Supporting Online Material

Materials and Methods

Viruses and cells

Virus work was performed in WHO-approved elevated BSL2+ areas. Poliovirus (serotype 1, Mahoney) cell culture infections and plaque assays were performed using HeLa cells grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum (Hyclone) and antibiotics (10,000 U/ml penicillin and 10,000 μg/ml streptomycin; Thermo Scientific). Poliovirus infections also were performed using PVRtg mouse embryonic fibroblasts (MEFs) grown in DMEM supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) and antibiotics (10,000 U/ml penicillin and 10,000 μg/ml streptomycin). PVRtg MEFs were generated from day 13-16 embryos and isolated as described (*15*). PVRtg MEFs were passaged for less than four weeks post-isolation to maintain a primary state. Reovirus T3SA+ was amplified and purified as described (*12*) and quantified by plaque assay using murine L929 cells maintained in DMEM supplemented with 5% FBS and antibiotics (10,000 U/ml penicillin and 10,000 μg/ml streptomycin) (*16*). All cells were incubated at 37°C with 5% CO_2.

Mice, treatments, and inoculations

Animal work was performed in accordance with University of Texas Southwestern Medical Center IACUC-approved protocols. C57BL/6 PVR-Tg21 (PVRtg) and C57BL/6 PVRtg-*Ifnar1-/-* (PVRtg-*Ifnar1-/-*) mice, obtained from S. Koike (Tokyo Metropolitan Institute for Neuroscience), were maintained in specific pathogen-free

conditions at University of Texas Southwestern Medical Center. Microbiologically-sterile germ-free C57BL/6 (non-PVR) mice were maintained in gnotobiotic chambers (*17*) until the point of poliovirus infection. For poliovirus infections, germ-free mice were housed in the BSL2+ facility in sterile cages with autoclaved bedding, food, and water. Feces were plated (see below) to monitor intestinal colonization, and fecal bacterial counts were undetectable through 48 hours post-infection. After 48 hours, mice became colonized.

Six-week old C57BL/6 (non-PVR; obtained from Jackson Laboratory), PVRtg or PVRtg-*Ifnar1-/-* mice were orally administered a combination of four antibiotics: ampicillin, neomycin, metronidazole, and vancomycin (Sigma-Aldrich, Research Products International) via oral gavage for 5 days (10 mg of each antibiotic per mouse per day) followed by *ad libitum* administration in drinking water (ampicillin, neomycin, and metronidazole: 1 g/L; vancomycin: 500 mg/L) (*18*) for the duration of the experiment. Mice were treated with antibiotics for 10 days prior to peroral inoculation with poliovirus or reovirus, or mice were treated with antibiotics for 8 days prior to recolonization with a fecal suspension. Antibiotics were administered for at least 7 days to ensure that bacterial detritus was cleared from the intestinal tracts of mice. Recolonized mice were perorally-administered 20-25 μl of untreated mouse feces suspended in PBS (2-4 pellets in 500 μl of PBS). Colonization was reestablished within 48 hours. Mouse feces were plated on brain heart infusion agar (BHI, Sigma-Aldrich) plates supplemented with 10% calf blood (Colorado Serum Company) to assess bacterial loads. Plates were incubated for 48 hours in anaerobic chambers with oxygen-reducing, carbon dioxide-generating sachets and anaerobic indicator strips (BD). Only culturable microbes were quantified by this method. Untreated mice were either mock

treated by oral gavage with sterile water or untreated. Both groups yielded similar results.

For all oral poliovirus infections (untreated, antibiotic-treated, or antibiotic-treated/recolonized mice), 7-9 week old mice were perorally inoculated with 2 x 10^7 PFU of poliovirus as previously described (*10*). Survival was assessed in mice inoculated with poliovirus by the oral or intraperitoneal (IP) routes. For IP survival experiments, PVRtg-*Ifnar1-/-* mice were inoculated with 2 x 10^7 PFU of poliovirus and PVRtg mice were inoculated with 10^8 PFU of poliovirus. Disease was monitored until day 12 post-inoculation for survival experiments. In all cases, mice were euthanized upon severe disease onset, which manifests as paralysis, encephalitis, or severe dehydration and lethargy. Once mice reach this stage of severe disease onset, they do not survive more than 1 to 12 hours. Therefore, throughout this study, mice were examined twice per day and euthanized upon disease symptom manifestation.

For shedding and replication experiments, PVRtg mice were orally inoculated with 2 x 10^7 PFU of standard poliovirus or light-sensitive poliovirus (to measure replication), and feces were collected at multiple times post-inoculation from each mouse and processed as outlined below. Gastrointestinal motility was measured in PVRtg-*Ifnar1-/-* mice by monitoring Evan's blue dye (MP Biomedicals, LLC) transit as described (*10*) (Supp. Figure 6).

To isolate poliovirus-containing lower small intestine lumenal contents (Supp. Fig. 10 experiments), C57BL/6 or PVRtg mice were orally inoculated with 2 x 10^7 PFU of poliovirus, and mice were euthanized at 1.5-2 hours post-infection. Lumenal materials

from the lower half of the small intestine were extracted from each mouse and processed as described below.

To assess *in vivo* effects of antibiotics on poliovirus, light-sensitive poliovirus was mixed with the four antibiotics (ampicillin, neomycin, and metronidazole: 1 g/L; vancomycin: 500 mg/L) prior to oral inoculation of untreated PVRtg-*Ifnar1-/-* mice. Feces were collected from each mouse at multiple times post-inoculation for titer analysis, and survival was monitored for 12 days post-inoculation.

Pathogenesis of poliovirus in antibiotic-treated mice harboring antibiotic-resistant bacteria was examined in PVRtg-*Ifnar1-/-* mice by selection of antibiotic-resistant bacteria. Four-week-old PVRtg-*Ifnar1-/-* mice were administered low-dose antibiotics in drinking water (ampicillin, neomycin, and metronidazole: 500 mg/L; vancomycin: 250 mg/L) for two weeks, and depletion of gastrointestinal microbes was confirmed by plating feces. The concentration of antibiotics in water was increased to the standard treatment regimen (ampicillin, neomycin, and metronidazole: 1g/L; vancomycin: 500 mg/L), and feces were collected periodically and plated on BHI/blood agar plates without or with antibiotics (ampicillin: 100 μg/ml, neomycin: 50 μg/ml, metronidazole: 25 μg/ml, and vancomycin: 5 μg/ml) to assess bacterial loads. When antibiotic-resistant fecal bacteria were present at similar abundance as bacteria in untreated mouse feces, mice were orally inoculated with light-sensitive poliovirus, feces were collected for viral replication assessment, and survival was monitored.

Isolation of tissues from poliovirus-infected mice was performed by orally inoculating 8 week-old PVRtg mice with 2×10^7 PFU of poliovirus. At 10, 24, and 48 hours post-inoculation (hpi), small intestine, colon, mesenteric lymph nodes, blood, and

spleen were harvested from each mouse. Small intestine and colon were flushed with PBS prior to processing (see below).

Reovirus infections were performed using 8 week-old untreated or antibiotic-treated PVRtg-*Ifnar1-/-* mice by peroral inoculation with 10^8 PFU of strain T3SA+ (*12*). Mouse feces were monitored up to day 4 post-inoculation for fecal pathology (see Supp. Table 1 for scoring system). At day 4 post-inoculation, mice were euthanized and tissues were collected for viral titer analysis (see below). Intestines were flushed with cold PBS for gross analysis, Peyer's patch measurements and viral titer assays.

Sample processing and titer analyses

Feces and lower small intestine lumenal contents from infected mice were resuspended in 1-5 volumes of PBS+ (PBS supplemented with 100 μg/ml $MgCl_2$ and 100 μg/ml $CaCl_2$) and freeze-thawed 3 times. The suspension was clarified by centrifugation at 13,000 rpm for 5 minutes, supernatants were extracted with 10% chloroform to eliminate bacteria, and samples were subjected to centrifugation for 3.5 minutes. Virus in supernatants was quantified by plaque assay using HeLa cells (*15*). Feces containing neutral red/light-sensitive poliovirus were processed as above, but in the dark under a red safety light, and a portion of each sample was light exposed (*10*). Light-exposed and unexposed viruses were quantified by plaque assay to determine the amount of replicated virus by dividing light-exposed PFU/ml by light unexposed PFU/ml and multiplying by 100.

Infectious center assays, modified from (*19*), were performed by comparing HeLa cell-derived poliovirus (TC) to lower small intestine lumenal content poliovirus isolated

from untreated, antibiotic-treated, and germ-free mice. HeLa cells and PVRtg MEFs (5 x 10^5 cells/well) were infected with each virus (3000-5000 PFU) for 10 minutes at 37°C, after which, virus was removed and cells were washed thoroughly with PBS. Cells were trypsinized in 500 μl and added to 500 μl of DMEM with 10% FBS, and 300 μl of undiluted, 1:10, or 1:100 cells in medium were plated on a monolayer of HeLa cells, which were incubated 3.5 hours at 37°C for cellular attachment to occur. Agar overlays were added, and plates were incubated at 37°C for two days to generate plaques. The PVRtg MEF viral titers were expressed as a percentage of the HeLa cell viral titers to normalize for different amounts of virus in individual mice and to reflect relative infectivity.

Visualization of intestinal architecture was performed following hematoxylin and eosin staining. PVRtg mice were treated with or without antibiotics and remained uninfected or were orally inoculated with 2 x 10^7 PFU poliovirus (n=3 each). Whole small intestines were isolated from mice at 48 hours post-infection. Intestines were flushed with cold PBS, transected, and arranged with the villi facing upward. To immobilize intestines prior to additional PBS washes, tissues were pinned every ~1-1.5 inches and washed to remove all lumenal contents. Tissues were submerged in 10% neutral-buffered formalin overnight at 4°C. The following day, intestines were concentrically rolled, using a wooden probe, and the villi were outwardly exposed. Each tissue roll was stabilized in a biopsy mega-cassette (Tissue Tek) using formalin-soaked biopsy sponges with cut holes in which to place each tissue. All tissue-containing cassettes were soaked in 10% neutral-buffered formalin at 4°C for at least 48 hours. Whole tissue rolls were paraffin-embedded, cut into 6 μm sections and stained with hematoxylin and

eosin (UT Southwestern Histology Core). Images were obtained using a Zeiss Axio Imager.M1 microscope equipped with Axio Vision Release 4.8.2 software.

Tissues collected from mice infected with poliovirus or reovirus were suspended in 1-5 volumes (wt/vol) of PBS+, freeze-thawed 3-5 times, and homogenized using a Bullet Blender Tissue Homogenizer (Next Advanced Inc) or sonication. Homogenized intestines, mesenteric lymph nodes, and spleens from poliovirus-infected mice were clarified by centrifugation and chloroform-extracted (10%). Poliovirus from tissue samples was quantified by plaque assay using HeLa cells. Reovirus from clarified supernatants was quantified by plaque assay using L929 cells as described (*16*).

In vitro viral replication kinetics

Poliovirus growth curves were performed using HeLa cells and PVRtg MEFs in the absence and presence of antibiotics (ampicillin, neomycin, and metronidazole: 1 g/L; vancomycin: 500 mg/L). Poliovirus (10^6 PFU) was suspended in PBS or PBS supplemented with all four antibiotics, and 10^5 cells were infected and harvested at various times post-infection. Viral yields were quantified by plaque assay as described (*15*).

Identification of antibiotic-resistant bacteria

Feces were collected from antibiotic-treated mice that harbored antibiotic-resistant bacteria. Feces were stored at -20°C prior to isolation of bacterial DNA via QIAamp DNA Stool Mini Kit (Qiagen). Genomic DNA from fecal bacteria and bacterial colonies from mouse feces plated on BHI-blood agar plates were amplified by PCR

using universal 16S rDNA primers (forward: 5'-agagtttgatymtggctcag-3', reverse: 5'-acggytaccttgttacgactt-3'). PCR products were purified using the QIAquick Nucleotide Removal Kit (Qiagen) and then cloned into a TOPO vector according to the manufacturer's instructions (TOPO TA Cloning Kit, Invitrogen). Plasmids were heat-shock transformed into *E. coli* DH5α competent cells and plated on LB agar plates supplemented with 10 μg/ml of ampicillin (Research Products International) and 40 μg/ml of X-Gal (Fisher Scientific). *E. coli* colonies were amplified to purify plasmid DNA using the QIAprep Spin Miniprep Kit (Qiagen), and plasmid DNA was sequenced (McDermott Sequencing Core, UT Southwestern Medical Center, Dallas, TX). Sequences of seven feces-derived clones and two colony-derived clones were queried via BLAST (NCBI) for identification of the antibiotic-resistant bacterium. In all nine clones, sequences aligned with the 16S rRNA region of *Ochrobactrum intermedium* (strain CCUG 43465, AM490610, NCBI database by Clustal W method).

Poliovirus *ex vivo*/*in vitro* infectivity assays

The experimental strategy is briefly outlined in Figure 4A. For all infectivity experiments, an inoculum of 10^4 to 10^5 PFU of poliovirus was used. After incubation, samples were quantified by standard plaque assay using HeLa cells (~ 10^6 cells) and compared to titers from poliovirus inputs at the zero time point to determine the percent of input PFU as an indicator of yield. Poliovirus infectivity was determined after incubation in PBS in glass tubes at 37°C and 42°C for various times to establish kinetics of poliovirus infectivity loss due to thermal inactivation. Assays involving bacteria (feces or pure cultures; Fig. 4CD, Supp. Fig. 11) were performed at 37°C to avoid bacterial

transcriptional and translational alterations and/or stress responses that may influence experimental outcome and to mimic physiological temperatures. Assays involving purified components such as LPS (Fig. 4E,4F) were performed at 42°C to accentuate effects over a short time course.

For incubations with fecal suspensions, poliovirus was added to PBS, untreated mouse feces, antibiotic-treated mouse feces, germ-free mouse feces, germ-free mouse feces with 1-5 x 10^9 CFU of *Bacillus cereus* (Abx-UK1; provided by L. Hooper) or germ-free mouse feces plus 1 mg/ml of *E. coli* LPS (Sigma). *B. cereus* was cultured aerobically overnight (~14-16 hours; from an isolated colony) in brain heart infusion (BHI) broth. The following day, bacteria were pelleted at 3750 rpm for 30 minutes, washed with PBS, re-pelleted at 3750 rpm for 30 minutes, and resuspended in PBS. Feces were suspended in 3-5 volumes (wt/vol) of PBS, and additional treatments or poliovirus was added. Samples were incubated in glass tubes for 6 hours at 37°C and then processed to extract virus. Chloroform (10-20%) was added to each sample, and samples were clarified by centrifugation at 13,000 rpm for 5 minutes. Poliovirus was quantified from supernatants using HeLa cell plaque assays.

To examine the influence of pure bacterial strains on poliovirus, *Escherichia coli* K12 (provided by L. Hooper), *Ochrobactrum intermedium* (abx^R isolate), *B. cereus* or *Enterococcus faecalis* V583 (provided by L. Hooper) were grown aerobically overnight (~14-16 hours; from an isolated colony) in Luria broth (LB; *E. coli*) or BHI broth (*O. intermedium*, *B. cereus*, and *E. faecalis*). The next day, bacteria were pelleted and washed as described above. Bacteria were resuspended in 500μl of Dulbecco's Modified Eagle's Medium (DME) and diluted in DME to particular cell densities (10^7, 10^8,

10^9 CFU) according to the original bacterial culture optical density value determined at 600 nm. UV-inactivated bacteria (10^7 CFU) were exposed to UV light (312 nm on high; Fotodyne: FOTO/UV 26) for 10 minutes prior to virus addition. Poliovirus was added, and samples were incubated for 6 hours at 37°C in glass tubes. After incubation, samples were subjected to 10% chloroform extraction, and poliovirus in supernatants was quantified by plaque assay. Bacteria were plated on LB (*E. coli* only) or BHI agar and grown aerobically overnight to confirm the number of CFU used and efficiency of UV inactivation. Bacteria inactivated by heating at 65°C or 100°C enhanced poliovirus yield to a similar extent as bacteria inactivated by UV exposure (data not shown).

The capacity of various compounds to affect poliovirus infectivity (Fig. 4E) was assessed by incubating 10^5 PFU poliovirus with 1000 μg/ml of the following for 6 hours at 42°C in a total volume of 200 μl in glass tubes: PBS, BSA (bovine serum albumin), milk (Kroger non-fat dry milk resuspended in water), mucin from porcine stomach, LPS (from *Escherichia coli, Salmonella enterica, or Klebsiella pneumoniae*), lipoteichoic acid from *Bacillus subtilis* (a Gram-positive cell wall component), peptidoglycan from *Bacillus subtilis*, glucose, sucrose (glucose-fructose disaccharide), stachyose (oligosaccharide containing galactose, fructose, and glucose), maltoheptaose (7-glucose oligosaccharide), dextran (glucose polysaccharide), N-acetylglucosamine (GlcNAc monosaccharide), chitin (insoluble GlcNAc polysaccharide), hyaluronic acid (polysaccharide containing GlcNAc and glucuronic acid), agarose (polysaccharide containing galactose and galactopyranose), amylose (glucose polysaccharide), cellulose (glucose polysaccharide), and mannan (mannose polysaccharide). All

compounds are from Sigma unless noted otherwise. After incubation, samples were diluted, and poliovirus was quantified by HeLa cell plaque assay.

To confirm that exposure of HeLa cells to compounds such as LPS and peptidoglycan does not enhance the capacity of poliovirus to infect cells, two experiments were performed. First, 10^6 HeLa cells were exposed to PBS or 1 μg/ml solutions of LPS or PG to mimic the final concentration in diluted virus-containing solutions plated on HeLa cells in Fig. 4E experiments. Following a 30-minute exposure at 37°C, cells were washed and infected with 100 PFU of poliovirus, followed by standard plaque assay. Second, PBS or 1000 μg/ml LPS or PG was added to 10^5 PFU poliovirus, and the samples were immediately diluted one thousand fold and plated on HeLa cells, followed by standard plaque assay.

LPS binding assay

Poliovirus (10^7 PFU) was either mixed with 50 μg of biotinylated LPS (*E. coli* O111:B4; InvivoGen) or water and incubated for 1 hour at 37°C in glass tubes. The mixture was then added to a column containing monomeric avidin conjugated to agarose beads, prepared according to the manufacturer's protocol (Pierce Monomeric Avidin Kit). After samples were pipetted onto the column, PBS was added up to 2 ml, and fraction 1 was collected. Subsequent 2 ml PBS washes were performed, and 6 total 2 ml PBS wash fractions were collected and stored on ice. Fractions 7 through 12 were collected using Biotin Blocking and Elution Buffer to compete the binding of biotin-LPS to the avidin beads. As with the first 6 fractions, 2 ml fractions were collected and stored

on ice. Each fraction was extracted with chloroform (10%) and diluted. Poliovirus infectivity was quantified by HeLa cell plaque assay.

Cell binding assay

To determine whether exposure to bacteria increased poliovirus binding to cells, we quantified the capacity of ^{35}S-labeled poliovirus incubated with PBS or *B. cereus* to bind HeLa cells. ^{35}S-labeled poliovirus was generated by growing virus in medium lacking methionine/cysteine in the presence of ^{35}S-labeled methionine/cysteine. At 6 hours post-infection, cell-associated virus was released by freeze-thawing, and the virus was purified by cesium chloride gradient centrifugation (10 ml 1.2g/cm^3 CsCl in PBS gently layered on 10 ml 1.4g/cm^3 CsCl in PBS, followed by ultracentrifugation for 4 hours at 25,000 rpm). Virus-containing fractions were concentrated and desalted using Amicon filters (Millipore) according to the manufacturer's instructions. The stock used for the experiment shown in Fig. 4H had a specific infectivity of 1300 PFU/counts per minute (CPM). For this experiment, 4 x 10^6 PFU of ^{35}S-labeled virus was incubated with PBS or 2 x 10^8 CFU of washed *B. cereus* in 250 μl for 1 hour at 37^{o}C in glass tubes. Following incubation, 250 μl of PBS or *B. cereus* was added, and 200 μl of each sample was added to duplicate plates containing 10^6 PBS-washed HeLa cells. Plates were incubated for 20 minutes at 37^{o}C for viral binding/entry. There was no visible change in cell morphology in the presence of *B. cereus* for the 20-minute incubation period. Cells were washed three times with PBS, harvested by trypsinization, and after centrifugation, cell pellets were resuspended in cell lysis buffer. Radioactivity in cell lysates and plated

solutions were quantified by scintillation counting. Data are expressed as percent cell-associated virus (cell-associated CPM/plated solution CPM x 100%).

References

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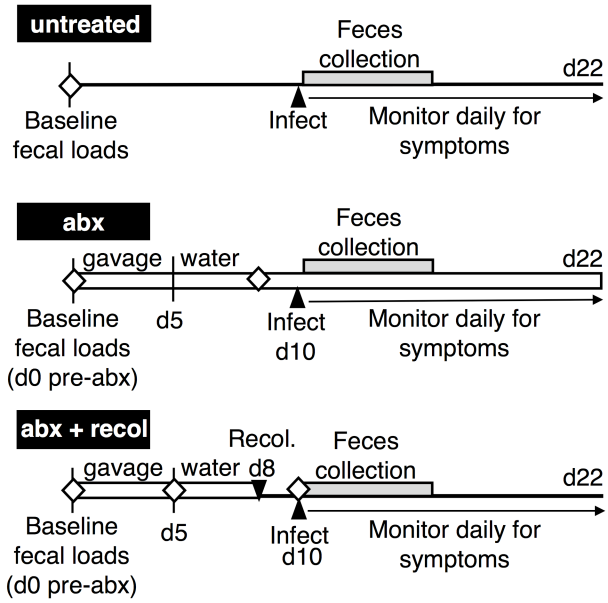
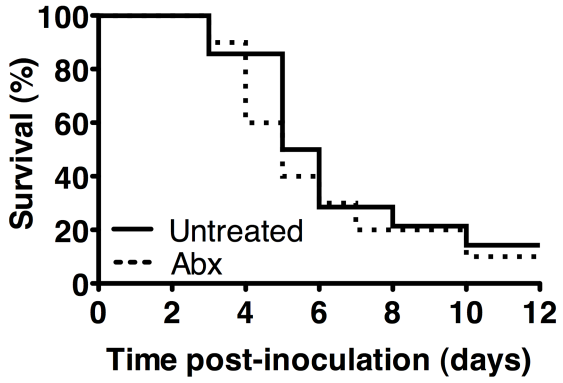
Supplemental Figure 1



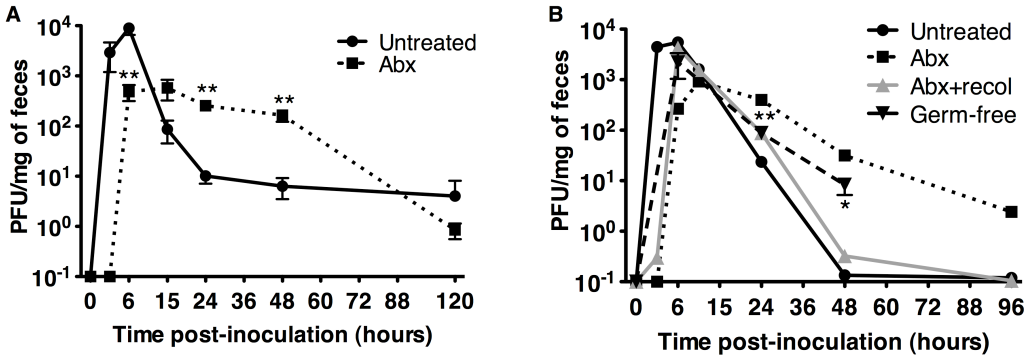
Supp. Figure 1. Treatment strategy used for microbiota depletion and viral infection. Treatment timelines for untreated, antibiotic-treated (abx), and antibiotic-treated/recolonized (abx + recol) mice. Fecal bacterial loads were determined (◊) prior to and after treatments. White bars denote extent of antibiotic treatment, and gray bars represent feces collection times post-infection.

Supplemental Figure 2



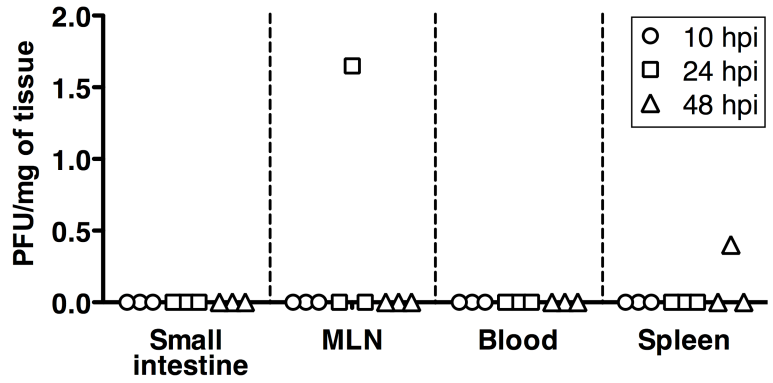
Supp. Figure 2. Poliovirus pathogenesis in intraperitoneally inoculated PVRtg mice. Survival of untreated and antibiotic-treated (Abx) immune competent PVRtg mice inoculated intraperitoneally with 1 x 10^8 PFU of poliovirus (untreated: n=14, Abx: n=10). N=2-3 experiments.

Supplemental Figure 3



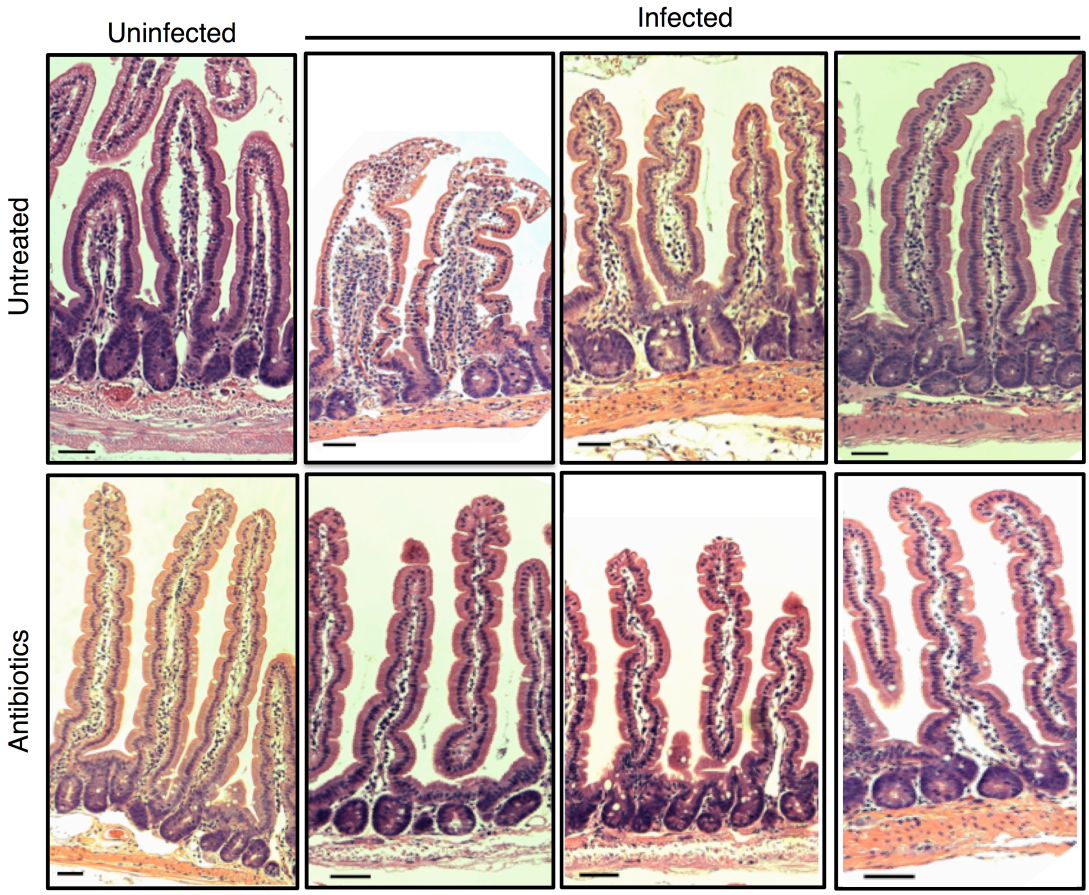
Supp. Figure 3. Poliovirus fecal shedding kinetics. (**A**) Poliovirus shedding from immune competent PVRtg mice. After oral inoculation with poliovirus, feces were collected from untreated and antibiotic-treated (Abx) mice (n=2-18 at each interval). Poliovirus was isolated from feces and quantified by plaque assay, yielding PFU per milligram of feces. N=2-6 experiments. (**B**) Poliovirus shedding in feces from non-PVR C57BL/6 mice (n=5 per treatment group) orally inoculated with poliovirus. Mice were untreated, antibiotic-treated, antibiotic-treated/recolonized (Abx + recol), or germ-free (GF, n=6). Germ-free mice became colonized after 48 hours of housing outside of gnotobiotic chambers. Therefore, only data from the first 48 hours is shown. Data are from a representative experiment (non-PVRtg C57BL/6 mice, symbols indicate the mean) or are means from two experiments (GF mice, symbols represent mean + SEM). **p*<0.05, ***p*<0.01 compared to untreated, Student's t-test.

Supplemental Figure 4



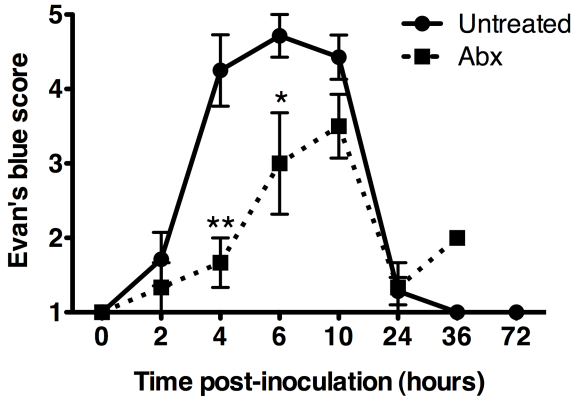
Supp. Figure 4. Quantification of poliovirus in tissues. PVRtg mice were orally inoculated with poliovirus, and tissues were harvested at 10, 24, or 48 hours post-inoculation (hpi) (n=3 each). Virus was extracted from tissues and quantified by plaque assay. MLN = mesenteric lymph node. Data are representative of two experiments.

Supplemental Figure 5



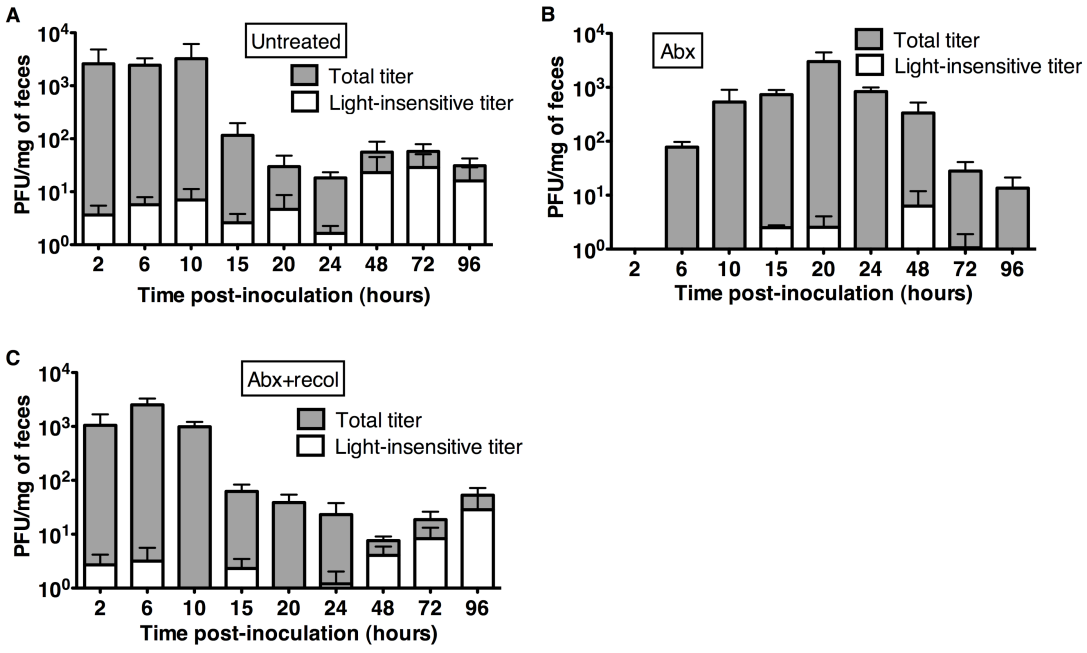
Supp. Figure 5. Intestine pathology. Intestinal architecture and cellular changes were analyzed following hematoxylin and eosin staining of tissue sections from uninfected PVRtg mice (untreated or antibiotic-treated) and poliovirus-infected PVRtg mice (untreated or antibiotic-treated; 48 hours post-infection by the oral route) (n=3 each). Scale bars = 50μm. Data are representative of two experiments.

Supplemental Figure 6



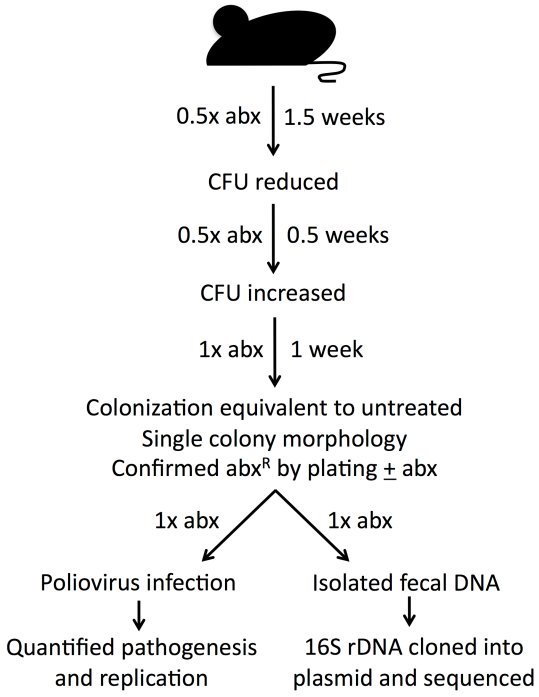
Supp. Figure 6. Intestinal transit time in mice. Untreated or antibiotic-treated (Abx) PVRtg-*Ifnar1-/-* mice were orally administered Evan's blue dye, and feces were collected at the times shown post-inoculation. Feces were suspended in PBS and the amount of dye excreted was scored. Symbols represent the mean + SEM. **p*<0.05, ***p*<0.01, Student's t-test. N=3 experiments.

Supplemental Figure 7



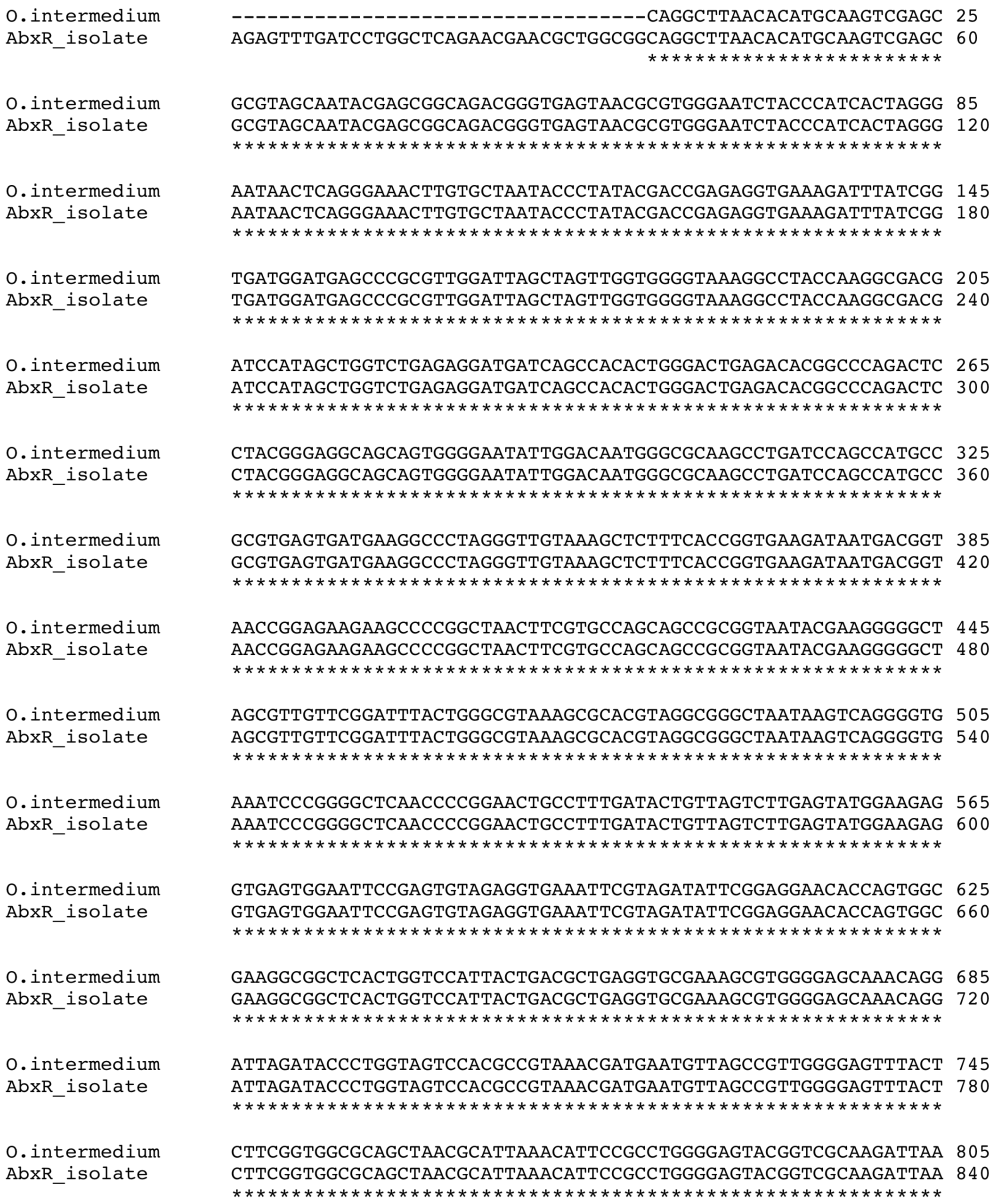
Supp. Figure 7. Poliovirus fecal titer data following oral inoculation with light sensitive virus. (**A,B,C**) Poliovirus titer data for unexposed (total) and light-exposed (light-insensitive) fecal samples from untreated (**A**), antibiotic-treated (Abx) (**B**), and antibiotic-treated/recolonized (Abx+recol) (**C**) PVRtg-*Ifnar1-/-* mice. These data were used to calculate the percent replication results shown in Fig. 1E. N=2-6 experiments.

Supplemental Figure 8



Supp. Figure 8. Strategy for isolating and identifying antibiotic resistant (abx^R) bacteria.

Supplemental Figure 9



Supplemental Figure 9 (cont)

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O.intermedium      AACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGTTAGTGGAGACACTTTCCT 925
AbxR_isolate       AACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGTTAGTGGAGACACTTTCCT 960
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O.intermedium      TCAGTTCGGCTGGATCGGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT 985
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O.intermedium      CACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACGTCAAGTCCTC 1105
AbxR_isolate       CACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACGTCAAGTCCTC 1140
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O.intermedium      ATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTGGGCAGCGAGC 1165
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O.intermedium      GGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTTTACCCGAAGGCGCTGTGC 1345
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O.intermedium      TAACCGCAAGGAGGCAGGCGACCACGGTAGGGTCAGCGACTGGGG 1390
AbxR_isolate       T---------------------------------------------- 1381
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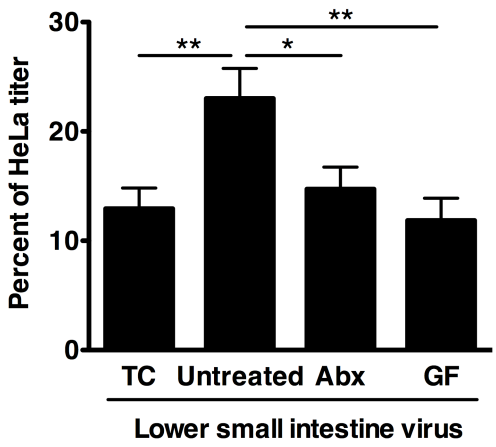
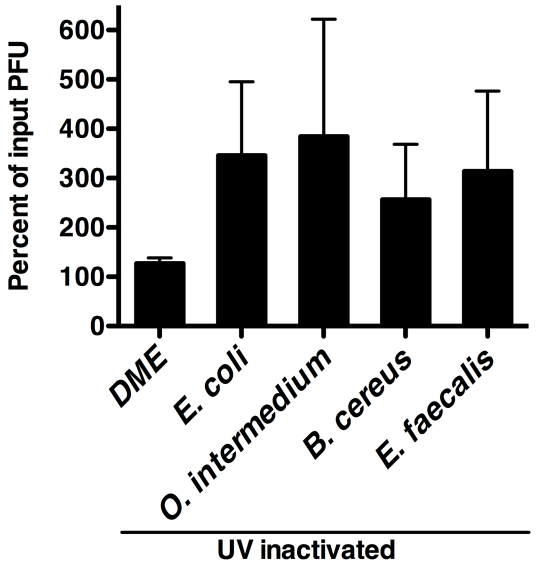
Supp. Figure 9. Identification of antibiotic-resistant (AbxR) bacteria in antibiotic treated mice. Feces were harvested from antibiotic-treated (Abx) mice harboring AbxR bacteria, genomic DNA was isolated, and the 16S rDNA region was PCR amplified, cloned into plasmids, and sequenced. One representative sequence is shown aligned to *Ochrobactrum intermedium,* with 96-100% sequence identity for all seven clones derived from fecal PCR products and both clones derived from AbxR colony PCR products.

Supplemental Figure 10



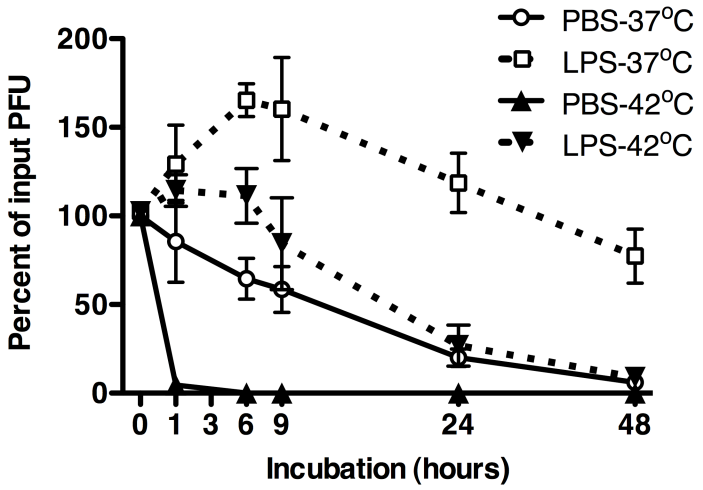
Supp. Figure 10. Poliovirus infectivity *ex vivo*. Infectivity of tissue culture-derived (TC) or mouse intestine lumenal content-derived poliovirus. Intestine lumenal contents were collected from the lower small intestine of untreated (n=5) or antibiotic-treated (Abx) (n=4) PVRtg mice or germ-free (GF) non-PVR C57BL/6 (n=3) mice two hours following oral inoculation with poliovirus. Since intestinal cells are minimally susceptible to poliovirus, and differences in viral infectivity may be more apparent in minimally susceptible cells, poliovirus infectivity was compared using minimally susceptible, freshly harvested PVRtg mouse embryonic fibroblasts (MEFs) and highly susceptible HeLa cells. Seven infectious center assays were performed, and MEF titers are presented as a percentage of HeLa cell titers to reflect relative infectivity. Bars denote the mean + SEM. *p<0.05, **p<0.01, Student's t-test. N=7 experiments.

Supplemental Figure 11



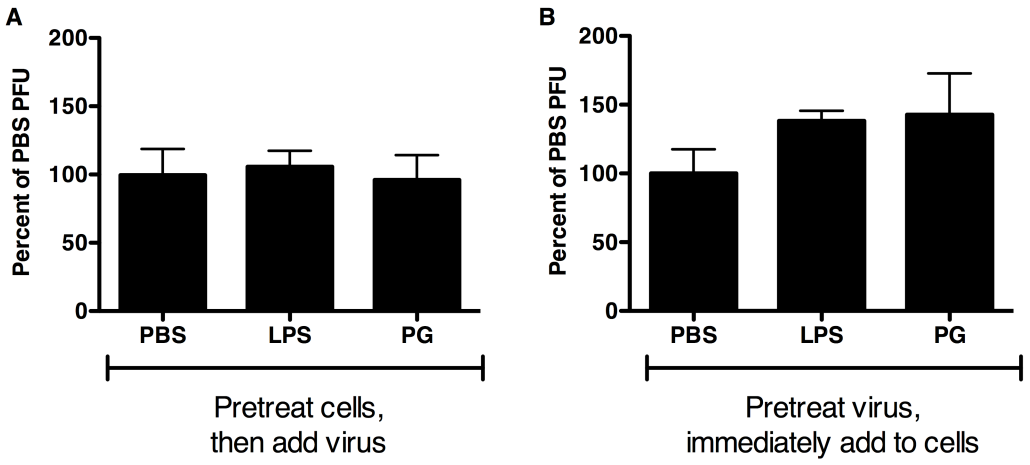
Supp. Figure 11. Poliovirus recovery after exposure to inactivated bacteria. Poliovirus was incubated with UV-inactivated bacteria (10^7 CFU per strain) or minimal medium (DME) for 6 hours at 37°C, followed by plaque assay on HeLa cells. Bars denote the mean + SEM. N=2 experiments. Similar results were obtained with heat-killed bacterial strains (data not shown).

Supplemental Figure 12



Supp. Figure 12. Poliovirus infectivity in the presence or absence of LPS. Poliovirus was exposed to 1000 µg/ml *E. coli* LPS at 37°C or 42°C for the times shown, and viable virus was quantified by plaque assay using HeLa cells. Symbols indicate the mean + SEM. N=2-4 experiments.

Supplemental Figure 13



Supp. Figure 13. Effect of LPS or peptidoglycan (PG) treatment on HeLa cells. (**A**) Pretreatment of HeLa cells with *E. coli* LPS or *B. subtilis* PG. To control for potential cell changes induced by LPS or PG exposure, HeLa cells were treated for 30 minutes with LPS or PG at the same concentration plated in the experiments shown in Fig. 4E after thousand-fold virus dilution (1 µg/ml final concentration). Cells were then washed, poliovirus was added and plaque assays were performed. (**B**) Poliovirus was treated with 1000 µg/ml LPS or PG, followed by immediate thousand-fold dilution and virus was quantified by a plaque assay. Data are displayed as the percentage of control (PBS)-treated plaque forming units. Bars denote the mean + SEM. N=2 experiments.

Supplementary Table 1

Supplemental Table 1. Fecal pathology scoring schema

	Color		Consistency	Example
0	normal		normal	brown, firm
1	slight discoloration	or	slight alteration	brown, soft
2	discoloration	and	alteration	tan, hard
3	extreme discoloration	and	extreme alteration	yellow, hard

Supp. Table 1. Scoring strategy for reovirus-infected mouse feces. Scores were based on color and consistency. See Fig. 3B for graphical representation.