Supplemental Figure 1 IL-20 signalling is altered in IBD

(A) Microarray results for three different IL20RA probes (219115_s_at, 222829_s_at, 222828_at) displayed as fold change of mean values in IECs isolated from the inflamed (n=5, right side) vs non-inflamed (n=4, left side) ileal tissue of Crohn’s disease patients. (B) The expression of IFNG and TNF (relative to GAPDH) was assessed in samples from patients with IBD with different inflammation scores and in samples from non-IBD control patients by quantitative polymerase chain reaction. (C) IL-20+ cells counted in 2-4 high power fields/biopsy in patients with mild and severe inflammation (n=4/group). (D) IL20 expression levels in a cohort of anti-TNF responders and non-responders from a publicly available dataset GSE16879. (E-F) Phosphorylated STAT3 in two IBD organoids after 30 minutes of stimulation with 250 ng/ml IL-20 (E) and one IBD organoid in flow-cytometry (F). (G-H) Tissue sections from patients with IBD and non-IBD control patients were stained with antibodies against IL-20 and the epithelial cell marker EpCAM. Hoechst indicates nuclear staining. Detailed images of single channels and a magnification with arrows indicating IL-20-positive staining in the epithelial compartment and arrowheads indicating positive staining in the non-epithelial compartment of a patient with IBD (G); side-by-side overview in non-IBD controls patients and patients with IBD (H). (I) RNAScope was performed in tissue from patients with IBD using specific ZZ probes for human IL-20. Hoechst was used to counterstain nuclei. Scale bars, 50 µm. Statistics: Analysis of variance corrected with Kruskal-Wallis for multiple comparisons in B. Welch’s t test in C and t test in D. Mean with CI 95% is displayed. Abbreviations: IBD, inflammatory bowel disease; HPF, high power field; ns, not significant.

Supplemental Figure 2 RNA-seq data indicate IL-20 activation during experimental colitis

(A) RNA-Seq normalized counts of Il20ra and Il20rb in WT mice at various time points after receiving DSS (n=2-3/time point). (B) Log2FoldChange representation of Il20ra levels in mice from A. (C) Heatmap clustering and volcano plot of differentially expressed genes in Il20ra (+) (n=3) and Il20rb (+) (n=3) compared to WT (n=5) mice in the RNA-Seq data from one DSS experiment. Statistics: mean + SD is displayed in A. Benjamini-Hochberg for padj in B with mean of log2FoldInduction being displayed. Abbreviations: DSS, Dextran sulfate sodium; WT, wildtype.

Supplemental Figure 3 IL-20 is predominately expressed by intestinal epithelial cells in mice with DSS-induced colitis

(A) RNAseq using mouse specific IL-20 ZZ probes was employed to reveal the sources of IL-20 production in colon cross sections from mice with DSS-induced colitis. (B) Partly consecutive slides were used for the co-staining of IL-20 with markers for epithelial cells (EpCAM), immune cells (F4/80, MPO), fibroblasts (vimentin) and glial cells (GFAP). Arrows indicate positive cells in the IEC-compartment, arrowheads indicate positive cells in the non-IEC compartment. Scale bars: 100µM in A and B, right-side panels; 50µM in B left-side rows. Abbreviations: DSS, Dextran sulfate sodium; IEC, intestinal epithelial cell.

Supplemental Figure 4 IL-20 signals protect mice in different experimental colitis models

(A) The preventive value of IL-20 in experimental colitis was evaluated in WT mice that received an intravenous injection of a vector encoding IL20 or a control vector (n=6/group) prior to DSS administration. Body weight curve and representative endoscopies pictures taken at day 5 and day 10 are presented along with histology findings at the end of the experiment. (B) Heatmap clustering and volcano plot of differentially expressed genes in Il20ra− (n=4) and WT (n=5) mice in the RNA-Seq data from one DSS experiment. (C) Selection of processes in the KEGG pathway analysis based on the RNA-Seq of CD and UC cohorts. Red bars indicate the same processes that were upregulated in Il20ra− mice (see Figure 3G), black bars indicate other processes and grey bars indicate processes that did not reach significance. The dashed red line indicates padj = 0.05 (D) Representative results from an oxazolone-induced colitis model in WT and Il20ra− mice. Scale bar: 250µm. Statistics: Welch’s t test in A, mean with SEM or CI 95% is displayed; Benjamini-Hochberg for padj in C; Welch’s t test in D, mean with CI...
95% is displayed. Abbreviations: CD, Crohn’s disease; DSS, Dextran sulfate sodium; UC, ulcerative colitis; WT, wildtype.

**Supplemental Figure 5** IL-20 can interfere with STAT2 signals in epithelial cells of murine organoids. (A) Expression levels of two IFN target genes in colon organoids (derived from WT mice with DSS-induced colitis) that have been stimulated with either 25 ng/ml IFN-β or a combination of 250 ng/ml IL-20 (20 hours) and 25 ng/ml IFN-β (6 hours) or were left untreated. (B) Representative pictures of murine colon organoids left untreated or treated with 100 ng/ml IFN-β for 36 hours (n=3/group). Metabolically active cells produce a dark formazan precipitate in this assay whereas dead organoids appear brighter. Arrows indicate dead areas. (C) Quantification of results from MTT assays after IFN-β stimulation. (D) Quantification of results from the MTT-formazan experiments conducted with organoids stimulated with IFN-β in the presence of IL-10 or IL-20. (E) Comparison between the growth of intestinal organoids (arrows point at buds) from WT and Stat2^-/- mice over a period of 10 days. (F) Quantification of the organoid budding is presented on two different days. Scale bars, 500 µm in B, 200µm in E. Statistics: Welch’s t test in C and F, mean with SD is displayed; Analysis of variance in D. Abbreviations: WT, wildtype.

**Supplemental Figure 6** Implication of STAT2 signalling in organoids after IFN-β stimulation and in mice during DSS-induced colitis. (A) Heatmap clustering of RNA-Seq results from WT organoids stimulated for 20 hours with 25 ng/ml IFN-β as compared with unstimulated organoids (three biological replicates, pairwise design). Volcano plot comparison of significantly upregulated and downregulated genes in WT organoids stimulated with IFN-β vs their unstimulated controls and Stat2^-/- organoids with IFN-β compared to unstimulated Stat2^-/- organoids (n=3 biological replicates/condition). (B) The gene ontology analysis of biological processes in the set of significantly upregulated genes revealed that most processes reach significance only in WT but not Stat2^-/- after IFN-β stimulation (marked in red in the second panel); the only two processes that are significant in Stat2^-/- organoids are marked in black in the lower panel. (C) Heatmap clustering of RNA-Seq results in WT and Stat2^-/- mice from one DSS experiment (n=4/group). Gene ontology of biological processes upregulated in Stat2^-/- mice with colour scale indicating the padj values. Statistics: Benjamini-Hochberg for padj in B. The dashed red line indicates padj = 0.05 in B. Abbreviations: WT, wildtype.

**Supplemental Figure 7** IFN/STAT2 signalling is upregulated in IBD and it induces necroptosis in IEC. RNA-Seq results for the differential expression of the IFNAR2 chain (A) and a typical IFN-stimulated target gene i.e. ISG20 (B) in patients suffering from CD or UC and non-IBD controls (human IBDome cohorts). (C) Effects of IFN-β stimulation on the morphology and vitality of IBD-derived organoids. Characteristic morphology of dead organoids can be seen as large light-dense areas (red arrows). Viable organoids show the typical structure with many buds surrounding a central lumen (green arrows). Yellow arrows indicate stressed organoids. (D) Organoids from patients with IBD were stimulated with either IFN-β or a combination of 250 ng/ml IL-10 or IL-20 plus 25 ng/ml IFN-β or were left untreated and the induction of cell death was followed by propidium iodide/Hoechst and the MTT-formazan assay. (E, F) Quantification of results as presented in D. (G) Fresh biopsies from patients with IBD were incubated for 60 minutes in the presence or absence of 250 ng/ml IL-20 and subsequently for 30 minutes with 25 ng/ml IFN-β. Representative confocal staining for pSTAT2 is presented. Arrows indicate positive cells in the epithelium. Scale bars, 500 µm in C and in D. Statistics: Welch’s t Test in A, B and G, mean with 95% CI is displayed; Analysis of variance in E and F. Abbreviations: CD, Crohn’s disease; HPF, high power field; IBD, inflammatory bowel disease; ns, not significant; RFU, relative fluorescence units; UC, ulcerative colitis.