

The endogenous peptide GPR15L shapes the intestinal microbiota to counteract colitis

- Supplemental data -

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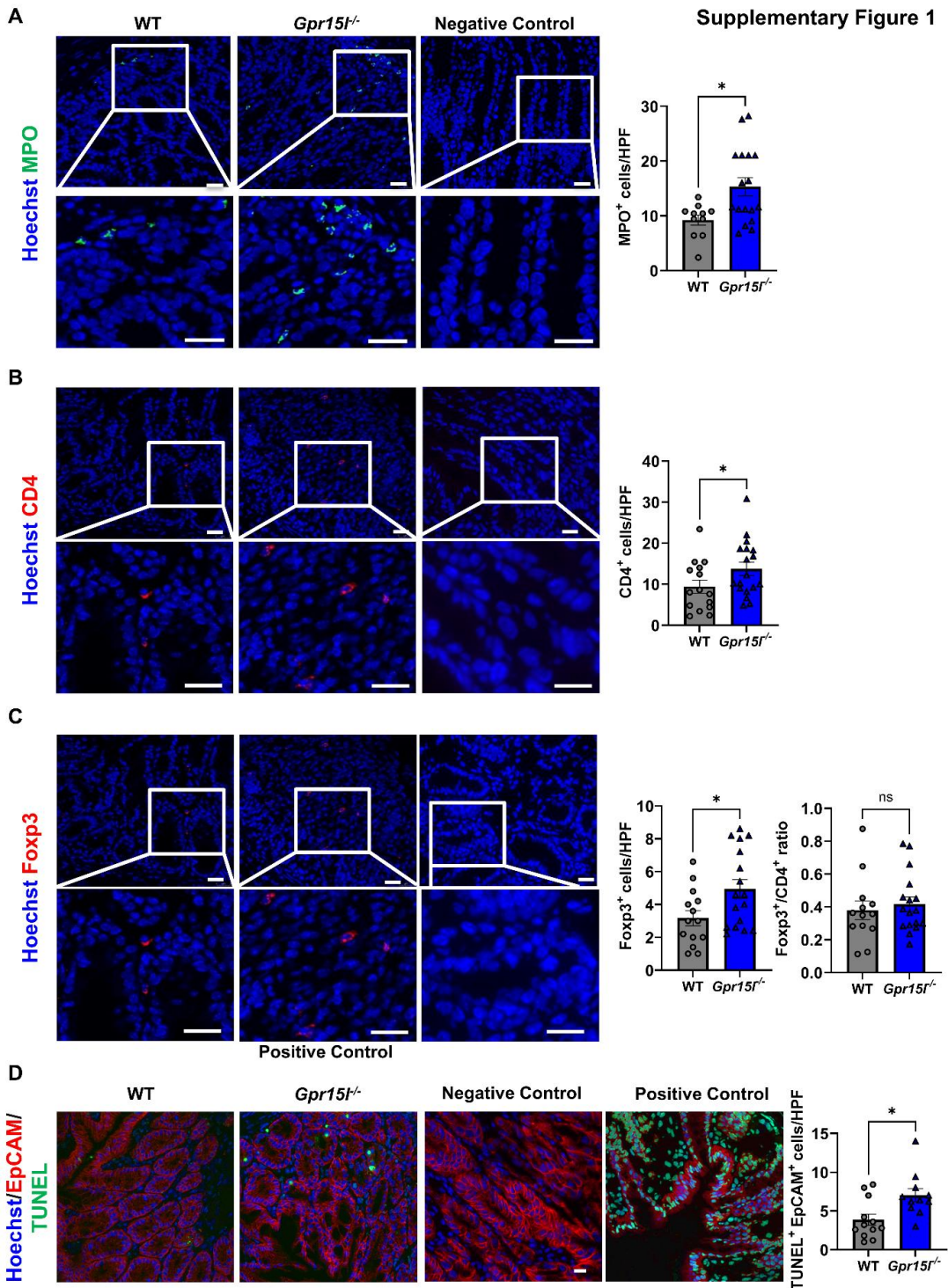
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Supplementary Figures 1-11

Supplementary Tables 1-6

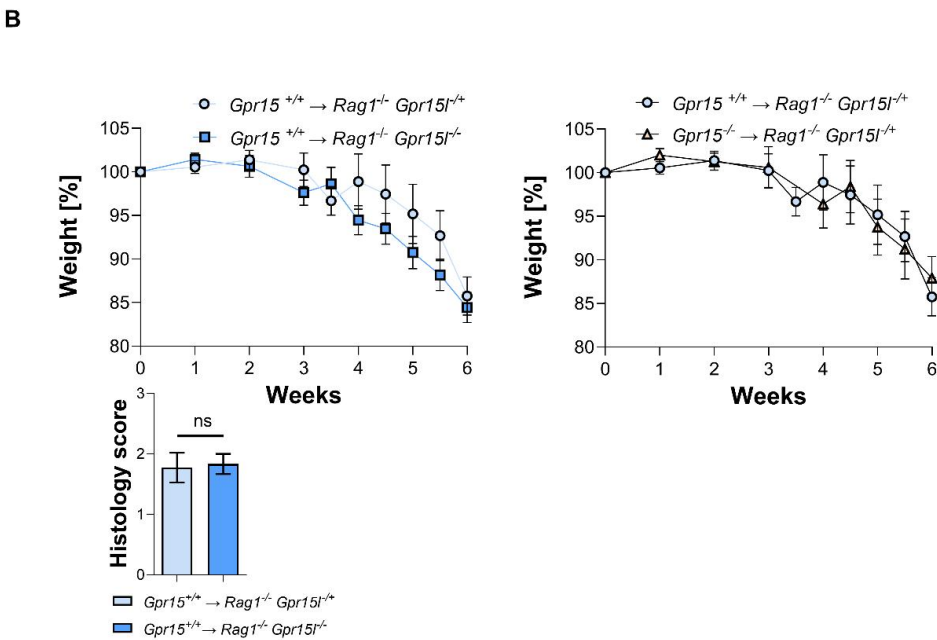
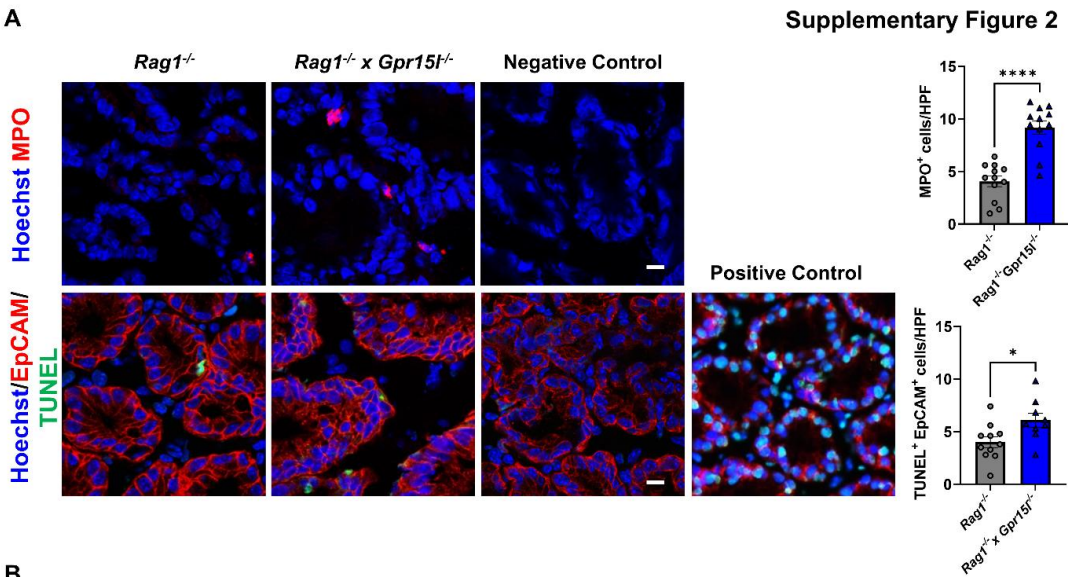


Supplementary Figure 1

Representative (left panels) and quantitative (right panels) immunofluorescence on large intestinal samples from *Gpr15l*^{-/-} and wild-type (WT) mice with acute dextran sodium sulfate colitis: **A** Myeloperoxidase (MPO); **B** CD4; **C** Foxp3, also showing the ratio of Foxp3⁺/CD4⁺ cells; **D** EpCAM and TUNEL (apoptotic cells).

n = 11-17 per group. Scale bars: 25 µm (MPO+, CD4⁺ T, Foxp3+), 10 µm (TUNEL).

*p < 0.05; ns – not significant.



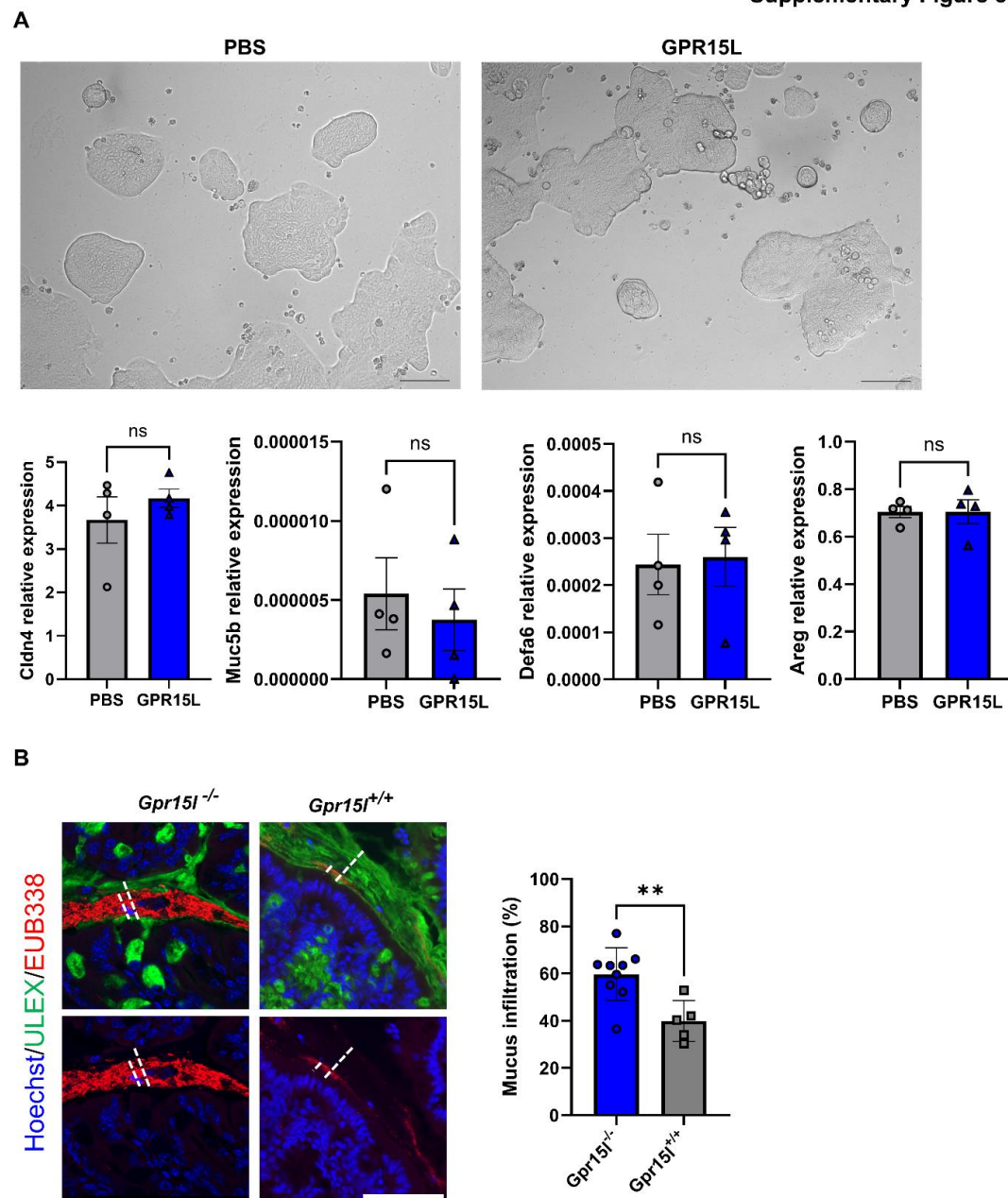
Supplementary Figure 2

A Representative (left panels) and quantitative (right panels) immunofluorescence on large intestinal samples from *Rag1*^{-/-} and *Rag1*^{-/-}*Gpr15*^{-/-} mice with acute dextran sodium sulfate colitis for Myeloperoxidase (MPO) and EpCAM with TUNEL (apoptotic cells) as indicated. n = 9 - 12 per group; Scale bars:10 µm.

B T cell transfer colitis model with *Gpr15*-proficient or -deficient donor CD4⁺CD45RB^{high} T cells adoptively transferred to *Rag1*^{-/-}*Gpr15*^{+/+} or *Rag1*^{-/-}*Gpr15*^{-/-} littermate mice. Upper panels: Weight course of mice transferred with *Gpr15*^{+/+} T cells (left) and of *Rag1*^{-/-}*Gpr15*^{-/-} recipients (right). Lower panel: Comparison of histologic disease activity in mice transferred with *Gpr15*^{+/+} T cells.

*p < 0.05; ****p<0.0001; ns – not significant.

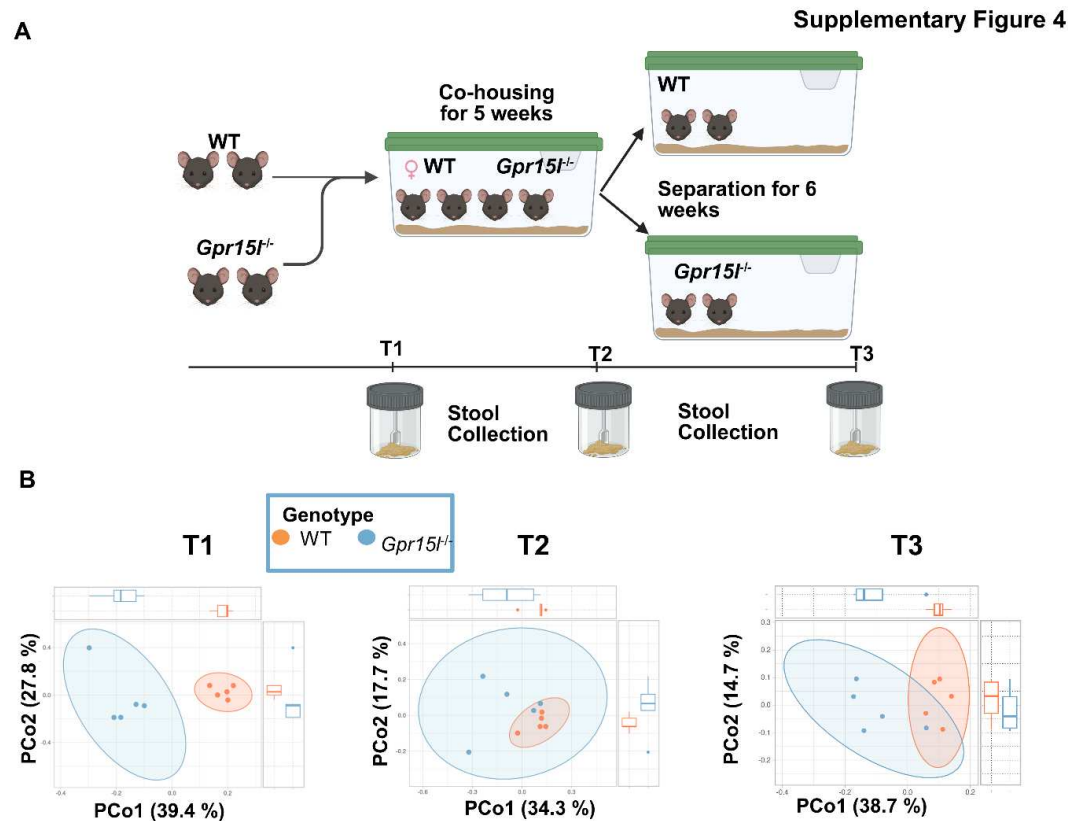
Supplementary Figure 3



Supplementary Figure 3

A 2D cultures of large intestinal epithelial organoids from wild-type mice treated with or without GPR15L for 48 hours. Upper panels: Representative microscopy of organoids after culture. Lower panels: QPCR of the expression of the indicated genes in organoids treated as indicated. n = 4 per group. n.s. – not significant. Scale bars: 100 μ m.

B Representative (left panels) and quantitative (right panel) fluorescence in situ hybridization (FISH) of colon tissue from *Gpr15l*^{-/-} and WT mice with acute DSS colitis. Staining for nuclei (Hoechst, blue), mucus (ULEX, green) and bacterial rRNA (EUB388, red). Quantitative analysis of the relative depth of bacterial mucus layer infiltration. n= 5-9 per group. ** p < 0.01.



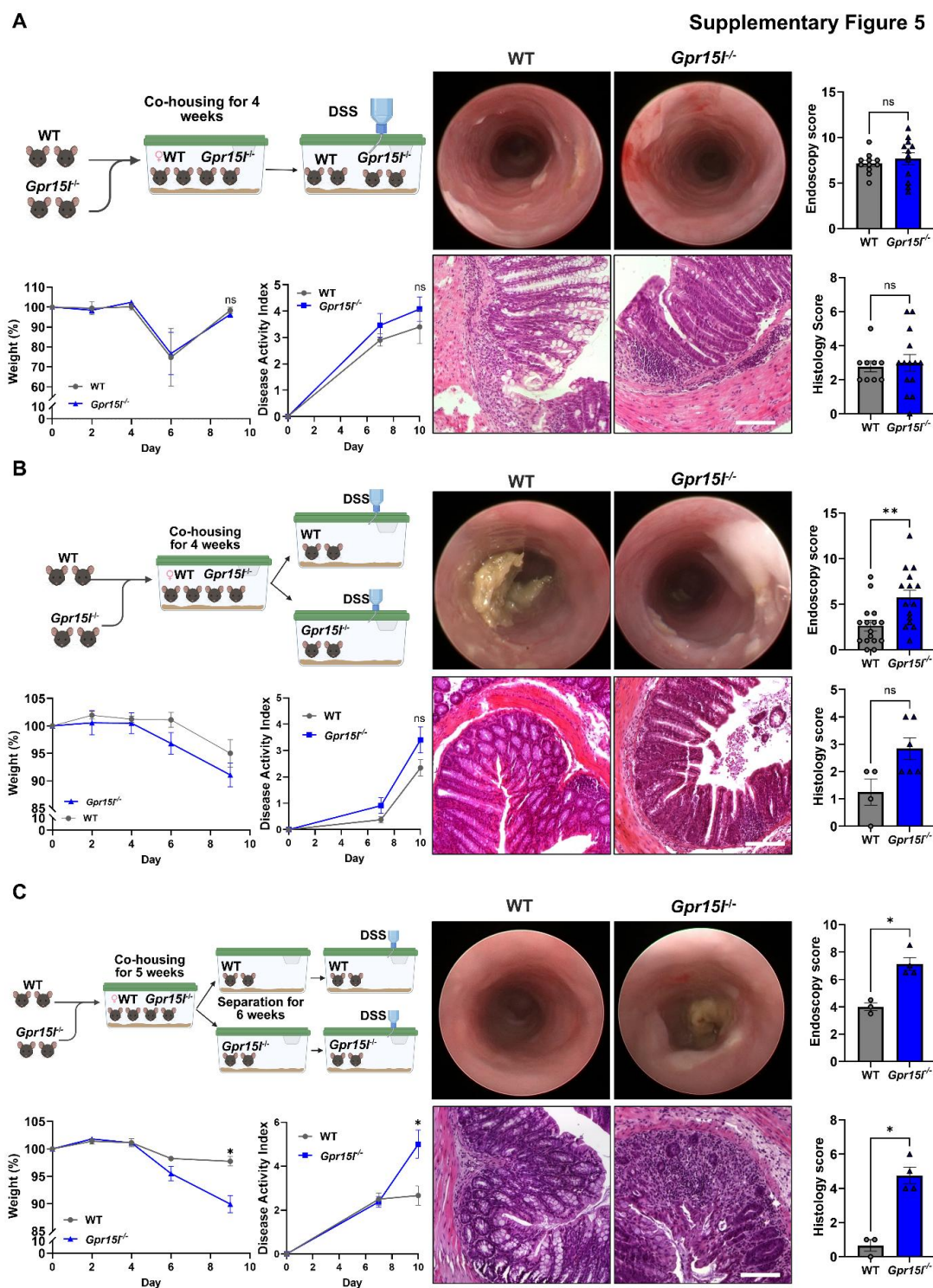
Supplementary Figure 4

Co-housing/re-separation and fecal microbiome in wild-type and *Gpr15^{-/-}* mice.

A Schematic representation of design of the experimental design.

B Microbiome analysis using 16S rRNA gene sequencing. Beta diversity in fecal samples collected at time point 1 (T1, before co-housing, $p = 0.007$), time point 2 (T2, after 5 weeks of co-housing, $p = 0.03$), and time point 3 (T3, after 6 weeks of genotype-specific re-separation, $p = 0.009$) as indicated. Beta diversity was measured using Bray–Curtis dissimilarity.

Alpha diversity in fecal samples at T1, T2 and T3 as indicated.



Supplementary Figure 5

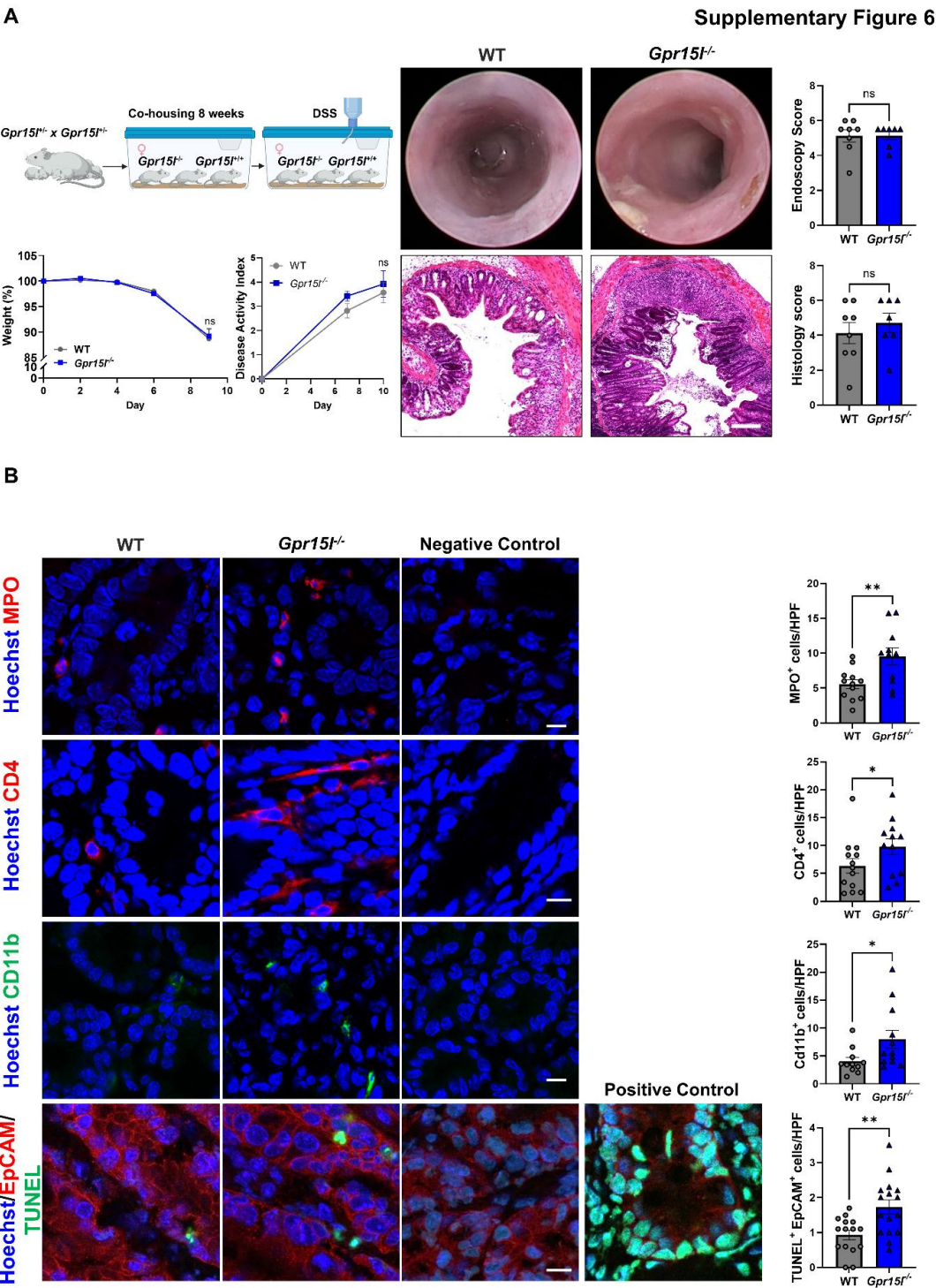
Co-housing/re-separation and phenotype of acute dextran sodium sulfate colitis (DSS) in wild-type and *Gpr15l*^{-/-} mice.

A Upper left panel: Schematic representation of the experimental design (4 weeks co-housing and subsequent DSS colitis. Lower left panels: Weight course and clinical disease activity. Right upper panels: Representative and quantitative endoscopic disease activity. Right lower panels: Representative and quantitative histologic disease activity. n = 9-14 per group.

B Upper left panel: Schematic representation of the experimental design (4 weeks co-housing and subsequent re-separation with direct induction of DSS colitis. Lower left panels: Weight course and clinical disease activity. Right upper panels: Representative and quantitative endoscopic disease activity. Right lower panels: Representative and quantitative histologic disease activity. n = 4-16 per group.

C Upper left panel: Schematic representation of the experimental design (4 weeks co-housing and subsequent re-separation for six weeks before induction of DSS colitis. Lower left panels: Weight course and clinical disease activity. Right upper panels: Representative and quantitative endoscopic disease activity. Right lower panels: Representative and quantitative histologic disease activity. n = 3-4 per group.

Scale bars: 100 μ m. *p < 0.05; **p < 0.01; ns – not significant.

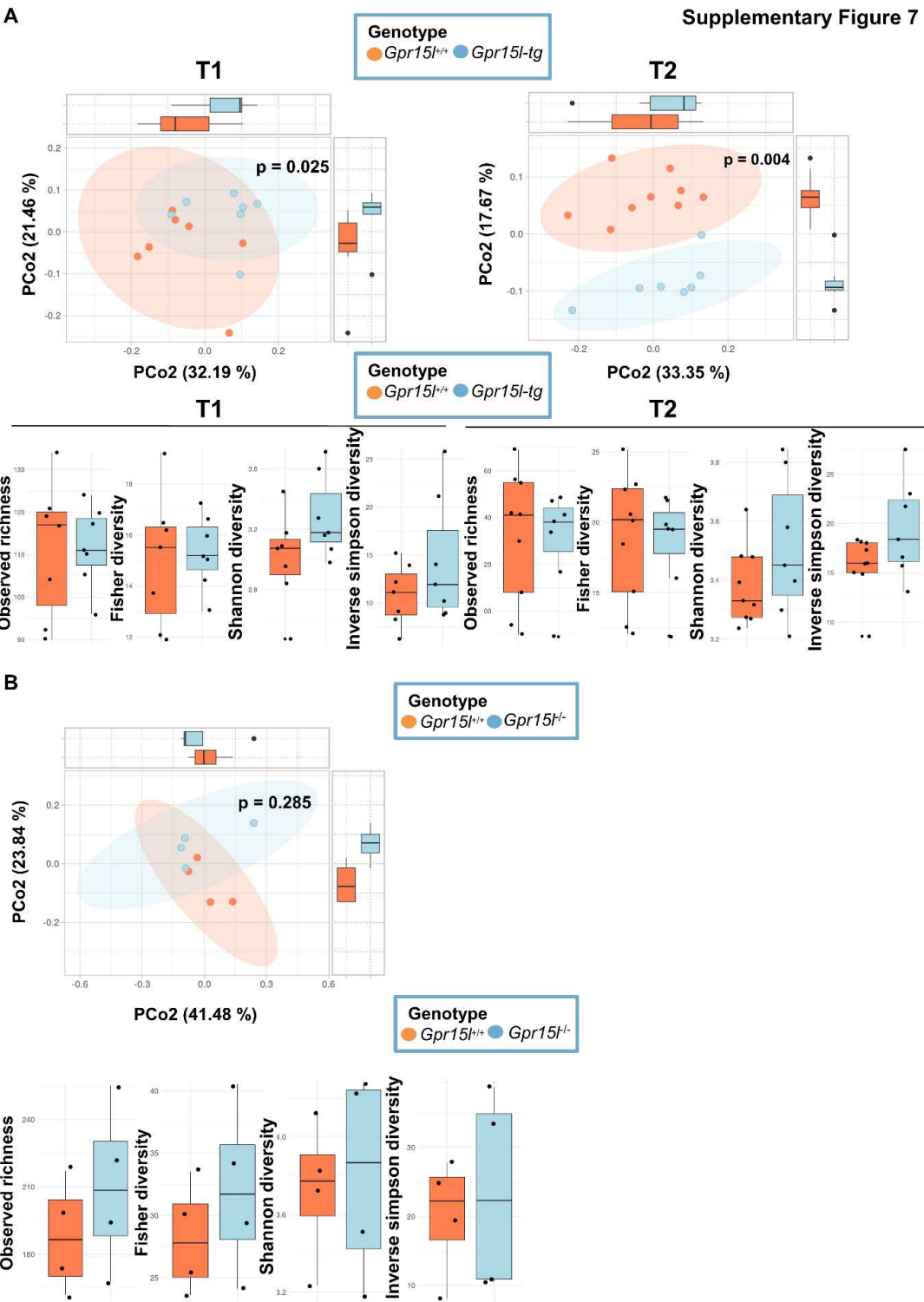


Supplementary Figure 6

A Acute dextran sodium sulfate (DSS) colitis in co-housed *Gpr15^{-/-}* and littermate *Gpr15^{+/+}* mice. Upper left panel: Schematic representation of the experimental design. Lower left panels: Weight course and Disease Activity Index. Right upper panels: Representative and quantitative endoscopic disease activity. Right lower panels: Representative and quantitative histologic disease activity. n = 7-8 per group. Scale bars: 100 μ m.

B Representative (left panels) and quantitative (right panels) immunofluorescence on large intestinal samples from *Gpr15^{-/-}* and littermate *Gpr15^{+/+}* mice with DSS colitis induced after separation for eight weeks: **A** Myeloperoxidase (MPO); **B** CD4; **C** CD11b; **D** EpCAM and TUNEL (apoptotic cells). n = 15-16 per group. Scale bars: 10 μ m.

*p < 0.05; **p < 0.01; ns – not significant.

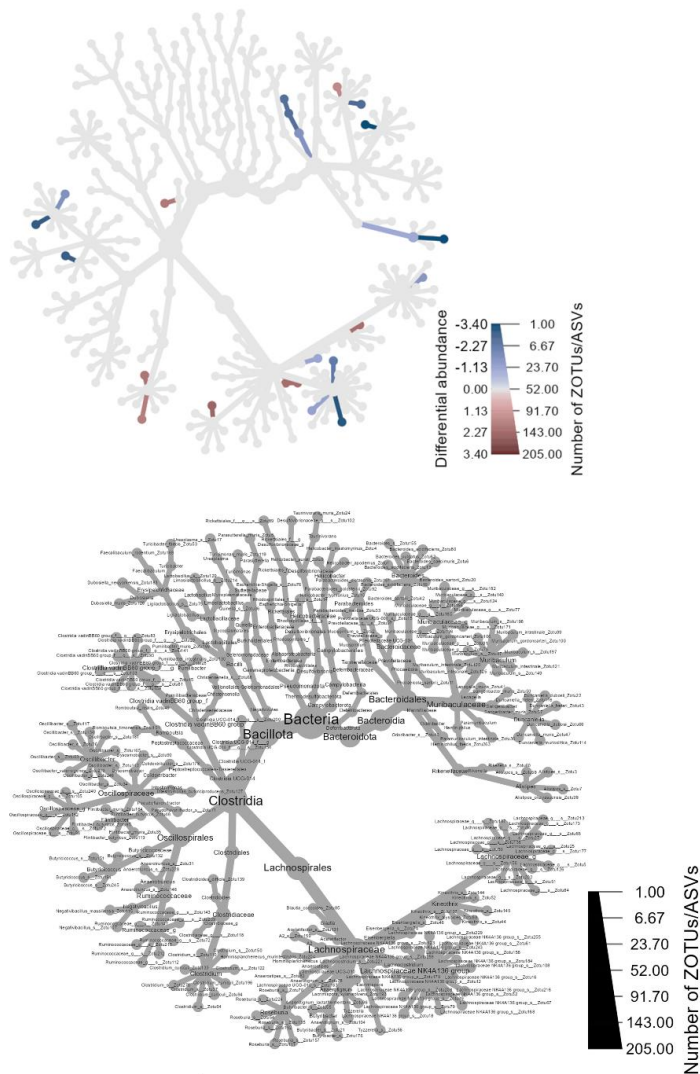


Supplementary Figure 7

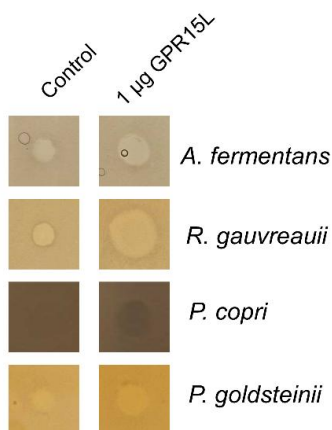
A-B Fecal microbiome in **(A)** *Gpr15l*-transgenic and littermate controls at weaning (T1) and after eight weeks of genotype-specific separation (T2) and in **(B)** co-housed *Gpr15l*^{-/-} and littermate *Gpr15l*^{+/+} mice before initiation of DSS colitis. Upper panels: Beta diversity (Bray-Curtis dissimilarity); lower panels: Alpha diversity (measures as indicated).

Supplementary Figure 8

A



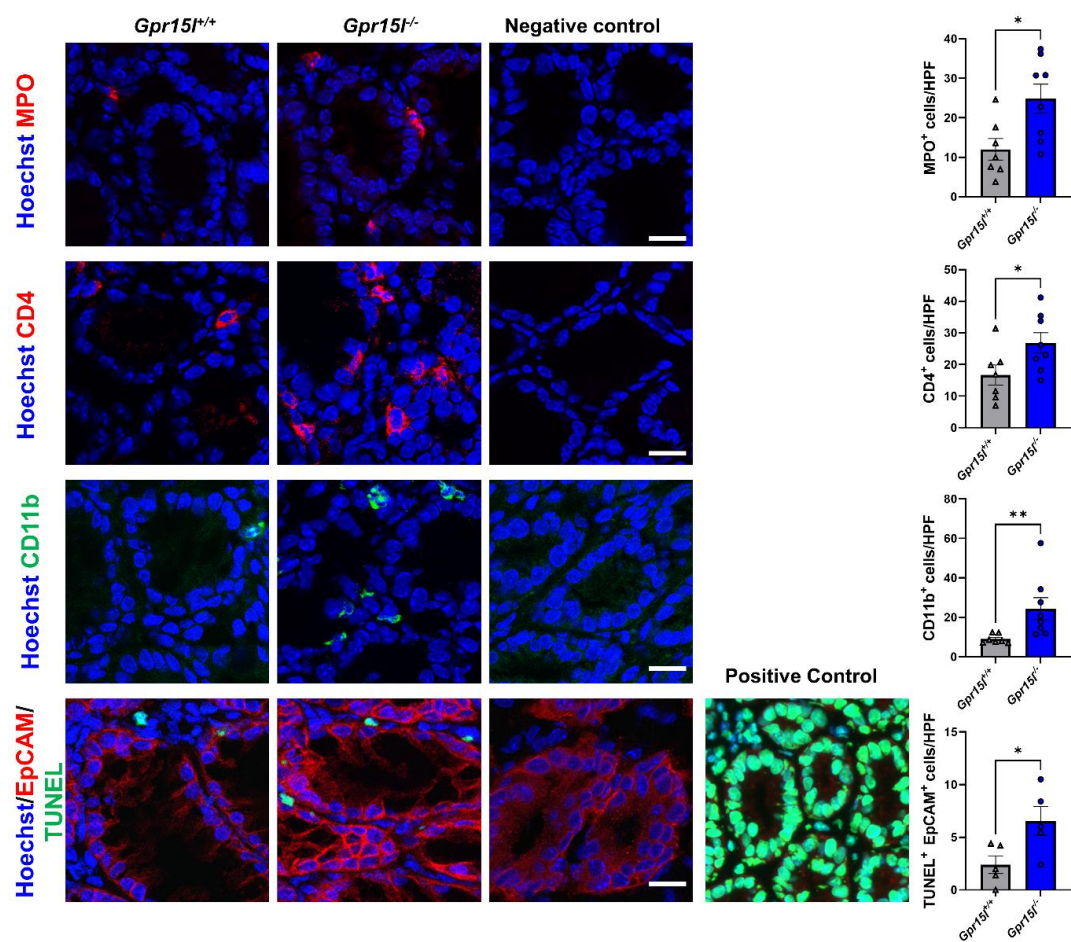
B



Supplementary Figure 8

16S rRNA sequencing of the fecal microbiome of littermate Gpr15l^{-/-} and Gpr15l^{+/+} mice after eight weeks of genotype-specific separation. **A** Differential abundance (Limma (voom)) illustrated in a taxonomy tree. **B** Radial diffusion assays with selected intestinal bacteria and with or without GPR15L treatment under anaerobic conditions. Representative analysis of antimicrobial activity of GPR15L.

Supplementary Figure 9

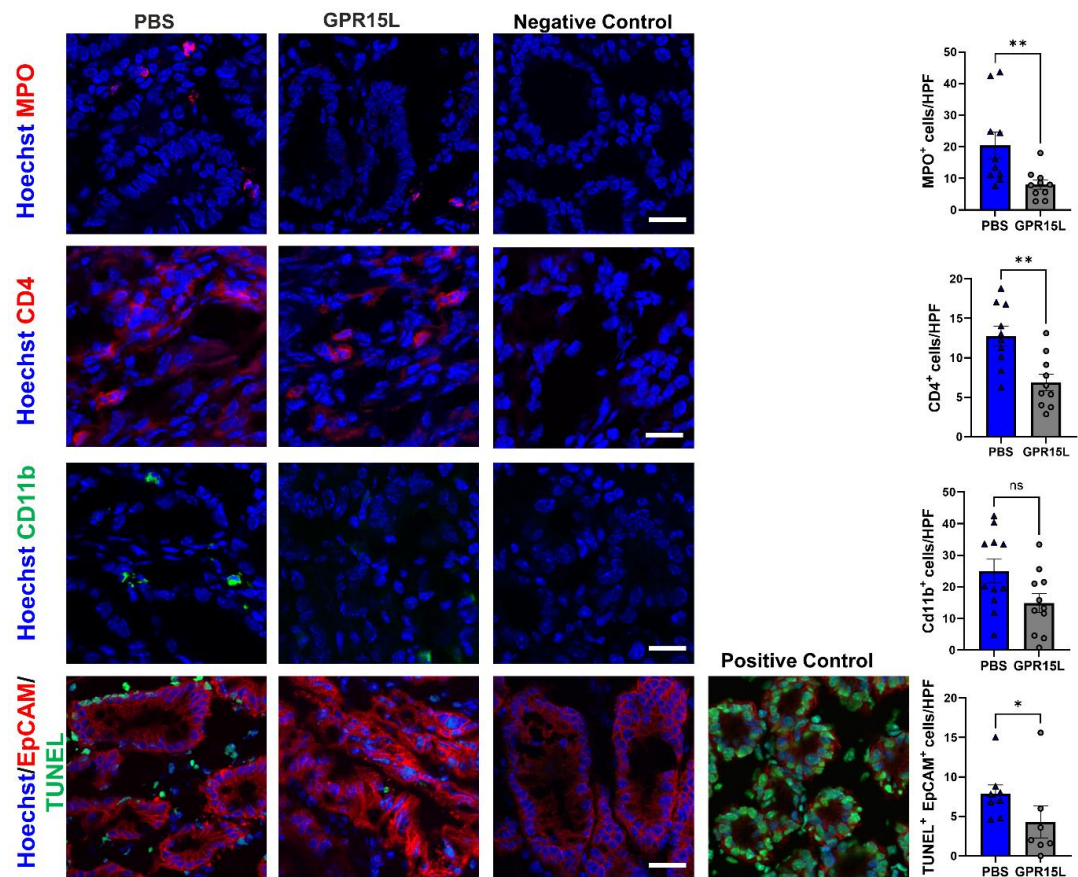


Supplementary Figure 9

Representative (left panels) and quantitative (right panels) immunofluorescence on large intestinal samples from WT mice with acute dextran sulfate colitis after broad-spectrum antibiotic treatment and fecal microbiota transfer from *Gpr15*^{-/-} or *Gpr15*^{+/+} mice: Myeloperoxidase (MPO), CD4, CD11b and EpCAM with TUNEL (apoptotic cells) as indicated. n = 5-8 per group.

Scale bars: 10 μ m. *p < 0.05, ** p < 0.01.

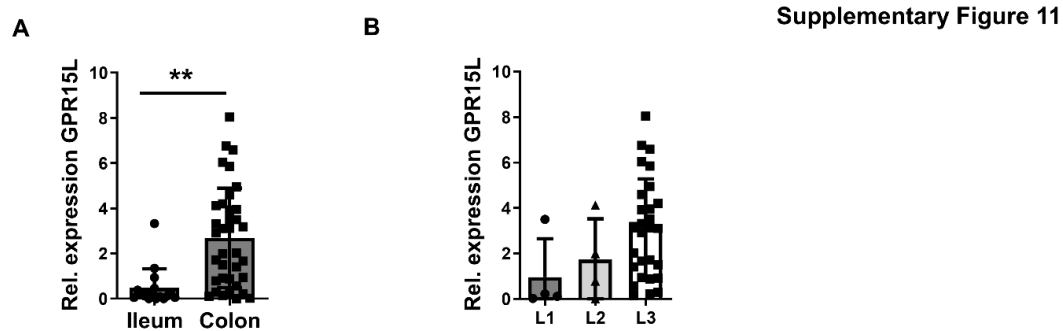
Supplementary Figure 10



Supplementary Figure 10

Representative (left panels) and quantitative (right panels) immunofluorescence on large intestinal samples from *Gpr15*^{-/-} with DSS colitis and treated with recombinant GPR15L or placebo: **A** Myeloperoxidase (MPO); **B** CD4; **C** CD11b; **D** EpCAM and TUNEL (apoptotic cells). n = 8-11 per group.

Scale bars: 10 μ m. *p < 0.05; **p < 0.01; ns – not significant.



Supplementary Figure 11

A QPCR analysis of *GPR15L* mRNA expression in colon (n = 36) and ileum (n = 16) biopsies from patients with Crohn's disease. **B** QPCR analysis of *GPR15L* mRNA expression in colon biopsies from patients with CD stratified according to the L category of the Montreal classification (L1, ileitis; L2, colitis; L3, ileocolitis; n = 4-28 per group).

** p < 0.01.

Supplementary Table 1: Characteristics of the donors for *Fig. 6E-G*

Number	7
Age (Ø, range)	37 (29 – 71)
Female [%]	71

Supplementary Table 2: Characteristics of the donors for *Fig. 7A,B* and

Supplementary Figure 11

	Non-IBD	CD	UC
Number	20	52	64
Age (Ø, range)	64 (27 – 86)	39 (17 – 72)	40 (20 – 74)
Female [%]	37	52	34
Endoscopic disease activity [%]		48	52
Disease localization [%]		L1: 15	E1: 2
		L2: 8	E2: 28
		L3: 77	E3: 70
		L4: 0	
		L4+: 33	

Supplementary Table 3: Characteristics of the donors for *Fig. 7C*

	Non-IBD	CD	UC
Number	13	7	14
Age (Ø, range)	62 (47 – 85)	38 (26 – 50)	35 (20 – 66)
Female [%]	15	57	57
Clinical disease activity [%]		100	100
Disease localization [%]		L1: 14	E1: 0
		L2: 14	E2: 14
		L3: 58	E3: 86
		L4: 0	
		L4+: 14	

Supplementary Table 4: Characteristics of the donors for *Fig. 7D,E*

	UC
Number	30
Age (Ø, range)	43 (22 – 67)
Female [%]	27
Clinical disease activity [%]	53
Disease localization [%]	E1: 4
	E2: 15
	E3: 81

Supplementary Table 5: Characteristics of the donors for *Fig. 7F*

	UC
Number	61
Age (Ø, range)	42 (19-79)
Female [%]	46
Clinical and/or endoscopic disease activity [%]	38
Disease localization [%]	E1: 10
	E2: 44
	E3: 46

Supplementary Table 6: Characteristics of the donors for *Fig. 7G*

Patient #	Diagnosis	Gender	Age [y]	Disease duration [y]	Concomitant treatment	Disease localization
1	UC	m	58	8.1	5-ASA, anti-TNF	E3
2	UC	m	58	3.0	5-ASA, steroids	E3
3	UC	m	50	4.5	5-ASA, budesonide	E3
4	UC	m	32	5.3	Thiopurine, steroids	E3
5	UC	f	29	1.9	Thiopurine	E3
6	UC	m	74	6.8	5-ASA, steroids	E3
7	UC	f	64	37.6	5-ASA, thiopurine	E3
8	UC	m	24	11.2	anti-TNF	E3
9	UC	m	25	2.6	5-ASA, thiopurine, steroids	E3
10	UC	m	39	6.6	5-ASA, steroids	E3
11	UC	m	49	26.4	5-ASA, anti-TNF	E3
12	UC	f	37	6.1	5-ASA, thiopurine	E3
13	UC	m	24	6.8	5-ASA, thiopurine, steroids	E1
14	UC	m	38	2.4	5-ASA, thiopurine	E2
15	UC	f	64	2.3	anti-TNF, methotrexate	E3
16	UC	m	54	23.4	anti-TNF	E2
17	UC	m	40	19.1	5-ASA	E3
18	UC	f	39	17.1	5-ASA, anti-TNF	E2
19	UC	m	50	27.0	5-ASA, anti-TNF	E2
20	UC	m	40	14.8	5-ASA, anti-TNF	E3
21	UC	m	51	27.7	5-ASA, anti-TNF	E3
22	UC	m	42	8.6	Vedolizumab	E2
23	UC	f	19	6.2	anti-TNF	E3
24	UC	m	36	6.8	Vedolizumab	E3
25	UC	m	22	3.6	-	E3
26	UC	m	64	2.2	Vedolizumab	E3
27	UC	f	24	2.8	Vedolizumab, 5-ASA	E2
28	UC	m	31	26.7	Vedolizumab	E2
29	UC	f	54	12.2	Vedolizumab	E3