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Supplemental Information

Distinct Microbial Communities Trigger Colitis

Development upon Intestinal Barrier Damage

via Innate or Adaptive Immune Cells

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Supplemental information

Supplemental experimental procedures:

Microbiota manipulation: Alteration of microbiota composition was conducted by cohousing or fecal transplantation (Thiemann et al., 2017). For cohousing both recipient and donor mice were cohoused at least 4 weeks prior starting experiment. For fecal transplantation donor mice were euthanized, intestinal content was collected in BBL thioglycollate media (BD Bioscience) and homogenized by vortexing. To remove coarse particle under anaerobic conditions the content was filtered through 70µm sterile filter. After centrifugation (10min, 500g, 4°C), the pellet containing fecal bacteria was resuspended in BHI medium (Sigma-Aldrich). After 2-hr of starving recipient mice were orally gavaged with a total 200µl of fecal bacterial content. Again a 4 week time period was given for a successful establishment of fecal transplanted bacteria (unless mentioned otherwise). Every microbiota manipulation was further confirmed by 16S rRNA sequencing of fecal bacteria.

Histology: At day 5 of DSS-treatment colon samples were collected, rolled up to “swiss roles”, fixed in 4% neutrally buffered formaldehyde and embedded in paraffin according to standard histological procedures. Sections of 3µm thickness were stained with hematoxylin-eosin (HE) and evaluated by light microscopy blinded to the experimental groups.

The histological scoring used to evaluate the severity of colitis in DSS treated mice microscopically, was adapted from the TJL-score, which was developed for scoring colitis in mice by The Jackson Laboratory (Mähler et al., 1998). The alteration of the score has been previously described (Pils et al., 2010). The colon was divided into a proximal (oral), middle and distal (aboral) section, each of about the same size. The three sections were scored for the general criteria: severity (0-3), ulceration (0-3), oedema (0-3), goblet cell metaplasia (0-3), and area involved (0-3) where score 0 depicted no alteration to score 3 massive alteration in the given parameters.

The scores were added up to a total of up to 15 per section and the scores of the three sections to a total of up to 45 per colon sample.

Colonoscopy: Colonoscopy was performed using a high-resolution mouse video endoscopic system (‘Coloview’, Carl Storz, Tuttlingen, Germany). The severity of colitis was blindly scored using MEICS (Murine Endoscopic Index of Colitis Severity), which is based on five parameters: granularity of mucosal surface (0-3); vascular pattern (0-3); translucency of the colon mucosa (0-3); visible fibrin (0-3); and stool consistency (0-3) (Becker et al., 2007).

Isolation of colonic lamina propria leukocytes (cLPL) and flow cytometry: To isolate cLPL, density gradient centrifugation using Percoll was done as previously described (Weigmann et al., 2007). In brief, colons were collected during steady state and at d5 of DSS treatment. Fecal content was removed, tissues were opened longitudinally, washed with PBS and then shaken in HBSS containing 2 mM EDTA for 20 min at 37°C. Tissues were cut into small pieces and incubated with digestion solution (DMEM containing 1% fetal bovine serum (FBS), 0.25 mg/ml collagenase D, 0.5 U/ml dispase and 5 µg/ml DNase I) in a shaker for 20 min at 37°C. Digested tissues were filtered through 70µm cell strainer (Falcon) and DMEM + 5% FBS was added to inactivate enzymes. The last two steps were repeated until all tissue was digested. After centrifugation, cells were resuspended in 4 ml of 40% Percoll (GE Healthcare) and overlaid on 4 ml of 80% Percoll. Percoll gradient separation was performed by centrifugation at 450 g for 25 min at 25°C. Cells in the interphase were collected and used as LPL. The collected cells were then suspended in staining buffer containing PBS, 1% FBS and 2 mM EDTA. The following antibodies were used: anti-CD45 (30-F11), anti-CD3 (17A2), anti-B220 (RA3-6B2), anti-CD4 (RM4-5, GK1.5), anti-CD8a (53-6.7), anti-TCR-gamma/delta (GL3), anti-CD44 (IM7), anti-CD62L (MEL-14), anti-MHC class II (M5/114.15.2), anti-CD11b (M1/70), anti-CD11c (N418), anti-Ly6G (IA8), anti-Ly6C (HK1.4) (Biolegend). To distinguish live dead cells AlexaFluor-350 NHS Ester (Life Technologies) was used. Flow cytometry analysis was performed using a BD LSR (BD Biosciences) and data were analyzed with FlowJo software (TreeStar Inc.).

In-vitro T cell activation of cLPL: For T cell activation freshly isolated cLPL (200,000/well) were cultured in 96-well round-bottom plates in complete culture medium containing soluble, plate-bound, anti-CD3 (1mg/ml) and soluble anti CD28 (5mg/ml) (Biolegend) for 3 days. Supernatant were collected after 3 days for cytokines and chemokines measurement.

Collection of colonic tissue homogenate: Colonic tissue homogenate was collected from euthanized mice during steady state and d7 colitis. Colons were excised into proximal and distal colon. Each part was cut longitudinally and cleaned by washing with autoclaved 1x PBS. Parts of proximal or distal colon were weighed and homogenized mechanically using Mini-Beadbeater-96 (Biospec) in NP-40 lysis buffer containing protease inhibitors (Complete Mini EDTA-free, Roche). Protein extracts were centrifuged (10,000 r.p.m. for 5 min at 4°C) and the supernatants were collected as tissue homogenate samples and stored at -80°C.

Cytokine detection- ELISA, multiplex and Legendplex: Concentration of IL-18 in the tissue homogenates was measured using the following commercial ELISA kits: IL-18 (MBL) according to manufacturer's instruction. Different other cytokines and chemokines were measured by using the ProcartaPlex Multiplex Immunoassay (eBioscience) and FACS based Legendplex kit (Biolegend) according to the manufacturer's instructions.

RNA isolation and quantitative PCR: Tissues were preserved in RNAlater solution (Ambion) and subsequently homogenized in Trizol reagent (Invitrogen). One microgram of total RNA was used to generate cDNA by the protocol for first strand cDNA synthesis using RevertAid RT (Thermo Scientific). RealTime-PCR was performed using gene-specific primer sets (Applied Biosystems) of *Cd4* primer (F: 5'-TAGCAACTCTAAGGTCTCTAAC and R: 5'-GATAGCTGTGCTCTGAAAAC) and Kapa Sybr Fast qPCR kit (Kapa Biosystems) on a LightCycler 480 instrument (Roche). PCR conditions were 95°C for 60 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s. Data were analyzed using the the deltaCt method with *hprt* (F: CTGGTGAAAAGGACCTCTCG and R: TGAAGTACTCATTATAGTCAAGGGCA) serving as the reference housekeeping gene.

RNA-Seq Analysis: Total RNA isolation from distal colonic tissue was performed as described at the RNA isolation section. RNA integrity was measured in a Bioanalyzer (Agilent Technologies, USA) and samples were selected according to RNA Integrity Number (RIN) > 9. Isolation of mRNA was performed with Dynabeads mRNA DIRECT Micro Kit (Ambion, USA) using 1ug of total RNA. Furthermore, cDNA synthesis, fragmentation and sequencing library preparation were done using ScriptSeq v2 RNA-Seq Kit (PCR 15 cycles) (Illumina, USA). Sequencing was performed through Illumina Hi-seq 2000 platform in single end mode for 50bp. Raw data is available in the SRA under accession number: PRJNA407361

We obtained an average of 52,2Mio of reads per sample (n=16). Reads were quality filtered using Trimmomatic with the following parameters (LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:35 HEADCROP:3). After quality control reads were aligned to the mouse reference genome (mm10) using STAR. Reads count to each gene was evaluated using HTseq. Normalization and differential expression were quantified using the DEseq2 package. Differentially expressed gene networks were analyzed with Consensus Path DB-mouse webserver. Data was visualized using ggplot2 R library.

Antibody-mediated depletion: Anti-CD4 (clone GK1.5) and anti-CD8a (2.43) neutralizing antibodies and an isotype control mAb (clone LTF-2) (Bio X Cell) were used. For each antibody, 200ug injections were given intraperitoneally (i.p.) at day -1, day 3 and day 7 of DSS treatment. Isolating cLPL and splenic lymphocytes followed by flow cytometry assessed depletion efficiency.

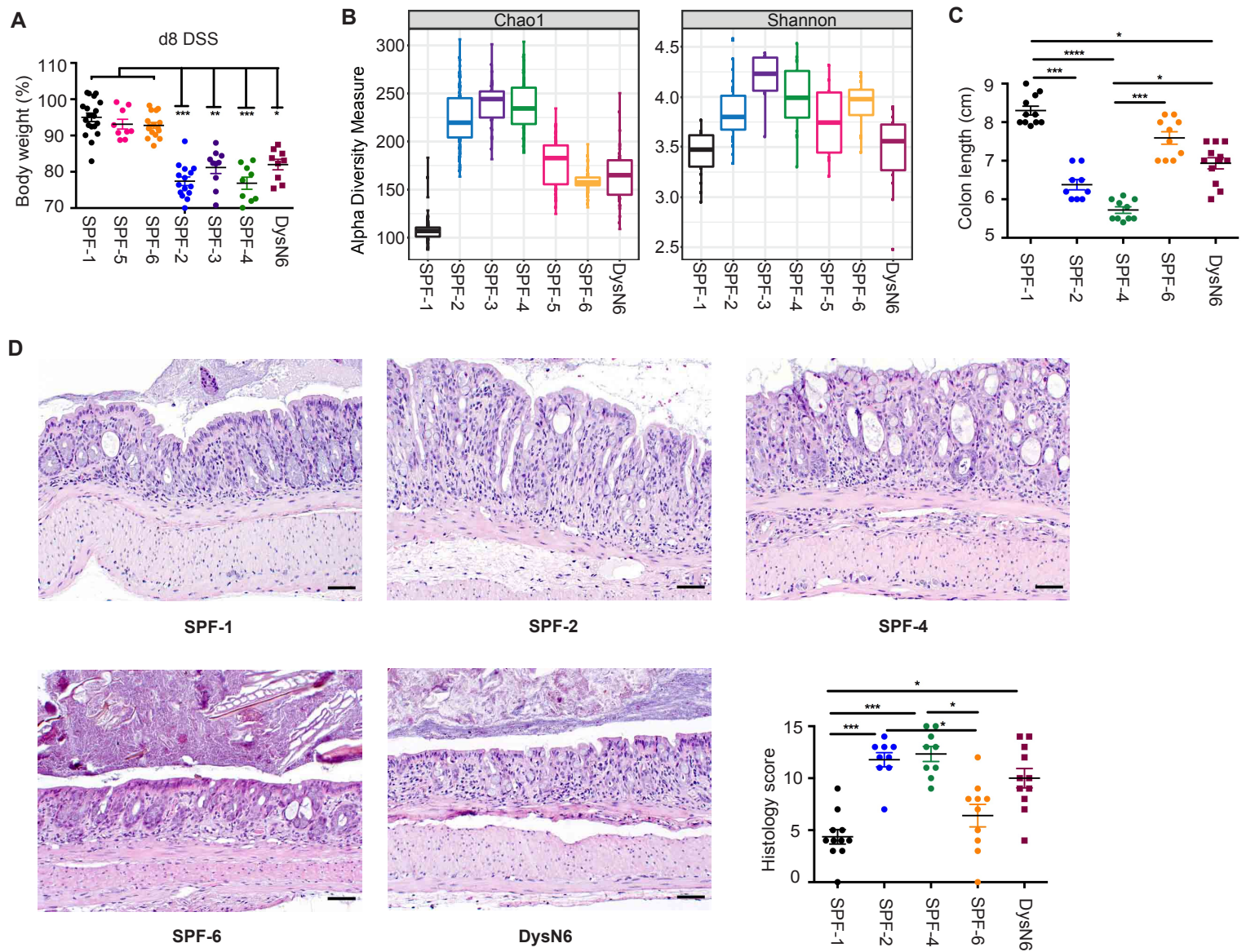


Figure S1

Figure S1. Differences in microbiota composition of SPF mice regulate severity of acute inflammation upon barrier breakdown. *Related to Figure 1*

(A) Body weight on d8 of acute DSS colitis of SPF WT (SPF-1 – SPF-6) and dysbiotic *Nlrp6*^{-/-} (DysN6) mice described in Figure 1A .

(B) Analysis of fecal microbiota composition in SPF WT (SPF-1 – SPF-6) and dysbiotic *Nlrp6*^{-/-} (DysN6) mice before induction of DSS colitis using 16S rRNA sequencing. Analysis of α -diversity (Chao1 and Shannon).

(C-D) Colon length was measured 5 days after induction of DSS colitis of SPF-1, SPF-2, SPF-4, SPF-6 and DysN6 (C). Histological analysis of distal colon was performed 5 days after induction of DSS colitis (D). Representative pictures of H&E-stained colon sections. Bar represents approx. 50 μ m.

Data represent n=6-18 mice/group as mean \pm SEM from at least two independent experiments. P values indicated represent a unpaired Student's t test. *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.

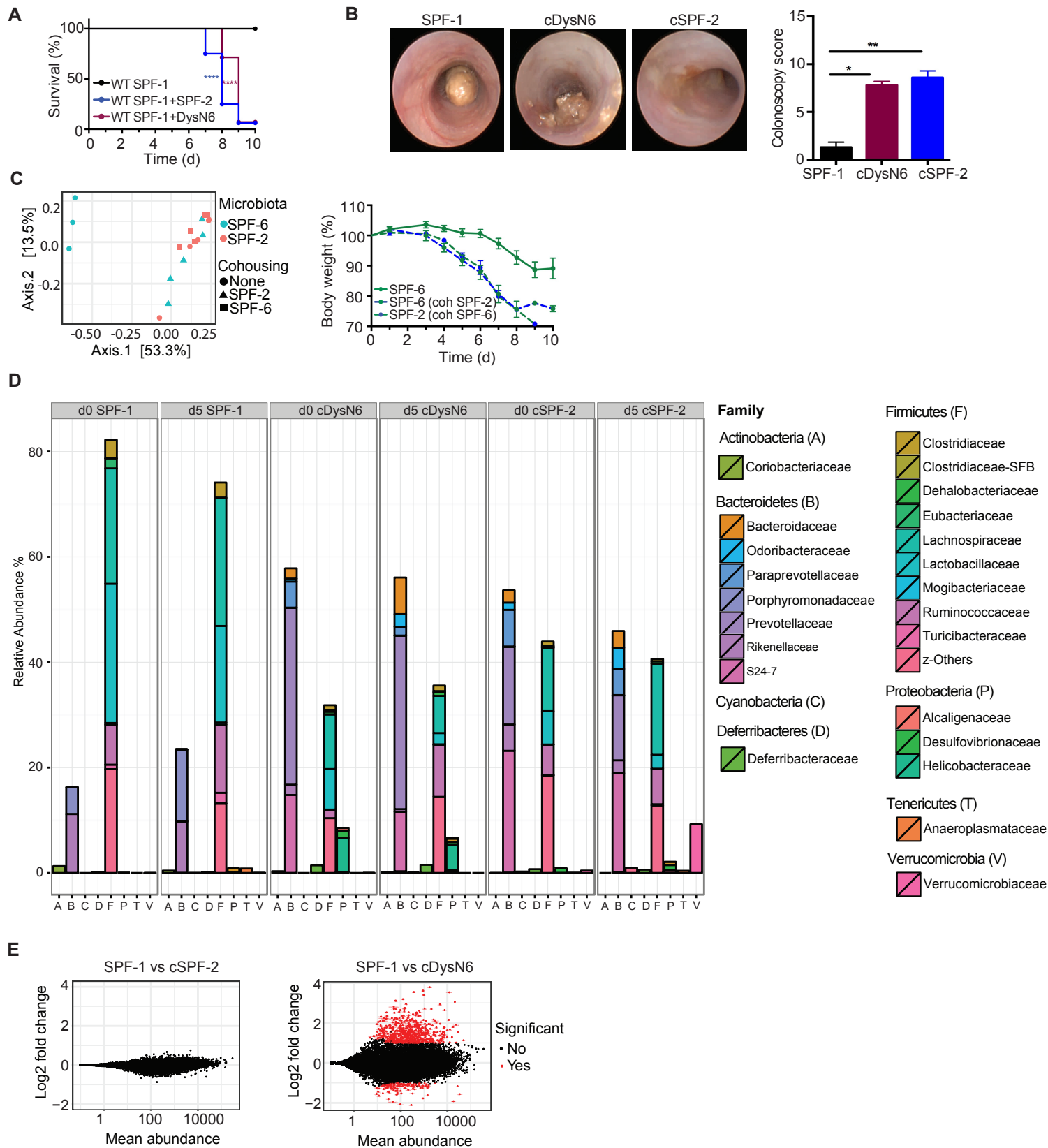


Figure S2

Figure S2. Alteration of colitis susceptibility and distinct host response driven by colitogenic microbiota. Related to Figure 2

(A-B) Acute colitis was induced in SPF-1 WT mice cohoused with either SPF-2 WT or DysN6 *Nlrp6*^{-/-} (SPF-1 + DysN6) mice resulting in SPF-1 + SPF-2 and SPF-1 + DysN6 mice, respectively. Survival of mice was monitored (A). Representative pictures and colitis severity score by colonoscopy performed on day 6 after colitis induction (B).

(C) SPF-6 WT mice were cohoused with SPF-2 WT resulting in SPF-6 (coh SPF-2) and SPF-2 (coh SPF-6) mice. Analysis of β -diversity (PCoA) of non-cohoused SPF-6 and cohoused SPF-6 (coh SPF-2) and SPF-2 (coh SPF-6) mice before induction of DSS colitis. Acute DSS colitis was induced and body weight of mice was monitored for 10 days.

(D) 16S rRNA sequencing of fecal microbiota from WT SPF-1, cSPF-2 and cDysN6 at d0 and d5 of DSS colitis was performed. Relative abundances of different microbial families are displayed.

(E) RNAseq analysis from total colonic tissue of WT mice colonized with SPF-1, cSPF-2 or cDysN6. DEseq analysis compares significant up/down-regulation of genes (fold change > 2) in different microbiota conditions.

Data represent n=4-16 mice/group as mean \pm SEM from at least two independent experiments. P values indicated represent a unpaired Student's t test (A) and nonparametric Kruskal-Wallis test (B) *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

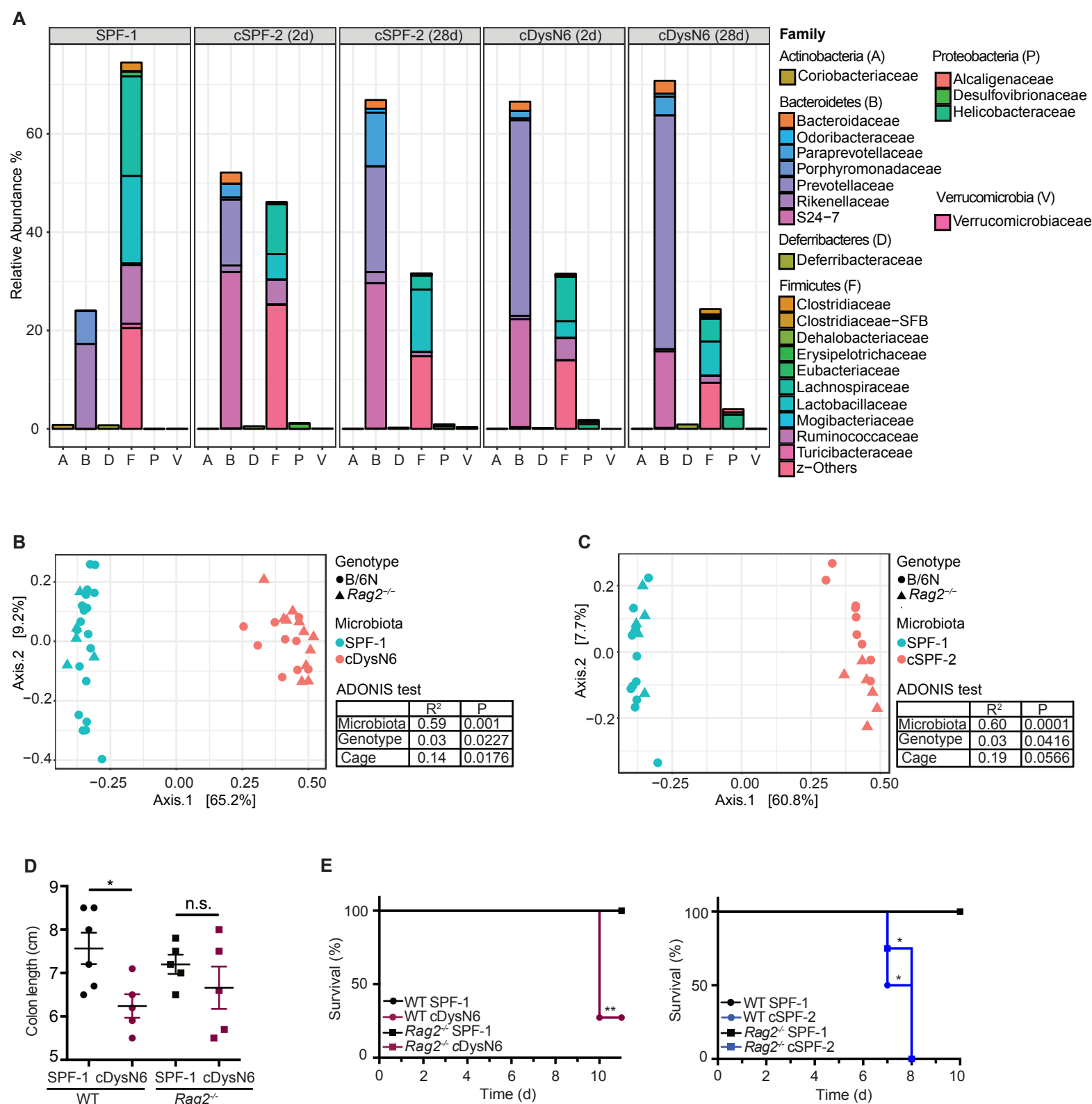


Figure S3

Figure S3. Adaptive immune system is important for DysN6 mediated colitis. Related to Figure 3

(A) SPF-1 WT recipient mice were transferred with fecal content from *Nlrp6*^{-/-} DysN6 or SPF-2 WT donor mice. Relative abundance of different bacterial families in fecal microbiota in mice with short (2d) and prolonged (28d) exposure to DysN6 and SPF-2 before DSS induction.

(B-C) SPF-1 WT and *Rag2*^{-/-} recipients were cohoused with donor DysN6 or SPF-2 mice. Analysis of β -diversity (PCoA) of DysN6 recipients (B) and SPF-2 recipients (C) is shown along with multivariate analysis of variance (ADONIS test) of variables 'microbiota composition', 'genotype' and 'cage'.

(D-E) SPF-1 and cDysN6 WT and *Rag2*^{-/-} were sacrificed on d5 of DSS colitis and colons were excised. Colon length was measured (D) and survival of mice with different microbiota are given (E).

Data represent n=3-15 mice/group as mean \pm SEM from at least two independent experiments. P values indicated represent a unpaired Student's t test *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.

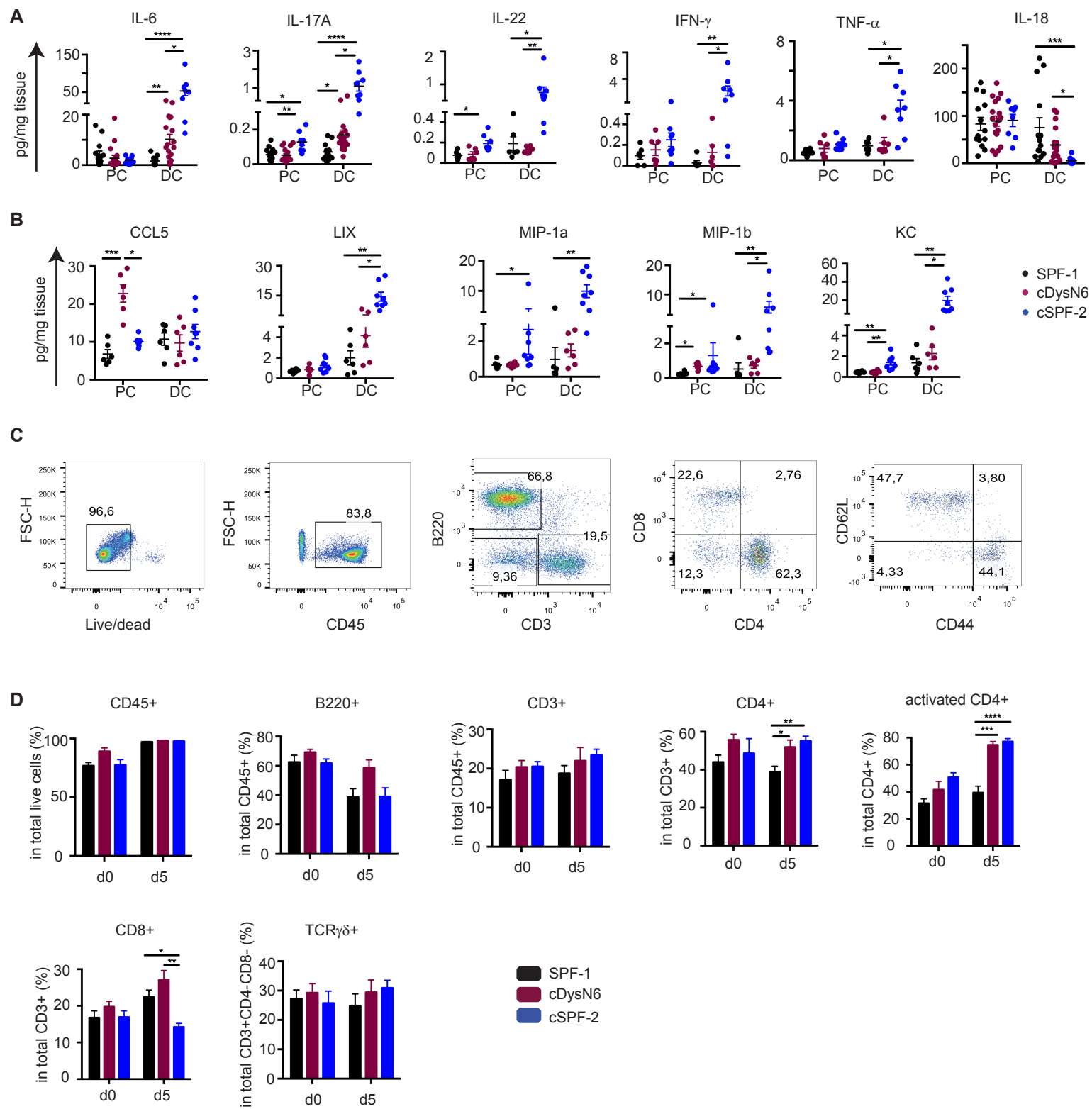


Figure S4

Figure S4. Colitis driven by DysN6 and SPF-2 are characterized by distinct local infiltration of innate and adaptive immune cells and cytokine profile. *Related to Figure 4*

(A-B) DSS colitis was induced in WT mice harboring SPF-1, cDysN6 or cSPF-2 communities. Colon samples were collected at day 7 of DSS and divided into two parts as proximal (PC) and distal (DC) colon. Cytokines (A) and chemokines (B) were measured using Multiplex/LEGENDplex kit from colonic tissue homogenates.

(C-D) Colonic lamina propria leukocytes (cLPL) were isolated from WT mice harboring SPF-1, cDysN6 or cSPF-2 communities during steady state (d0) and d5 of DSS colitis and analyzed by FACS. Gating strategy of FACS data is displayed (C). Frequencies of different immune cells at d0 and d5 of DSS (D).

Data represent n=5-17 mice/group as mean \pm SEM from at least two independent experiments. P values indicated represent a nonparametric Kruskal-Wallis test *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.

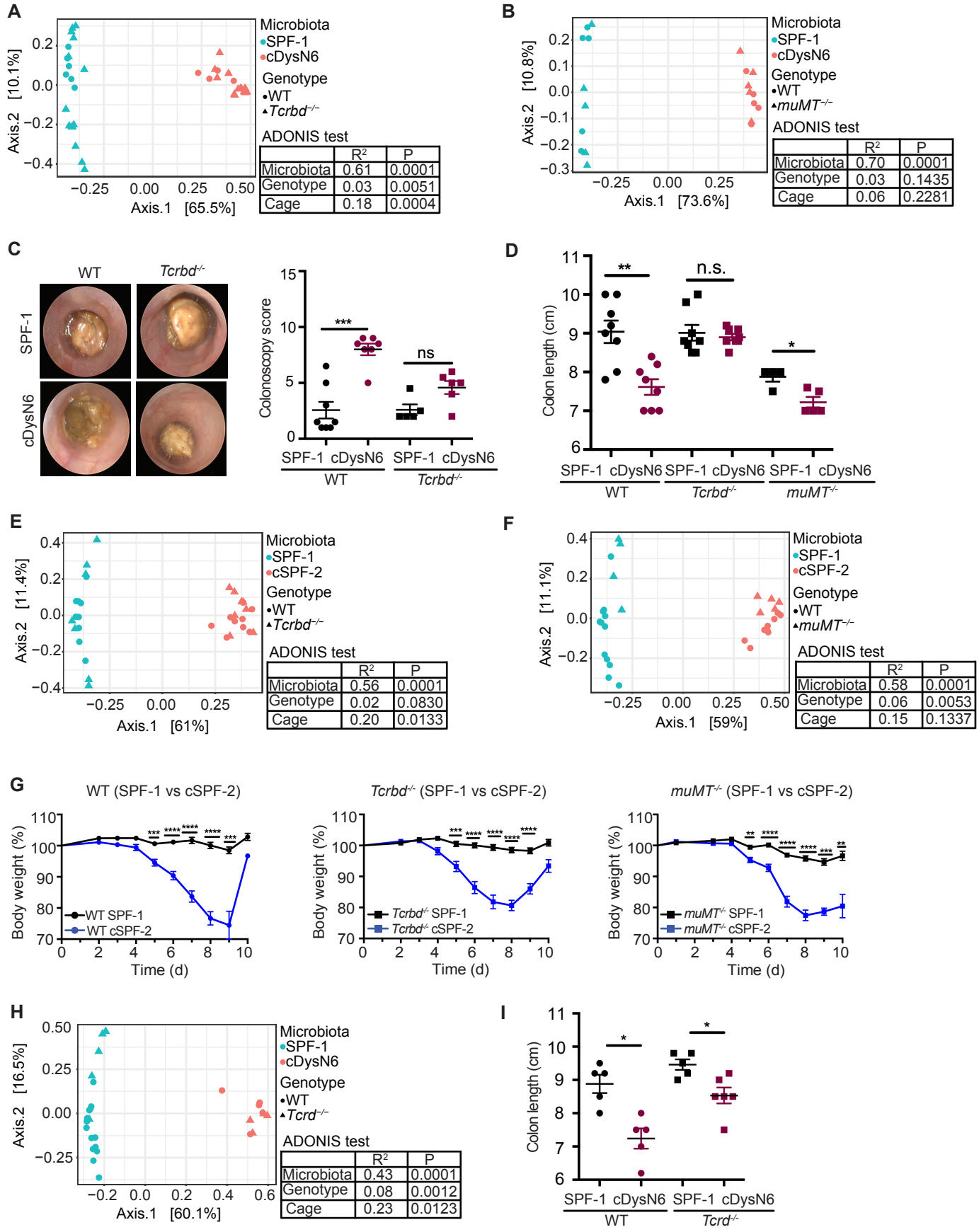


Figure S5

Figure S5. $\alpha\beta$ T cells are required for DysN6 but not SPF-2 mediated colitis. Related to Figure 5

(A-D) SPF-1 WT, *Tcrbd*^{-/-} and *muMT*^{-/-} mice were cohoused with DysN6 donor mice. Fecal microbiota composition was analyzed by 16S rRNA sequencing before induction of DSS colitis. Analysis of β -diversity (PCoA) of SPF-1 and cDysN6 WT and *Tcrbd*^{-/-} mice (A) or *muMT*^{-/-} mice (B) is shown along with multivariate analysis of variance (ADONIS test) of variables 'microbiota composition', 'genotype' and 'cage'. Representative pictures and colitis severity score by colonoscopy performed on day 6 after colitis induction in SPF-1 and cDysN6 WT and *Tcrbd*^{-/-} mice (C). Colon shortening was measured 5 days after colitis induction in SPF-1 and cDysN6 WT, *Tcrbd*^{-/-} and *muMT*^{-/-} mice (D).

(E-F) SPF-1 WT, *Tcrbd*^{-/-} and *muMT*^{-/-} were cohoused with SPF-2 donor mice. Fecal microbiota composition was analyzed by 16S rRNA sequencing before induction of DSS colitis. Analysis of β -diversity (PCoA) of SPF-1 and cSPF-2 WT and *Tcrbd*^{-/-} mice (E) or *muMT*^{-/-} mice (F) is shown along with multivariate analysis of variance (ADONIS test) of variables 'microbiota composition', 'genotype' and 'cage'.

(G) DSS colitis was induced in SPF-1 and cSPF-2 WT, *Tcrbd*^{-/-} and *muMT*^{-/-} mice. Body weight was monitored over 10 days after DSS induction.

(H-I) SPF-1 WT and *Tcrd*^{-/-} mice were cohoused with DysN6 donor mice. Analysis of β -diversity (PCoA) of fecal microbiota from SPF-1 and cDysN6 WT and *Tcrd*^{-/-} mice (H) is shown along with multivariate analysis of variance (ADONIS test) of variables 'microbiota composition', 'genotype' and 'cage'. Colon lengths of WT and *Tcrd*^{-/-} mice 5 days after colitis induction were measured (I).

Data represent n=5-26 mice/group as mean \pm SEM from at least two independent experiments. P values indicated represent a unpaired Student's t test *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.

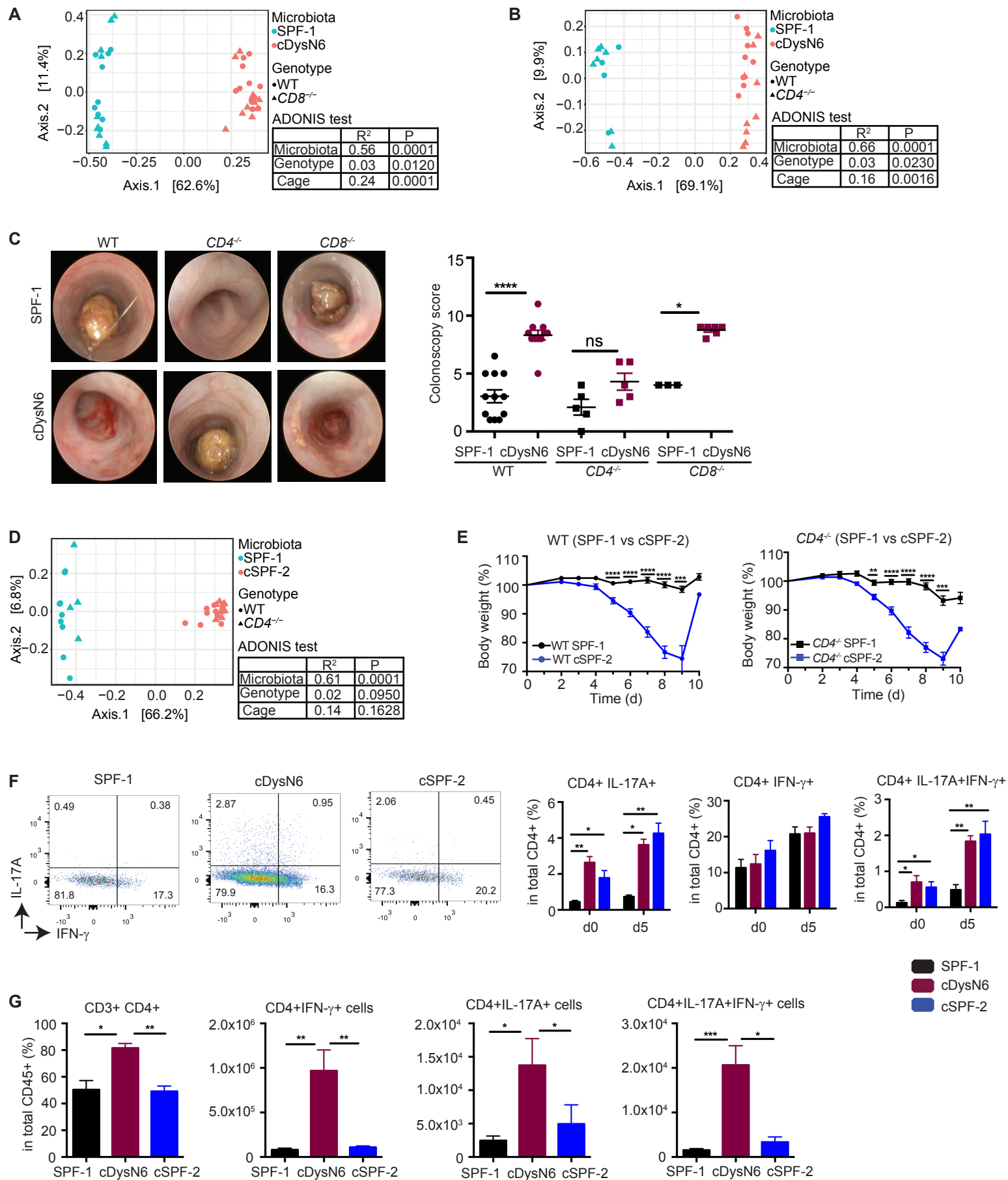


Figure S6

Figure S6. Pathogenic CD4⁺ T cells are crucial for DysN6 but not SPF-2 to enhance colitis severity. Related to Figure 6

(A-C) SPF-1 WT, *CD8^{-/-}* and *CD4^{-/-}* mice were cohoused with DysN6 donor mice. Fecal microbiota was analyzed by 16S rRNA sequencing before DSS induction and analysis of β -diversity (PCoA) of WT control, *CD8^{-/-}* mice (A) and *CD4^{-/-}* mice (B) is shown along with multivariate analysis of variance (ADONIS test) of variables 'microbiota composition', 'genotype' and 'cage'. Representative pictures and total score after colonoscopy performed on day 6 after colitis induction in WT, *CD8^{-/-}* and *CD4^{-/-}* mice harboring SPF-1 and cDysN6 communities (C).

(D-E) SPF-1 WT and *CD4^{-/-}* mice were cohoused with SPF-2 donor mice for 4 weeks. Fecal microbiota was analyzed by 16S rRNA before DSS induction and analysis of β -diversity (PCoA) of WT control, and *CD4^{-/-}* mice is shown along with multivariate analysis of variance (ADONIS test) of variables 'microbiota composition', 'genotype' and 'cage' (D). Body weight was monitored for 10 days after inducing DSS (E).

(F) Colonic lamina propria leukocytes (cLPL) were isolated from SPF-1, cSPF-2 and cDysN6 IL-17A^{GFP} IFN- γ ^{Katushka} FoxP3^{RFP} triple reporter mice during steady state (d0) and d5 of DSS colitis and analyzed by FACS. Representative FACS plots and percentage of different cytokine producing CD4⁺ T cells are displayed.

(G) T cell transfer colitis was induced by injecting CD4⁺Foxp3⁻CD45RB(high) T cells into SPF-1, cDysN6 or cSPF-2 *Rag2^{-/-}* recipients. Mice were sacrificed 16 days after T cell transfer. Lymphocytes were isolated from colon and analyzed by FACS. Infiltration of different cytokine producing cell population in colon is displayed.

Data represent n=5-20 mice/group as mean \pm SEM from at least two independent experiments. P values indicated represent a unpaired Student's t test (C,E) and nonparametric Kruskal-Wallis test (F,G) *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.

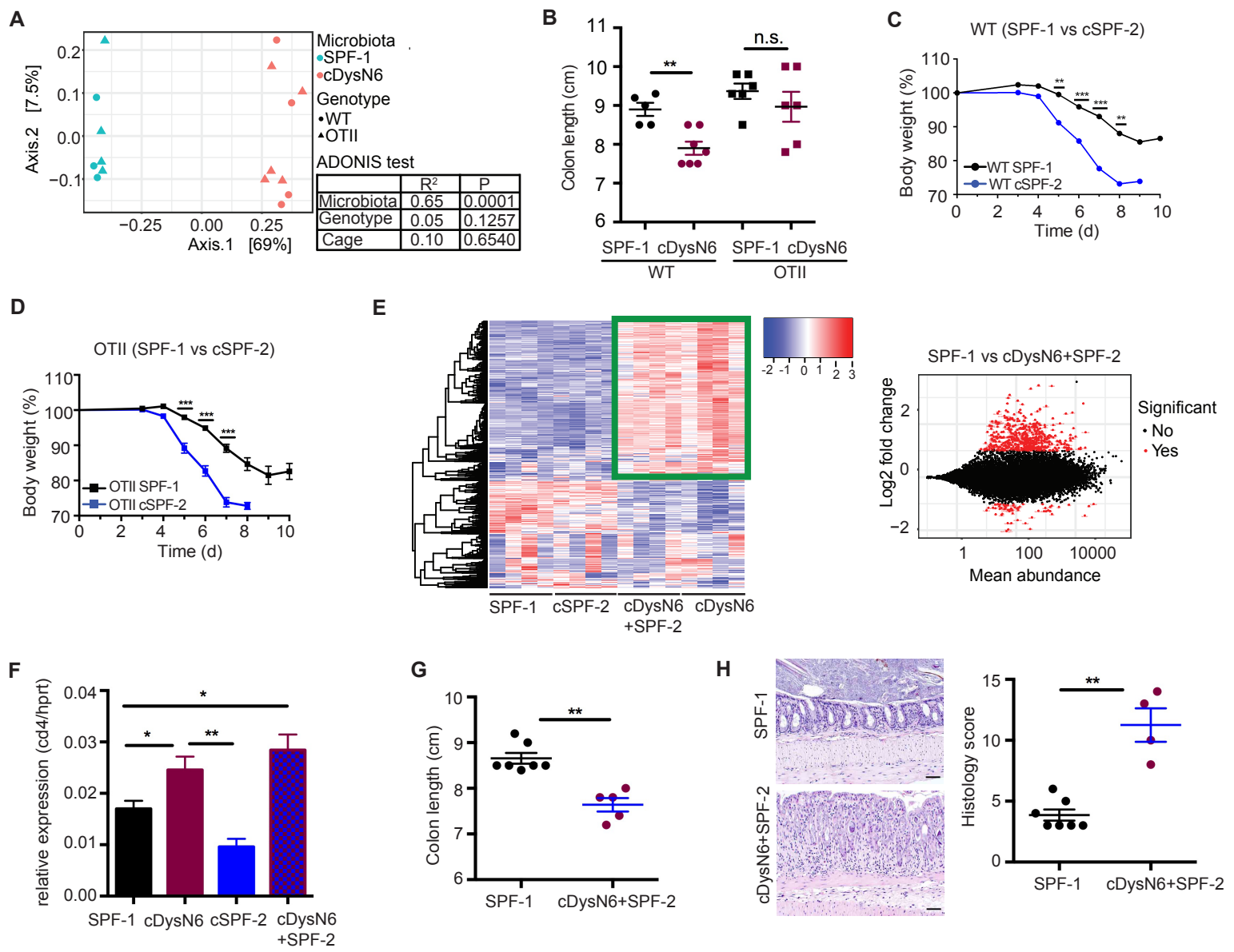


Figure S7

Figure S7. CD4⁺ T cells drives DSS colitis severity in DysN6 but not SPF-2 mice by recognizing antigens from dominant microbial members. *Related to Figure 7*

(A-B) SPF-1 WT and OTII transgenic mice were cohoused with DysN6 donor mice for 4 weeks. Analysis of β -diversity (PCoA) of fecal microbiota from SPF-1 and cDysN6 WT and OTII transgenic mice is shown along with multivariate analysis of variance (ADONIS test) of variables 'microbiota composition', 'genotype' and 'cage' (A). Colon length of SPF-1 and cDysN6 WT and OTII transgenic mice after 5 days of DSS induction were measured (B).

(C-D) DSS colitis was induced in SPF-1 and cSPF-2 WT and OTII transgenic mice and body weight was monitored for 10 days.

(E) RNAseq analysis from total colonic tissue of WT mice harboring different communities. Heatmap shows quantification of RNA reads and DEseq analysis to identify significant up/down-regulation (fold change >2) of genes in SPF-1 and cDysN6+SPF-2 conditions.

(F) Relative *Cd4* expression in colonic tissue of mice harboring different microbiota.

(G-H) DSS colitis was induced in WT mice colonized with SPF-1 and cDysN6+SPF-2 communities. At d5 of DSS mice were sacrificed and colon length was measured (G). Histological analysis of distal colon was performed 5 days after induction of DSS colitis (H). Representative pictures of H&E-stained colon sections. Bar represents approx. 50 μ m.

Data represent n=5-20 mice/group as mean \pm SEM from at least two independent experiments. P values indicated represent a unpaired Student's t test (B-D, G, H) and nonparametric Kruskal-Wallis test (F) *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.

Supplemental Tables:

Table S1: Provider of mice. *Related to Figure 1*

Table S2: Microbial signatures of experimental mice. *Related to Figure 1*

Table S1: Provider of mice

Mouse line:	Genotype	Source		Commercial:
		Breeder	Barrier	
SPF-1	C57Bl/6NCrl	HZI	T2	N
SPF-2	C57Bl/6NRj	Janvier	A1	Y
SPF-3	C57Bl/6NRj	Janvier	C10	Y
SPF-4	C57Bl/6NHsd	Envigo	2	Y
SPF-5	C57Bl/6NTac	Taconic	1C-HC	Y
SPF-6	C57Bl/6NTac	Taconic	EBU401	Y
DysN6	B6.Cg-Nlrp6tm1Flv	HZI	T1	N

Table S2: Microbial signatures of experimental mice

OTU numbers	SPF-1	SPF-2	SPF-3	SPF-4	SPF-5	SPF-6	DysM	Kingdom	Phylum	Class	Order	Family
681370	0.000	0.000	0.000	0.000	0.096	0.000	0.000	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae
4393532	0.240	0.000	0.000	0.000	0.156	0.000	0.232	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae
181719	0.000	2.015	2.527	1.269	0.793	2.102	1.817	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae
835900	0.000	1.270	0.742	0.986	0.412	0.000	0.986	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Odoribacteraceae
4449525	0.000	5.752	5.325	3.660	0.000	0.000	4.123	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae
4372003	9.702	0.097	0.000	0.000	0.359	0.442	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae
4378740	0.000	13.268	7.778	1.973	8.634	0.082	28.874	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
4449518	13.321	2.520	2.502	1.403	2.806	0.121	1.888	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae
2212505	0.000	26.084	27.303	37.901	23.072	20.639	13.292	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7
4405128	0.000	0.255	0.569	0.168	0.095	0.000	0.000	Bacteria	Cyanobacteria	4C0d-2	YS2	z-Others
1136443	1.493	0.463	0.000	0.208	0.111	1.210	1.829	Bacteria	Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae
179575	0.848	0.547	1.050	0.907	0.672	0.438	2.184	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae
376862	0.000	0.000	0.423	0.113	0.388	0.000	0.239	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae-SFB
176868	0.171	0.281	0.295	0.211	0.243	0.296	0.629	Bacteria	Firmicutes	Clostridia	Clostridiales	Dehalobacteriaceae
4426641	0.000	0.462	1.143	0.375	0.358	0.000	0.000	Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae
170248	0.138	0.141	0.060	1.251	1.317	1.216	0.147	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae
186043	24.499	11.461	18.245	11.438	13.987	22.135	12.357	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae
135956	15.271	11.556	8.692	19.410	13.736	7.097	5.508	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae
228140	0.268	0.091	0.000	0.087	0.078	0.136	0.000	Bacteria	Firmicutes	Clostridia	Clostridiales	Mogibacteriaceae
263895	0.000	0.000	0.000	0.000	0.000	0.385	0.000	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae
179547	10.430	3.060	4.588	3.145	5.668	9.615	1.810	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae
180944	17.893	5.999	10.792	5.217	10.968	16.115	8.156	Bacteria	Firmicutes	Clostridia	Clostridiales	z-Others
1105376	0.000	0.268	0.340	0.457	0.328	0.111	0.440	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae
1108599	0.000	1.032	0.761	0.280	0.250	0.000	1.098	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae
782953	0.000	0.000	0.000	0.066	1.830	0.000	0.000	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
1141335	0.000	0.000	0.000	0.000	0.000	0.000	7.751	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacteriales	Helicobacteraceae
4390755	0.366	0.000	0.000	0.000	0.000	0.681	0.000	Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae
4306262	0.000	1.025	0.373	0.205	2.025	0.086	0.000	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae

Supplemental references:

- Becker, C., Fantini, M.C., and Neurath, M.F. (2007). High resolution colonoscopy in live mice. *Nat. Protoc.* 1, 2900–2904.
- Mähler, M., Bristol, I.J., Leiter, E.H., Workman, A.E., Birkenmeier, E.H., Elson, C.O., and Sundberg, J.P. (1998). Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis. *Am. J. Physiol.* 274, G544–51.
- Pils, M.C., Pisano, F., Fasnacht, N., Heinrich, J.-M., Groebe, L., Schippers, A., Rozell, B., Jack, R.S., and Müller, W. (2010). Monocytes/macrophages and/or neutrophils are the target of IL-10 in the LPS endotoxemia model. *Eur. J. Immunol.* 40, 443–448.
- Thiemann, S., Smit, N., Roy, U., Lesker, T.R., Gálvez, E.J.C., Helmecke, J., Basic, M., Bleich, A., Goodman, A.L., Kalinke, U., et al. (2017). Enhancement of IFN γ Production by Distinct Commensals Ameliorates Salmonella-Induced Disease. *Cell Host Microbe* 21, 682–694.e5.
- Weigmann, B., Tubbe, I., Seidel, D., Nicolaev, A., Becker, C., and Neurath, M.F. (2007). Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue. *Nat. Protoc.* 2, 2307–2311.