

Imbalance of the intestinal virome caused by a conditional deletion of the vitamin D receptor from epithelial cells, Paneth cells, or myeloid cells in murine models

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Abstract

Background Vitamin D receptor (VDR) is highly conserved in mammals, and its deficiency is associated with various diseases (e.g., cancer, infection, and chronic inflammation). Prior research has described the effect of VDR on bacteria; however, little is known regarding the effects of VDR on viruses. We hypothesize that VDR is a regulator of the virome and virus-bacterial interactions. We conditionally deleted VDR from intestinal epithelial cells (VDR^{ΔIEC}), Paneth cells (VDR^{ΔPC}), and myeloid cells (VDR^{ΔLyZ}) in mice. Feces were collected for shotgun metagenomic sequencing and metabolite profiling. To test the functional changes, we evaluated pattern recognition receptors (PRRs) and analyzed microbial metabolites.

Results *Vibrio* phages, *Lactobacillus* phages and *Escherichia coli* typing phages were significantly enriched in all three conditional VDR-knockout mice compared to VDR^{LoxP} mice. In the VDR^{ΔLyZ} mice, the levels of eight more virus species (2 enriched, 6 depleted) were significantly changed. These significantly altered virus species were primarily observed in female VDR^{ΔLyZ} (2 enriched, 3 depleted) versus male VDR^{ΔLyZ} (1 enriched, 1 depleted). Altered alpha and beta diversity were found in VDR^{ΔLyZ} (family to species). In VDR^{ΔIEC} mice, bovine viral diarrhea virus 1 was significantly enriched. Overall, these significantly altered virus species were more often observed in female VDR^{ΔIEC} mice (2 enriched, 5 depleted) versus male mice (none). A significant correlation between viral and bacterial alterations was found in conditional VDR knockout mice. There was a positive correlation between *Vibrio* phage JSF5 and *Cutibacterium acnes* in VDR^{ΔPC} and VDR^{ΔLyZ} mice. Also, there were more altered viral species in female conditional VDR knockout mice. Notably, there were significant changes in PRRs: upregulated TLR3, TLR7, and NOD2 in VDR^{ΔLyZ} mice and increased CLEC4L expression in VDR^{ΔIEC} and VDR^{ΔPC} mice. Furthermore, we identified metabolites related to virus infection: decreased glucose in VDR^{ΔIEC} mice, increased ribulose/xylulose and xylose in VDR^{ΔLyZ} mice, and increased long-chain fatty acids in VDR^{ΔIEC} and VDR^{ΔLyZ} female mice.

Conclusion Tissue-specific deletion of VDR changes the virome and functionally changes viral receptors, which leads to dysbiosis, metabolic dysfunction, and infection risk. This study helps to elucidate VDR regulation of the virome in a tissue-specific and sex-specific manner.

Background

During the pandemic, the status of vitamin D and risk of coronavirus disease 2019 (COVID19) are of great interests for prevention and treatment [1, 2]. Vitamin D acts through the VDR, an ancient nuclear receptor and a transcription factor highly conserved in mammals [3, 4]. The expression level of VDR is very high in the small intestine and colon and plays an important role in local and systemic immunity, host defense, and host-microbial interactions [5-11]. Vitamin D/VDR deficiency is not only associated with various digestive diseases but also plays an important role in viral infection, including influenza infections [12, 13], Epstein-Barr virus infection [14], varicella-zoster virus infection [15], cytomegalovirus

infection [14], and hepatitis C virus infection [16]. An epidemiological study showed an inverse association between vitamin D levels in the serum and infections of the upper and lower respiratory tracts [17]. Vitamin D deficiency also contributes to the pathogenesis of HIV infection by negatively modulating innate and adaptive immune responses [18, 19]. At the gene level, a meta-analysis determined that the *VDR* gene polymorphism *FokI* is consistently associated with host susceptibility to infection by respiratory syncytial virus [20]. Gene variation in *VDR* was also reported to correlate with persistent hepatitis B infection in clinical patient samples [21]. Consistent with the epidemiological data, activation of the vitamin D/*VDR* pathway in response to respiratory syncytial virus infection occurs in lung cells via TLR3 signaling, which results in the induction of the *CAMP* gene [22, 23].

The microbiota consist of bacteria, viruses, fungi, multicellular parasites and archaea. The virome is a collection of nucleic acids, both RNA and DNA that compose the viral community associated with a particular ecosystem of microbiota. The virome includes eukaryotic viruses, endogenous retroviruses, bacterial viruses (i.e., bacteriophages), and archaeal viruses and is one of the least understood components of the microbiota [24]. Dysbiosis of the microbiome not only leads to intestinal inflammatory and infectious diseases but also to diseases beyond the gastrointestinal tract [5, 25]. At present, we are beginning to understand the influence of *VDR* on microbial homeostasis, which is critical in various diseases [5, 26-29]. However, the effects and mechanisms of *VDR* on the virome have not been fully elucidated.

In this study, we hypothesize that host factors (e.g., *VDR* status in specific tissues) are regulators of the intestinal virome, virus-bacterial interactions, and microbial metabolites. Specifically, we used novel animal models and statistical and bioinformatic tools and models to understand host factors and aspects of metabolites and the microbiome, including bacteria and viruses, *in vivo*. We conditionally deleted *VDR* from intestinal epithelial cells (*VDR*^{ΔIEC}), Paneth cells (*VDR*^{ΔPC}), and myeloid cells (*VDR*^{ΔLyz}) of mice. Fecal samples were collected for microbial DNA extraction and shotgun metagenomic sequencing. From a functional perspective, we investigated the virus infection-associated metabolites and virome-related PRRs of colonic epithelial cells. Understanding the role of *VDR* in altering the multi-kingdom aspects of the microbiome, including bacteria and viruses, may help to elucidate the mechanisms by which *VDR* regulates microbial homeostasis in terms of health and disease.

Materials And Methods

Experimental animals

We conditionally deleted vitamin D receptor from intestinal epithelial cells, Paneth cells, and myeloid cells of the mice by crossbreeding *VDR*^{LoxP} mouse and cre mouse. The *VDR*^{LoxP} mice were formerly developed by Dr. Geert Carmeliet [30]. *VDR*^{ΔIEC}, *VDR*^{ΔPC} and *VDR*^{ΔLyz} mice were obtained by crossing the *VDR*^{LoxP} mice with villin-cre, Defa6-cre and Lyz-cre mice, respectively. Villin-cre and Lyz-cre mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). DEFA6-cre mice were from Dr. Richard Blumberg [31]. Conditional knockout (KO) mice were derived from heterozygous mating pairs such that wild-type and

conditional KO mice came from the same litter. The same breeding method was used for the VDR^{LoxP} mice.

The mice aged 6 to 8 weeks were chosen from each group and cohoused until the experiments were performed. All the animals were housed in the Biologic Resources Laboratory (BRL) at the University of Illinois at Chicago (UIC) and utilized in accordance with the UIC Animal Care Committee (ACC) and Office of Animal Care and Institutional Biosafety (OACIB) guidelines. The animal work was approved by the UIC Office of Animal Care (ACC 15231,17-218, and 18-216).

Real-time quantitative PCR

Total RNAs were extracted from colon epithelial cells of four genotype mice of VDR^{LoxP}, VDR^{ΔIEC}, VDR^{ΔPC} and VDR^{Δlyz}, using TRIzol reagent (Life technologies, Carlsbad, CA, USA). RNAs were first reverse-transcribed into cDNA with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's manual. The CFX 96 Real-time system (Bio-Rad, Hercules, CA, USA) and SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) were used for the quantitative real-time PCR with the RT-cDNA reaction products according to the manufacturer's directions. All expression levels were normalized to β-actin levels of the same sample. Percent expression was calculated as the ratio of the normalized value of each sample to that of the corresponding untreated ones. All real-time PCR reactions were performed in triplicate. Optimal primer sequences target for PRRs were obtained from Primer Bank [32] (<http://pga.mgh.harvard.edu/primerbank/>) as listed in **Table 1**.

Fecal sample collection and shotgun metagenomic sequencing

We used whole-genome shotgun sequencing to sequence fecal samples. Fresh fecal samples from each group (VDR^{LoxP}: male n=3 and female n=7; VDR^{ΔIEC}: male n=5 and female n=5; VDR^{ΔPC}: male n=5 and female n=5; VDR^{Δlyz}: male n=5 and female n=5) were collected from the colon and placed into the sterile tubes. The samples were kept at low temperature with dry ice and were sent to the University of Illinois at Chicago Research Resources Center for genomic sequencing. The DNAs of samples were extracted using DNeasy Power Fecal Kit (Qiagen, Hilden, Germany) based on manufacturer's instructions with a slight modification. The samples were heated at 65°C for 10 min before homogenizing with FastPre-24 5G bead-beating device (MP Biomedicals, Solon, OH, USA) at 6 m/s for 40 s. These genomic DNA was fragmented into relatively small pieces (generally 250-600 bp fragments) prior sequencing. Sequencing was performed using a Illumina HiSeq system, as in our previous publications [31] [33]. Following quality

checking, filtering the reads, removing noisy sequences, metagenomic assembly [34], the resulting assemblies were filtered to exclude contigs shorter than 1,000 nucleotides and all remaining set of DNA reads (or contigs) were classified with Centrifuge [35], an efficient metagenomic classifier capable of indexing the entirety of nucleotide (nt). Next, the taxonomic annotation of each contig (genes) was obtained by searching for the comprehensive NCBI GenBank non-redundant nucleotide database (as described in <https://merenlab.org/2016/06/18/importing-taxonomy>) ([36]). . After removing the identical sequences with $\geq 99\%$ identity of each other to make it nonredundant, 289,629,756 readable sequences were yielded in 40 studied samples with an average of 7,240,744 reads per sample. Of these sequences, a total of 100,013,480 reads were taxonomic alignments with an average of 2,500,337 reads per sample.

Metabolite sample preparation and metabolomic data analysis

Sample preparation and process. As in our previous report [33], metabolite profiling was performed on Metabolon platform (Metabolon, Inc., Durham, NC, USA). Briefly, fecal samples from mouse groups (VDR^{LoxP}: male n=6 and female n=10; VDR^{ΔIEC}: male n=8 and female n=9; VDR^{ΔLyz}: male n=5 and female n=5) were maintained at -80°C and were prepared using the automated MicroLab STAR® system from Hamilton Company. Several recovery standards were added prior to the first step in the extraction process for QC purposes. The proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation aim to remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites. Next, samples were placed on a TurboVap® (Zymark) briefly to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis. The resulting extract was divided into five fractions for analysis by four methods using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS) (see details in the bioinformatic analysis below) and one for backup.

Bioinformatic analysis of metabolomic data. Bioinformatic analysis was performed using the Metabolon informatic system (the LAN backbone, and a database server running Oracle 10.2.0.1 Enterprise Edition), which consists of four major components, including the Laboratory Information Management System (LIMS), the data extraction and peak-identification software, data processing tools for QC and compound identification, and a collection of information interpretation and visualization tools for use by data analysis. The hardware and software foundations for these informatic components were the LAN backbone and a database server running Oracle 10.2.0.1 Enterprise Edition.

Data extraction and compound identification. After raw data extraction, peak-identification and QC process using Metabolon's hardware and software, compounds were identified by comparison to library entries of more than 3300 currently commercially available purified standard compounds or recurrent unknown entities. Metabolon maintains a library based on authenticated standards that contains the retention time/index (RI), mass to charge ratio (m/z), and chromatographic data (including MS/MS

spectral data) on all molecules present in the library. Furthermore, biochemical identifications are based on three criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards.

Metabolite quantification and data normalization. Peaks were quantified using area-under-the-curve. A data normalization step was performed to correct variation resulting from instrument inter-day tuning differences as necessary or for purposes of data visualization. Additionally, biochemical data were normalized to other factors (e.g., cell counts, total protein as determined by Bradford assay, and osmolality) to account for differences in metabolite levels due to differences in the amount of material present in each sample.

Statistical analysis

All the tests as specified in related analysis performed in study were two-sided. A P-value ≤ 0.05 was considered statistically significant. For a large number of tests, the P-values were adjusted to account for false positives using the False Discovery Rate (FDR) [37] and the q-value (FDR-corrected p-value) ≤ 0.05 was reported to be significant. For all the data, we ran Shapiro-Wilk test to determine whether parametric ANOVA or non-parametric Kruskal-Wallis test is used to group comparisons.

Alpha (within-sample) diversity (e.g., Shannon diversity) measures the number (richness) and/or distribution (evenness) of species within a single sample, while beta (between-sample) diversity (e.g., Bray-Curtis dissimilarity) measures the differences of microbial composition in one sample compared to another [38]. For the metagenomic sequencing data, the raw read counts were normalized to Counts Per Million (CPM). Shannon diversity was used to examine the diversity of the gut microbiome in conditional VDR KO and control mice and the differences between groups were tested with Kruskal-Wallis test.

Bray-Curtis dissimilarity index was used to detect the differences or dissimilarities among studied groups. We first performed principal coordinate analysis (PCoA) to visualize the Bray-Curtis dissimilarities among groups. Next, we performed the nonparametric PERMANOVA to evaluate whether the group (VDR^{ΔIEC}, VDR^{ΔPC}, and VDR^{ΔLyz} mice vs. VDR^{LoxP} mice) has a significant effect on overall gut microbiota composition. Next, we performed pairwise PERMANOVA using RVAideMemoire package. Finally, we conducted analysis of similarity (ANOSIM), another nonparametric procedure, based on a permutation test for rank dissimilarities among between- and within-groups to confirm the hypothesis testing results from those of PERMANOVA and obtained the pairwise-comparison results. The Spearman correlation analysis of viruses and bacteria was performed via the **Hmisc** package [39].

The statistical analysis of metabolites was performed using Welch's two-sample *t*-test on log transformed data. Welch's two-sample *t*-test is used to test whether two unknown means are different from two independent populations, which enables unequal variances and has an *approximate t*-distribution with degrees of freedom estimated using Satterthwaite's approximation. The \log_2 fold-changes were reported as the effect sizes in comparisons between groups. The statistical analyses for microbiome and metabolomic data were performed using R [40], R packages of ampvis2, microbiome, phyloseq and vegan [41], as well as ggplot2 [42] and ggpubr packages [43].

Results

Overall virome community composition in conditional VDR knockout mice

In all fecal samples, 4,048 viral species were identified. Distinct viral compositions and communities, which differed in both diversity and composition, were present between the control mice and the conditional VDR knockout mice (Figure 1). We presented the 10 most abundant viral species for individual samples in each group (Figure 1). The most abundant species in all the groups were *Vibrio* phage JSF5, *Vibrio* phage JSF6, bovine viral diarrhea virus 1, *Escherichia coli* O157 typing phage 7, human alphaherpesvirus 1, *Lactobacillus* prophage Lj771, influenza A virus, *Lactobacillus* phage KC5a, and viruses *incertae sedis* unidentified phage (Figure 1A). When individually analyzing the species, *Vibrio* phages JSF5 and JSF6 were significantly more abundant in all three VDR KO mouse models compared to control VDR^{LoxP} mice. *Escherichia coli* O157 typing phage 7 was found to be significantly more abundant in VDR^{ΔPC} mice, compared to the control mice. Meanwhile, *Lactobacillus* phage phiadh was considerably less abundant in VDR^{ΔLyz} mice compared to control VDR^{LoxP} mice (Figure 1B) (P<0.01).

Altered diversity of the virome in conditional VDR knockout mice

Shannon diversity is commonly used to characterize species diversity in a community [38]. We found that the three conditional VDR knockout groups had lower Shannon diversities than the control mice. The Shannon diversity at the viral species level was significantly lower in VDR^{ΔIEC}, VDR^{ΔPC}, and VDR^{ΔLyz} mice than in VDR^{LoxP} control mice (P=0.05, P=0.05, and P=0.04, respectively) (Figure 2A).

The Bray-Curtis dissimilarity index was used in this study to measure the dissimilarities of samples. We first performed PCoA and found viral dissimilarities between conditional VDR knockout mice and control mice (Figure 2B). VDR^{ΔIEC} mice partially overlapped with control VDR^{LoxP} mice, whereas VDR^{ΔPC} and VDR^{ΔLyz} mice were completely separated from VDR^{LoxP} mice. The group differences can be explained by a total of 37.2% (29.3% + 7.9%) of the variations among the animals.

Next, we performed nonparametric PERMANOVA to evaluate whether VDR status impacts the overall intestinal viral profile. The sequential test "Group and Gender" showed that the dissimilarities among groups were significantly different (P=0.011). Because the overall dissimilarity among groups was

significantly different, we performed a pairwise PERMANOVA and found that the Bray-Curtis dissimilarities of viruses of the VDR^{ΔIEC}, VDR^{ΔPC}, and VDR^{ΔLyz} mice were significantly different from the dissimilarity in the VDR^{LoxP} mice. Furthermore, the differences in dissimilarity among groups were confirmed by ANOSIM (analysis of similarity), where the rank dissimilarities between and within groups were significantly different (P=0.001). VDR^{ΔIEC}, VDR^{ΔPC}, and VDR^{ΔLyz} mice had higher dissimilarities (i.e., lower similarity) than VDR^{LoxP} mice (Figure 2C).

Altered abundance of the virome in conditional VDR knockout mice

We observed that a total of 12 viral species were differential in the conditional VDR knockout mice compared to the control VDR^{LoxP} mice, of which 6 had q-values <0.001, 2 had q-values <0.01 and 4 had q-values <0.05 (Figure 3A). The log-ratio of fold change with significant differences (q<0.05) is shown with colored histograms. There were two enriched viral species in the comparison of VDR^{ΔIEC}/VDR^{LoxP}, *Vibrio* phage JSF5 (q<0.001) and bovine viral diarrhea virus 1 (q<0.05), and one enriched viral species in the comparison of VDR^{ΔPC}/VDR^{LoxP}, *Vibrio* phage JSF5 (q<0.01). *Vibrio* phage JSF5 was more abundant in both VDR^{ΔIEC} and VDR^{ΔPC} mice, whereas enriched BVDV1 was only found in VDR^{ΔIEC} compared with the other groups (Figure 3A). For the comparison of VDR^{ΔLyz} and VDR^{LoxP}, nine virus species were found to be significantly altered (5 with a q value <0.001, 1 with q<0.01, and 3 with a q value <0.05). Of these species, three were enriched (*Vibrio* phage JSF5, bovine viral diarrhea virus 1 and *Vibrio* phage JSF6), while six were depleted (*Lactobacillus* prophage phiadh, Cherry green ring mottle virus, *Lactobacillus* phage KC5a, avian avulavirus 1, *Mycobacterium* virus Phayonce, and one unidentified species in the *Podoviridae* family) (Figure 3A).

Sex-based differences in the gut virome altered by VDR status

To investigate the impact of sex on the alteration of the virome community in the intestines of the studied mice, we illustrated significantly different virus species abundances in male and female mice with logarithmic fold-changes and q-values (Figure 3B). Seven species were detected to be significantly altered in the female VDR^{ΔIEC}/VDR^{LoxP} comparison but not when comparing males of the same two groups, the altered species included 2 enriched species, *Vibrio* phages JSF5 and BVDV1, and 5 depleted species, *Lactobacillus* prophage phiadh, *Lactobacillus* prophage KC5a, *Lactobacillus* prophage Lj771, macacine alphaherpesvirus 1 and Catovirus CTV1. However, only *Vibrio* phage JSF5 was enriched in female VDR^{ΔPC} mice compared to control VDR^{LoxP} mice (Figure 3B), which is the same as in the group-factor analysis. Five species in the female VDR^{ΔLyz}/VDR^{LoxP} comparison were found to be significantly differential (q<0.05), including 2 enriched species: *Vibrio* phage JSF5 and bovine viral diarrhea virus 1, and 3 depleted species, *Lactobacillus* prophage phiadh, Cherry green ring mottle virus and *Lactobacillus* prophage KC5a, while in males, only *Vibrio* phage JSF5 was enriched and only *Lactobacillus* prophage

phiadh was depleted (Figure 3B). Overall, more altered viral species in all conditional VDR knockout mice were found in the female mice than in male mice, which indicated the impact of VDR status on sex differences.

VDR deletion led to significantly enriched *Vibrio* phages

Vibrio phages target *Vibrio cholerae* bacteria, which can secrete cholera toxin and cause watery diarrhea in patients [44, 45]. In this study, we found a markedly enriched abundance of *Vibrio* phage JSF5 and *Vibrio* phage JSF6 in VDR^{ΔIEC}, VDR^{ΔPC}, and VDR^{ΔLyz} mice compared with control VDR^{LoxP} mice (Figure 1). Moreover, differential analysis found that *Vibrio* phage JSF5 was significantly enriched in all three conditional VDR-knockout mice ($q < 0.01$) compared to the control mice, and *Vibrio* phage JSF6 was also more enriched in VDR^{ΔLyz} mice in comparison to VDR^{LoxP} mice ($q < 0.01$) (Figure 3). When the sex factor was included in the analysis, the fold-changes of *Vibrio* phage JSF5 were found to be significant in both female and male VDR^{ΔLyz} mice but only in female VDR^{ΔIEC} and VDR^{ΔPC} mice (Figure 3).

Altered bacterial abundance in conditional VDR knockout mice

We reasoned that the abundance of bacteria in the intestines of the mice should be altered due to the dysbiosis of bacteriophages. In this study, we found that *Vibrio cholerae*, the host of *Vibrio* phage JSF5 and *Vibrio* phage JSF6, which were largely enriched in conditional VDR knockout mice (Figure 1B), was detected at low concentrations in knockout mice compared with control mice (Figure 4A). Significantly lower abundance of this bacterium was found in VDR^{ΔLyz} mice, which matches the finding that both *Vibrio* phage JSF5 and *Vibrio* phage JSF6 were depleted in VDR^{ΔLyz} mice. A similar situation was also found in *Lactobacillus gasseri*, the bacterial host of *Lactobacillus* prophage phiadh and *Lactobacillus* prophage KC5a (Figure 1B; Figure 4A). Furthermore, as the target host of *Escherichia coli* O157 typing phage 7, *E. coli* was found to have the opposite phage species abundance in the mice studied here (Figure 1B; Figure 4A). The altered bacterial abundance in the microbial community supports our findings of virome changes.

To further evaluate the altered bacteria, we showed the altered bacterial species with q -values < 0.1 in differential analysis in our three mouse models (Figure 4B). We found that *Haemophilus ducreyi* and *Mesorhizobium huakuii* were significantly depleted in VDR^{ΔIEC} mice compared with control VDR^{LoxP} mice (Figure 4B). Meanwhile, five bacterial species were significantly depleted, and three bacterial species were significantly enriched in VDR^{ΔPC} mice, compared with the control. These depleted species were *Haemophilus ducreyi*, *Kushneria konosiri*, *Microbacterium* sp. LKL04, *Isosphaera pallida*, and *Actinomyces radingae*. Three enriched bacterial species were *Bacteroides uniformis*, *Faecalibaculum rodentium*, and *Cutibacterium acnes* ($q < 0.01$, Figure 4B). Furthermore, we found that nine bacterial species were significantly depleted, and three bacterial species were enriched in VDR^{ΔLyz} mice. These

depleted bacterial species were *Bifidobacterium pseudolongum*, *Bifidobacterium choerinum*, *Bifidobacterium animalis*, *Bordetella pseudohinzii*, *Haemophilus ducreyi*, *Clostridium perfringens*, *Streptomyces peucetius*, and *Bacteroides acidifaciens*. The three enriched bacterial species were *Ralstonia solanacearum*, *Chroococcidiopsis thermalis*, and *Cutibacterium acnes* (Figure 4B). The less abundant *Haemophilus ducreyi* is a gram-negative bacterium and causative agent of genital ulcer disease chancroid [46] and was detected in the three conditional VDR knockout mice. Similar to viruses, more bacterial species were changed in VDR^{ΔPC} and VDR^{ΔLyz} mice compared to the control mice (Figure 4B).

Correlation of viral and bacterial alterations in conditional VDR KO mice

Bacteria and viruses are essential for protective, metabolic, and physiological functions. We found that the viral (consisting mostly of bacteriophages) and bacterial species abundances were altered in conditional VDR KO mice. To investigate the interactions between bacteria and viruses, we performed a correlation analysis of viruses and bacteria. All the bacterial and viral species with significant fold changes in the differential analysis ($q < 0.05$) were included in the correlation analysis (Figure 4C and Supplement Table 1).

In VDR^{ΔIEC} mice, bovine viral diarrhea virus 1 was significantly negatively correlated with the bacteria *Mesorhizobium huakuii* (correlation coefficient (r)=-0.63; $P=0.05$) (Figure 4C). *M. huakuii* induces the formation of nitrogen-fixation nodules on its host plant *Astragalus sinicus* and has been assigned to a new biovariety based on its host range and taxonomic characteristics [47]. *M. huakuii* isolates were also found to have endotoxic activity against lipopolysaccharides [48]. In VDR^{ΔPC} mice, the *Vibrio* phage JSF5 was dramatically positively correlated with the bacteria *Cutibacterium acnes* ($r=0.98$; $P<0.0001$) (Figure 4C). *Cutibacterium acnes*, formerly known as *Propionibacterium acnes*, is an anaerobic, aerotolerant, bacillus-shaped bacterium. It is ubiquitously found as a commensal on the surface of skin in areas rich in oleic and palmitic acids [49]. Moreover, variable results for the association between *C. acnes* and ulcerative colitis were found [50]. In VDR^{ΔLyz} mice, 11 pairs of virus-bacteria were found to be significantly correlated, including *Vibrio* phage JSF5 and *Bordetella pseudohinzii* ($r=0.65$; $P=0.04$), *Vibrio* phage JSF5 and *Cutibacterium acnes* ($r=0.68$; $P=0.03$), bovine viral diarrhea virus 1 and *Bifidobacterium animalis* ($r=-0.64$; $P=0.05$), bovine viral diarrhea virus 1 and *Haemophilus ducreyi* ($r=0.65$; $P=0.04$), bovine viral diarrhea virus 1 and *Bacteroides acidifaciens* ($r=-0.66$; $P=0.04$), bovine viral diarrhea virus 1 and *Bifidobacterium* sp. ($r=-0.66$; $P=0.04$), Lactobacillus phage phiadh and *Clostridium perfringens* ($r=-0.71$; $P=0.02$), *Vibrio* phage JSF6 and *Bifidobacterium animalis* ($r=-0.67$; $P=0.03$), *Vibrio* phage JSF6 and *Ralstonia solanacearum* ($r=0.65$; $P=0.04$), *Vibrio* phage JSF6 and *Haemophilus ducreyi* ($r=0.75$; $P=0.01$), and *Podoviridae* (family level) and *Chroococcidiopsis thermalis* ($r=-0.75$; $P=0.01$) (Figure 4C). In addition, we found a positive correlation between *Vibrio* phage JSF5 and *Cutibacterium acnes* in VDR^{ΔPC} mice and VDR^{ΔLyz} mice. Taken together, these data indicate the critical role of viral and bacterial interactions in intestinal microbial homeostasis with the support of VDR.

VDR status altered the expression of PRRs in colonic epithelial cells

To examine the impact of virome dysbiosis, virus-related receptors in the colon were evaluated (Figure 5). TLR3 and TLR7 are transmembrane PRRs located in endosomes that recognize nucleic acids and mediate cell extrinsic virus recognition [51]. We observed that expression of TLR3 and TLR7 was upregulated in the conditional VDR knockout mice compared with the control mice, with a significant difference in the VDR^{ΔLyz} group vs. the control group ($P < 0.05$) (Figure 5).

NLRs characterized by the presence of a conserved NOD motif, comprise a large receptor family, including NOD1, NOD2, and NLRPs [52, 53]. NLRs are activated not only in response to viruses but are also important modulators of other virus sensing pathways [51]. Here, we observed upregulated expression of NOD1 and NLRP6 and significantly increased NOD2 RNA in VDR^{ΔLyz} mice compared with control VDR^{LoxP} mice ($P < 0.05$) (Figure 5). C-type lectin receptors (CLRs) also mediate cell-extrinsic sensing of specific viruses by binding viral glycans [54]. Compared with the control VDR group, the expression of CLEC4L, one of the CLRs, was significantly upregulated in all three conditional VDR knockout mice, especially in VDR^{ΔIEC} and VDR^{ΔPC} mice (Figure 5). Overall, the significant alterations of PRRs in conditional VDR knockout mice suggest the influence of VDR on intestinal homeostasis and expression of PRRs.

VDR status altered metabolites related to bacteriophage infection in feces

Microbiota-derived metabolites are chemical messengers that elicit a profound impact on host physiology. Thus, we investigated bacterial metabolites that are related to bacteriophage infection (Figure 6). We found that glycolysis glucose was significantly decreased in VDR^{ΔIEC} mice compared with the control ($P < 0.05$), while ribulose/xylulose and xylose were significantly increased in VDR^{ΔLyz} mice compared with the control ($P < 0.05$) (Figure 6A). Moreover, most of the long-chain fatty acids were significantly increased in both VDR^{ΔIEC} and VDR^{ΔLyz} female mice compared to control mice, while some fatty acids were only increased in VDR^{ΔIEC} mice. 10-Hydroxystearate, which is related to phage infection, was decreased in VDR^{ΔLyz} mice and increased in female VDR^{ΔIEC} mice compared to control mice ($P < 0.05$) (Figure 6B). Metabolic alterations of nucleotides were also detected in both conditional knockout mice, such as uridine in VDR^{ΔIEC} mice and 3-ureidoisobutyrate in VDR^{ΔLyz} mice ($P < 0.05$) (Figure 6C). Similarly, we observed amino acid alterations in the feces of conditional VDR knockout mice when compared to those of the control mice. For instance, we observed decreased phage infection-related serine in both VDR^{ΔIEC} and VDR^{ΔLyz} female mice and decreased cellular virus infection-related glutamine in VDR^{ΔIEC} and VDR^{ΔLyz} female mice compared to the control (Figure 6D).

Discussion

In the current study, we report that conditionally deleted VDR from intestinal epithelial cells, Paneth cells, and myeloid cells significantly altered the virome profile and virome-bacterial interactions in mice. The changes in *Vibrio* phages, *Lactobacillus* phages, and *E. coli* typing phage were significantly related to VDR status. Seven more virus species were significantly changed in VDR^{ΔLyz} mice compared to control mice, while BVDV1 was significantly enriched in VDR^{ΔIEC} mice. The altered viral abundance was greater in female mice than in male mice. Bacteriophages modulate their hosts directly by affecting their mortality and horizontal gene transfer or indirectly by impacting target/host bacteria and hence may alter the microbiome. After conditionally deleting VDR, the virome and other aspects of the microbiota were changed, which further led to microbial dysbiosis. From a functional perspective, the significant changes in PRRs and metabolites related to infection suggest the influence of VDR on intestinal virus homeostasis.

Vibrio phages JSF5 and JSF6 are organisms hosted by *Vibrio cholera*, which can secrete cholera toxin and cause watery diarrhea in patients [44, 45]. The *Lactobacillus* phages phiadh and KC5a are members of the viral family *Siphoviridae* and can preferentially infect the bacteria *Lactobacillus gasseri*, which is a common component of intestinal mucosae and plays an important role in modulating the gut immune system [55]. These observations were consistent with our results that VDR deletion in myeloid cells had a more severe influence on the intestinal virome balance. Similarly, the interactions in the microbial community are important to maintain homeostasis and host health. For instance, viruses can bind to bacterial products (e.g., lipopolysaccharide or HBGA (human blood group antigen)-like substances), increase virome stability and protect virome from physical stresses [56].

We found that the *Lactobacillus* phage KC5a and *E. coli* O157 typing phage 7, which belong to the family *Myoviridae*, and *Lactobacillus* phage phiadh, which belongs to the family *Siphoviridae*, were altered in our conditional VDR knockout mouse models compared with the control mice. A study indicated that the CD susceptibility gene *ATG16L1* was phenocopied in mice infected with murine norovirus [57-59]. Meanwhile, we have demonstrated that VDR transcriptionally regulates *ATG16L1*, and VDR status may be a determinant of IBD risk through its actions on *ATG16L1* [26, 60]. However, these results may support a model in which bacteriophages contribute to the development of bacterial dysbiosis associated with IBD. Bacteriophages modulate their hosts directly by affecting their mortality and horizontal gene transfer and by further altering the components of the intestinal community and contributing to dysbiosis. The virome could be a candidate biomarker for human IBD [61] because CD and UC were associated with a significant expansion of *Caudovirales* bacteriophages. The phage families of the *Caudovirales* order, including the *Siphoviridae*, *Myoviridae* and *Podoviridae*, were found to be altered in murine colitis and human IBD patients [62]. Therefore, further studies are needed to elucidate the important and real role of the intestinal virome in the development and progression of IBD.

Paneth cells play an integral role in shaping the microbiome and host defenses. The absence of VDR in Paneth cells impairs antimicrobial function, affects microbial assemblage, and increases susceptibility to colitis and infection [26, 31]. The altered virome in $VDR^{\Delta PC}$ mice further indicated the important role of VDR and Paneth cells in shaping the intestinal microbiome and secreting antimicrobial peptides or metabolites. Paneth cells may indirectly impact the intestinal microbiota or the outcome of a viral infection by adjusting the population of bacteria. Paneth cells also play a key role in host defense by sensing microorganisms through TLRs [63]. It is clear from our data that Paneth cell dysfunction leads to dysbiosis and a compromised epithelial barrier. Our previous reports demonstrated that VDR could promote healthy microbial metabolites and a healthy microbiome to prevent obesity [33]. Furthermore, it has been reported that increased serum 25(OH)D was associated with increased beneficial bacteria, such as *Parabacteroides*, which were suppressed in IBD patients and were altered in an obesity mouse model [33, 64]. We have reported that human *VDR* gene variation determines the abundance of *Parabacteroides* [28]. In this study, we also found a decreased abundance of *Parabacteroides* in three VDR knockout mice ($VDR^{\Delta PC}$, $VDR^{\Delta IEC}$ and $VDR^{\Delta Lyz}$) compared with VDR^{LoxP} controls. This further confirms the critical role of VDR in shaping the microbiome at the genetic level.

Eukaryotic viruses, such as adenoviruses, hepatitis B virus, hepatitis C virus and human immunodeficiency virus [65], are also present in the intestinal viromes of some individuals, which indicates the potential infectious capability of these viruses in the host. BVDV1 can infect mice and cause significant histopathological damage [66]. The significantly increased abundance of intestinal BVDV1 in VDR conditional knockout mice suggested the importance of VDR in viral infection and survival inside the host. Furthermore, the association between vitamin D deficiency and the pathogenesis and course of HIV disease has also been recognized recently. Infants born from HIV-infected women with vitamin D deficiency are at an increased risk of infection and a decreased survival rate [67]. In addition, the VDR polymorphism *FokI* was evaluated using a meta-analysis to be consistently associated with susceptibility to infection to respiratory syncytial virus [20].

The innate immune response is nonspecific, is the first line of defense against infectious agents and initiates antigen presentation, including responses to viral infection. PRRs and related pathways involved in intestinal virus sensing, such as TLRs, are mainly mediated by cell-extrinsic virus recognition [68]. We found significant alterations in PRRs, including upregulated expression of TLR3, TLR7 and NOD2 in $VDR^{\Delta Lyz}$ mice and increased expression of CLEC4L in $VDR^{\Delta IEC}$ and $VDR^{\Delta PC}$ mice, suggesting the influence of VDR on intestinal virus homeostasis. Our data further suggest that the impacts of tissue-specific VDR deletion on intestinal receptors are different. For instance, VDR deletion in myeloid cells would significantly increase TLR3, TLR7 and NOD2, while VDR deletion in intestinal epithelial cells would more likely increase CLEC4L levels. TLRs are known to be affected by VDR in monocytes and epidermal keratinocytes [12]. In mice administered an antiviral drug cocktail, the depletion of gut viral entities could

increase susceptibility to DSS via TLR3 and TLR7 signaling, with a final increased level of *Caudovirales*, which is observed in IBD patients [61, 69]. Viral dysbiosis in the intestine is consistent with our reports that VDR deletion leads to a higher risk of infection and chronic inflammation [27, 31]. Vitamin D metabolites have long been known to support innate antiviral effector mechanisms, including induction of antimicrobial peptides and autophagy. Laboratory data relating to effects of vitamin D on host responses to SARS-CoV-2 specifically are scarce [1, 2].

The diversity and abundance of bacteriophages have been proposed to affect bacterial communities in the gut [70]. We found a significant correlation between the virome and bacteria altered by VDR status. The enrichment of bacteriophages and related bacteria were consistent, such as *Vibrio* phages and their host *Vibrio cholerae*. *Bifidobacterium animalis*, one of the bacteria widely used for probiotics in clinical trials [71, 72], was negatively correlated with BVDV1 and *Vibrio* phage JSF6. The impaired protection of *B. animalis* in the intestinal barrier may further enhance infection with pathogenic viruses, such as BVDV1 and *Vibrio* phage JSF6. Similarly, *Bacteroides acidifaciens*, which was found to have strong protective effects against colitis [73], was found to be less abundant and thus negatively correlated with BVDV1. Meanwhile, *Bordetella pseudohinzii* showed a positive correlation with *Vibrio* phage JSF5. As a new member of the genus *Bordetella*, *B. pseudohinzii* was first identified and isolated from laboratory-raised mice and has now been detected in mouse facilities worldwide [74]. The infected mice presented elevated numbers of neutrophils in bronchoalveolar lavage fluid and inflammatory signs in histopathology, although no obvious clinical symptoms were shown [75]. These causative agents may induce more severe inflammation through cooperation with intestinal microbes. Similarly, the abundances of *Vibrio* phage JSF5 and *Bordetella pseudohinzii* [50], were changed in VDR^{ΔPC} and VDR^{ΔLyz} mice compared to control mice.

Microbial metabolites are important players in diverse cellular processes and functions. We found marked changes in virus-related metabolites and pathways, such as fatty acid metabolism. It has been suggested that most viruses require lipids or intermediates of lipid synthesis to replicate, and many of them actively induce lipid metabolic pathways to sustain a favorable replication environment [76]. Pathogenic viruses use lipid droplets as a platform for viral replication, and many non-oncogenic viruses are related to various metabolic alterations during infection, such as glycolysis, nucleotide synthesis, fatty acid biosynthesis and glutaminolysis [65, 77]. This finding may explain our results that many long-chain fatty acid and fatty acid metabolites were increased in VDR^{ΔIEC} and VDR^{ΔLyz} mice. Although among the most abundant microbes in the gut, phages are also among the least understood [78]. Using gnotobiotic mice, Hsu et al. found that phage predation not only directly impacts susceptible bacteria but also leads to cascading effects on other bacterial species via interbacterial interactions. Moreover, the shifts in the microbiome caused by phage predation have a direct consequence on the intestinal metabolome as revealed by metabolomic profiling [79]. We also found that the levels some phage

infection-related metabolites were altered in the conditional VDR knockout mice compared to the control. For example, VDR deletion decreased fecal serine amino acids, which is consistent with a previous study [79]. Even though various aspects of host central carbon metabolism have been shown to be related to virus infection, several viruses were also found to increase the consumption of key nutrients such as glucose and glutamine and converge on similar metabolic pathways for anabolism [65], which was different from our results. It should be mentioned that all these reports are based on host cell metabolic data, while our analysis is based on intestinal microbial metabolites. Furthermore, it is noteworthy that the precise metabolic changes induced by specific viruses are often context-dependent and can vary even within the same viral family or largely depend on the individual host [65]. However, the regulatory complexity of viral metabolism in chronic diseases is an area of investigation in the future.

Conclusions

The virome includes viruses that infect host cells, virus-derived elements in the genome, and viruses that infect the broad array of other types of microorganisms that inhabit the host [80]. Dysbiosis is associated with the treatment efficiency of vitamin D supplements [81, 82]. Our data demonstrate that conditional VDR knockout causes changes in the abundance and diversity of the virome and functional changes in viral intestinal receptors, which may further induce intestinal dysbiosis and the risk of infection. We also found that the related fecal metabolites were altered in the mice with tissue-specific deletion, which further confirmed the consequences of dysbiosis. The marked alteration of gut viruses (especially bacteriophages) by VDR status may aid the development of intestinal phage-bacteria or intestinal virus-bacteria therapy against pathobionts [56, 83]. Our study fills the knowledge gaps of how the virome is affected by VDR in a tissue-specific manner. The physiological relevance of these changes will be assessed in the future in digestive disease and infectious models. Notably, there is a growing body of evidence suggesting that VDR activation has a regulatory role in mutualistic intestinal virome-host interactions, and more information on the interactions between the sensing of vitamin D and VDR is needed [84].

List Of Abbreviations

ATG16L1: Autophagy Related 16 Like 1

BVDV 1: Bovine viral diarrhea virus 1

CD: Crohn's disease

CLEC4L: C-type lectin domain family 4

CLRs: C-type lectin receptors

COVID19: coronavirus disease 2019

CRC: Colorectal cancer

HBGA: human blood group antigen

IBD: Inflammatory bowel disease

LPS: Lipopolysaccharide

TLR: Toll-like receptor

NOD: nucleotide-binding oligomerization domain

NLRs: nucleotide-binding oligomerization domain-like receptor

PCoA: performed principal coordinate analysis

PERMANOVA: nonparametric permutational multivariate analysis of variance

PRR: Pattern recognition receptors

UC: Ulcerative colitis

Declarations

Ethics approval and consent to participate:

No human study. All animal studies were performed following ACC guidelines at the University of Illinois at Chicago (UIC), IL, U.S.A.

Consent for publication:

Not applicable.

Availability of data and material:

data and material will be available by request.

Competing interests:

There are no competing interests. The authors declare no conflict of interest.

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Authors' contributions:

Jun Sun obtained funds, designed the study, and directed the project. Yinglin Xia designed the study and directed the project for the statistical analysis of the microbiome, metabolite data, and other data. Jilei Zhang performed PCR, metabolites analysis, detailed analysis, and metagenomic analysis of the microbiome and prepared the figures and draft. Yongguo Zhang participated in the animal studies. All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

References

1. Martineau AR, Forouhi NG: **Vitamin D for COVID-19: a case to answer?** *Lancet Diabetes Endocrinol* 2020, **8**(9):735-736.
2. Zhang JG, S.; Sun, J: **Gastrointestinal symptoms, pathophysiology, and treatment in COVID-19.** *Genes & Diseases* 2020(<https://doi.org/10.1016/j.gendis.2020.08.013>)
3. Bakke D, Chatterjee I, Agrawal A, Dai Y, Sun J: **Regulation of Microbiota by Vitamin D Receptor: A Nuclear Weapon in Metabolic Diseases.** *Nuclear receptor research* 2018, **5**.
4. Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh J-C, Jurutka PW: **Molecular mechanisms of vitamin D action.** *Calcified tissue international* 2013, **92**(2):77-98.
5. Bakke D, Sun J: **Ancient Nuclear Receptor VDR With New Functions: Microbiome and Inflammation.** *Inflammatory bowel diseases* 2018, **24**(6):1149-1154.
6. Barbáchano A, Fernández-Barral A, Ferrer-Mayorga G, Costales-Carrera A, Larriba MJ, Muñoz A: **The endocrine vitamin D system in the gut.** *Molecular and cellular endocrinology* 2017, **453**:79-87.
7. Barragan M, Good M, Kolls JK: **Regulation of dendritic cell function by vitamin D.** *Nutrients* 2015, **7**(9):8127-8151.
8. Bashir M, Prietl B, Tauschmann M, Mautner SI, Kump PK, Treiber G, Wurm P, Gorkiewicz G, Högenauer C, Pieber TR: **Effects of high doses of vitamin D 3 on mucosa-associated gut microbiome vary between regions of the human gastrointestinal tract.** *European journal of nutrition* 2016, **55**(4):1479-1489.
9. Korf H, Wenes M, Stijlemans B, Takiishi T, Robert S, Miani M, Eizirik DL, Gysemans C, Mathieu C: **1, 25-Dihydroxyvitamin D3 curtails the inflammatory and T cell stimulatory capacity of macrophages through an IL-10-dependent mechanism.** *Immunobiology* 2012, **217**(12):1292-1300.
10. Ooi JH, Li Y, Rogers CJ, Cantorna MT: **Vitamin D regulates the gut microbiome and protects mice from dextran sodium sulfate–induced colitis.** *The Journal of nutrition* 2013, **143**(10):1679-1686.
11. Young VB, Kahn SA, Schmidt TM, Chang EB: **Studying the enteric microbiome in inflammatory bowel diseases: getting through the growing pains and moving forward.** *Frontiers in microbiology* 2011, **2**:144.
12. Gruber-Bzura B: **Vitamin D and Influenza—Prevention or Therapy?** *International journal of molecular sciences* 2018, **19**(8):2419.
13. Cannell J, Vieth R, Umhau J, Holick M, Grant W, Madronich S, Garland C, Giovannucci E: **Epidemic influenza and vitamin D.** *Epidemiology & Infection* 2006, **134**(6):1129-1140.

14. Mowry EM, James JA, Krupp LB, Waubant E: **Vitamin D status and antibody levels to common viruses in pediatric-onset multiple sclerosis.** *Multiple Sclerosis Journal* 2011, **17**(6):666-671.
15. Chao C-T, Lee S-Y, Yang W-S, Yen C-J, Chiang C-K, Huang J-W, Hung K-Y: **Serum vitamin D levels are positively associated with varicella zoster immunity in chronic dialysis patients.** *Scientific reports* 2014, **4**(1):1-8.
16. Villar LM, Del Campo JA, Ranchal I, Lampe E, Romero-Gomez M: **Association between vitamin D and hepatitis C virus infection: a meta-analysis.** *World Journal of Gastroenterology: WJG* 2013, **19**(35):5917.
17. Jolliffe DA, Greiller CL, Mein CA, Hoti M, Bakhsoliani E, Telcian AG, Simpson A, Barnes NC, Curtin JA, Custovic A: **Vitamin D receptor genotype influences risk of upper respiratory infection.** *British Journal of Nutrition* 2018, **120**(8):891-900.
18. Jiménez-Sousa MÁ, Martínez I, Medrano LM, Fernández-Rodríguez A, Resino S: **Vitamin D in human immunodeficiency virus infection: influence on immunity and disease.** *Frontiers in immunology* 2018, **9**:458.
19. Villamor E: **A potential role for vitamin D on HIV infection?** *Nutrition reviews* 2006, **64**(5):226-233.
20. Laplana M, Royo JL, Fibla J: **Vitamin D Receptor polymorphisms and risk of enveloped virus infection: A meta-analysis.** *Gene* 2018, **678**:384-394.
21. Bellamy R, Ruwende C, Corrah T, McAdam K, Thursz M, Whittle H, Hill A: **Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene.** *The Journal of infectious diseases* 1999, **179**(3):721-724.
22. Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW: **Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense.** *The Journal of Immunology* 2008, **181**(10):7090-7099.
23. Gombart AF: **The vitamin D–antimicrobial peptide pathway and its role in protection against infection.** *Future microbiology* 2009, **4**(9):1151-1165.
24. Mukhopadhyay I, Segal JP, Carding SR, Hart AL, Hold GL: **The gut virome: the 'missing link' between gut bacteria and host immunity?** *Therapeutic advances in gastroenterology* 2019, **12**:1756284819836620.
25. Zhang YG, Wu S, Yi J, Xia Y, Jin D, Zhou J, Sun J: **Target Intestinal Microbiota to Alleviate Disease Progression in Amyotrophic Lateral Sclerosis.** *Clinical therapeutics* 2017, **39**(2):322-336.
26. Wu S, Zhang Y-g, Lu R, Xia Y, Zhou D, Petrof EO, Claud EC, Chen D, Chang EB, Carmeliet G: **Intestinal epithelial vitamin D receptor deletion leads to defective autophagy in colitis.** *Gut* 2015, **64**(7):1082-1094.
27. Wu S, Liao AP, Xia Y, Li YC, Li J-D, Sartor RB, Sun J: **Vitamin D receptor negatively regulates bacterial-stimulated NF-κB activity in intestine.** *The American journal of pathology* 2010, **177**(2):686-697.
28. Wang J, Thingholm LB, Skiecevičienė J, Rausch P, Kummen M, Hov JR, Degenhardt F, Heinsen F-A, Rühlemann MC, Szymczak S: **Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota.** *Nature genetics* 2016, **48**(11):1396.

29. Jun S: **Ethnicity May Be Important for Studying the Role of the Microbiome and Vitamin D Receptor in IBD.** *Inflammatory bowel diseases* 2019, **25**(5):e54-e54.
30. Van Cromphaut SJ, Dewerchin M, Hoenderop JG, Stockmans I, Van Herck E, Kato S, Bindels RJ, Collen D, Carmeliet P, Bouillon R: **Duodenal calcium absorption in vitamin D receptor–knockout mice: functional and molecular aspects.** *Proceedings of the National Academy of Sciences* 2001, **98**(23):13324-13329.
31. Lu R, Zhang Y, Xia Y, Zhang J, Kaser A, Blumberg R, Sun J: **Paneth cell alertness to pathogens maintained by vitamin D receptors.** *Gastroenterology* 2020.
32. **PrimerBank: PCR Primers for Gene Expression Detection and Quantification** [<https://pga.mgh.harvard.edu/primerbank/index.html>]
33. Chatterjee I, Lu R, Zhang Y, Zhang J, Dai Y, Xia Y, Sun J: **Vitamin D receptor promotes healthy microbial metabolites and microbiome.** *Scientific Reports* 2020, **10**(1):7340.
34. Xia Y, Sun J, Chen D-G: **Bioinformatic analysis of microbiome data.** In: *Statistical Analysis of Microbiome Data with R.* edn.: Springer; 2018: 1-27.
35. Kim D, Song L, Breitwieser FP, Salzberg SL: **Centrifuge: rapid and sensitive classification of metagenomic sequences.** *Genome research* 2016, **26**(12):1721-1729.
36. **Importing taxonomy into contigs database** [<https://merenlab.org/2016/06/18/importing-taxonomy/>]
37. Storey JD, Tibshirani R: **Statistical significance for genomewide studies.** *Proceedings of the National Academy of Sciences* 2003, **100**(16):9440-9445.
38. Xia Y, Sun J, Chen D-G: **Statistical analysis of microbiome data with R:** Springer; 2018.
39. **Hmisc: Harrell Miscellaneous. R package version 4.4-1.** <https://CRAN.R-project.org/package=Hmisc> [<https://CRAN.R-project.org/package=Hmisc>]
40. R Core Team: **R: A language and environment for statistical computing.** In. Vienna, Austria: R Foundation for Statistical Computing; 2019.
41. Xia Y, Sun J, Chen D-G: **Statistical analysis of microbiome data with R.** In.: Springer; 2018.
42. Wickham. H: **ggplot2: Elegant Graphics for Data Analysis.** New York: Springer-Verlag; 2016.
43. **ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0.** <https://CRAN.R-project.org/package=ggpubr> [<https://CRAN.R-project.org/package=ggpubr>]
44. Naser IB, Hoque MM, Nahid MA, Tareq TM, Rocky MK, Faruque SM: **Analysis of the CRISPR-Cas system in bacteriophages active on epidemic strains of Vibrio cholerae in Bangladesh.** *Scientific reports* 2017, **7**(1):14880.
45. hun Yoon S, Waters CM: **Vibrio cholerae.** *Trends in microbiology* 2019, **27**(9):806-807.
46. González-Beiras C, Marks M, Chen CY, Roberts S, Mitjà O: **Epidemiology of Haemophilus ducreyi Infections.** *Emerging infectious diseases* 2016, **22**(1):1-8.
47. Luo S, Yin J, Peng Y, Xie J, Wu H, He D, Li X, Cheng G: **Glutathione is Involved in Detoxification of Peroxide and Root Nodule Symbiosis of Mesorhizobium huakuii.** *Current microbiology* 2020, **77**(1):1-

- 10.
48. Komanięcka I, Zdzisinska B, Kandefer-Szerszen M, Choma A: **Low endotoxic activity of lipopolysaccharides isolated from Bradyrhizobium, Mesorhizobium, and Azospirillum strains.** *Microbiology and immunology* 2010, **54**(12):717-725.
49. Fournięre M, Latire T, Souak D, Feuilloley MGJ, Bedoux G: **Staphylococcus epidermidis and Cutibacterium acnes: Two Major Sentinels of Skin Microbiota and the Influence of Cosmetics.** *Microorganisms* 2020, **8**(11).
50. Sakhamuru S, Kambampati S, Wasim S, Kukkar V, Malik BH: **The Role of Propionibacterium acnes in the Pathogenesis of Sarcoidosis and Ulcerative Colitis: How This Connection May Inspire Novel Management of These Conditions.** *Cureus* 2020, **12**(10):e10812.
51. Metzger RN, Krug AB, Eisenächer K: **Enteric virome sensing—its role in intestinal homeostasis and immunity.** *Viruses* 2018, **10**(4):146.
52. Elinav E, Strowig T, Henao-Mejia J, Flavell RA: **Regulation of the antimicrobial response by NLR proteins.** *Immunity* 2011, **34**(5):665-679.
53. Wang T-T, Dabbas B, Laperriere D, Bitton AJ, Soualhine H, Tavera-Mendoza LE, Dionne S, Servant MJ, Bitton A, Seidman EG: **Direct and indirect induction by 1, 25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin β 2 innate immune pathway defective in Crohn disease.** *Journal of Biological Chemistry* 2010, **285**(4):2227-2231.
54. Monteiro J, Lepenies B: **Myeloid C-type lectin receptors in viral recognition and antiviral immunity.** *Viruses* 2017, **9**(3):59.
55. Yolken RH, Severance EG, Sabunciyani S, Gressitt KL, Chen O, Stallings C, Origeni A, Katsafanas E, Schweinfurth LA, Savage CL: **Metagenomic sequencing indicates that the oropharyngeal phageome of individuals with schizophrenia differs from that of controls.** *Schizophrenia bulletin* 2015, **41**(5):1153-1161.
56. Monedero V, Collado MC, Rodríguez-Díaz J: **Therapeutic Opportunities in Intestinal Microbiota-Virus Interactions.** *Trends in biotechnology* 2018, **36**(7):645-648.
57. Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Kc W, Carrero JA, Hunt S: **A key role for autophagy and the autophagy gene Atg1611 in mouse and human intestinal Paneth cells.** *Nature* 2008, **456**(7219):259-263.
58. Cadwell K, Patel KK, Maloney NS, Liu T-C, Ng AC, Storer CE, Head RD, Xavier R, Stappenbeck TS, Virgin HW: **Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine.** *Cell* 2010, **141**(7):1135-1145.
59. Sartor RB, Wu GD: **Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches.** *Gastroenterology* 2017, **152**(2):327-339. e324.
60. Sun J: **VDR/vitamin D receptor regulates autophagic activity through ATG16L1.** *Autophagy* 2016, **12**(6):1057-1058.
61. Norman JM, Handley SA, Baldrige MT, Droit L, Liu CY, Keller BC, Kambal A, Monaco CL, Zhao G, Fleshner P *et al.*: **Disease-specific alterations in the enteric virome in inflammatory bowel disease.** *Cell*

- 2015, **160**(3):447-460.
62. Duerkop BA, Kleiner M, Paez-Espino D, Zhu W, Bushnell B, Hassell B, Winter SE, Kyrpides NC, Hooper LV: **Murine colitis reveals a disease-associated bacteriophage community.** *Nature microbiology* 2018, **3**(9):1023.
63. Holly MK, Smith JG: **Paneth cells during viral infection and pathogenesis.** *Viruses* 2018, **10**(5):225.
64. CHAROENNGAM N, SHIRVANI A, KALAJIAN TA, SONG A, HOLICK MF: **The Effect of Various Doses of Oral Vitamin D3 Supplementation on Gut Microbiota in Healthy Adults: A Randomized, Double-blinded, Dose-response Study.** *Anticancer Research* 2020, **40**(1):551-556.
65. Thaker SK, Ch'ng J, Christofk HR: **Viral hijacking of cellular metabolism.** *BMC biology* 2019, **17**(1):59.
66. Seong G, Oem J-K, Lee K-H, Choi K-S: **Experimental infection of mice with bovine viral diarrhea virus.** *Archives of virology* 2015, **160**(6):1565-1571.
67. Spector SA: **Vitamin D and HIV: letting the sun shine in.** *Topics in antiviral medicine* 2011, **19**(1):6.
68. Iwasaki A, Medzhitov R: **Control of adaptive immunity by the innate immune system.** *Nature immunology* 2015, **16**(4):343-353.
69. Yang JY, Kim MS, Kim E, Cheon JH, Lee YS, Kim Y, Lee SH, Seo SU, Shin SH, Choi SS *et al*: **Enteric Viruses Ameliorate Gut Inflammation via Toll-like Receptor 3 and Toll-like Receptor 7-Mediated Interferon- β Production.** *Immunity* 2016, **44**(4):889-900.
70. Rodriguez-Brito B, Li L, Wegley L, Furlan M, Angly F, Breitbart M, Buchanan J, Desnues C, Dinsdale E, Edwards R: **Viral and microbial community dynamics in four aquatic environments.** *The ISME journal* 2010, **4**(6):739-751.
71. Boontun C, Vatanyoopaisarn S, Hankla S, Eisuke K, Yasutomo T: **Modification of media using food-grade components for the fermentation of Bifidobacterium and Lactobacillus strains in large-scale bioreactors.** *Preparative biochemistry & biotechnology* 2021:1-11.
72. Penhasi A, Reuveni A, Baluashvili I: **Microencapsulation May Preserve the Viability of Probiotic Bacteria During a Baking Process and Digestion: A Case Study with Bifidobacterium animalis Subsp. lactis in Bread.** *Current microbiology* 2021.
73. Han R, Ma Y, Xiao J, You L, Pedisić S, Liao L: **The possible mechanism of the protective effect of a sulfated polysaccharide from Gracilaria Lemaneiformis against colitis induced by dextran sulfate sodium in mice.** *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 2021:112001.
74. Ma L, Huang S, Luo Y, Min F, He L, Chen M, Pan J, Zhang Y, Wang J: **Isolation and characterization of Bordetella pseudohinzii in mice in China.** *Animal models and experimental medicine* 2019, **2**(3):217-221.
75. Perniss A, Schmidt N, Gurtner C, Dietert K, Schwengers O, Weigel M, Hempe J, Ewers C, Pfeil U, Gärtner U *et al*: **Bordetella pseudohinzii targets cilia and impairs tracheal cilia-driven transport in naturally acquired infection in mice.** *Sci Rep* 2018, **8**(1):5681.

76. Lange PT, Lagunoff M, Tarakanova VL: **Chewing the Fat: The Conserved Ability of DNA Viruses to Hijack Cellular Lipid Metabolism.** *Viruses* 2019, **11**(2):119.
77. Wong AC, Vanhove AS, Watnick PI: **The interplay between intestinal bacteria and host metabolism in health and disease: lessons from *Drosophila melanogaster*.** *Disease models & mechanisms* 2016, **9**(3):271-281.
78. Keen EC, Dantas G: **Close encounters of three kinds: bacteriophages, commensal bacteria, and host immunity.** *Trends in microbiology* 2018, **26**(11):943-954.
79. Hsu BB, Gibson TE, Yeliseyev V, Liu Q, Lyon L, Bry L, Silver PA, Gerber GK: **Dynamic Modulation of the Gut Microbiota and Metabolome by Bacteriophages in a Mouse Model.** *Cell host & microbe* 2019.
80. Virgin Herbert W: **The Virome in Mammalian Physiology and Disease.** *Cell* 2014, **157**(1):142-150.
81. Jadhav P, Jiang Y, Jarr K, Layton C, Ashouri JF, Sinha SR: **Efficacy of Dietary Supplements in Inflammatory Bowel Disease and Related Autoimmune Diseases.** *Nutrients* 2020, **12**(7).
82. Kanhere M, He J, Chassaing B, Ziegler TR, Alvarez JA, Ivie EA, Hao L, Hanfelt J, Gewirtz AT, Tangpricha V: **Bolus Weekly Vitamin D3 Supplementation Impacts Gut and Airway Microbiota in Adults With Cystic Fibrosis: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial.** *The Journal of clinical endocrinology and metabolism* 2018, **103**(2):564-574.
83. Fujimoto K, Kimura Y, Shimohigoshi M, Satoh T, Sato S, Tremmel G, Uematsu M, Kawaguchi Y, Usui Y, Nakano Y *et al*: **Metagenome Data on Intestinal Phage-Bacteria Associations Aids the Development of Phage Therapy against Pathobionts.** *Cell Host Microbe* 2020, **28**(3):380-389.e389.
84. Waldschmitt N, Chamaillard M: **Time for epithelial sensing of vitamin D to step into the limelight.** *Gut* 2015, **64**(7):1013-1014.
85. Kuss SK, Best GT, Etheredge CA, Pruijssers AJ, Frierson JM, Hooper LV, Dermody TS, Pfeiffer JK: **Intestinal microbiota promote enteric virus replication and systemic pathogenesis.** *Science* 2011, **334**(6053):249-252.

Tables

Table 1. Primers for real-time PCR used in this study.

Primer	Nucleotides
TLR3-F	GTGAGATACAACGTAGCTGACTG
TLR3-R	TCCTGCATCCAAGATAGCAAGT
TLR7-F	ATGTGGACACGGAAGAGACAA
TLR7-R	GGTAAGGGTAAGATTGGTGGTG
NOD1-F	GAAGGCACCCCATTGGGTT
NOD1-R	AATCTCTGCATCTTCGGCTGA
NOD2-F	CAGGTCTCCGAGAGGGTACTG
NOD2-R	GCTACGGATGAGCCAAATGAAG
NLRp6-F	CTCGCTTGCTAGTGACTACAC
NLRp6-R	AGTGCAAACAGCGTCTCGTT
CLEC4L-F	CCTGAACACAAGTGCTGGTTA
CLEC4L-R	CACATCGTTCCCAATCTTGGT

Figures

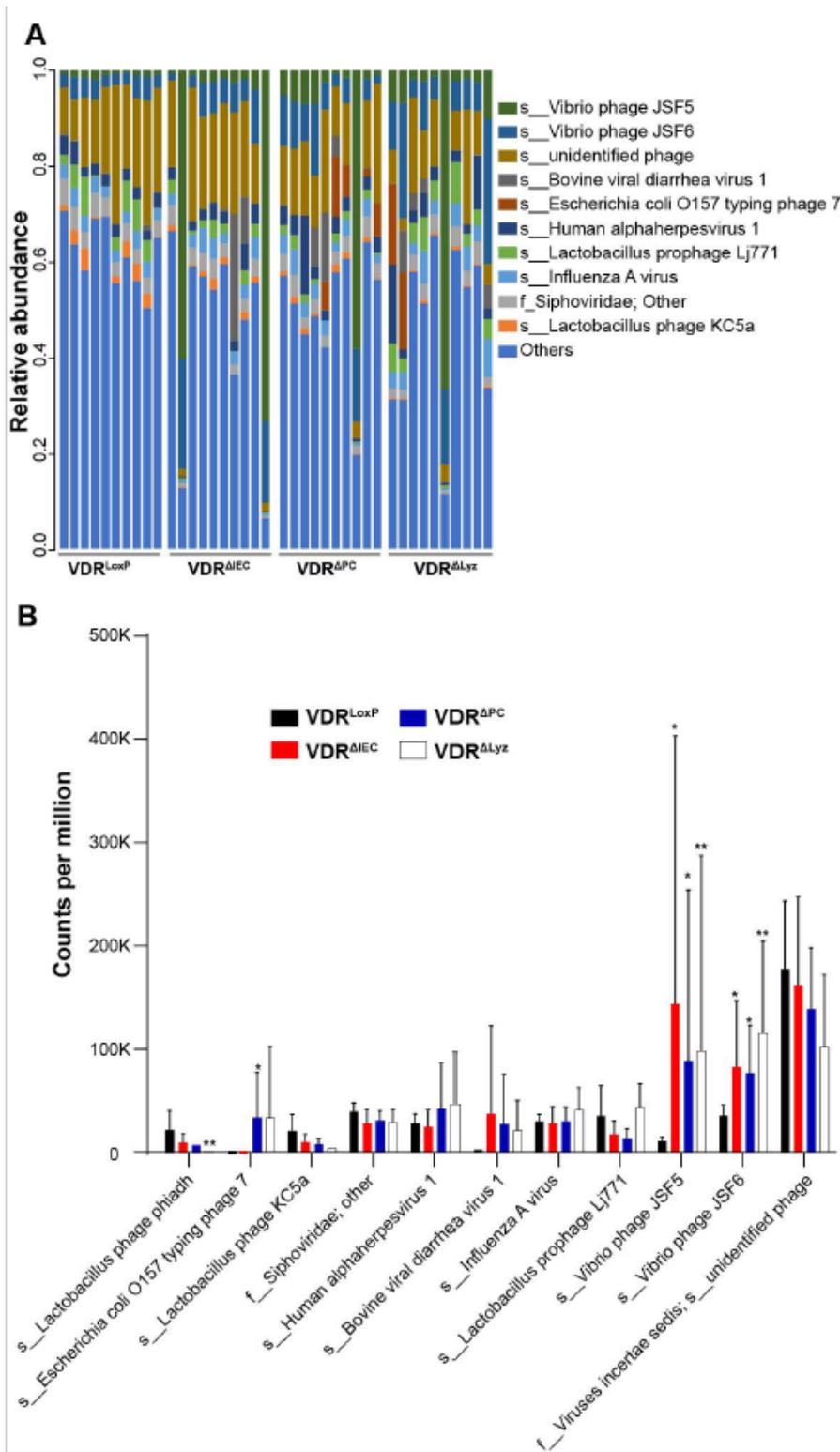


Figure 1

Altered taxa abundance of viruses in feces of the three conditional VDR-knockout and control mice. (A) Relative viral abundance at the species level (family/f.; s.; the unidentified species were named with super level and other) is shown with the top 10 species, and less abundant species were grouped as “others”. Species were colored using the key as listed on the right side of the figure. Each bar represents an individual mouse (n=10 each group). (B) The virus differential abundance at count per million of the

top 10 species in four studied mouse genotypes are illustrated with different colors. Statistical analysis in each group was compared to control VDR^{Loxp} mice. Mean \pm SD, n=10 per group; ** P-value < 0.01, * P-value < 0.05, Kruskal–Wallis test by ranks.

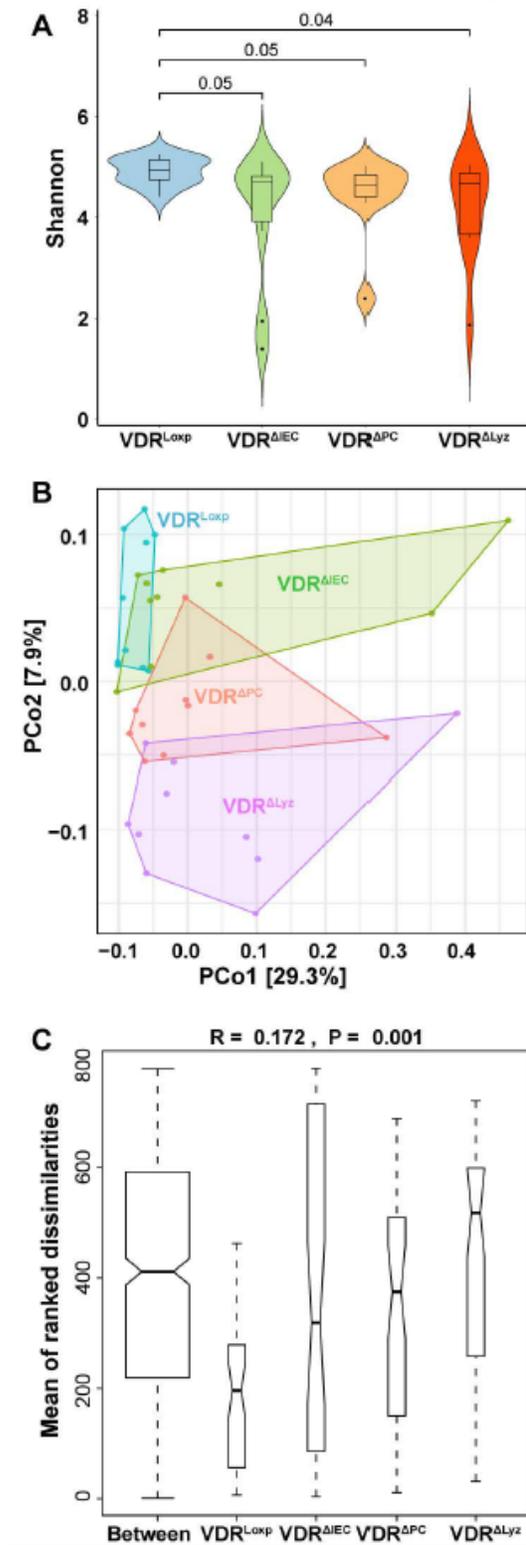
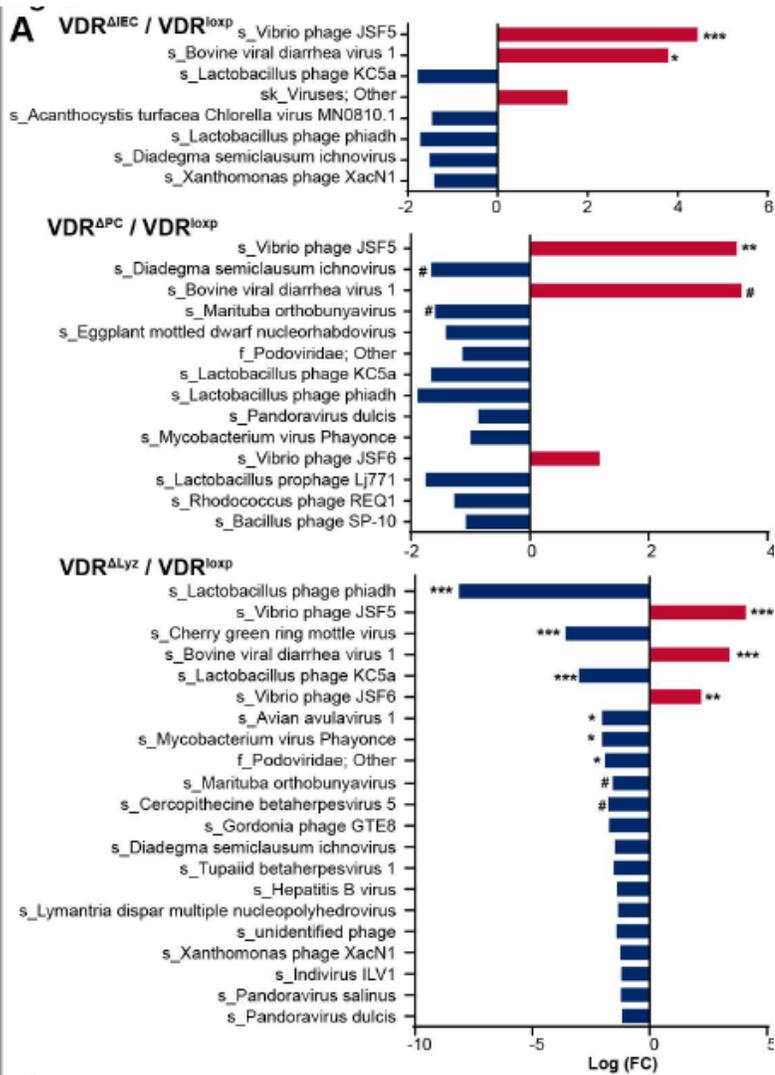


Figure 2

Alpha and beta diversity of viral abundance in feces from the four genotypes of mice. (A) Violin plots presenting alpha diversity measurements. The Shannon diversity index was used to determine the alpha

diversity differences of intestinal viruses between the control group (VDR^{LoxP}) and conditional VDR-knockout mice (VDR Δ IEC, VDR Δ PC and VDR Δ Lyz), n=10 each group. P-value as indicated above the plots. (B) The principal coordinates analysis (PCoA) plot of the mouse fecal samples was produced to inspect the homogeneity multivariate dispersions. The samples collected from different mouse genotypes are colored in the illustration, n=10 per group. (C) Plots of between- and within-means Bray-Curtis dissimilarity. Analysis of similarity (ANOSIM) was performed to compare between dissimilarity and within dissimilarity of VDR^{LoxP}, VDR Δ IEC, VDR Δ PC and VDR Δ Lyz mice based on Bray-Curtis dissimilarity, n=10 each group.



B

VDR ^{ΔIEC} / VDR ^{Loxp}	Female / Female		Male / Male	
	LogFC	q-value	LogFC	q-value
Vibrio phage JSF5	5.19	4.24E-04	3.80	0.41
Bovine viral diarrhea virus 1	4.99	4.24E-04	-0.40	0.92
Lactobacillus phage phiadh	-6.27	8.08E-04	-1.42	0.88
Lactobacillus phage KC5a	-5.31	3.47E-03	-1.55	0.88
Lactobacillus prophage Lj771	-5.40	4.75E-03	-1.36	0.88
Macacine alphaherpesvirus 1	-3.89	0.05	-0.83	0.88
Catovirus CTV1	-3.84	0.05	-0.61	0.88

VDR ^{ΔPC} / VDR ^{Loxp}	Female / Female		Male / Male	
	LogFC	q-value	LogFC	q-value
Vibrio phage JSF5	4.29	0.02	2.35	0.24

VDR ^{ΔLyz} / VDR ^{Loxp}	Female / Female		Male / Male	
	LogFC	q-value	LogFC	q-value
Lactobacillus phage phiadh	-8.44	5.74E-06	-7.80	3.64E-03
Vibrio phage JSF5	2.36	7.77E-03	5.03	0.04
Cherry green ring mottle virus	-5.44	7.77E-03	-3.73	0.09
Bovine viral diarrhea virus 1	3.30	0.03	4.10	0.09
Lactobacillus phage KC5a	-2.76	0.03	-3.40	0.16

LogFC

>0

<0

q < 0.01

q < 0.05

Figure 3

Differential analysis of viral genes in the feces of mice of four genotypes. (A) Log₂-fold changes (FC) of viral species with a P-value ≤ 0.05 are shown by increasing [85] or decreasing (blue) in three pairwise comparisons: VDR^{ΔIEC}/VDR^{Loxp}, VDR^{ΔPC}/VDR^{Loxp} and VDR^{ΔLyz}/VDR^{Loxp}, n=10 each group. *** indicates q value (FDR-corrected p-value) <0.001, ** indicates q-value < 0.01, * indicates q-value < 0.05, # indicates q-value < 0.1. (B) Viral species with q-values ≤0.05 in both female and male pairwise

comparisons are shown with fold-changes (logFCs) and q-values in three comparisons between the conditional VDR knockout groups (VDR Δ IEC, VDR Δ PC, and VDR Δ Lyz) and control mice (VDR^{LoxP}). Both fold-change and q-value were colored using the key as listed on the right side of the figure, n=10 each group.

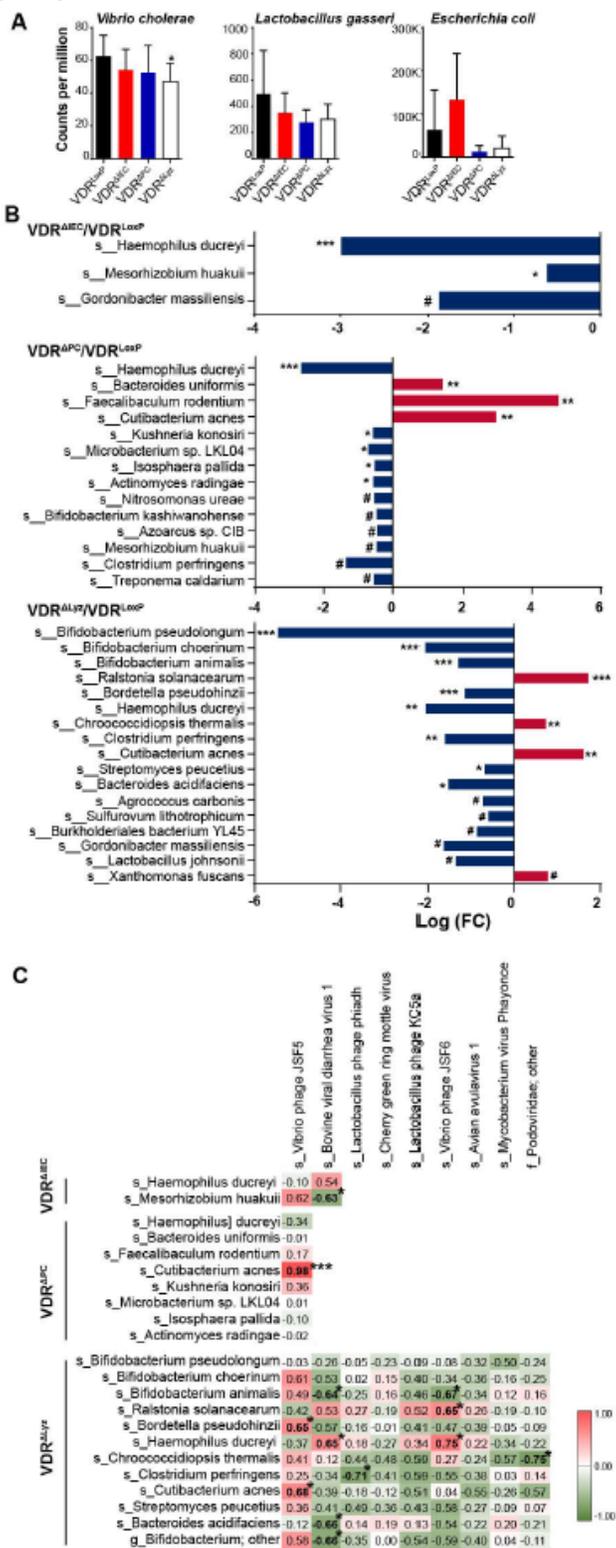


Figure 4

Altered bacterial abundance and correlations with viral alteration. (A) Bacterial species related to the altered bacteriophages were shown with bacterial differential abundance at count per million, including *Vibrio cholerae*, *Lactobacillus gasseri* and *Escherichia coli*. Mean \pm SD, n=10 per group; Kruskal-Wallis test. (B) Log₂-fold changes (FCs) of bacterial species with q-values \leq 0.1 are shown by increasing [85] or decreasing (blue) in three pairwise comparisons: VDR Δ IEC/VDRLoxP, VDR Δ PC/VDRLoxP and VDR Δ Lyz/VDRLoxP, n=10 each group. *** indicates q value (FDR-corrected p-value) <0.001, ** indicates q < 0.01, * indicates q < 0.05, # indicates q < 0.1. (C) Correlation matrix of bacteria and viruses in the feces of conditional VDR knockout mice. The correlation of viral (vertical) and bacterial (horizontal) species with a q-value \leq 0.05 in differential analysis was analyzed using the Hmisc package in R. The positive-correlation (red background) and negative-correlation (green background) values are shown in the map. *** indicates P<0.001, * indicates P \leq 0.05, n=10 each group.

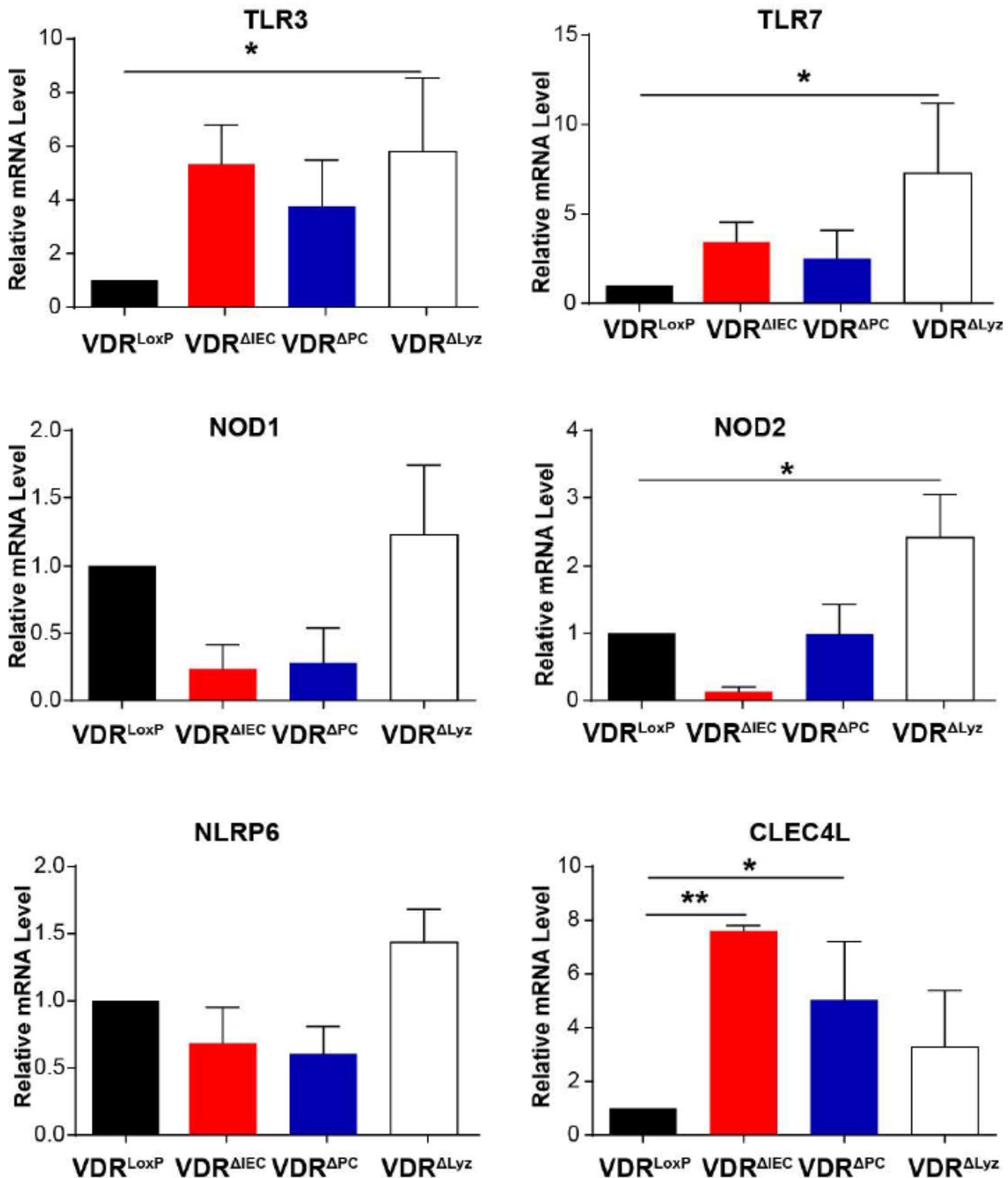


Figure 5

Altered pattern recognition receptors in the colon of VDR conditional knockout mice. Expression of virus translocation-related PRRs in the intestine, including TLR3, TLR7, NOD1, NOD2, NLRP6, and CLEC4L, was performed with colon epithelial cells using real-time quantitative PCR. Mean \pm SD, $n=3$; ** P-value < 0.01, * P-value < 0.05, one-way ANOVA.

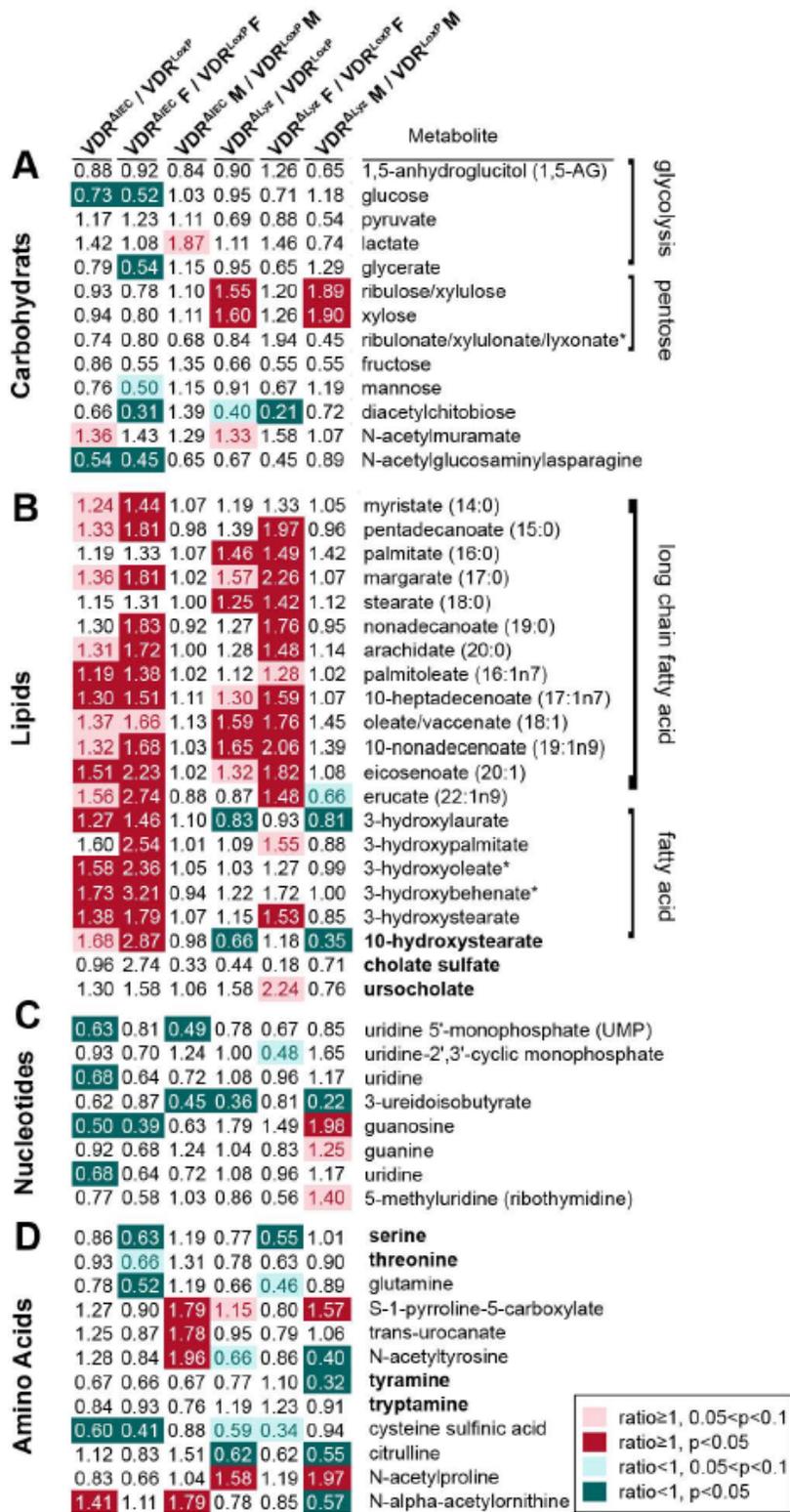


Figure 6

Altered virus infection-related metabolites in the feces of VDR conditional knockout mice and controls. A heatmap of fold changes of metabolites related to virus infection (regular font) and bacteriophage infection (bold font) is shown in both male and female VDR Δ IEC, VDR Δ PC, VDR Δ Lyz and control mice (VDR Δ LoxP) with different pathways, including carbohydrates (A), lipids (B), nucleotides (C) and amino

acids (D). Mean \pm SD, n=5-10; * P-value indicated as the color bar on the right and together with the number inside the blocks, two-way ANOVA.

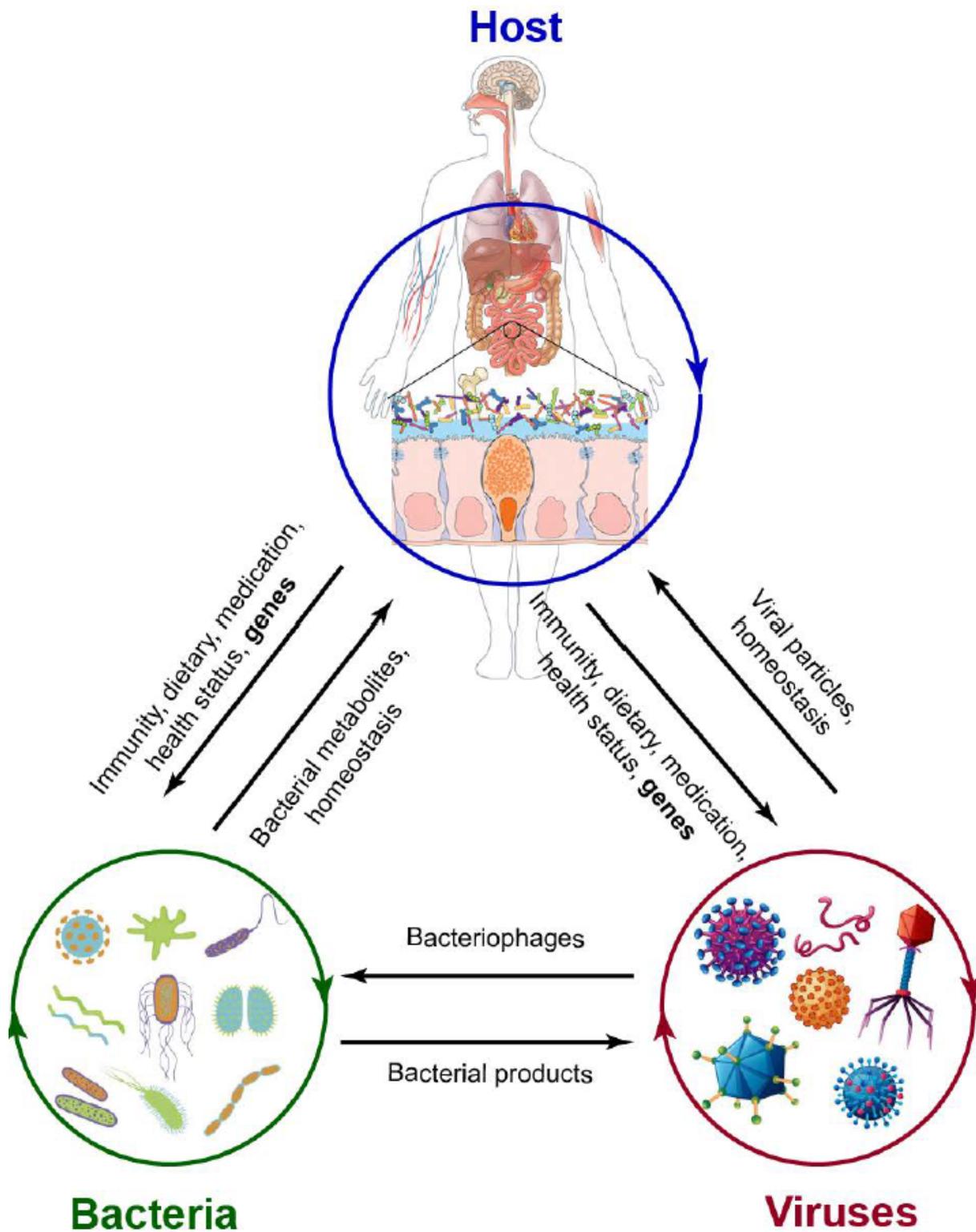


Figure 7

The interrelations of the host, bacteria, and virus. In the host, microbiota, including both bacteria and viruses, could be affected by immune activities, diet, health status and genetic background (e.g., *vdr* gene). In turn, the homeostasis of bacteria and viruses is essential to keep the host healthy. Moreover,

bacterial metabolites and viral particles play an important role in host health and the progression of diseases. As one of the viruses, bacteriophages can infect host bacteria and impact bacterial homeostasis. Bacterial products, such as LPS, could be used by the virus for replication. However, the interactions between eukaryotic viruses and bacteria need more study.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Suppl.Table128Feb2021.xlsx](#)