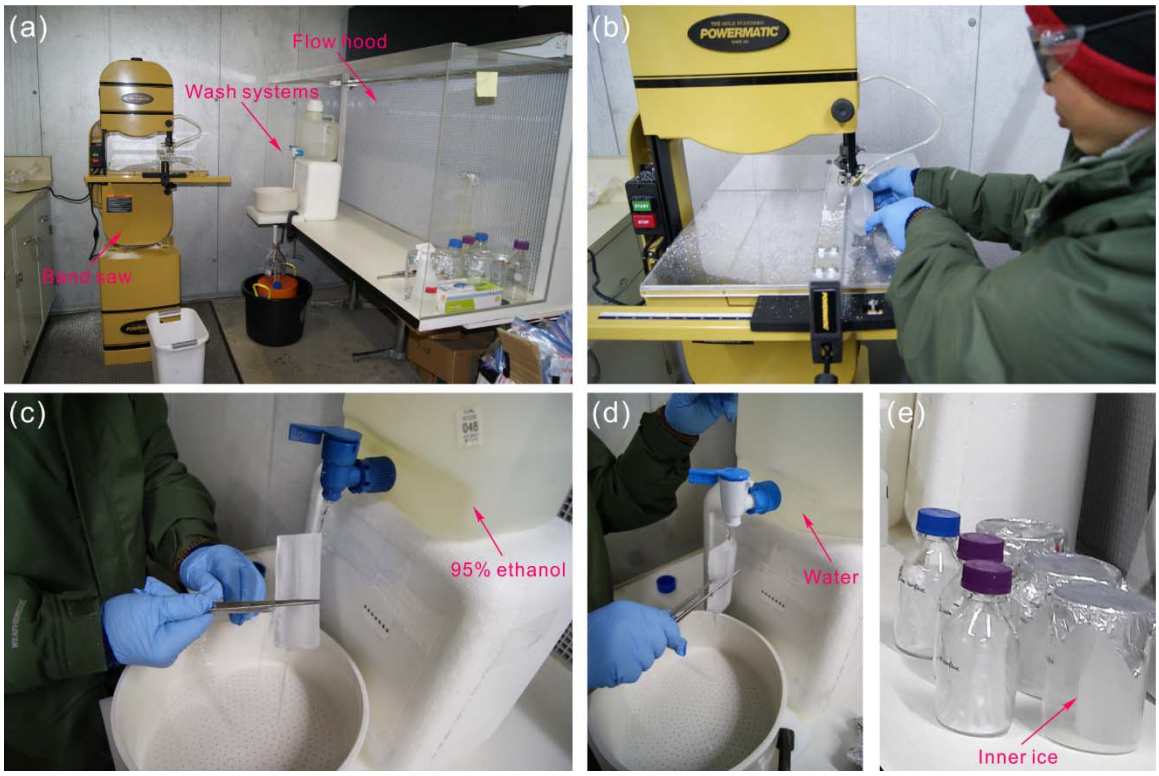
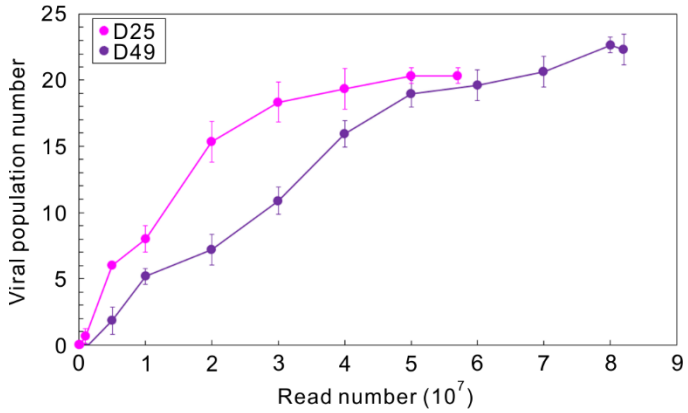
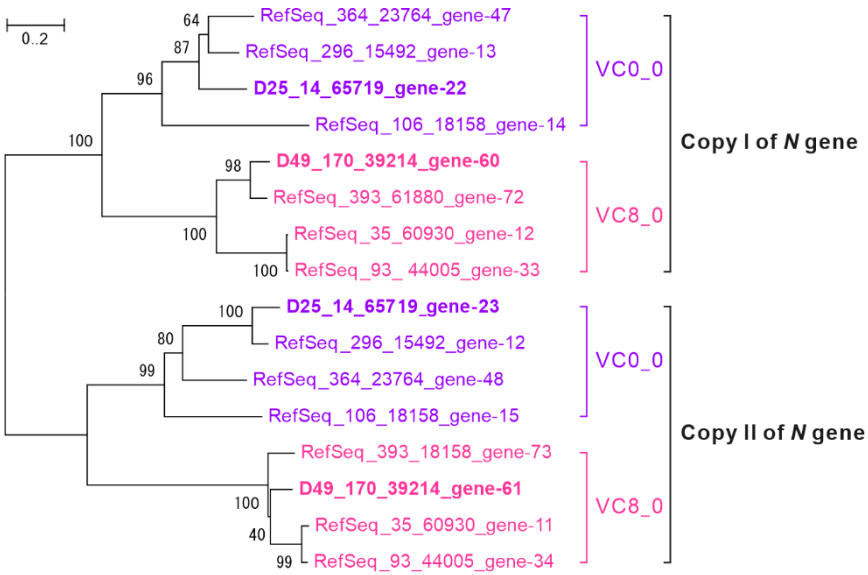


Supplementary Figure S1 Ice core sampling and preparation in the laboratory. (**a**) The cold work room (−5ºC) with band saw, BioGard laminar flow hood and wash systems. (**b**) the outer layer of the ice section being removed by the band saw. (**c**) The ice section being washed with 95% ethanol and (**d**) with water. (**e**) The "clean" inner ice is preserved in the autoclaved beakers or bottles.

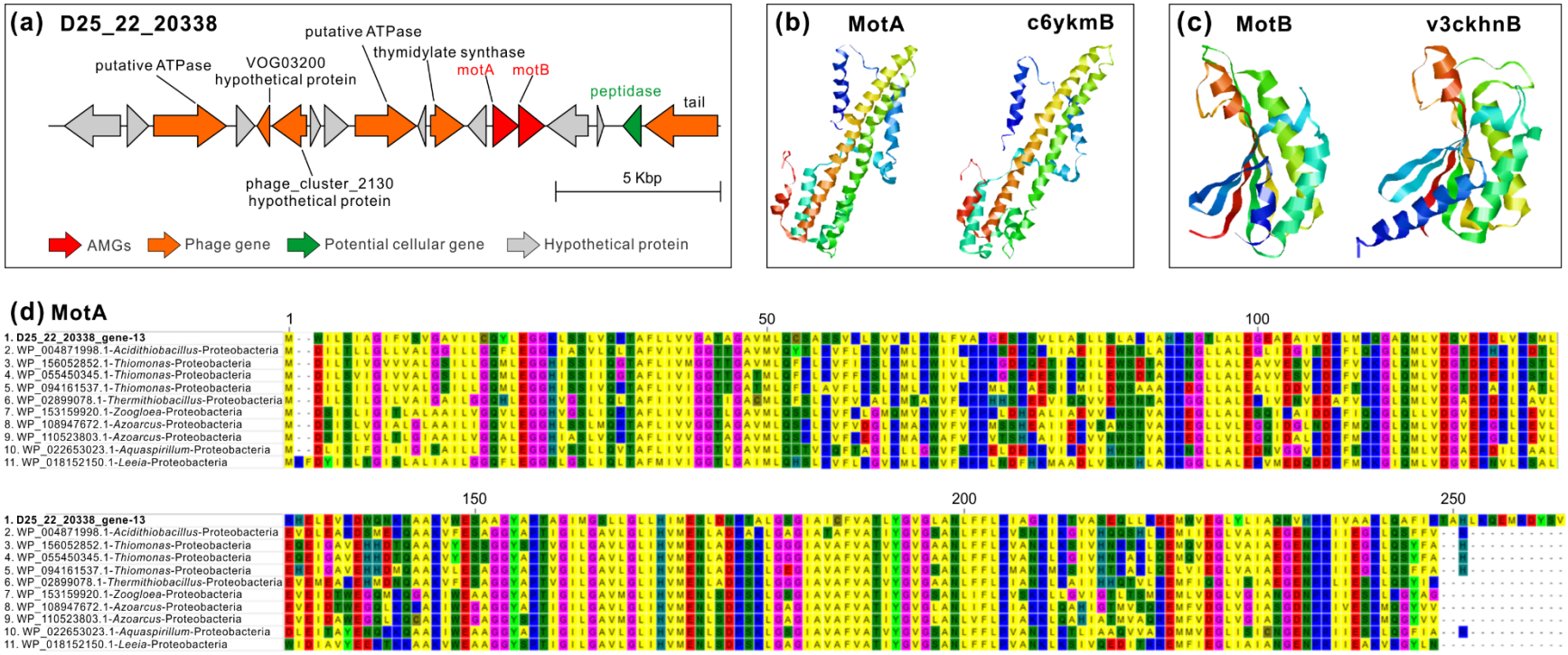
Supplementary Figure S2 Microbial communities at genus level (a) and overlapped OTUs (b) of removed and inner ice samples collected during decontamination procedures. The most abundant genera (n = 30) and OTUs (n = 33) are illustrated. Cut, Wash and Inner represent ice samples collected from band saw scrapping, water washing and the inner ice, respectively.

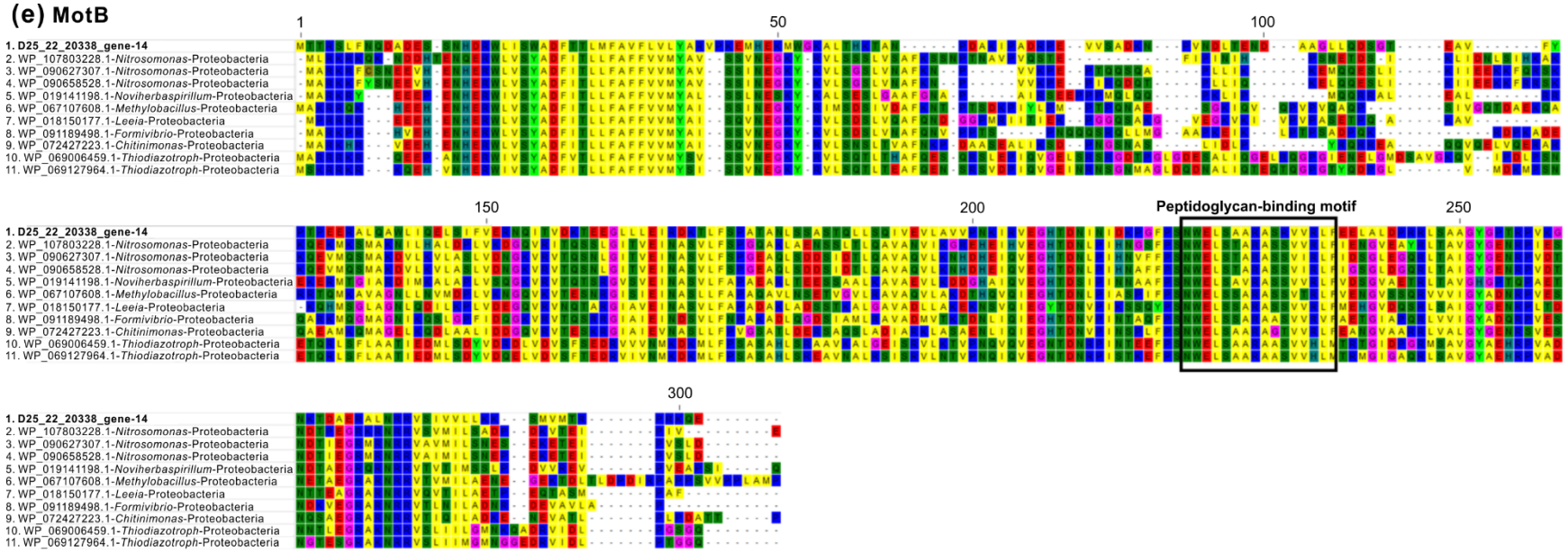


Supplementary Figure S3 Rarefaction curves of two glacier-ice viromes by vOTU numbers. Rarefaction curves were constructed by the change of vOTUs (≥10 kb) number along sequencing depth (i.e., read number) obtained by subsampling quality-controlled reads.



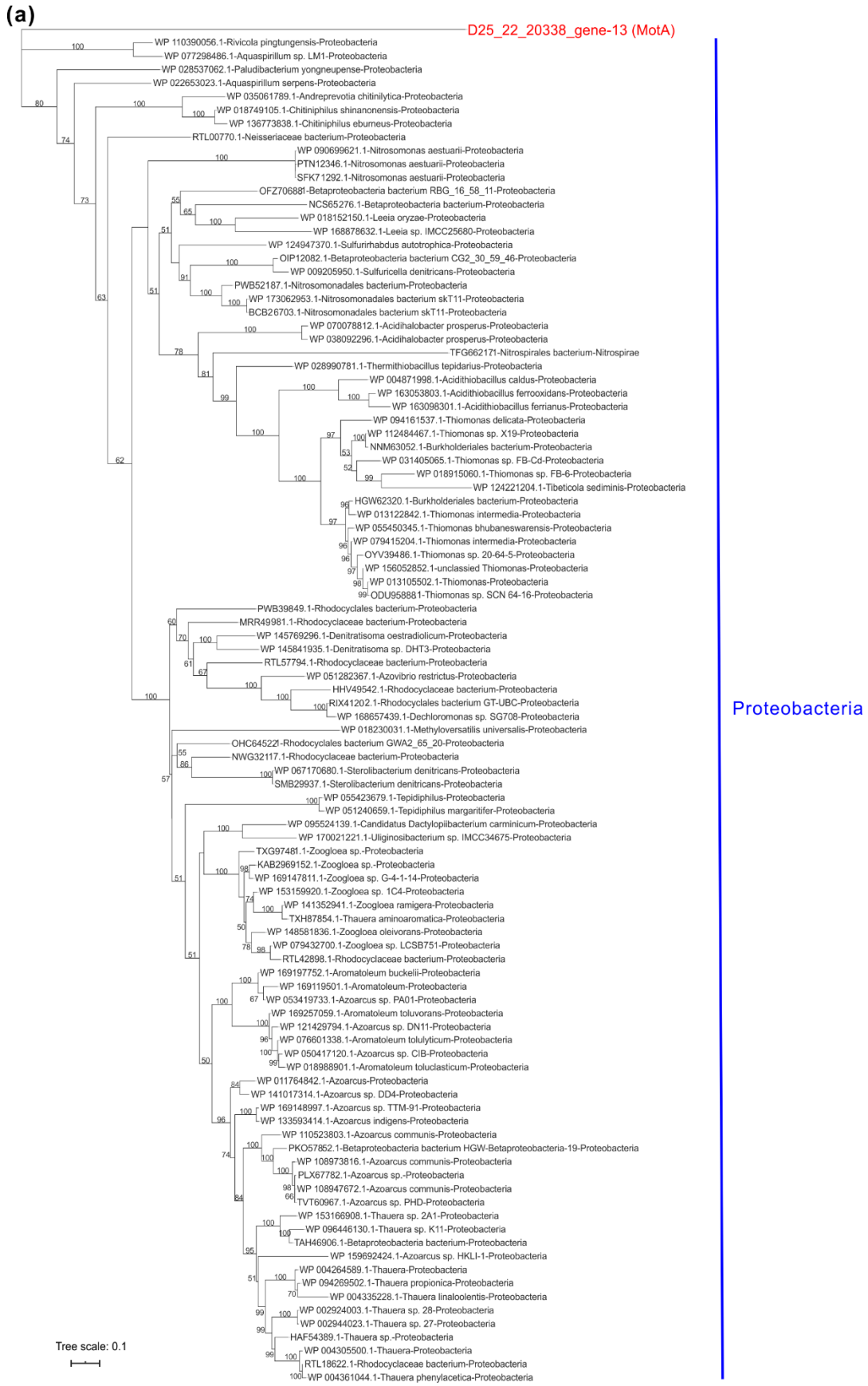
Supplementary Figure S4 The unrooted neighbor-joining phylogenetic tree of Mu *N* genes from eight *Methylobacterium* viruses. The tree was constructed using the predicted amino acid sequences of the *N* genes from two glacier ice viruses (i.e., D25_14_65719 and D49_170_39214; in bold font) and six prophages identified from bacterial genomes. Each viral contig contains two copies of *N* genes. Viruses belonged to the same VC (i.e., VC0_0 or VC8_0) are indicated in the same color. Bootstrap values (expressed as percentages of 1,000 replications) are shown at the branch points. The scale bar indicates a distance of 0.2.


(a) D25_22_20338
putative ATPase
putative ATPase
VOG03200
hypothetical protein
thymidylate synthase
motA
motB
peptidase
tail
phage_cluster_2130
hypothetical protein
5 Kbp
AMGs
Phage gene
Potential cellular gene
Hypothetical protein
(b)
MotA
c6ykmB
(c)
MotB
v3ckhnB
(d) MotA
1
50
100
150
200
250
1. D25_22_20338_gene-13
2. WP_004871998.1-Acidithiobacillus-Proteobacteria
3. WP_156052852.1-Thiomonas-Proteobacteria
4. WP_055450345.1-Thiomonas-Proteobacteria
5. WP_094161537.1-Thiomonas-Proteobacteria
6. WP_02899078.1-Thermithiobacillus-Proteobacteria
7. WP_153159920.1-Zoogloea-Proteobacteria
8. WP_108947672.1-Azoarcus-Proteobacteria
9. WP_110523803.1-Azoarcus-Proteobacteria
10. WP_022653023.1-Aquaspirillum-Proteobacteria
11. WP_018152150.1-Leeia-Proteobacteria



Supplementary Figure S5 Characterization of virus-encoded auxiliary metabolic genes (AMGs). (**a**) Genome map of glacier-ice virus D25_22_20338 encoding AMGs (motility genes *motA* and *motB*). CheckV was used to assess host-virus boundaries and remove potential host fractions on the viral contig (See Materials and Methods). Genes were marked by four colors to illustrate AMGs (red), phage genes (orange), potential cellular genes (green), and unaffiliated genes (grey). AMGs were detected by DRAM-v and following manual inspection; The latter three groups of genes were classified by comparing their predicted protein sequences to those of a large database of 15,958 profile hidden Markov models by CheckV and of viral genes in the extended RefSeqABVir database by VirSorter v1 in virome decontamination mode. Genes were marked as “phage genes” if they were matched to the genes of viruses in

RefSeqABVir database or CheckV databases. Genes were marked as “potential cellular genes” if they were matched to the genes of bacteria or archaea by CheckV. Genes were considered “unaffiliated” if they had no hit to a sequence in RefSeqABVir or CheckV databases. (**b-c**) Predicted three-dimensional (3D) structures of AMG products and templates. The 3D structure of template protein for each AMG is at the right (i.e., c6ykmB and v3ckhnB). Both AMG products are linked to their closest template protein with 100% confidence score by phyre2. (**d-e**) Multiple alignments of protein sequences for two AMGs and 10 closest related bacteria-originated genes. The AMG and 10 closest related bacteria-related genes are numbered as 1 and 2-11, respectively. Conserved motif of the MotB was indicated by black boxes and notes (i.e., conserved peptidoglycan-binding motif). MotA does not have a conserved motif. ‘h’ indicates hydrophobic amino acid and ‘x’ indicates any amino acid. The protein sequences were aligned using MAFFT (v.7.017) with the E-INS-I strategy for 1000 iteration. The position numbers of aligned sequences are indicated at the top.


(a)
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WP 077298486.1-Aquaspirillum sp. LM1-Proteobacteria
WP 028537062.1-Paludibacterium yongneupense-Proteobacteria
WP 022653023.1-Aquaspirillum serpens-Proteobacteria
WP 035061789.1-Andreprevotia chitinilytica-Proteobacteria
WP 018749105.1-Chitiniphilus shinanonensis-Proteobacteria
WP 136773838.1-Chitiniphilus eburneus-Proteobacteria
RTL00770.1-Neisseriaceae bacterium-Proteobacteria
WP 090699621.1-Nitrosomonas aestuarii-Proteobacteria
PTN12346.1-Nitrosomonas aestuarii-Proteobacteria
SFK71292.1-Nitrosomonas aestuarii-Proteobacteria
OFZ70688.1-Betaproteobacteria bacterium RBG_16_58_11-Proteobacteria
NCS65276.1-Betaproteobacteria bacterium-Proteobacteria
WP 018152150.1-Leeia oryzae-Proteobacteria
WP 168878632.1-Leeia sp. IMCC25680-Proteobacteria
WP 124947370.1-Sulfurirhabdus autotrophica-Proteobacteria
OIP12082.1-Betaproteobacteria bacterium CG2_30_59_46-Proteobacteria
WP 009205950.1-Sulfuricella denitricans-Proteobacteria
PWB52187.1-Nitrosomonadales bacterium-Proteobacteria
WP 173062953.1-Nitrosomonadales bacterium skT11-Proteobacteria
BCB26703.1-Nitrosomonadales bacterium skT11-Proteobacteria
WP 070078812.1-Acidihalobacter prosperus-Proteobacteria
WP 038092296.1-Acidihalobacter prosperus-Proteobacteria
TFG66217.1-Nitrospirales bacterium-Nitrospirae
WP 028990781.1-Thermithiobacillus tepidarius-Proteobacteria
WP 004871998.1-Acidithiobacillus caldus-Proteobacteria
WP 163053803.1-Acidithiobacillus ferrooxidans-Proteobacteria
WP 163098301.1-Acidithiobacillus ferrianus-Proteobacteria
WP 094161537.1-Thiomonas delicata-Proteobacteria
WP 112484467.1-Thiomonas sp. X19-Proteobacteria
NNM63052.1-Burkholderiales bacterium-Proteobacteria
WP 031405065.1-Thiomonas sp. FB-Cd-Proteobacteria
WP 018915060.1-Thiomonas sp. FB-6-Proteobacteria
WP 124221204.1-Tibeticola sediminis-Proteobacteria
HGW62320.1-Burkholderiales bacterium-Proteobacteria
WP 013122842.1-Thiomonas intermedia-Proteobacteria
WP 055450345.1-Thiomonas bhubaneswarensis-Proteobacteria
WP 079415204.1-Thiomonas intermedia-Proteobacteria
OYV39486.1-Thiomonas sp. 20-64-5-Proteobacteria
WP 156052852.1-unclassied Thiomonas-Proteobacteria
WP 013105502.1-Thiomonas-Proteobacteria
ODU95888.1-Thiomonas sp. SCN 64-16-Proteobacteria
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WP 145841935.1-Denitratisoma sp. DHT3-Proteobacteria
RTL57794.1-Rhodocyclaceae bacterium-Proteobacteria
WP 051282367.1-Azovibrio restrictus-Proteobacteria
HHV49542.1-Rhodocyclaceae bacterium-Proteobacteria
RIX41202.1-Rhodocyclales bacterium GT-UBC-Proteobacteria
WP 168657439.1-Dechloromonas sp. SG708-Proteobacteria
WP 018230031.1-Methyloversatilis universalis-Proteobacteria
OHC64522.1-Rhodocyclales bacterium GWA2_65_20-Proteobacteria
NWG32117.1-Rhodocyclaceae bacterium-Proteobacteria
WP 067170680.1-Sterolibacterium denitricans-Proteobacteria
SMB29937.1-Sterolibacterium denitricans-Proteobacteria
WP 055423679.1-Tepidiphilus-Proteobacteria
WP 051240659.1-Tepidiphilus margaritifer-Proteobacteria
WP 095524139.1-Candidatus Dactylopiibacterium carminicum-Proteobacteria
WP 170021221.1-Uliginosibacterium sp. IMCC34675-Proteobacteria
TXG97481.1-Zoogloea sp.-Proteobacteria
KAB2969152.1-Zoogloea sp.-Proteobacteria
WP 169147811.1-Zoogloea sp. G-4-1-14-Proteobacteria
WP 153159920.1-Zoogloea sp. 1C4-Proteobacteria
WP 141352941.1-Zoogloea ramigera-Proteobacteria
TXH87854.1-Thauera aminoaromatica-Proteobacteria
WP 148581836.1-Zoogloea oleivorans-Proteobacteria
WP 079432700.1-Zoogloea sp. LCSB751-Proteobacteria
RTL42898.1-Rhodocyclaceae bacterium-Proteobacteria
WP 169197752.1-Aromatoleum buckelii-Proteobacteria
WP 169119501.1-Aromatoleum-Proteobacteria
WP 053419733.1-Azoarcus sp. PA01-Proteobacteria
WP 169257059.1-Aromatoleum toluvorans-Proteobacteria
WP 121429794.1-Azoarcus sp. DN11-Proteobacteria
WP 076601338.1-Aromatoleum tolulyticum-Proteobacteria
WP 050417120.1-Azoarcus sp. CIB-Proteobacteria
WP 018988901.1-Aromatoleum toluclasticum-Proteobacteria
WP 011764842.1-Azoarcus-Proteobacteria
WP 141017314.1-Azoarcus sp. DD4-Proteobacteria
WP 169148997.1-Azoarcus sp. TTM-91-Proteobacteria
WP 133593414.1-Azoarcus indigens-Proteobacteria
WP 110523803.1-Azoarcus communis-Proteobacteria
PKO57852.1-Betaproteobacteria bacterium HGW-Betaproteobacteria-19-Proteobacteria
WP 108973816.1-Azoarcus communis-Proteobacteria
PLX67782.1-Azoarcus sp.-Proteobacteria
WP 108947672.1-Azoarcus communis-Proteobacteria
TVT60967.1-Azoarcus sp. PHD-Proteobacteria
WP 153166908.1-Thauera sp. 2A1-Proteobacteria
WP 096446130.1-Thauera sp. K11-Proteobacteria
TAH46906.1-Betaproteobacteria bacterium-Proteobacteria
WP 159692424.1-Azoarcus sp. HKLI-1-Proteobacteria
WP 004264589.1-Thauera-Proteobacteria
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WP 004335228.1-Thauera linaloolentis-Proteobacteria
WP 002924003.1-Thauera sp. 28-Proteobacteria
WP 002944023.1-Thauera sp. 27-Proteobacteria
HAF54389.1-Thauera sp.-Proteobacteria
WP 004305500.1-Thauera-Proteobacteria
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Proteobacteria
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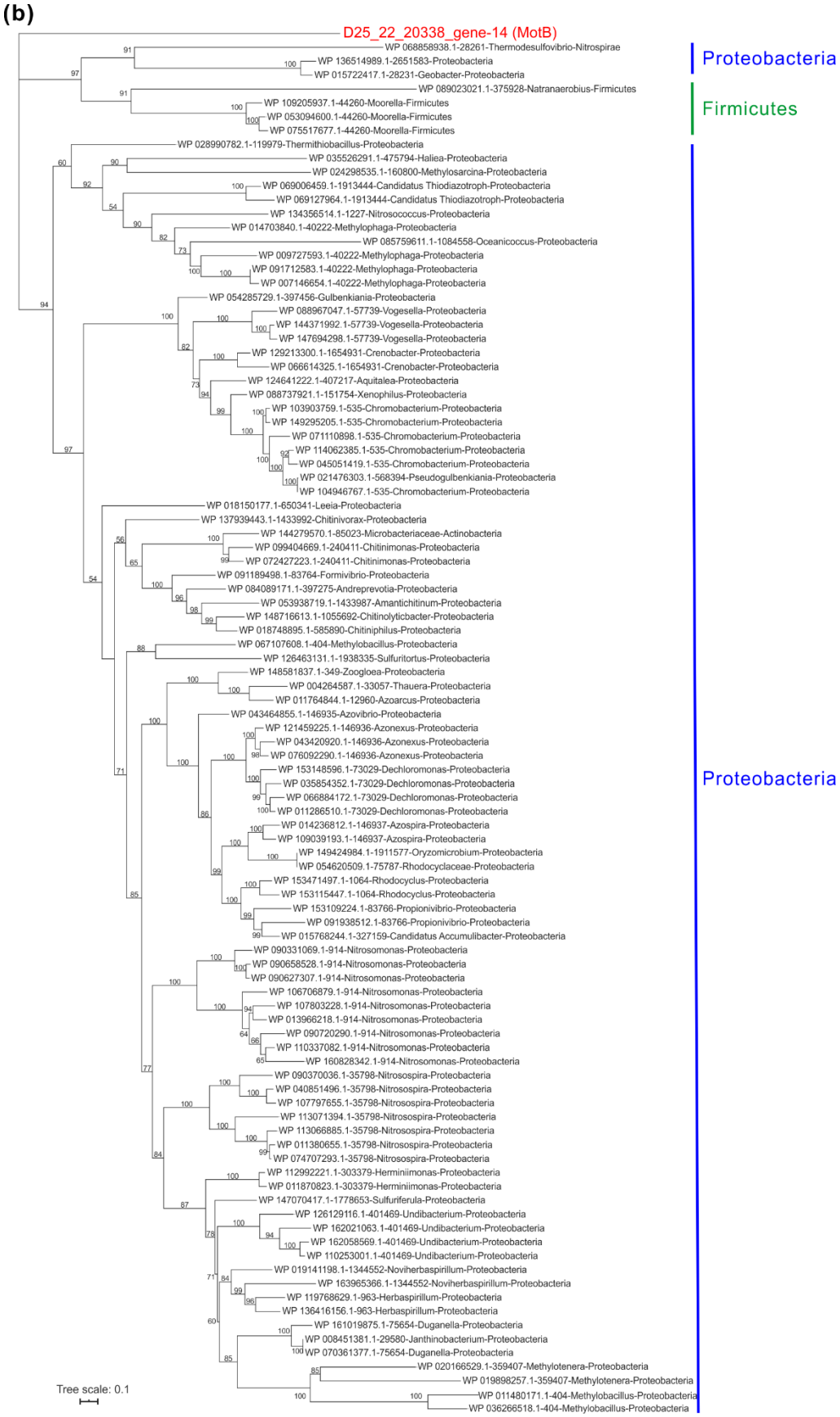
(b)
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WP 089023021.1-375928-Natranaerobius-Firmicutes
WP 109205937.1-44260-Moorella-Firmicutes
WP 053094600.1-44260-Moorella-Firmicutes
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WP 028990782.1-119979-Thermithiobacillus-Proteobacteria
WP 035526291.1-475794-Haliea-Proteobacteria
WP 024298535.1-160800-Methylosarcina-Proteobacteria
WP 069006459.1-1913444-Candidatus Thiodiazotroph-Proteobacteria
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WP 134356514.1-1227-Nitrosococcus-Proteobacteria
WP 014703840.1-40222-Methylophaga-Proteobacteria
WP 085759611.1-1084558-Oceanicoccus-Proteobacteria
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WP 129213300.1-1654931-Crenobacter-Proteobacteria
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WP 011764844.1-12960-Azoarcus-Proteobacteria
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WP 121459225.1-146936-Azonexus-Proteobacteria
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WP 136416156.1-963-Herbaspirillum-Proteobacteria
WP 161019875.1-75654-Duganella-Proteobacteria
WP 008451381.1-29580-Janthinobacterium-Proteobacteria
WP 070361377.1-75654-Duganella-Proteobacteria
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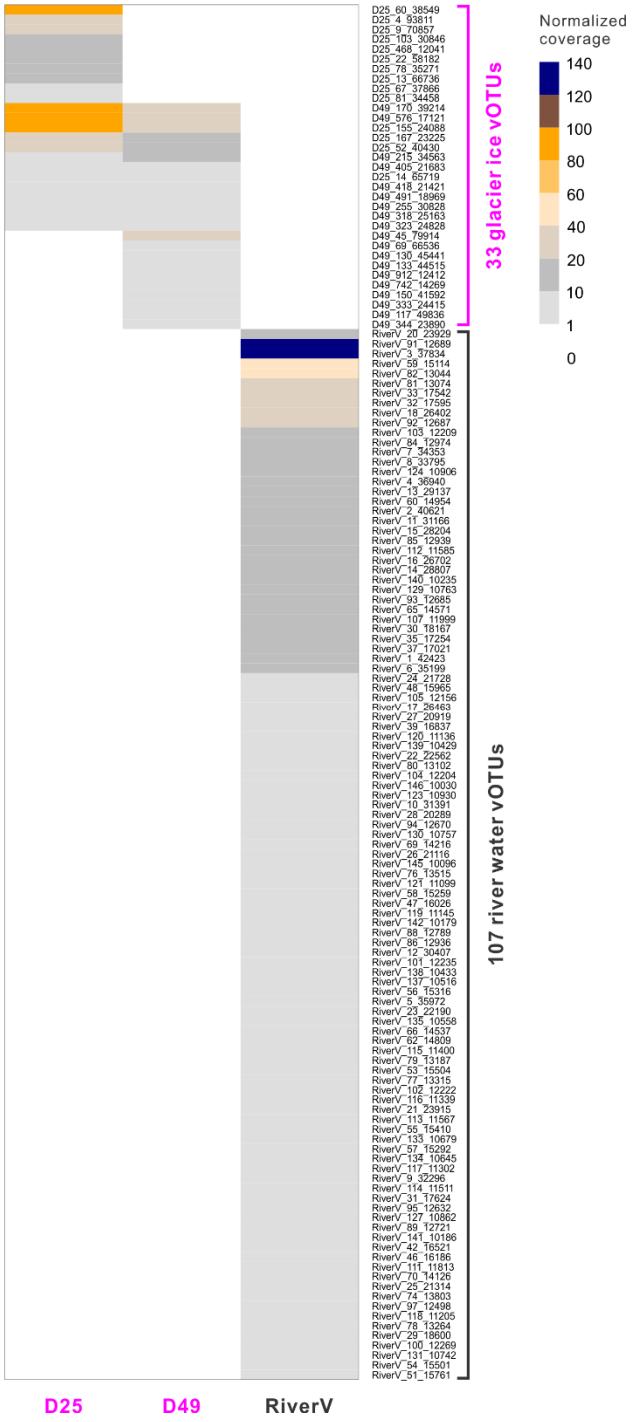
WP 036266518.1-404-Methylobacillus-Proteobacteria
Proteobacteria
Firmicutes
Proteobacteria
Tree scale: 0.1

Supplementary Figure S6 Phylogenetic analysis of two novel AMG products MotA (A) and MotB (B). Phylogenetic trees are inferred using maximum likelihood method with amino acid sequences (see Materials and Methods). The genes from glacier-ice virus (i.e., AMGs) and the NCBI RefSeq database (release v99) are colored in red and black, respectively. The scale bars indicate a distance of 0.1. Bootstrap values (expressed as percentages of 1000 replications) ≥50 are shown at the branch points.



Supplementary Figure S7 Heatmap showing the viral community compositions of two glacier-ice and one river-water viromes. Glacier ice samples: D25 and D49; River water sample: RiverV. The coverages of 140 vOTUs (>10 kb; 33 and 107 vOTUs from glacier ice and river water, respectively) were normalized to per gigabase of metagenome.