SUPPLEMENTARY METHODS

Reagents and cell culture

Human recombinant cytokines and antibody against IL-27RA were purchased from R&D Systems (Wiesbaden, Germany). Antibodies against phosphorylated and total ERK, p38, Akt and STAT6 were from New England Biolabs (Frankfurt/Main, Germany) as well as antibodies against gp130 and beta-actin. Antibodies against total STAT1 and STAT3 and DMBT1 were from Santa Cruz Biotechnology (Heidelberg, Germany). Antibodies against phospho-STAT1 and phospho-STAT3 were from BD Biosciences (Heidelberg, Germany) and Millipore (Schwalbach, Germany). Horseradish peroxidase linked anti-rabbit, anti-mouse and anti-goat secondary antibodies were purchased from GE Healthcare (Freiburg, Germany) and Santa Cruz Biotechnology. Secondary antibodies for immunohistochemistry were from Dako and Sigma-Aldrich (Taufkirchen, Germany). Inhibitors against MEK-1 (PD980959) and p38 (SB203580) inhibitors were from Sigma-Aldrich, PI3 kinase inhibitor wortmannin was from Tocris (Bristol, UK) and STAT3 Inhibitor S3I-201 was from Merck (Darmstadt, Germany). Validated siRNA against STAT1, STAT3, STAT6 or control siRNA was purchased from Applied Biosystems (Darmstadt, Germany) and Lipofectamine RNAiMAX was from Invitrogen (Karlsruhe, Germany). Kynurenine was from Sigma-Aldrich.

Human colorectal cancer derived IEC lines HT-29, HCT116, DLD-1, SW480 and T84 were obtained from LGC Standards (Wesel, Germany). They were grown in Dulbecco's modified Eagle medium (DMEM) containing 100 IU/ml penicillin, 100 µg/ml streptomycin and 10% defined fetal calf serum (FCS) from PAA (Pasching, Austria) in a humidified 5% CO_2 atmosphere at 37˚C.

Escherichia coli strains WP2 (trp^-) and K12 (trp+) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany).

siRNA tranfection

DLD-1 cells were transfected with siRNA using Lipofectamine RNAiMAX (Invitrogen) following the manufacturer's guidelines. Briefly, 50,000 cells per well of a 24-well plate were reverse transfected with 10 nM siRNA against STAT1, STAT3, STAT6 or a non-specific control siRNA. Total mRNA and protein was isolated and specific knockdown was assessed by qPCR and Western Blot, respectively (Suppl. Fig. S1A and B). IL-27 stimulation experiments were performed 48 hours post transfection.

Nuclear extract preparation and electrophoretic mobility shift assay (EMSA)

DLD-1 cells were incubated overnight with serum-free medium and were then stimulated with 50 ng/ml IL-27 for 30 minutes or were left unstimulated. The cells were harvested in ice-cold PBS and the pellet was resuspended in hypotonic buffer (10 mM HEPES ph 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM PMFS, 0.5 mM DTT) containing protease and phosphatase inhibitors (Roche). After incubation on ice for 15 minutes, NP-40 was added to a final concentration of 0.6 % and cells were immediately vortexed for 10 seconds. The nuclei were pelleted by centrifugation and nuclear protein was extracted for 15 minutes in high salt buffer (20 mM HEPES pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 25 % glycerol, 1 mM DTT, 1 mM PMSF) with protease and phosphatase inhibitor. Nuclear extracts were dialyzed for 1 hour against a 1000-fold volume of dialysis buffer (20 mM HEPES pH 7.9, 100 mM KCl, 1 mM EDTA, 10 % glycerol, 0.2 mM DTT, 0.2 mM PMSF) and were stored at -80°C until further use.

Promoter regions of *IDO1* and *DMBT1* were analyzed with the TFBIND (http://tfbind.hgc.jp/) and TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) programs for putative STAT binding sites and biotin-labeled oligonucleotides were synthesized accordingly.

Cell proliferation assay

DLD-1 cells were seeded into 96-well plates at a density of 5,000 cells per well and grown for one day. Following serum starvation overnight, cells were incubated with or without IL-27 in medium containing 0.1% FCS. Cell proliferation was determined after 48 hours using the CyQuant NF Cell Proliferation Assay (Invitrogen) following the manufacturer's guidelines.

Cell wounding assay

For *in vitro* cell regeneration assays, DLD-1 cells were seeded in 96 well plates at a density of 50,000 cells per well and were

allowed to attach for one day. With a sterile pipette tip, a standardized wound in the confluent cell layer was created. Detached cells were removed by two washes with PBS. The cells were stimulated with IL-27 (50 ng/ml) or PBS. Photographs were taken immediately after stimulation and after 24 hours. The cell free area was photographed under the microscope and was measured using AxioVision Software (Carl Zeiss, Jena). For each group, at least 8 different wells were analyzed in one experiment.

Alpha-2-macroglobulin (A2M) enzyme-linked immunosorbent assay (ELISA)

Human alpha-2-macroglobulin ELISA Kit from AssayPro (St. Charles, MO) was used to determine the concentration of A2M in cell culture supernatants according to the manufacturer's guidelines.

Microarray analysis

After reaching 70% confluency, DLD-1 cells were incubated overnight with serum-reduced medium containing 1% FCS. The next day, cells were stimulated in triplicates with 50 ng/ml IL-27 or were left unstimulated. RNA was isolated after six hours.

For the analysis of the IL-27-induced gene expression, Agilent Whole Human Genome Oligo Microarrays were used in combination with a One-Color based hybridization protocol. Signals on the microarrays were detected with the Agilent DNA Microarray Scanner. Differential gene expression was identified within the human cells by applying appropriate biostatistics to the data set. GeneSpring GX 10 analysis software (Agilent Technologies, Santa Clara, CA) was used to normalize and analyze the raw data. Cytokine-induced gene expression was calculated in comparison to unstimulated cells at the same time point. Welch's approximate t-test ("unpaired unequal variance", parametric) was applied to the comparison of the different groups. Resulting *P*-values were corrected for multiple testing applying the algorithm of Benjamini and Hochberg (1).
The microarray data were deposited in the Array Express databank (http://www.ebi.ac.uk/arrayexpress/). The accession number is available on request.

Immunohistochemistry

Formalin-fixed, paraffin-embedded sections were deparaffinized and the antigens were unmasked by heating according to standard procedures. Endogenous peroxidases were blocked with H_2O_2. The slides were then blocked with 10% normal serum and avidin / biotin. Following incubation with the primary antibody (anti-IL-27RA or anti-gp130) overnight at 4°C, the slides were incubated with the secondary, biotin-conjugated antibody. Then HRP-coupled streptavidin was added, followed by an incubation with the peroxidase substrate 3'-diaminobenzidine (DAB). The slides were counterstained with hematoxylin. In the negative controls, the primary antibody was omitted.

Assessment of colitis activity

DSS-treated mice were monitored daily for body weight, stool consistency, and stool blood with disease activity index determined as previously described (2). At euthanasia, the cecum and proximal and distal parts of the colon were removed and fixed in 10% buffered formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Histological assessment was performed by a pathologist blinded to the treatment. Histological severity of acute colitis of DSS-treated mice was determined using a combined score of crypt damage (0–4), polymorphonuclear neutrophil (PMN) infiltrate (0–3), edema (0–3), erosion/ulceration (0–3), and epithelial regeneration (0–3).

Preparation of murine intestinal epithelial cells

The colons were removed, opened longitudinally and washed in PBS with 5% penicillin-streptomycin to remove the feces. The intestines were cut into 1-2 mm pieces and incubated with Ca^{2+}- and Mg^{2+}-free HBSS containing 1M DTT for 10 min on a shaker to remove the mucus. Tissue pieces were then incubated while being shaken in complete medium containing 5% FCS, 0.02 mol/l HEPES, L-glutamine, 5% penicillin and streptomycin with 1U/ml dispase in RPMI 1640 at 37°C for 60 min. The cell suspension was collected, washed two times with RPMI 1640 containing 5% penicillin and streptomycin and afterwards centrifuged on a Percoll (Pharmacia, Piscataway, NJ) density gradient (800 g for 20 min). The IECs, located at the 0%–30% layer interface, were collected.

References

1. Benjamini Y., Drai D., Elmer G., Kafkafi N., Golani I. (2001) *Behav Brain Res* **125**, 279-84.
2. Cooper H. S., Murthy S. N., Shah R. S., Sedergran D. J. (1993) *Lab Invest* **69**, 238-49.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. **Silencing of STAT1, STAT3 and STAT6 expression by specific siRNAs.** (A) Silencing of STAT1, STAT3 and STAT6 expression was assessed by qPCR 48 hours after transfection with the respective siRNAs. * $p<0.01$ vs. control. Expression in control siRNA transfected cells was arbitrarily set as 1.0 for each group. (B) Protein knockdown of STAT1, STAT3 and STAT6 was determined by Western blot 48 hours post transfection with the respective siRNAs. C=control, S1=STAT1, S3=STAT3, S6=STAT6.

Supplemental Figure S2. IL-27 expression is increased in inflamed colonic biopsies from patients with Crohn's disease. IL-27 mRNA expression was determined in 11 patients by qPCR. From each patient, two inflamed and two non-inflamed biopsies were analyzed. Expression of IL-27 was normalized to beta-actin expression in the respective samples. Median expression levels in each group are depicted by the red line.

Supplemental Figure S3. Overview of the signaling pathways and functions mediated by IL-27 in IEC. IL-27 induces phosphorylation of the MAP kinases p38 and ERK1/2 (via MEK-1) as well as PI3 kinase and Akt. STAT transcripton factor activation is necessary for the regulation of IDO1 and DMBT1 expression as well as for mediating cell proliferation and migration. Kinases are depicted in yellow, transcription factors in orange; P=phosphorylation

SUPPLEMENTAL TABLES

Primer	Primer sequence
hIL-27R forward:	5'-GCCTTCTGCTCCAAAAGATG-3'
hIL-27R reverse:	5'-GGAGCAGCAGCAGGTAATTC-3'
hgp130 forward:	5'-TCAACTTGGAGCCAGATTCC-3'
hgp130 reverse:	5'-CCCACTTGCTTCTTCACTCC-3'
hIL-27 forward:	5'-GAGCAGCTCCCTGATGTTTC-3'
hIL-27 reverse:	5'-AGCTGCATCCTCTCCATGTT-3'
hA2M forward:	5'-AGCTGCCTGGGGAATACAGC-3'
hA2M reverse:	5'-CAGAGCGGCTCCCTGTGTAA-3'
hBATF3 forward:	5'-AGCAGCAGAGCCCTGAGGA-3'
hBATF3 reverse:	5'-TCTCTCCGCAGCATGGTGTTT-3'
hDMBT1 forward:	5'-TGCTCTGTCTGCCAAATCAC-3'
hDMBT1 reverse:	5'-GTCATTGTCTGCCTGCTTGA-3'
hIDO1 forward:	5'-GGCAAAGGTCATGGAGATGT-3'
hIDO1 reverse:	5'-CTGCAGTCTCCATCACGAAA-3'
hSTAT1 forward:	5'-TCGGCAGCAGCTTAAAAAGT-3'
hSTAT1 reverse:	5'-CACCACAAACGAGCTCTGAA-3'
hSTAT3 forward:	5'-AGCTGCACCTGATCACCTTT-3'
hSTAT3 reverse:	5'-AATTGGGGGCTTGGTAAAAA-3'
hSTAT6 forward:	5'-TTGGCTTCATCAGCAAACAG-3'
hSTAT6 reverse:	5'-GGTCCCTTTCCACGGTCA-3'
hIL-8 forward:	5'-CCAGGAAGAAACCACCGGA-3'
hIL-8 reverse:	5'-GAAATCAGGAAGGCTGCCAAG-3'
hSOCS-3 forward:	5'-TTCTGATCCGCGACAGCTC-3'
hSOCS-3 reverse:	5'-TGCAGAGAGAAGCTGCCCC-3'
hβ-actin: forward:	5'-GCCAACCGCGAGAAGATGA-3'
hβ-actin reverse:	5'-CATCACGATGCCAGTGGTA-3'
mIL-27 forward:	CACCTCCGCTTTCAGGTGC-3'
mIL-27 reverse:	AGGTATAGAGCAGCTGGGGC-3'
mA2M forward:	5'-GGAGCCAGCGGCAACCATGT-3'
mA2M reverse:	5'-ACCTCAGCCGAGGGAGCCTG-3'
mBATF3 forward:	5'-CGTGCTGCAGAGAAGCGTGGA-3'
mBATF3 reverse:	5'-TCCTCTGAGCTGCAACCCGGT-3'
mDMBT1 forward:	5'-TGTTTCCAGTGACCAGCAGC-3'
mDMBT1 reverse:	5'-GGGCGAGGAGTAGGATTGGT-3'
mIDO1 forward:	5'-GTTCGAAAGGTGCTGCCCCGC-3'
mIDO1 reverse:	5'-AGAAGCCCTTGTCGCAGTCCCC-3'
mSTAT1 forward:	5'-CCCGGATAGTGGGCCCCGAA-3'
mSTAT1 reverse:	5'-ACCAGCAGTGCTCAGCAAATGTG-3'
mSTAT3 forward:	5'-CGACCCAGGTGCTGCCCCGTA-3'
mSTAT3 reverse:	5'-ATGGGGGAGGTAGCACACTCCGA-3'
mSOCS-3 forward:	5'-TAGGAGGCGCAGCCCCAAGG-3'
mSOCS-3 reverse:	5'-GCGGCGGGAAACTTGCTGTG-3'
mβ-actin: forward:	5'-GATGCTCCCCGGGCTGTATT-3'
mβ-actin reverse:	5'-GGGGTACTTCAGGGTCAGGA-3'

Supplemental Table S1. PCR primers (h=human, m=murine) used for RT-PCR analysis.

Primer	Primer sequence	promoter region included (relative to transcription start site)
DMBT1 forward_1 DMBT1 reverse_1	5'-ATGAACAGTGCATGGCAAAG-3' 5'-TTGATGGCCAGTAACTCTTGG-3'	-944 to -798
DMBT1 forward_2 DMBT1 reverse_2	5'-CTCGTTCTTCCAAGGTGAGG-3' 5'-TACAGGACACAGTGCCAAGG-3'	-393 to -283
DMBT1 forward_3 DMBT1 reverse_3	5'-CCTTGGCACTGTGTCCTGTA-3' 5'-GTTTCCTGAATACGGGCTTG-3'	-303 to -179
DMBT1 forward_4 DMBT1 reverse_4	5'-ATCCTGTTCTTCCCCAAACC-3' 5'-ACTTCCCTCTCGGTGAACCT-3'	-266 to -144

Supplemental Table S2. PCR primers used for quantification of DNA pulled down by ChIP. Primers were designed to span regions with putative STAT3 binding sites as determined by analyzing the DMBT1 promoter region sequence with the TFBIND (http://tfbind.hgc.jp/) and TFSEARCH (http://www.cbrc.jp/research /db/TFSEARCH.html) programs.

Probe	Probe sequence	promoter region included (relative to transcription start site)
IDO1_#1	5'-AAAGAAGCCAAAGTTTGAGGAAGTTAAGTGGCTA-3'	-1776 to -1743
IDO1_#2	5'-TTTGTTTGTTTTCCTTGAACTGATTCCCAAAG-3'	-1143 to -1111
IDO1_#3	5'-CTATTCAAGGAACAT-3'	-1020 to -1007
IDO1_#4	5'-TTTTCCTGTAAAAT-3'	-1007 to -994
IDO1_#5	5'-CAAGGCAATCTTCCTAAAAGTTACTTATTA-3'	-561 to -532
IDO1_#6	5'-ATTTAAAAGTTTCCATAAAGTAAAATG-3'	-250 to -224
STAT1 mutant (unspecific competitor oligo)	5'-CATGTTATGCATATTGGAGTAAGTG-3'	

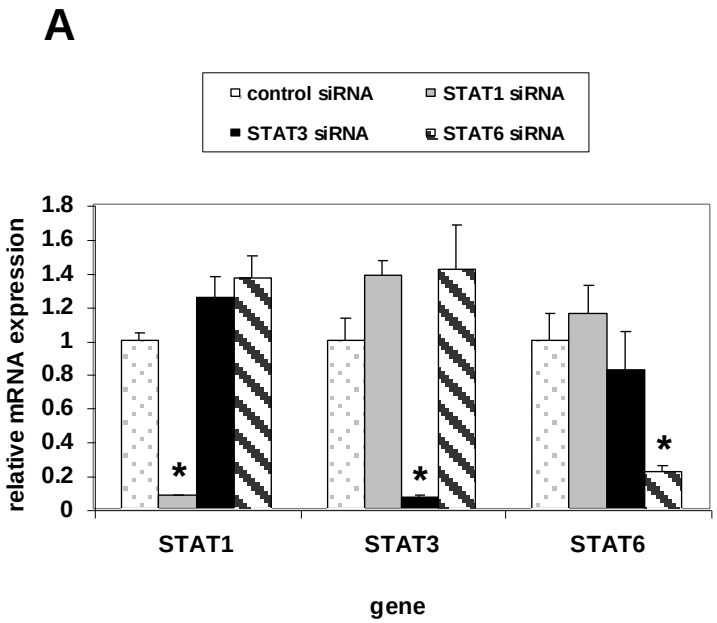
Supplemental Table S3. Oligonucleotides used for EMSA. All oligonucleotide sequences given represent the sense orientation and were annealed with the respective antisense oligonucleotides prior to EMSA. All oligos (except the unspecific competitor) were biotin-labeled at the 3'-end. The STAT1 binding site is underlined.

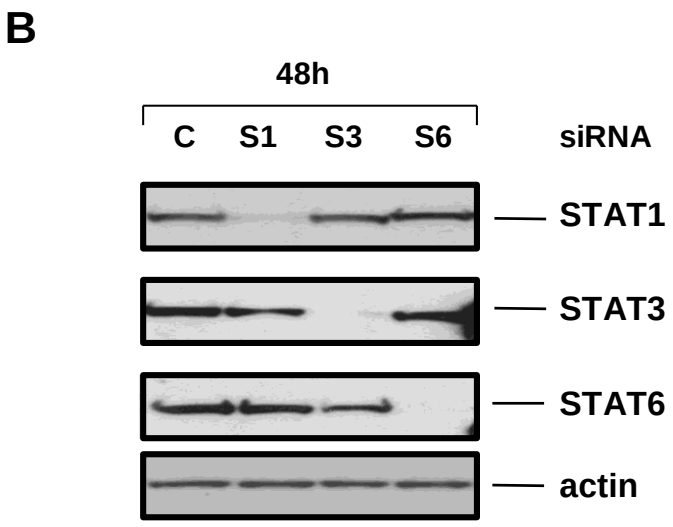
Patient number	gender	age at diagnosis (years)	disease duration (years)	anatomic site of biopsy sampling non-inflamed samples	anatomic site of biopsy sampling inflamed samples	current medication	IL-27 expression vs. non-inflamed
1	male	18	16	descending colon	descending colon	mesalazine, AZA	4.0
2	female	25	15	colon	colon	none	38.1
3	male	36	17	transverse colon	transverse colon	AZA	7.2
4	male	50	1	ascending colon	terminal ileum	none	1.6
5	male	26	7	cecum	cecum	AZA	0.25
6	female	18	5	cecum*	terminal ileum / ileocecal valve*	MTX	421.4
7	female	29	1	cecum*	terminal ileum*	mesalazine, corticosteroids	2.1
8	female	29	22	descending colon	descending colon	none	2.6
9	male	36	17	cecum*	terminal ileum*	corticosteroids	16.1
10	male	25	6	colon	colon	AZA	0.26
11	female	28	1	cecum	terminal ileum*	AZA	21.7

Supplemental Table S4. Characteristics of the Crohn's disease patients from which intestinal biopsies were collected. Biopsies from inflamed and non-inflamed lesions were intended to originate from the same colonic or ileal segment. Patients marked with an asterisk (*) had severe inflammation in a whole anatomic segment. Therefore, biopsies from a bordering (non-inflamed) segment were included for comparison. Abbreviations: AZA, azathioprine; IFX, infliximab; MTX, methotrexate; 6-MP, 6-mercaptopurine

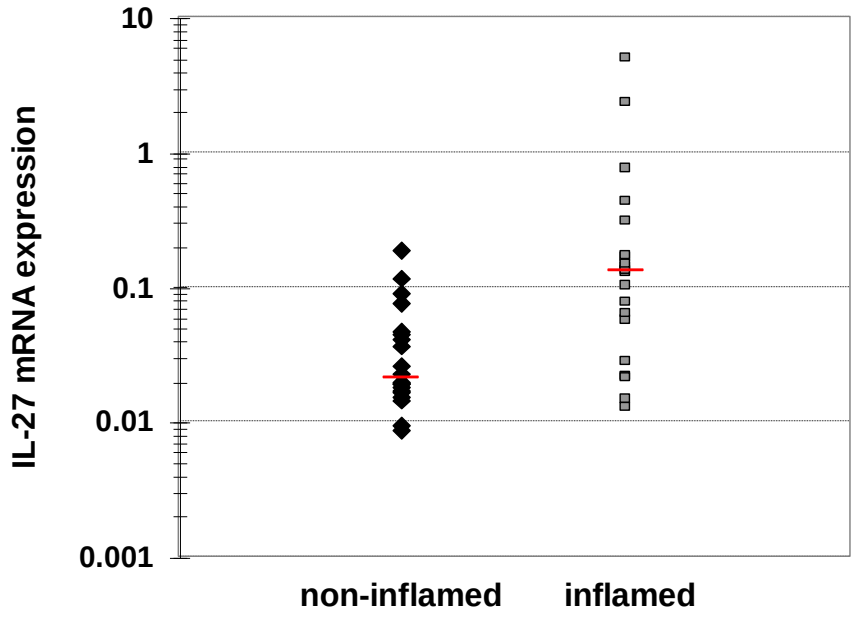
SUPPLEMENTAL FIGURES

Supplemental Figure S1





Supplemental Figure S2



Supplemental Figure S3