

Supplementary Information

Extrusion of BMP2+ surface colonocytes promotes stromal remodeling and tissue regeneration

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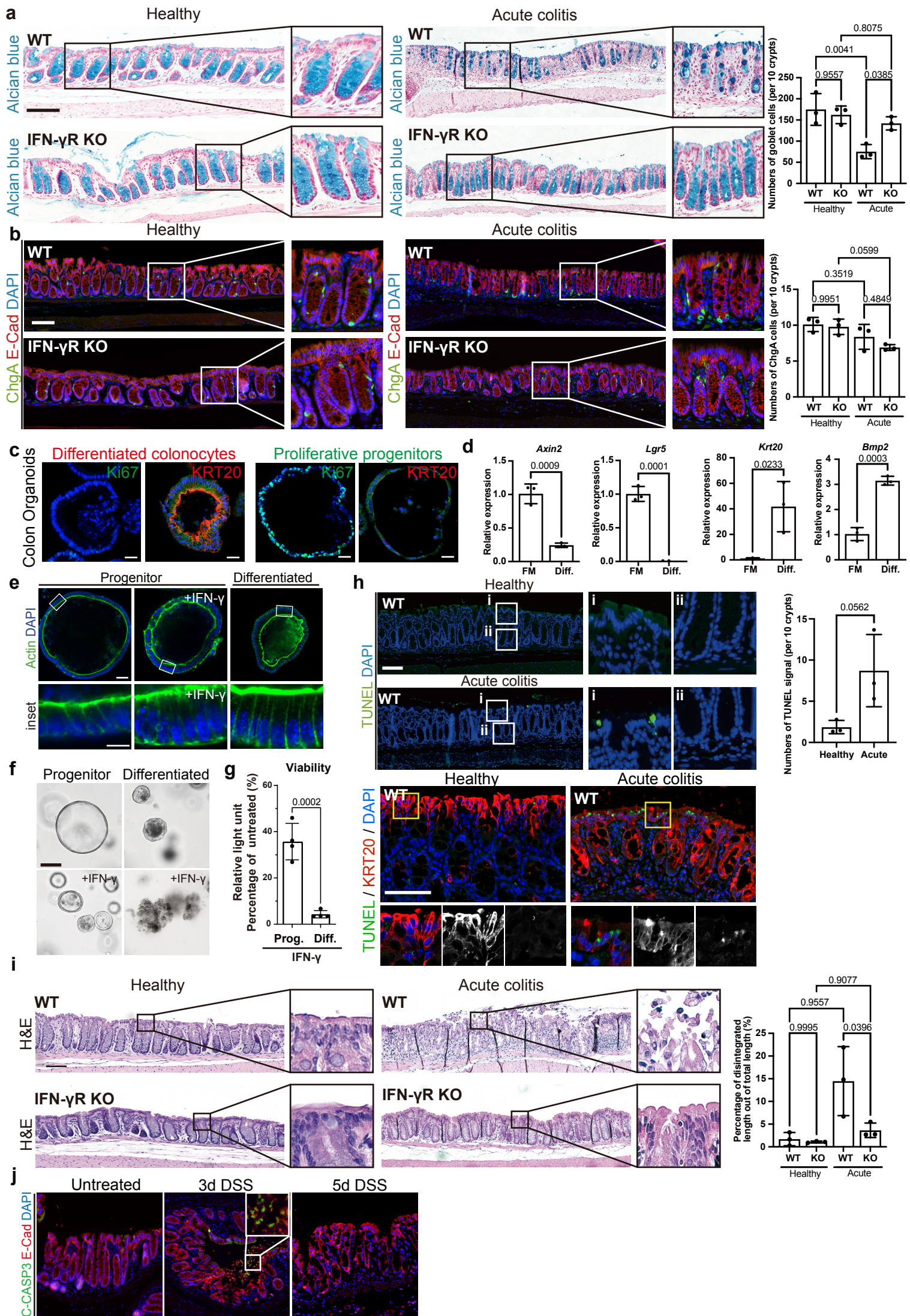
Supplementary Table 1, Supplementary Figures 1-8

Supplementary Table 1

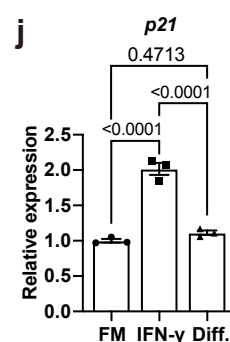
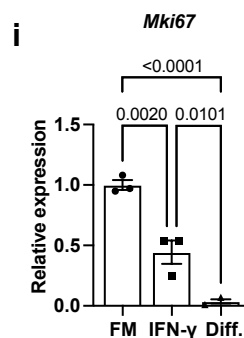
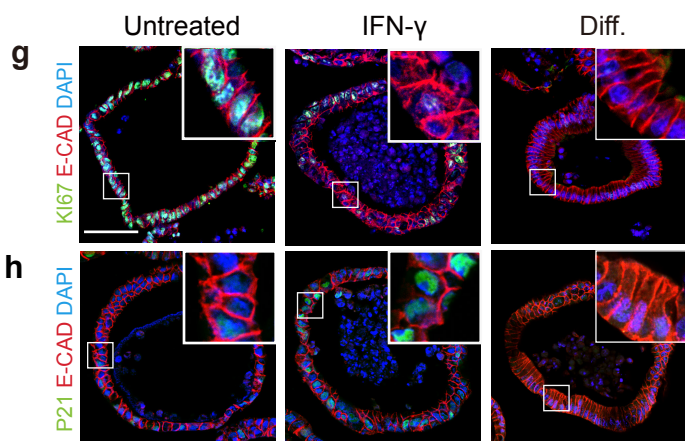
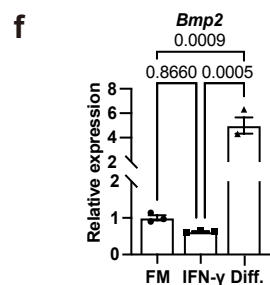
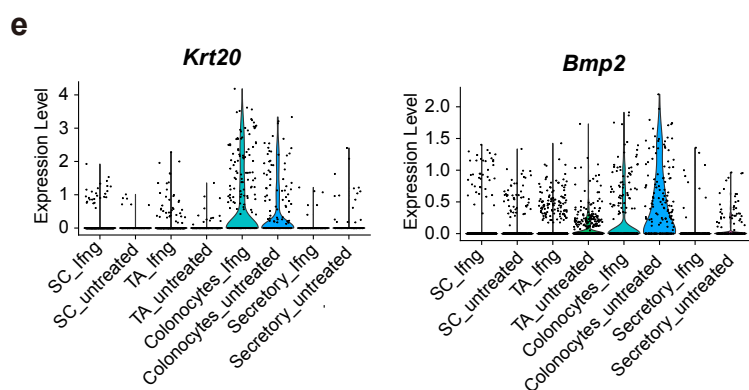
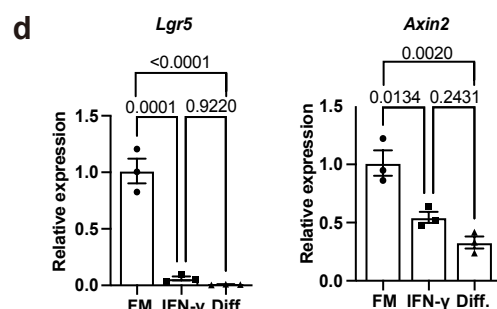
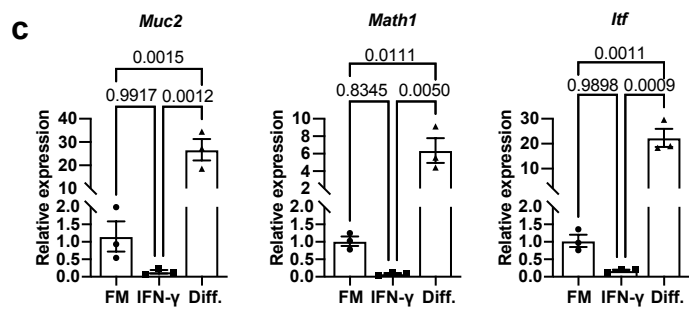
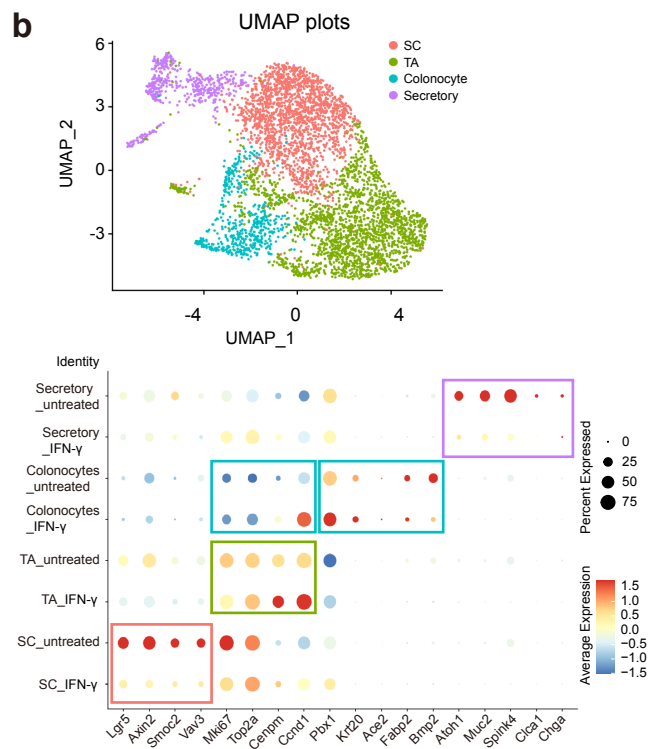
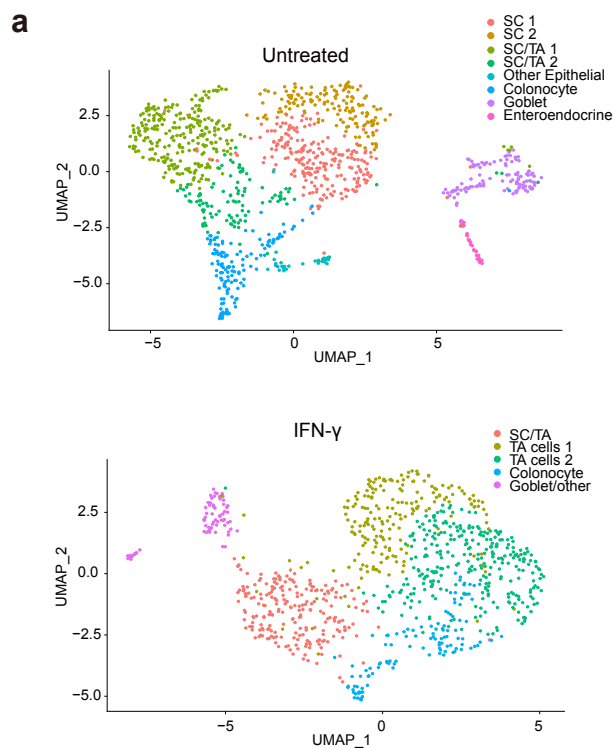
Corresponding P-values:

celltype	GeneSymbol	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Colonocytes	Bmp2	1.063813E-08	-1.0327533	0.277	0.592	0.0002419535
SC	Bmp2	1.801412E-17	-1.4318483	0.03	0.15	4.097131E-13
Secretory	Bmp2	1.346806E-10	-1.2795962	0.038	0.256	3.063175E-06
TA	Bmp2	1.184192E-19	-0.9140337	0.112	0.34	2.693326E-15
Colonocytes	Krt20	0.3164751	0.9638114	0.375	0.374	1
SC	Krt20	0.8088588	0.6159561	0.02	0.018	1
Secretory	Krt20	0.0007944942	-2.5921778	0.023	0.098	1
TA	Krt20	0.7998547	1.1080759	0.037	0.035	1

Supplementary Table 1: Corresponding P-values of Bmp2 and Krt20 expression in the scRNA-seq dataset from progenitors with or without IFN- γ treatment, related to Extended Data Figure 2. The p-values were determined using one-way ANOVA (two-sided) with no adjustments for multiple comparisons.

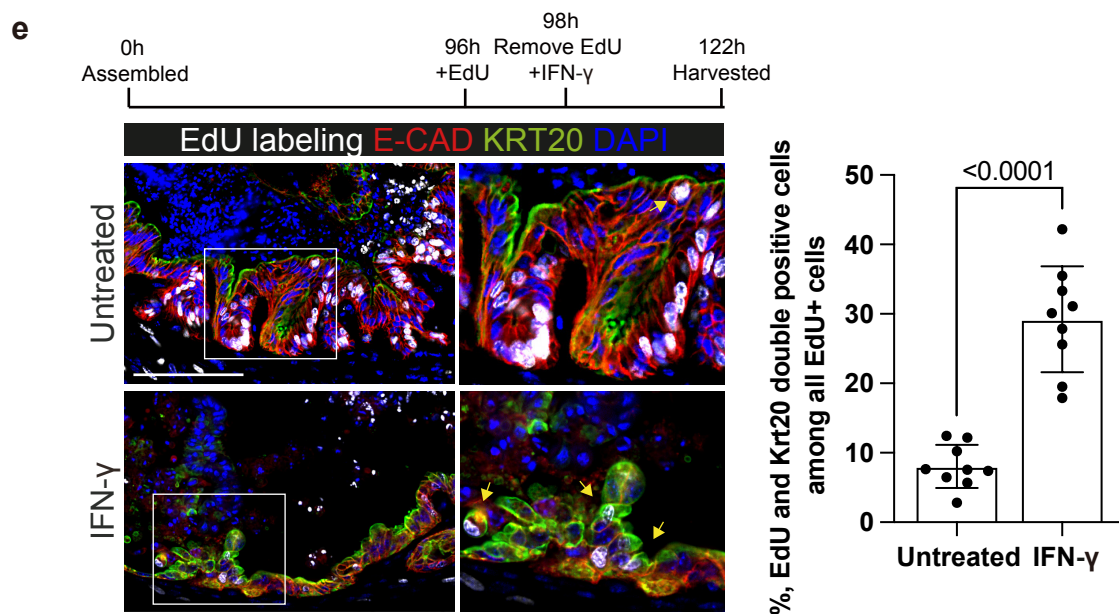
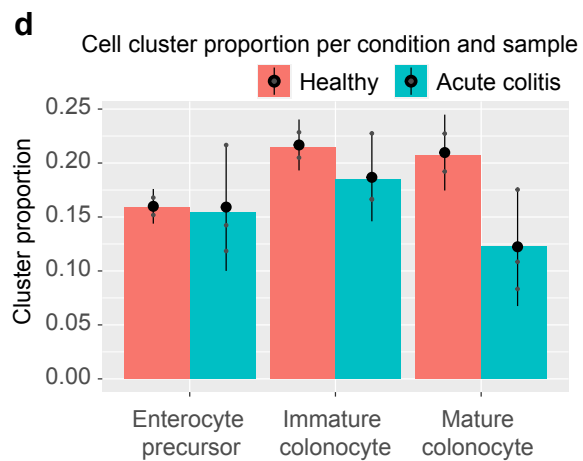
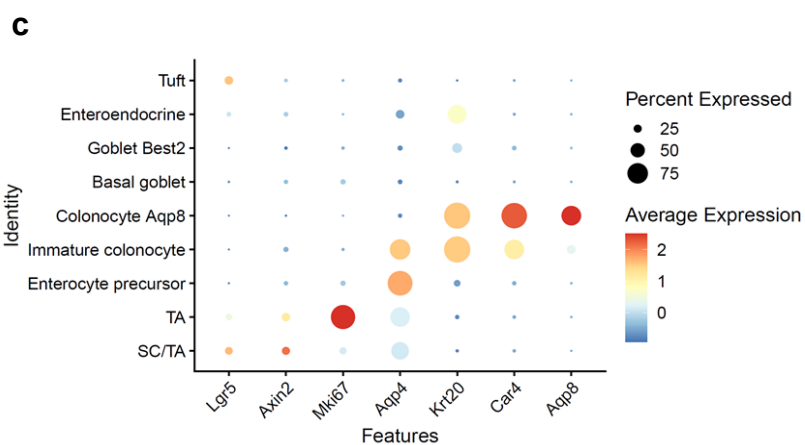
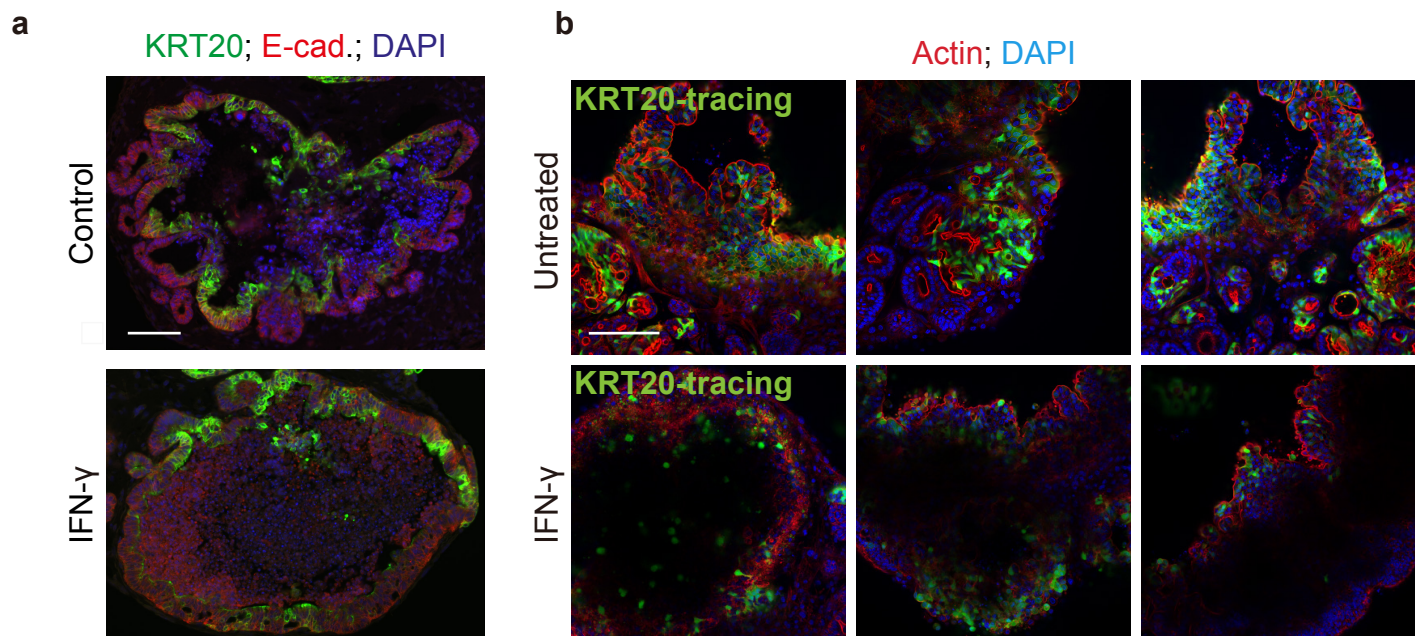


Supplementary Fig.1: IFN- γ -induced loss of goblet cells and colonocyte death, related to Figure 1. (a) Alcian blue staining ($\times 200$ magnification) showing goblet cells in acute colitis mice compared to untreated controls, as well as in WT and IFN- γ R KO mice, quantification on the right. Scale bar: 100 μ m. n=3 mice. (b) Neuroendocrine cells were analyzed by IF staining ($\times 200$ magnification) of chromogranin A (ChgA) in acute colitis and healthy WT and IFN- γ R KO mice, quantification on the right. Scale bar: 100 μ m. n=3 mice. (c) IF staining ($\times 600$ magnification) of Ki67 and KRT20 was performed to distinguish between proliferative progenitors and differentiated colonocytes. Scale bars: 30 μ m. (d) qPCR was used to compare the expression of *Axin2*, *Lgr5*, *Krt20*, and *Bmp2* between proliferative progenitors (FM) and differentiated colonocytes (Diff.). n=3 mice. (e) Actin/phalloidin staining ($\times 600$ magnification) was performed to indicate cell polarity in progenitors with or without IFN- γ treatment and differentiated colonocytes. Scale bars: 30 μ m. (f) Progenitor organoids and differentiated organoids were treated with 50 ng/ml IFN- γ for 48 h. Scale bar: 100 μ m. ($\times 200$ magnification) (g) Progenitors (Prog.) and differentiated organoids were treated with IFN- γ for 20 h, and apoptotic cells were detected using the CellTiter-Glo[®] Luminescent Cell Viability Assay. n=4 mice. (h) Apoptotic cells detected by TUNEL staining ($\times 200$ magnification) in healthy and acute colitis tissue of WT mice, with quantification on the right. Co-staining of TUNEL and KRT20 ($\times 600$ magnification) was shown on the lower panel. Scale bar: 100 μ m. n=3 mice. (i) H&E staining ($\times 200$ magnification) indicating the disintegrated area in healthy and acute colitis colon tissue of WT and IFN- γ R KO mice; quantification on the right. Scale bar: 100 μ m. n=3 mice. (j) C-CASP3 staining ($\times 200$ magnification) was performed at different time points (3 and 5 days DSS) of DSS colitis samples. Scale bar: 100 μ m. Data are represented as mean \pm SD. The p-values in (a), (b), and (i) were determined using one-way ANOVA (two-sided) with no adjustments for multiple comparisons. The p-values in (d), (g), and (h) were determined using unpaired Student's t-tests (two-sided).

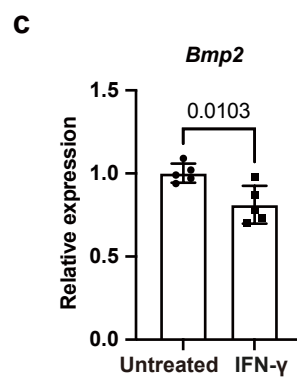
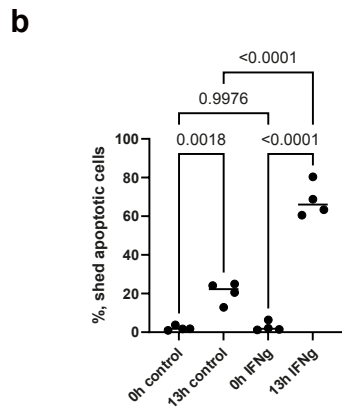
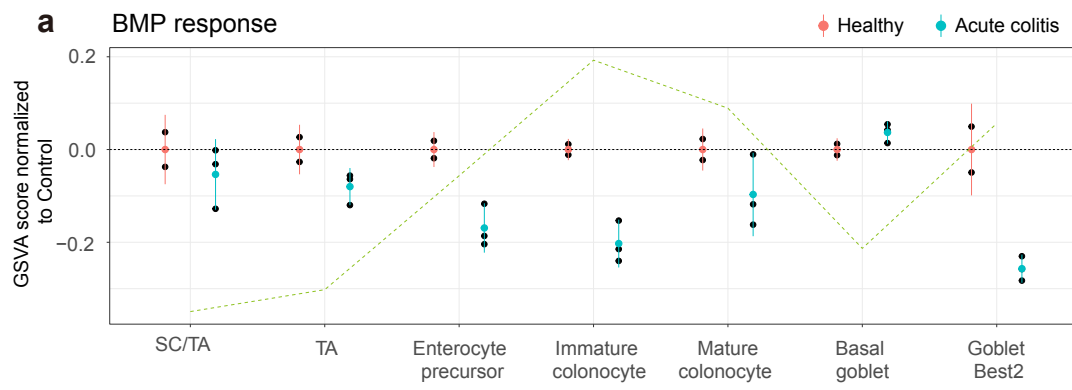


Supplementary Fig.2: IFN- γ -induced colonocytes are distinct from homeostatic colonocytes

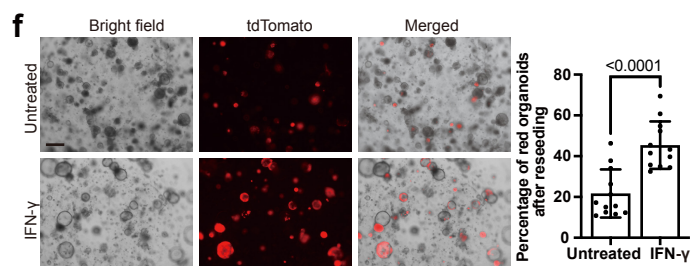
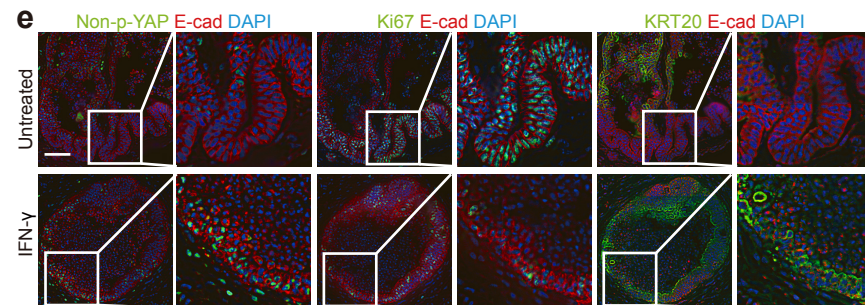
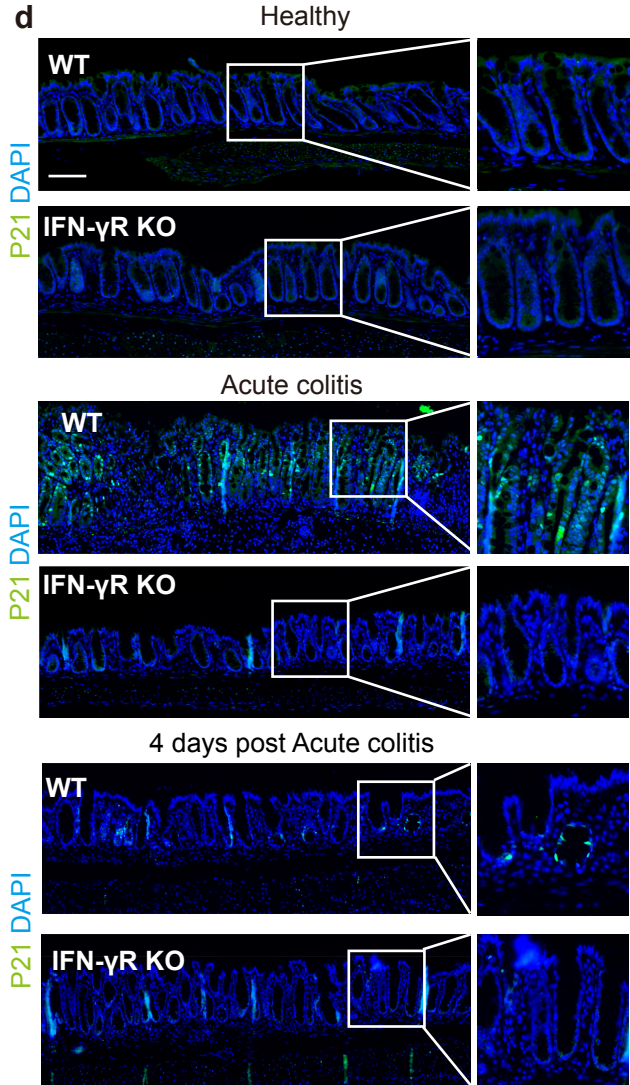
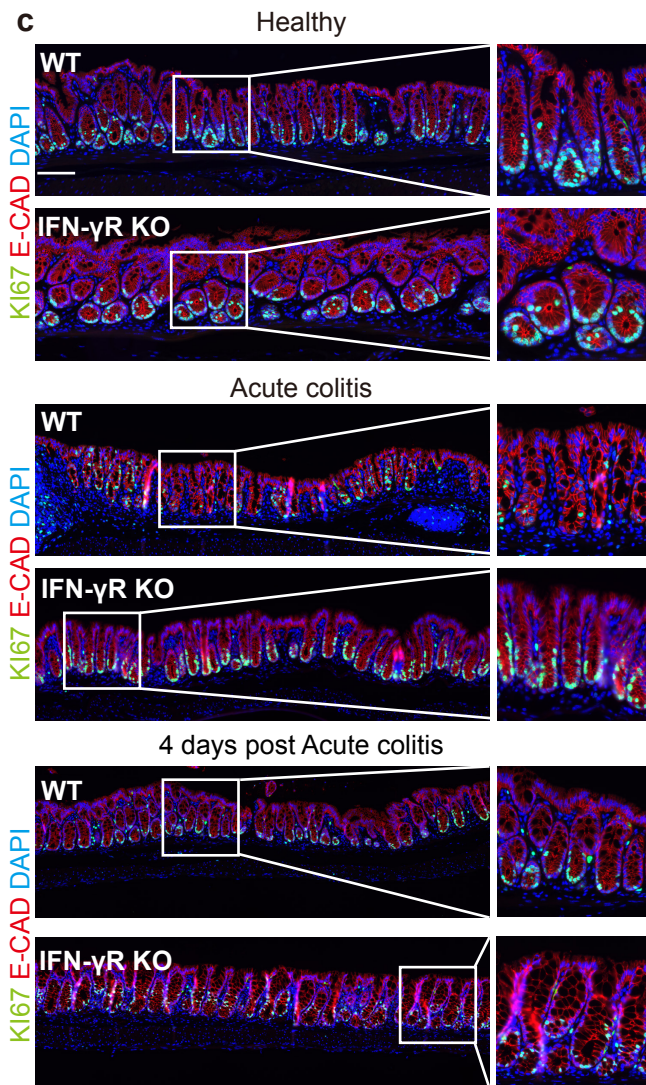
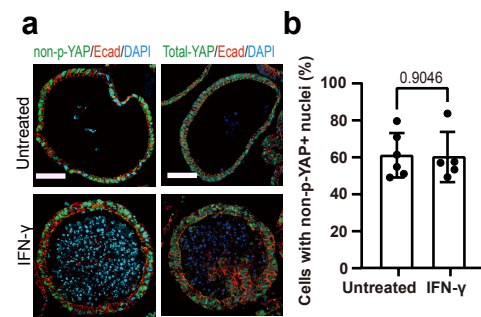
(a) UMAP plot illustrating different cell clusters in sc-RNAseq dataset from untreated progenitors (upper) and IFN- γ treated progenitors (lower). SC: stem cells. TA: Transit-amplifying cells. **(b)** UMAP and Dot plot illustrating the sc-RNAseq dataset from progenitors with or without IFN- γ treatment as well as the expression of known marker genes against detected epithelial cell clusters in FM organoids identified by sc-RNAseq. Circle size represents the within-cluster probability of gene detection, while fill color represents the normalized average expression level. **(c)** mRNA expression of goblet cell markers (*Muc2*, *Math1*, and *Itf*) as assessed by RT-PCR in organoids grown in FM with, without IFN- γ treatment, or in differentiation medium; n = 3 mice. **(d)** qPCR comparing progenitor markers *Axin2* and *Lgr5*. **(e)** Violin plots showing the expression levels of *Krt20* and *Bmp2* in progenitor cells, comparing untreated and IFN- γ -treated conditions. **(f)**, mRNA expression of the differentiation marker *Bmp2* assessed by RT-PCR in organoids grown in FM with or without IFN- γ treatment or in differentiation medium; n = 3 mice. **(g, h)** IF staining ($\times 600$ magnification) illustrating the differential expression Ki67 (proliferation marker) and p21 (cell cycle arrest target) in progenitors, IFN- γ -treated progenitors, and differentiated colonocytes. Scale bars: 40 μ m. **(i)** RT-PCR for the proliferation marker *mKi67* in the indicated groups as in **(c)**; n = 3 mice per group. **(j)** RT-PCR for the cell cycle arrest marker *p21* in the indicated groups as in **(c)**; n = 3 mice per group. (c, d, g, j and k) p-values were determined using one-way ANOVA (two-sided) with no adjustments for multiple comparisons. (All data are represented as mean \pm SD).



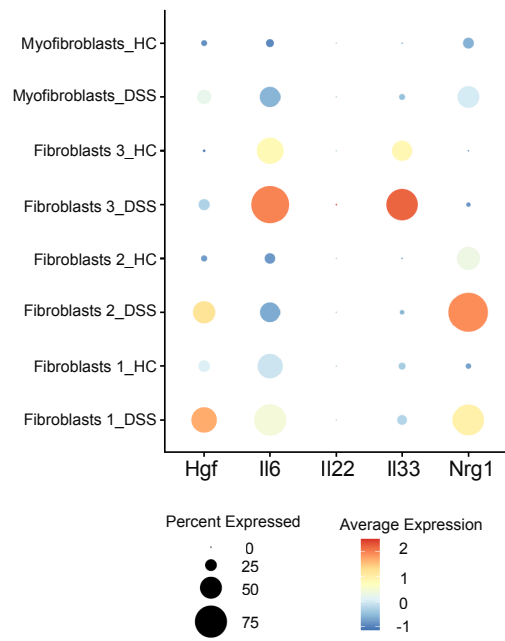
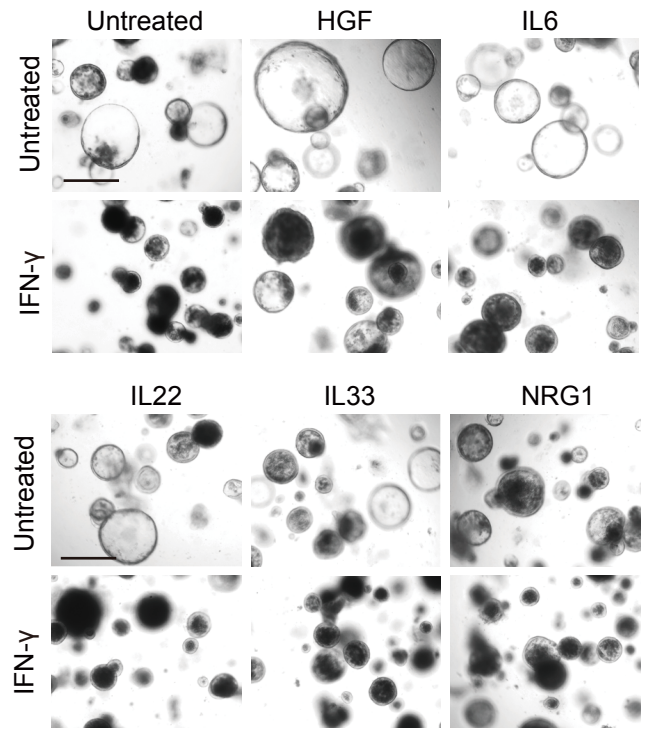
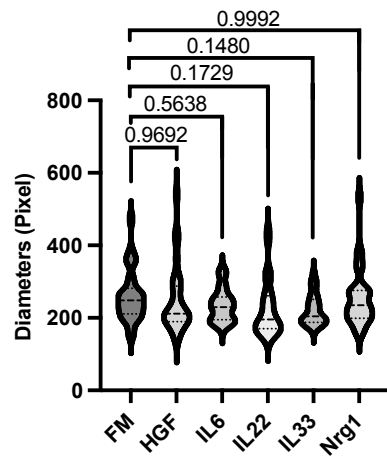
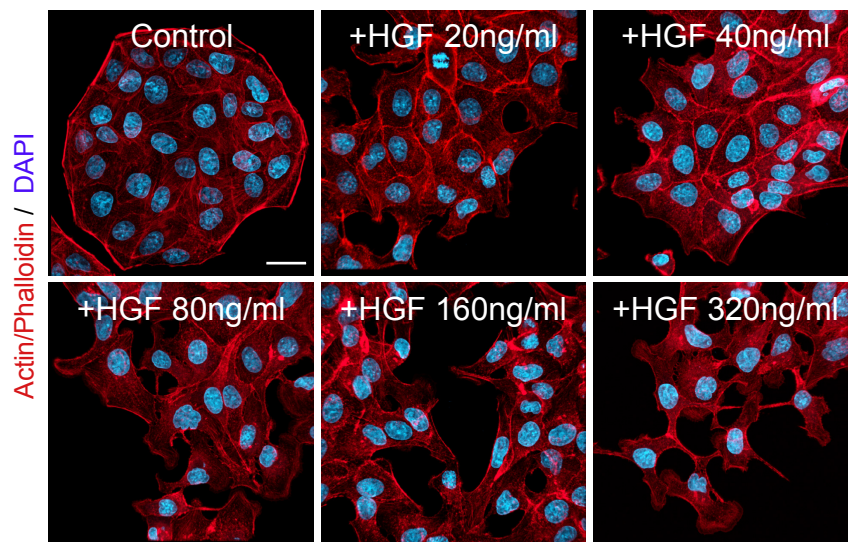
Supplementary Fig.3: IFN- γ decreases the proportion of mature colonocytes in vivo and accelerates the differentiation of progenitors in assembloids, related to Figure 2. (a) Overview images ($\times 200$ magnification) of assembloids stained for KRT20 and E-cadherin in the untreated state (left) and after treatment with IFN- γ (right). Scale bar: 100 μm . (b) Additional images ($\times 600$ magnification) of whole-mount staining of assembloids co-stained with actin/phalloidin derived from *Krt20CreERT2/Rosa26-tdTomato* mice. Krt20 was traced using 800 nM tamoxifen to label the differentiated lineages. Scale bar: 100 μm . (c) Dot blot illustrating the definition of cell populations based on their marker gene expression. SC: stem cells. TA: Transit-amplifying cells. (d) The proportion of enterocyte precursors, immature and mature colonocytes in mice with 6 days of DSS treatment (acute colitis) and their associated controls (healthy), as determined by sc-RNAseq from an online dataset (GSE201723), presented as absolute numbers. Data are represented as mean \pm 2 SEM. $n=2$ control mice, $n=3$ DSS mice. (e) Proliferative cells were labeled with EdU and co-stained with KRT20 and E-cadherin. Images were taken via Observer 7 with $\times 200$ magnification. The scheme of EdU labeling is presented in the upper left panel and quantifications are shown in the right panel. $n=9$ individual assembloids from 3 biological replicates. Scale bar: 100 μm . The p-value was determined using Student's t-test (two-sided). Data are represented as mean \pm SD.



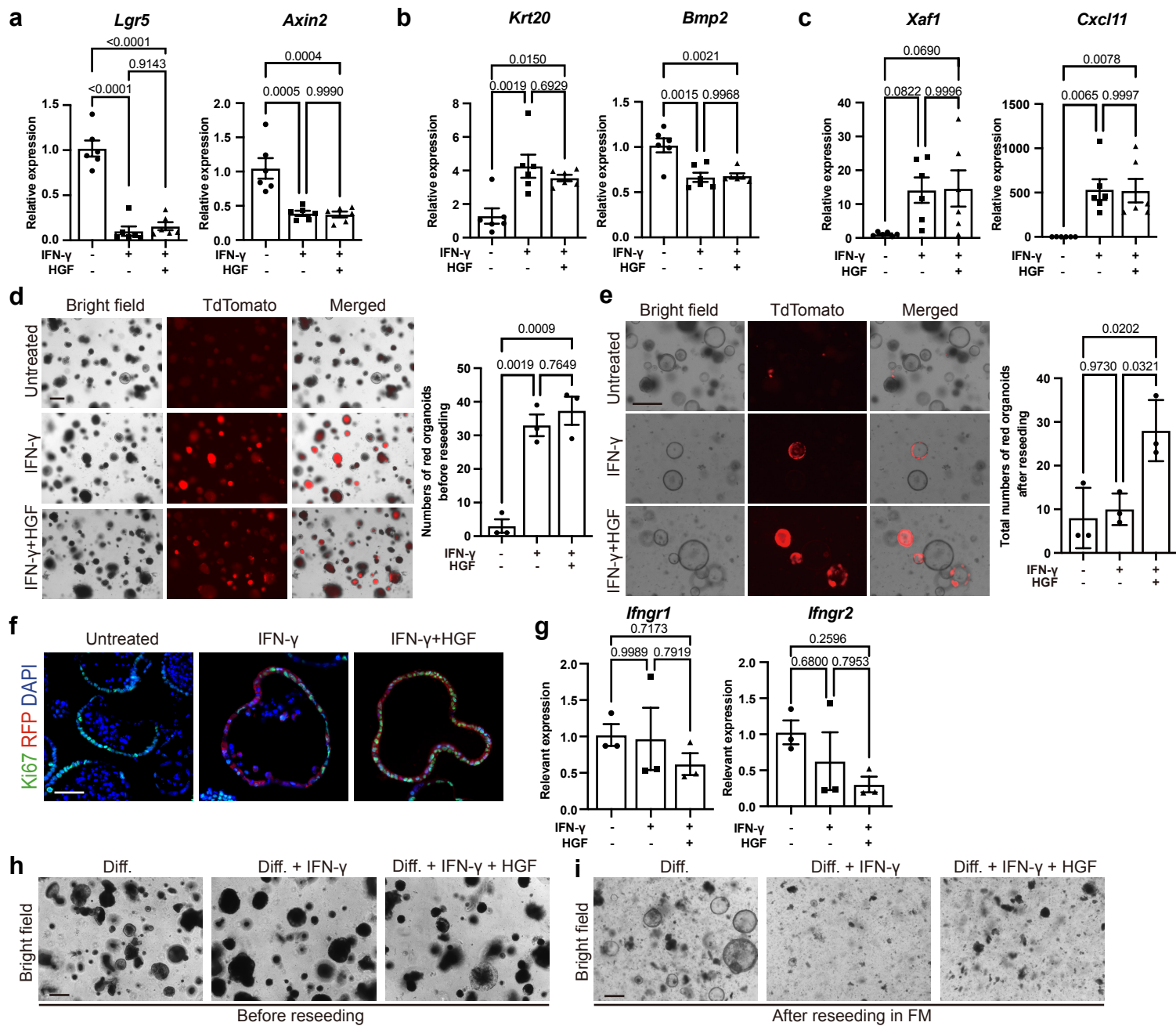
Supplementary Fig.4: BMP signaling is inhibited in colitis and IFN- γ decreases BMP2 expression in assembloids, related to Figure 4. (a) BMP2 coexpressed gene signature activity by cell population upon DSS (acute colitis) *in vivo*, as measured by GSVA enrichment score above the average level in control (healthy) mice from an online sc-RNAseq dataset (GSE201723). Dashed line indicates average control level for each population. SC: stem cells. TA: Transit-amplifying cells. n=3 mice per group. Data are represented as mean \pm 2 SEM. **(b)** Quantification of apoptotic and shed cells of control and IFN- γ treated organoids, p-values were determined using one-way ANOVA (two-sided) with no adjustments for multiple comparisons. n=4 mice per group. **(c)** qRT-PCR of *Bmp2* in assembloids with or without IFN- γ treatment. n=5 mice. The p-value was determined by Student's t-test (two-sided). Data are represented as mean \pm SD.



Supplementary Fig.5: IFN- γ inhibits colonic epithelial proliferation and induces cell cycle arrest, related to Figure 5. (a) IF staining ($\times 600$ magnification) of non-phosphorylated YAP (non-p-YAP) and total YAP was performed in progenitor organoids with IFN- γ treatment and non-p-YAP was quantified **(b)**; Scale bar: 50 μm ; Control: n=6 mice; IFN- γ -treated: n=5 mice. **(c)** Proliferative activity was assessed by Ki67 via IF staining ($\times 200$ magnification) in healthy control, acute colitis, and repair phase of WT and IFN- γ R KO mice. Scale bar: 100 μm . **(d)** Cell cycle arrest was evaluated by p21 staining via IF ($\times 200$ magnification) in healthy control, acute colitis, and repair phase of WT and IFN- γ R KO mice. Scale bar: 100 μm . **(e)** The effect of IFN- γ on non-p-YAP, Ki67, and KRT20 was visualized using multiplex IF staining ($\times 200$ magnification) in assembloids. Scale bar: 100 μm . **(f)** Regrowth of organoids ($\times 200$ magnification) in FM derived from KRT20-traced assembloids, either untreated or treated with IFN- γ , originating from *Krt20CreERT2/Rosa26-tdTomato* mice, accompanied by quantification of the percentage of KRT20-traced organoids. n=3 mice. Scale bar: 100 μm . All p-values were determined by Student's t-test (two side). Data are represented as mean \pm SD.

a**b****c****d**

Supplementary Fig.6: Growth factors (HGF, IL-6, IL-22, IL-33, and Nrg1) are overexpressed in DSS-induced colitis but do not much affect the growth of colonic organoids in FM, related to Figure 6. (a) Single-cell RNA sequencing analysis of alterations in the expression of healing factors in stromal cells in healthy and colitis conditions obtained from an online data set (GSE114374). **(b)** Images (×200 magnification) of progenitor organoids treated with various growth factors (HGF, IL-6, IL-22, IL-33, and NRG1) in FM, and quantifications of organoids diameters were shown in **(c)**. n=30 organoids from 3 biological replicates. Scale bar: 1 mm. The p-values were determined using one-way ANOVA (two-sided) with no adjustments for multiple comparisons. **(d)** MDCK-scatter assay (×600 magnification) to validate the activity of self-produced HGF used at the indicated concentrations for 24 h. MDCK stained with actin-phalloidin and DAPI; scale bar: 25 μm. Data are represented as mean ± SD.



Supplementary Fig.7: Evaluation of the combined effect of HGF and IFN- γ treatment on progenitor and differentiated colonic cells, related to Figure 7. (a) qPCR for expression of the stem cell marker *Lgr5* and the WNT-target gene *Axin2* in progenitors treated with IFN- γ or IFN- γ + HGF. n=3 mice. **(b)** qPCR for expression of *Krt20* and *Bmp2* in progenitors treated with IFN- γ or IFN- γ + HGF. n=6 mice. **(c)** qPCR for expression of the IFN- γ targets *Xaf1* and *Cxcl11* in progenitors treated with IFN- γ or IFN- γ + HGF. n=6 mice. **(d)** Images of KRT20 tracing in organoids derived from *Krt20CreERT2/Rosa26-tdTomato* mice treated with IFN- γ or IFN- γ + HGF together with 800 nM 4OHT-tamoxifen for 48 h, with the number of traced organoids quantified. Scale bar: 100 μ m. n=3 mice. **(e)** Organoids from **(d)** were reseeded into FM. Images of regrowing organoids ($\times 200$ magnification) are shown and numbers of KRT20-traced organoids quantified. Scale bar: 100 μ m. n=3 mice. **(f)** Co-staining of KI67 and RFP ($\times 200$ magnification) in organoids harvested from **(e)** to evaluate proliferation of KRT20-traced cells. Scale bar: 100 μ m. **(g)** qPCR for expression of IFN- γ receptors, *Ifngr1* and *Ifngr2*, in progenitors treated with IFN- γ or IFN- γ + HGF. n=3 mice. **(h)** Brightfield images ($\times 200$ magnification) of differentiated organoids (Diff.) with IFN- γ or IFN- γ + HGF treatment for 48 h, followed by reseeded into FM **(i)**; Scale bar: 100 μ m. All p-values were determined using one-way ANOVA (two-sided) with no adjustments for multiple comparisons. Data are represented as mean \pm SD.

a



Supplementary Fig.8: Purification of HGF produced in CHO Lec3.2.8.1 cells. (a) Elution from HGF/SF from MonoS GL column Sample pooled were F17 to F36 range from 620 mM to 770 mM NaCl, demonstrating high purity of produced tc-HGF.