Desmoplakin Maintains Transcellular Keratin Scaffolding and Protects From Intestinal Injury

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SUMMARY
Analysis of intestine-specific mice lacking desmoplakin or both desmoplakin/desmoglein 2 show that these proteins are dispensable under basal conditions. However, desmoplakin is essential for cell adhesion, mechanical resilience, and proper keratin network organization, and protects from intestinal injury.

BACKGROUND & AIMS: 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. Although the importance of the desmosome-keratin network is well established in mechanically challenged tissues, we aimed to assess the currently understudied function of desmosomal proteins in intestinal epithelia.

METHODS: We analyzed the intestine-specific villin-Cre DSP (ΔDSP) knockout mice. Cross-breeding with keratin 8-yellow fluorescent protein knock-in mice and generation of organoids was performed to visualize the keratin network. A Dsp-deficient colorectal carcinoma HT29-derived cell line was generated and the role of Dsp in adhesion and mechanical stress was studied in dispase assays, after exposure to uniaxial cell stretching and during scratch assay.

RESULTS: The intestine of ΔDSP mice was histopathologically inconspicuous. Intestinal epithelial cells, however, showed an accelerated migration along the crypt and an enhanced shedding into the lumen. Increased intestinal permeability and altered levels of desmosomal proteins were detected. An inconspicuous phenotype also was seen in ΔDsg2/Dsp mice. After dextran sodium sulfate treatment, ΔDSP mice developed more pronounced colitis. A retracted keratin network was seen in the intestinal epithelium of ΔDSP mice and organoids derived from these mice presented a collapsed keratin network. The level, phosphorylation status, and solubility of keratins were not affected. ΔDSP-deficient HT29 cells had an impaired cell adhesion and suffered from increased cellular damage after stretch.
CONCLUSIONS: Our results show that Dsp is required for proper keratin network architecture in intestinal epithelia, mechanical resilience, and adhesion, thereby protecting from injury. (Cell Mol Gastroenterol Hepatol 2022;13:1181–1200; https://doi.org/10.1016/j.jcmgh.2021.12.009)

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The results show that Dsp is required for keratin network architecture in intestinal epithelia, mechanical resilience, and adhesion, thereby protecting from injury. To that end, DSPΔIEC mice were cross-bred with the reporter K8-yellow fluorescent protein (YFP) knock-in mouse or 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 epithelium–specific Dsp knockout mice (DSPΔIEC). In line with previous findings, DSPΔIEC mice showed 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 (Figures 1A and B and 2). Immunofluorescence staining of colonic tissue confirmed the loss of Dsp and showed a normal distribution of other desmosomal proteins (Figure 1C). Biochemical analysis showed decreased levels of Dsg2 and plakoglobin (PG), while the amounts of other desmosomal proteins were unaltered (Figure 1D and E). These changes seemed to occur post-transcriptionally given that there were no differences in the Dsg2/PG messenger RNA (mRNA) levels (Figure 3). DSPΔIEC mice developed normally; displayed normal body weight, colonic and small intestinal length; and had no diarrhea (Figure 4A). No inflammation was seen and this finding was supported by unaltered expression of the proinflammatory cytokines tumor necrosis factor α, interleukin (IL)1β, and IL6 (Figure 4B and C). Histologic evaluation showed a morphologically inconspicuous small and large intestine (Figure 5A shows large intestine; pictures from small intestine are not shown). Electron microscopy showed normal-appearing desmosomal plaques in the colon (Figure 5B). Notably, DSPΔIEC animals showed somewhat increased intestinal permeability for 4 kilodaltons fluorescein isothiocyanate (FITC) dextran (Figure 5C). Accelerated migration of 5-bromo-2-deoxyuridine (BrdU)-labeled colonic cells along the crypt axis was seen 24 hours after BrdU injection (Figure 6A). 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 K8.
The analysis of selected differentiation/lineage markers showed an inapparent stem cell differentiation pattern (Figure 7). To explore the impact of aging, we systematically analyzed 52-week-old animals. DSPΔIEC mice had normal body weights, colon lengths, and small intestinal lengths (Figure 8A). Histologic staining showed a regular colonic structure, while periodic acid–Schiff (PAS) staining and immunohistochemical staining for anterior gradient 2 (Agr2) showed an unaltered number of goblet cells (Figure 8B). No colonic inflammation was noted within the groups as confirmed by unchanged levels of cytokines tumor necrosis factor α and IL1β (Figure 8C). Because neither a loss of a desmosomal cadherin 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 deletion of both Dsg2 and Dsp in the intestinal epithelia (ΔDsg2/ΔDsp). Biochemical analysis confirmed the efficient deletion of both desmosomal proteins (Figure 9). ΔDsg2/ΔDsp animals (age, 28 wk) developed normally and no changes in

(Figure 6B).
body weight or in the colon and small intestinal lengths were detected (Figure 10A). 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 mucin 2 (Figure 10B). Furthermore, no inflammation was noted as shown by similar levels of proinflammatory cytokines (Figure 10C). Gavage with 4 kilodaltons FITC-labeled dextran showed only a moderate increase in intestinal permeability (Figure 10D).

To test the importance of Dsp during intestinal stress, we challenged DSPΔIEC mice and their floxed littermates with DSS. Compared with DSPfl/fl mice, DSPΔIEC animals experienced increased weight loss with profound fecal bleeding and a significantly reduced colon length (Figure 11A–C). Histologic examination showed 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 (Figure 11D). The profoundly intensified inflammation was corroborated by increased levels of the analyzed proinflammatory cytokines (Figure 11E).

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 showed similar mRNA and protein levels of K7, K8, K18, and K19 (Figure 12A and B). No differences in K8 solubility were noted (Figure 12C). In line with that, phosphorylation of K8 at S79 and S432 did not differ significantly among the phenotypes (Figure 12C and data not shown). To better delineate keratin network organization in vivo, DSPΔIEC and DSPfl/fl mice were cross-bred with knock-in animals expressing the YFP-tagged version of K8.19 Confocal laser scanning microscopy showed a normal-appearing K8 network in the colon and jejunum of DSPfl/fl mice, with K8...
being located in close contact with the plasma membrane. Loss of Dsp resulted in a retracted network that became apparent as a wider distance between the keratin rings, and was even more pronounced in the jejunum (Figure 13A–C). To further explore keratin distribution in rapidly growing intestinal epithelia, we turned to small intestinal organoids. Although 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 showed a profoundly disorganized, collapsed network (Figure 13D), which was in strong contrast to the cortical pattern seen in DSPfl/fl organoids.

Given the known importance of keratins for mechanical stability, we compared the mechanical resilience of wildtype colorectal carcinoma–derived HT29 cells and HT29 cells with a deleted Dsp exon 8 (ΔDSP). The complete loss of Dsp was confirmed on both the mRNA and protein level (Figure 14A and B), and the efficient expression of the targeting vector was corroborated by the incorporated green fluorescent protein (GFP) fluorescence (Figure 14C). No changes in cell growth or morphology compared with wildtype (WT) HT29 cells were observed (Figure 14C and not shown). An inconspicuous cellular monolayer was seen in ΔDSP cells by H&E and phalloidin stainings (Figure 15A and data not shown). Immunofluorescence staining showed an unperturbed localization of the desmosomal cadherin Dsg2 (Figure 14D). Nevertheless, mechanical stress resulted in a more profound fragmentation of the epithelial sheets in Dsp-deficient cells compared with their WT counterparts.
Figure 5. Loss of Dsp leads to increased intestinal permeability. (A) H&E staining highlights the overall colon morphology in 10-week-old, sex-matched DSP ΔIEC (ΔIEC) mice and their floxed littermates (fl/fl). Scale bar: 100 μm. (B) Desmosomal ultrastructure was assessed in both groups by electron microscopy. Scale bar: 100 nm. (C) Serum levels of 4-kilodalton FITC-dextran were quantified in 10-week-old, sex-matched mice 4 hours after the gavage (n = 4). The data are represented as dot plots. A 2-tailed Student t test was used for statistical analyses. *P < .05. Similar results were obtained in male and female mice.

Figure 6. DSP-deficient animals (DSP ΔIEC) showed an accelerated epithelial migration and a higher epithelial loss. (A) Ten-week-old, sex-matched DSP ΔIEC (ΔIEC) mice and their floxed littermates (fl/fl) were injected with 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 K8 in the colonic luminal content of 10-week-old, sex-matched mice was performed as a marker of epithelial extrusion (n = 4). Coomassie staining was used as a loading control. A 2-tailed Student t test was used for statistical analyses. **P < .01. Similar results were obtained in male and female mice.
Similarly, uniaxial cyclic cell stretching led to a more obvious monolayer disruption in DSP vs WT cells (Figure 14F). Moreover, Dsp-decient cells showed a stronger release of the cellular damage marker lactate dehydrogenase into the cell supernatant (Figure 14F). In contrast, loss of Dsp did not affect the wound healing response determined by a scratch assay (Figure 15B). In summary, our results show that 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 (Figure 16).

Discussion

Our study analyzed the role of the 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. The fact that Dsp is necessary for desmosomal integrity in the epidermis but less so in the intestine suggests that it is more important in mechanically challenged tissues. This is not surprising because Dsp becomes mechanically loaded only when cells are exposed to external mechanical stresses. Although no intestinal injury was noted, DSPAIEC mice showed decreased Dsg2 and PG protein levels. These data are in line with observations in Dsg2-decient animals and indicate that alterations in desmosomal proteins affect the post-translational regulation of other desmosomal components. Similarly, cardiac-specific ablation of Dsp resulted in decreased levels of cytosolic PG. Further studies are needed to delineate the underlying molecular mechanisms.

The alterations observed in unchallenged DSPAIEC mice included an increased intestinal permeability, a faster migration along the crypt–villus axis, and a stronger epithelial turnover, which indicates the importance for epithelial adhesion. Similar findings were made after the loss of desmosomal components Dsc2 and Dsg2, which lead to impaired intestinal adhesion. The increased epithelial shedding into the intestinal lumen that was observed in DSPAIEC mice is compatible with the animals with intestine-specific plectin deletion that show increased cellular turnover and a trend toward higher epithelial detachment. The fact that Dsp is crucial for cellular adhesion was supported further by our in vitro studies highlighting a higher cell mechanical fragility of Dsp-decient cells. In addition to Dsp, keratins constitute important mechanical stabilizers and keratin mutations result in cellular fragility. Despite that, neither an isolated Dsp loss nor a combined deletion of Dsp and Dsg2 resulted in a spontaneous intestinal injury. This finding extends earlier observations and suggests that loss of desmosomal proteins can be functionally compensated in unchallenged intestinal epithelia. These rather minor functional defects were somewhat surprising because the cross-breeding of DSPAIEC animals with K8–YFP mice showed that Dsp loss results in a profoundly disorganized keratin filament network in the small and large intestine. Even stronger alterations were seen in the rapidly growing intestinal organoids. Further studies are needed to dissect the importance of Dsp in these situations as well as to delineate its role in the small vs large intestine.

Collectively, these data indicate that Dsp is essential for the tethering of keratins in these cells and cannot be

![Figure 7](image-url)
compensated by other cytolinkers. In line with that, Dsp absence or mutation in keratinocytes led to a retracted keratin network after mechanical stress. Furthermore, it has been shown that modifications in the keratin–desmosome interaction alter cell stiffness in human epithelial cells. However, despite the lost transcellular connection, the retained keratins still seem to fulfill important cellular functions because the phenotype of DSPΔIEC mice is markedly less severe than the phenotype seen in K8 knockout mice. Notably, keratins are multifunctional proteins fulfilling various nonmechanical functions and these retained functions likely are responsible for the comparably mild phenotype of DSPΔIEC animals. Finally, our data show that desmoplakin is more dispensable than its related cytolinker plectin because intestinal deletion of plectin led to spontaneous colitis. This is not surprising because plectin fulfills a much broader spectrum of functions than desmoplakin and its deletion results in dysfunctional hemidesmosomes and intercellular junctions that are not affected by desmoplakin loss. On the other hand, the DSPΔIEC animals showed no obvious phenotype under basal conditions. (A) The body weights, colon lengths, and small intestinal (SI) lengths were analyzed in 52-week-old, sex-matched DSPΔIEC (ΔIEC) and DSP+/+ (fl/fl) mice. The data are shown as dot plots (n = 19). (B) H&E staining showed the overall colonic architecture. PAS staining and Agr2 immunohistochemical staining visualize the goblet cells. Scale bar: 100 μm. (C) Real-time reverse-transcription polymerase chain reaction quantifies the colonic levels of the cytokines tumor necrosis factor α (TNFα) and IL-1β (n = 6–7) as a surrogate of inflammation. The L7 (mouse ribosomal protein) gene was used as an internal control. Average mRNA expression in fl/fl mice was set arbitrarily as 1 and levels in ΔIEC mice represent a ratio. Similar results were obtained in male and female mice.
other hand, deletion of epiplakin, a cytolinker with more restricted cellular junctions, did not lead to an obvious intestinal phenotype either.\textsuperscript{31} Although the moderate intestinal permeability seen in untreated DSP animals is not sufficient to induce epithelial injury, it may promote the disruption of the intestinal barrier during DSS colitis. As an underlying mechanism, proinflammatory cytokines are known to weaken the epithelial junctions\textsuperscript{32} and thereby may perpetuate the vicious cycle of disturbed epithelial barrier and injury.\textsuperscript{33} A similar mechanism was postulated in DSG2\textsuperscript{ΔIEC} mice\textsuperscript{13,19} and multiple cellular models.\textsuperscript{13}

In summary, our findings support an important role of Dsp for epithelial tissue integrity. Because 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. Although desmosomal proteins are dispensable under basal conditions, they may constitute an important second line of defense during intestinal stress. Previous data from patients with idiopathic pulmonary fibrosis suggest that decreased expression of Dsp caused by intrinsic variant rs2076295 may predispose to development of injury in single-layered epithelia.\textsuperscript{31} Together with our data, these findings should spur a systematic analysis of this variant in individuals with digestive disorders.

Materials and Methods

**Mouse Experiments**

Mice with intestine-specific deletion of Dsp and Dsg2, as well as combined deletion of both genes (ΔDsg2/Dsp), were generated by crossing previously described DSG2 exons 4/5 floxed (DSG2\textsuperscript{ΔIEC}/) and DSP exon 2 floxed (DSP\textsuperscript{ΔIEC}/) mice with animals expressing Cre under the control of the villin promoter (DSG2\textsuperscript{ΔIEC}/DSP\textsuperscript{ΔIEC}).\textsuperscript{13,20} DSP\textsuperscript{ΔIEC} animals were further cross-bred with previously described K8–YFP knock-in mice.\textsuperscript{19} All mice were on a C57BL/6 background, were co-housed, and kept under standardized conditions (12 hours day/night cycle; 21°C–24°C; humidity, ~50%) with free access to food and water. To induce colitis, 10-week-old sex-matched mice were exposed to 2% DSS (MP Biochemicals, Heidelberg, Germany) in drinking water for 5 days followed by a switch to normal water. The animals were killed with an isoflurane overdose on day 7. Untreated, co-housed, age- and sex-matched littermates were used as controls. Rectal bleeding was evaluated using a commercial hemoCare fecal occult blood guaiac test (Care diagnostica, Voerde, Germany). Semiquantitative scoring from 0 to 3 (0, no bleeding; 1, mild bleeding; 2, moderate bleeding; and 3, severe bleeding) was performed. All intestinal parts were washed with 1× phosphate-buffered saline (PBS). Proximal parts were stored as Swiss rolls in 4% formaldehyde overnight for histologic evaluation or frozen in OCT 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 3 hours and subsequently gavaged with 0.6 mg/g of body weight 4-kilodalton FITC-labeled 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 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. Afterward, they were incubated in 1 mmol/L EDTA/PBS solution for 30 minutes at 4°C on a tube roller and transferred to 5 mmol/L 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 × g for 5 minutes at 4°C. The crypt-containing pellet was resuspended in a Matrigel matrix (Corning, Kaiserslautern, Germany) and seeded into a prewarmed 48-well plate. Matrigel was allowed to polymerize for 15 minutes at 37°C and the crypts were overlayed with Advanced Dulbecco’s modified Eagle medium/F12 supplemented with 1% Glutamax, 1% 1 mol/L HEPES, and 1% penicillin/ streptomycin, containing 1× N2, 1× B27 supplement (both from Invitrogen, Waltham, MA), 1.25 mmol/L n-acetylcysteine (Sigma-Aldrich), 0.05 μg/mL mouse epidermal growth factor (Invitrogen), 0.1 μg/mL murine Noggin (Peprotech, Hamburg, Germany), and 1 μg/mL recombinant human R-Spondin 1 (R&D Systems, Minneapolis, MN). The medium was changed every 3 days and...
the development was recorded with the EVOS FL Cell Imaging System (Thermo Scientific, Waltham, MA).

**Biochemical Methods**

To obtain the luminal content, the colon was removed and opened longitudinally. The tissue was vigorously inverted 20 times in 1/2 PBS. The solution was centrifuged at 5000 rpm for 10 minutes at 4°C, and the pellet was homogenized in 3% sodium dodecyl sulfate (SDS)-containing buffer supplemented with protease and phosphatase inhibitors. The protein content of the obtained luminal lysates was determined by Coomassie brilliant blue staining. 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-100 (Thermo Scientific, Waltham, MA) buffer and centrifuged to obtain the supernatants constituting the soluble fraction. The pellet was homogenized in high-salt buffer (10 mmol/L Tris, pH 7.6; 140 mmol/L NaCl, 1.5 mol/L KCl; 5 mmol/L 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).\(^3^6\) The same amounts of proteins were separated by SDS-polyacrylamide gel electrophoresis followed by transfer to polyvinylidene difluoride membranes. The membranes were incubated with specific primary and horseradish-peroxidase–coupled secondary antibodies. Finally, antigen–antibody complexes were visualized by an

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**Figure 10.** DSG2/DSP-deficient animals (ΔDsg2/Dsp) showed no obvious basal phenotype, but showed an increase in intestinal permeability. (A) The body weights and colon/small intestinal (SI) lengths of 28-week-old, sex-matched double-knockout DSG2/DSP\(^{ΔIEC}\) (ΔDsg2/Dsp) mice, single-knockout DSG2\(^{ΔIEC}\) (ΔDsg2) and DSP\(^{ΔIEC}\) (ΔDsp) animals, as well as their floxed littermates (fl/fl) were measured (n = 11–12). (B) The colonic architecture was assessed after H&E staining. PAS staining shows the goblet cells. The expression of the goblet cell product mucin 2 (MUC2) was quantified by real-time reverse-transcription polymerase chain reaction (n = 5–6). Scale bars: 100 μm. (C) The levels of inflammatory cytokines tumor necrosis factor α (TNFα) and IL-1β in colonic tissues were evaluated in 28-week-old, sex-matched mice by real-time reverse-transcription polymerase chain reaction (n = 5). The L7 (mouse ribosomal protein) gene was used as an internal control. Average mRNA expression in fl/fl mice was set arbitrarily as 1 and levels in other genotypes represent a ratio. (D) Serum levels of 4-kilodalton FITC dextran were measured in 28-week-old ΔDsg2/Dsp animals and the corresponding floxed mice 4 hours after the gavage (n = 3–4). Average FITC dextran level in fl/fl mice was set arbitrarily as 1 and levels in ΔIEC mice were presented as a ratio. All data are represented as dot plots. Similar results were obtained in male and female mice.
enhanced chemiluminescence detection kit (GE Healthcare/Amersham Biosciences, Chicago, IL). The relative protein amounts were quantified by densitometry via ImageJ software (National Institutes of Health, Bethesda, MD) and depicted as optical density values. The antibodies used are summarized in Table 1.

**Histologic Analysis**

Formaldehyde-fixed tissues were embedded in paraffin, cut into 3-μm-thick sections, and deparaffinized for H&E and 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 1 mol/L 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 a mean from at least 20 assessed crypts per mouse by ImageJ software. H&E-stained, DSS-treated sections were evaluated by a previously described scoring system with minor modifications:13 (1) submucosal thickening/edema, (2) inflammatory cell infiltration, (3) goblet cell loss (each parameter with a score of 0 to 3, as follows: 0, normal; 1, mild; 2, moderate; and 3, severe), (4) 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 P.B. (certified pathologist) and A.G.

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**Figure 11.** DSP-deficient animals (DSP^ΔIEC) showed an enhanced susceptibility toward DSS-induced colitis. (A) Relative body weights of 10-week-old, sex-matched DSP^ΔIEC (grey rectangles) and DSP^fl/fl (black circles, n = 6 each) were evaluated daily starting at the day of first DSS administration (day 0). (B–D) Seven days after the first DSS administration, the severity of colitis was assessed by measuring colonic length (n = 6), semiquantitative scoring of stool blood content with guaiac test (n = 5), and H&E staining of colon sections with histologic scoring (n = 6). Scale bar: 100 μm. (E) To assess colonic inflammation, cytokines tumor necrosis factor α (TNFα), IL-1β, and IL-6 were quantified by real-time reverse-transcription polymerase chain reaction (n = 4–5). The cytokine expression in nontreated animals (ctrl) was set arbitrarily as 1. The L7 (mouse ribosomal protein) gene was used as an internal control. A 2-tailed Student t test was used for statistical analyses. *P < .05, **P < .01, ***P < .001. The data are represented as dot plots. Similar results were obtained in male and female mice.
Immunohistochemistry

Immunohistochemistry staining and visualization of BrdU and Agr2 was performed on paraffin specimens, which were cut into 5-μm-thick sections. Deparaffinized slides were boiled in citrate-based antigen unmasking solution at pH 6 (Vector Laboratories, Burlingame, CA). 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. For BrdU staining, an additional treatment with 2 N HCl for 30 minutes was performed to denature DNA, followed by neutralization with 0.1 mol/L sodium borate (pH 8) for 9 minutes. Afterward, samples were incubated with anti-BrdU or anti-Agr2 antibody overnight at 4°C. After washing, a species-specific biotinylated secondary antibody (Vector Laboratories) was applied for 1 hour, after incubation with Vectastain working solutions (Vectastain ABC Kit; Vector Laboratories). 3,3′-diaminobenzidine (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 20 different crypts per mouse.

Immunofluorescence Staining

Immunofluorescence staining was performed on frozen, OCT-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 precooled acetone or precooled methanol for 10 minutes, respectively. Blocking was performed for 1 hour in 2% normal goat serum, 1% bovine serum albumin (BSA), 0.1% cold fish skin gelatin, 0.1% Triton X-100, 0.05% Tween 20 in 1× PBS (tissue) or 2% BSA in phosphate-buffered saline with Tween (cells). Subsequently, samples were incubated with the following antibodies overnight at 4°C: anti-Dsg2, anti-Dsc2 (AG Leube, RWTH Aachen, Aachen, Germany), anti-Dsp (CBL173; Millipore, Darmstadt, Germany) and anti-β-catenin (Plakoglobin) (sc30997 K-20; Santa Cruz, Heidelberg, Germany). After washing, specimens were subjected to anti-goat Alexa-Fluor 488/568-conjugated secondary antibodies (Invitrogen, Molecular Probes, Eugene, OR) for 1 hour at room temperature and mounted with ProLong Gold antifade reagent containing 4′,6-diamidino-2-phenylindole (P36935; Thermo Scientific).

Figure 12. Loss of DSP does not affect the expression and solubility of keratins. (A and B) The mRNA and protein levels of K7, K8, K18, and K19 were assessed in the colons of 10-week-old, sex-matched DSPΔIEC (ΔIEC) mice and their floxed littermates (fl/fl) by real-time reverse-transcription polymerase chain reaction (n = 3) 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 (OD) values were normalized to the OD values of β-actin (n = 5). Average levels in fl/fl mice were set arbitrarily as 1 and the amounts in ΔIEC mice were presented as a ratio. The L7 (mouse ribosomal protein) gene is an internal control and (B) β-tubulin and (C) β-actin were used as loading controls. The data are shown as dot plots. A 2-tailed Student t test was used for statistical analyses. Similar results were obtained in male and female mice.
GmbH, Schwerte, Germany). Images were acquired with a Zeiss microscope Axio Imager Z1 (Zeiss).

**Quantitative Real-Time Polymerase Chain Reaction**

Total RNA was isolated from tissues and HT29 cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. A total of 1 μg RNA was reverse-transcribed into complementary DNA with the M-MLV Reverse Transcriptase Kit (Promega, Mannheim, Germany) and quantitative real-time reverse-transcription polymerase chain reaction was performed using the 7300 Fast Real-Time Polymerase Chain Reaction System (Applied Biosystems, Waltham, MA). All samples were measured in duplicate and quantified with the ΔΔCt method in relation to the manufacturer’s instructions. A total of 1 μg RNA was reverse-transcribed into complementary DNA with the M-MLV Reverse Transcriptase Kit (Promega, Mannheim, Germany) and quantitative real-time reverse-transcription polymerase chain reaction was performed using the 7300 Fast Real-Time Polymerase Chain Reaction System (Applied Biosystems, Waltham, MA). All samples were measured in duplicate and quantified with the ΔΔCt method in relation to the manufacturer’s instructions. A total of 1 μg RNA was reverse-transcribed into complementary DNA with the M-MLV Reverse Transcriptase Kit (Promega, Mannheim, Germany) and quantitative real-time reverse-transcription polymerase chain reaction was performed using the 7300 Fast Real-Time Polymerase Chain Reaction System (Applied Biosystems, Waltham, MA). All samples were measured in duplicate and quantified with the ΔΔCt method in relation to the manufacturer’s instructions.
to the internal control (ribosomal protein L7). The primers used in the experiments are summarized in Table 2. All expression levels are represented as means ± SEM.

**Transmission Electron Microscopy**
Colonic tissue was cut into ~1 mm³ pieces and fixed at room temperature with the following 3 fixatives: (1) 3.7% formaldehyde, 1% glutaraldehyde, 11.6 g NaH₂PO₄·H₂O and 2.7 g NaOH per liter ddH₂O for 2 hours; (2) 1% OsO₄ for 1 hour; and (3) 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 mmol/L lead citrate for 3 minutes. Images were acquired using a transmission electron microscope.
Ex Vivo Microscopy

Colons and jejunae from DSP<sup>K8</sup>-YFP knock-in mice were flushed with PBS, opened longitudinally, and transferred to glass-bottom dishes (MatTek, Ashland, MA) containing prewarmed Krebs-Henseleit buffer (114 mmol/L NaCl, 5 mmol/L KCl, 24 mmol/L NaHCO<sub>3</sub>, 1 mmol/L MgCl<sub>2</sub>, 2.2 mmol/L CaCl<sub>2</sub>, 10 mmol/L HEPES, 0.25% BSA, pH 7.35). A total of 2.5 μg/mL Hoechst33342 was added for staining of the nuclei in colonic tissue. 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 63×/1.4 Numerical aperture DIC M27 oil immersion objective at 37°C. In addition, the Airyscan detector in super-resolution mode was used. Images were deconvoluted using Zen black software (Zeiss, Wetzlar, Germany) and processed using Fiji. The distance between the keratin rings of individual cells was quantified via Fiji.

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. Briefly, short guide RNA, which targets exon 8 of the DSP gene (for additional information see Table 2), was designed using the Broad Institute (Cambridge, MA) platform and integrated into the vector pL-CRISPR.EFS.GFP (Addgene, Watertown, MA) for lentiviral delivery. The construct was amplified in competent Stbl3<i> Escherichia coli</i> (Invitrogen) and the GeneJET plasmid miniprep and maxiprep kits were used for its isolation (Thermo Scientific). For the 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 48 hours, the lentiviral particles were collected by centrifugation of the cell culture supernatant at 1500 rpm for 5 minutes and filtration with a 45-μm pore size filter. Finally, target HT29 cells were transduced with the isolated particles. Fluorescence-activated cell sorting was used to select transduced, GFP-expressing cells. HT29 cells were cultured in a complete culture medium (RPMI 1640; PAN Biotech, Bavaria, Germany) containing 10% fetal bovine serum and 1% (50 U/mL) penicillin-streptomycin (PAN biotech) in a 5% CO<sub>2</sub> atmosphere at 37°C until they reached confluence. For H&E staining, WT and GFP-expressing Dsp-deficient HT29 cells were seeded on chamber slides (Thermo Scientific) and fixed in 4% paraformaldehyde. Images were acquired with an 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 (P04-

Figure 15. DSP-deficient animals (DSP<sup>DIEC</sup>) showed no alterations in wound healing. (A) Monolayer formation was confirmed via H&E staining in Dsp-deficient (ΔDSP) and WT HT29 cells. Scale bars: 20 μm. (B) Cell migration was assessed by wound healing assay with subsequent quantification of the wound closure area (%) after 48 hours in both groups (n = 5). Cells were visualized 24 and 48 hours after wound scratching by bright-field microscopy. Scale bars: 200 μm. The data are represented as dot plots. A 2-tailed Student t test was used for statistical analyses.
34500; PAN Biotech). Afterward, incubation with 3.6 U/mL dispase II in Hank’s balanced salt solution (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 (444-0500; VWR, Radnor, PA) for 5 minutes at 18 rpm and the resulting fragments were counted by an ImageQuant AS 4000 camera system equipped with ImageQuant software (GE Healthcare Europe GmbH, Freiburg, Germany).

Cell Stretching

To perform cyclic stretch experiments, 0.3 × 10^6 Dsp-deficient or WT HT29 cells were seeded on elastic polydimethylsiloxane chambers (silicone elastomers, SYLGARD, 184; Dow Chemical Company, Midland, MI) that were coated with 100 μg/mL fibronectin. After reaching more than 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 for 10 hours. To analyze the impact of stretching on cellular adhesion,

**Table 1. Antibodies Used for Western Blot**

| Antibody | Host | Company |
|----------|------|---------|
| Anterior gradient 2 (EPR20164-278) | Rabbit | ab209224; Abcam, Cambridge, UK |
| 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/I | Rabbit | sc33555 (H-300); Santa Cruz |
| Desmoplakin I/I (clone DP 2.15) | Mouse | CBL173; Millipore |
| Keratin 7 (RCK105) | Mouse | ab9021; Abcam |
| Keratin 8 (clone Ks.8.7) | Mouse | 61038; Progen, Heidelberg, Germany |
| Keratin 8 (S79) | Mouse | LJ47 |
| Keratin 18 (clone Ks 18.04) | Mouse | 61028; Progen |
| Keratin 19 (TROMAIII) | Rat | Developmental Studies Hybridoma Bank; Iowa City, IA |
| Plakophilin 2 | Goat | ab189323 |
| β-actin | Mouse | A2228; Sigma-Aldrich |
| β-tubulin | Mouse | T8328; Sigma-Aldrich |
| γ-catenin (PG) | Goat | sc30997 (K-20); Santa Cruz |
monolayers were examined by bright-field microscopy before and after stretching. To quantify the extent of cellular damage, lactate dehydrogenase levels were measured in the supernatant.

**Wound Healing Assay**

Dsp-deficient and WT HT29 cells were seeded into 12-well plates. After reaching confluency, a pipette tip was used to scratch a wound (straight line) into the cell

### Table 2. Primers Used for Genotyping, Quantitative Real-Time Polymerase Chain Reaction, and CRISPR/Cas

| Genotyping polymerase chain reaction primer | Forward | Reverse |
|--------------------------------------------|---------|---------|
| mDsg2                                      | GGTAATGCAGACGGGATCAG | TGGGCCAACCTACATAGGAAG |
| mDsp                                       | GTCTTGTGCACTGATGATGCC | GACCTGGGCTGTGCCTGTC |
| mVillin-Cre                                 | CCAACACAGTGACGACGAAT | TCAGGATCATCGACTACCC |
| mK8YFP                                     | AGTAAGCAGGCCAACCA   | AAAGCTGCTGCTTCCTC |

| Quantitative real-time polymerase chain reaction primer | Forward | Reverse |
|---------------------------------------------------------|---------|---------|
| mutE4/E5-mDsg2                                          | ACCGGGAAGAAACACCATATT | AGGGCTTTTTCAGGTGTTTT |
| mDsc2                                                   | GCACCTGCCGTGTAAGCAGT | CTCTGGCCTACATCCCTGTC |
| mPG/JUP                                                  | TCCGTCAGACACCTCTTCAC | ACTAGACATTGCGACTAGGA |
| mDSP                                                    | CGCCCAACAGGAACAAATCA | GATGCCACGCTGACTTCA |
| mPkp2                                                   | GGAGAGATCCAGTTGACAA | TCAGGACTGCTCGAATAGGTT |
| mK7                                                     | AGGGCTGCTGAGAATGATCT | CGTGAAGGGCTCAGGAGAG |
| mK8                                                     | GACATGAGAGATACCACTAC | TGAAGCCAGAGCTAGTAGGA |
| mK18                                                    | CAACGCCAGAGCTAGTAGG | ACCTACCTTGCTCGAATAGG |
| mK19                                                    | TCCAGATGGTGTCTGTTT | CGTGACTTCGGCTGCTTC |
| mMuc2                                                   | CGTGACTTCGGCTGCTTC | CGTGACTTCGGCTGCTTC |
| mSpdef                                                  | CGTGACTTCGGCTGCTTC | CGTGACTTCGGCTGCTTC |
| mGfl1                                                   | CGTGACTTCGGCTGCTTC | CGTGACTTCGGCTGCTTC |
| mAtoh1                                                  | CTGGTGCGATCATCGCTGT | CGTGACTTCGGCTGCTTC |
| mHes1                                                   | CTTGTGCCGATAAGCGAGA | CGTGACTTCGGCTGCTTC |
| hTNFa                                                   | AGGGCATCTAGTACGCTGT | CGTGACTTCGGCTGCTTC |
| mL1b                                                    | TAAAGGCACGACTTCACTCAGT | CGTGACTTCGGCTGCTTC |
| mIL6                                                    | AGGGCATCTAGTACGCTGT | CGTGACTTCGGCTGCTTC |
| mL7                                                     | AATCCTAGTCCGCTACCTGT | CGTGACTTCGGCTGCTTC |

CRISPR/Cas primer

| hDSP (exon 8)                                           | CAACG+ forward | CTGGCAACACAGAACAAATCA |
| NM_001008844                                          | AAAC + reverse | GATGCCAGCTGACGTTCATA |

*h*, human; *m*, mouse.
monolayer followed by a washing step in 1× PBS to remove detached cells. To analyze cell migration, wound closure was tracked by bright-field microscope before and 24/48 hours after scratching. Surface area measurements (wound closure %) were conducted via ImageJ software.

**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. Data were analyzed with an unpaired 2-tailed Student t test or 1-way analysis of variance. Two-tailed P values less than .05 were considered statistically significant. All authors had access to the study data and reviewed and approved the final manuscript.

**References**

1. Etienne-Manneville S. Cytoplasmic intermediate filaments in cell biology. Annu Rev Cell Dev Biol 2018; 34:1–28.
2. Jacob JT, Coulombe PA, Kwan R, Omary MB. Types I and II keratin intermediate filaments. Cold Spring Harb Perspect Biol 2018;10:a018275.
3. Hatzfeld M, Keil R, Magin TM. Desmosomes and intermediate filaments: their consequences for tissue mechanics. Cold Spring Harb Perspect Biol 2017;9:a029157.
4. Rubsam M, Broussard JA, Wickstrom SA, Nekrasova O, Green KJ, Niessen CM. Adherens junctions and desmosomes coordinate mechanics and signaling to orchestrate tissue morphogenesis and function: an evolutionary perspective. Cold Spring Harb Perspect Biol 2018;10:a029207.
5. Broussard JA, Jaiganesh A, Zarkoob H, Conway DE, Dunn AR, Espinosa HD, Janmey PA, Green KJ. Scaling up single-cell mechanics to multicellular tissues - the role of the intermediate filament-desmosome network. J Cell Sci 2020;133:jcs228031.
6. Holthofer B, Windoffer R, Troyanovsky S, Leube RE. Structure and function of desmosomes. Int Rev Cytol 2007;264:65–163.
7. Coulombe PA. The molecular revolution in cutaneous biology: keratin genes and their associated disease: diversity, opportunities, and challenges. J Invest Dermatol 2017;137:e67–e71.
8. Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkus PE, Whittock N, Leigh IM, Stevens HP, Kelsell DP. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. Hum Mol Genet 2000; 9:2761–2766.
9. Spindler V, Eming R, Schmidt E, Amagai M, Grando S, Jonkman MF, Kowalczyk AP, Muller EJ, Payne AS, Pincelli C, Sinha AA, Sprecher E, Zillikens D, Hertl M, Waschke J. Mechanisms causing loss of keratinocyte cohesion in pemphigus. J Invest Dermatol 2018; 138:32–37.
10. Ku NO, Strnad P, Bantel H, Omary MB. Keratins: biomarkers and modulators of apoptotic and necrotic cell death in the liver. Hepatology 2016;64:966–976.
11. Mathai SK, Pedersen BS, Smith K, Russell P, Schwarz MI, Brown KK, Steele MP, Loyd JE, Crapo JD, Silverman EK, Nickerson D, Fingerlin TE, Yang IV, Schwartz DA. Desmoplakin variants are associated with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2016;193:1151–1160.
12. Polari L, Alam CM, Nyström JH, Heikkila T, Tayyab M, Baghestani S, Toivola DM. Keratin intermediate filaments in the colon: guardians of epithelial homeostasis. Int J Biochem Cell Biol 2020;129:105878.
13. Gross A, Pack LAP, Schacht GM, Kant S, Ungewiss H, Meir M, Schlegel N, Preisinger C, Boor P, Guldiken N, Kruische CA, Sellge G, Trautwein C, Waschke J, Heuser A, Leube RE, Strnad P, Desmoglein 2, but not desmocollin 2, protects intestinal epithelia from injury. Mucosal Immunol 2018;11:1630–1639.
14. Schlegel N, Boerner K, Waschke J. Targeting desmosomal adhesion and signalling for intestinal barrier stabilization in inflammatory bowel diseases-lessons from experimental models and patients. Acta Physiol (Oxf) 2021;231:e13492.
15. Raya-Sandino A, Luissint AC, Kusters DHM, Narayanan V, Fleming S, Garcia-Hernandez V, Godsie LM, Green KJ, Hagen SJ, Conway DE, Parkos CA, Nusrat A. Regulation of intestinal epithelial intercellular adhesion and signaling by desmosomal cadherin desmocollin-2. Mol Biol Cell 2021;32:753–768.
16. Habtezion A, Toivola DM, Butler EC, Omary MB. Keratin-8-deficient mice develop chronic spontaneous Th2 colitis amenable to antibiotic treatment. J Cell Sci 2005; 118:1971–1980.
17. Vasioukhin V, Bowers E, Bauer C, Degenstein L, Fuchs E. Desmoplakin is essential in epidermal sheet formation. Nat Cell Biol 2001;3:1076–1085.
18. Krausova A, Buresova P, Samova L, Oyman-Eyrilmex G, Skarda J, Wohl P, Bajer L, Sticova E, Bartonova L, Pacha J, Koubkova G, Prochazka J, Sporrer M, Durrbeck C, Stehlikova Z, Vit M, Ziolkowska N, Sedlacek R, Jirak D, Kverka M, Wiche G, Fabry B, Korinek V, Gregor M. Plectin ensures intestinal epithelial integrity and protects colon against colitis. Mucosal Immunol 2021;14:691–702.
19. Schwarz N, Windoffer R, Magin TM, Leube RE. Dissection of keratin network formation, turnover and reorganization in living murine embryos. Sci Rep 2015; 5:9007.
20. Sumigray KD, Lechler T. Desmoplakin controls microvilli length but not cell adhesion or keratin organization in the intestinal epithelium. Mol Biol Cell 2012;23:792–799.
21. Price AJ, Cost AL, Ungewiss H, Waschke J, Dunn AR, Grashoff C. Mechanical loading of desmosomes depends on the magnitude and orientation of external stress. Nat Commun 2018;9:5284.

22. Garcia-Gras E, Lombardi R, Giocono MJ, Willerson JT, Schneider MD, Khoury DS, Marijn AJ. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. J Clin Invest 2006; 116:2012–2012.

23. Coulombe PA, Kems ML, Fuchs E. Epidermolysis bullosa simplex: a paradigm for disorders of tissue fragility. J Clin Invest 2009;119:1784–1793.

24. Flemming S, Luijssint AC, Kusters DHM, Harnstein V, Garcia AJ, Parkos CA, Nusrat A. Desmocollin-2 promotes intestinal mucosal repair by controlling integrin-dependent cell adhesion and migration. Mol Biol Cell 2020;31:407–418.

25. Wanuske MT, Brantschen D, Schinner C, Studle C, Flemming S, Luissint AC, Kusters DHM, Raya-Sanchez MM, Walter E, Hiermaier M, Vielmuth F, Waschke J, Spindler V. Clustering of desmosomal cadherins by desmoplakin is essential for cell-cell adhesion. Acta Physiol (Oxf) 2021;231:e13609.

26. Cabral RM, Tattersall D, Patel V, McPhail GD, Hatzimasoura E, Abrams DJ, South AP, Kelsell DP. The DSPII splice variant is crucial for desmosome-mediated adhesion in HaCaT keratinocytes. J Cell Sci 2012;125:2853–2861.

27. Norgett EE, Lucke TW, Bowers B, Munro CS, Leigh IM, Sandino A, Fan S, Zhou DW, Hasegawa M, Garcia-Gras E, Lombardi R, Giocondo MJ, Willerson JT, Hernandez V, Garcia AJ, Parkos CA, Nusrat A. Desmocollin-2 promotes intestinal mucosal repair by controlling integrin-dependent cell adhesion and migration. Mol Biol Cell 2020;31:407–418.

28. Broussard JA, Yang R, Huang C, Nathangari SSP, Beese AM, Godsell LM, Hegazy MH, Lee S, Zhou F, Sniadecki NJ, Green KJ, Espinosa HD. The desmoplakin-intermediate filament linkage regulates cell mechanics. Mol Biol Cell 2017;28:3156–3164.

29. Snider NT, Omary MB. Post-translational modifications of intermediate filament proteins: mechanisms and functions. Nat Rev Mol Cell Biol 2014;15:163–177.

30. Geisler F, Leube RE. Epithelial intermediate filaments: guardians against microbial infection? Cells 2016;5:29.

31. Spazierer D, Fuchs P, Reipert S, Fischer I, Schmuth M, Lassmann H, Wiche G. Epilakin is dispensable for skin barrier function and for integrity of keratin network cytoarchitecture in simple and stratified epithelia. Mol Cell Biol 2006;26:559–568.

32. Kojouharoff G, Hans W, Obermeier F, Mannel DN, Andus T, Scholmerich J, Gross V, Falk W. Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. Clin Exp Immunol 1997; 107:353–358.

33. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol 2009;9:799–809.

34. Capaldo CT, Nusrat A. Cytokine regulation of tight junctions. Biochim Biophys Acta 2009;1788:864–871.

35. Spindler V, Meir M, Vigh B, Flemming S, Hutz K, Germer CT, Waschke J, Schlegel N. Loss of desmoglein 2 contributes to the pathogenesis of Crohn’s disease. Inflamm Bowel Dis 2015;21:2349–2359.

36. Strnad P, Guldiken N, Helenius TO. Simple Epithelial Keratins. Methods Enzymol 2016;568:351–388.

37. Liao J, Ku NO, Omary MB. Stress, apoptosis, and mitosis induce phosphorylation of human keratin 8 at Ser-73 in tissues and cultured cells. J Biol Chem 1997; 272:17565–17573.

38. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A, Fiji: an open-source platform for biological-image analysis. Nat Methods 2012;9:676–682.

39. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. Science 2013;339:819–823.

40. Faust U, Hampe N, Rubner W, Kirchgeessner N, Saffran S, Hoffmann B, Merkel R. Cyclic stress at mHz frequencies aligns fibroblasts in direction of zero strain. PLoS One 2011;6:e28963.
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