STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit Anti-Rotavirus Group-A Ab	Biorad	Cat.# AHP1360
Hyperimmune guinea pig anti-RRV serum	Prfal	N/A
HRP Donkey Anti-Guinea Pig Antibody	Jacksonimmuno	Cat.# 706-035-148
IL-22 Neutralizing Ab	Genentech	N/A
IL-17 Neutralizing Ab	Genentech	N/A
IFN-λ Neutralizing Ab	R&D	Cat.# MAB17892
Villin-1 Antibody	Cell Signal	Cat.# 2369
Donkey Anti-Guinea Pig IgG	Jacksonimmuno	Cat.# 706-586-148
Donkey Anti-Rabbit IgG	Jacksonimmuno	Cat.# 711-095-152
Rabbit IgG HRP Linked Whole Ab	GE Healthcare	Cat.# NA934V
Bacterial and Virus Strains		
Murine RV EC strain	Dr. Mary Estes	N/A
Cell-culture adapted RRV	Dr. Mary Estes	N/A
Reovirus strain type 1 Lang	Dr. Terence Dermody	N/A
Influenza virus A/WSN/1933 (H1N1)	Dr. Richard Plemper	N/A
Recombinant vesicular stomatitis virus	Dr. Richard Plemper	N/A
SFB (Pasteur)	Dr. Nadine Cerf-Bensussan	N/A
SFB (GSU)	This paper	N/A
Chemicals, Peptides, and Recombinant Proteins		
TMB ELISA Substrate Solution	Invitrogen	Cat.# 00420156
TMB Stop Solution	KPL	Cat.# 50-85-04
Ampicillin	Sigma	Cat.# A6140
Neomycin	Sigma	Cat.# N6386
Streptomycin	Sigma	Cat.# S9137
Kanamycin	Sigma	Cat.# K1377
amphotericin	Sigma	Cat.# A9528
5-fluorocytosine	Sigma	Cat.# F7129
Fluconazole	Sigma	Cat.# F8929
Western blot detection reagents	GE healthcare	Cat.# RPN2106V1
Critical Commercial Assays		
Whole bacterial genome sequencing	CHOP Microbiome	N/A
RNA-seq (ileum)	GA Tech	N/A
16S rRNA Sequencing	Cornell University	N/A
DNeasy Blood & Tissue Kit	QIAGEN	Cat.# 69504
QIAamp DNA Stool Mini Kit	QIAGEN	Cat.# 51504
Biorad iScriptTM One-Step RT-PCR Kit	Biorad	Cat.# 170-8893
QuantiFast SYBR Green PCR Kit	QIAGEN	Cat.# 204054
TRIzol Reagent	Thermofisher	Cat.# 15596026
Lamina Propria Dissociation Kit	Miltenyi biotec	Cat.# 130-097-410
Deposited Data		
Raw 16S sequencing data	ENA	PRJEB34108
Raw SFB genome sequencing data	ENA	PRJEB34149
Raw ileum RNA-seq data	ENA	PRJEB34133

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Cell Lines		
HT-29	ATCC	Cat.# HTB38
MA-104	ATCC	Cat.# CRL2378.1
Experimental Models: Organisms/Strains		
Mouse: WT C57BL/6	Jackson Laboratories	Cat. # 000664
Mouse: WT C57BL/6 (SFB positive)	Taconic biosciences	Cat. # B6NTac MPF
Mouse: WT C57BL/6 (SFB negative)	Taconic biosciences	Cat. # B6NTac EF
Mouse: Rag1-KO	Jackson Laboratories	Cat. # 002216
Mouse: IFNαR1-KO	MMRRC	Cat. # 032045-JAX
Mouse: Il2rγ-KO	Jackson Laboratories	Cat. # 003174
Mouse: Rag1/Il2rγ-DKO	This paper	N/A
Mouse: Tcrβ-KO	Jackson Laboratories	Cat. # 002118
Mouse: Ighm-KO	Jackson Laboratories	Cat. # 002288
Mouse: Rag1/IFN-λ-R1-DKO	Dr. Megan Baldridge	N/A
Mouse: GF C57BL/6	Taconic farms	Cat. # B6NTac GF
Mouse: GF Swiss-Webster	Taconic farms	Cat. # Tac:SW GF
Mouse: GF Rag1-KO	Dr. Lora Hooper	N/A
Oligonucleotides		
EC.C 5′-GTTCGTTGTGCCTCATTCG-3′ and 5′-TCGGAA CGTACTTCTGGAC-3′	Invitrogen	N/A
36B4 5′-TCCAGGCTTTGGGCATCA-3′ and 5′-CTTTATT CAGCTGCACATCACTCAGA-3′	Invitrogen	N/A
IFN-λ 5′-AGCTGCAGGCCTTCAAAAAG-3′ and 5′-TGGGA GTGAATGTGGCTCAG-3′	Invitrogen	N/A
IL-22 5′-GTGCTCAACTTCACCCTGGA-3′ and 5′-TGGAT GTTCTGGTCGTCACC-3′	Invitrogen	N/A
IL-17 5′-TGAGCTTCCCAGATCACAGA 3′ and 5′ TCCAG AAGGCCCTCAGACTA 3′	Invitrogen	N/A
16S rRNA: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-CT GCTGCCTCCCGTAGGAGT-3′	Invitrogen	N/A
16S rRNA sequencing: 5′-AATGATACGGCGACCACCGA GATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGG TAA-3′ and 5′-CAAGCAGAAGACGGCATACGAGATXXX XXXXXXXXXAGTCAGTCAGCCGGACTACHVGGGTWTC TAAT-3′	Invitrogen	N/A
IFN-γ 5′- GTCTCTTCTTGGATATCTGGAGGAACT-3′ and 5′ GTAGTAATCAGGTGTGATTCAATGACGC −3′	Invitrogen	N/A
SFB: 5′-GACGCTGAGGCATGAGAGCAT-3′ and 5′-GACG GCACGGATTGTTATTCA-3′	Invitrogen	N/A
Reovirus 5′-CGCTTTTGAAGGTCGTGTATCA-3′ and 5′-CTGGCTGTGCTGAGATTGTTTT-3′	Invitrogen	N/A
Software and Algorithms		
FlowJo	BD	https://www.flowjo.com/
Microbial Ecology software	QIIME	http://qiime.org/
ImageJ	NIH	https://imagej.nih.gov/ij/
Prism	GraphPad	https://www.graphpad.com/
FastQC	Babraham Bioinformatics	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Galaxy	This paper	https://usegalaxy.org/

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
HISAT2	Johns Hopkins university center for computational biology	https://ccb.jhu.edu/software/hisat2/
HTSeq	Stanford University and EMBL Heidelberg	https://htseq.readthedocs.io/en/release_0.11.1/
Sunbeam pipeline	Clarke et al., 2019	https://sunbeam.readthedocs.io/en/latest/
BWA	Li and Durbin, 2009	http://bio-bwa.sourceforge.net/
SPAdes	Bankevich et al., 2012	http://cab.spbu.ru/files/release3.11.1/manual.html
CheckM	Parks et al., 2015	https://ecogenomics.github.io/CheckM
Patric	Wattam et al., 2014	https://www.patricbrc.org/
FastTree	Price et al., 2009	http://www.microbesonline.org/fasttree/
Roary	Page et al., 2015	https://sanger-pathogens.github.io/Roary/
RStudio	RStudio, Inc.	https://www.rstudio.com/

LEAD CONTACT AND MATERIALS AVAILABILITY

The mouse line, Rag1/IL2Rγ-DKO was generated and remains housed at GSU. These mice, feces from GSU-RAG and SFB-G mono-associated mice are available subject to a standard academic MTA. Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Andrew T. Gewirtz (agewirtz@gsu.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

All mice used in this study were 6 to 8-weeks-old adult mice of C57BL/6 background, unless otherwise specified (diarrhea studies used 7-8 days old mice, early FT studies used 3-weeks-old mice). All individual experiments used gender-matched mice with overall study using approximately similar amounts of male and female mice. Breeding colonies of WT C57BL/6 and Rag1-KO (RAG) and IFNαR1-KO, and Il2rγ-KO were established from mice purchased from Jackson Laboratories (Bar Harbor, ME) and maintained at GSU by vivarium staff following American Association for Laboratory Animal Care procedures. Mice referred to as ''JAX-RAG'' were bred at GSU using autoclaved cages, chow (LabDiet 5010/5021) and water by the authors of this manuscript. C57 BL/6 Tcrβ-KO, Ighm-KO mice (commonly referred to as μMT) were not bred at GSU but purchased directly from Jackson Labs and used for experiments within two weeks of arrival at GSU. Rag1/IFN-λ-R1-DKO, along with control Rag1-KO mice, were bred at Wash-U. GF mice (C57BL/6 and Swiss-Webster) were purchased from Taconic Biosciences (Rensselaer, NY). GF C57BL/6 Rag1-KO mice were generously provided by Dr. Lora Hooper (UT Southwestern). GF mice were maintained in glovebox isolators (Park Bioservices, Groveland MA) and IsoCages (Techniplast Inc. Buguggiate Italy). Genetic authenticity of all KO mice breeding colonies was confirmed by a commercial provider (Transnetyx, Memphis TN). All experiments using mice were conducted under the surveillance of the Georgia State University Institutional Animal Care and Use Committee. As part of such surveillance, sentinel mice that were exposed to used bedding from GSU-RAG mice were subjected to serologic testing by IDEXX Analytics (Columbia MO), specifically their ''Edx Comprehensive Profile,'' and found to be negative for all microbes except mouse norovirus, which is commonly present in many mouse vivaria.

Viruses

Murine RV EC strain, which was used for all mouse studies, and cell-culture adapted RRV, which was used for *in vitro* infections, were propagated and titered as previously described (Burns et al., 1995; Zhang et al., 2014). Mouse RV strain EC was kindly provided by Dr. Mary Estes (Baylor College of Medicine). Reovirus strain type 1 Lang (T1L) was propagated as described (Brown et al., 2018). Recombinant vesicular stomatitis virus harboring a nano luciferase ORF as an additional transcription unit and influenza virus A/WSN/1933 (H1N1) encoding nano luciferase in the NS1 segment were engineered and propagated as described (Yan et al., 2015; Yoon et al., 2018). Viral titers were determined by plaque assays using Vero-E6 and MDCK cells, respectively.

Cell lines

HT29 (HTB38) and MA-104 (CRL2378.1) cell lines were obtained from ATCC® and maintained with 10% heat-inactivated FBS (cellgro 35-010-CV) in DMEM (cellgro 10-013-CV).

METHOD DETAILS

Virus Infection

Acute RV infection: Age and sex-matched mice were orally administered 100 μL 1.33% sodium bicarbonate (sigma S5761) followed by oral inoculation with 10^4-10^5 SD50 of murine rotavirus EC strain in 100 μL PBS. Chronic RV infection: 3-week-old Rag1-KO mice were orally administered 100 μL 1.33% sodium bicarbonate (sigma S5761) followed by oral inoculation with 10^5 SD50 in 100 μL PBS. RV fecal antigens were measured by specific ELISA 3-weeks post RV challenge (4) (Jiang et al., 2008). Diarrhea model: 8-day-old pups were inoculated with 500 SD50 in 50 μL PBS. Acute reovirus infection: mice were orally inoculated with 1-4 × 10^8 PFU of reovirus strain T1L in 200 μL PBS (5) (Johansson et al., 2007). *In vitro* RRV infection: HT-29 cells were washed with serum-free RPMI-1640 medium 3 times and incubated in serum-free RPMI-1640 medium for 1 hour before viral challenge. RRV was trypsin-activated (10 μg/ml) for 1 hour in 37°C water bath. Then, virus media of a MOI = 1 was prepared in serum-free RPMI-1640 (cellgro 10-040) and used to inoculate the HT-29 cells. 1-hour post-inoculation, the viral media was removed, and fresh serum-free RPMI-1640 was applied to the infected cells (Frias et al., 2010). For virus treatment with microbiota, trypsin-treated RRV or reovirus were incubated for 4 hours at 37°C with 5 μm- or 0.2 μm-filtered mice feces; such RV-microbiota suspensions were passed through 0.2 μm filters to remove bacterial cells and then used for *in vitro* and *ex vivo* experiments. VSV and IAV WSN were co-incubated with 5 μm-prefiltered mouse feces for 4 hours at 37°C, followed by 0.2 μm filtration and transfer of the filtrates to human respiratory BEAS-2B cells in a 96-well plate format. After 24-hour incubation at 37°C, luciferase activities were determined in a Synergy H1 microplate reader (BioTek) using Nano-Glo luciferase substrate (Promega). The substrate was directly added to each well and bioluminescence quantified after a 3-minute incubation for signal stabilization.

RV-Antigen-Specific ELISA

A sandwich enzyme-linked immunosorbent assay (ELISA) was performed to detect rotavirus antigen in mouse feces as previously described (Zhang et al., 2014). Briefly, mouse Fecal Rotavirus shedding was analyzed via Sandwich Elisa. Ninety-six well EIA/RIA plates (Costar, 3590) were coated with Rabbit Anti-Rotavirus Group-A (Biorad, AHP1360) capture antibody at 1:1000 dilution in PBS overnight RT, and subsequently blocked by 1% BSA PBS. Mouse fecal homogenates were prepared at 100 mg/mL concentration and centrifuged at 10,000 g to remove debris. Supernatants of the homogenates were then incubated in test tubes with serial dilutions of stock mouse Rotavirus utilized as control. Hyperimmune guinea pig anti-RRV serum (Prfal) at 1:1000 dilution in 1% BSA PBS was incubated as detection antibody followed by the incubation of HRP Donkey Anti-Guinea Pig Antibody (Jackson ImmunoResearch, 706-035-148) secondary antibody at 1:10000 dilution in 1% BSA PBS. All incubation steps following capture antibody were at one hour at room temperature. TMB ELISA Substrate Solution (Invitrogen, 00420156) was utilized to develop the signal, followed by addition of TMB Stop Solution (KPL, 50-85-04) after ten minutes of substrate incubation. OD readings were taken at 450nm with 540nm OD subtracted as a correction (Zhang et al., 2014).

Microbiota Transplantation

Donor fecal samples were freshly collected and then suspended in 20% glycerol PBS (21-040-CV) solution at a concentration of 20 mg/ml, passed through a 5 μm filter, aliquoted, and stored in −80°C. Such frozen fecal suspensions were administered to recipient mice by oral gavage using 400 μL per adult mouse and 50 μL per suckling mice. Adult recipients received a single transplant at 6-8 weeks of age. Suckling mice received transplants 2 and 7 days after birth at which times their dams were transplanted via oral gavage and spread of fecal suspension (200 μl) on their belly fur, as a potential means of reducing incidence of maternal rejection of their pups. For antibiotic treatment, before FT, fecal microbiota was first prepared as described above, and then incubated with Ampicillin Sigma A6140 50 mg/ml, Neomycin Sigma N6386 50 mg/ml, Streptomycin Sigma S9137 50 mg/ml, Kanamycin Sigma K1377 50mg/ml, or the 4 antibiotics combined at 37°C for 4 hours. For anti-fungal treatment, before FT, fecal microbiota was first prepared as described above, and then incubated with anti-fungal cocktail (amphotericin Sigma A9528 2 mg/ml, 5-fluorocytosine Sigma F7129 2 mg/ml, fluconazole Sigma F8929 2 mg/ml) at 37°C for 4 hours.

Antibiotic and Antifungal Drinking Water Treatment

Antibiotic cocktail drinking water (ampicillin, Sigma A6140, 1 g/L, neomycin, Sigma N6386, 1 g/L, streptomycin, Sigma S9137, 1g/L, and kanamycin, Sigma K1377, 1g/L) and/or anti-fungal cocktail drinking water (amphotericin, Sigma A9528, 0.2 g/L, fluconazole, Sigma F8929, 0.5 g/L, and 5-fluorocytosine, Sigma F7129, 0.5 g/L) were prepared and provided for consumption for 3 weeks before RV inoculation.

Neutralization of IL-17, IL-22, and IFN-λ

IL- 17 (100 μg), IL-22 (150 μg) neutralizing mAb (Genentech, Inc) were administered by intraperitoneal inoculation shortly prior to RV inoculation and thereafter every other day until day 10 post-infection. Neutralizing mAb against IFN-λ (R&D MAB17892, 1 μg/gram of body weight) was administered by intraperitoneal inoculation to mice one day prior to RV inoculation or fecal transplantation and continued for the entire experiment. The dosing regimen of these antibodies neutralizes bioactivity of these cytokines (Basu et al., 2012; Crotta et al., 2013; Kezic et al., 2012).

Fluorescence Immunohistochemistry

Intestine tissue was immediately isolated after euthanasia and imbedded into O.C.T. compound on dry ice. The samples were then sliced into 6 μm thickness onto glass slides, which were then incubated in 4% paraformaldehyde for 15 minutes, followed by 5 minutes washing of PBS twice. The slides were then incubated in 100% methanol for 5 minutes, followed by 5 minutes PBS washing 3 times. Then, 3% BSA-PBS was used to block the samples for 1 hour at RT. The slides were then washed with PBS for 5 minutes, followed by incubation of primary antibodies cocktail (Villin-1 Antibody, Cell Signal 2369 1:100, Hyperimmune guinea pig anti-RRV serum, Prfal, 1:100), in 1% BSA-PBS overnight at 4°C. The slides were then washed 3 times with PBS, 5 minutes each time. Then, secondary antibodies cocktail (Jacksonimmuno 706-586-148 Amax: 591 nm Emax: 614 nm 1:500; 711-095-152 Amax: 492 nm Emax: 520 nm) 1%BSA-PBS was applied for 1-2 hours at RT. The samples were then 3 X washed with PBS. Images were generated using a UV microscope under the relative excitation wavelengths as given by the manufacturer.

H&E and BrdU Staining

Mouse ileum was fixed in 10% buffered formalin and then embedded in paraffin. The tissues were sectioned at 5 μm thickness and stained with hematoxylin and eosin (H&E). For BrdU staining, the mice were I.P. injected with BrdU (50 μg of BrdU/g). One day post-injection, ileum was harvested and embedded in OCT. The tissues were divided into 5 μm sections and fixed with 4% formaldehyde for 30 min at room temperature. Villi and crypts, as well as epithelial cell migration and villi length, were determined using ImageJ.

Bacterial and Viral Binding/Degradation Assay

Fecal samples from mice were collected and suspended in HEPES (20mM)-Glycerol (20%)-PBS into a concentration of 10mg/ml. The fecal suspensions were then filtered through 5 μm filter to remove large food debris. Purified RRV was then incubated with the fecal filtrate for various times, and total RNAs were extracted for RV genome detection.

qRT-PCR

Intestine tissue was homogenized in TRIzol Reagent, and mRNA was isolated according to the manufacturer's instruction (Thermofisher 15596026). Intestine DNA was also isolated using DNeasy Blood & Tissue Kits (QIAGEN 69504). Stool bacterial DNA was isolated with QIAamp DNA Stool Mini Kit (QIAGEN 51504). Stool total RNA was isolated using RNeasy PowerMicrobiome Kit (Mobio 26000-50), and qRT-PCR was performed using the Biorad iScriptTM One-Step RT-PCR Kit with SYBR green (Bio-Rad 170-8893), and qPCR was performed with QuantiFast SYBR Green PCR Kit (QIAGEN 204054) in Bio-Rad CFX96 apparatus (Bio-Rad 170-8892). For quantitation of luminal enterocytes (i.e., cell shedding), the ileum content was collected. Total RNA was then extracted, and qRT-PCR was performed on the 36B4 gene to determine relative shedding for different groups. The sense and antisense oligonucleotides primers used were:

EC.C 5′-GTTCGTTGTGCCTCATTCG-3′ and 5′-TCGGAACGTACTTCTGGAC-3′
36B4 5′-TCCAGGCTTTGGGCATCA-3′ and 5′-CTTTATTCAGCTGCACATCACTCAGA-3′
IFN-λ 5′-AGCTGCAGGCCTTCAAAAAG-3′ and 5′-TGGGAGTGAATGTGGCTCAG-3′
IL-22 5′-GTGCTCAACTTCACCCTGGA-3′ and 5′-TGGATGTTCTGGTCGTCACC-3′
IL-17 5′-TGAGCTTCCCAGATCACAGA 3′ and 5′ TCCAGAAGGCCCTCAGACTA 3′
16S rRNA: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-CTGCTGCCTCCCGTAGGAGT-3′
16S rRNA sequencing:
5′-AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3′ and 5′-CAAGCAGAAGACG GCATACGAGATXXXXXXXXXXXXAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3′
IFN-γ 5′- GTCTCTTCTTGGATATCTGGAGGAACT-3′ and 5′ GTAGTAATCAGGTGTGATTCAATGACGC –3′
SFB: 5′-GACGCTGAGGCATGAGAGCAT-3′ and 5′-GACGGCACGGATTGTTATTCA-3′
Reovirus 5′-CGCTTTTGAAGGTCGTGTATCA-3′ and 5′-CTGGCTGTGCTGAGATTGTTTT-3′

The quantification results were analyzed to relative housing keeping genes (36B4) or feces weight, as previously described (Zhang et al., 2014).

Lamina Propria Transplantation and Flow Cytometry Analysis

Mouse intestinal lamina propria cells were isolated using a Lamina Propria Dissociation Kit (130-097-410) purchased from Miltenyi biotec (Bergisch, Gladbach, Germany) according to the manufacturer's protocol. The prepared cells were then injected into recipient mice intravenously. For flow cytometry, the prepared cells were then blocked with 1 μg/million cells 2.4G2 (anti-CD16/anti-CD32 ATCC) in 100 μL FACS buffer for 5 minutes at 4°C, followed by 1-time wash of FACS buffer to remove residue 2.4G2 and then incubated in conjugated mAbs Alexa Fluor 700-CD45.2, FITC- CD103, PE- F4/80, PerCP-cy5.5- CD11b, APC-MHC class II, PE-Cy7-CD11c, and Pacific blue-CD103 (BD Biosciences). The labeled cells were then washed 3 times in FACS buffer and then analyzed using a Becton Dickinson LSR II flow cytometer. Data were analyzed using FlowJo (TreeStar, Ashland, OR) (Zhang et al., 2014).

SDS-PAGE and Immunoblotting

Cells were harvested in RIPA buffer. Rabbit anti-rotavirus group-A (Biorad, AHP1360) at 1/1000 dilution in blocking buffer was used as a primary antibody. 2nd antibody (GE healthcare NA934V) was used at 1/5000 dilution to detect the primary antibody. Western blot membrane was developed using detection reagents (GE healthcare RPN2106V1) (Zhang et al., 2014).

16S rRNA Sequencing

Bacterial 16S rRNA sequencing was performed as previously described (Chassaing et al., 2015). Briefly, fecal microbiome DNA was extracted with QIAamp DNA Stool Mini Kit (QIAGEN 51504). Then, the stool DNA was amplified by 4 rounds of PCR targeting the V4 region located within 16S rRNA gene. The intensified examples were then consolidated and purified with Ampure attractive XP beads (Beckman/Agencourt). The samples were then evaluated using gel electrophoresis for examining sample quality and quantified by utilizing a Quant-iT PicoGreen dsDNA assay (BIOTEK Fluorescence Spectrophotometer). The generated DNA pool was then sequenced on Illumina MiSeq sequencer with paired-end reads (Cornell University, Ithaca). The sequencing data was analyzed using Quantitative Insights into Microbial Ecology software (QIIME, version 1.8.0) (Chassaing et al., 2015).

SFB Genome Sequencing and Analysis

SFB genome sequencing was performed by The CHOP Microbiome Center. Briefly, DNA was extracted from cecal and fecal material using the DNeasy PowerSoil kit (QIAGEN, Germantown, MD). Sequencing libraries were generated using the Nextera XT DNA Library Preparation Kit and were sequenced on the HiSeq 2500 using 2x125 bp chemistry (Illumina, San Diego, CA). Raw sequencing data were processed using the Sunbeam pipeline (Clarke et al., 2019), including quality control, removing host sequences, and mask low-complexity regions. Due to the high host mouse DNA percentage, we merged the processed reads from 2 cecum samples and 1 fecal sample for better coverage of the SFB genomes. We then aligned reads to all 13 SFB genomes in NCBI and collected all the mapped reads, using BWA (Li and Durbin, 2009), as "SFB reads," and did *de novo* assembly using Spades (Bankevich et al., 2012). To evaluate the assembled contigs, we analyzed all the available SFB genomes on NBCI and as well as the assembled draft genomes for GSU and Pasteur-SFB strains using CheckM (Parks et al., 2015), and the estimated completeness for our assembled Pasteur and GSU-SFB strains are 99.01% and 93.89% (five reference genomes were removed from the phylogenetic analysis due to poor genome quality and completeness < 90%). Comparative genome analysis: Pairwise proteome comparison was carried out using Patric (Wattam et al., 2014). The pangenome analysis was carried out for two assembled genomes and eight SFB genomes using Roary (Page et al., 2015). The phylogenetic tree was built on the core genomes using FastTree (Price et al., 2009).

Ileum RNaseq

Total RNA from ileum was extracted using TRIzol Reagent (Thermofisher 15596026) and was further purified using QIAGEN RNeasy MinElute Cleanup Kit (Cat No./ID: 74204). The prepared RNA samples were then sent to Molecular Evolution Core of Georgia Institute of Technology for library preparation and sequencing on the NextSeq instrument utilizing a high output 2x75 bp run. FASTQC was used for sequencing reads quality screening. The RNaseq data were then analyzed using the Galaxy server. Briefly, the alignment of RNaseq data was performed using HISAT2, and the aligned data were then assigned to the host genome (Ensembl mouse GTF) using HTSeq. The processed data were then analyzed on software R for preparations of linear gene expression comparison, PCA, and heatmap figures.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data were plotted in GraphPad Prism (La Jolla, CA). Statistical significance was assessed by 2-way ANOVA, 1-way ANOVA, Student's t test, and Chi-square analysis.

DATA AND CODE AVAILABILITY

Unprocessed 16S sequencing data are deposited in the European Nucleotide Archive under accession numbers PRJEB34108. Unprocessed raw SFB genome sequencing data are deposited in the European Nucleotide Archive under accession numbers PRJEB34149. Unprocessed raw Ileum RNaseq sequencing data are deposited in the European Nucleotide Archive under accession numbers PRJEB34133.

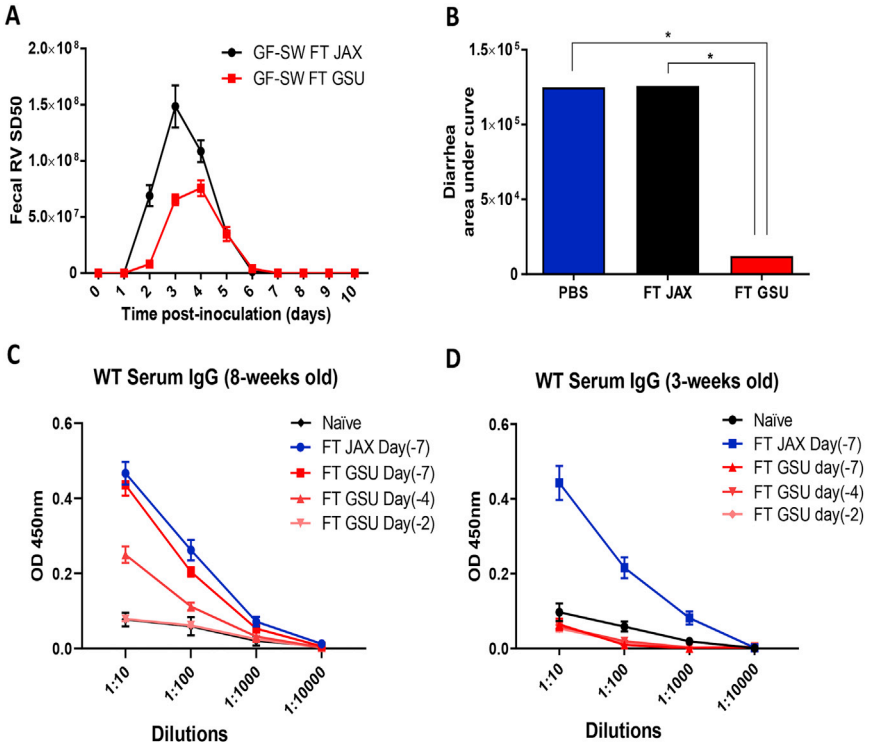


Figure S1. GSU Rag1-KO Mouse Fecal Microbiota Partially Protects GF Swiss-Webster Mice from RV Infection, Related to Figure 2

(A) Two groups of GF Swiss-Webster mice groups received FT from JAX or GSU Rag1-KO mice. One-week post FT, the 2 groups of mice were challenged with RV. Feces were collected daily and fecal RV antigens measured by ELISA.

(B) Seven-day-old WT C57BL/6J mice pups received PBS or FT from JAX or GSU Rag1-KO mice twice 6 d and 1 d prior to RV inoculation. Result shows the area under curve of incidence of diarrhea.

(C-D) Groups of (C) 8-week-old or (D) 3-week-old WT C57BL/6 mice received FT from JAX one week prior to RV inoculation or FT from GSU Rag1-KO mice 7 days, 4 days, or 2 days prior to RV inoculation. Serum was collected on day 14 post RV inoculation and RV antigen-specific IgG measured by ELISA (Naive mice were WT mice not subjected to any treatment).

Results are shown as mean ± SEM (two-way ANOVA), A; n = 4, $p < 0.0001$. C-D; C, n = 3, D, n = 5 $p < 0.0001$ for all groups relative to FT JAX (control). B; FT GSU differed significantly from both FT JAX and the control (PBS) groups by $\chi 2$ test, n = 12, *$p < 0.01$ by $\chi 2$ test.

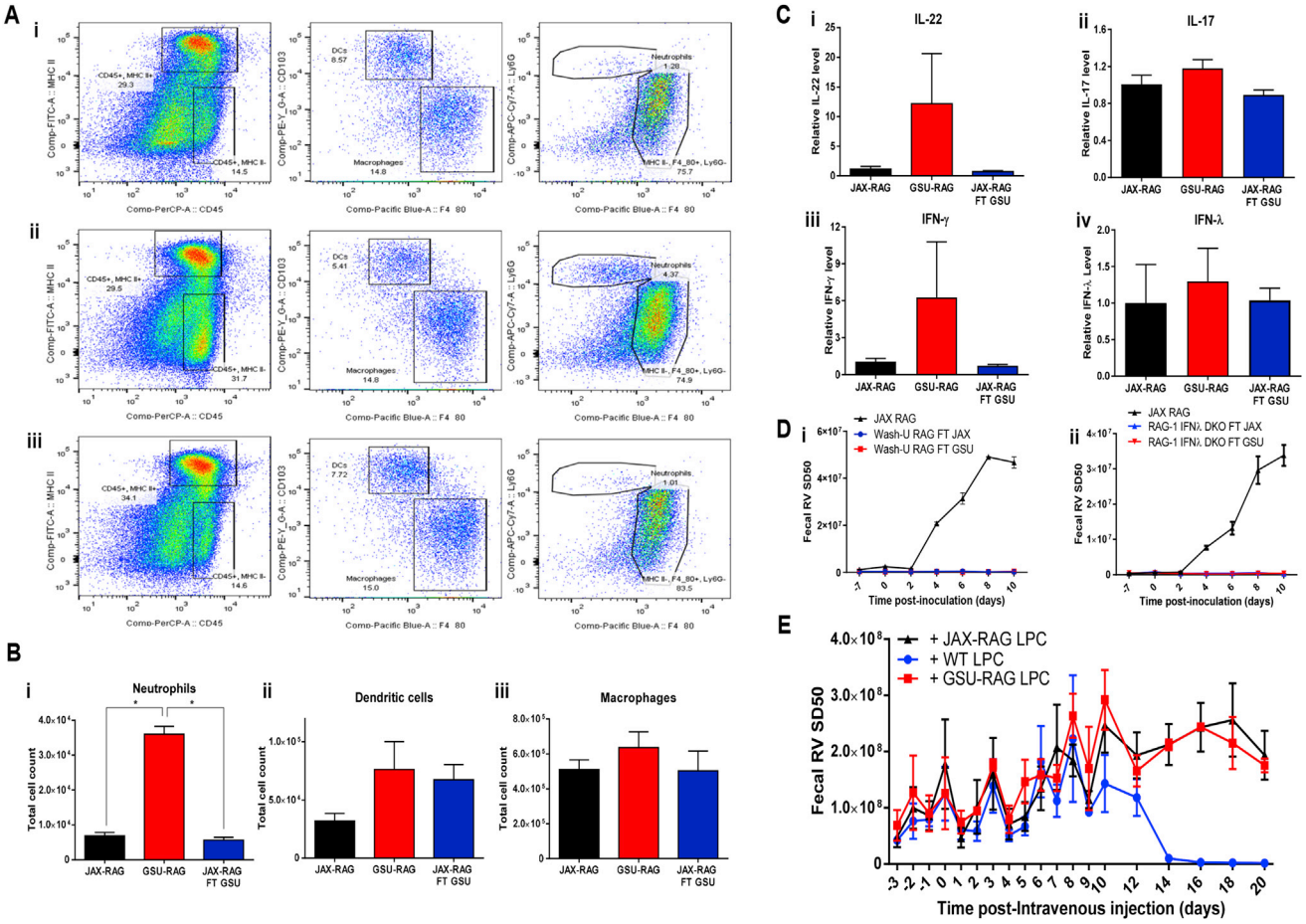


Figure S2. GSU Rag1-KO Mouse Microbiotas Do Not Induce Significant Anti-Viral Immune Responses in the Small Intestines of Recipient Rag1-KO Mice after FT, Related to Figure 2

(A-C) FT JAX Rag1-KO mice from donor JAX or GSU Rag1-KO mice for 1 week. Lamina propria cells and Ileum were collected 7 days post-FT, and cell population and cytokines expression levels were assayed by flow cytometry and qRT-PCR, respectively. (A) Lamina propria cells flow cytometry of JAX Rag1-KO mice, GSU Rag1-KO mice, and recipient JAX Rag1-KO mice that transferred with GSU Rag1-KO mice fecal samples. (i) JAX Rag1-KO mice, (ii) GSU Rag1-KO mice, and (iii) recipient JAX Rag1-KO mice. (B) Cell populations in lamina propria. (i) Neutrophils (ii) Dendritic cells (iii) Macrophages. (C) Cytokine mRNA levels in the recipient mice ileum. (i) IL-22 (ii) IL-17 (iii) IFN-γ (iv) IFN-λ.

(D) RV infection test of Rag1-KO mice obtained from JAX, or Rag1-KO mice (i) and Rag1-IFN-λ DKO mice (ii) (bred at Washington University) transplanted with either JAX-RAG or GSU-RAG microbiota.

(E) Lamina propria cells from GSU Rag1-KO mice did not reduce chronic RV shedding after I.V. injection. Three groups of RV chronically infected Rag1-KO mice received I.V. injection with total lamina propria cells from JAX Rag1-KO mice, WT C57BL/6J mice, or GSU Rag1-KO mice.

Results are shown as mean ± SEM. B-C; Student's t test, n = 3, *$p < 0.01$. D-E; two-way ANOVA, n = 4, $p < 0.0001$. Each experiment was performed 2-3 times and yielded an identical pattern of results.

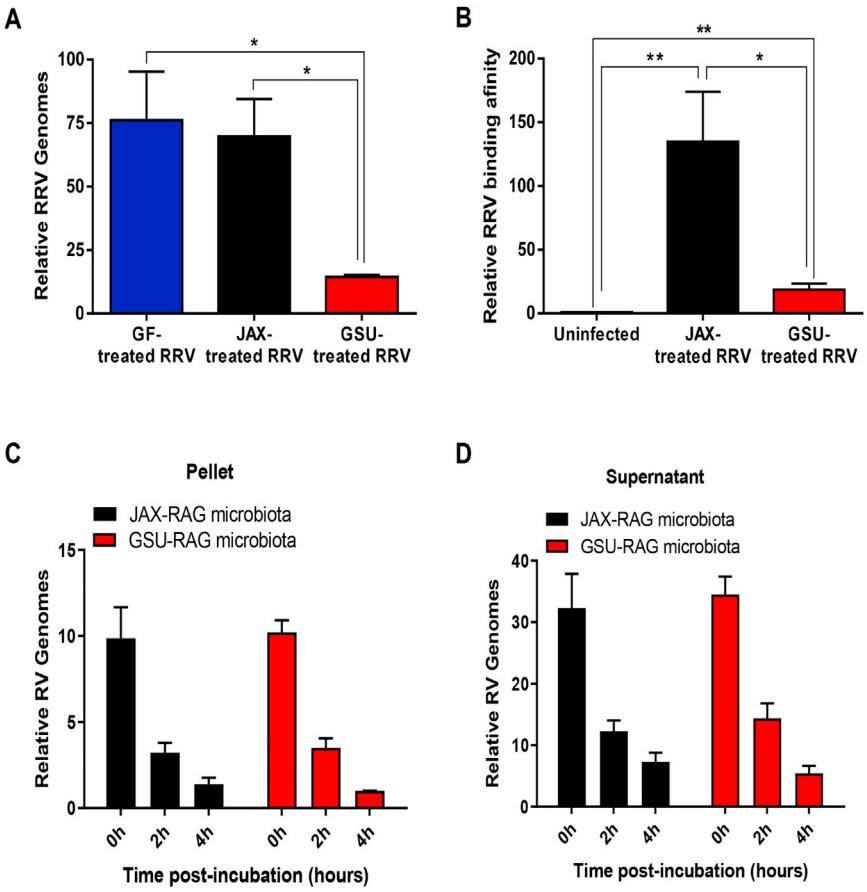


Figure S3. The GSU-RAG Microbiota May Directly Interact with Viruses to Reduce Infectivity, Related to Figure 3

(A-B) RRV viral particles were pre-incubated with 5μm filtered GF, JAX or GSU Rag1-KO mice fecal samples at 37°C for 4 hours. The mixtures were then filtered through 0.2μm to remove the bacteria cells and then used for *in vitro* studies. (A) qRT-PCR results at 20 hours post-inoculation. (B) qRT-PCR results at 1-hour post-inoculation of RRV with HT-29 cells at 4°C.

(C-D) RRV viral particles were incubated with JAX-RAG or GSU-RAG microbiota at 37°C. The mixtures were then centrifuged at 800 g for 10 mins to pellet bacteria, and viral genomes in the pellet (C) and supernatant (D) quantified by qPCR at 0 h, 2 h, and 4 h. Results are shown as mean ± SEM. n = 3 for A,C, and D, n=4 for B. *$p < 0.05$, **$p < 0.01$) by Student's t test. Each experiment was performed 2-3 times and yielded an identical pattern of results.

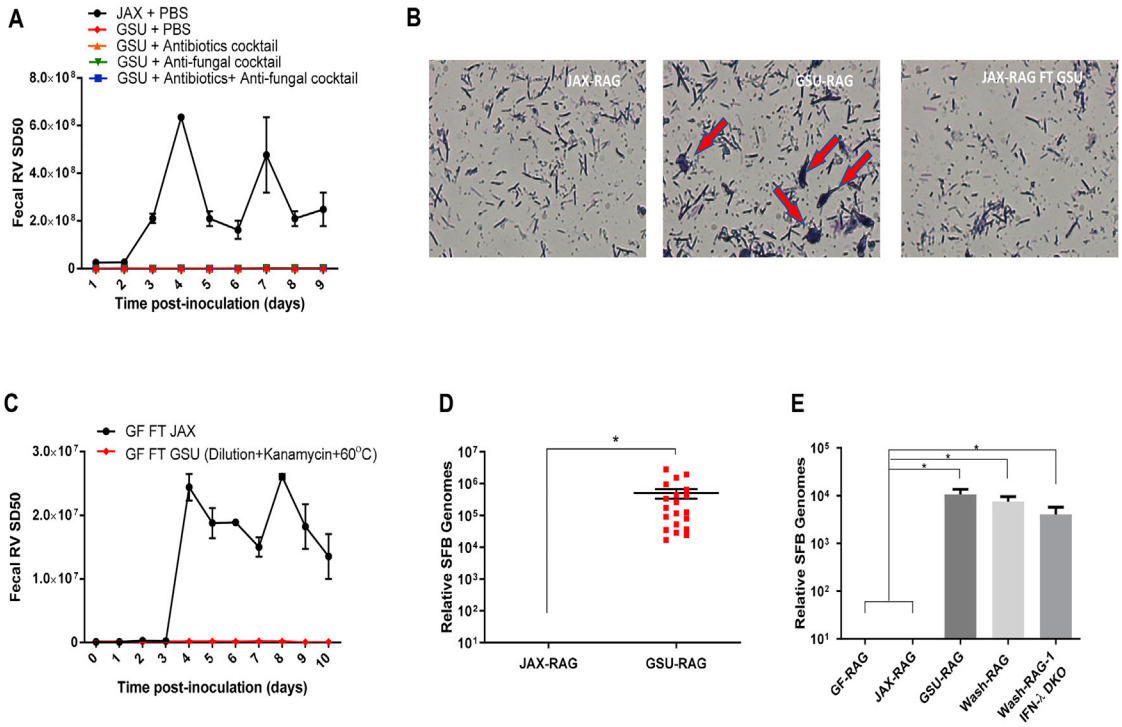


Figure S4. RV Resistance Is Associated with Transfer of SFB, Related to Figure 4

(A) GSU Rag1-KO mice received water with PBS, antibiotic cocktail, anti-fungal cocktail, or both for 4 weeks. The mice were then challenged with RV.

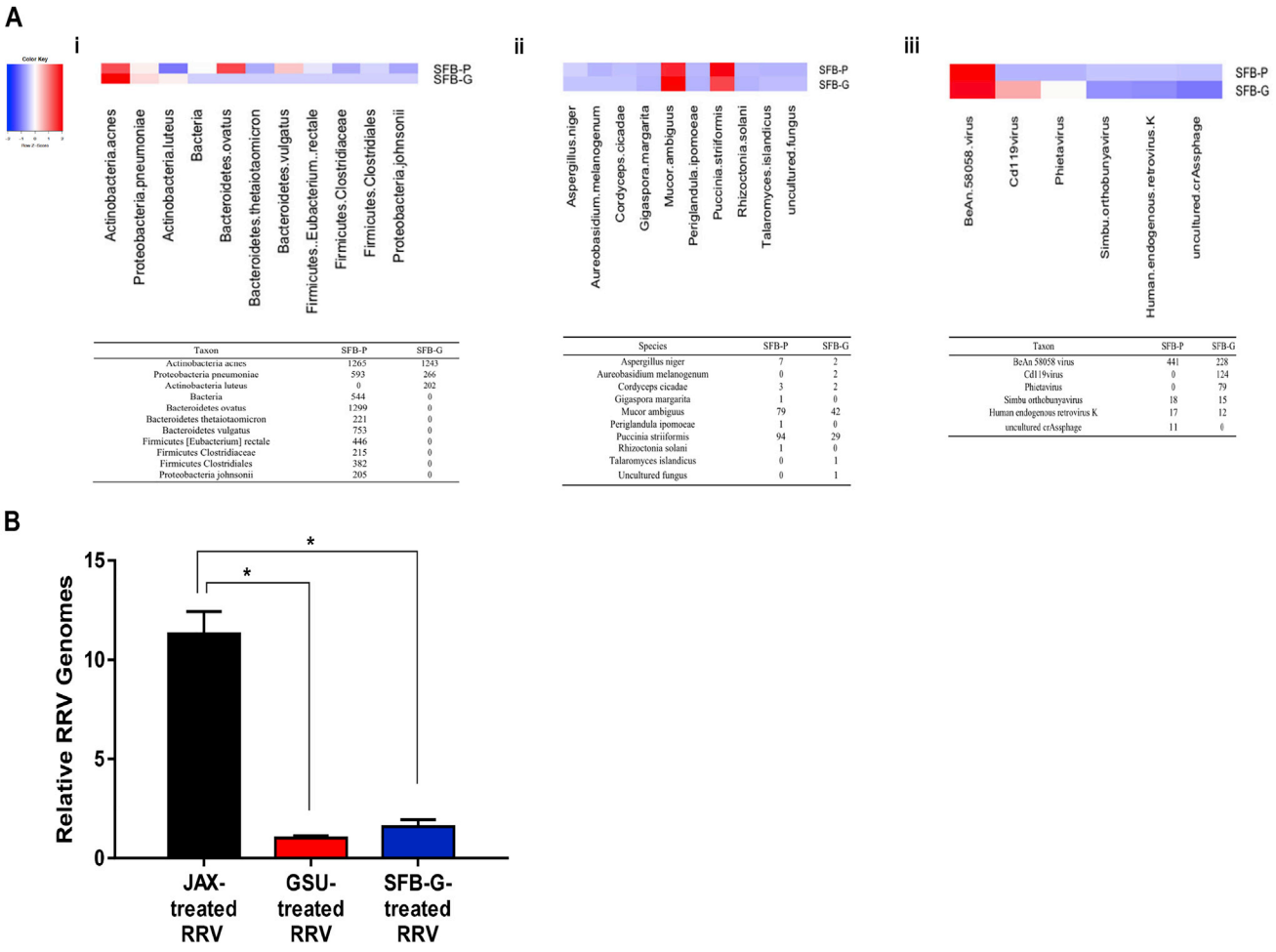
(B) Microscopies of fecal suspension of JAX-RAG, GSU-RAG, and GSU-RAG microbiota transplanted JAX-RAG (1-week post-FT). GSU-RAG were colonized by protozoa (red arrows), FT did not transfer visualizable parasites, might due to sizes of the parasites and their eggs are larger than 5 microns.

(C) GF Rag1-KO mice were transplanted with diluted, Kanamycin, and heat (60°C 10 minutes)-treated JAX or GSU Rag1-KO mice fecal samples. The mice were then challenged with RV, and fecal samples were collected from day 0 through day 10 post-inoculation.

(D) Fecal SFB level by qPCR showed GSU Rag1-KO mice generally carry SFB.

(E) Fecal SFB levels by qPCR showed RV uninfectable Rag1-KO mice and Rag1 IFN-λ-R1 DKO mice, both bred at Wash-U, were also colonized by SFB.

Results are shown as mean ± SEM. A; n = 3, two-way ANOVA, $p < 0.001$. C; two-way ANOVA, $p < 0.001$. D; Student's t test, n = 3, *$p < 0.01$. E; Student's t test, n = 8, *$p < 0.001$. For D & E, values below 10^1 were considered inauthentic amplification and therefore not plotted. Each experiment was performed 2-3 times and yielded an identical pattern of results.



Taxon	SFB-P	SFB-G
Actinobacteria acnes	1265	1243
Proteobacteria pneumoniae	593	266
Actinobacteria luteus	0	202
Bacteria	544	0
Bacteroidetes ovatus	1299	0
Bacteroidetes thetaiotaomicron	221	0
Bacteroidetes vulgatus	753	0
Firmicutes [Eubacterium] rectale	446	0
Firmicutes Clostridiaceae	215	0
Firmicutes Clostridiales	382	0
Proteobacteria johnsonii	205	0

Species	SFB-P	SFB-G
Aspergillus niger	7	2
Aureobasidium melanogenum	0	2
Cordyceps cicadae	3	2
Gigaspora margarita	1	0
Mucor ambiguus	79	42
Periglandula ipomoeae	1	0
Puccinia striiformis	94	29
Rhizoctonia solani	1	0
Talaromyces islandicus	0	1
Uncultured fungus	0	1

Taxon	SFB-P	SFB-G
BeAn 58058 virus	441	228
Cd119virus	0	124
Phietavirus	0	79
Simbu orthobunyavirus	18	15
Human endogenous retrovirus K	17	12
uncultured crAssphage	11	0

Figure S5. Limited Other Microbiome Populations in SFB-P and SFB-G, Related to Figure 4

(A) SFB-P and SFB-G samples were subjected to deep genome sequencing (Figure 4H) yielded minimal other microbiome populations. (i) Bacteria population other than SFB. (ii) Fungus population. (iii) Virus population.

(B) *Ex-vivo* treatment (37°C 4 hours) of RRV with GSU or SFB-G samples significantly reduced viral infection on HT-29 cells.

Results are shown as mean ± SEM.n=3 *$p < 0.05$, by Student's t test.

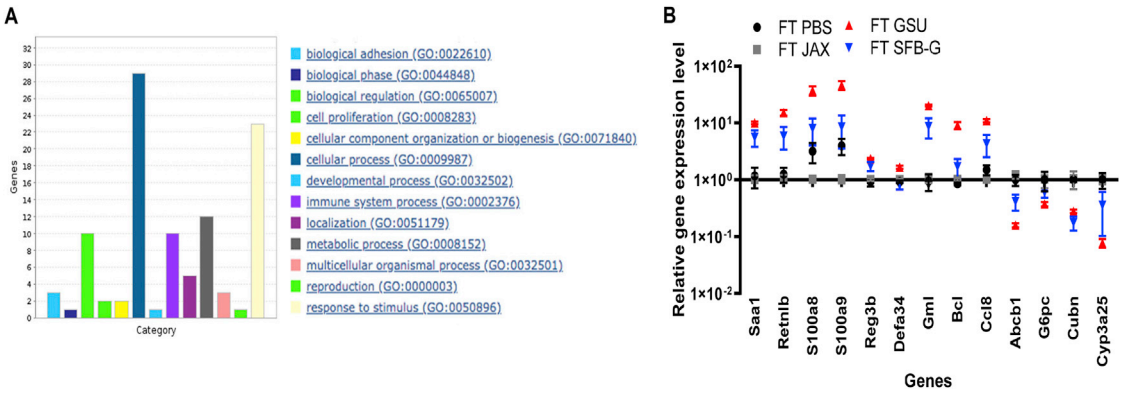


Figure S6. The GSU-RAG Microbiota and SFB-G Alter Host Gene Expression, Related to Figure 5

Rag1-KO mice received PBS, or feces from JAX-RAG, GSU-RAG, or SFB-G monoassociated mice. Ileum was harvested at 2 weeks post-FT for RNaseq analysis.

(A) Biological functions that elevated by GSU-RAG microbiota and/or SFB-G in the host.

(B) qRT-PCR results of certain gene expression in the 4 groups, as verification of RNaseq results.

Results are shown as mean ± SEM n = 3. Each experiment was performed 2 times and yielded an identical pattern of results.

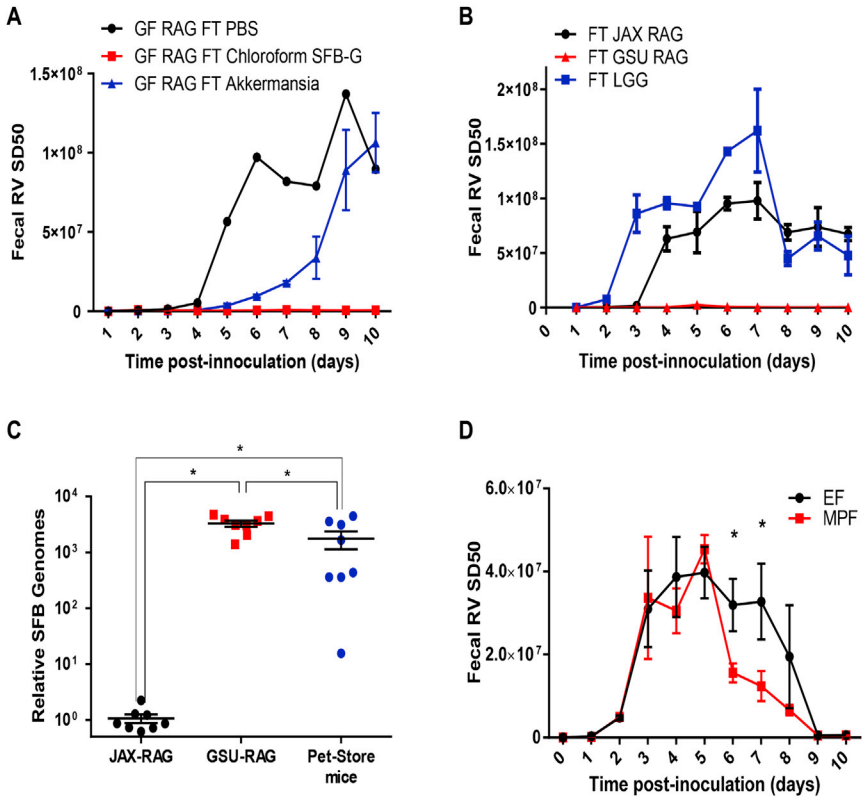


Figure S7. SFB Treat Chronic RV Infection in Immunodeficient Mice and Protect WT Mice from Diarrhea, Related to Figure 7

(A) RV antigens in feces of GF-RAG mice received PBS, chloroform-treated SFB-G, or feces from Akkermansia monoassociated mice.

(B) RV antigens in feces of JAX-RAG mice administered feces from JAX-RAG, GSU-RAG mice or in vitro-cultured Lactobacillus GG.

(C) Fecal samples isolated from JAX Rag1-KO mice, GSU Rag1-KO mice, and Pet store mice were assayed for relative SFB level using RT-PCR.

(D) C57BL/6 mice of excluded flora (EF) or murine pathogen free flora (MPF) obtained from Taconic farm were challenged with RV, fecal samples were collected from day 0 through day 10 post-inoculation.

Results are shown as mean ± SEM. A; n = 3, B; n = 4, $p < 0.05$ by two-way ANOVA. C; n = 8, D; n=5, *$p < 0.05$ by Student's t test Each experiment was performed 2-3 times and yielded an identical pattern of results.