binding to LPS. Poliovirus was incubated +/- biotinylated LPS for 1 hour at 37°C. A monomeric avidin column was loaded with samples and washed with PBS to collect fractions 1-6. Excess biotin was added to elute (fractions 7-12). Poliovirus was quantified yielding PFU per fraction, $p<0.0001$, 2-way ANOVA. (**H**) Binding of radiolabeled poliovirus to HeLa cells. ^{35}S-labeled poliovirus was incubated with PBS or 10^8 CFU *B. cereus* for 1 hour at 37°C. An equal volume of PBS or *B. cereus* was added followed by immediate incubation with HeLa cells. After washing, cell-associated radioactivity was quantified. For all experiments, N=2-8 and bars and symbols denote mean + SEM, *$p<0.05$, **$p<0.01$, Student's t-test.

Supplemental Figure Legends

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.

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.

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 are 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.

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.

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.

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.

Supp. Figure 7. Poliovirus fecal titer data following oral inoculation with lightsensitive 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. Bars denote the mean + SEM. N=2-6 experiments.

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

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.

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.

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).

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.

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 virus was quantified by a plaque assay. (**B**) Poliovirus was treated with 1000 μg/ml LPS or PG, followed by immediate thousand-fold dilution and plating on HeLa cells for a plaque assay. Data are displayed as the percentage of control (PBS)- treated plaque forming units. Bars denote the mean + SEM. N=2 experiments.

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