

Supplementary Information for

Viral diversity and ecological functions in the world's largest deep-sea mineral exploration region

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Supplementary Results

Overview of prokaryotic communities

Based on 16S rRNA gene amplicon sequencing, this study assessed the composition, diversity, and spatial distribution of prokaryotic communities in sediments from the CCZ. Within each sediment depth layer, prokaryotic communities based on centered log-ratio (CLR)-transformed 16S rRNA gene counts showed no significant distance–decay relationship among sampling sites (Mantel test, $P > 0.05$; Supplementary Fig. 1a). Across the vertical sediment profile of the CCZ, principal component analysis (PCA) revealed clear depth-stratified clustering of prokaryotic communities (Supplementary Fig. 2a), and permutational multivariate analysis of variance (PERMANOVA) confirmed significant differences among sediment depths (pseudo- $F = 4.46$, $R^2 = 0.21$, $P = 0.001$). Prokaryotic richness, Shannon diversity, Shannon evenness, and phylogenetic diversity all showed significant declines with depth (Supplementary Fig. 2b). Further, Prokaryotic community composition was significantly correlated with TOC, Se, Ni, and Mn in the 0–2 cmbsf layer, whereas significant correlations with Se and Mo were observed in the 4–6 and 14–16 cmbsf layers, respectively ($P < 0.05$; Supplementary Fig. 15b). Taken together, these results suggest that prokaryotic community structure in the CCZ may be influenced by depth-related environmental variation. At the horizontal scale, relatively homogeneous environmental conditions may lead to convergence of communities among sampling sites. In contrast, along the vertical profile, environmental differentiation and depth-specific microbe–environment associations may jointly promote vertical differentiation of prokaryotic communities, consistent with an environmental filtering effect¹.

In contrast, the composition of dominant prokaryotic phyla remained largely similar across depths. Pseudomonadota (25.6–29.1% on average) was the most abundant phylum, followed by Chloroflexota (11.1–13.4%), Acidobacteriota (4.5–5.7%), Gemmatimonadota (3.3–4.0%), NB1-j (1.7–2.5%), Nitrospirota (1.5–2.0%), and Myxococcota (1.3–2.0%) (Supplementary Fig. 2c). Interestingly, a large fraction of

prokaryotic taxa (33.4–40.7%) were found not to be assigned to any known taxonomic groups, a proportion substantially higher than that reported in previous global sediment surveys². This finding indicated the remarkable novelty of prokaryotic communities in the CCZ and the presence of abundant "dark matter" awaiting exploration. Consistently, recent investigation of benthic metazoans in the same region revealed that approximately 88% of the observed biodiversity had yet to be formally described³. Together, these findings highlight the exceptional biological novelty and unexplored biodiversity of deep-sea benthic ecosystems in the CCZ.

Depth-related viral populations and proteins

We identified shared and depth-enriched vOTUs across sediment depth layers. A total of 723 vOTUs (8%) were shared across all depth layers (Supplementary Fig. 8a). A total of 117 dominant vOTUs were identified using prevalence (> 90%) and relative-abundance (> 0.1%) thresholds (Supplementary Fig. 8b), accounting for 44.5% of the total viral relative abundance. Depth-associated vOTUs were then identified using DESeq2 and grouped into four depth-related trend groups according to their mean normalized abundance patterns across depth layers (Supplementary Fig. 9). The 0–2 cmbsf-associated group contained 75 vOTUs and declined progressively with depth, whereas the 14–16 cmbsf-associated group contained 90 vOTUs and showed the opposite pattern. Together, these results suggest that the viral community in CCZ sediments contained both core viral populations shared across sediment depth layers and viral populations enriched in specific depth layers.

Depth-related variation was further assessed at the vPC level using a read recruitment approach. The number of detected vPCs varied from 48,571 to 108,320 across sediment depth layers, corresponding to 27.0–60.3% of all vPCs, while layer-specific vPCs ranged from 9,371 to 57,268, corresponding to 19.3–52.9% of detected vPCs within each layer (Supplementary Fig. 10). In total, 65,674 vPCs (36.6%) were shared by more than one depth layer, including 11,803 vPCs (6.6%) shared across all

layers. vPC sharing was generally higher between adjacent sediment layers than between more distant layers. Functional annotation indicated that most vPCs across depth layers were either unannotated (no hit) or assigned to unknown functions, and the distribution of high-level functional categories was broadly similar across layers (Supplementary Fig. 11). Overall, vPC distribution varied among sediment depth layers, whereas high-level functional categories were broadly similar.

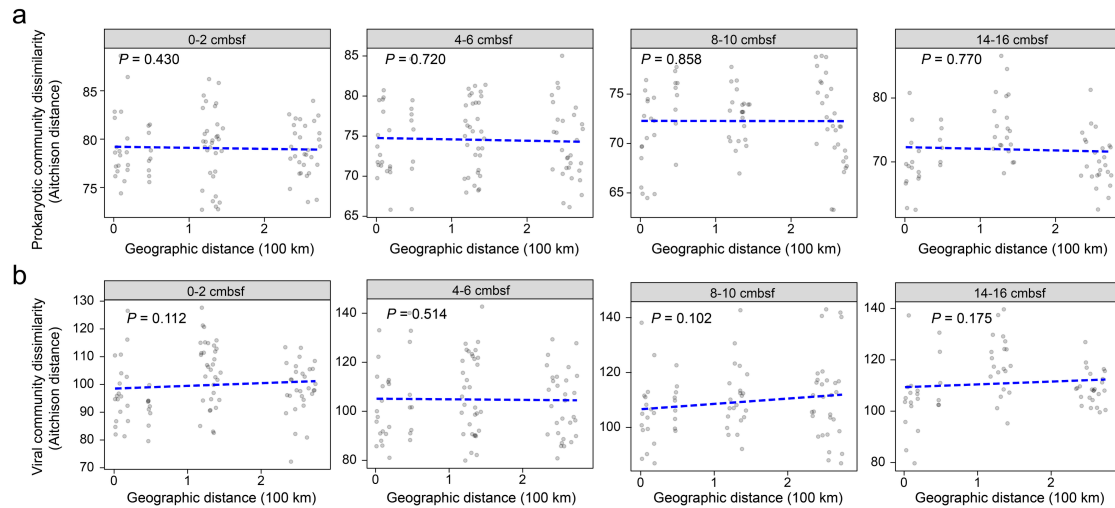
Environmental differences across depth

A total of 10 environmental variables were measured in this study, including Fe, Mn, Cu, Ni, Zn, Se, Mo, Si, Total organic carbon (TOC), and Total nitrogen (TN; Supplementary Data 1). Overall, CCZ sediments were characterized by a metal-enriched and low-organic-carbon background. Si was the most abundant component of the sediments, whereas Fe and Mn were the most abundant among the measured metallic elements. The average Fe content was approximately ninefold higher than that of Mn, consistent with previous reports⁴. The mean TOC content was 0.44 dry wt %, indicating a low-organic-carbon background comparable to that reported previously for this region⁵.

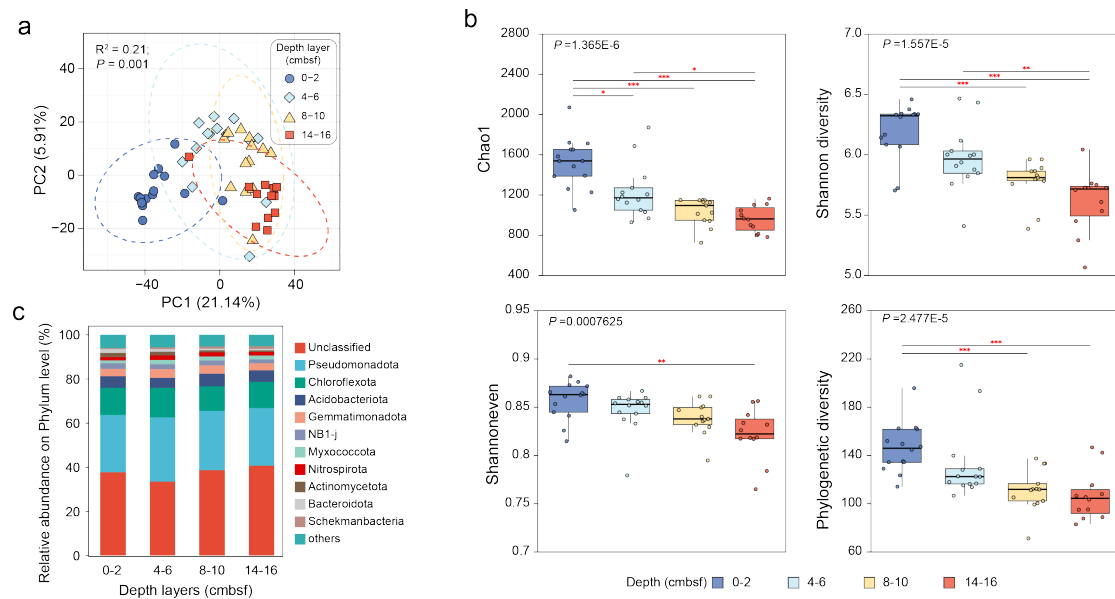
Environmental variation among sampling sites within each sediment depth layer did not show a significant distance–decay relationship with geographic distance ($P > 0.05$; Supplementary Fig. 12), indicating that the measured environmental variables were relatively homogeneous across sites at the same depth. In contrast, environmental variables varied significantly along the vertical sediment profile. Principal component analysis (PCA) revealed clear depth-related stratification of samples based on environmental variables (Supplementary Fig. 13), and permutational multivariate analysis of variance (PERMANOVA) further supported significant differences among depth layers (pseudo- $F = 3.41$, $R^2 = 0.20$, $P = 0.001$).

To characterize depth-related variation in environmental variables, individual variables were evaluated using linear mixed-effects models, with sampling site included

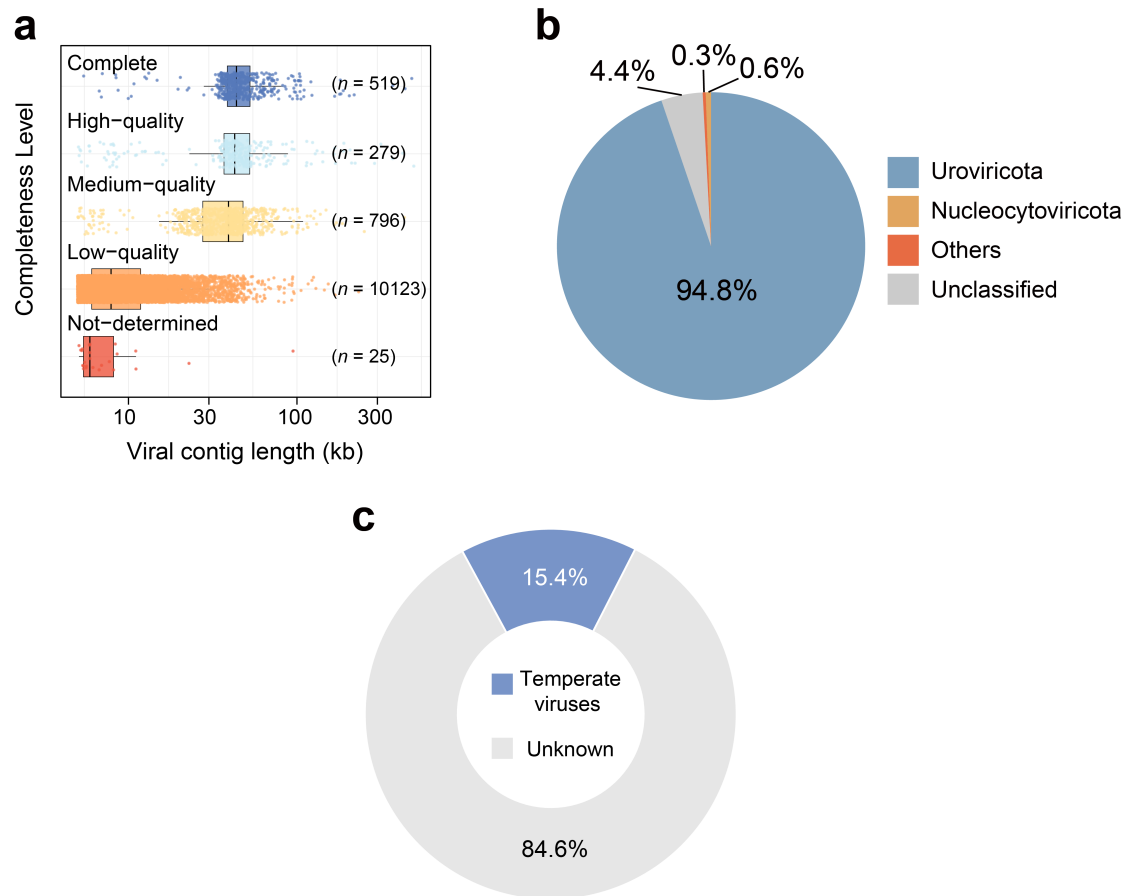
104 as a random effect. Significant depth-related differences were detected for Fe, Mn, Cu,
105 Ni, Si, Zn, and Se ($P < 0.05$; Supplementary Fig. 14). Notably, Cu, Ni, Zn, and Se are
106 essential micronutrients for microbial metabolism but may also exert toxic effects at
107 elevated concentrations. Among them, Cu, Ni, and Se decreased downcore, and the
108 combined concentration of these four elements was highest in 0–2 cmbsf layer. TOC
109 did not vary significantly with depth, although mean values tended to decrease
110 downcore. Overall, geochemical characteristics differed significantly among sediment
111 depth layers in the CCZ.



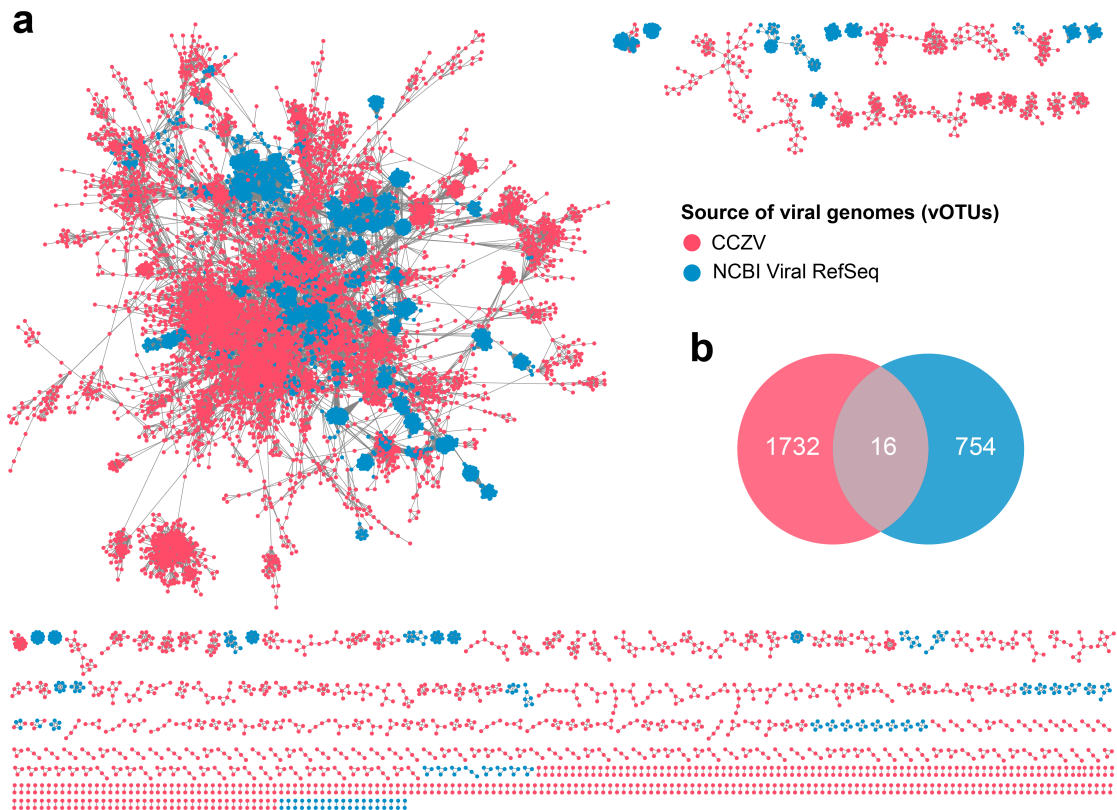
Supplementary Fig. 1. Distance–decay patterns of prokaryotic and viral community dissimilarity across sediment depth layers in the CCZ. a, Prokaryotic distance–decay relationships within each sediment depth layer: 0–2, 4–6, 8–10 and 14–16 cmbsf. b, Viral distance–decay relationships within each sediment depth layer: 0–2, 4–6, 8–10 and 14–16 cmbsf. Prokaryotic and viral community dissimilarities between sites were quantified using Aitchison distance, calculated as Euclidean distance on CLR-transformed read-count data. Associations between geographic distance and prokaryotic or viral community dissimilarity were assessed using Mantel tests based on Spearman’s ρ with 9,999 permutations. P values were adjusted across depth layers using the FDR procedure. Each point represents a pairwise comparison between sediment samples (0–2 cmbsf, $n = 91$ sample pairs; 4–6 cmbsf, $n = 91$; 8–10 cmbsf, $n = 78$; 14–16 cmbsf, $n = 66$). Source data are provided as a Source Data file.



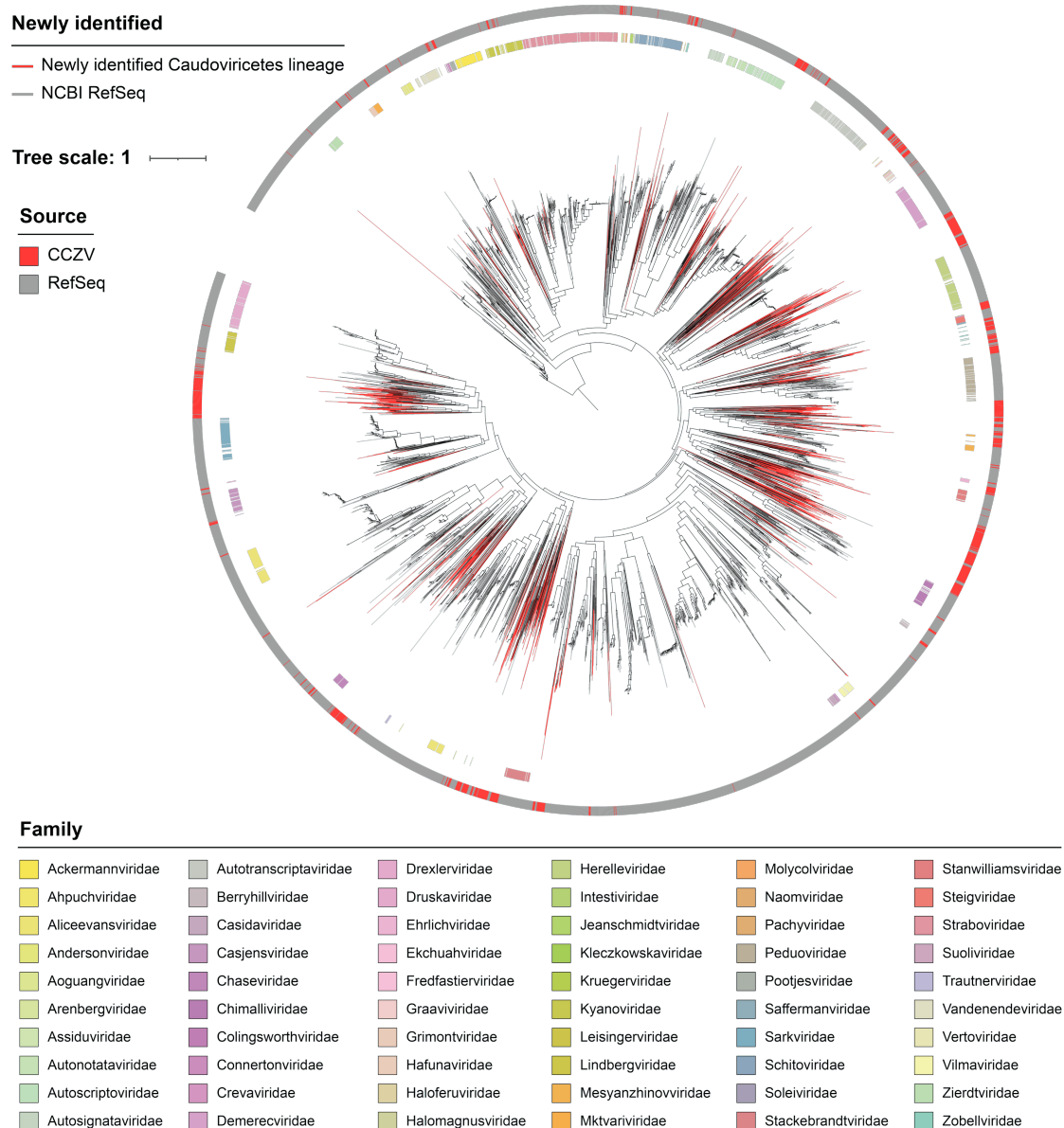
Supplementary Fig. 2. Distribution of prokaryotic composition and diversity across different sediment depths. a, Principal component analysis (PCA) of centered log-ratio (CLR)-transformed prokaryotic relative abundance profiles in the CCZ sediment. Ellipses indicate 95% confidence intervals around the centroids of each depth layer. PERMANOVA based on CLR-transformed prokaryotic relative abundance profiles showed that prokaryotic community structure differed significantly among depth layers. b, Distribution of prokaryotic diversity across different sediment depths. Chao1, Shannon diversity, Shannoneven, and Phylogenetic diversity indices of prokaryotic community diversity. Box plots show the median (centre line), the interquartile range (spanning from the 25th to the 75th percentiles), and whiskers extending to values within $1.5 \times$ the interquartile range. Individual data points are shown as jittered points. Pairwise differences were assessed using two-sided Wilcoxon tests, with ***, **, *, and none represent $P < 0.001$, $P < 0.01$, $P < 0.05$, and $P > 0.05$, respectively. Overall differences were assessed using the two-sided Kruskal–Wallis test. c, Taxonomic compositions of prokaryotes across different sediment depths. Sample sizes were as follows: 0–2 cmbsf, $n = 14$; 4–6 cmbsf, $n = 14$; 8–10 cmbsf, $n = 13$; and 14–16 cmbsf, $n = 12$. Source data are provided as a Source Data file.



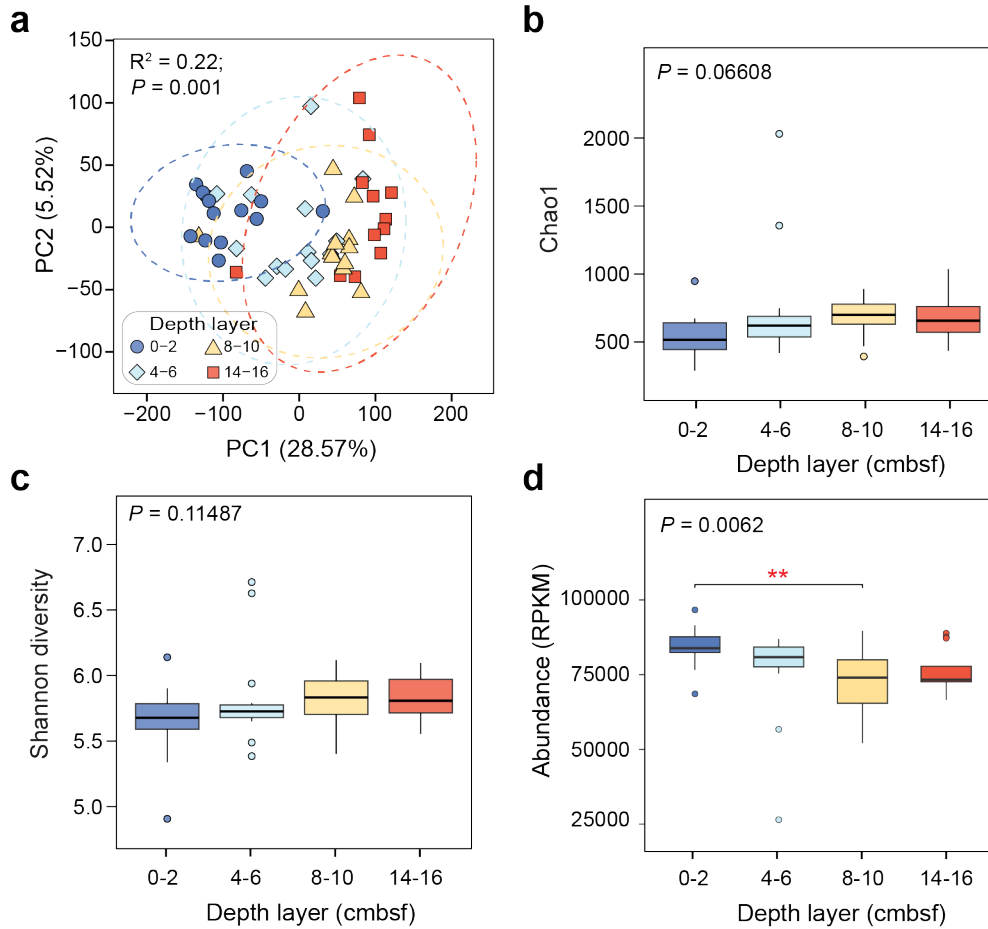
Supplementary Fig. 3. Completeness, taxonomic composition, and lifestyle of the Clarion–Clipperton Zone virome (CCZV). a, Completeness and length of vOTUs. Completeness range: complete (100%); high-quality (90.0–100.0%); medium-quality (50.0–89.99%); low-quality (0.01–49.99%); not-determined. n indicates the number of vOTUs. b, Taxonomic compositions of CCZV viruses. c, Predicted lifestyles of vOTUs (completeness $\geq 90\%$) within the CCZV. Pie plot shows the proportion of unknown lifestyle (grey) and temperate (blue) viruses. Source data are provided as a Source Data file.



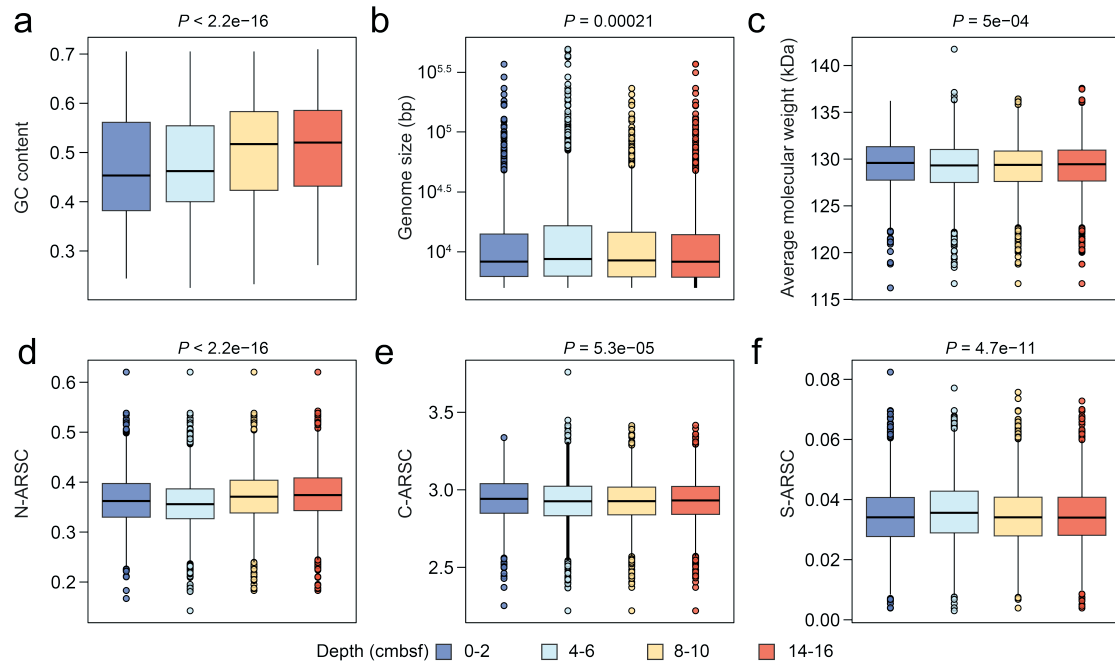
Supplementary Fig. 4. Taxonomic diversity of viruses in the CCZV. a, Gene-sharing network of viral sequences (≥ 5 kb) from sediments of deep-sea polymetallic nodule provinces and Viral RefSeq database. Nodes represent viral sequences, and edges connect significantly similar viral sequences. Node colors denote the origin environment of viral sequences. b, Venn diagram showing the number of shared viral clusters between the CCZV and Viral RefSeq database.



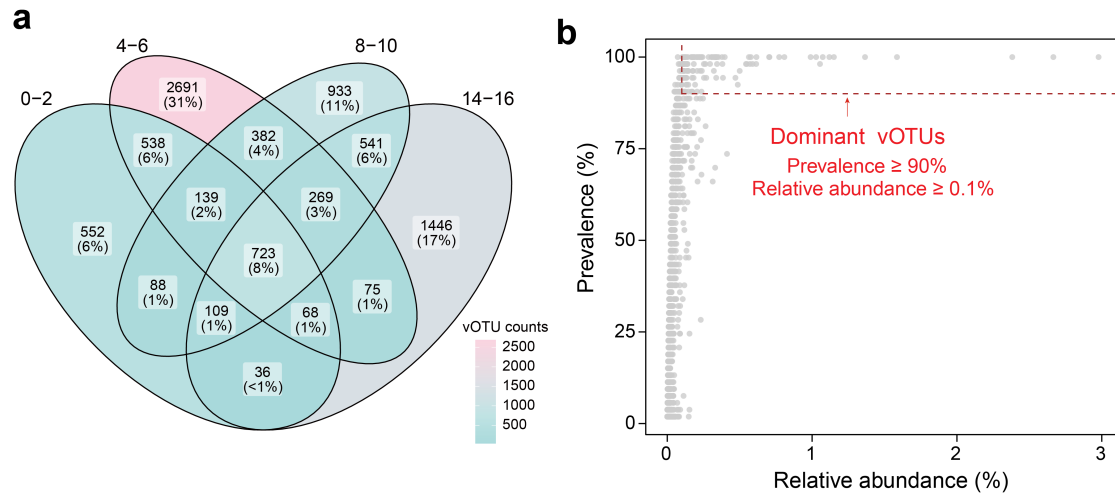
Supplementary Fig. 5. The Caudoviricetes phylogenetic tree of CCZV vOTUs over medium-quality and NCBI Refseq viruses inferred from concatenation of 77 marker proteins. Ring colors indicate viral family annotations (NCBI RefSeq) and virus sources, from the innermost to the outermost rings. The phylogenetic tree is rooted at the midpoint.



Supplementary Fig. 6. Depth-associated variation in viral functional composition, diversity, and abundance. a, PCA of CLR-transformed read count data for viral protein clusters (vPCs) in CCZ sediments. Ellipses indicate 95% confidence intervals around the centroids of each depth layer. PERMANOVA based on CLR-transformed read-count data for vPCs showed that viral functional structure differed significantly among depth layers (cmbsf). b, Chao1; c, Shannon diversity; d, relative abundance of the viral community. Sample sizes were as follows: 0–2 cmbsf, $n = 14$; 4–6 cmbsf, $n = 14$; 8–10 cmbsf, $n = 13$; and 14–16 cmbsf, $n = 12$. Box plots show the median (centre line), the interquartile range (spanning from the 25th to the 75th percentiles), and whiskers extending to values within $1.5 \times$ the interquartile range. Points beyond the whiskers represent outliers. Pairwise differences were assessed using two-sided Wilcoxon tests, with ***, **, *, and none represent $P < 0.001$, $P < 0.01$, $P < 0.05$, and $P > 0.05$, respectively. Overall differences were assessed using the two-sided Kruskal–Wallis test. Source data are provided as a Source Data file.



Supplementary Fig. 7. Viral genomic characteristics across different sediment depths. a, GC content; b, genome size; c, average molecular weight; d, N-ARSC; e, C-ARSC; f, S-ARSC of viral genomes across sediment depths. Sample sizes were as follows: 0–2 cmbsf, $n = 2,252$; 4–6 cmbsf, $n = 4,885$; 8–10 cmbsf, $n = 3,183$; and 14–16 cmbsf, $n = 3,266$. Box plots show the median (centre line), the interquartile range (spanning from the 25th to the 75th percentiles), and whiskers extending to values within $1.5 \times$ the interquartile range. Points beyond the whiskers represent outliers. Overall differences were assessed using the two-sided Kruskal–Wallis test. Source data are provided as a Source Data file.



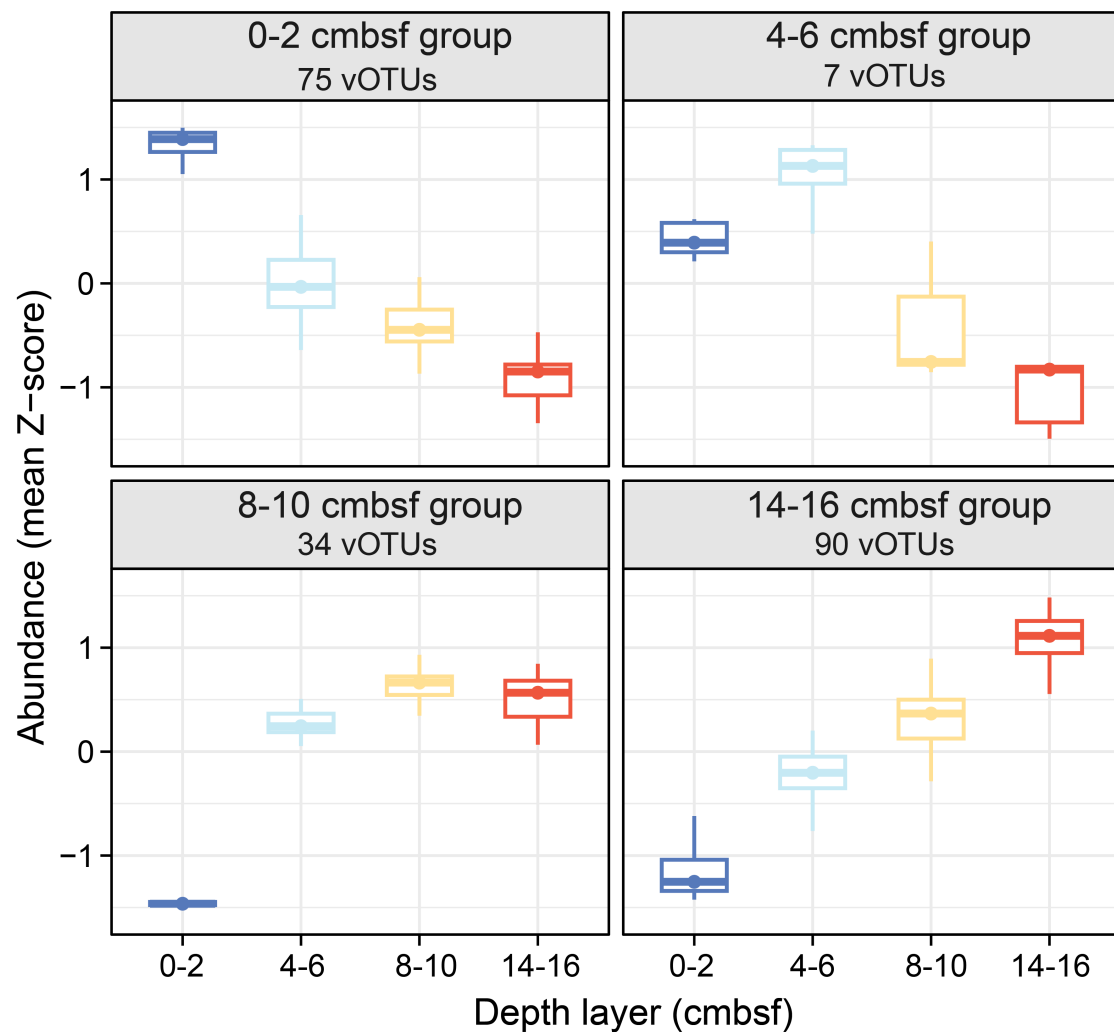
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194 Supplementary Fig. 8. Distribution of dominant vOTUs in the CCZ sediments. a,

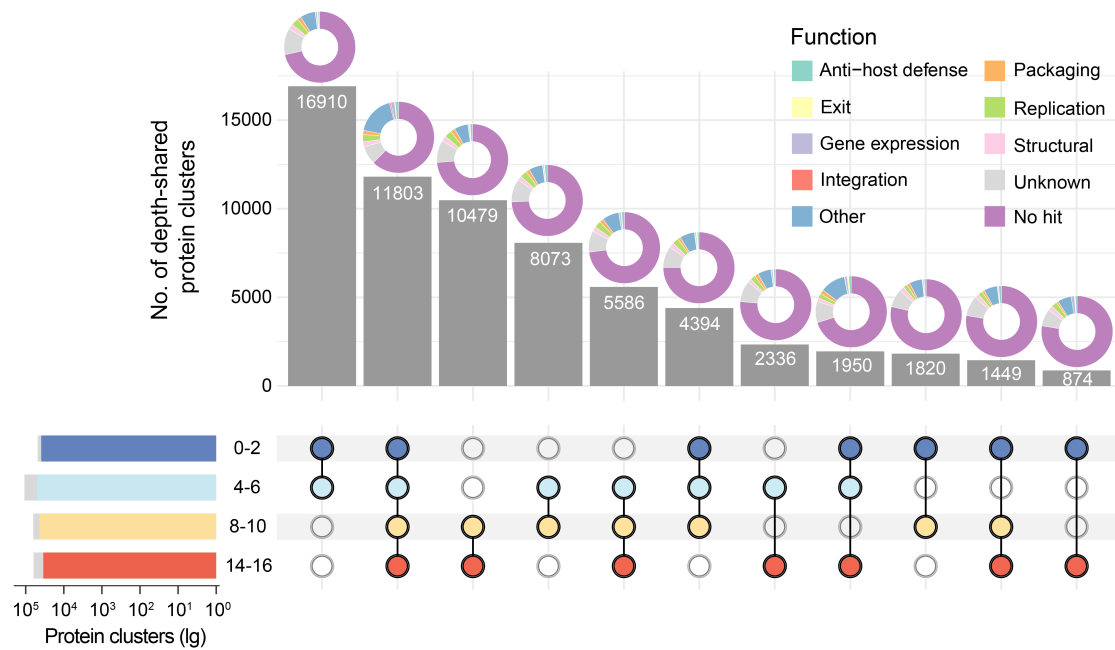
195 Shared vOTUs across different sediment depths. b, Prevalence and abundance pattern

196 of vOTUs. Source data are provided as a Source Data file.

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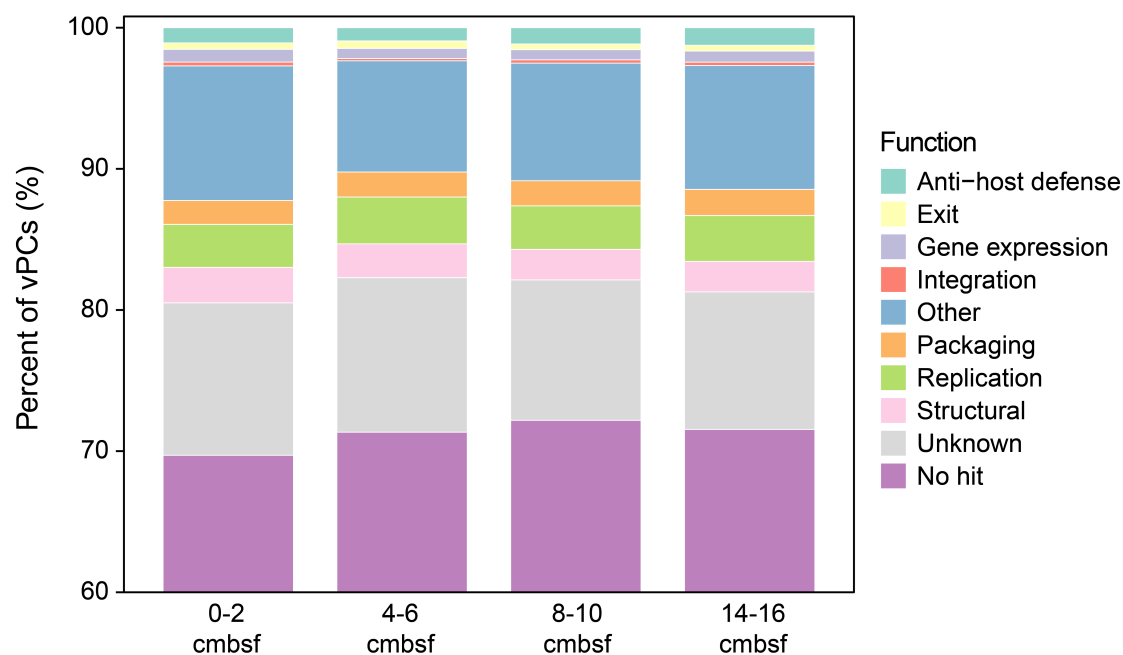


Supplementary Fig. 9. Depth-associated abundance trend groups of depth-enriched vOTUs in CCZ sediments. Depth-enriched vOTUs identified by DESeq2 were clustered into four groups based on mean Z-score-standardized relative abundance patterns across four depth layers (0–2, 4–6, 8–10, and 14–16 cmbsf). Boxplots show the distribution of mean Z-scores for vOTUs within each group across depth layers, with the number of vOTUs in each group indicated in the panel titles. Source data are provided as a Source Data file.



Supplementary Fig. 10. Shared and depth-specific viral protein clusters across the CCZ sediment profile. The UpSet plot summarizes overlap in vPCs across four depth layers (0–2, 4–6, 8–10, and 14–16 cmbsf). Connected intersection points indicate vPCs shared across the corresponding depth layer(s). Grey bars above indicate the number of shared vPCs for each intersection, and the accompanying donut charts show the functional composition of vPCs. Bars on the left depict the total number of protein clusters in each depth layer (grey) and the subset shared across depth layers (colored). Source data are provided as a Source Data file.

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219 Supplementary Fig. 11. Relative composition of functional categories of viral proteins

220 across four depth layers. Stacked bars show the percentage of vPCs assigned to each

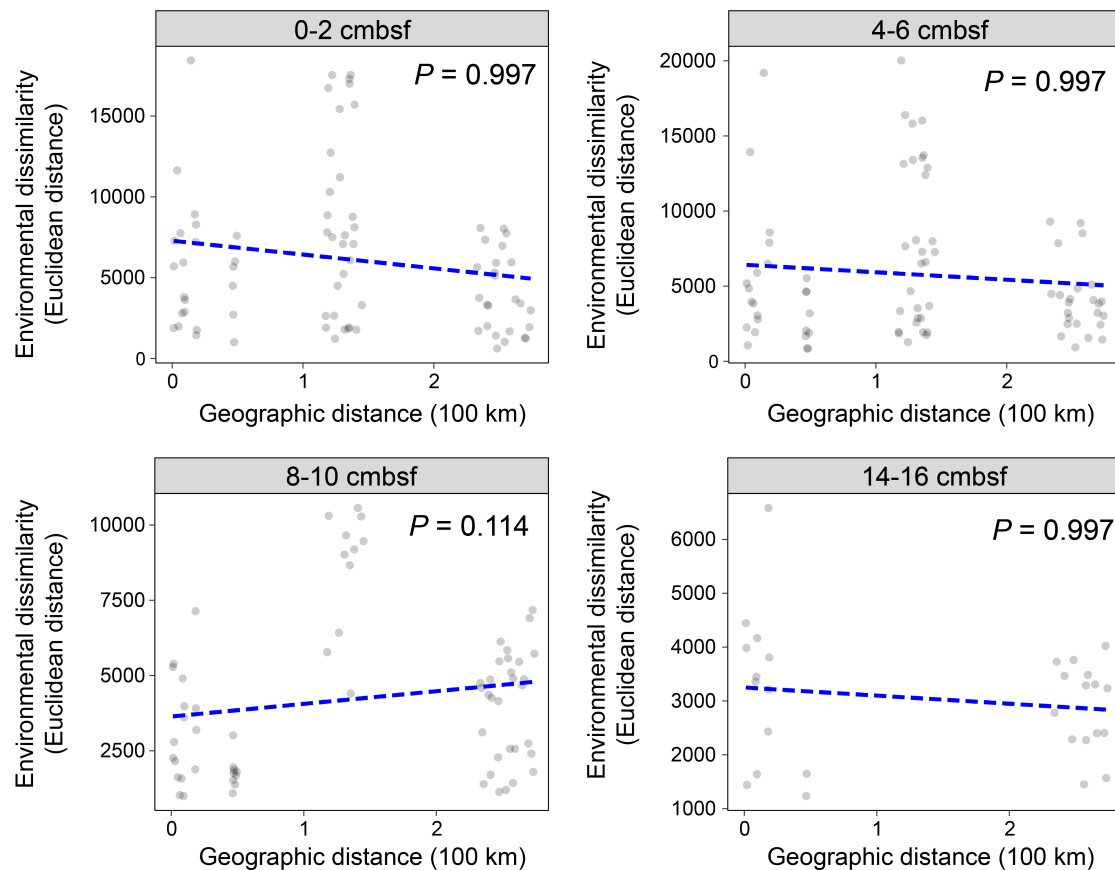
221 functional category in 0–2, 4–6, 8–10, and 14–16 cmbsf layers based on a unified

222 annotation workflow. “Unknown” indicates proteins with database hits but without an

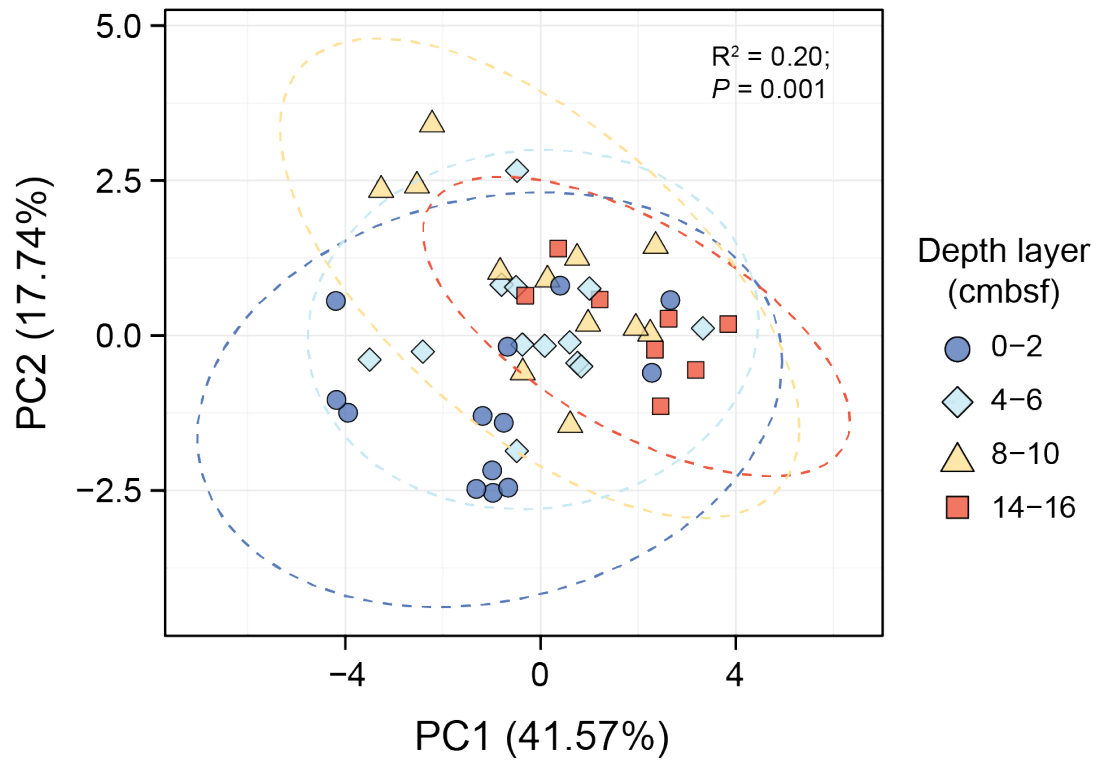
223 assigned function, whereas “No hit” indicates proteins without significant matches in

224 the queried databases. Source data are provided as a Source Data file.

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Supplementary Fig. 12. Distance-decay patterns of environmental similarity across sediment depth layers in the CCZ. Environmental distance-decay relationships were evaluated within each depth layer: 0–2 cmbsf; 4–6 cmbsf; 8–10 cmbsf; and 14–16 cmbsf. Environmental dissimilarity between sites was quantified as Euclidean distance based on environmental variables. The relationship between geographic distance and environmental dissimilarity was assessed using Mantel tests (Spearman's ρ , 9999 permutations), with P values adjusted across depth layers using the FDR procedure. Each point represents a pairwise comparison between sediment samples (0–2 cmbsf, $n = 91$ sample pairs; 4–6 cmbsf, $n = 91$; 8–10 cmbsf, $n = 78$; 14–16 cmbsf, $n = 66$). Source data are provided as a Source Data file.



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239 Supplementary Fig. 13. PCA of environmental profiles across sediment depth layers.

240 Environmental variables were $\log_{10}(x + 1)$ transformed and z-score standardized prior

241 to ordination. Each sampling site was colored and shaped according to depth layer

242 (cmbsf), and 95% confidence ellipses were drawn around the centroid of each layer.

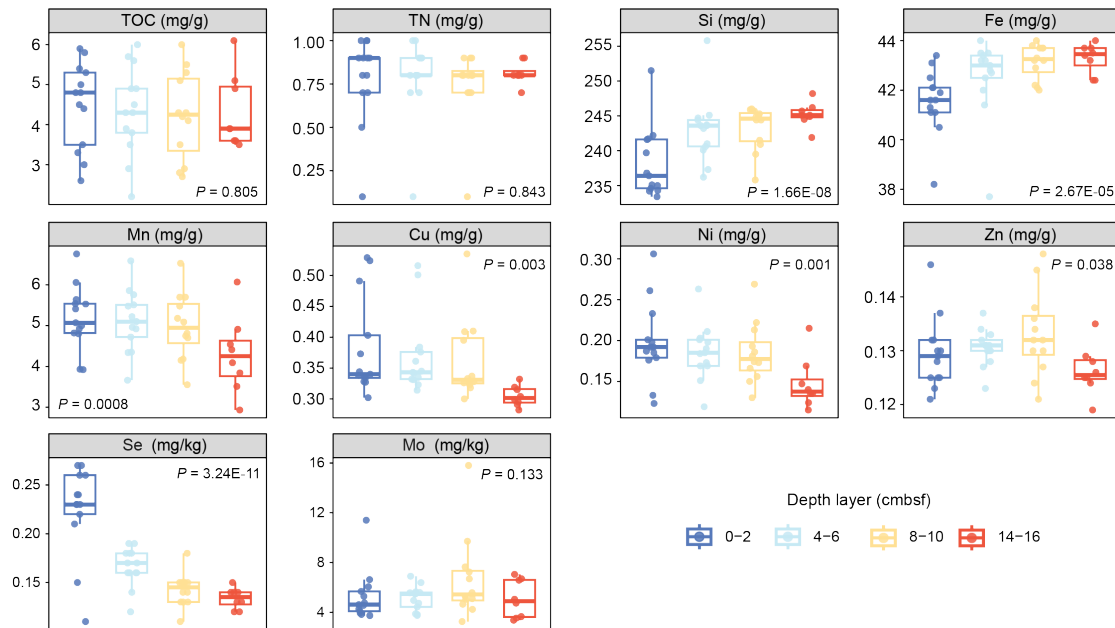
243 PERMANOVA based on Euclidean distances of the transformed environmental data

244 indicated significant depth-related differences in environmental conditions among

245 layers ($P = 0.001$). Each data point represents an independent sediment sample

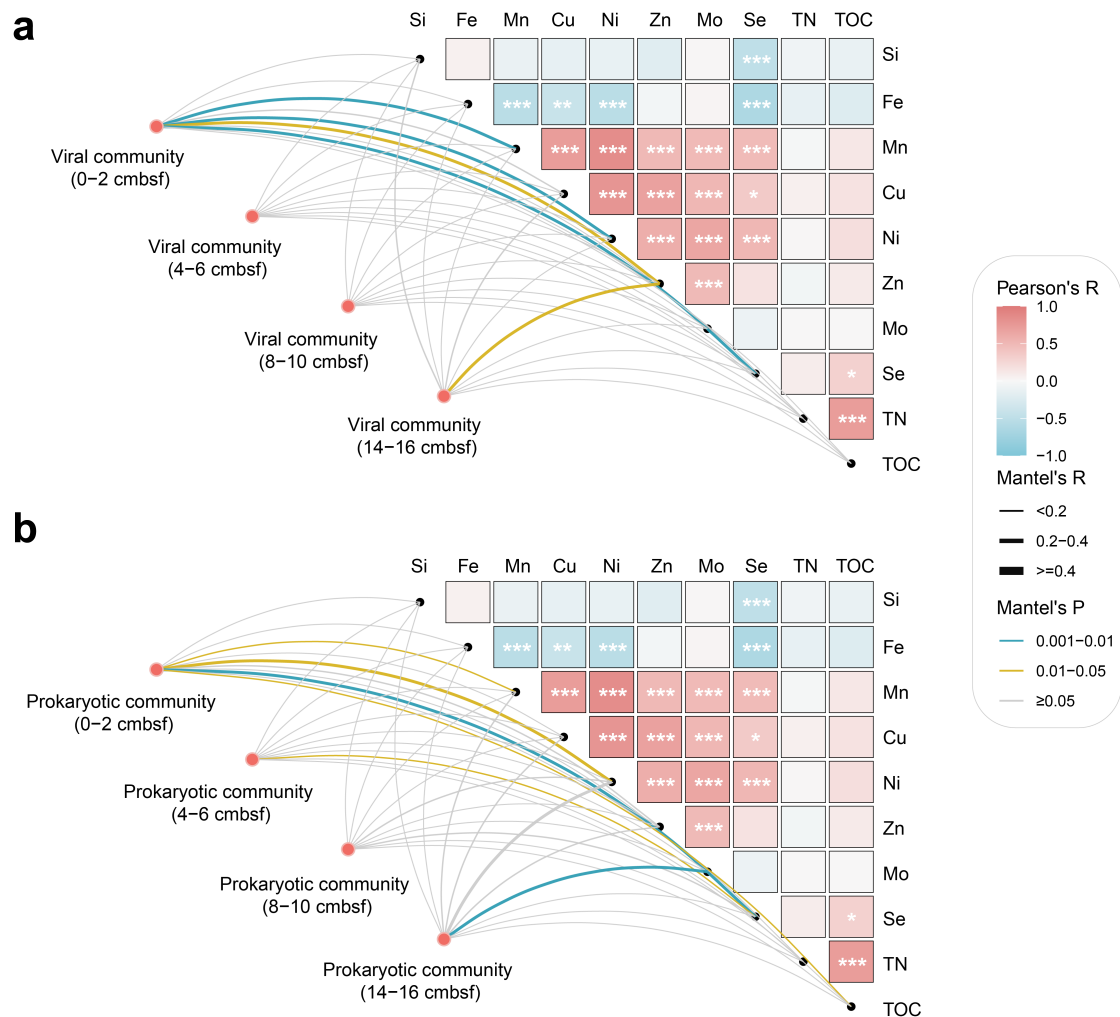
246 collected from a separate sampling station (0–2 cmbsf, $n = 14$; 4–6 cmbsf, $n = 14$; 8–

247 10 cmbsf, $n = 13$; 14–16 cmbsf, $n = 12$). Source data are provided as a Source Data file.

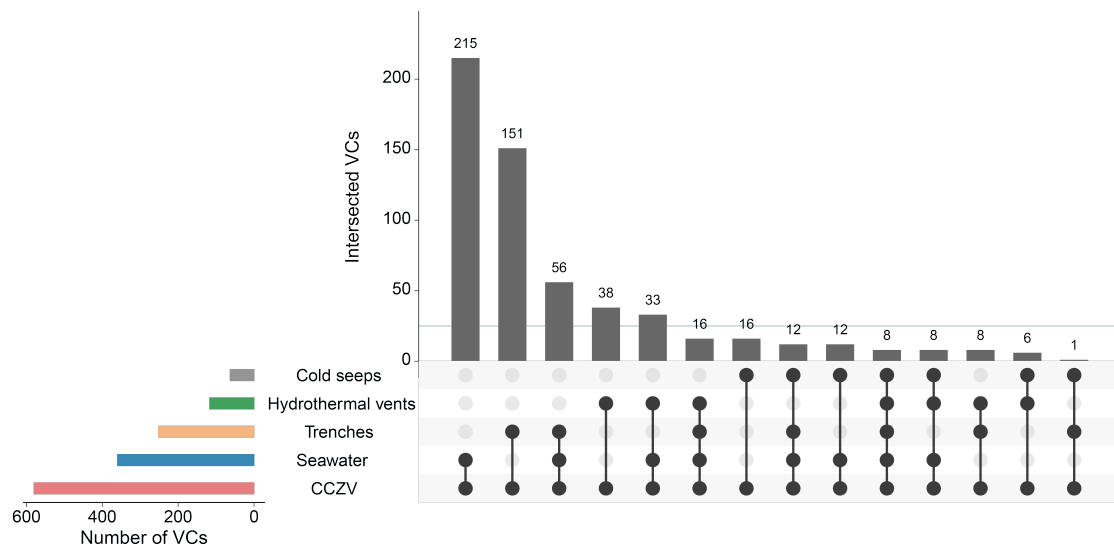


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249 Supplementary Fig. 14. Depth-dependent variation in environmental variables across
 250 sediment layers. Boxplots show the distributions of selected environmental variables
 251 across sediment depth layers (0–2, 4–6, 8–10, and 14–16 cmbsf). Sample sizes were as
 252 follows: 0–2 cmbsf, $n = 14$; 4–6 cmbsf, $n = 14$; 8–10 cmbsf, $n = 13$; and 14–16 cmbsf,
 253 $n = 12$. Box plots show the median (centre line), the interquartile range (spanning from
 254 the 25th to the 75th percentiles), and whiskers extending to values within $1.5 \times$ the
 255 interquartile range. Individual data points are shown as jittered points. Depth effects
 256 were tested using linear mixed-effects models (LMMs) with sampling site included as
 257 a random intercept to account for site-level non-independence. P values shown in each
 258 panel were adjusted for multiple comparisons using the FDR procedure. Source data
 259 are provided as a Source Data file.



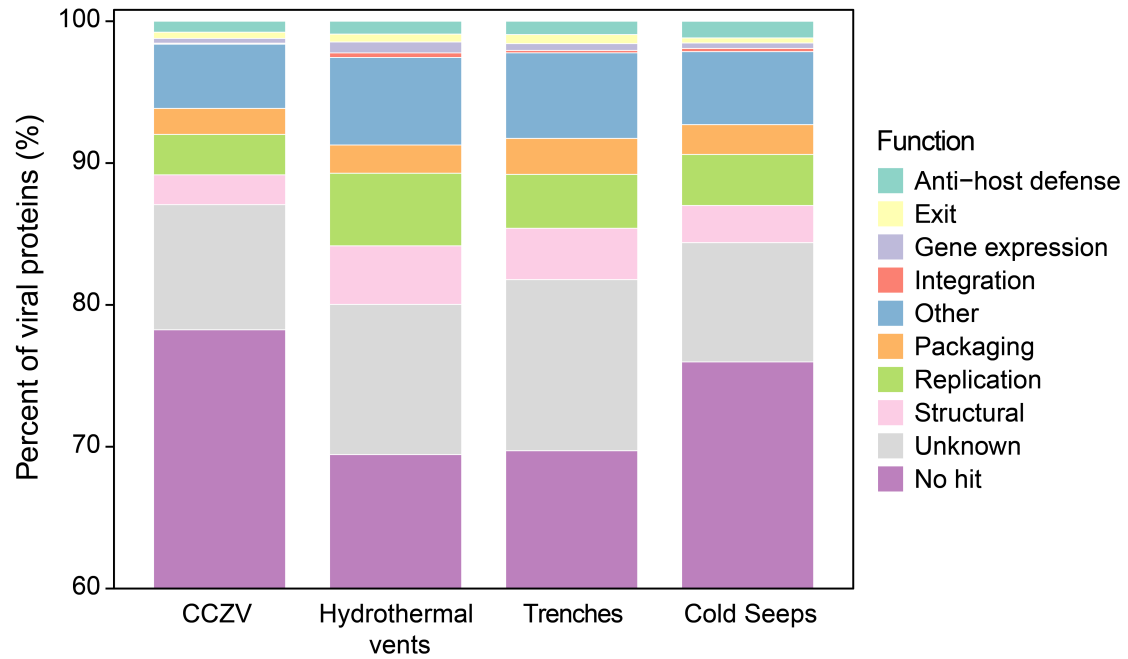
Supplementary Fig. 15. Mantel correlation analysis of environmental variables with viral and prokaryotic communities across different sediment depth layers in the CCZ. a, Viral community composition was tested for associations with individual environmental variables within each sediment depth layer. b, Prokaryotic community composition was tested for associations with individual environmental variables within each sediment depth layer. Line thickness indicates the strength of the Mantel correlation, and significant correlations are shown for $P < 0.05$. Pairwise relationships among environmental variables are shown by a color gradient representing Pearson's correlation coefficients were assessed using two-sided tests ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Sample sizes were as follows: 0–2 cmbsf, $n = 14$; 4–6 cmbsf, $n = 14$; 8–10 cmbsf, $n = 13$; and 14–16 cmbsf, $n = 12$. Source data are provided as a Source Data file.



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274 Supplementary Fig. 16. Upsets plot showing shared viral clusters among different

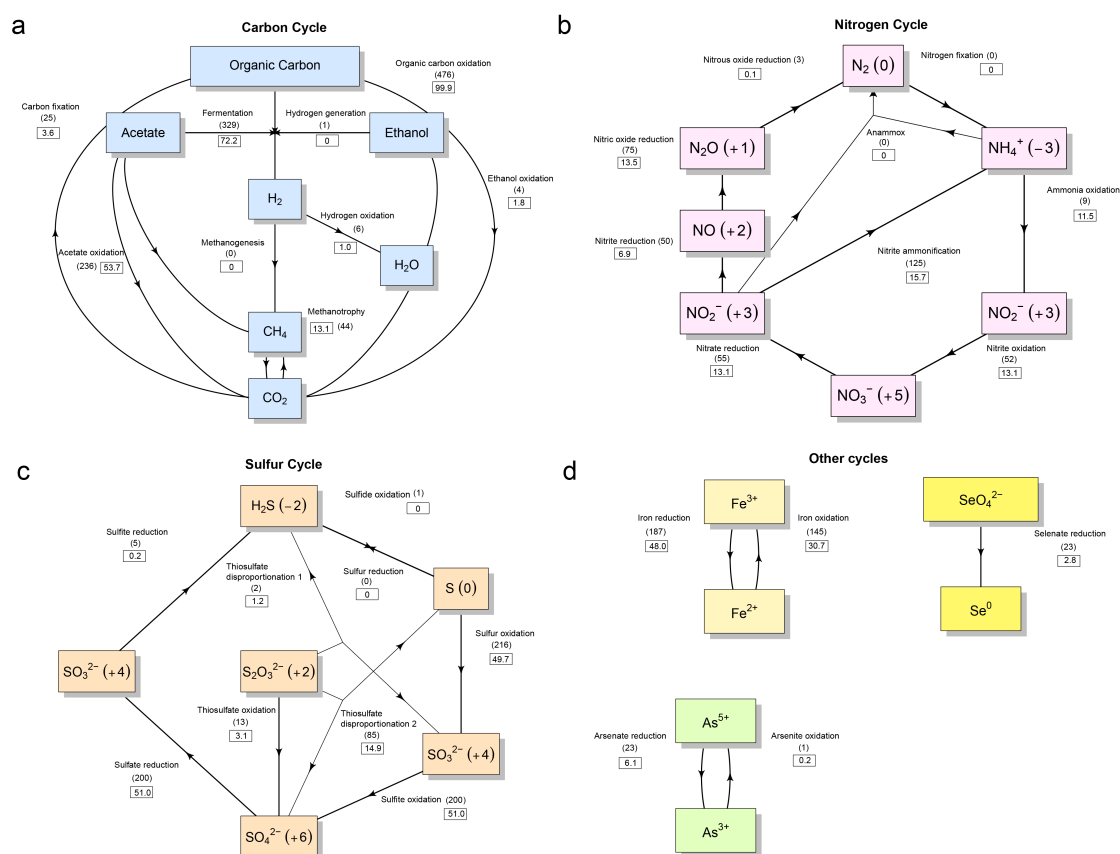
275 habitats. Source data are provided as a Source Data file.



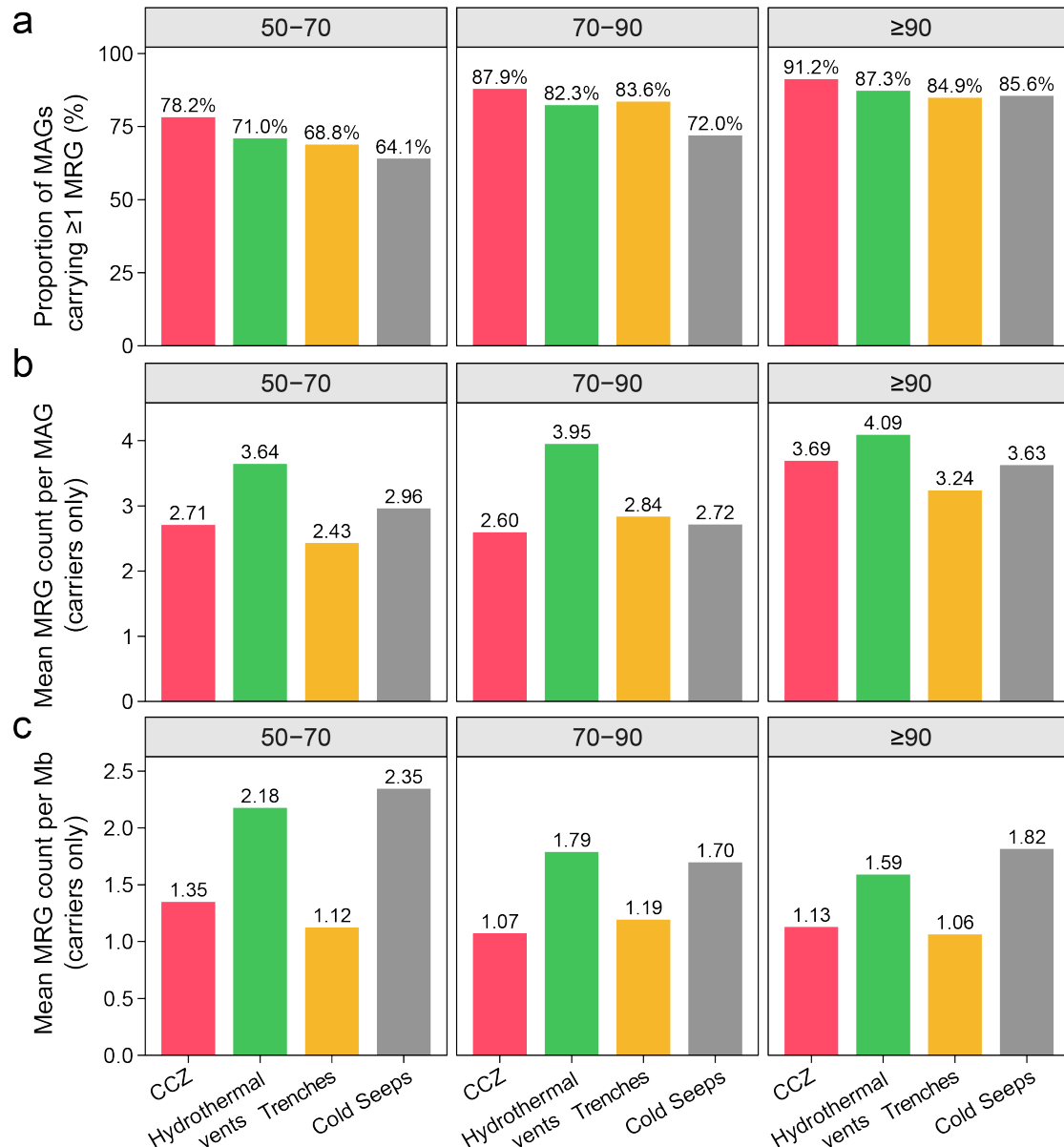
Supplementary Fig. 17. Relative composition of functional categories of viral proteins across deep-sea ecosystems. Stacked bars show the percentage of viral proteins assigned to each functional category in CCZV, hydrothermal vents, trenches, and cold seeps based on a unified annotation workflow. “Unknown” indicates proteins with database hits but without an assigned function, whereas “No hit” indicates proteins without significant matches in the queried databases. Source data are provided as a Source Data file.

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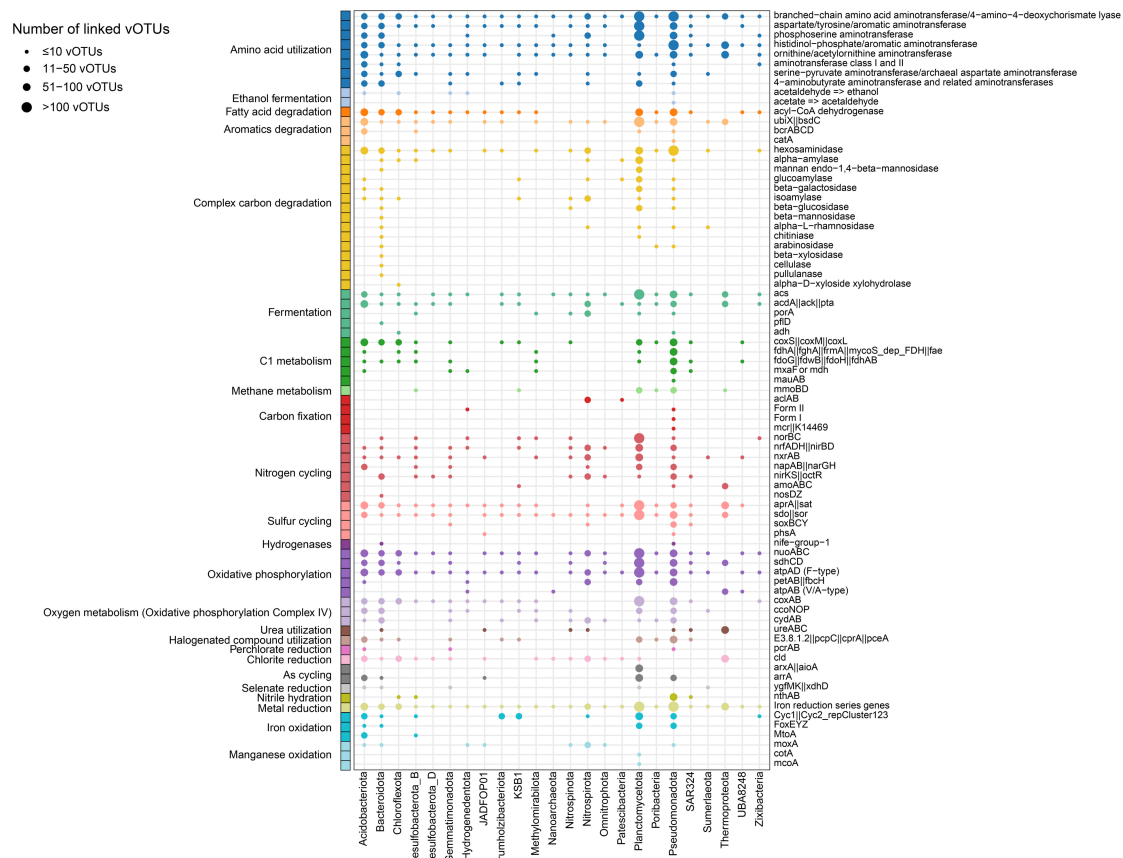
Supplementary Fig. 19. Genomic prevalence of metabolic pathways associated with major biogeochemical processes in CCZ MAGs. The pathway panels summarize the number and proportion of CCZ MAGs in which key genes for each pathway were detected. a, carbon cycling; b, nitrogen cycling; c, sulfur cycling; d, other metal cycling. Functional potentials of MAGs were annotated and predicted with METABOLIC v4.0. Source data are provided as a Source Data file.



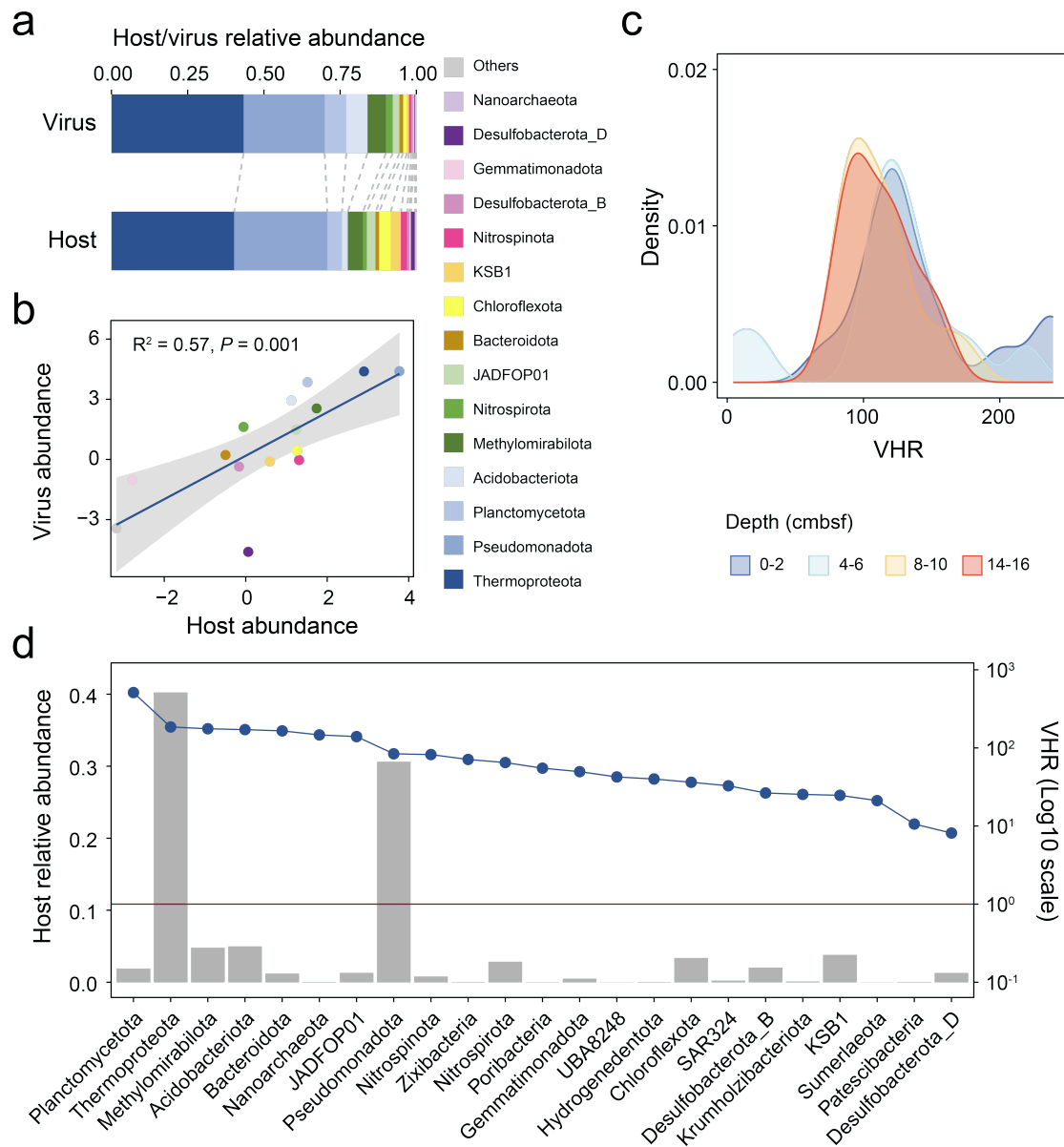
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300 Supplementary Fig. 20. Comparison of metal resistance gene (MRG) carriage in MAGs
 301 from the CCZ and other deep-sea habitats. a, prevalence of MRG carriage across MAGs;
 302 b, MRG density per genome among MRG-carrying MAGs; c, MRG density per
 303 megabase (Mb) among MRG-carrying MAGs. MAGs were grouped into three
 304 completeness categories (%): 50–70, 70–90, and ≥ 90 . Source data are provided as a
 305 Source Data file.

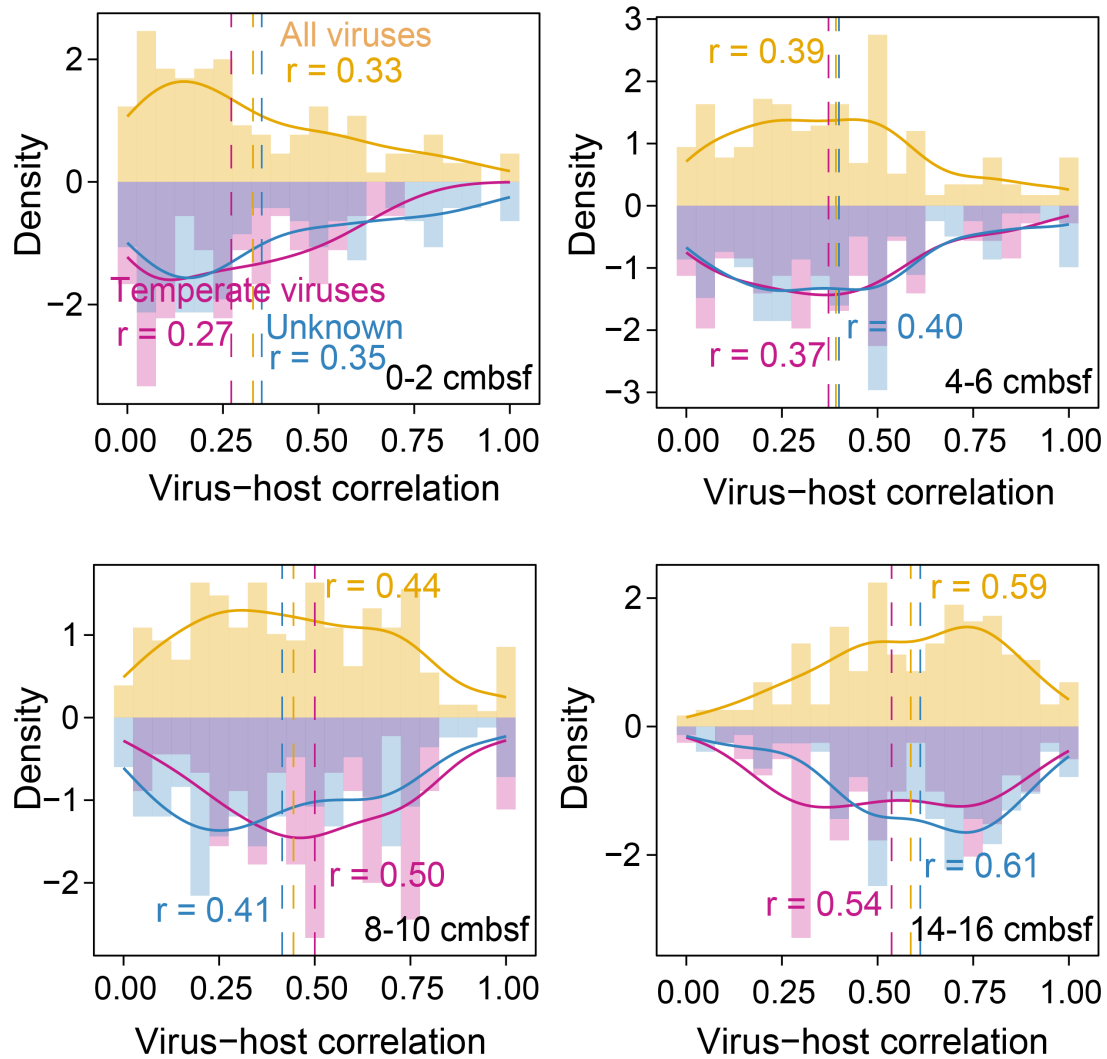
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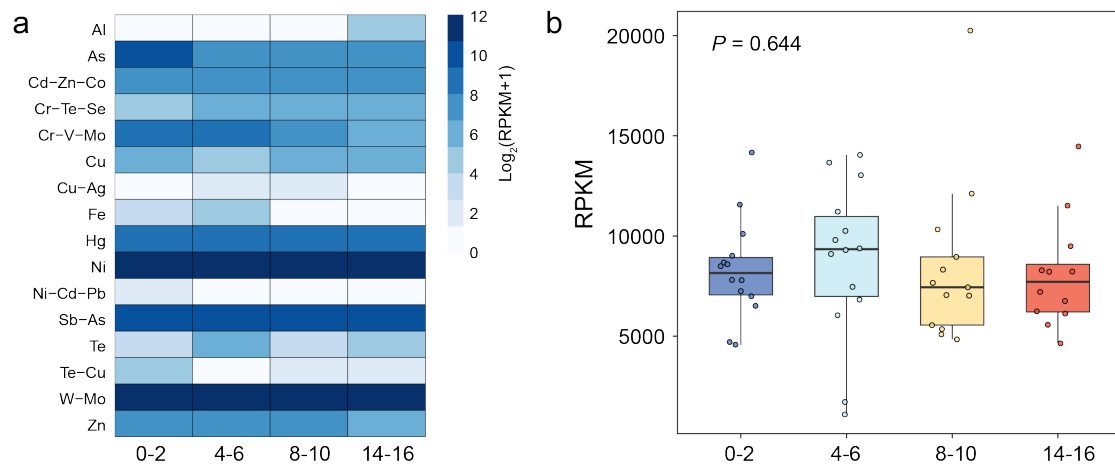
Supplementary Fig. 21. Bubble chart showing virus-host connections for key prokaryotes involved in metabolic processes. Functional potentials of host MAGs were annotated and predicted with METABOLIC v4.0. Source data are provided as a Source Data file.



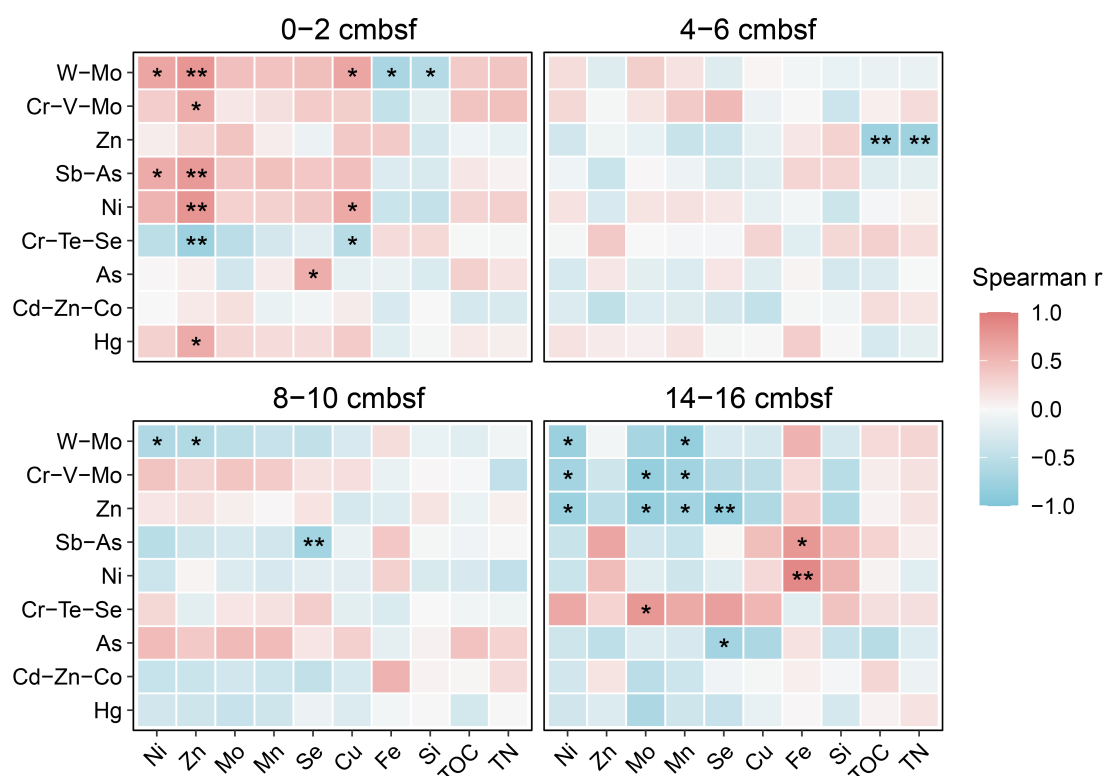
Supplementary Fig. 22. Relative abundance patterns of viruses and their predicted hosts in CCZ sediments. a, Relative abundances of vOTUs and their predicted hosts, grouped by the host taxonomy. b, Significant Pearson correlations between relative abundances of viruses and corresponding host taxa. Statistical significance was assessed using two-sided tests. c, Density plots showing variation in virus–host abundance ratios (VHR) across different sediment depths. d, Lineage-specific VHR for all predicted prokaryotic hosts. The red line indicates a 1:1 ratio. Bar plots represent relative abundances of hosts, while line plots indicate VHR values. Source data are provided as a Source Data file.



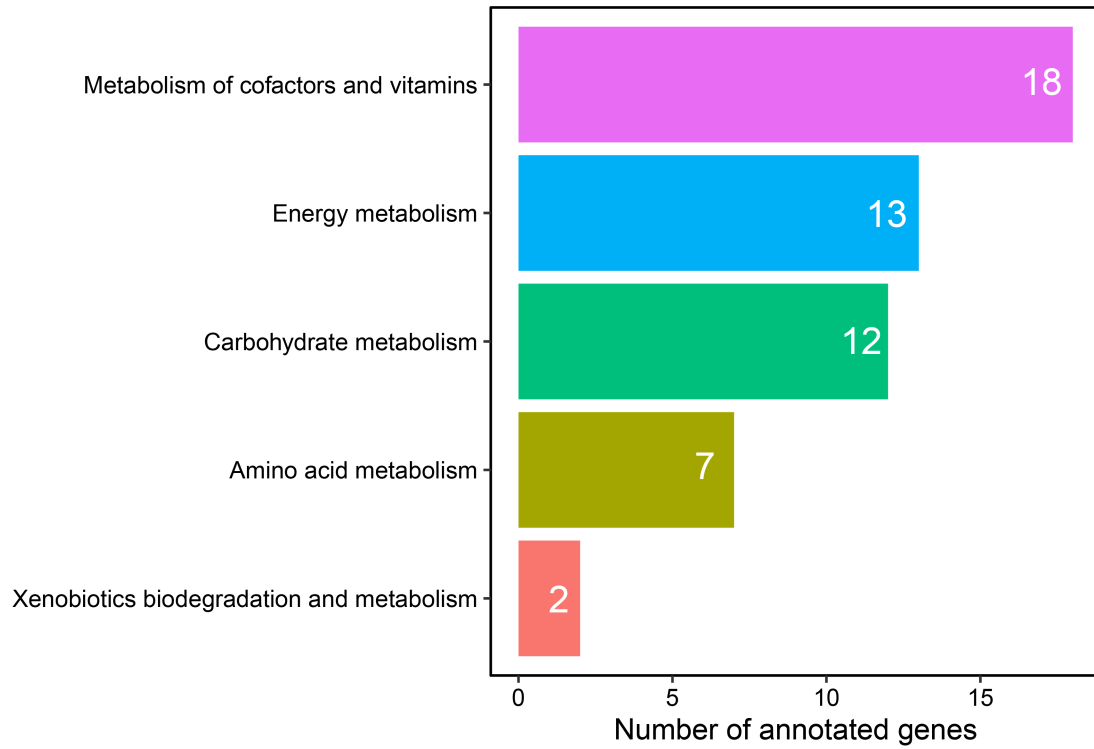
Supplementary Fig. 23. Histogram and kernel density distributions of virus-host correlations in CCZ sediment across different depth layers. For each sediment depth layer, two-sided Spearman's rank correlations were calculated between the CLR-transformed abundance profiles of each vOTU and its predicted host across sampling sites. Colored histograms show the probability density of correlation coefficients for each viral lifestyle group: orange, pink, and blue represent all viruses, temperate viruses, and viruses with unknown lifestyle, respectively. Higher density values indicate that more virus-host pairs have correlation coefficients within that range. Solid curves denote kernel density estimates. Vertical dashed lines indicate the mean correlation (r) for each distribution. Source data are provided as a Source Data file.



Supplementary Fig. 24. Distribution of metal resistance genes (MRGs) across different sediment depths. a, Relative abundances of various MRG types. b, Total MRG abundance across sediment depth layers. Sample sizes were as follows: 0–2 cmbsf, $n = 14$; 4–6 cmbsf, $n = 14$; 8–10 cmbsf, $n = 13$; and 14–16 cmbsf, $n = 12$. Box plots show the median (centre line), the interquartile range (spanning from the 25th to the 75th percentiles), and whiskers extending to values within $1.5 \times$ the interquartile range. Individual data points are shown as jittered points. Overall differences were assessed using the two-sided Kruskal–Wallis test. Source data are provided as a Source Data file.



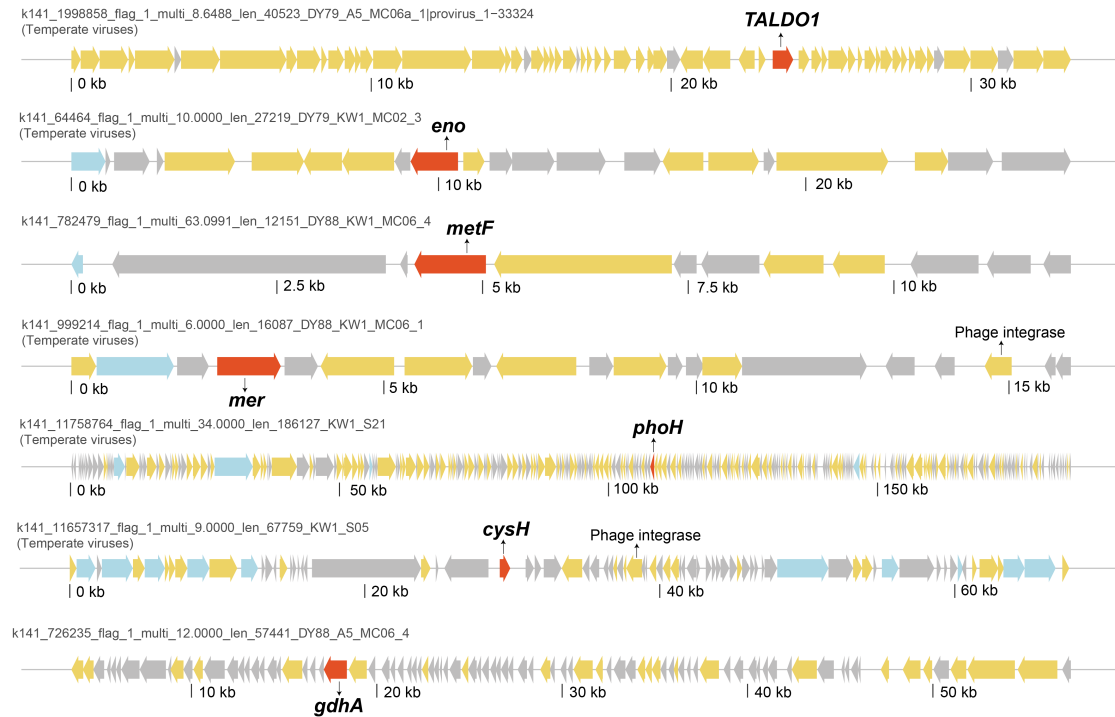
Supplementary Fig. 25. Spearman correlations between virus-encoded metal resistance genes (MRGs) and environmental variables across sediment depth layers in the CCZ. Spearman correlation coefficients were calculated separately for each depth layer using CLR-transformed read counts of virus-encoded MRGs and log_{1p}-transformed environmental variables. Color intensity indicates the Spearman correlation coefficient (r), and asterisks denote significant correlations based on two-sided tests (* $P < 0.05$, $P < 0.01$). Source data are provided as a Source Data file.



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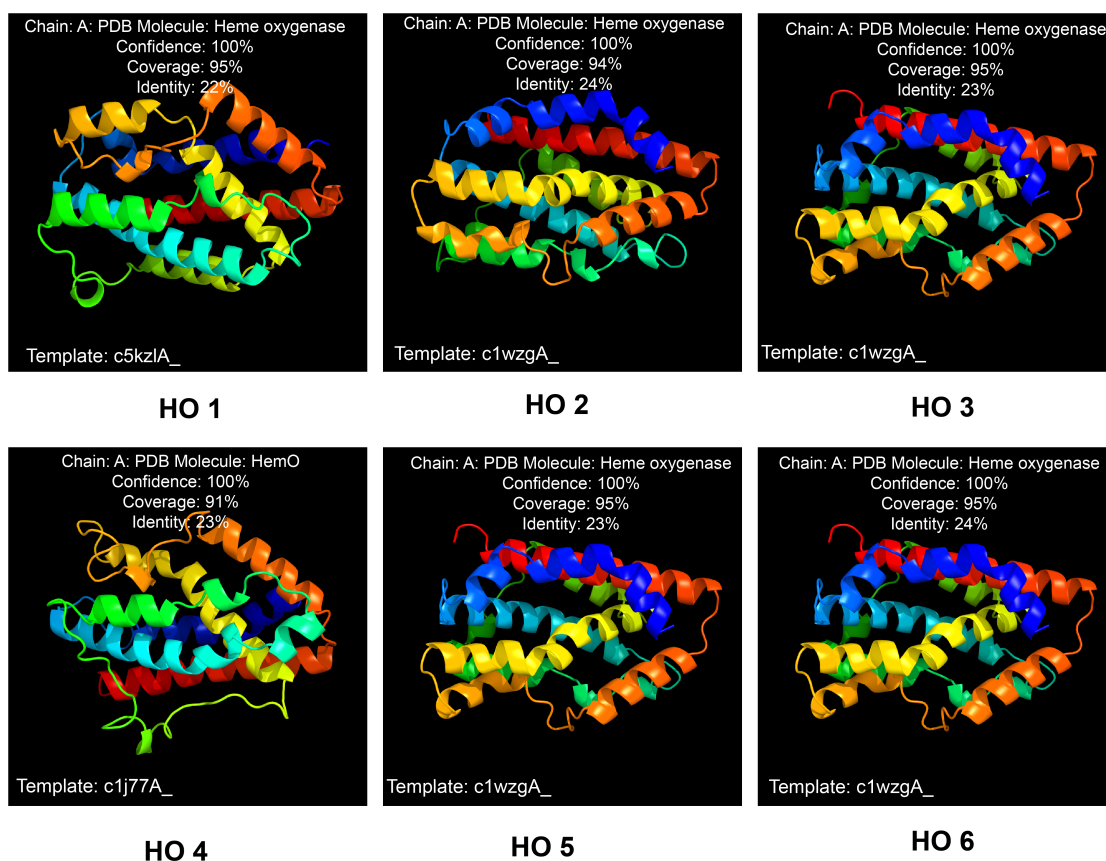
353 Supplementary Fig. 26. Number of AMGs in each KEGG metabolic pathway. Source
354 data are provided as a Source Data file.

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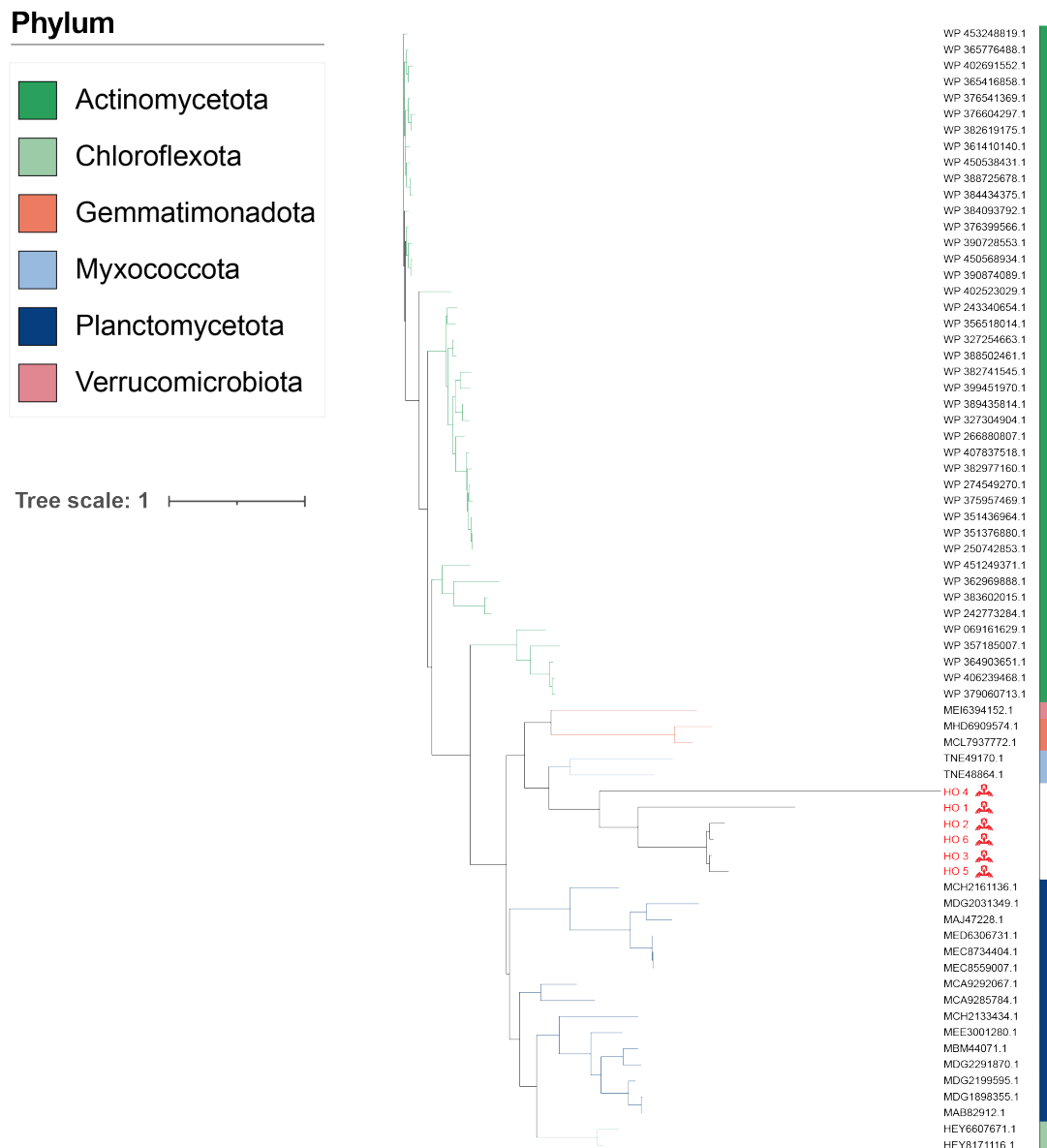


Genes ■ Viral hallmark genes ■ Viral-like genes ■ Others ■ Putative AMGs

Supplementary Fig. 27. Genome map of representative AMGs encoding virus. Source data are provided as a Source Data file.



Supplementary Fig. 28. Predicted three-dimensional structures of six virus-encoded heme oxygenase (HO)-like proteins (HO1–HO6). Structural models were generated using Phyre2 and matched against templates in the Protein Data Bank (PDB). All six proteins showed 100% confidence structural similarity to known heme oxygenase.



Supplementary Fig. 29. Maximum-likelihood tree including the 6 viral heme oxygenase sequences and reference microbial homologs from the NCBI nr database. The tree was constructed using FastTree and visualized with iTOL. Virus-encoded heme oxygenase sequences are highlighted in red.

Supplementary References

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