Role of Csk in intestinal epithelial barrier function and protection against colitis

Sun, Chunxiao ; Murata, Yoji ; Imada, Shinya ; Konno, Tasuku ; Kotani, Takenori ; Saito, Yasuyuki ; Yamada, Hideto ; Matozaki, Takashi

(Citation)
Biochemical and Biophysical Research Communications,504(1):109-114

(Issue Date)
2018-08-31

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90006598

Role of Csk in intestinal epithelial barrier function and protection against colitis

Chunxiao Sun[a,b], Yoji Murata[a*], Shinya Imada[a], Tasuku Konno[a], Takenori Kotani[a], Yasuyuki Saito[a], Hideto Yamada[b], and Takashi Matozaki[a*]

[1]Division of Molecular and Cellular Signaling, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe, Japan.
[2]Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan.

[*]To whom correspondence should be addressed: Takashi Matozaki and Yoji Murata, Division of Molecular and Cellular Signaling, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku,
Kobe 650-0017, Japan. Tel.: +81-78-382-5600. Fax: +81-78-382-5619.
E-mail: matozaki@med.kobe-u.ac.jp and ymurata@med.kobe-u.ac.jp

Word counts: 4580/4600

Abstract

Intestinal epithelial cells (IECs) play a pivotal role in the maintenance of the integrity and barrier function of the intestinal epithelium. Dysfunctions of IECs are thought to participate in the disruption of the intestinal epithelial barrier, resulting in gastrointestinal diseases, such as colitis and colorectal cancer. Here we show that IEC-specific COOH-terminal Src kinase (Csk)-deficient mice (Csk CKO mice) manifested the increased susceptibility to dextran sodium sulfate (DSS)-induced colitis, a model of inflammatory bowel disease. DSS-treated Csk CKO mice also exhibited the significantly elevated intestinal permeability. Following DSS treatment, Csk CKO mice exhibited the higher proliferative activity of colonic epithelial cells and the increased number of apoptotic cells in the colon compared with that apparent for control mice. Moreover, the abundance of the tight junction protein occludin, which regulates cell-cell adhesion as well as epithelial permeability, was markedly reduced in the colon of DSS-treated Csk CKO mice. These results thus suggest that Csk in IECs plays important roles in the regulation of the intestinal epithelial barrier function and protection against colitis.

Keywords: colitis, Csk, intestinal epithelial barrier, intestinal epithelial cell, tight junction

Abbreviations

Ab: antibody;

ANOVA, analysis of variance analysis;

CC3, cleaved caspase-3;

DAI, disease activity index;

DAPI, 4', 6-diamidino-2-phenylindole;

DSS, dextran sodium sulfate;

FITC, fluorescein isothiocyanate;

mAb: monoclonal antibody;

H&E, hematoxylin and eosin;

IBD; inflammatory bowel disease;

IEC, intestinal epithelial cell;

ISC, intestinal stem cell;

mAb: monoclonal antibody;

OCT, optical cutting temperature;

pAb: polyclonal antibody;

PBS, phosphate buffered saline;

SEM, standard error of the mean;

SFK, Src family kinase

1. Introduction

The intestinal epithelium consists of a variety of epithelial cells, such as enterocytes, goblet cells, Paneth cells, and enteroendocrine cells, which arise from intestinal stem cells (ISCs) located at the base of crypts in the intestine and undergo continuous and rapid renewal [1, 2]. Whereas the intestinal epithelium enables the absorption of nutrients, electrolytes, and water by the intestine, it also provides a physical barrier that protects the inner body from the external environment including commensal and pathogenic microorganisms present in the intestinal lumen [3, 4]. This selective barrier function of the intestinal epithelium is achieved in part through proper proliferation and death of intestinal epithelial cells (IECs) as well as their intercellular adhesion mediated by tight junctions, adherens junctions, and desmosomes [3-5]. Disruption of the intestinal epithelial barrier is thought to participate in the development of inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis [3-6]. Indeed, the promotion of epithelial cell death in mouse colon due to IEC-specific deficiency of NEMO, a component of the IκB kinase complex, caused a breakdown of the intestinal epithelial barrier and the development of spontaneous colitis [7]. Moreover, mice lacking the tight junction component claudin-7 in IECs showed the enhancement of paracellular permeability for small organic solutes and mucosal inflammation in the colon [8]. The importance of IEC proliferation, death, and intercellular adhesion for the intestinal epithelial barrier is further supported by the evidence that the increased cell apoptosis and the altered expression of tight junction components were observed in the colon of individuals with IBDs [9, 10]. The molecular mechanism underlying the maintenance of the intestinal epithelial barrier by IECs remains unclear, however.

The COOH-terminal Src kinase (Csk) is a tyrosine kinase and negatively regulates Src family kinases (SFKs)—including c-Src, Fyn, and c-Yes—are nonreceptor protein tyrosine kinases that play essential roles in the regulation of cell proliferation, survival,

and cell-cell adhesion [11]. We previously demonstrated that IEC-specific Csk-deficient mice (Csk CKO mice) manifested the increased proliferative activity and turnover of IECs, with the activity of Src, Fyn, and c-Yes being elevated in epithelial cells of the small intestine [12]. By contrast, the significant reduction of ISCs was observed at the base of crypts in the small intestine of the mutant mice. The number of Paneth cells was decreased, whereas that of goblet cells was increased, in the small intestine of the mutant mice. In addition, Cordero et al. also demonstrated that IEC-specific ablation of c-Src, as well as of Fyn and c-Yes together, led to an increase in the extent of IEC apoptosis in villi of the small intestine [13]. The lack of c-Src in combination with Fyn and c-Yes in IECs also resulted in the marked reduction in Paneth cells of the small intestine. These results thus suggested that Csk in IECs is important for homeostasis of the intestinal epithelium in the steady-state condition. By contrast, the role of intestinal epithelial Csk in the barrier function of, or inflammatory condition of, the intestinal epithelium remains unclear.

To address this issue, we have here examined the effect of dextran sodium sulfate (DSS) on Csk CKO mice. DSS acts as a toxic agent for the colonic epithelium and causes epithelial cell injury, leading to the disturbance of intestinal epithelial barrier function and then the development of colonic inflammation (DSS-induced colitis) as well as regeneration [14-16].

2. Material and Methods

2.1. Antibodies and reagents

Mouse monoclonal antibodies (mAbs) to β-tubulin (TUB 2.1) and to β-catenin (clone14) were obtained from Sigma-Aldrich (St. Louis, MO) and BD Biosciences (San Diego, CA), respectively. Rabbit polyclonal antibody (pAb) to cleaved caspase-3 (Asp175) (9661S) was obtained from Cell Signaling Technology (Beverly, MA). A rabbit mAb to Ki67 (DRM004) were from Acris (Herford, Germany). A rat mAb to E-cadherin (ECCD-2) was obtained from TaKaRa (Kyoto, Japan). Rabbit pAbs to occludin (17-1500) and ZO-1 (61-7300), as well as mouse mAbs to claudin-1(2H10D) and claudin-2 (12H12), were from Thermo Fisher Scientific (Waltham, MA). Horseradish peroxidase–conjugated goat secondary pAbs to rabbit, mouse, or rat IgG (111-035-144, 115-035-166, or 112-035-167) for immunoblot analysis were obtained from Jackson ImmunoResearch (West Grove, PA). Cy3- or Alexa Fluor 488–conjugated goat secondary pAbs to rabbit or mouse IgG (111-165-144 or A-11029) for immunofluorescence analysis were obtained from Jackson ImmunoResearch and Thermo Fisher Scientific, respectively. 4',6-diamidino-2-phenylindole (DAPI) was obtained from Nacalai Tesque (Kyoto, Japan). Tissue-Tek Hematoxylin 3G was from Sakura Finetek Japan (Tokyo, Japan), and eosin was from Wako (Osaka, Japan).

2.2. Mice

Csk$^{fl/fl}$ mice were kindly provided by M. Okada [17, 18], and villin-*cre* mice [B6.SJL-Tg(Vil-cre)997Gum/J] were obtained from Jackson Laboratory (Bar Harbor, ME). Villin-*cre* mice were crossed with *Csk*$^{fl/fl}$ mice to generate *Csk*$^{fl/+}$;villin-*cre* offspring, which were then crossed with *Csk*$^{fl/fl}$ mice to obtain *Csk*$^{fl/fl}$;villin-*cre* (Csk CKO) and *Csk*$^{fl/fl}$ (control) animals. All mice were maintained at the Institute for Experimental Animals at Kobe University Graduate School of Medicine under specific

pathogen–free conditions. All animal experiments were performed according to Kobe University Animal Experimentation Regulations.

2.3. DSS-induced colitis

Male mice at 8-10 weeks of age were treated with 2% (wt/vol) DSS (molecular mass = 36–50 kD, MP Biomedicals, Santa Ana, CA) in drinking water for 7 days, followed by 2 days of regular drinking water.

2.4. Clinical and histological assessment of colitis

Mice were monitored daily for weight loss, stool consistency, and stool blood, and disease activity was scored as described previously [19]. Weight loss was scored as follows: 0 = <1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–20%, or 4 = >20%. Stool consistency was scored as: 0 = normal, 2 = loose stools, or 4 = liquid stools. Blood in the stool was scored as: 0 = no blood as revealed with the use of the guaiac occult blood test (ColoScreen-ES, Helena, Beaumont, TX), 2 = positive occult blood test, and 4 = gross bleeding. The total score for weight loss, diarrhea, and blood in the stool, ranging from 0 (normal) to 12 (severe), was determined. For scoring of colonic inflammation by histological examination, the colon was excised and then immediately fixed with 4% (wt/vol) paraformaldehyde in phosphate buffered-saline (PBS). Paraffin-embedded sections (5 μm) were stained with Mayer's hematoxylin and eosin (H&E), and a combined score for inflammatory cell infiltration, tissue damage, and crypt structure was determined in a blinded manner as described previously [20]. Inflammatory cell infiltration was scored as follows: 0 = presence of occasional inflammatory cells in the lamina propria, 1 = presence of an increased number of inflammatory cells in the lamina propria, 2 = confluence of inflammatory cells extending into the submucosa, 3 = transmural extension of the infiltrate. Tissue damage was scored as: 0 = no mucosal damage, 1 = lymphoepithelial lesions, 2 = surface mucosal erosion, and 3 = extensive

mucosal damage and extension into deeper structures of the colonic wall. Crypt structure was scored as: 0 = normal structure, 1 = occasional hyperplasia without depletion of goblet cells, 2 = hyperplasia with depletion of goblet cells, and 3 = distortion of crypts and the presence of crypt abscesses. Each section was given a score based on the three criteria, ranging from 0 (no change) to 9 (severe).

2.6. Intestinal permeability assay

Mice administered with 2% DSS for 0, 3, and 6 days were fasted for 4 hours and gavaged with 4-kDa fluorescein isothiocyanate (FITC)–dextran (FD4, 600 mg/kg body weight, Sigma-Aldrich). Four hours after gavage, blood was then collected by cardiac puncture, and serum was separated by the use of Capiject Capillary Blood Collection Tubes (Terumo, Tokyo, Japan). The concentration of FITC-dextran in the serum was determined by measuring fluorescence at 535nm/485nm using spectrophotofluorometry (2030 ARVO X4, PerkinElmer, Waltham, MA) and then calculated according to a standard curve plotted by serially diluted FITC-dextran.

2.7. Immunofluorescence analysis

The colon was fixed for 6 h at 4°C with 4% paraformaldehyde in PBS, transferred to a series of sucrose solutions [7, 20, and 30% (w/v), sequentially] in PBS for cryoprotection, embedded in optical cutting temperature (OCT) compound (Sakura Finetek Japan), and rapidly frozen in liquid nitrogen. Frozen sections with a thickness of 5 μm were prepared using a cryostat (CM3050S, Leica Microsystems, Wetzlar, Germany), mounted on glass slides, and air-dried. The sections were then stained with primary antibodies (Abs) and fluorescent dye–labeled secondary Abs as described previously [20]. Images were obtained with a confocal laser-scanning microscope (LSM710; Carl Zeiss, Oberkochen, Germany).

2.8. Immunoblot analysis

The colon was washed with ice-cold PBS and then homogenized in SDS buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1% sodium dodecyl sulfate] containing 1 mM sodium vanadate, 50 mM NaF, and a protease inhibitor cocktail (04080-11, Nacalai Tesque). The lysates were heated at 95°C for 5 min and centrifuged at 17,500 × g for 15 min at room temperature, and the resulting supernatants were subjected to immunoblot analysis as previously described [20].

2.9. Statistical analysis

Quantitative data are presented as means ± SEM and were analyzed with Student's *t*-test, Mann-Whitney *U* test, or by ANOVA followed by Sidak's test or Tukey's test. A P value of <0.05 was considered statistically significant. Analysis was performed with the use of GraphPad Prism software (GraphPad Software, La Jolla, CA).

3. Results

3.1. Impacts of Csk deficiency in IECs on DSS-induced colitis

To investigate the role of Csk in the regulation of the intestinal epithelial barrier, we crossed mice homozygous for a floxed (fl) *Csk* allele [17, 18] with those harboring a transgene for Cre recombinase under the control of the villin gene promoter (villin-*cre*) [21], thereby obtaining $Csk^{fl/fl}$;villin-*cre* mice (Csk CKO mice) [12], in which *Csk* was efficiently and specifically deleted in IECs. As we previously reported [12], Csk CKO mice manifested the significant reduction in Csk protein in IECs from the colon (**data not shown**). Moreover, the marked difference in the morphology of the intestinal epithelium between control and Csk CKO mice at 8 to 12 weeks of age was undetectable (**data not show)**. We then gave Csk CKO and control mice drinking water with 2% DSS, a chemical disrupting the epithelium, for 7 days, followed by 2 days of regular drinking water. Following DSS treatment, Csk CKO mice showed a significant reduction in body weight compared with control mice (**Fig. 1A**). On day 6 of DSS treatment, the colon length of Csk CKO mice was significantly shorter than that of control mice (**Fig. 1B)**. To analyze the severity of colitis, we monitored the disease activity, which was scored by the extents of body weight loss, stool consistency, and stool blood with Csk CKO mice as well as control mice. The disease activity in Csk CKO mice at 9 days after the start of DSS treatment was markedly increased, compared with that in control mice (**Fig. 1C**). Microscopic examination revealed that transmural inflammation, epithelial erosion, immune cell infiltration, and crypt disappearance were marked in the colon of Csk CKO mice on day 9 of DSS treatment (**Fig. 1D**). Thus, the histological score for colonic injury and inflammation was markedly greater for Csk CKO mice than for control mice (**Fig. 1E**). Moreover, when Csk CKO and control mice were gavaged with 4-kDa FITC-dextran on day 0, 3, and 6 of DSS administration, the fluorescence intensity of FITC in the serum of Csk CKO mice was significantly higher than that apparent for control mice on day 6 of DSS treatment (**Fig. 2**). Collectively,

these results suggested that Csk in IECs protects against DSS-induced colonic injury and acute colitis.

3.2. Increased proliferation activity and apoptosis of IECs in DSS-treated Csk CKO mice

Disruption of the intestinal epithelial barrier is thought to participate in the development of DSS-induced colitis [14-16]. The maintenance of the barrier function of the intestinal epithelium requires the proper regulation of IEC proliferation and death [5, 7, 14]. We thus examined the proliferative activity and apoptosis of colonic epithelial cells in Csk CKO mice following DSS administration. Immunostaining of the colon with Abs to Ki67, a marker for cell proliferation [22], showed a significant increase in the number of Ki67-positive cells in the colon of Csk CKO mice treated with or without DSS, compared with that apparent for control mice (**Fig. 3A**). Moreover, immunostaining for the cleaved form of caspase-3, a maker of cell apoptosis, showed that the number of cells positive for cleaved caspase-3 was significantly higher in Csk CKO mice than in control mice on day 6 of DSS treatment, whereas it did not differ between two genotypes before the treatment (**Fig. 3B**).

3.3. Effects of Csk deficiency in IECs on the expression of tight junction and adherens junction proteins in the colon of mice treated with DSS

Tight junction and adherens junction proteins, such as occludin, claudin-1, claudin-2, ZO-1, E-cadherin, and β-catenin are thought to be key players in the maintenance of the intestinal epithelial barrier function [8, 23, 24]. We thus determined the expression levels of these junctional proteins in the colon at 0 and 6 days after the start of DSS treatment. Immunoblot analysis showed that the expression levels of occludin, claudin-1, claudin-2, ZO-1, E-cadherin, and β-catenin in the colon were similar between Csk CKO and control mice at baseline (**Fig. 4A and B)**. However, DSS

treatment resulted in the marked reduction in the abundance of occludin in the colon of Csk CKO mice (**Fig. 4A and B)**. By contrast, DSS treatment failed to reduce the abundance of occludin in the colon of control mice (**Fig. 4A and B)**. The amount of claudin-2 was slightly decreased by DSS treatment in the colon of Csk CKO mice, whereas such a reduction was not statistically significant (**Fig. 4A and B)**. By contrast, the amounts of claudin-1, ZO-1, E-cadherin, and β-catenin in the colon of Csk CKO and control mice were not significantly changed by DSS treatment (**Fig. 4A and B)**. These results thus indicated that DSS treatment did not affect the abundance of tight junction and adherens junction proteins in the colon of the control mice. In contrast, Csk likely participated in the regulation of the abundance of occludin protein in the colon exposed to DSS.

4. Discussion

In the present study, we have demonstrated that IEC-specific Csk deficiency in mice results in the increased susceptibility to DSS-induced colitis. In addition, following DSS treatment, intestinal permeability in Csk CKO mice was significantly increased compared with that in control mice, suggestive of the impairment of the intestinal epithelial barrier by Csk deficiency. Our results thus suggest that Csk in IECs contributes to protection against the development of DSS-induced colitis through the maintenance of the intestinal epithelial barrier.

We also found that Csk CKO mice treated with or without DSS manifested the increased proliferative activity of IECs in the colon. Consistently, the number of apoptotic cells in the colon of the DSS-treated mutant mice was higher than that for DSS-treated control mice. By contrast, the abundance of occludin in the colon was significantly reduced for DSS-treated Csk CKO mice. Given that the reduction in occludin was observed in the colon of patients with IBDs [23] and is thought to contribute to the development of DSS-induced colitis [25], Csk in IECs likely participates in the maintenance of the intestinal epithelial barrier in part through the regulation of the abundance of occludin in the colon after DSS-induced colonic injury.

The molecular mechanism by which Csk in IECs modulates the expression of occludin in the DSS-treated colon remains to be fully elucidated. It was demonstrated that treatment of mice with DSS in drinking water induced tyrosine phosphorylation of occludin in the colonic epithelial cells [25, 26]. c-Src was shown to mediate the tyrosine phosphorylation of occludin in cultured IECs upon DSS exposure [25, 26]. The DSS-induced tyrosine phosphorylation of occludin caused its proteasome-dependent degradation and increased paracellular permeability in cultured IECs [25]. Moreover, we previously showed that deficiency of Csk in IECs resulted in the elevated activation of the Src family tyrosine kinases c-Src, c-Yes, and Fyn in IECs [12]. In addition, it has been demonstrated that c-Src is implicated in the pathogenesis of DSS-induced colitis

[25, 27]. It is thus likely that intestinal epithelial Csk modulates the intestinal epithelial barrier in the colonic epithelium by controlling the c-Src-mediated degradation of occludin. However, further investigation is certainly necessary to clarify the molecular mechanisms by which intestinal epithelial Csk regulates the intestinal epithelial barrier in the colon after tissue damage.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

We thank M. Okada (Osaka University) for *Csk*$^{fl/fl}$ mice and D. Tanaka for technical assistance. This work was in part supported by a Grant-in-Aid for challenging Exploratory Research from JSPS (16K15219 to T.M.), a Grant-in-Aid for Scientific Research (C) from JSPS (16K08624 to Y.M.), and Yakult Bio-Science Foundation.

References

[1] N. Barker, Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration, Nat Rev Mol Cell Biol 15 (2014) 19-33.

[2] H. Clevers, The intestinal crypt, a prototype stem cell compartment, Cell 154 (2013) 274-284.

[3] M.A. Odenwald, J.R. Turner, The intestinal epithelial barrier: a therapeutic target?, Nat Rev Gastroenterol Hepatol 14 (2017) 9-21.

[4] J.R. Turner, Intestinal mucosal barrier function in health and disease, Nat Rev Immunol 9 (2009) 799-809.

[5] C. Günther, H. Neumann, M.F. Neurath, C. Becker, Apoptosis, necrosis and necroptosis: cell death regulation in the intestinal epithelium, Gut 62 (2013) 1062-1071.

[6] A.H. Gitter, F. Wullstein, M. Fromm, J.D. Schulzke, Epithelial barrier defects in ulcerative colitis: characterization and quantification by electrophysiological imaging, Gastroenterology 121 (2001) 1320-1328.

[7] A. Nenci, C. Becker, A. Wullaert, R. Gareus, G. van Loo, S. Danese, M. Huth, A. Nikolaev, C. Neufert, B. Madison, D. Gumucio, M.F. Neurath, M. Pasparakis, Epithelial NEMO links innate immunity to chronic intestinal inflammation, Nature 446 (2007) 557-561.

[8] H. Tanaka, M. Takechi, H. Kiyonari, G. Shioi, A. Tamura, S. Tsukita, Intestinal deletion of *Claudin-7* enhances paracellular organic solute flux and initiates colonic inflammation in mice, Gut 64 (2015) 1529-1538.

[9] J.M. Blander, Death in the intestinal epithelium–basic biology and implications for inflammatory bowel disease, FEBS J 283 (2016) 2720-2730.

[10] J. Landy, E. Ronde, N. English, S.K. Clark, A.L. Hart, S.C. Knight, P.J. Ciclitira, H.O. Al-Hassi, Tight junctions in inflammatory bowel diseases and inflammatory

bowel disease associated colorectal cancer, World J Gastroenterol 22 (2016) 3117-3126.

[11] M. Okada, Regulation of the Src family kinases by Csk, Int J Biol Sci 8 (2012) 1385-1397.

[12] S. Imada, Y. Murata, T. Kotani, M. Hatano, C. Sun, T. Konno, J.H. Park, Y. Kitamura, Y. Saito, H. Ohdan, T. Matozaki, Role of Src family kinases in regulation of intestinal epithelial homeostasis, Mol Cell Biol (2016).

[13] J.B. Cordero, R.A. Ridgway, N. Valeri, C. Nixon, M.C. Frame, W.J. Muller, M. Vidal, O.J. Sansom, c-Src drives intestinal regeneration and transformation, EMBO J 33 (2014) 1474-1491.

[14] M. Perše, A. Cerar, Dextran sodium sulphate colitis mouse model: traps and tricks, J Biomed Biotechnol 2012 (2012) 718617.

[15] S. Kitajima, S. Takuma, M. Morimoto, Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium, Exp Anim 48 (1999) 137-143.

[16] P. Kiesler, I.J. Fuss, W. Strober, Experimental models of inflammatory bowel diseases, Cell Mol Gastroenterol Hepatol 1 (2015) 154-170.

[17] C. Schmedt, K. Saijo, T. Niidome, R. Kuhn, S. Aizawa, A. Tarakhovsky, Csk controls antigen receptor-mediated development and selection of T-lineage cells, Nature 394 (1998) 901-904.

[18] R. Yagi, S. Waguri, Y. Sumikawa, S. Nada, C. Oneyama, S. Itami, C. Schmedt, Y. Uchiyama, M. Okada, C-terminal Src kinase controls development and maintenance of mouse squamous epithelia, EMBO J 26 (2007) 1234-1244.

[19] B. Siegmund, G. Fantuzzi, F. Rieder, F. Gamboni-Robertson, H.A. Lehr, G. Hartmann, C.A. Dinarello, S. Endres, A. Eigler, Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-γ and TNF-α production, Am J Physiol Regul Integr Comp Physiol 281 (2001) R1264-1273.

[20] Y. Murata, T. Kotani, Y. Supriatna, Y. Kitamura, S. Imada, K. Kawahara, M. Nishio, E.W. Daniwijaya, H. Sadakata, S. Kusakari, M. Mori, Y. Kanazawa, Y. Saito, K. Okawa, M. Takeda-Morishita, H. Okazawa, H. Ohnishi, T. Azuma, A. Suzuki, T. Matozaki, Protein tyrosine phosphatase SAP-1 protects against colitis through regulation of CEACAM20 in the intestinal epithelium, Proc Natl Acad Sci U S A 112 (2015) E4264-4271.

[21] B.B. Madison, L. Dunbar, X.T. Qiao, K. Braunstein, E. Braunstein, D.L. Gumucio, Cis elements of the villin gene control expression in restricted domains of the vertical (crypt) and horizontal (duodenum, cecum) axes of the intestine, J Biol Chem 277 (2002) 33275-33283.

[22] P.R. Holt, S.F. Moss, A.M. Kapetanakis, A. Petrotos, S. Wang, Is Ki-67 a better proliferative marker in the colon than proliferating cell nuclear antigen?, Cancer Epidemiol Biomarkers Prev 6 (1997) 131-135.

[23] R. Ahmad, M.F. Sorrell, S.K. Batra, P. Dhawan, A.B. Singh, Gut permeability and mucosal inflammation: bad, good or context dependent, Mucosal Immunol 10 (2017) 307-317.

[24] M.L. Hermiston, J.I. Gordon, Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin, Science 270 (1995) 1203-1207.

[25] C. Chelakkot, J. Ghim, N. Rajasekaran, J.S. Choi, J.H. Kim, M.H. Jang, Y.K. Shin, P.G. Suh, S.H. Ryu, Intestinal epithelial cell-specific deletion of PLD2 alleviates DSS-induced Colitis by regulating occludin, Sci Rep 7 (2017) 1573.

[26] G. Samak, K.K. Chaudhry, R. Gangwar, D. Narayanan, J.H. Jaggar, R. Rao, Calcium/Ask1/MKK7/JNK2/c-Src signalling cascade mediates disruption of intestinal epithelial tight junctions by dextran sulfate sodium, Biochem J 465 (2015) 503-515.

[27] K. Taniguchi, L.W. Wu, S.I. Grivennikov, P.R. de Jong, I. Lian, F.X. Yu, K. Wang, S.B. Ho, B.S. Boland, J.T. Chang, W.J. Sandborn, G. Hardiman, E. Raz, Y.

Maehara, A. Yoshimura, J. Zucman-Rossi, K.L. Guan, M. Karin, A gp130-Src-YAP module links inflammation to epithelial regeneration, Nature 519 (2015) 57-62.

Figure legends

Fig. 1. Impacts of Csk deficiency in IECs on DSS-induced colitis. (A-E) Control (Ctrl) and Csk CKO (CKO) mice were given 2% DSS in drinking water for 7 days, followed by 2 days of regular drinking water. Body weight curves of control (n = 20) and Csk CKO (n = 18) mice from 0 to 9 days after the start of DSS treatment (**A**). The colon length of control and Csk CKO mice on day 0 or 6 of DSS treatment (n = 13-15 per group) (**B**). Disease activity index (DAI) of control (n = 9) and Csk CKO (n = 10) mice on day 0 or 9 of DSS treatment (**C**). Representative images of H&E stained paraffin-embedded colon sections from control and Csk CKO mice at 9 days after the start of 2% DSS treatment (**D**). Histological score of control (n = 5) and Csk CKO mice (n = 4) was examined for such sections (**E**). Quantitative data in (**A**, **B**, **E**) are means ± SEM from two or three separate experiments. Bars in (**C**) indicate median values. *P < 0.05; **P < 0.01; ***P < 0.001 [Two-way repeated ANOVA and Sidak's test in (**A**), One-way ANOVA and Tukey's test in (**B**), Mann-Whitney U test in (**C**), Student's t-test in (**E**)]. Scale bars, 200 μm.

Fig. 2. Increased intestinal permeability in DSS-treated Csk CKO mice. Control (Ctrl) and Csk CKO (CKO) mice administered with 2% DSS for 0, 3, or 6 days (n = 6 per group) were fasted for 4 hours and gavaged with 4-kDa FITC-dextran (600 mg/kg body weight). Four hours after gavage, blood was collected by cardiac puncture, and serum was separated by centrifugation. The concentration of FITC-dextran in the serum was determined by spectrophotofluorometry. Quantitative data are means ± SEM from two separate experiments. *P < 0.05; ***P < 0.001 (One-way ANOVA and Tukey's test).

Fig. 3. Increased proliferation activity and apoptosis of IECs in DSS-treated Csk CKO mice. (**A**) Frozen sections of the distal colon from control (Ctrl) and Csk CKO

(CKO) mice on day 0 or 6 of DSS treatment were subjected to immunostaining with mAbs to Ki67 (red) and to β-catenin (green), and to staining of nuclei with DAPI (blue) (left panel). The number of Ki67-positive cells per crypt was also determined (right panel). (**B**) Frozen sections prepared from the distal colon of control and Csk CKO mice on day 0 or 6 of DSS treatment were subjected to immunostaining with pAbs to cleaved caspase-3 (CC3, red), a mAb to β-catenin (green), and to staining of nuclei with DAPI (blue) (left panel). Arrows indicate cleaved caspase-3–positive cells. The number of cleaved caspase-3–positive cells per field (magnification 200x) was also determined (right panel). Quantitative data are means ± SEM for 100 crypts (**A**) or 150-180 random optical fields (**B**) from a total of 5 or 6 mice per group examined in two separate experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (One-way ANOVA and Tukey's test). Scale bars, 50 μm.

Fig. 4. Effects of Csk deficiency in IECs on the expression of tight junction and adherens junction proteins in the colon of mice treated with DSS. (**A, B**) Lysates of the distal colon from control (Ctrl) and Csk CKO (CKO) mice at 0 or 6 days after the start of 2% DSS treatment were subjected to immunoblot analysis with Abs to the indicated proteins (**A**). The band intensity for each protein in such blots was also normalized by that of β-tubulin and expressed relative to the normalized value for control mice (day 0) (**B**). Quantitative data are means ± SEM for a total of 6 or 7 mice per group examined in three separate experiments. $*P < 0.05$; $**P < 0.01$ (One-way ANOVA and Tukey's test).

Highlights

1. IEC-specific Csk-deficient mice manifested the increased susceptibility to DSS-induced colitis.
2. DSS treatment significantly increased intestinal permeability in the mutant mice.
3. DSS treatment markedly reduced occludin protein in the colon of the mutant mice.

Figure 1
C. Sun *et al.*

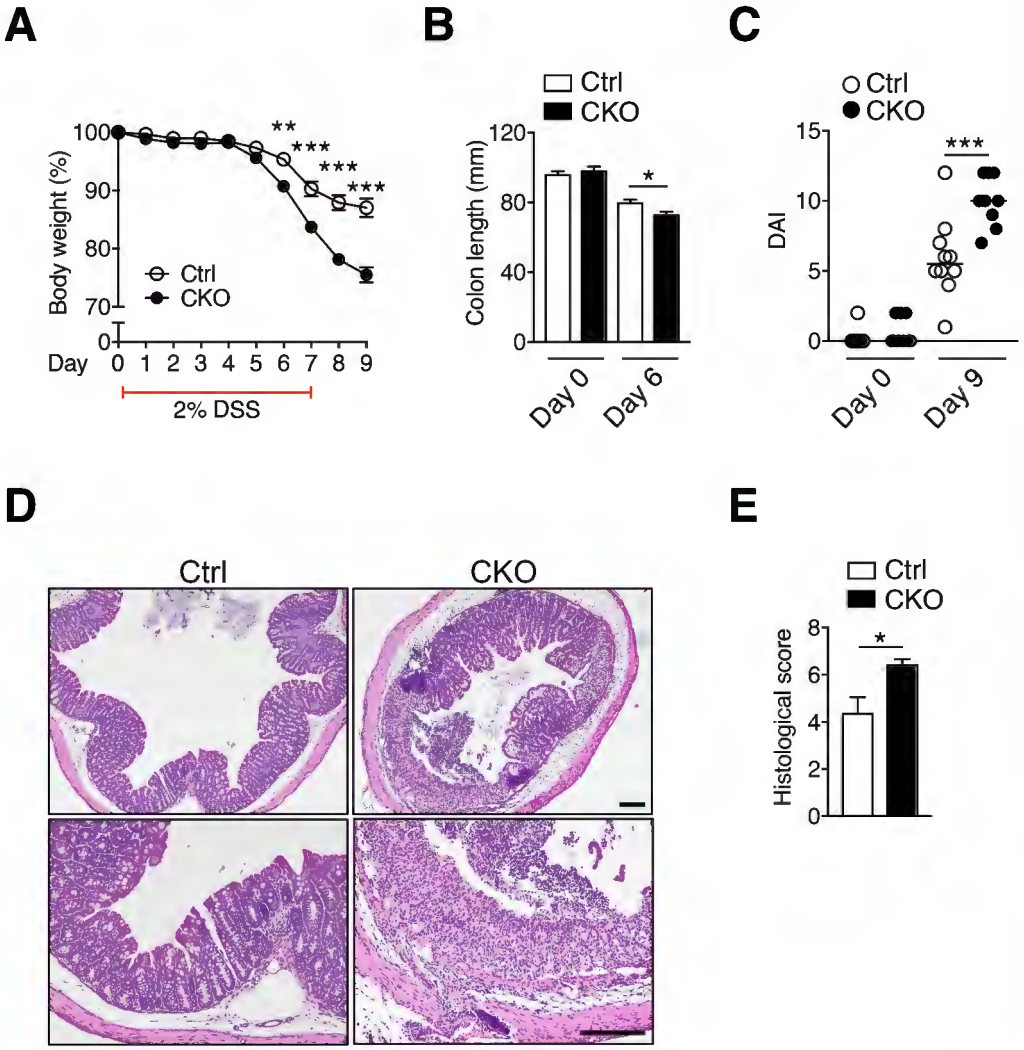


Figure 2

C. Sun *et al.*

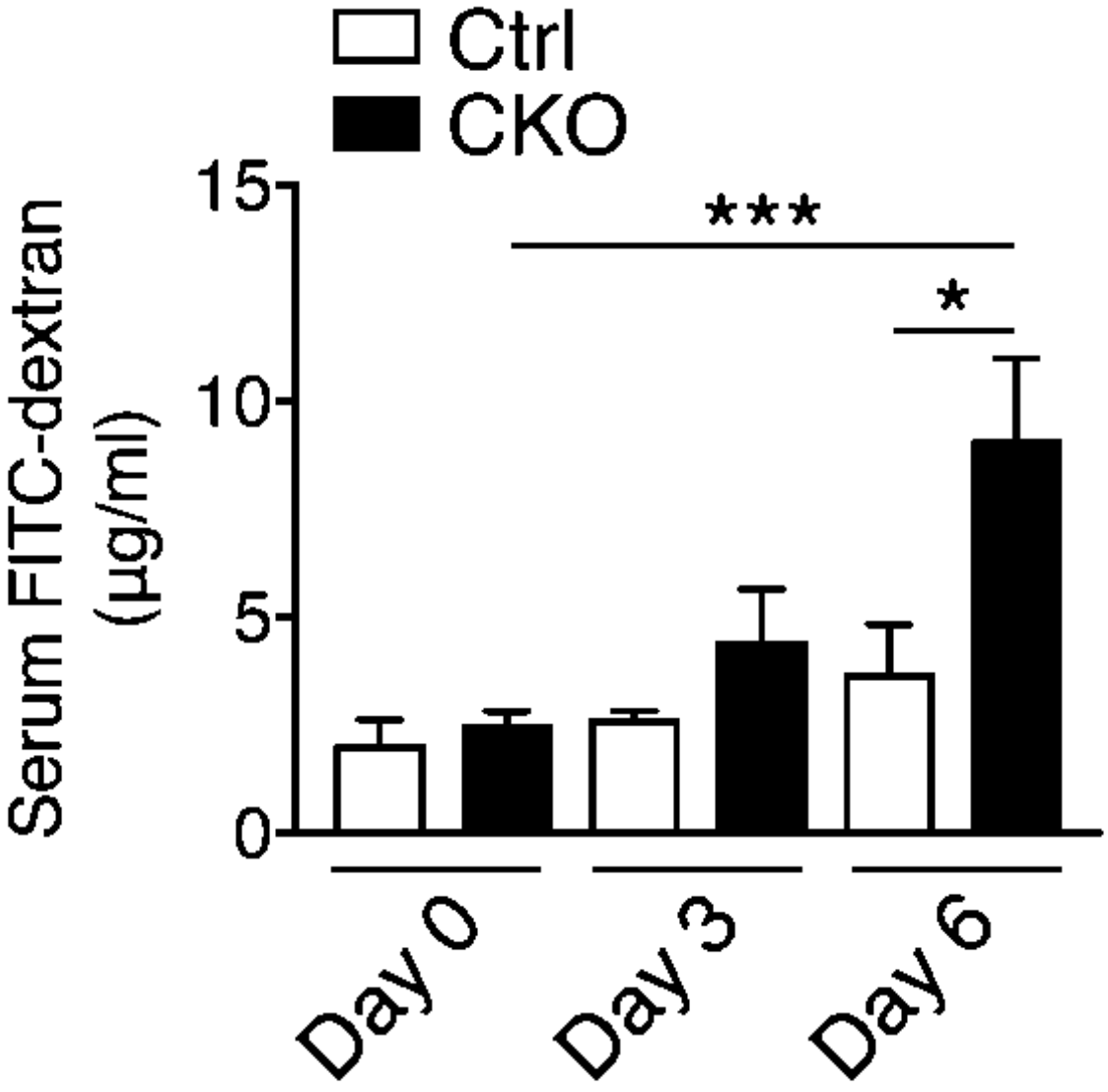
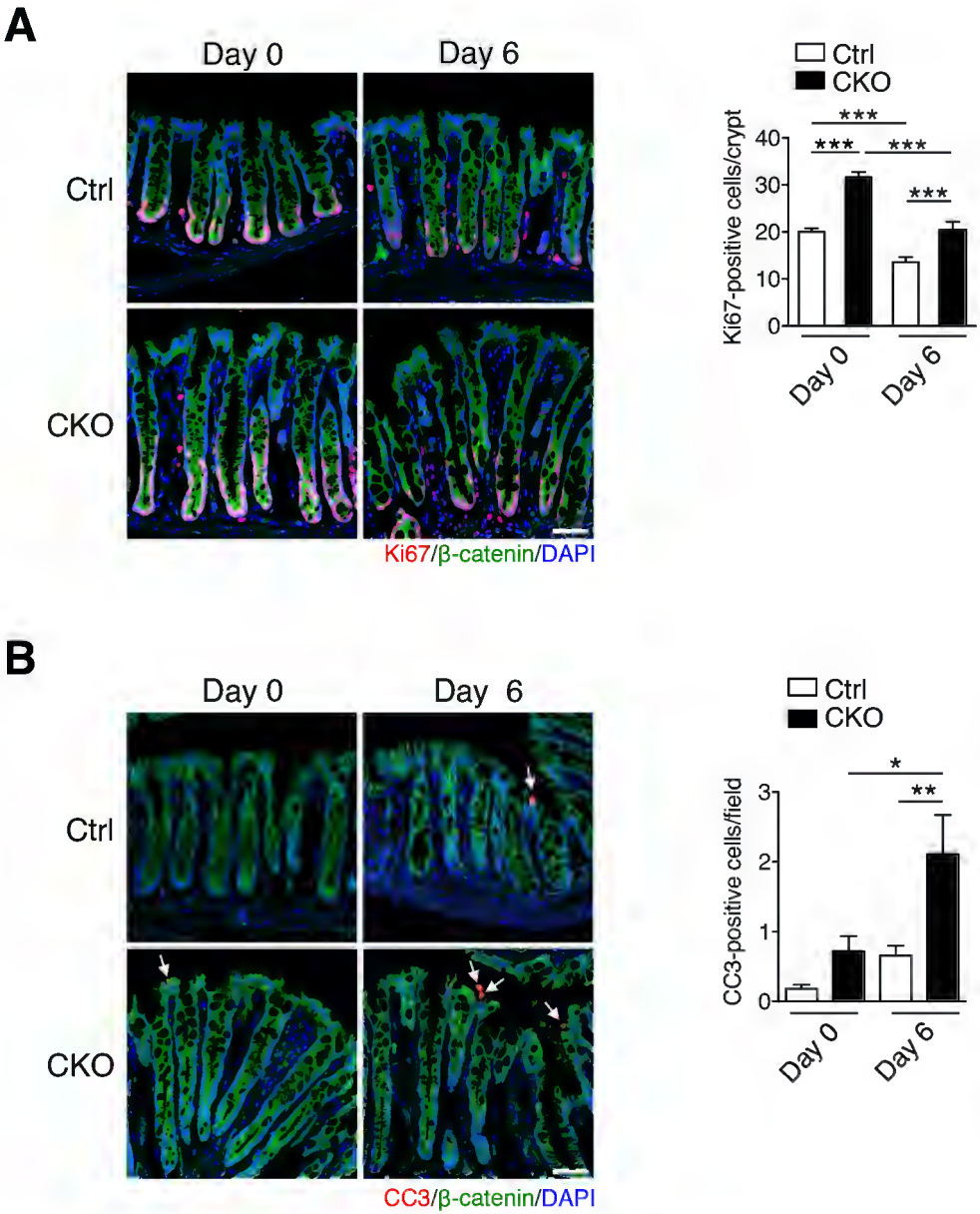
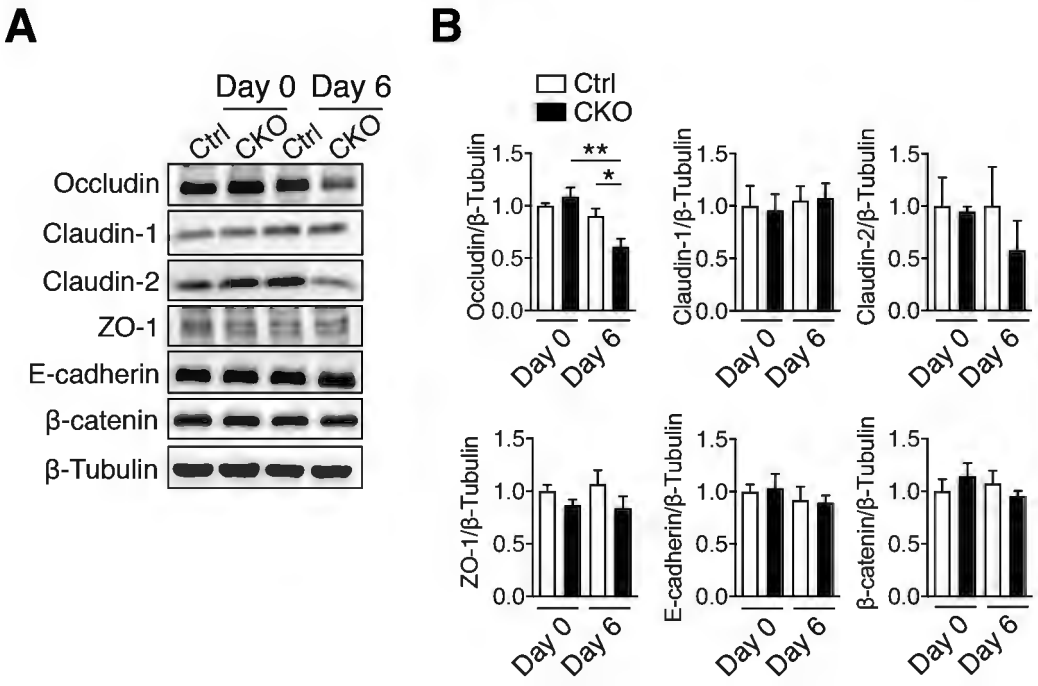


Figure 3
C. Sun *et al.*




A
Day 0
Day 6
Ctrl
CKO
Ctrl
CKO
Occludin
Claudin-1
Claudin-2
ZO-1
E-cadherin
β-catenin
β-Tubulin
B
Ctrl
CKO
Occludin/β-Tubulin
Claudin-1/β-Tubulin
Claudin-2/β-Tubulin
ZO-1/β-Tubulin
E-cadherin/β-Tubulin
β-catenin/β-Tubulin
1.5
1.0
0.5
0.0
**
*
Day 0
Day 6