Supplemental Material

Supplemental Tables

Suppl. Table 1

Primer name	*Sequence*
hDSG2-1 f	caccgCTTTGGCGCCCTTTCCGCAA
hDSG2-1 r	aaacTTGCGGAAAGGGCGCCAAAGc
hDSG2-2 f	caccgCTAAACATCCTCATTTAGTG
hDSG2-2 r	aaacCACTAAATGAGGATGTTTAGc
qPCR primer	*Sequence*
DSG2 f	Aattgcgctcatgattttgg
DSG2 r	Gcaatggcacatcagcagta

Primer list of used in for the present study as outlined in Methods

Suppl. Table 2

Antibody	Source	Catalogue Number	Dilution	
Primary			**WB**	**IF**
p38 MAPK	Cell Signaling, Danvers, USA	9212	1:1000	
Phospho-p38 MAPK (Thr180/Tyr182)	Cell Signaling, Danvers, USA	9211	1:1000	
GFRα1	Abcam, Cambridge, UK	ab186855	1:1000	
GFRα2	Abcam, Cambridge, UK	ab19146	1:1000	
GFRα3	Abcam, Cambridge, UK	ab89343	1:1000	
Ret	Abcam, Cambridge, UK	ab184189	1:1000	
Phospho-Ret (Tyr905)	Cell Signaling, Danvers, USA	3221	1:1000	
GDNF	R&D Systems, Abingdon, UK	AF-212-NA	1:200	
Phospho-Cytokeratin18 (Serin52)	Abcam, Cambridge, UK	ab63393	1:1000	
Phospho-Cytokeratin18 (Serin33)	SAB Signalway Antibody, College Park, USA	11306-2	1:1000	
Cytokeratin18	Santa Cruz Biotechnology, Heidelberg, Germany	Sc-6259	1:1000	
Phospho-Cytokeratin8 (Serin74)	Thermo Fisher Scientific, Waltham, USA	PA5-37623	1:1000	
Cytokeratin8	Santa Cruz Biotechnology, Heidelberg, Germany	Sc-8020	1:1000	
Cleaved Caspase-3 (Asp175)	Cell Signaling, Danvers, USA	9661	1:600	
Caspase-3	Cell Signaling, Danvers, USA	9662	1:600	
Desmoglein-2 (m)	Thermo Fisher Scientific, Waltham, USA	32-6100	1:1000	1:100
Desmoglein-2 (rb)	Millipore/Merck, Darmstadt, Germany	ABT290	1:1000	1:100
E-Cadherin	BD Transduction Laboratories, Heidelberg, Germany	610182	1:1000	1:100
Claudin-1	Thermo Fisher Scientific, Waltham, USA	51-9000	1:500	1:50
GAPDH-Peroxidase	Sigma-Aldrich, Munich, Germany	G92950	1:10000	
ß-Actin-Peroxidase	Sigma-Aldrich, Munich, Germany	A3854	1:10000	
Alexa Fluor 488 Phalloidin	Thermo Fisher Scientific, Waltham, USA	A-12379		1:60

Secondary				
horseradish peroxidase-labelled goat anti-mouse IgG	Dianova, Hamburg, Germany	115-035-003	1:3000	
horseradish peroxidase-labelled goat anti-rabbit IgG	Dianova, Hamburg, Germany	111-035-003	1:3000	
horseradish peroxidase-labelled donkey anti-goat IgG	Dianova, Hamburg, Germany	705-035-003	1:1000	
dam Alexa Fluor 488	Thermo Fisher Scientific, Waltham, USA	A-21201		1:200
darb Alexa Fluor 488	Thermo Fisher Scientific, Waltham, USA	A-21206		1:200
goat-anti-mouse Cy3	Dianova, Hamburg, Germany	115-165-003		1:600
goat-anti-rabbit Cy3	Dianova, Hamburg, Germany	111-165-003		1:600
donkey-anti-goat Cy3	Dianova, Hamburg, Germany	705-035-003		1:600

List of all antibodies with catalogue and/or clone numbers are shown. On the right side the dilutions used for Western blot or immunostaining are indicated.

Supplemental Figure legends

Supplemental Figure 1

A As shown in Figure 1F measurements of the optical density of DSG2 western blots of CD patients demonstrated a reduction of DSG2 in the inflamed parts of the specimens to 0.33± 0.10 (equates 0.21± 0.05 of controls) compared to 1.91± 0.56 in control specimens. In the uninflamed resection margins of the specimens no changes compared to controls were observed when OD was 0.66 ± 0.22; (OD = optical density, n=9).

B As shown in Figure 1F for CD patients quantification of the optical density resulted in a significant increase of phosphorylation of p38MAPK to 2.37± 0.42 (-fold of controls) in the inflamed areas of the tissue that was not seen in the non-inflamed specimens when OD was 0.76± 0.22 (OD = optical density, n=9).

C As shown in Figure 1F for CD patients in inflamed tissue phosphorylation of cytokeratin18 at serin52 was increased to 1.92± 0.15 compared to 1.29± 0.36 in the non-inflamed tissue (OD = optical density, n=9).

D As shown in Figure 1F for CD patients increased phosphorylation of cytokeratin8 at serin74 was observed in inflamed tissue specimen (OD = optical density, n=9).

E, F Representative Western blots for E-cadherin (E) and claudin1 are shown from specimens of patients with CD compared to healthy controls to verify the presence of epithelial markers and to exclude loss of the mucosa (n= 3)

Supplemental Figure 2

A As shown in Figure 1F analysis of optical density of DSG2 demonstrated a significant reduction of DSG2 in inflamed colon specimen of patients suffering from ulcerative colitis (OD = optical density, n=9)

B-D As shown in Figure 1F the loss of Desmoglein2 in specimen of ulcerative colitis was paralleled by an increase in phosphorylation of p38MAPK (B), cytokeratin18 at serin52 (C) and

cytokeratin8 at serin74 (D) as shown by an augmented optical density of western blots (OD = optical density, n=9).

E, F Representative Western blots for E-cadherin (E) and claudin1 are shown from specimens of patients with UC compared to healthy controls to verify the presence of epithelial markers and to exclude complete loss of the mucosa (n= 3). Western blot shown for β-actin to verify equal protein loading is the same in E and F since the same gel was used for E-cadherin and claudin1 blots after stripping.

Supplemental Figure 3

A, B Western blot signaling of DSG2 and quantification of the optical densities in Caco2 cells in Triton assays are shown. TNFα led to a reduced signal in the triton-insoluble fraction. In contrast incubation with GDNF increased the intensity of DSG2 in the triton-insoluble fraction and blocked the effects of TNFα (OD = optical density, n=5).

C Measurements of the permeability coefficient in a transwell filter system of a Caco2 monolayers demonstrated permeability of 70kDa FITC-Dextran was not altered in Dsg2 deficient cells compared to a DSG2 wild type monolayer. Permeability was not changed following incubation of TNFα and TNFα + GNDF under both conditions (n=8); ordinary one way ANOVA.

D Analyses of cytokeratin immunostaining as shown in Figure 3D revealed a shift of the intensity peak away from the cell border after incubation with TNFα. Co-incubation with GDNF attenuated the effect of TNFα (n = 6); ordinary one way ANOVA for each time point

E The distance of keratin retraction in all immunostaining for cytokeratin18 in Caco2 cells from the cell borders is shown; representative images are shown in Figure 3D. The distance between the highest intensities and the cell borders was determined. TNFα increased the keratin retraction to 43.52 ± 4.48 pixel compared to 9.84 ± 1.14 after co-incubation of TNFα with GDNF (n=6); ordinary one way ANOVA.

Supplemental Figure 4

A, B Immunostaining of Propidiumiodide (pink) and DAPI (blue) in Caco2 cells demonstrated that application of TNFα did not increase ratio of propidiumiodide positive cells. Quantification of apoptotic cells / HPF showed no significant difference between controls, TNFα, TNFα and GDNF and GDNF. Apoptosis inductor staurosporine served as a positive control; (HPF= high power field, n=10)

C Cell viability assays in Caco2 cells were performed. Cell viability was not significantly changed compared to controls following the incubation with TNFα, TNFα and GDNF and GDNF. The cell viability was significantly reduced after application of staurosporine (n=5); ordinary one way ANOVA.

D Following crystal violet staining, the colorimetric signal of crystal violet dye did not change after the application of TNFα, TNFα and GDNF+GDNF compared to controls (n= 8). Ordinary one way ANOVA.

E Western Blots for Caspase3 showed that TNFα-induced inflammation did not lead to an increase in cleaved Caspase3 signal in contrast to incubation with Staurosporine in Caco2 cells (representative blots are shown for n=5).

F The ratio of cleaved Caspase3 / Caspase3 was also not altered in DSS mice compared to mice that were treated with GDNF simultaneously (representative blots are shown for n=5).

Supplemental Figure 5

A, C, E Measurements of the optical density in Western Blots after application of TNFα and GDNF in Caco2 cells demonstrate that TNFα showed increased phosphorylation of p38MAPK, cytokeratin18 at serin 52 and cytokeratin8 at serin 74 as shown in Figure 3E. This was attenuated following co-incubation of TNFα with GDNF, where phosphorylation patterns were comparable to control levels (OD = optical density, n=6); ordinary one way ANOVA.

B, D, F TNFα enhanced phosphorylation of p38 MAPK and cytokeratin18 at serin52 in human enteroids as shown in Figure 4B which was not that strong for cytokeratin8 at serin74. Application of GDNF reduced TNFα-induced phosphorylation (OD = optical density, n=6); ordinary one way ANOVA.

Supplemental Figure 6

A, B Western Blots of Caco2 cells showed that TNFα and GDNF did not affect the phosphorylation of cytokeratin18 at serin33 (OD = optical density, n=4; ordinary one way ANOVA).

C Western Blots of Caco2 cells, lysates of human intestinal tissue, lysates of mouse tissue and human enteroids revealed the expression of RET and GFRα1-3 receptor. While Caco2 cells express RET and GFRα1, lysates of human specimen and human enteroids also show a signal of GFRα2. In lysates of mouse colon, Western Blots showed an expression of RET and GFRα3; β-actin blots shown are loading controls from RET blots which are representative for sufficient protein loading of all blots shown here (representative blots are shown for n=3).

D Representative Western blot of murine full wall intestinal lysates for GDNF is shown on the left for control, DSS-colitis and DSS-colitis with GDNF treatment. Quantifications of all Western blot experiments are shown on the right. (OD = optical density, n=5; ordinary one way ANOVA).

Supplemental Figure 7

A Quantification of all western blot experiments of murine colon lysates as shown in Figure 7D is shown (n=6); Kruskal-Wallis Test (ANOVA)

B Quantification of all western blot experiments for the phosphorylation of p38MAPK is shown as shown in Figure 7D. In DSS animals phosphorylation of p38MAPK was significantly increased which was blocked by treatment with GDNF (OD = optical density, n=6); Kruskal-Wallis Test (ANOVA)

C, D Measurements of the optical density of phosphorylation of all Western blot experiments of cytokeratin18 and 8 in Western blots of murine colon lysates demonstrated a significantly

elevated signal of phosphorylated cytokeratin18 an 8 (OD = optical density, n=6); Kruskal-Wallis Test (ANOVA).