Delta IS}$ mice (Fig. 3) suggest that in the colonic epithelium the role of Klf4 in mediating NF-κB signaling is limited to the inflammatory response. The results also suggest that Klf4 might play a role in the regulation of IκB expression during the inflammatory response because the IκB level was greatly increased in DSS-treated $Klf4^{\Delta IS}$ mice compared with DSS-treated $Klf4^{fl/fl}$ mice. Taken together, our results strongly suggest a central role for

Klf4 in mediating the proinflammatory response through NF-κB pathway, at the intestinal epithelium level, in response to DSS treatment, and that it is required for NF-κB to maintain its nuclear localization in response to inflammatory signals. It is not yet clear whether the Klf4 mediation of NF-κB pathway in the colonic epithelium is by a direct[33] or an indirect interaction[32] of the 2 factors.

The increased proliferative response observed in DSS-treated *Klf4*$^{\Delta IS}$ mice (see Fig., Supplemental Digital Content 3, http://links.lww.com/IBD/A443) compared with suppressed proliferation in DSS-treated *Klf4*$^{fl/fl}$ mice and given the role of Klf4 as antiproliferation, highlights the importance of colonic epithelium regeneration as a factor in reducing the sensitivity to DSS-induced colitis. Klf4 expression is known to be induced by oxidative stress[50,57] and in response to DNA damage.[47,48] DSS has been shown to cause oxidative stress to colonic epithelial cells[9–11,51] that can induce DNA damage.[12,52] Here, we have demonstrated the occurrence of DNA double-strand breaks in the colonic epithelial cells after DSS treatment in both *Klf4*$^{fl/fl}$ and *Klf4*$^{\Delta IS}$ mice (see Fig., Supplemental Digital Content 4, http://links.lww.com/IBD/A444). Our data suggest that the increased expression of Klf4 and the extension of its expression to the proliferative zone of the colonic crypts are at least in part because of oxidative stress-induced DNA damage in the colonic epithelium after DSS treatment.

For clinical relevance, the effect of temporarily suppressing Klf4 in the colonic epithelium during the course of DSS-induced colitis was then tested. The siRNA knockdown of Klf4 at the mRNA level has been chosen for this study because of the lack of drugs known to specifically suppress Klf4 expression. In vitro, Klf4-siRNA showed high efficiency in suppressing Klf4 expression in mouse embryonic fibroblasts as shown in Figure A, Supplemental Digital Content 5, http://links.lww.com/IBD/A445. For in vivo use, to overcome the low penetration of naked siRNA across cell membranes,[58] we resorted to NPs that have previously shown high potential in binding and delivering siRNA.[39,41,59–61] Here, we used biodegradable noncytotoxic NPs for targeting of Klf4-siRNA with the aim to inhibit Klf4 expression in the colonic epithelium. This method has been used successfully before to target the colonic epithelial cells for load delivery.[39,61,62] There was no significant difference in clinical scoring between WT mice given water and NP/SC-siRNA or NP/Klf4-siRNA (Fig. 5) indicating that treatment with NP/Klf4-siRNA has no negative side effects on the mice. The susceptibility of WT mice to DSS-induced colitis when given DSS plus NP/SC-siRNA (see Fig. 5 and Fig. B(1), Supplemental Digital Content 5, http://links.lww.com/IBD/A445) indicates that giving NP/SC-siRNA confers no protection to the mice. However, the resistance of WT mice to DSS-induced colitis when given DSS plus NPs/Klf4-siRNA, (see Fig. 5 and Fig. B(2), Supplemental Digital Content 5, http://links.lww.com/IBD/A445) is in line with the findings observed with DSS-treated *Klf4*$^{\Delta IS}$ mice and strongly confirming a role for Klf4 in mediating colonic inflammation.

It is worth noting that there was a marked reduction in Klf4 expression in mice given DSS plus NP/Klf4-siRNA when compared with WT given NP/Klf4-siRNA only (see Fig. 6 and Fig. A, Supplemental Digital Content 6, http://links.lww.com/IBD/A446), in particular in the nonproliferating zone of the colonic epithelium (Fig. 6). This could be attributed to an enhanced uptake of the NP/Klf4-siRNA by the colonic epithelium as a result of DSS

treatment because DSS has been shown to cause nano-lipocomplexes in colonic epithelial cells and affect cell membrane permeability[13]; thus, possibly enhancing NPs uptake by colonic epithelial cells. Also when compared with WT mice given DSS plus NP/SC-siRNA, mice given DSS plus NP/Klf4-siRNA had no Klf4 expression extending to the proliferative zone (Fig. 6), indicating a more robust suppression of Klf4 expression in the proliferative zone than in the nonproliferative zone. This could be attributed, in addition to the DSS-effect mentioned above, to the fact that proliferating cells uptake NPs much more efficiently than nonproliferating cells.[63,64] Additionally, mice given DSS plus NP/Klf4-siRNA also had sustained proliferation (see Fig. B, Supplemental Digital Content 6, http://links.lww.com/IBD/A446) consistent with previous findings that Klf4 is antiproliferative.

In conclusion, results from our study indicate a role for Klf4 in the colonic epithelium in promoting colonic inflammation and strongly suggest that it does so by modulating the activity of NF-κB pathway.

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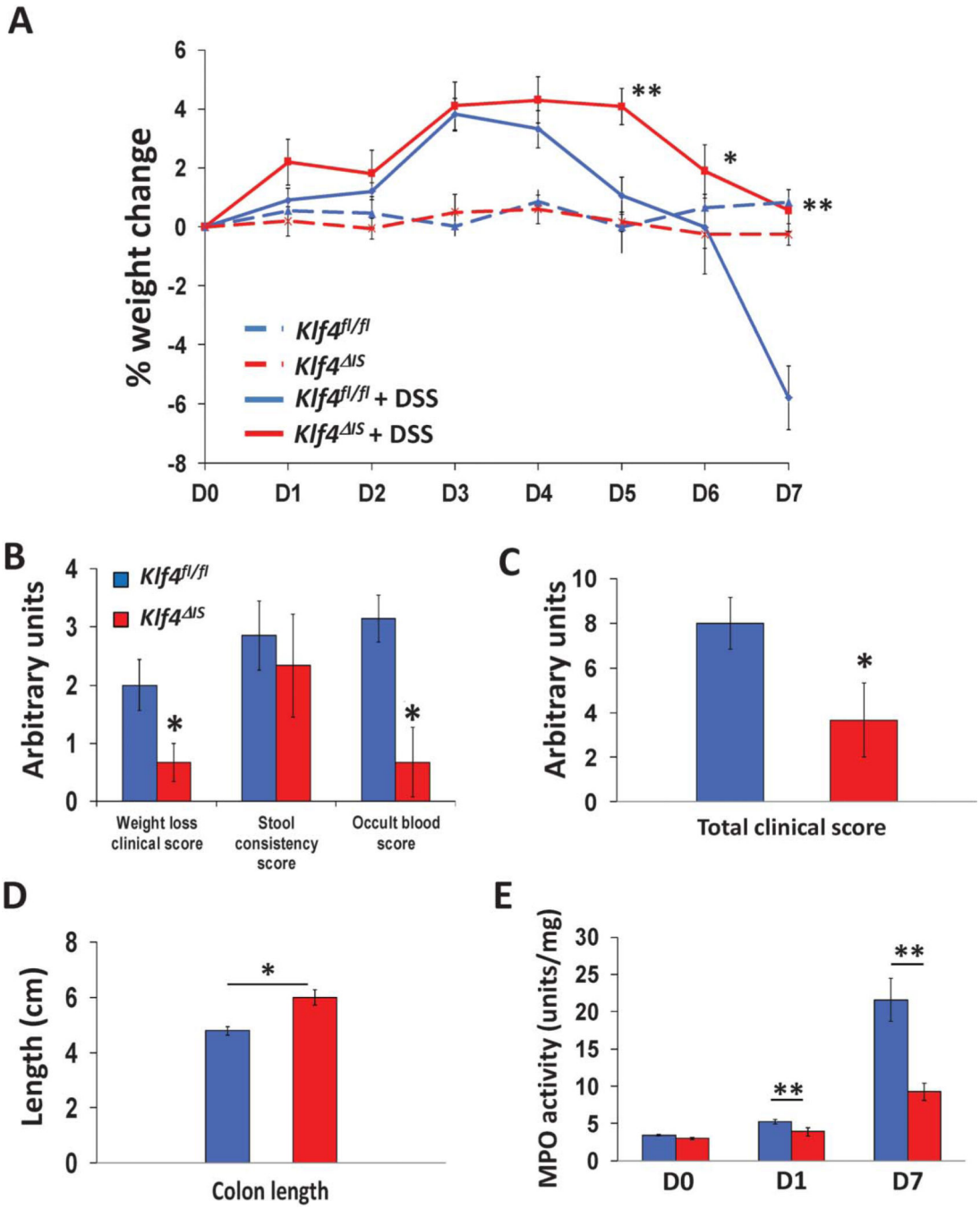

FIGURE 1.

Resistance of *Klf4*$^{\Delta IS}$ mice to symptoms of DSS-induced colitis. A, Mice with intestinal deletion of Klf4 (*Klf4*$^{\Delta IS}$) showed significant resistance to weight loss after treatment with DSS compared with DSS-treated mice with intact intestinal Klf4 (*Klf4*$^{fl/fl}$). B and C, DSS-treated *Klf4*$^{\Delta IS}$ mice had significantly lower clinical scores compared with DSS-treated *Klf4*$^{fl/fl}$ mice. D, DSS-treated *Klf4*$^{\Delta IS}$ mice maintained significantly longer colon lengths compared with DSS-treated *Klf4*$^{fl/fl}$ mice. E, DSS-treated *Klf4*$^{\Delta IS}$ mice had significantly

lower myeloperoxidase (MPO) activity compared with DSS-treated *Klf4*$^{fl/fl}$ mice. N = 8 mice per group. ±SE. **P* < 0.05, ***P* < 0.01.

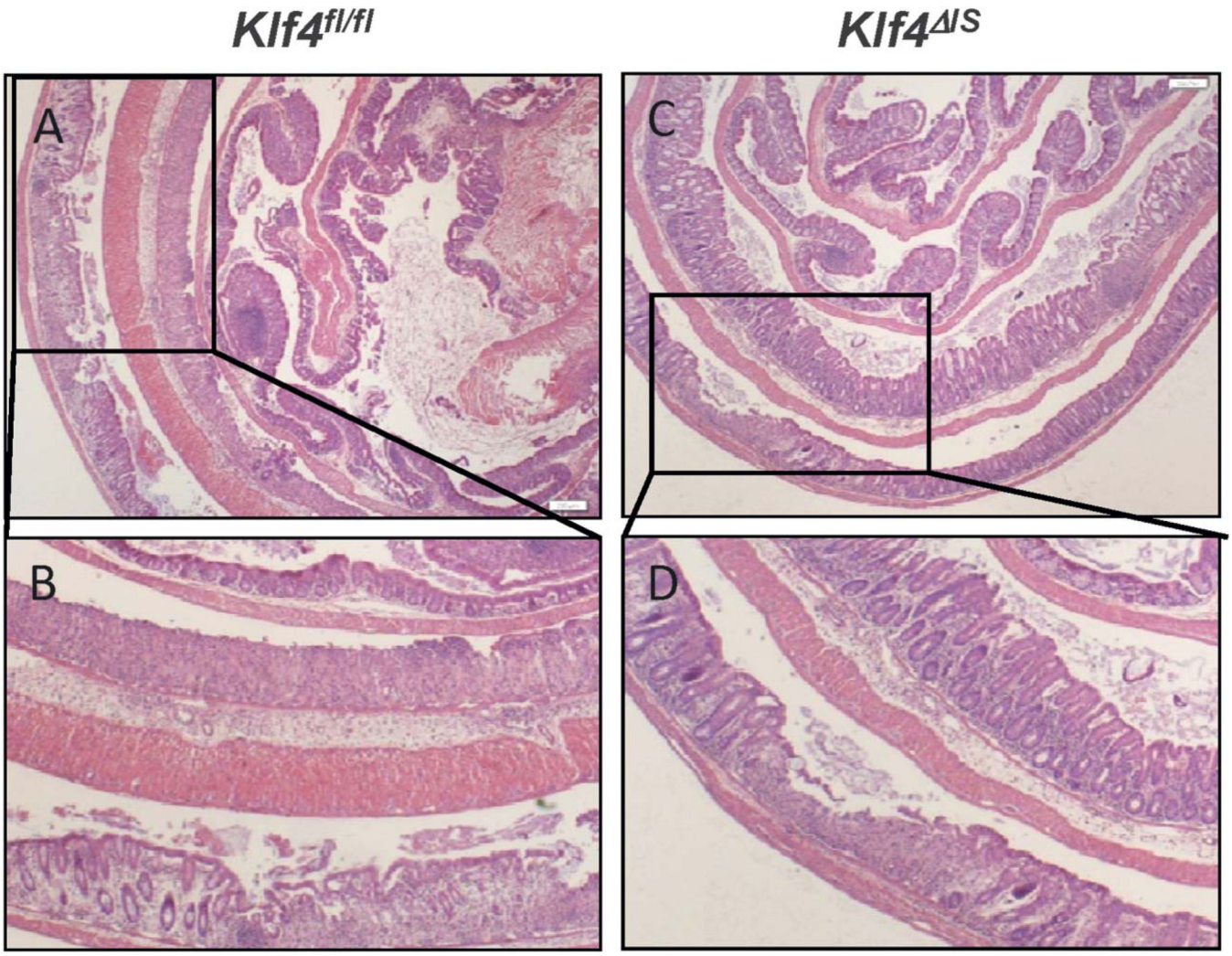

FIGURE 2.

Minimal colonic epithelium loss and inflammation in *Klf4*$^{\Delta IS}$ mice after DSS treatment. A and B, H&E staining of DSS-treated *Klf4*$^{fl/fl}$ mice colon showed extensive colonic epithelium loss. C and D, H&E staining of DSS-treated *Klf4*$^{\Delta IS}$ mice showed minimal loss of colonic epithelium and ulceration regions.

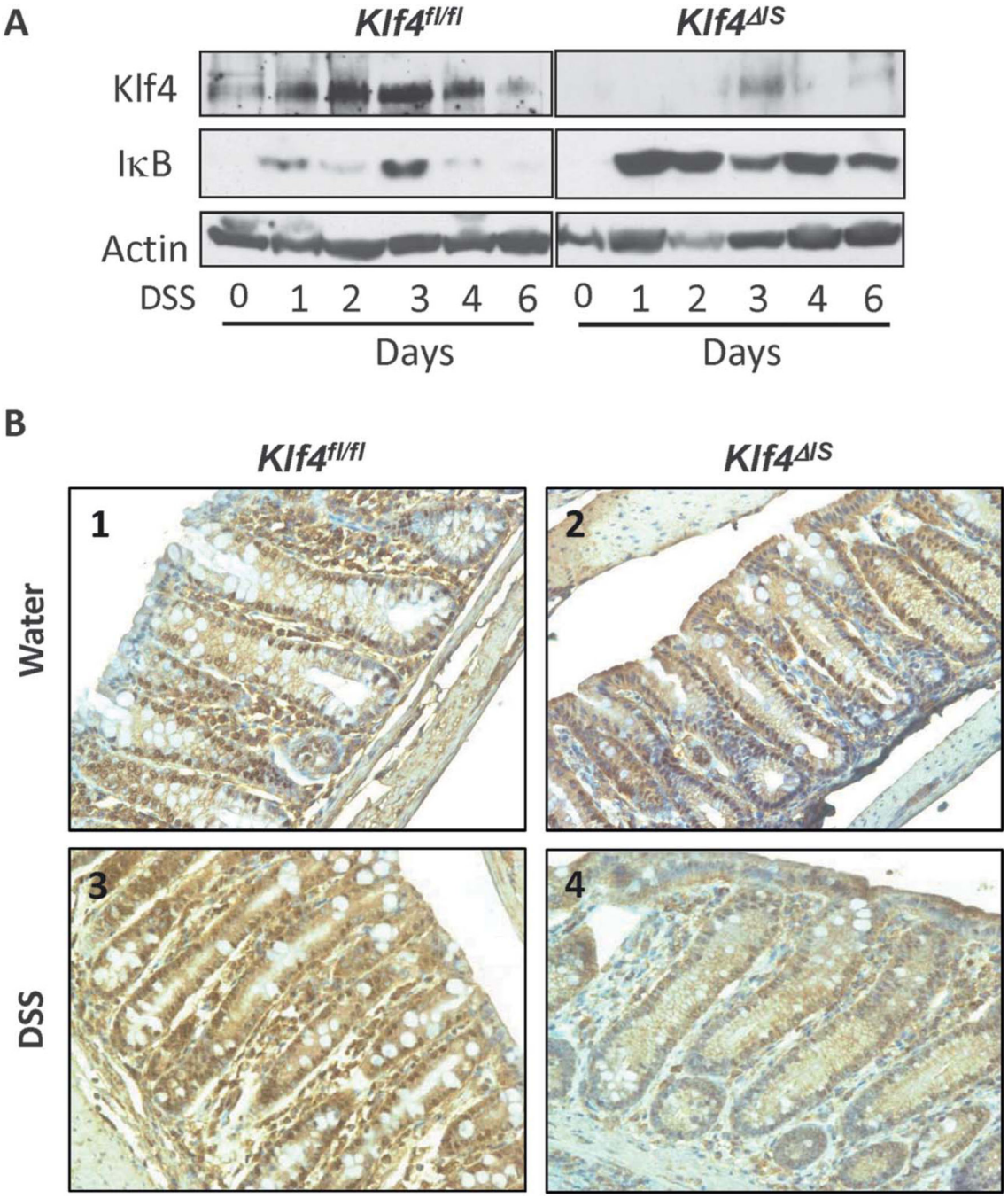

FIGURE 3.

Suppression of NF-κB signaling in the colonic epithelium of *Klf4*$^{\Delta IS}$ mice after DSS treatment. A, Western blot showing increase in Klf4 and low levels of IκB protein levels in *Klf4*$^{fl/fl}$ mice in response to DSS treatment, whereas DSS-treated *Klf4*$^{\Delta IS}$ mice showed absence of Klf4 expression and very high levels of IκB protein level. B, IHC staining of NF-κB (p65). Both *Klf4*$^{fl/fl}$ and *Klf4*$^{\Delta IS}$ mice had similar staining level and localization for NF-κB (p65) at basal level (B1 and B2, respectively). After DSS treatment, *Klf4*$^{fl/fl}$ showed

increased staining of NF-κB (p65) (B3), whereas *Klf4*$^{\Delta IS}$ mice showed much reduced staining (B4).

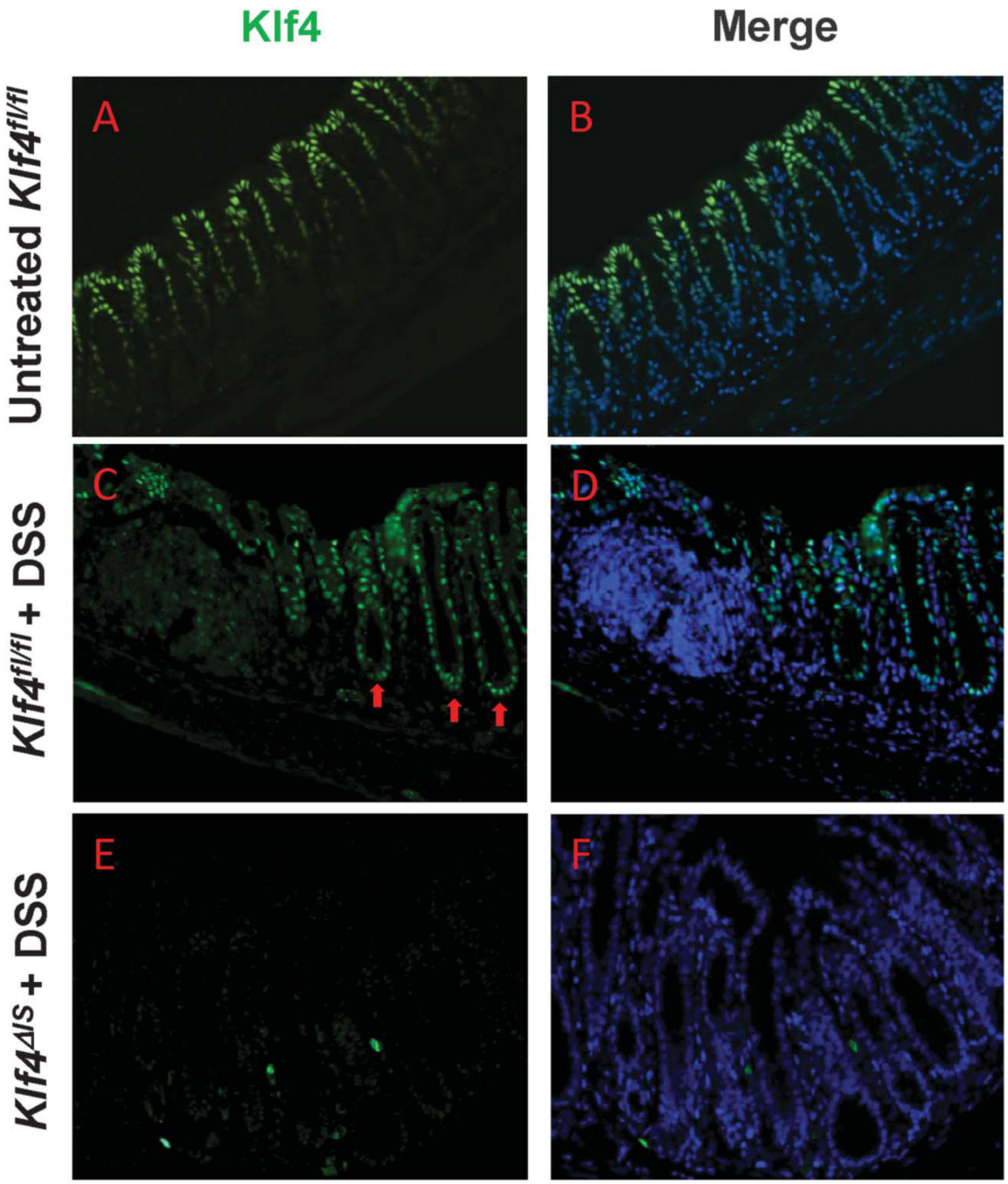

FIGURE 4.

DSS treatment induces the expression of Klf4 in the colonic crypts proliferative zone. Immunofluoroscence staining of Klf4 in the colonic epithelium. A and B, Expression of Klf4 in the normal colonic epithelium of untreated *Klf4*$^{fl/fl}$ mice is limited to the nonproliferating differentiated cells in the upper half of the colonic crypts. C and D, Klf4 expression is extended into the proliferative zone (arrows) of *Klf4*$^{fl/fl}$ mice after DSS treatment. E and F, Absence of Klf4 staining in the colonic epithelium of *Klf4*$^{\Delta IS}$ mice.

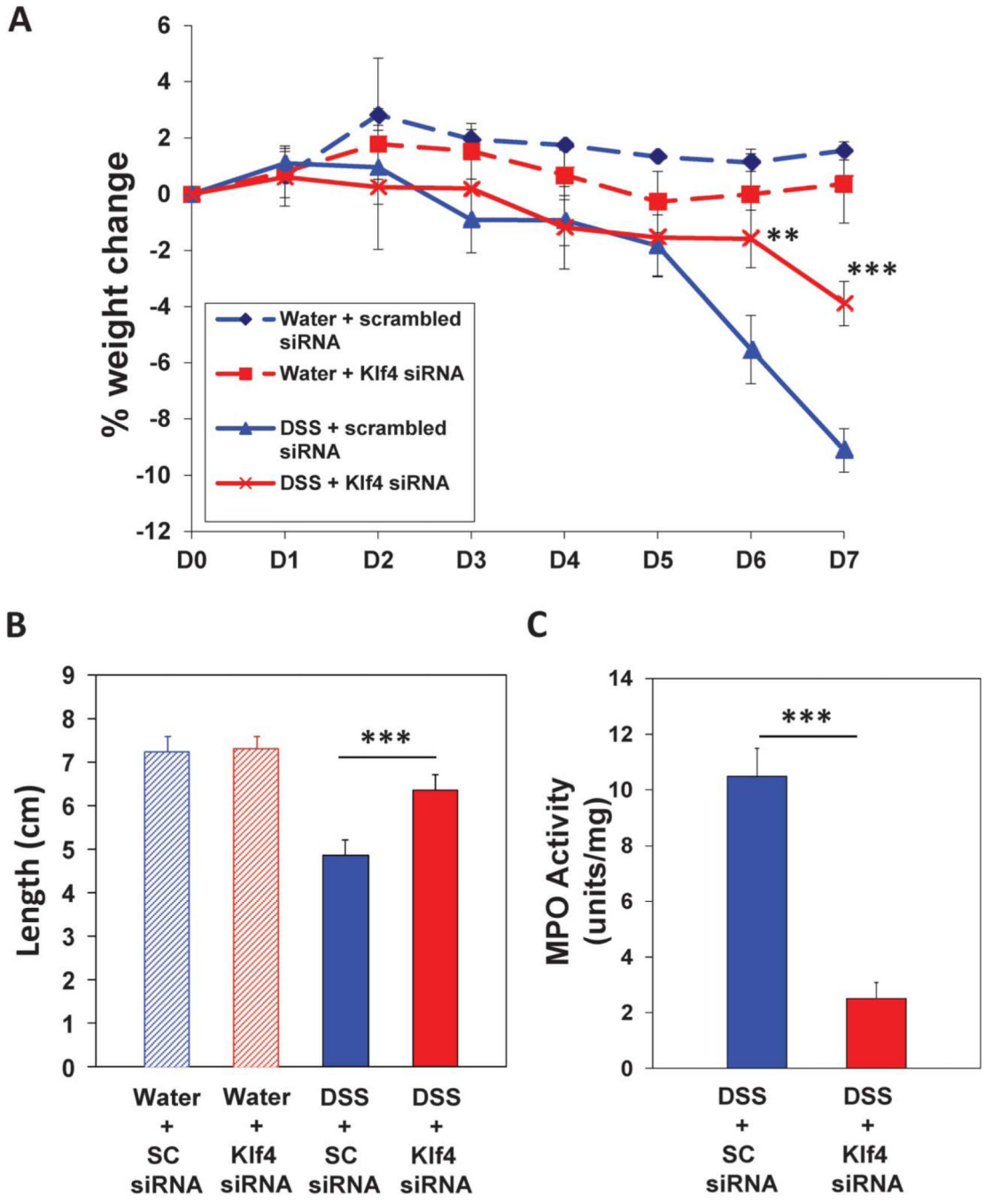

FIGURE 5.

Resistance of mice treated with NP and Klf4-siRNA to symptoms of DSS-induced colitis. A, Mice treated with NP/Klf4-siRNA showed significant resistance to weight loss after treatment with DSS compared with DSS-treated mice given NP/scrambled RNA. B, DSS-treated mice given NP/Klf4-siRNA had significantly lower clinical scores compared with DSS-treated mice given NP/scrambled RNA. C, DSS-treated mice given NP/Klf4-siRNA had significantly lower MPO activity compared with DSS-treated mice given NP/scrambled RNA. N = 8 mice per group. ±SE. $^{**}P < 0.01$.

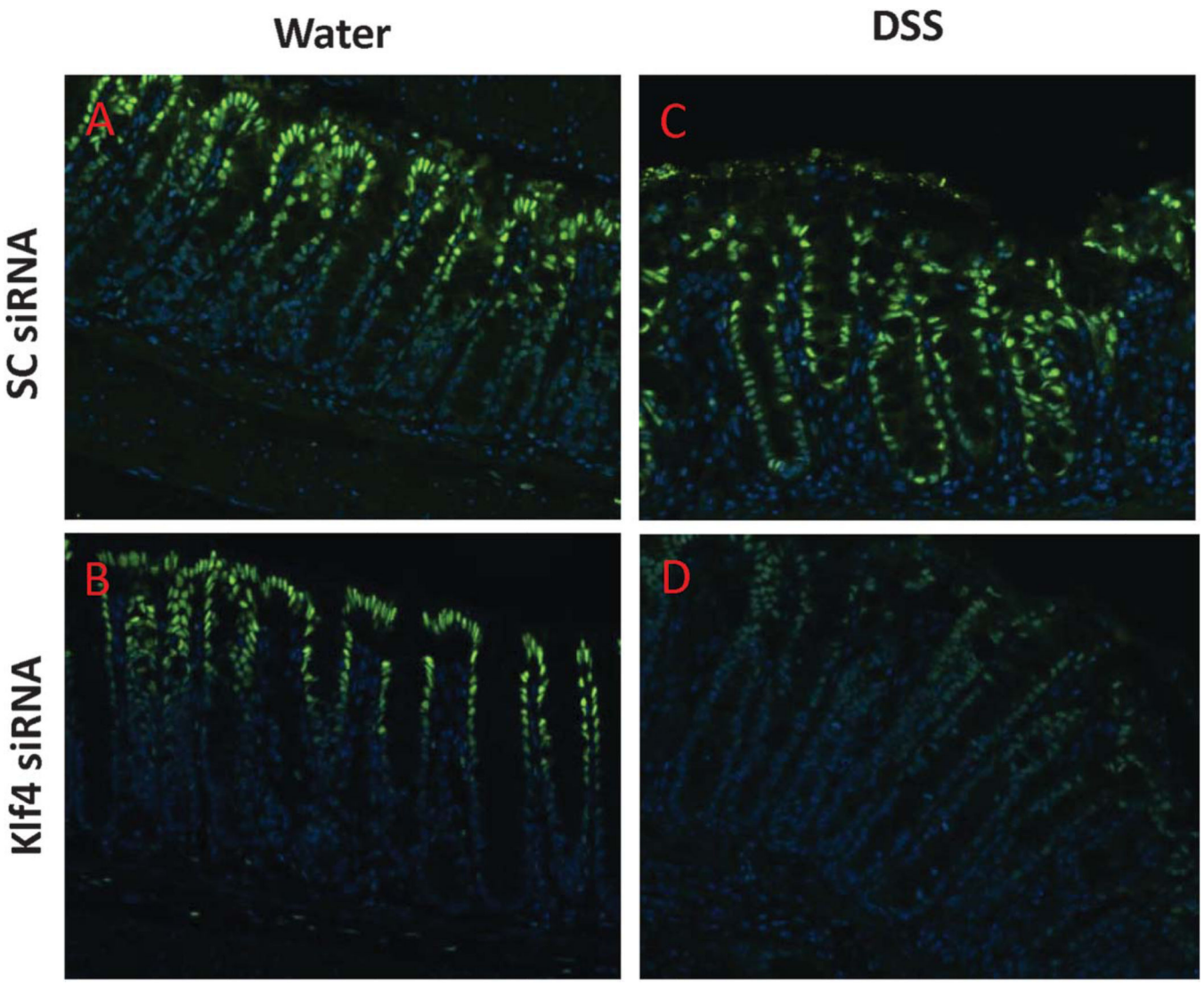

FIGURE 6.

Reduced Klf4 expression in the colonic epithelium of mice treated with DSS and given NP and Klf4-siRNA. Immunoflouroscence staining for Klf4 (Klf4 [green] and Hoechst [blue]) in the colonic epithelium of mice given NP/scrambled RNA (A), NP/Klf4-siRNA (B), DSS and NP/scrambled RNA (C), or DSS and NP/Klf4-siRNA (D).