Supplemental Figure Legends

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**) RNA-scope 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**) RNAScope 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 endoscopy 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 *Il20$^{-/-}$* (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 *Il20$^{-/-}$* mice (see Figure 3G), black bars indicate other processes and grey bars indicate processes that did not reach significance. The dashed red line indicates pdaj = 0.05 (**D**) Representative results from an oxazolone-induced colitis model in WT and *Il20$^{-/-}$* mice. Scale bar: 250µm. Statistics: Welch's *t* test in A, mean with SEM or CI 95% is displayed; Benjamini-Hochberg for pdaj 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 pdaj = 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.