**Title: Desmoplakin is essential for transcellular keratin scaffolding and protects from intestinal injury**

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**Running title: The role of desmoplakin in the intestine**

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**Abstract:** Desmosomes are intercellular junctions connecting keratin intermediate filaments of neighboring cells. The cadherins desmoglein 2 (Dsg2) and desmocollin 2 mediate cell-cell adhesion, whereas desmoplakin (Dsp) provides the attachment of desmosomes to keratins. While the importance of the desmosome-keratin-network is well established in mechanically challenged tissues, its function in intestinal epithelia is less understood. **Methods:**We analyzed the intestine-specific villin-Cre DSP (DSPΔIEC) and the combined DSG2/DSPΔIEC (ΔDsg2/Dsp) knockout mice. Cross-breeding with K8-YFP knock-in mice and generation of organoids was performed to assess the impact on the keratin network. A Dsp-deficient HT29 cell line was generated and the role of Dsp in adhesion and mechanical stress was studied in dispase assay and after exposure to uniaxial cell stretching. **Results:**The intestine of DSPΔIEC mice was histopathologically inconspicuous, however, the epithelial cells displayed an accelerated migration along the crypt and an enhanced shedding into the lumen. An increased intestinal permeability and altered levels of desmosomal proteins were detected. Similar, an inconspicuous phenotype was seen in ΔDsg2/Dsp mice. After dextran sodium sulfate treatment, DSPΔIEC mice developed a more-pronounced colitis. DSPΔIEC/K8-YFP mice and the corresponding organoids harbored a collapsed keratin network with loss of desmosomal attachment. In contrast, keratin levels, phosphorylation status and solubility remained unaltered. Dsp-deficient HT29 cells had an impaired cell-adhesion and suffered from increased cellular damage after stretching. **Conclusions:**Our results demonstrate that in intestinal epithelia, Dsp is required for a proper keratin network architecture mechanical resilience and adhesion and thereby protects from intestinal injury.

Keywords: desmosome/ keratin/apical junctional complex/intestinal epithelial barrier/cell adhesion

Abbreviations:

βTub, β-tubulin; AJ, adherens junction; AJC, apical junctional complex; BF, bright field; BrdU, 5-bromo-2-deoxyuridine; CD, Crohn’s Disease; Dsc, desmocollin; Dsg, desmoglein; Dsp, desmoplakin; DSS, dextran sodium sulphate; EM, electron microscopy; FITC, fluorescein isothiocyanate; fl, floxed; FL, fluorescence; GFP, green fluorescent protein; H&E, hematoxylin and eosin; IEC, intestinal epithelial cells; IL-1β, interleukin 1β; KO, knockout; K, keratin; LDH, lactate dehydrogenase, LI, large intestine; mRNA, messenger RNA; PAS, periodic acid-Schiff; PG, plakoglobin (γ-catenin); Pkp2, plakophilin 2; RT-PCR, reverse transcriptase polymerase chain reaction; SI, small intestine; TJ, tight junction; TNFα, tumor necrosis factor alpha; WT, wildtype; YFP, yellow fluorescent protein

**INTRODUCTION**

Keratin intermediate filaments are multifunctional stress-protectors expressed primarily in epithelial cells [1, 2]. They are connected to the transcellular networks through cell-cell junctions termed desmosomes [3, 4]. Desmosomes consist of transmembrane components from the desmosomal cadherin families of desmogleins (Dsg) and desmocollins (Dsc) that mediate cell-cell adhesion. In the cytoplasm, they are associated with the armadillo proteins plakophilin and plakoglobin and the plakin member desmoplakin (Dsp) that mediates the attachment to the keratin filament network [5]. The desmosome-keratin system is mainly responsible for the stability of epithelial tissues and its function is particularly prominent in mechanically challenged tissues such as epidermis. In the latter, mutations in keratins lead to a large variety of skin disorders such as epidermolysis bullosa or palmoplantar keratoderma [2, 6]. Similarly, auto-antibodies against Dsg/Dsc cause the autoimmune blistering disease pemphigus vulgaris, while Dsp mutations were implicated in keratoderma [7, 8]. In addition to that, an increasing evidence demonstrates the importance of the desmosome-keratin system in mechanically less challenged glandular and single-layered epithelia. For example, mutations in keratins 8/18 (K8/K18), the major keratin family members expressed in simple epithelia, increased the susceptibility to advanced liver disease [9]. An intronic variant in the Dsp gene constitutes one of the most established genetic factors predisposing to idiopathic pulmonary fibrosis [10]. While the biological role of K8/K18 variants in inflammatory bowel disease (IBD) remains to be clarified [11], altered desmosomal protein levels are seen in individuals with inflammatory bowel disease (IBD) and these changes may contribute to the impaired intestinal barrier observed in Crohn’s Disease [12-14]. The human data are supported by findings from multiple transgenic models. Among them, K8 knockout mice display a spontaneous colitis [15], while loss of Dsg2, the only Dsg produced in intestinal epithelial cells, is well tolerated under basal conditions, but leads to increased susceptibility to both chemical and microbial injury [12]. To further elucidate the biological role of the keratin-desmosome system in the intestine, we turned to Dsp knockout animals. While Dsp is essential for epidermal sheet formation [16], intestine-specific Dsp knockout (DSPΔIEC) mice did not display an obvious phenotype under basal conditions. This was somewhat surprising given that intestinal epithelial specific loss of plectin, another cytolinker connecting keratin filaments with cell junctions, led to a spontaneous colitis [17]. Therefore, we decided to systematically study the impact of Dsp loss on keratin network architecture as well as the susceptibility to intestinal injury. To that end, DSPΔIEC mice were cross-bred with the reporter K8-yellow fluorescent protein (YFP) knock-in mouse [18] and subjected to dextran sodium sulfate (DSS)-induced colitis. Mating of DSPΔIEC mice with an intestinal-specific Dsg2 knockout (DSG2ΔIEC) was used to evaluate the consequence of a combined desmosomal defect. In summary, we show that Dsp is required for keratin network organization, epithelial adhesion and the protection of intestinal epithelial cells from mechanical and chemical injury*.*

**RESULTS**
To study the biological relevance of Dsp in the intestine, we generated intestinal-specific Dsp knockout mice (DSPΔIEC). In line with previous findings [19], DSPΔIEC mice displayed an efficient deletion of Dsp in both jejunum and colon while no Dsp loss was observed in other organs such as stomach, liver and heart (Fig. 1; Suppl. Fig.1). Immunofluorescence staining of colonic tissue manifested the loss of Dsp and a normal distribution of other desmosomal proteins (Fig. 2A). DSPΔIEC mice developed normally, displayed normal body weight, colonic and small intestinal length and no diarrhea (Suppl. Fig. 2A). Histological evaluation demonstrated a morphologically inconspicuous small and large intestine (Fig. 2B and not shown). No inflammation was seen and the finding was supported by unaltered expression of pro-inflammatory cytokines TNFa, IL-1β and IL-6 (Suppl. Fig. 2B,C). Biochemical analysis revealed decreased levels of Dsg2 and PG while the amounts of other desmosomal proteins were unaltered (Fig. 2C,D). These changes seemed to occur posttranscriptionally given that there were no differences in the Dsg2/PG mRNA levels (Suppl. Fig. 3). Electron microscopy depicted normal appearing desmosomal plaques in the colon (Fig. 2E). Notably, DSPΔIEC animals displayed somewhat increased intestinal permeability for 4 kDa FITC dextran (Fig. 2F). An accelerated migration of BrdU-labelled colonic cells along the crypt axis was seen
24 hours after BrdU injection (Fig. 3A). In line with the increased cellular turnover, Dsp-deficient animals harbored a higher epithelial cell content in the intestinal lumen as indicated by the increased amount of the epithelial cell marker keratin 8 (K8) (Fig. 3B). To explore the impact of aging, we systematically analyzed 52 weeks old animals. DSPΔIEC mice had normal body weights, colon and small intestinal lengths (Suppl. Fig. 4A). Histological staining illustrated a regular colonic structure, while PAS staining revealed an unaltered number of goblet cells (Suppl. Fig. 4B). No colonic inflammation was noted within the groups as confirmed by unchanged levels of cytokines TNFa and IL-1β (Suppl. Fig. 4C). Since neither a loss of a desmosomal cadherin [12] nor a Dsp deficiency in intestinal epithelial cells led to an obvious phenotype under basal conditions, we wondered about an impact of a combined defect. To that end, we generated mice with a specific deletion of both Dsg2 and Dsp in the intestinal epithelia (ΔDsg2/Dsp). 28 weeks old ΔDsg2/Dsp animals developed normally and no changes in body weight nor in the colon and small intestinal lengths were detected (Suppl. Fig. 5). Histology illustrated an unaltered colon architecture and a comparable amount of goblet cells in all analyzed genotypes. The latter observation was confirmed by similar mRNA expression of the goblet cell marker mucin2 (MUC2) (Suppl. Fig. 6A). Furthermore, no inflammation was noted as demonstrated by similar levels of pro-inflammatory cytokines (Suppl. Fig. 6B). Biochemical analysis confirmed the efficient deletion of both desmosomal proteins (Suppl. Fig. 7A). Despite that, gavage with 4kD FITC-labeled dextran revealed only a moderate increase in intestinal permeability (Suppl. Fig. 7B). To test the importance of Dsp during intestinal stress, we challenged DSPΔIEC mice and their floxed littermates with DSS. Compared to DSPfl/fl mice, DSPΔIEC animals experienced an increased weight loss with profound fecal bleeding and a significantly reduced colon length (Fig. 4A-C). Histological examination depicted a massive tissue destruction in DSS-treated Dsp-deficient mice with marked epithelial cell loss, edema and inflammatory cell infiltration that translated into increased injury scores (Fig. 4D). The profoundly intensified inflammation was corroborated by increased levels of the analysed pro-inflammatory cytokines (Fig. 4E). Given that Dsp mediates the connection between desmosomes and keratin intermediate filaments, we assessed the consequences of Dsp loss on keratin organization. Under basal conditions, DSPΔIEC and DSPfl/fl mice displayed similar mRNA and protein levels of K7, K8, K18 and K19 (Fig. 5A,B) and no differences in K8 solubility were noted (Fig. 5C). In line with that, the K8 phosphorylation at S79 and S432 did not significantly differ among the phenotypes (Fig. 5C and data not shown). To better delineate keratin network organization *in vivo*, DSPΔIEC and DSPfl/fl mice were crossbred with knock-in animals expressing the YFP-tagged version of K8 [18]. Confocal laser scanning microscopy revealed a normal appearing K8 network in DSPfl/fl mice with K8 being located in a close contact to the plasma membrane. Loss of Dsp resulted in a “collapsed” network that became apparent as a wider space between the keratin rings (Fig. 6A). To further explore keratin distribution in rapidly growing intestinal epithelia, we turned to small intestinal organoids. While the loss of Dsp did not visibly alter the growth and development of the organoids, a dramatic disruption of the keratin network occurred in DSPΔIEC organoids. They displayed an even more profoundly disorganized, “collapsed” network (Fig. 6B), which was in a strong contrast with the perimembraneous pattern seen in DSPfl/fl organoids. Given the known importance of keratins for mechanical stability, we compared the mechanical resilience of wild-type HT29 cells and cells with a deleted Dsp exon 8 (ΔDSP). The complete loss of Dsp was confirmed on both mRNA and protein level (Fig. 7A,B) and the efficient expression of the targeting vector was corroborated by the incorporated green fluorescent protein (GFP) fluorescence (Fig. 7C). No changes in cell growth and morphology compared to WT HT29 cells were observed (Fig. 7C and not shown). Immunofluorescence staining revealed an unperturbed localization of the desmosomal cadherin Dsg2 (Fig. 7D). Nevertheless, mechanical stress resulted in a more profound fragmentation of the epithelial sheets in Dsp-deficient cells compared to their WT counterparts (Fig. 7E). Similarly, an uniaxial cyclic cell stretching led to a more obvious monolayer disruption in ΔDSP vs. WT cells (Fig. 7F). Moreover, Dsp-deficient cells displayed a stronger release of the cellular damage marker lactate dehydrogenase (LDH) into the cell supernatant (Fig. 7F). In summary, our results demonstrate that while Dsp is largely dispensable in unstressed intestinal epithelia, but it is crucial for keratin network organization, cellular adhesion and tissue integrity and thereby for coping with intestinal stress.

**DISCUSSION**

Our study analyzed the role of desmosome-keratin system in the intestine. We showed that loss of Dsp did not influence the formation of normal appearing desmosomes, which is in line with previous data [19]. The fact that Dsp is necessary for desmosomal assembly during epithelial sheet formation in the epidermis but not in the intestine [16, 19] suggests that it is more important in mechanically challenged tissues. This is not surprising since Dsp was shown to become mechanically loaded when cells are exposed to external mechanical stresses [20]. While no intestinal injury was noted, DSPΔIEC mice displayed decreased Dsg2 and PG protein levels. The data are consistent with observations in Dsg2-deficient animals [12] and indicate that alterations in desmosomal proteins affect the posttranslational regulation of other desmosomal components. In line with that, cardiac-specific ablation of Dsp resulted in decreased levels of cytosolic PG [21]. Further studies are needed to delineate the underlying molecular mechanisms. The alterations observed in unchallenged DSPΔIEC mice included an increased intestinal permeability, a faster migration along the crypt-villus axis and a stronger epithelial turnover, which indicates an importance for epithelial adhesion. This is reminiscent of loss of several desmosomal components including Dsc2 and Dsg2 that lead to impaired intestinal adhesion as well [13, 14]. In line with that, an increased epithelial shedding into the intestinal lumen was observed in DSPΔIEC mice, which is compatible with the animals with intestine-specific plectin deletion that display an increased cellular turnover and a trend towards higher epithelial detachment [17]. The fact that Dsp is crucial for cellular adhesion was further supported by our *in vitro* studies highlighting a higher cell fragility after mechanical stress in Dsp-deficient cells. Moreover, keratins constitute important mechanical stabilizers and keratin mutations result in cellular fragility [22]. Despite that, neither an isolated Dsp loss nor a combined deletion of Dsp and Dsg2 resulted in a spontaneous intestinal injury. This finding complements earlier observations [12, 23, 24] and further demonstrates that loss of desmosomal proteins can be functionally compensated in the unchallenged intestinal epithelia. Despite these rather minor functional defects, the cross-breeding of DSPΔIEC animals with K8-YFP mice clearly demonstrated that Dsp loss results in a profoundly disorganized keratin filament network. This demonstrates that Dsp is essential for tethering of keratins in these cells and it cannot be compensated by other cytolinkers. Similarly, Dsp absence or mutation in keratinocytes led to retracted keratin network after mechanical stress [24-26]. Furthermore, it has been shown that modifications in the keratin-desmosome interaction alter cell stiffness in human epithelial cells [27]. However, despite the lost transcellular connection, the retained keratins still seem to fulfill important cellular functions since the phenotype of DSPΔIEC mice is markedly less severe than the spontaneous colitis seen in K8 knockout mice [11]. Notably, keratins are multifunctional proteins fulfilling various non-mechanical functions [2, 28, 29] and these retained functions are likely responsible for the comparably mild phenotype of DSPΔIEC animals. Finally, our data demonstrate that desmoplakin is more dispensable than its related cytolinker plectin since intestinal deletion of plectin led to spontaneous colitis [17]. This is not surprising since plectin fulfills much broader spectrum of functions than desmoplakin and its deletion results in dysfunctional hemidesmosomes and intercellular junctions [17]. On the other hand, a deletion of epiplakin, a cytolinker with more restricted cellular junctions, did not lead to an obvious intestinal phenotype either [30]. Although the moderate intestinal permeability seen in untreated DSPΔIEC animals is not sufficient to induce epithelial injury, it may promote the disruption of the intestinal barrier during DSS colitis. As an underlying mechanism, pro-inflammatory cytokines are known to weaken the epithelial junctions [31] and may thereby perpetuate the vicious cycle of disturbed epithelial barrier and injury [32]. A similar mechanism was postulated in DSG2ΔIEC mice [12] and multiple cellular models [33, 34].

In summary, our findings support an important role of Dsp for epithelial tissue integrity. As its loss results in impaired attachment of keratins to desmosomes as well as alterations in desmosomal protein levels, Dsp seems to be important for both. While desmosomal proteins are dispensable under basal conditions, they may constitute an important second line of defense during intestinal stress.

**MATERIALS AND METHODS**

**Mouse experiments**

Micewith intestine-specific deletion of Desmoplakin (Dsp) and Desmoglein 2 (Dsg2) as well as combined deletion of both genes (ΔDsg2/Dsp) were generated by crossing previously described DSG2 exon 4/5 floxed (DSG2fl/fl) and DSP exon 2 floxed (DSPfl/fl) mice with animals expressing Cre under the control of the villin promotor (DSG2ΔIEC/DSPΔIEC) [12, 19]. DSPΔIEC animals were further cross-bred with previously described K8-YFP knock-in mice [18]. All mice were on C57BL/6 background and kept under standardized conditions (12 hours day/night cycle, 21-24°C, humidity ~50%) with free access to food and water. To induce colitis, 10 weeks old sex-matched mice were exposed to 2% dextran sodium sulfate (DSS, MP Biochemicals, Heidelberg, Germany) in the drinking water for five days following by a switch to normal water. The animals were sacrificed with an isoflurane overdose at day 7. Untreated, age- and sex-matched littermates were used as controls. Rectal bleeding was evaluated using a commercial hemoCARE fecal occult blood Guajak test. A semi-quantitative scoring from 0 to 3 (0 - no bleeding, 1 - mild bleeding, 2 - moderate bleeding, 3 - severe bleeding) was performed.  All intestinal parts were washed with 1x phosphate-buffered saline (PBS). Proximal parts were stored as swiss rolls in 4% formaldehyde overnight for histological evaluation or frozen in O.C.T compound (Tissue-Tek, Sakura, Staufen, Germany) for cryosectioning. Distal parts and samples from other organs were snap frozen in liquid nitrogen for protein and RNA analysis. To examine intestinal permeability, mice were fasted for three hours and subsequently gavaged with 0.6 mg/g of body weight 4kD FITC-labelled dextran (Sigma-Aldrich, Steinheim, Germany). Four hours later, blood was collected retroorbitally and the fluorescence intensity in serum was quantified (excitation: 492 nm; emission: 525 nm, Cytation3 imaging reader, BioTek, Bad Friedrichshall, Germany). The samples were prepared in duplicates and the results were calculated according to the standard curve. To label proliferating cells, 50 µg/g of body weight 5-bromo-2-deoxyuridine (BrdU, Sigma-Aldrich) was injected intraperitoneally.

**Generation of organoids from isolated small intestinal stem cells**

Small intestines were removed, washed with ice-cold PBS and cut into 3 cm long pieces that were opened longitudinally. The villi were scraped off with a coverslip and the remaining tissue fragments were washed with PBS. Afterwards, they were incubated in 1 mM ethylenediaminetetraacetic acid (EDTA)/PBS solution for 30 minutes at 4°C on a tube roller and transferred to 5 mM EDTA/PBS for 1 hour at 4°C to enrich for small intestinal crypts. The crypt-containing solution was filtered through a 70 µm cell strainer, the crypts were counted and centrifuged at 300 x g for 5 minutes at 4°C. The crypt-containing pellet was resuspended in a matrigel matrix (Corning, Kaiserslautern, Germany) and seeded into a pre-warmed 48-well plate. Matrigel was allowed to polymerize for 15 minutes at 37°C and the crypts were overlayed with Ad-DF+++ medium (Advanced DMEM/F12 supplemented with 1% Glutamax, 1% 1M Hepes, 1% penicillin/ streptomycin) containing 1x N2, 1x B27 supplement (both from Invitrogen), 1.25 mM n-acetylcysteine (Sigma-Aldrich), 0.05 µg/ml mEGF (Invitrogen), 0.1 µg/ml mNoggin (Peprotech) and 1 µg/ml recombinant hRspondin1 (R&D Systems). The medium was changed every 3 days and the development was recorded with EVOS FL Cell Imaging System (Thermo Scientific).

**Biochemical methods**

To obtain the luminal content, colon was removed and opened longitudinally. The tissue was vigorously inverted twenty times in 1x PBS. The solution was centrifuged at 5000 rpm for 10 minutes at 4°C and the pellet was homogenized in 3% SDS-containing buffer supplemented with protease and phosphatase inhibitors. Total protein lysates were prepared by direct homogenization of murine tissues or HT29 cells in an appropriate volume of 3% SDS-containing buffer. Insoluble keratin extracts were generated via high salt extraction. Briefly, colonic tissue was homogenized in ice-cold 1% Triton-X buffer and centrifuged to obtain the supernatants constituting the soluble fraction. The pellet was homogenized in high salt buffer (10 mM Tris, pH 7.6; 140 mM NaCl, 1.5M KCl; 5 mM EDTA in 0.5% Triton-X) and washed to remove nucleic acids before being dissolved in 3% SDS-containing Laemmli buffer (Strnad et al., 2016). Same amounts of proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by transfer to PVDF membranes. The membranes were incubated with specific primary and horseradish peroxidase (HRP)-coupled secondary antibodies. Finally, antigen-antibody complexes were visualized by an enhanced chemiluminescence detection kit (GE Healthcare/Amersham Biosciences, UK). The relative protein amounts were quantified by densitometry via ImageJ software and depicted as optical density (O.D.) values. The used antibodies are summarized in Suppl. Table 1.

**Histological analysis**

Formaldehyde-fixed tissues were embedded in paraffin, cut into 3 µm thick sections and deparaffinized for hematoxylin & eosin (H&E) and periodic acid-Schiff (PAS) staining. For the latter, slides were oxidized in 2% periodic acid solution for 5 minutes. After washing in distilled water, Schiff reagent was applied for 15 minutes, followed by hematoxylin counterstaining. Subsequently, the sections were blued in 1M Tris buffer (pH 8.0). All images were acquired and examined with a Zeiss light microscope and AxioVision Rel 4.8 software (Zeiss, Jena, Germany). PAS-positive cells were counted and presented as mean from at least twenty assessed crypts per mouse by ImageJ software. H&E-stained DSS-treated sections were evaluated by a previously described scoring system with minor modifications (reference): (i) submucosa thickening/edema, (ii) inflammatory cell infiltration, (iii) goblet cell loss (each parameter with a score 0-3: 0: normal; 1: mild; 2: moderate; 3: severe), (iv) epithelial damage/erosion (0: normal; 2: <1/3 of total area with altered epithelial cell morphology; 4: >1/3 of total area with altered epithelial cell morphology and/or mild erosions; 6: <10% of ulcerative areas; 8: 10-20% of ulcerative areas, 10: >20% of ulcerative areas). Analysis was performed in a blinded manner by PB (certified pathologist) and AG.

**Immunohistochemistry**

Immunohistochemistry staining and visualization of BrdU was performed on paraffin specimen, which were cut into 5 µm thick sections. Deparaffinized slides were boiled in citrate-based antigen unmasking solution at pH 6 (Vector laboratories, Burlingame, USA). Before blocking in 5% normal goat serum in PBS for 30 minutes, sections were incubated with 3% H2O2 for 10 minutes to reduce the endogenous peroxidase activity. Additionally, a treatment with 2N HCl for 30 minutes was performed to denature DNA and was followed by neutralization with 0.1 M sodium borate (pH 8) for 9 minutes. Afterwards, samples were incubated with anti-BrdU antibody overnight at 4°C. After washing, a species-specific biotinylated secondary antibody (Vector laboratories) was applied for 1 hour, following an incubation with Vectastain working solutions (Vectastain ABC Kit, Vector laboratories). 3,3’-diaminobenzidine (DAB, Vector laboratories) was used to develop staining and hematoxylin was applied as a counterstain. BrdU-positive cells were counted as a mean from at least twenty different crypts per mouse.

**Immunofluorescence staining**

Immunofluorescence staining was performed on frozen, O.C.T.-embedded tissues cut into
5 µm thick sections or HT29 cells grown on glass slides (#354114, 4 wells, Falcon®, Kaiserslautern, Germany). Tissue specimen and cells were fixed in a pre-cooled acetone or pre-cooled methanol for 10 minutes, respectively. Blocking was carried out for 1 hour in 2% normal goat serum, 1% BSA, 0.1% cold fish skin gelatine, 0.1% Triton X-100, 0.05% Tween 20 in 1x PBS (tissue) or 2% BSA in PBST (cells). Subsequently, samples were incubated with following antibodies overnight at 4°C: anti-Desmoglein 2, anti-Desmocollin 2 (AG Leube, RWTH Aachen), anti-Desmoplakin (CBL173, Millipore, Darmstadt, Germany) and anti-γ-catenin (Plakoglobin) (sc30997 K-20, Santa Cruz, Heidelberg, Germany). Following washing, specimen were subjected to anti-goat Alexa–Fluor 488/568-conjugated secondary antibodies (Invitrogen, Molecular Probes, Eugene, OR, USA) for 1 hour at RT and mounted with ProLong Gold antifade reagent containing DAPI (#P36935, Thermo Scientific GmbH, Schwerte, Germany). Images were acquired with Zeiss microscope Axio Imager Z1 (Zeiss).

**Quantitative real-time PCR**

Total RNA was isolated from tissues and HT29 cells using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. 1 µg RNA was reverse-transcribed into cDNA with M-MLV Reverse Transcriptase Kit (Promega, Mannheim, Germany) and quantitative real-time RT PCR was performed using the 7300 Fast Real-Time PCR System (Applied Biosystems). All samples were measured in duplicates and quantified with the ΔΔCt method in relation to the internal control (ribosomal protein L7). The primers used in the experiments are summarized in Suppl. table 2. All expression levels are represented as means +/- SEM.

**Transmission electron microscopy**

Colonic tissue was cut into ~1 mm³ pieces and fixed at RT with following three fixatives: (i) 3.7% formaldehyde, 1% glutaraldehyde, 11.6 g NaH2PO4xH2O and 2.7 g NaOH per liter ddH2O for 2 hours; (ii) 1% OsO4 for 1 hour; (iii) 0.5% uranylacetate/0.05 N sodium hydrogen maleate (pH 5.2) for 2 hours. Subsequently, samples were dehydrated, embedded in araldite for 48 hours at 60°C and cut into 75 nm ultrathin sections. To enhance the contrast, sections were treated with 3% uranylacetate for 4 minutes and with 80 mM lead citrate for 3 minutes. Images were acquired with an EM 10 (Zeiss) plus digital camera (Olympus) and the corresponding iTEM software (Olympus).

***Ex vivo* microscopy**

Colons from DSPΔIEC/K8-YFP knock-in mice were flushed with PBS, opened longitudinally and transferred to glass-bottom dishes (MatTek) containing pre-warmed Krebs-Henseleit buffer (114 mM NaCl, 5 mM KCl, 24 mM NaHCO3, 1 mM MgCl2,2.2 mM CaCl2, 10 mM HEPES, 0.25% BSA, pH 7.35). 2.5 µg/ml Hoechst33342 was added for staining of the nuclei. Organoids were grown on glass bottom dishes and overlayed with the Hoechst33342-containing Krebs-Henseleit buffer. Images were acquired with a Zeiss LSM710 Duo microscope, a 405 nm diode laser, an argon ion laser at 488 nm and a 63x/1.4 N.A. DIC M27 oil immersion objective at 37°C. Additionally, the Airyscan detector in „super resolution” mode was used. Images were deconvoluted using Zen black software (Zeiss) and processed using Fiji [35].

**Cell culture experiments**
A human colon adenocarcinoma cell line (HT29, ATCC® HTB-38™, LGC Standards GmbH, Wesel, Germany) with a stable DSP knockdown was generated using the CRISPR/Cas system [36]. Briefly, short guide RNA (sgRNA) that targets exon 8 of the DSP gene (for additional information see Suppl. table 2) was designed using the Broad institute platform and integrated into the vector pL-CRISPR.EFS.GFP (Addgene, Massachusetts, USA) for lentiviral delivery. The construct was amplified in competent Stbl3 *Escherichia coli* (Invitrogen, USA) and the GeneJET plasmid miniprep and maxiprep kits were used for its isolation (Thermo Scientific, USA). For production of lentiviral particles, HEK293T cells were co-transfected with lentiviral envelope plasmid (pMD2.G, Addgene Europe, Teddington, UK), packaging plasmid (psPAX2, Addgene Europe) and the previously generated vector using TransIT-LT1 transfection reagent (Mirusbio, Goettingen, Germany). After 48h, the lentiviral particles were collected by centrifugation of the cell culture supernatant at 1500 rpm for 5 minutes and filtration with a 45μm pore size filter. Finally, target HT29 cells were transduced with the isolated particles. Fluorescence activated cell sorting (FACS) was used to select transfected, GFP-expressing cells. HT29 cells were cultured in complete culture medium (RPMI 1640, PAN biotech, Bavaria, Germany) containing 10% fetal bovine serum and 1% (50 U/ml) penicillin-streptomycin (PAN biotech) in 5% CO2 atmosphere at 37°C until they reached confluence. Images of WT and GFP-expressing Dsp-deficient HT29 cells were acquired with Axio Vert.A1 (Zeiss).

**Dispase assay**
Dsp-deficient and WT HT29 cells were seeded into 6-well plates. After reaching confluency, cells were washed in PBS and Hank`s balanced salt solution (HBSS, #P04-34500, PAN biotech). Afterwards, incubation with 3.6 U/ml dispase II in HBSS (Roche, Mannheim, Germany) at 37°C for 30 minutes was performed to release cellular monolayers from the plate bottom. The epithelial sheets were subjected to mechanical stress by inversion on a tube rotator (VWR, # 444-0500) for 5 minutes at 18 rpm and the resulting fragments were counted by ImageQuant AS 4000 camera system equipped with ImageQuant software (GE Healthcare Europe GmbH, Freiburg, Germany).

**Cell stretching**
To perform cyclic stretch experiments, 0.3 x 106 Dsp-deficient or WT HT29 cells were seeded on elastic polydimethylsiloxane (PDMS) chambers (silicone elastomers, SYLGARD®, 184, Dow chemical company, Michigan, USA) that were coated with 100 µg/ml fibronectin. After reaching >80% confluence, chambers were placed into an automatic cell chamber stretcher and a simultaneous, linear uniaxial stretch with 35% stretching strength and a frequency of 0.3 Hz was conducted over a period of 10 hours [37]. To analyze the impact of stretching on cellular adhesion, monolayers were examined by bright field microscopy before and after stretching. To quantify the extent of cellular damage, LDH levels were measured in the supernatant.

**Study approval**

The animal experiments were approved by the state of North Rhine-Westphalia in Germany and the University of Aachen animal care committee and were conducted in compliance with the German Law for Welfare of Laboratory Animals.

**Data analysis and statistical methods**

Image quantifications were performed with ImageJ (National Institutes of Health, Bethesda, USA). Data were analyzed with an unpaired two-tailed Student´s t-test or one-way ANOVA. Two-tailed p-values<0.05 were considered statistically significant.

Suppl. table 1: Antibodies used for Western Blotting

|  |  |  |
| --- | --- | --- |
| Antibody | Host | Company |
| Desmocollin 2 | guinea-pig | Institute of Molecular and Cellular Anatomy, RWTH Aachen, Germany |
| Desmoglein 2 | rabbit | Institute of Molecular and Cellular Anatomy, RWTH Aachen, Germany |
| Desmoplakin I/II | rabbit | sc33555 (H-300) Santa Cruz, Heidelberg, Germany |
| Desmoplakin I/II(Clone DP 2.15) | mouse |  CBL173, Millipore, Darmstadt, Germany |
| Keratin 7(RCK105) | mouse  | ab9021, Abcam, Cambridge, UK |
| Keratin 8 (Clone Ks.8.7) | mouse | 61038, Progen, Heidelberg, Germany |
| Keratin 8 (S79) | mouse | LJ4, Omary et al. 1997, USA |
| Keratin 18(Clone Ks 18.04) | mouse | 61028, Progen, Heidelberg, Germany |
| Keratin 19(TROMAIII) | rat | Developmental Studies Hybridoma Bank, USA |
| Plakophilin 2 | goat | ab189323, Abcam, Cambridge, UK |
| ß-Actin | mouse | A2228,Sigma-Aldrich, Steinheim, Germany |
| βTubulin | mouse | T8328, Sigma-Aldrich, Steinheim, Germany |
| γ-catenin (PG) | goat | sc30997 (K-20) Santa Cruz, Heidelberg, Germany |

Suppl. table 2: Primers used for genotyping, quantitative Real Time PCR and CRISPR/Cas

|  |
| --- |
| Genotyping PCR Primer |
| mDsg2 | Forward | GGTAAATGCAGACGGATCAG |
| Reverse | TGGGCTACACTCATAGGAAG |
| mDsp | Forward | TGT CTG TTG CCA TGT GAT GCC |
| Reverse | GAC TTG GAC GAT CGC CTT CTG |
| mVillin-Cre | Forward | CCACGACCAAGTGACAGCAAT |
| Reverse | TTCGGATCATCAGCTACACCA |
| mK8YFP | Forward | ACGTAAACGGCCACA |
|  | Reverse | AAGTCGTGCTGCTTC |
| Quantitative Real Time PCR Primer |
| mutE4/E5-mDsg2 | Forward | ACCGGGAAGAAACACCATATT |
| Reverse | AGGGCTTTTCCAGGTTGTTT |
| mDsc2 | Forward | GCACTGGTCGTGTAGATCGT |
| Reverse | CTCTGGCGTATACCCATCTG |
| mPG/JUP | Forward | TCCTGCACAACCTCTCTCAC |
| Reverse | ACTGAGCATTCGGACTAGGG |
| mDSP | Forward | CTGGCAAACGAGACAAATCA |
| Reverse | GATGCCAGCTGCAGTTCATA |
| mPkp2 | Forward | TCAGCATACACGGAAGATGC |
| Reverse | GGGAAAGATTCCGTGACAAA |
| mK7 | Forward | ACGGCTGCTGAGAATGAGTT  |
| Reverse | CGTGAAGGGTCTTGAGGAAG  |
| mK8 | Forward | GGACATCGAGATCACCACCT |
| Reverse | TGAAGCCAGGGCTAGTGAGT  |
| mK18 | Forward | CAAGTCTGCCGAAATCAGGGAC |
|  | Reverse | TCCAAGTTGATGTTCTGGTTTT |
| mK19 | Forward | ACCTACCTTGCTCGGATTGACGTGACTTCGGTCTTGCTTA |
|  | Reverse | CGTGACTTCGGTCTTGCTTA |
| mMuc2 | Forward | GCTGACGAGTGGTTCGTGAATG |
| Reverse | GATGAGGTGGCAGACAGGAGAC |
| mTNFa | Forward | TCAGCCTCTTCTCATTCCTGCTT |
| Reverse | AGGCCATTTGGGAACTTCTCATC |
| mIL1b | Forward | TGAAGCAGCTATGGCAACTG |
| Reverse | GGGTCCGTCAACTTCAAAGA |
| mIL6 | Forward | ACAAAGCCAGAGTCCTTCAGAGAGA |
| Reverse | TGGTCTTGGTCCTTAGCCACTCC |
| mL7 | Forward | GAAAGGCAAGGAGGAAGCTCATCT |
| Reverse | AATCTCAGTGCGGTACATCTGCCT |
| CRISPR/Cas Primer  |
| hDSP (Exon 8) | CAACG+Forward | CTGGCAAACGAGACAAATCA |
| NM\_001008844 | AAAC +Reverse |  GATGCCAGCTGCAGTTCATA |

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**Author contributions**

Study was planned and designed by AG and PS and the acquisition of data was performed by AG, BZ, NS, GMS, PB. Analysis and interpretation of data were conducted by AG, NS, PB, REL, PS. AG and PS drafted the manuscript and all authors contributed to the critical revision of the manuscript for important intellectual content. Statistical analysis was performed by AG and PS, who also obtained the funding and supervised the study. KH, BH, EF, RK, RM, REL, CT provided technical or material support.

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**FIGURE LEGENDS**

**Figure 1. DSP-deficient animals (DSPΔIEC) display an intestine-specific Dsp loss.**(A,B) Desmoplakin (DSP) mRNA and protein levels were evaluated by real time RT-PCR (n=4) and immunoblotting in the delineated organs of DSPΔIEC (ΔIEC) and DSPfl/fl (fl/fl) mice. L7 (mouse ribosomal protein) gene and β-tubulin (βTub) were used as an internal and a loading control, respectively. Two-tailed Student’s t test was used for statistical analyses. \*\*\*p<0.001.

**Figure 2. Dsp loss leads to altered desmosomal protein composition and an increased intestinal permeability.** (A) The distribution of desmoplakin (Dsp), desmoglein 2 (Dsg2), desmocollin 2 (Dsc2) and plakoglobin (PG) in the colon of DSPΔIEC (ΔIEC) mice and their floxed littermates (fl/fl) was visualized by immunofluorescence. Scale bar = 20 μm.
(B) Hematoxylin and eosin (H&E) staining highlights the overall colon morphology. Scale bar = 100 µm. (C,D) The impact of Dsp loss on colonic desmosomal composition was analysed by immunoblotting (n=5). β-tubulin was used as a loading control. The optical density (O.D.) values from immunoblots were normalized to the O.D. values of β-tubulin. Pkp2, plakophilin 2; K8, keratin 8; Tub, β-tubulin. (E) Desmosomal ultrastructure was assessed in both groups by electron microscopy. Scale bar = 200 nm (F) Serum levels of 4kD FITC-dextran were quantified 4 hours after the gavage (n=4). Two-tailed Student’s t test was used for statistical analyses. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

**Figure 3. DSP-deficient animals (DSPΔIEC) exhibit an accelerated epithelial migration and a higher epithelial loss.** (A) DSPΔIEC (ΔIEC) mice and their floxed littermates (fl/fl) were injected with bromodeoxyuridine (BrdU) and the amount of BrdU-positive cells was quantified 24 hours later (n=11). Scale bar = 200 µm (B) Immunoblotting for the epithelial cell marker keratin 8 (K8) in the colonic luminal content was used as a marker of epithelial extrusion. Two-tailed Student’s t test was used for statistical analyses. \*\*p<0.01

**Figure 4. DSP-deficient animals (DSPΔIEC) display an enhanced susceptibility towards dextran sodium sulfate (DSS)-induced colitis.** (A) Relative body weights of DSPΔIEC(ΔIEC) (grey rectangles) and DSPfl/fl (fl/fl) mice (black circles, n=6 each) were evaluated daily starting at the day of first DSS administration (day 0). (B-D) 7 days after the first DSS administration, the severity of colitis was assessed by measuring colonic length (n=6), semi-quantitative scoring of stool blood content with guaiac test (n=5) and hematoxylin and eosin (H&E) staining of colon sections with histological scoring. Scale bar = 100 µm (E) To assess colonic inflammation, cytokines TNFα, IL-1β and IL-6 were quantified by real time RT-PCR (n=5). The cytokine expression in non-treated animals (ctrl) was arbitrarily set as 1. L7 (mouse ribosomal protein) gene was used as an internal control.Two-tailed Student’s t test was used for statistical analyses of DSS-treated animals. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

**Figure 5. Loss of DSP does not affect the expression and solubility of keratins.** (A,B) The mRNA and protein levels of keratins 7 (K7), 8 (K8), 18 (K18) and 19 (K19) were assessed in the colon of 11 weeks old DSPΔIEC (ΔIEC) mice and their floxed littermates (fl/fl) by real time RT-PCR (n=5) and immunoblotting (n=6) (C) K8 solubility in 1% Triton X-containing buffer was evaluated in the colon of both groups by immunoblotting and subsequent densitometric quantification. The K8 optical density (O.D.) values were normalized to the O.D. values of β-actin (n=5). Average levels in fl/fl mice were arbitrarily set as 1 and the amounts in ΔIEC mice were presented as ratio. L7 (mouse ribosomal protein) gene and β-tubulin (B)/β-actin (C) were used as an internal and a loading control, respectively. Two-tailed Student’s t test was used for statistical analyses.  **Figure 6. DSP-deficient animals display an altered keratin network organization.**
(A) Keratin 8 structure was evaluated in the colon of Dsp-deficient mice (ΔIEC) and their floxed littermates (fl/fl) producing yellow fluorescent protein (YFP)-tagged keratin 8 (K8). Hoechst was used as a nuclear counterstain. Scale bar = 100 μm. (B) Organoids were grown from small intestinal stem cells of both genotypes and assessed at days 2 and 4 of culture by hematoxylin and eosin (H&E) staining. Organisation of the keratin network was visualized by fluorescence microscopy (FL). Scale bar H&E= 100 μm; fluorescent image = 20 µm  **Figure 7. DSP loss results in epithelial fragility**. (A,B) Desmoplakin (Dsp) mRNA and protein levels were assessed in Dsp-deficient (ΔDSP) and wildtype (WT) HT29 cells by real time RT-PCR and immunoblotting (n=3). hRPLPO (human large ribosomal protein) gene and β-actin were used as an internal and a loading control, respectively. (C) Autofluorescence of green fluorescent protein incorporated in the Dsp targeting construct was used to visualize the transduction efficiency. Scale bar = 100 µm (D) The distribution of Dsp and desmoglein 2 (Dsg2) was analysed by immunofluorescence. Scale bar = 20 μm (E,F) Epithelial adhesion was assessed by dispase test with subsequent quantification of the number of epithelial sheet fragments (n=3) and by 10 hours of cyclic stretching of cell monolayers in silicone chambers. Cells were visualized pre-stretch and post-stretch by bright-field microscopy. Scale bar = 20 µm. L-Lactatdehydrogenase (LDH) was measured to determine the extent of cellular damage (n=3). Two-tailed Student’s t test was used for statistical analyses.\*p<0.05, \*\*\*p<0.001. BF, bright-field; FL, fluorescence.
**Figure 8. Schematic summarizes the findings of the study.** Dsp- and Dsg2/Dsp-deficient mice show no basal phenotype, but an increased permeability, epithelial loss into the intestinal lumen and a faster migration. In DSPΔIEC mice, treatment with DSS leads to increased intestinal injury with strong inflammatory cell response. Cross-breeding with K8-YFP knock-in mice and assessment of the tissues as well as small intestinal organoids reveal a collapsed keratin network with loss of desmosomal anchorage. Dsp knockdown *in vitro* resulted in susceptibility to mechanical injury and impaired cell adhesion.
**Suppl. Figure 1. DSP-deficient animals (DSPΔIEC) show an efficient intestine-specific Dsp loss.** DSP mRNA levels were quantified in the highlighted mouse organs of DSPΔIEC (ΔIEC) and DSPfl/fl (fl/fl) mice by real time RT PCR. L7 (mouse ribosomal protein) gene was used as an internal control. Average mRNA expression in fl/fl mice was arbitrarily set as 1 and levels in ΔIEC mice were presented as ratio. Two-tailed Student’s t test was used for statistical analyses. \*\*\*p<0.001.
**Suppl. Figure 2. DSP-deficient animals (DSPΔIEC) develop normally and show no obvious intestinal inflammation under basal condition.** (A)The body weights as well as colon and small intestinal (SI) lengths of 11 weeks old DSPΔIEC (ΔIEC) mice and their floxed littermates (fl/fl) are shown as dot plots (n=7-9). (B) The inflammatory cytokines TNFα, IL-1β and IL-6 were assessed in the colon and jejunum of both groups by real time RT-PCR (n=3). L7 (mouse ribosomal protein) gene was used as an internal control. Average mRNA expression in fl/fl mice was arbitrarily set as 1 and levels in ΔIEC mice were presented as ratio.
**Suppl. Figure 3. DSP-deficient animals (DSPΔIEC) exhibit no alterations in the expression of desmosomal components.** The impact of Dsp loss on colonic desmosomal composition was analysed by real time RT-PCR (n=5). L7 (mouse ribosomal protein) gene was used as an internal control. Average mRNA expression in fl/fl mice was arbitrarily set as 1 and levels in ΔIEC mice were presented as ratio. Dsg2, Desmoglein 2; Dsc2, Desmocollin 2; PG, Plakoglobin; Pkp2, Plakophilin 2.
**Suppl. Figure 4. 52 weeks old DSP-deficient animals (DSPΔIEC) display no obvious phenotype under basal condition.** (A)The body weights, colon lengths and small intestinal (SI) lengths were analyzed in 52 weeks old DSPΔIEC (ΔIEC) and DSPfl/fl (fl/fl) mice. The data are shown as dot plots (n=19). (B) Hematoxylin and eosin (H&E) staining demonstrated the overall colonic architecture. Periodic acid-Schiff (PAS) staining visualizes the goblet cells. Scale bar = 100 µm (C) Real Time RT-PCR quantifies the colonic levels of the cytokines TNFα and IL-1β (n=7-8) as a surrogate of inflammation. L7 (mouse ribosomal protein) gene was used as an internal control. Average mRNA expression in fl/fl mice was arbitrarily set as 1 and levels in ΔIEC mice represent a ratio.
**Suppl. Figure 5. DSG2/DSP-deficient animals (ΔDsg2/Dsp) exhibit no abnormalities in their body weights and intestinal lengths.** The body weights and colon/small intestinal (SI) lengths of 28 weeks old double knockout DSG2/DSPΔIEC (ΔDsg2/Dsp) mice, single knockout DSG2ΔIEC (ΔDsg2) and DSPΔIEC (ΔDsp) animals as well as their floxed littermates (fl/fl) were measured (male, n=8-11; female, n=5-7). The data are represented as dot plots.
**Suppl. Figure 6. DSG2/DSP-deficient animals (ΔDsg2/Dsp) develop normally without colonic inflammation under basal condition.** (A) The colonic architecture was assessed after hematoxylin and eosin (H&E) staining in 28 weeks old DSG2/DSPΔIEC (ΔDsg2/Dsp) mice compared to DSG2ΔIEC (ΔDsg2), DSPΔIEC (ΔDsp) mice as well as floxed controls (fl/fl). Periodic acid-Schiff (PAS) staining depicts the goblet cells. The expression of the goblet cell product mucin 2 (Muc2) was quantified by real time RT-PCR (n=5). Scale bar = 100 µm
(B) The levels of inflammatory cytokines TNFα and IL-1β in colonic tissues were evaluated by real time RT-PCR (n=6). L7 (mouse ribosomal protein) gene was used as an internal control. Average mRNA expression in fl/fl mice was arbitrarily set as 1 and levels in other genotypes represent a ratio.
**Suppl. Figure 7. DSG2/DSP-deficient animals (ΔDsg2/Dsp) show an increase in intestinal permeability.** (A) The colonic levels of the depicted proteins were assessed in DSG2ΔIEC (ΔDsg2) or DSPΔIEC (ΔDsp) single knockout, DSG2/DSPΔIEC (ΔDsg2/Dsp) double knockout mice and their floxed littermates (fl/fl) by immunoblotting (n=3-5). Pkp2, plakophilin 2; K8, keratin 8; Tub, Tubulin. β-tubulin (Tub) was used as a loading control. (B) Serum levels of 4kD FITC dextran were measured in ΔDsg2/Dsp animals and the corresponding floxed mice 4 hours after the gavage (n=3-4).