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Supporting Information for

Deficiency of IL-22–binding protein enhances the ability of the gut microbiota to protect against enteric pathogens

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SI Appendix, Material and Methods

Generation of Il22ra2$^{-/-}$ mice

To generate *Il22ra2*tm1b (null LacZ) mice, *Il22ra2*tm2a(EUCOMM)Wtsi ES cell clones (ES line JM8A3.N1) were obtained from European Conditional Mouse Mutagenesis Consortium. The correctly targeted clone, confirmed by Southern blot analysis, was introduced into B6-albino (C57BL/6J-Tyrc-2J/J) eight-cell embryos by laser-assisted injection. Male chimeras were initially bred to B6-albino mice to assess germline transmission; those transmitting the mutant allele were bred to CMV-Cre transgenic mice (B6.CTg(CMV-Cre)1Cgn/J; >99% C57BL/6) to delete exon 3 of *Il22ra2* and the neomycin-resistance cassette. The CMV-Cre transgene was subsequently bred out of the lines by backcrossing these mice several times with C57BL/6 mice. Mice were subsequently accommodated in a shared facility room, with uniform bedding and water conditions. For co-housing conditions, pregnant mothers of both WT and *Il22ra2*$^{-/-}$ mice were housed together and with their litters until weaning.

Mice

C57BL/6 mice were purchased from Jackson Laboratory for back-crossing WT and *Il22ra2*$^{-/-}$ littermate mice. *TCRβγ*$^{-/-}$ x *Ahr*$^{fl/fl}$ *Rorγt*-Cre mice were previously described by our group (1). All strains were maintained in a C57BL/6 background and were kept in specific pathogen–free conditions at the animal facility of Washington University in St Louis. All animal studies were approved by the Washington University Animal Studies Committee.

C. difficile infection

C. difficile VPI 10463 strain was cultivated in BHI blood agar at 37°C in anaerobiosis (AnaeroGen, Oxoid; ThermoFisher Scientific). 6-8 weeks old age- and gender-matched mice were pre-treated with an antibiotic mixture (0.4 mg/mL kanamycin, 0.035 mg/mL gentamicin, 0.035 mg/mL colistin, 0.215 mg/mL metronidazole and 0.045 mg/mL vancomycin; Sigma) for 4 days via the drinking water, as previously described (2). Antibiotics were subsequently discontinued and mice received an intraperitoneal single dose of clindamycin (10 mg/kg) (Sigma Aldrich, St. Louis, MO, USA). The following day, mice were infected with 10^8 colony forming units (CFU) of *C. difficile* by gavage. Thereafter, all animals were weighted daily and were systematically monitored for clinical severity scores from 0 (normal) to 15 (dead) by an evaluator blinded to the treatment. The scores were calculated by summing values ranging from 0 to 3 for each of five parameters, which included activity levels, posture, coat condition, diarrhea, and eyes/nose discharge (**SI Appendix, Table S1**) (3).

Repeated C. difficile infection model

6-8 week-old mice were initially treated with the antibiotic cocktail described above for 4 days through drinking water supplementation. Antibiotics were then discontinued and mice received an intraperitoneal single dose of clindamycin (10 mg/kg) (Sigma Aldrich, St. Louis, MO, USA), and were then infected with 10^8 CFU of *C. difficile* by oral gavage. After 7 days, mice again received the antibiotic

cocktail treatment for 4 days, followed by an i.p. dose of clindamycin and re-challenge with 10^8 CFU of *C. difficile*. Animals were observed daily for symptoms of *C. difficile* infection (weight loss and clinical score) until day 19 after the first infection.

C. rodentium infection

C. rodentium, strain DBS100 (ATCC), was cultivated overnight in LB agar plate in a dry incubator at 37°C. Next, few isolated colonies were inoculated into 50 mL of LB broth medium in sterile flask and placed for 3-4 hours in a 200-rpm shaker at 37°C. Finally, a suspension of 10^{10} colony forming units (CFU) per mL of *C. rodentium* was prepared and a 200 µL aliquot was administrated to 8–12-week-old gender-matched mice by gavage (2 x 10^9 CFU/mouse), as previously described (4). Body weight was measured every 3 days during the first week of infection and once a week for the rest of the experiment until day 20 p.i. CFU burdens were measured at the end of the experiment.

Quantification of bacterial DNA

For analysis of intestinal bacterial translocation, total DNA was extracted from 50 mg liver and mesenteric lymph nodes harvested from *C. difficile*-infected mice using DNeasy Blood & Tissue Kit (Qiagen), according to manufacturer's indications. Bacterial DNA levels were quantified by qPCR using iTaq Universal SYBR Green Supermix (Bio-Rad) and primers directed at 16S rDNA V4 gene (**SI Appendix, Table S4**). Bacterial load was determined by constructing a standard

curve based on serial dilution of *Escherichia coli* genomic DNA; load expressed as16S rDNA gene copy number/tissue

Epithelial permeability assay

Food and water were removed for 4 hours after which time mice received an oral gavage of FITC-Dextran (70,000 Da; Sigma) (dose 250 mg/Kg, suspended in 200 µL of sterile PBS). After 4 hours, mice were anesthetized and blood was collected by cardiac puncture. FITC-Dextran fluorescence intensity was quantified in serum (excitation, 485 nm, emission, 528 nm; BioTek Synergy H1 Plate Reader; Synergy HT; Vermont, USA). A standard curve was prepared with serial dilutions of 100 µg/mL FITC-Dextran in PBS.

Histopathological analysis

Colons were harvested, washed with cold PBS, opened longitudinally, fixed in 4% phosphate-buffered formalin overnight, and then transferred to 70% ethanol. Fixed tissues were next paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Pictures were obtained using U-LH100HG Olympus Microscope. Histopathological analysis was performed using H&E sections and tissue inflammation was determined by a total score from 0 to +30, which represents the sum of 10 parameters evaluated from 0 (normal) to 3 (severe) (**SI Appendix, Table S2**). The frequency of goblet cells was quantified using a scoring system described in **SI Appendix, Table S3**. All measurements were conducted with the investigator blinded with respect to treatment group.

Cecal microbiota transplantation (CMT)

6-8-week-old mice were treated *ad libitum* for 3 weeks with an antibiotic cocktail – VNAM = 0.5 g/L vancomycin (V), 1 g/L neomycin (N), 1 g/L ampicillin (A), 1 g/L metronidazole (M), Sigma-Aldrich, St. Louis, MO, USA – supplemented in the drinking water with the addition of 25 g/L Grape Kool-Aid (Kraft Foods). Water bottles were changed every 4 days. After 2 days, the mice received and oral gavage of 200 µL of cecal microbiota suspension prepared from WT or *Il22ra2*$^{-/-}$ animals. To prepare this suspension, cecal contents were diluted 1:10 (wt/vol) with reduced PBS (PBS/ 0.05% L-cysteine-HCl) in 50mL conical plastic tubes. Tubes were gently vortexed and the resulting slurry was passed through a 100 µm-pore diameter nylon cell strainer (BD Falcon). The clarified sample was then combined with an equal volume of a solution of PBS/0.05% L-cysteine-HCl/30% glycerol and aliquoted into 1.8 mL glass vials (E-Z vials, Wheaton). Tubes were crimped with covers containing a PTFE/grey butyl liner (Wheaton) and stored at -80 °C until use.

IL-22 in vivo neutralization

C. difficile–infected WT and *Il22ra2*$^{-/-}$ mice received an i.p. dose of 50 µL PBS with 150 µg anti-mouse IL-22 neutralizing antibody (clone 8E11; Genentech) on day 1 and 3 p.i., as previous described (1, 5). Mice were clinically evaluated until day 5 post-infection.

Quantitative gene expression

Total RNA was extracted from snap frozen colonic tissue using the RNeasy Mini Kit (Qiagen, Inc.), following the supplier's instructions. RNA was converted to cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad) and qPCR were performed using iTaq Universal SYBR Green Supermix (Bio-Rad). Gene expression was normalized using the $2^{\Delta\Delta Ct}$ method with *Gapdh* as the reference gene. Primer sequences are provided in **SI Appendix, Table S4**.

Fecal lipocalin-2 quantification

Fecal samples were harvested, reconstituted in PBS containing 0.1% Tween 20 (100 mg/ml), and then vortexed for 20 seconds to obtain a homogenous suspension. Samples were centrifuged for 10 min at 8000 x g. The resulting supernatants were collected and stored at −20°C until analysis. Lipocalin-2 levels were quantified using Mouse Lipocalin-2/NGAL ELISA kit (R&D Systems, Minneapolis, MN); supernatants from the fecal samples were diluted 4- to 100-fold using the kit-recommended diluent (1% BSA in PBS). Sample absorbance was read at 450 nm.

Intestinal lamina propria isolation

Colon was harvested from mice, cut longitudinally, and washed twice with Hanks' Balanced Salt solution (Thermofisher) for removing the luminal content. Intraepithelial lymphocytes (IELs) were excluded by two 20-minutes washes with room temperature HBSS/HEPES + 5 mM EDTA + 10% bovine calf serum (BCS). Tissues were then placed in a 37°C shaker for 40 minutes with 1 mg/mL

collagenase IV (Sigma) in complete RPMI medium with 10% BCS. Immune cells were enriched over a 40%/70% Percoll gradient (GE Healthcare), washed, and then labeled with monoclonal antibodies for analysis by flow cytometry (**SI Appendix, Table S5**).

Flow cytometry

For FACS analysis, dead cells from single-cell preparations of colonic lamina propria were excluded using a live/dead cell viability assay Zombie Aqua™ Fixable Viability Kit (BioLegend). Surface staining was performed after blocking of Fc receptors with in-house produced and purified anti-CD16/CD32. Antibodies diluted in FACS-buffer were added to the samples on ice for 20 minutes in the dark. When needed, cells were fixed and stained intracellularly using the Foxp3 Staining Buffer Set (eBioscience) according to manufacturer's instructions. Samples were acquired in BD FACS-Symphony™ with BD FACSDiva™ Software (BD biosciences). All FACS data were analyzed using FlowJo v.9.5.2 software (Tree Star).

V4-16S rRNA Amplicon Sequencing

DNA was isolated from mouse fecal samples by bead beating in phenol:chloroform and purified on QiaQuick plates (QIAGEN). PCR amplification of variable region 4 (V4) of bacterial 16S rRNA genes present in fecal samples was performed using published primers and cycling conditions (6). Amplicons were sequenced using an Illumina MiSeq instrument (2x250 nt reads; 970,038 ± 75,587 reads/sample).

Amplicon sequences were oriented, trimmed of adaptor and primer sequences and paired using the bbtools (37.02) software package (https://sourceforge.net/projects/bbmap/; 'bbduk.sh' and 'repair.sh' tools). DADA2 (1.8.0) was used to remove chimeric sequences and to identify amplicon sequence variants (ASVs) (7). Representative sequences for ASVs were then used for taxonomic profiling using the following multi-taxonomic assignment (MTA) approach. Each representative sequence was aligned using NCBI BLAST toolkit version 2.10.0 to the 16S rRNA gene reference database compiled by joining unique sequences from two databases: Ribosomal Database Project (RDP) version 11.5 and the NCBI 16S ribosomal RNA Project. The "indicspecies v 1.7.14" R package was utilized for the indicator species analysis (8).

Measurement of short-chain fatty acids

Measurement of SCFA was performed as previously described (5). Cecal contents were weighed in 4ml PTFE screw cap vials. 10 ul of an internal standard mix (20mM of formic acid-13C, acetic acid-13C2,d4, propionic acid-d6, butyric acid-13C4, lactic acid-3,3,3-d3 and succinic acid-13C4) was added to each vial, followed by 20ul 33% HCl to acidify the mixture. 1 ml diethyl ether was added and vigorously vortexed for 10 mins. The two phases were separated by centrifugation (4000 x g, 5 minutes). The upper organic layer was transferred into another clear vial and the second 1 ml diethyl ether extraction was performed. After combining the two ether extracts, 60 ul and 20 ul N-tert.-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) were mixed in a GC auto-sampler vial with

a 100 ul glass insert and incubated for 2 hours at room temperature. Derivatized samples (1 ul) were injected with 15:1 split into an Agilent 7890A gas chromatography system coupled with 5977B mass spectrometer detector (Agilent, CA). Analyses were carried on a HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness, Agilent J & W Scientific, Folsom, CA) using electronic impact (70 eV) as ionization mode. Helium was used as a carrier gas at a constant flow rate of 1.26 mL/min and the solvent delay time was set to 3.5 min. The column head-pressure was 10 p.s.i. The temperatures of injector, transfer line, and quadrupole were 270, 280 and 150 °C, respectively. The GC oven was programmed as follow: 45 °C held for 2.25 min, increased to 200 °C at a rate of 20 °C/min, to 300 °C at a rate of 100 °C/min and finally held for 3 min.

Fiber diet treatment

Mice were fed diets containing different amounts of fiber for 21 days: a conventional mouse chow diet (Purina PicoLab Rodent diet 20), a diet containing 5% cellulose (low fiber content); and a diet containing 5% cellulose plus 10% soluble inulin (‘high fiber’ content). Low and high fiber diets were purchased from Research Diets, Inc. New Brunswick, New Jersey (D10012M and D19071901).

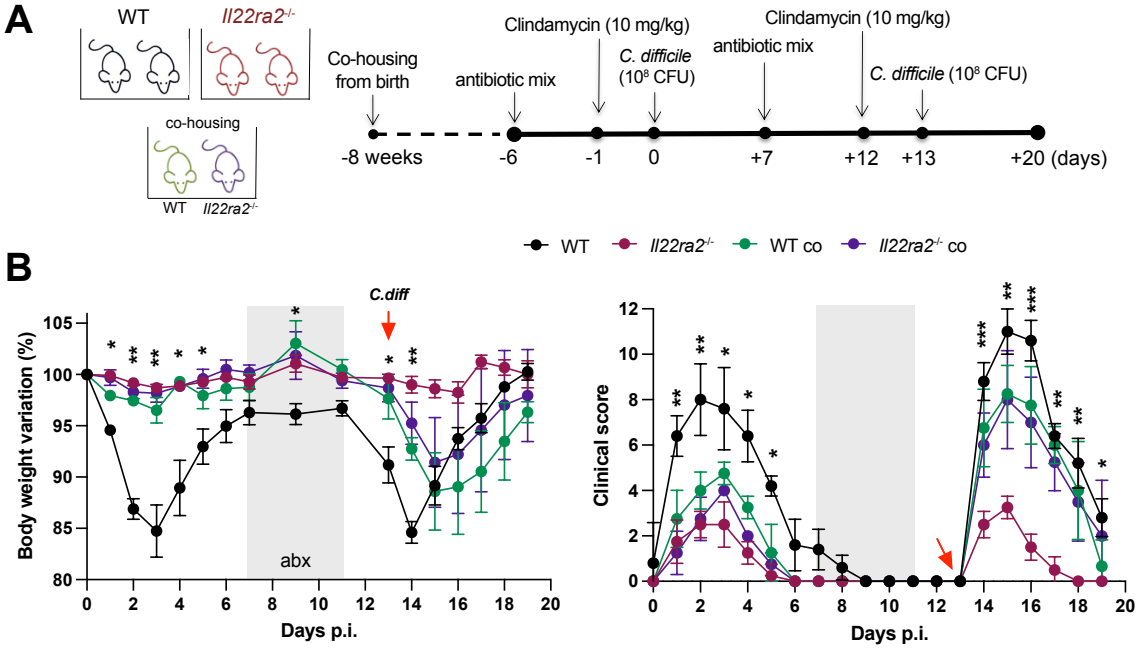
SCFA treatment

Mice received drinking water supplemented with acetate at 150 mM or placebo, as reported in other studies (5, 9). The pH of the SCFA drinking solution was adjusted to 7.2. After 2 weeks, administration of acetate was stopped; mice then received

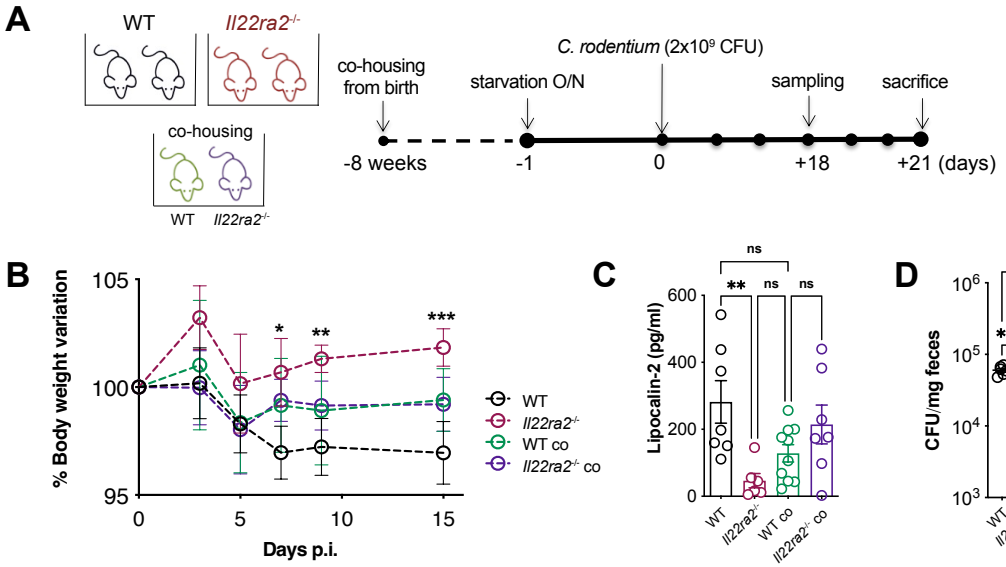
antibiotics for 5 days, and were subsequently infected with *C. difficile* by gavage (10^8 CFU per mouse).

Statistical analysis

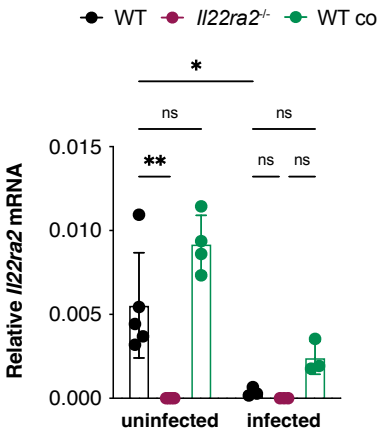
No statistical methods were used to predetermine sample size. Analyses were performed using GraphPad software 8.0 (San Diego, CA, USA). Statistical tests used to analyze experiments are described in the Figure Legends. Differences between samples/treatment groups were compared by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test.



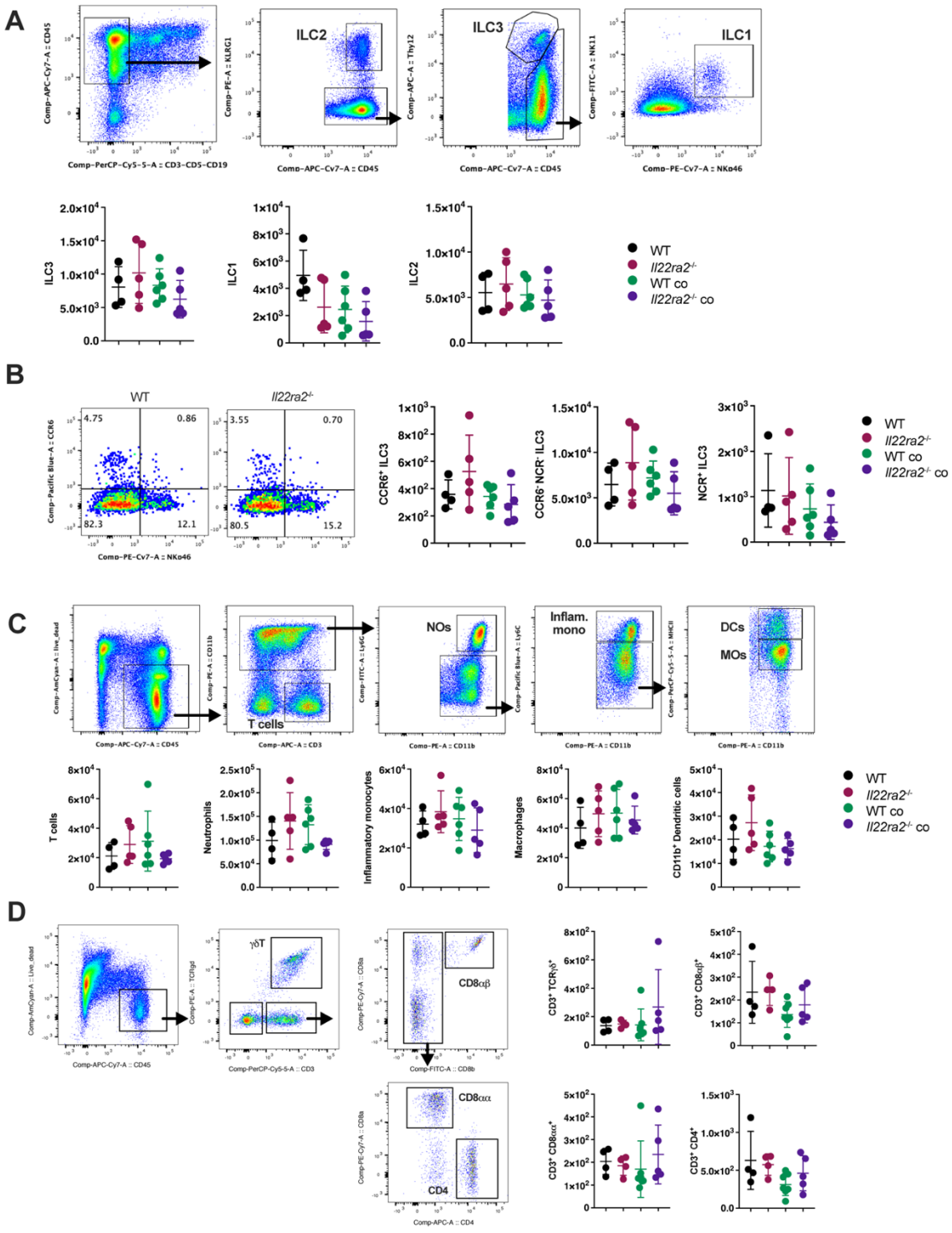
SI Appendix, Figure S1. Repeated *C. difficile* infection model in WT and *Il22ra2*$^{-/-}$ mice. (A) Experimental design. Mice received an antibiotic cocktail (0.4 mg/mL kanamycin, 0.035 mg/mL gentamicin, 0.035 mg/mL colistin, 0.215 mg/mL metronidazole and 0.045 mg/mL vancomycin; Sigma) for 4 days in drinking water and then clindamycin i.p. Next, mice were infected with 10^8 CFU of *C. difficile* and monitored daily. On day 7 post-infection, mice again received the antibiotic cocktail for 4 days and were then infected with 10^8 CFU of *C. difficile*. (B) Body weights (left) and clinical scores (right) of non-cohoused and cohoused WT and *Il22ra2*$^{-/-}$ after the first and second infection. (A, B) n = 6-9 mice per group. Data from two independent experiments are pooled together. Error bars represent mean ± SD. Normality was assessed by D'Agostino Pearson test; statistical analysis was performed using One-way ANOVA with post-hoc Tukey test. *p < 0.05; **p < 0.01; ***p < 0.001.



SI Appendix, Figure S2. *C. rodentium* infection of WT and *Il22ra2*$^{-/-}$ mice. (A) Schematic representation of *C. rodentium* infection of cohoused and non-cohoused WT and *Il22ra2*$^{-/-}$ mice. Mice in both housing conditions received 200 µL of 10^{10} CFU/mL *C. rodentium* in PBS by gavage. (B-D) Body weight (B), fecal lipocalin-2 content (C) and fecal *C. rodentium* burden (D) were measured on day 18 post-infection. (A-D) n = 7-9 mice per group. Data are combined from two independent experiments. Error bars represent mean ± SEM. Normality was assessed by D'Agostino Pearson test; statistical analysis was performed using One-way ANOVA with post-hoc Tukey test. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns = not significant.

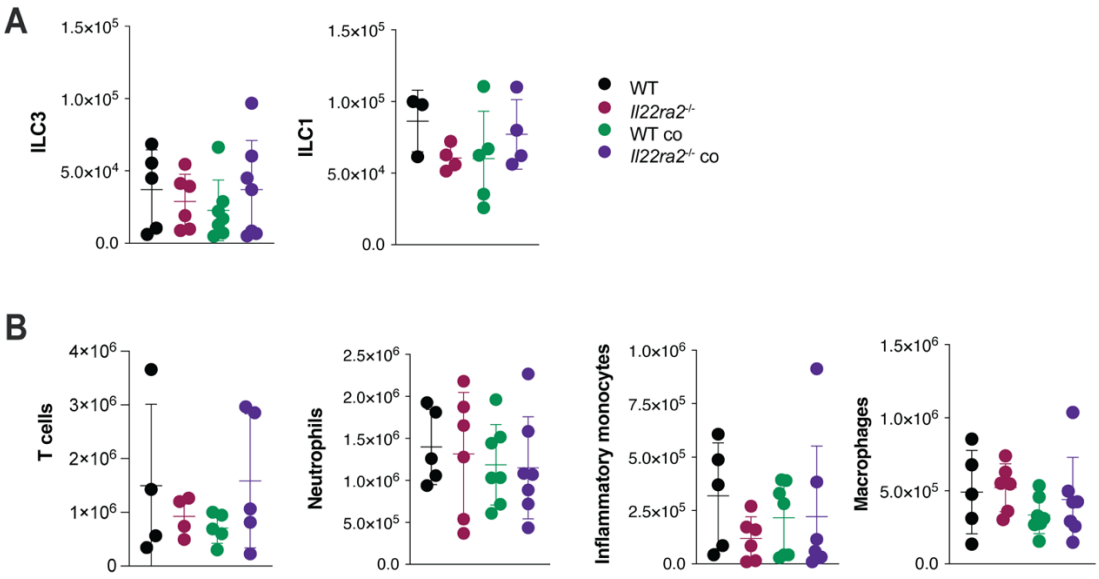


SI Appendix, Figure S3. Colonic expression of *IL22ra2* in the steady state and upon *C. difficile* infection. Relative expression of *Il22ra2* mRNA in total proximal colon of non-infected and *C. difficile* infected WT and *Il22ra2*$^{-/-}$ mice on day 4 p.i. normalized by *Gapdh*. N = 4-5 mice/group. Error bars represent mean values ± SD. Normality was assessed by D'Agostino Pearson test; statistical analysis by One-way ANOVA with post-hoc Tukey test. *p < 0.05; **p < 0.01; ns = not significant.

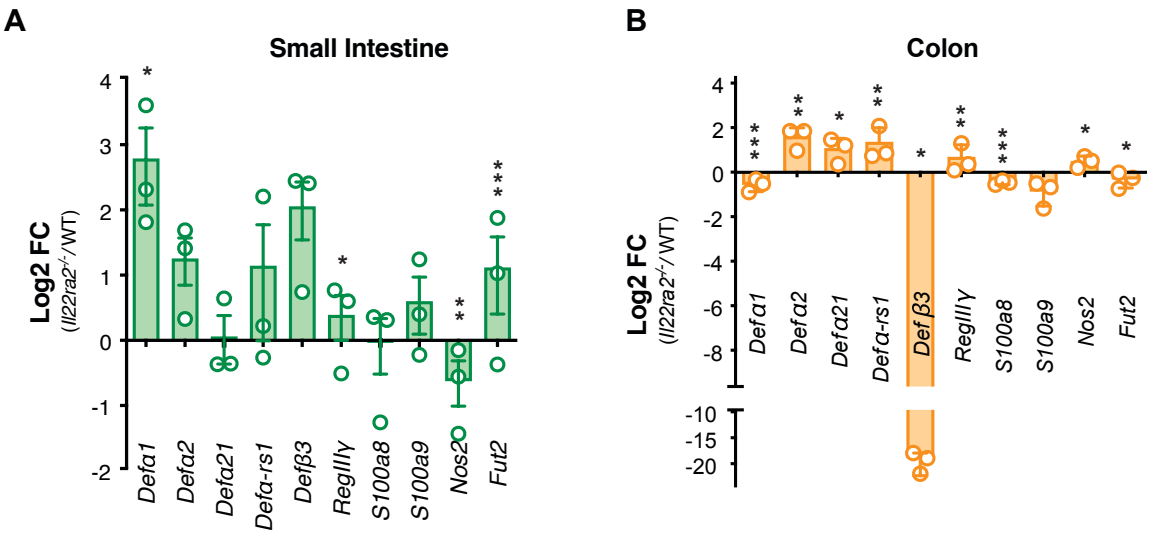


SI Appendix, Figure S4. Immune cell frequency in the colon of WT and *Il22ra2*$^{-/-}$ mice on day 2 post-*C. difficile* infection. (A-D) Frequency of (A) ILC3s, ILC1s and ILC2s; (B) CCR6^{+}, CCR6^{-} NKp46 (NCR)$^{-}$ and NCR^{+} ILC3 subtypes; (C)

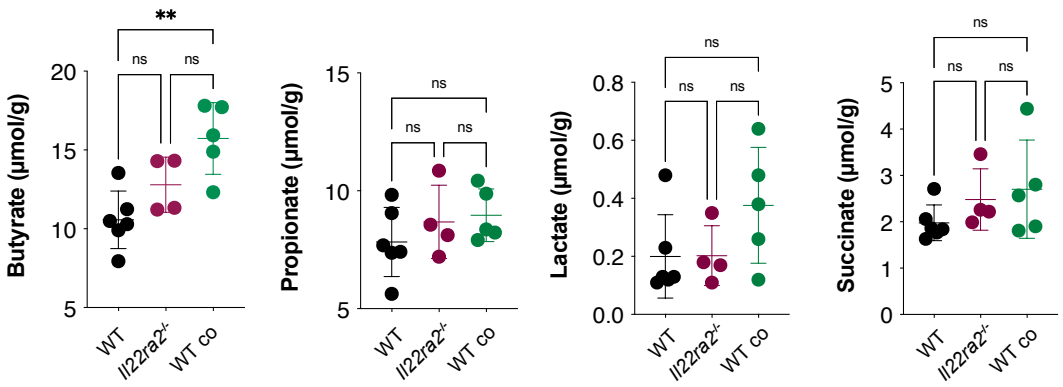
T cells, neutrophils, inflammatory monocytes, macrophages and dendritic cells; and (D) intraepithelial lymphocytes (including $CD4^+$ T cells, $CD8\alpha\alpha^+$ T cells, $CD8\alpha\beta^+$ T cells, and $\gamma\delta$ T cells). FACS plots show the gating strategy. n = 4-6 mice per group. One of two experiments is shown. Results from the second experiment are shown in **SI Appendix**, **Table S8**. Error bars represent mean ± SD. Normality was assessed by D'Agostino Pearson test; statistical analysis was performed using One-way ANOVA with post-hoc Tukey test. *$p < 0.05$.



SI Appendix, Figure S5. **Immune cells frequency in the colons of WT and *Il22ra2*$^{-/-}$ mice on day 4 post-*C. difficile* infection**. (A, B) Frequency of (A) ILC3s and ILC1s; (B) T cells, neutrophils, inflammatory monocytes and macrophages. N = 4-6 mice per group. One of three independent experiments is shown. Results from the other two experiments are reported in **SI Appendix**, **Table S8**. Error bars represent mean ± SD. Normality was assessed by D'Agostino Pearson test; statistical analysis was performed using One-way ANOVA with post-hoc Tukey test.



SI Appendix, Figure S6. Expression of intestinal antimicrobial peptides (AMP), inducible nitric oxide (*Nos2*) and fucosyltransferase 2 (*Fut2*) by *Il22ra2*$^{-/-}$ vs. WT mice. (A, B) Analysis of AMP mRNA expression in the distal small intestine (A) and proximal colon (B) of *Il22ra2*$^{-/-}$ mice relative to *Gapdh* and normalized by mRNA expression in WT mice (n = 3 mice per group). Data from one experiment are shown. Results from a second experiment are reported in **SI Appendix**, **Table S8**. Error bars represent mean ± SD. Unpaired multiple t-test analysis was performed comparing the relative expression of the indicated genes in the intestinal tissues of WT versus *Il22ra2*$^{-/-}$ mice. *p < 0.05; **p < 0.01; ***p < 0.001.



SI Appendix, Figure S7. Intestinal microbiota-derived SCFA in WT and *Il22ra2*$^{-/-}$ mice. Quantification of luminal SCFA in the proximal colon assessed by GC-MS. N = 4-6. Error bars represent mean ± SD. Normality was assessed by D'Agostino Pearson test; statistical analysis was performed using One-way ANOVA with post-hoc Tukey test. *p < 0.05; ns = not significant.

SI Appendix, Table S1. Clinical score for evaluating the severity of *C. difficile* infection in mice.

Category	Score*			
	0	1	2	3
Activity	Normal	Alert/Slow moving	Lethargic/Shaky	Inactive unless prodded
Posture	Normal	Back slanted	Hunched	Hunched/Nose down
Coat	Normal	Piloerection	Rough skin	Very ruffled Puff/Ungroomed
Diarrhea	Normal	Soft stool/Discolored (yellowish)	Wet stained tail/ mucus ± blood	Liquid/no stool
Eyes/Nose	Normal	Squinted $^1/_2$ closed	Squinted/Discharge	Closed/Discharge

*Clinical score = sum of all parameter scores. The total possible score was 15 (death).

SI Appendix, Table S2. Histopathological scoring system applied to colon.

Category		Score*			
		0 (none)	**1 (mild)**	**2 (moderate)**	**3 (severe)**
Mucosal epithelium	Ulceration	None	Mild surface	Moderate	Extensive full thickness
Crypts	Mitotic Activity	Lower third	Mild mid third	Moderate mild third	Upper third
	Mucus depletion	None	Mild	Moderate	Severe
Lamina propria	Mononuclear infiltrate	None	Mild	Moderate	Severe
	Granulocyte infiltrate	None	Mild	Moderate	Severe
	Vascularity	None	Mild	Moderate	Severe
	Fibrin deposition	None	Mucosal	Submucosal	Transmural
Sub-mucosal	Mononuclear infiltrate	None	Mild	Moderate	Severe
	Granulocyte infiltrate	None	Mild	Moderate	Severe
	Edema	None	Mild	Moderate	Severe

SI Appendix, Table S3. Goblet cell scoring.

SCORE	DESCRIPTION
0	Absent Alcian blue staining characterizing complete loss of goblet cells.
1	Faint Alcian blue staining characterizing marked-to-moderate multifocal-to-diffuse loss of goblet cells associated with low mucin content in the remaining goblet cells.
2	Moderate Alcian blue staining characterizing mild multifocal-to-diffuse loss of goblet cells associated with intermediate mucin content in the remaining goblet cells.
3	Intense Alcian blue staining characterizing adequate number of goblet cells associated with adequate mucin content in the goblet cells present (normal colonic mucosa).

SI Appendix, Table S4. qPCR primers.

Primer		Sequence (5' – 3')
***Universal Bacterial Primers* (rDNA 16S)**	Forward	CGGCAACGACGCCAACCC
	Reverse	CCATTGTAGCACGTGTGTAGCC
tcdB	Forward	ATATCAGAGACTGATGAG
	Reverse	TAGCATATTCAGAGAATATTGT
Il22	Forward	AGAATGTCAGAAGGCTGAAGG
	Reverse	AGGAGCAGTTCTTCGTTTTCTAG
Il22ra2	Forward	GAAGGTCCGATTTCAGTCCA
	Reverse	TCACCCTCCCGTAATACAGC
Defa1	Forward	TCAAGAGGCTGCAAAGGAAGAGAAC
	Reverse	TGGTCTCCATGTTCAGCGACAGC
Defa2	Forward	CCAGGCTGATCCTATCCAAA
	Reverse	GTCCCATTCATGCGTTCTCT
Defa21	Forward	CCAGGGGAAGATGACCAGGCTG
	Reverse	TGCAGCGACGATTTCTACAAAGGC
Defa-rs1	Forward	CACCACCCAAGCTCCAAATACACA
	Reverse	ATCGTGAGGACCAAAAGCAAATGG
Defb3	Forward	GTCTCCACCTGCAGCTTTTAG
	Reverse	ACTGCCAATCTGACGAGTGTT
Reg3g	Forward	AACAGAGGTGGATGGGAGTG
	Reverse	GGCCTTGAATTTGCAGACAT
S100a8	Forward	GGAAATCACCATGCCCTCTA
	Reverse	ATCACCATCCGAAGGAACTC
S100a9	Forward	GTCCAGGTCCTCCATGATGT
	Reverse	TCAGACAAATGGTGGAAGCA
Fut2	Forward	TGCACTGGCCAGGATGAA
	Reverse	GCGCTAGAGCGTTGTGCAT
Nos2	Forward	CCAAGCCCTCACCTACTTCC
	Reverse	CTCTGAGGGCTGACACAAGG
Gapdh	Forward	CATCACTGCCACCCAGAAGACTG
	Reverse	ATGCCAGTGAGCTTCCCGTTCAG

SI Appendix, Table S5 - Antibodies for analysis by flow cytometry.

ANTIBODY	SOURCE	CLONE	IDENTIFIER
Anti-mouse CD45 APC/Cy7	BioLegend	30-F11	Cat# 103116
Anti-mouse CD3, PercP-Cy5.5	BioLegend	17A2	Cat# 100218
Anti-mouse CD5, PercP-Cy5.5	BioLegend	53-7.3	Cat# 100624
Anti-mouse CD19, PercP-Cy5.5	BioLegend	6D5	Cat# 115534
Anti-mouse KLRG1, PE	BioLegend	2F1/KLRG1	Cat# 138408
Anti-mouse CD90.2 (Thy-1.2) APC	BioLegend	53-2.1	Cat# 140312
Anti-mouse CD335 (NKp46), PE-Cy7	BioLegend	29A1.4	Cat# 137618
Anti-mouse CD196 (CCR6), BV421	BioLegend	29-2L17	Cat# 129818
Anti-mouse NK1.1, FITC	BioLegend	S17016D	Cat# 156508
Anti-mouse CD11b, PE	BioLegend	M1/70	Cat# 101208
Anti-mouse CD3, APC	BioLegend	17A2	Cat# 100236
Anti-mouse Ly6C, BV421	BioLegend	HK1.4	Cat# 128032
Anti-mouse MHC2, PercP-Cy5.5	BioLegend	M5/114.15.2	Cat# 107624
Anti-mouse Ly6G, FITC	BioLegend	1A8	Cat# 127605
Anti-mouse TCR γ/δ, PE	BioLegend	GL3	Cat# 118108
Anti-mouse CD8a, PE-Cy7	BioLegend	53-6.7	Cat# 100722
Anti-mouse CD8b, FITC	BioLegend	YTS156.7.7	Cat# 126605
Anti-mouse CD4, APC	BioLegend	GK1.5	Cat# 100412
Anti-mouse NK1.1, BV421	BioLegend	PK136	Cat# 108741
Live/Dead™ Fixable Aqua Dead Cell Stain Kit, BV510	Invitrogen ™	-	Cat# L34957

SI Appendix, Dataset S1 (separate file). 13 Amplicon Sequence Variants (ASVs).

SI Appendix, Dataset S2 (separate file). V4 16S gene sequences of three ASVs aligned against the NCBI 16S ribosomal DNA database.

SI Appendix, Dataset S3 (separate file). Additional results related to experiments shown in Fig. 1, 5, S4, S5 and S6.

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