

Integrative structural analysis of *Pseudomonas* phage DEV reveals a genome ejection motor

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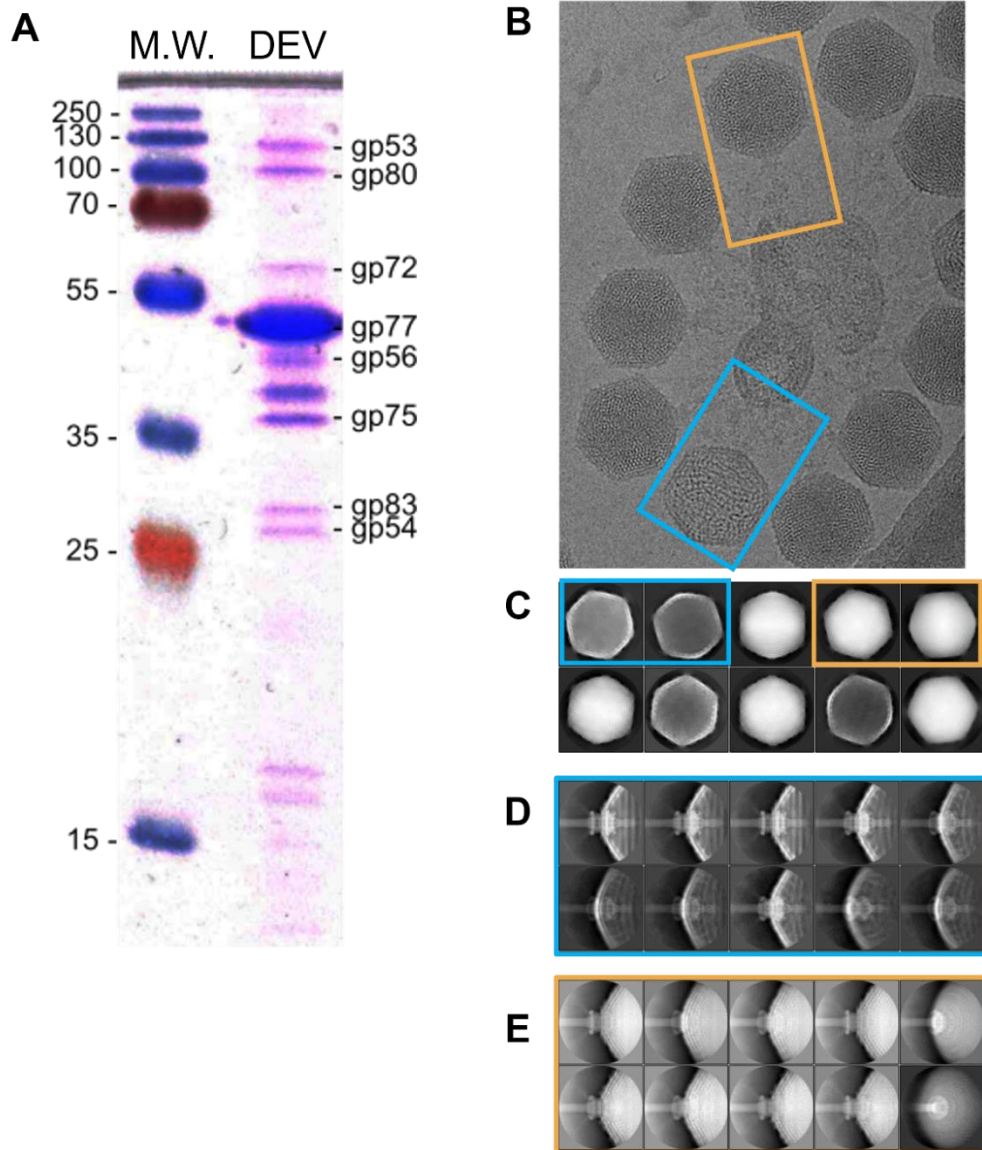
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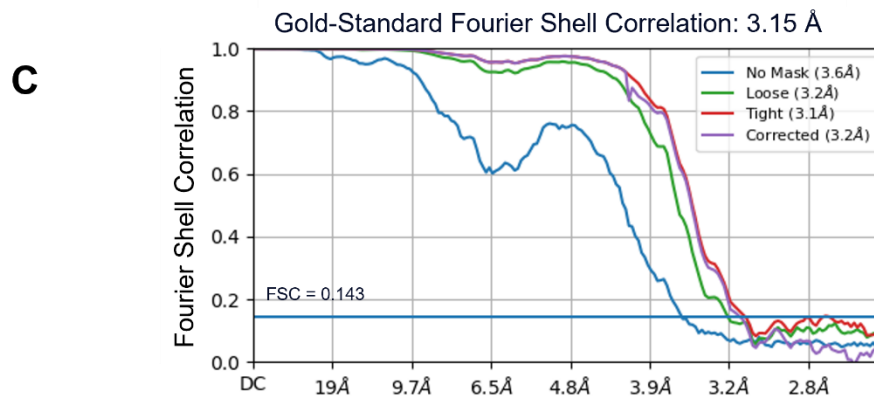
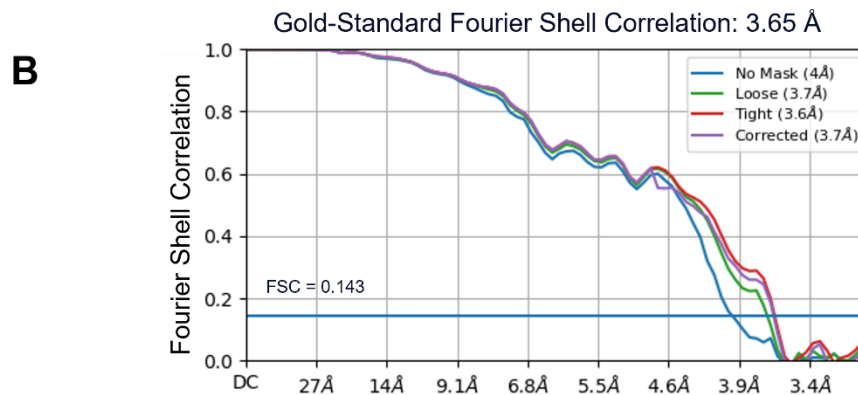
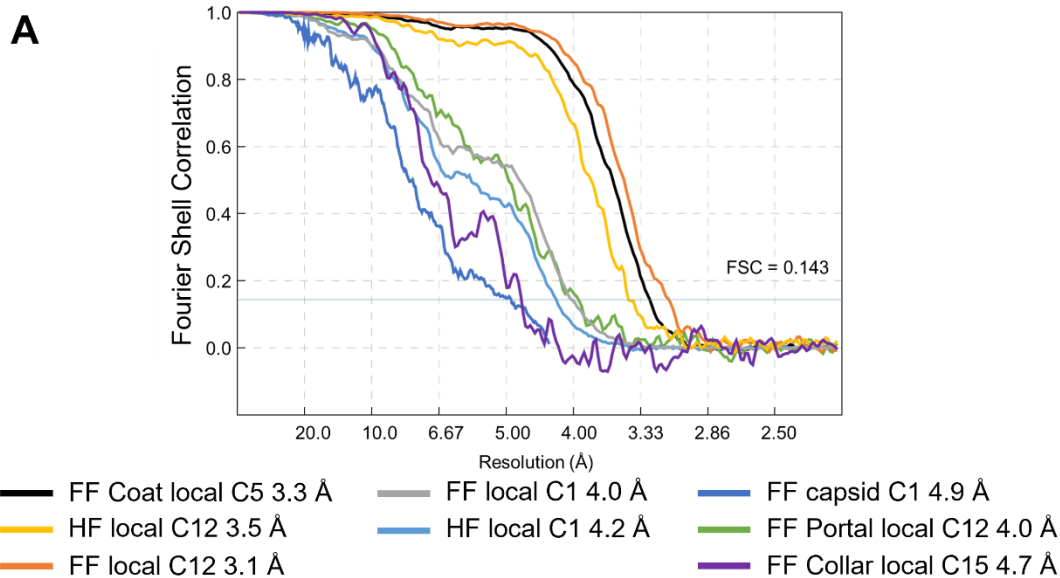
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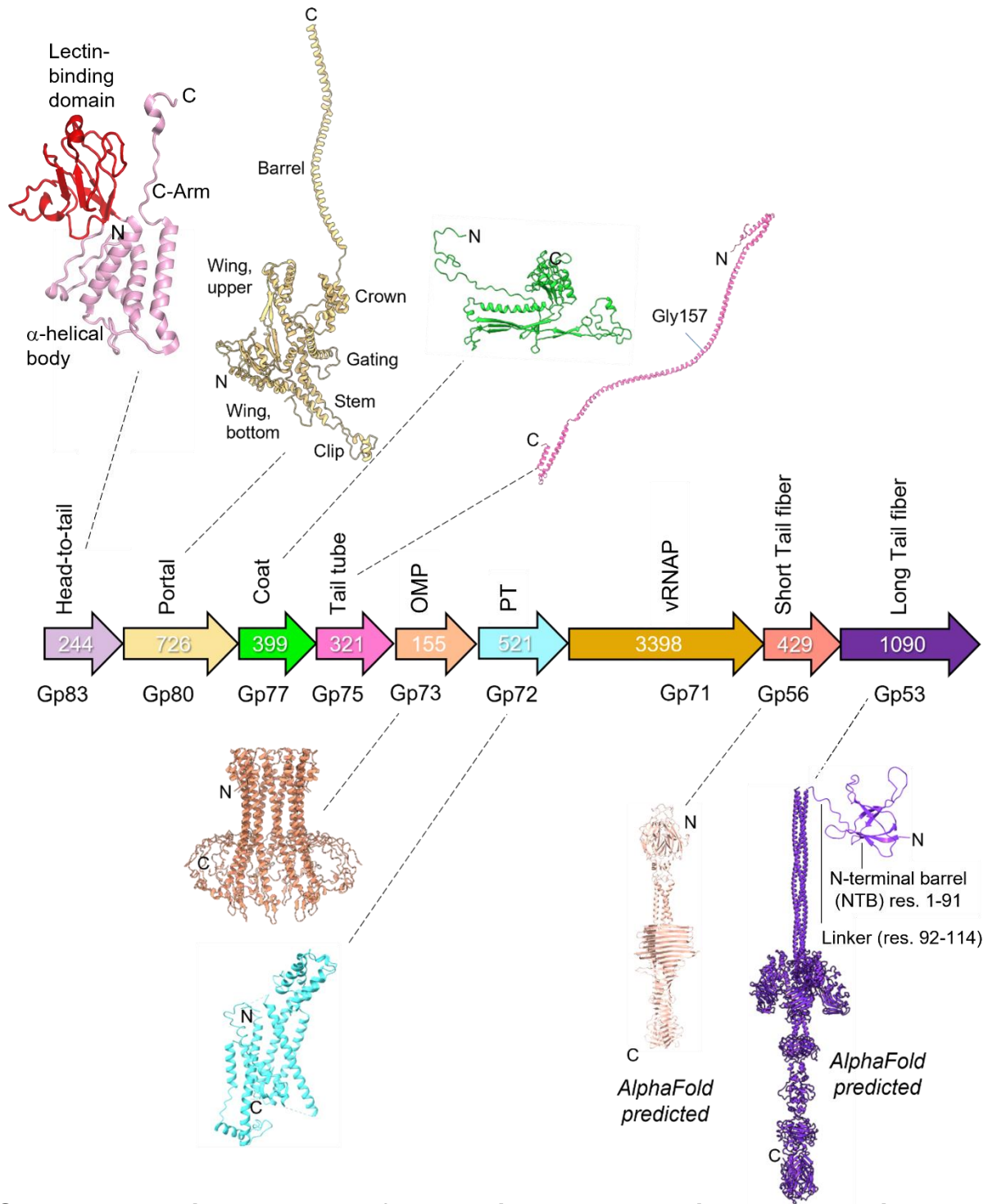
125-character teaser. Structural annotation of phage DEV structural components, host-absorption fibers, and ejection proteins.



Supplemental Figure 1. Structural characterization of phage DEV. (A) SDS-PAGE analysis of DEV infectious virions. Proteins extracted from CsCl₂ gradient-purified DEV particles were analyzed using 15 % SDS-PAGE and Coomassie staining. M.W. = molecular weight marker (kDa). Tentative identification of DEV proteins based on their M.W., assuming possible $\pm 10\%$ gel shifting, is reported on the right. Only proteins identified in the proteomic analyses of both DEV and LIT1 virions have been considered. The highly abundant gp77 (major coat protein; 44 kDa) migrates with an apparent size of ~ 50 kDa. The vRNAPol gp71 (350 kDa) is not visible, most likely because it does not enter the gel. (B) Representative cryo-micrograph of double CsCl₂ gradient-purified DEV particles. The orange and blue boxes cover a fully-filled (FF) virion and a half-filled (HF) particle. (C) Representative 2D class averages reveal the existence of HF particles (blue frame) and FF virions (orange frame). Nonetheless, both HF and FF had a tail apparatus. Representative localized reconstruction of 2D classes of the neck region for (D) HF particles (blue frame) and (E) FF virions (orange frame).



Supplemental Figure 2. Fourier Shell Correlation (FSC) resolution curves. (A) FSC curves for all reconstructions of FF virions and HF particles. (B) FSC curves for the cryo-EM reconstruction of recombinant gp72 in the post-ejection conformation. (C) FSC curves for the cryo-EM reconstruction of the recombinant gp72:gp73 complex in the post-ejection conformation. For all curves, the resolution is indicated at 0.143 cut-off.



Supplemental Figure 3. Atlas of all protein structures built in this work. All DEV proteins were built *de novo* in cryo-EM maps, except for the long-tail fiber gp53 and short-tail fiber gp56, which were predicted by AlphaFold.

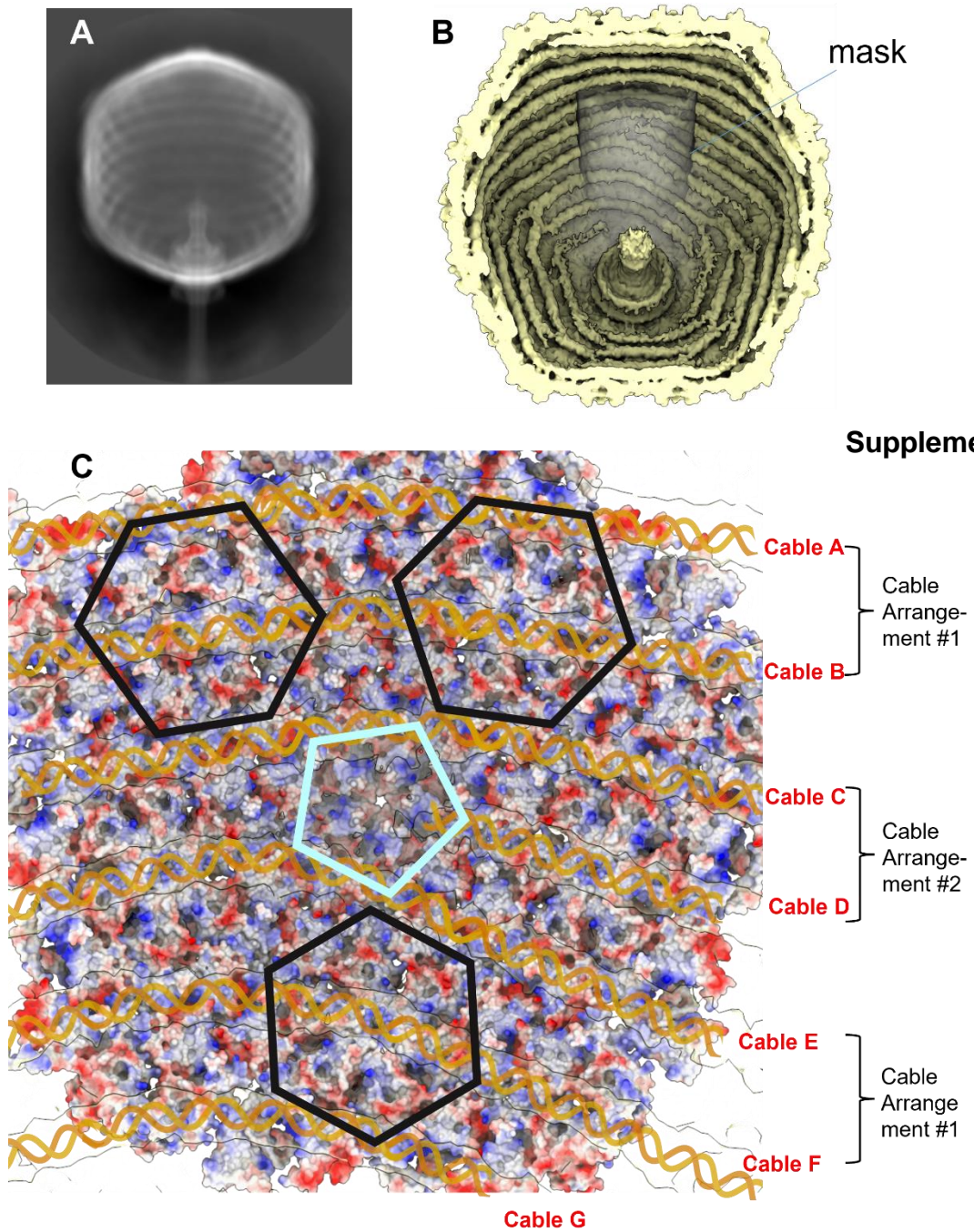
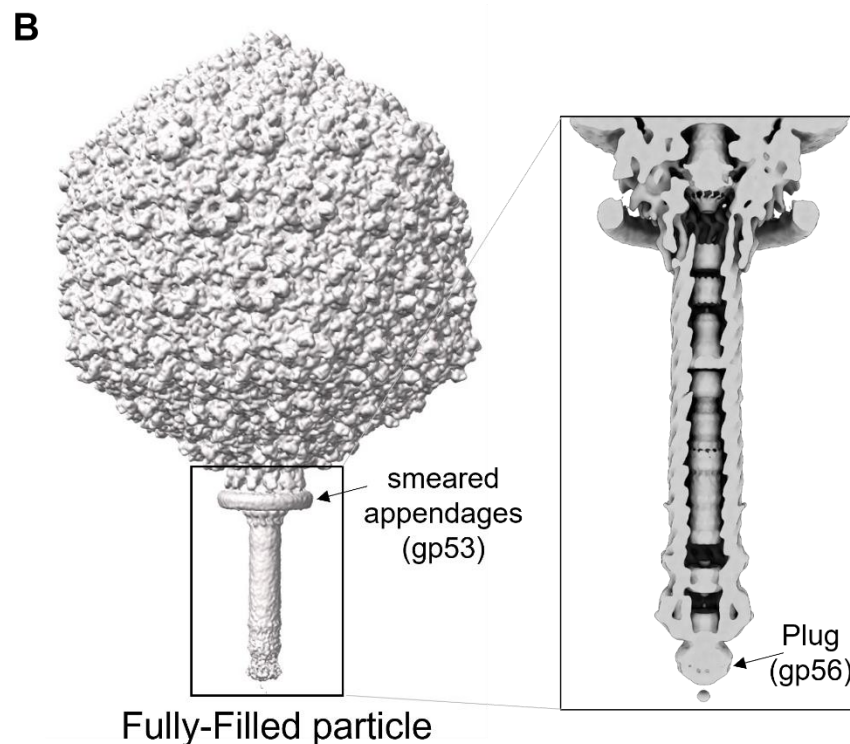
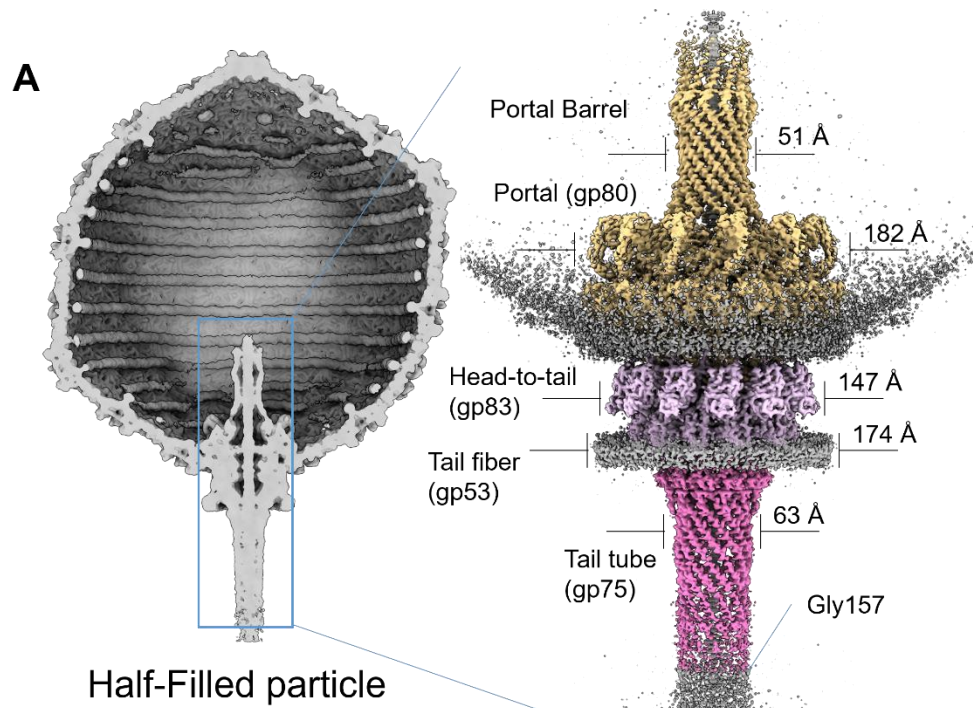
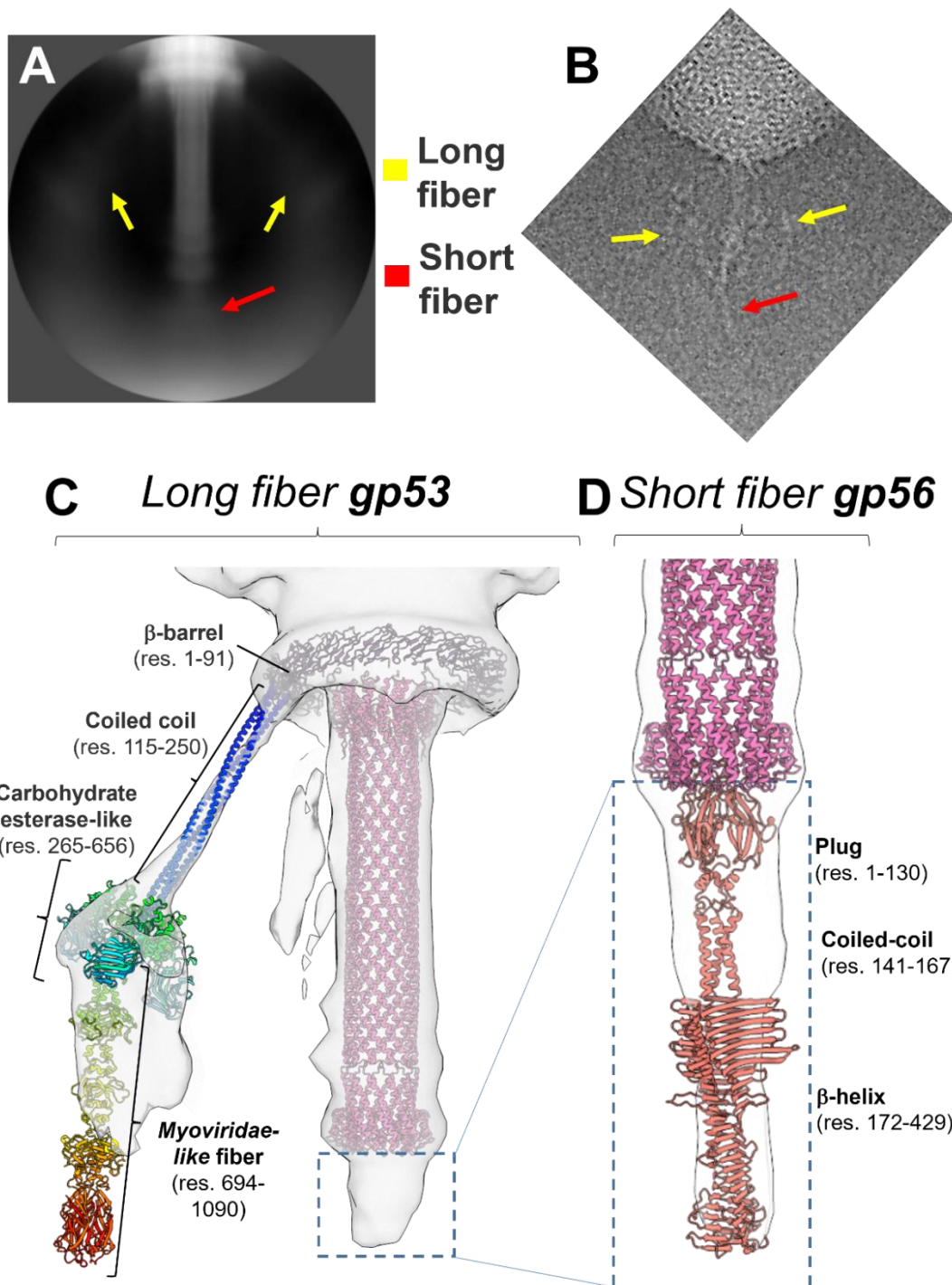


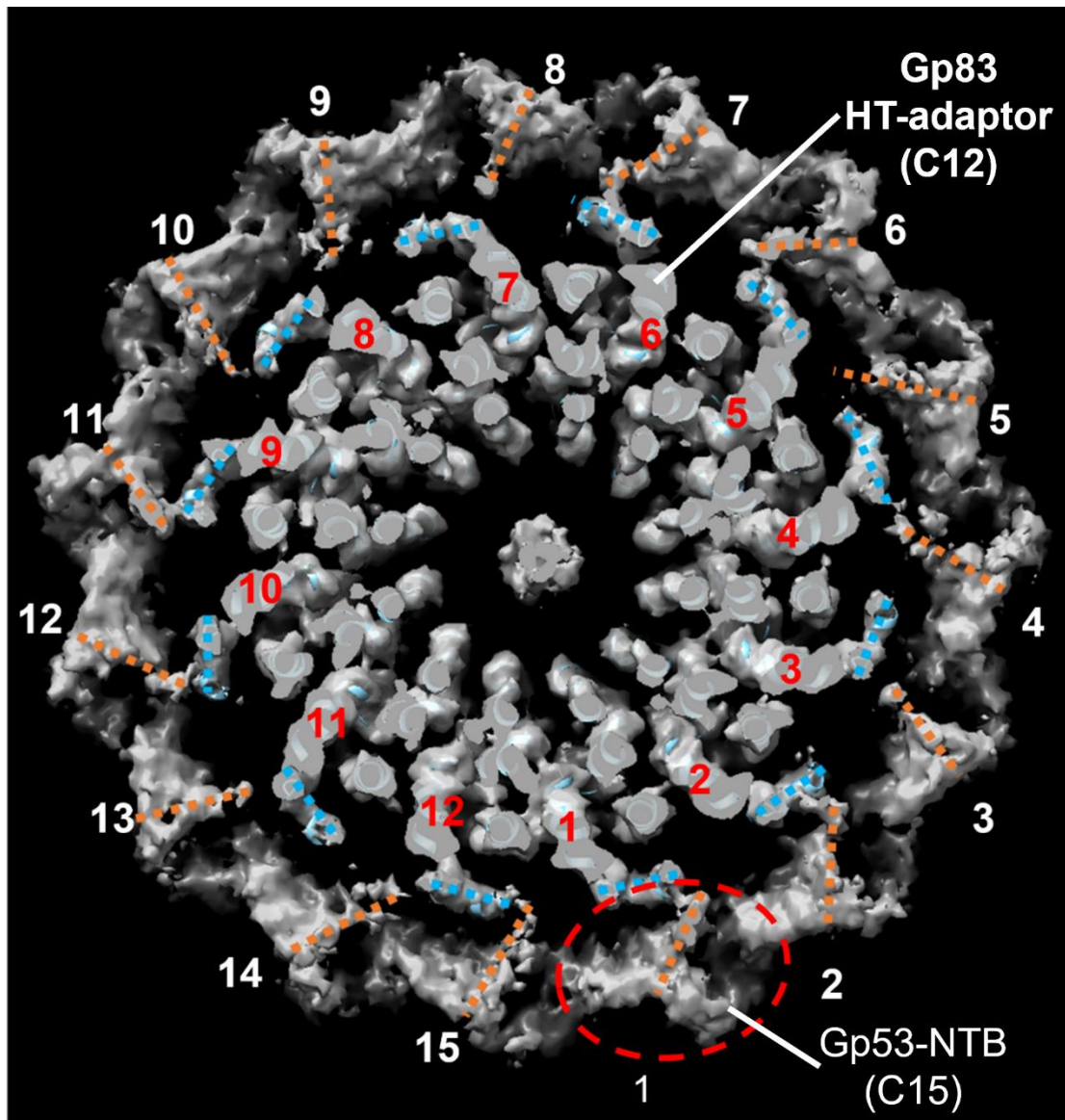
Figure 4. Localized reconstruction of DEV cables. (A) An averaged 2D projection from 13 expanded particles aligned locally with the tail (C5-C12 symmetry aligned). (B) A 3D reconstruction from C5-C12 aligned particles showing the interior of the capsid. (C) A focused reconstruction of one capsid five-fold vertex visualized from the inside of the capsid generated using the transparent cylindrical mask shown in panel (B). The density reveals an asymmetric pattern of seven cables (labeled A-G) relative to the capsid exons (black) and penton (gray). Double-stranded DNA helices were modeled inside the cable densities that are displayed onto an electrostatic surface representation of the DEV capsid interior.



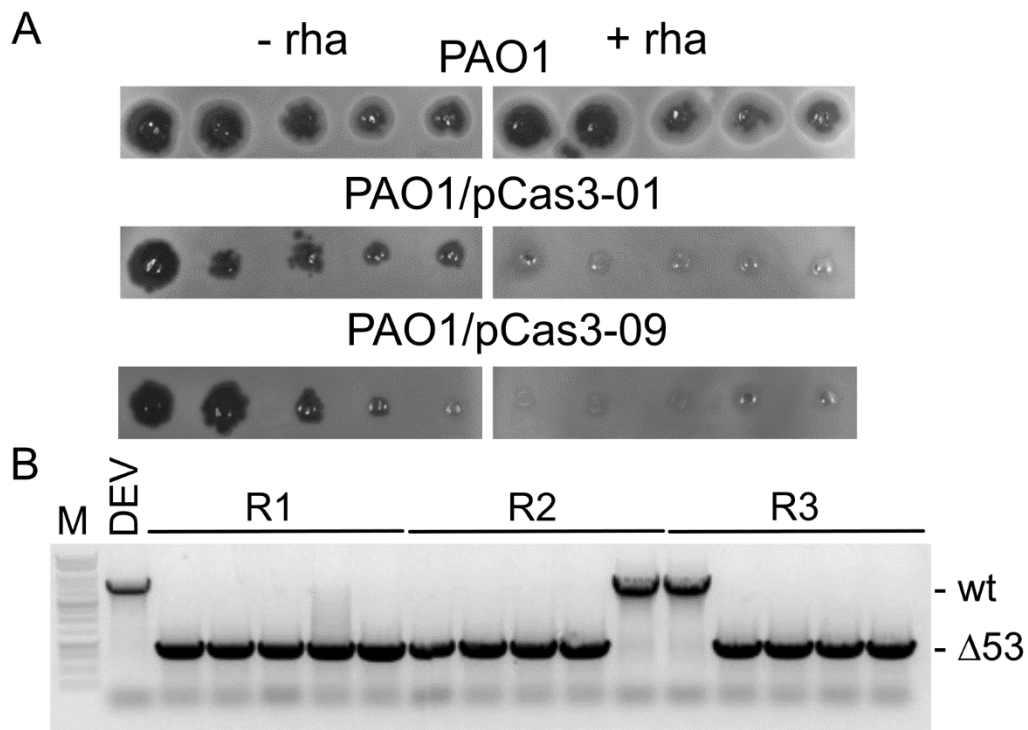
Supplemental Figure 5. Cryo-EM reconstructions. (A) DEV tail apparatus from Half-Filled particles. Section view of an HF particle, with a magnified view of the C12, symmetrized localized reconstruction of the unique vertex colored coded by component. The 3.5 Å map is contoured at 3.5σ . **(B) C1 reconstruction of DEV mature virion** calculated at 7 Å map and contoured at 2.0σ reveals a plug-like density at the tail tube tip, distal from the capsid, that we assigned to gp56.



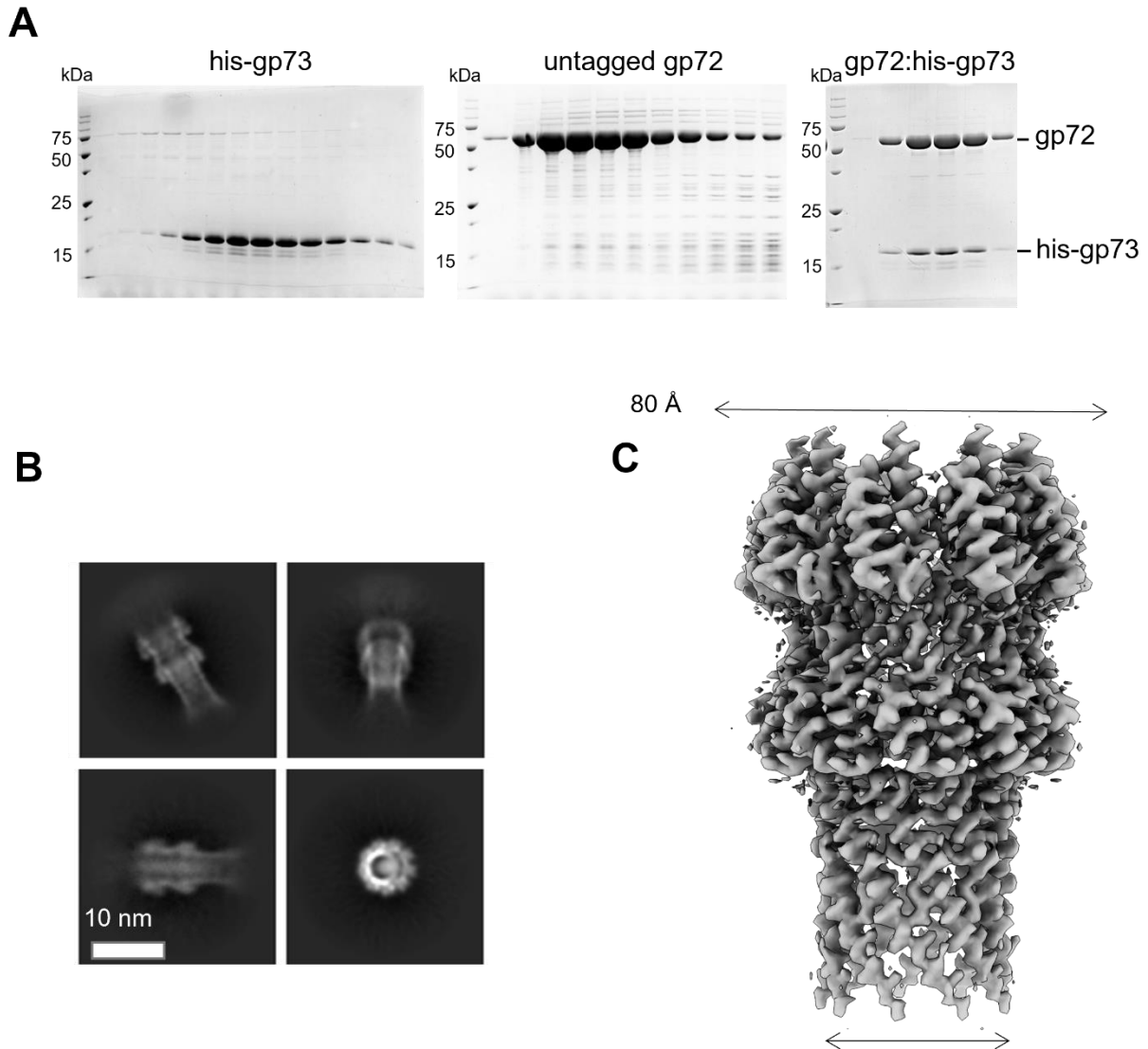
Supplemental Figure 6. DEV flexible fibers. (A) Diffused density for DEV fibers emanating from the neck collar is visible in 2D class averages from localized reconstruction (indicated by yellow arrows). A smeared signal is also visible at the distal tip of the tail tube (indicated by a red arrow). (B) The same features are visible in raw micrographs of vitrified DEV. (C-D) Low-resolution localized reconstruction (light gray) was overlaid to the AlphaFold2 models for gp53 (long-tail fiber) and gp56 (short-tail fiber).



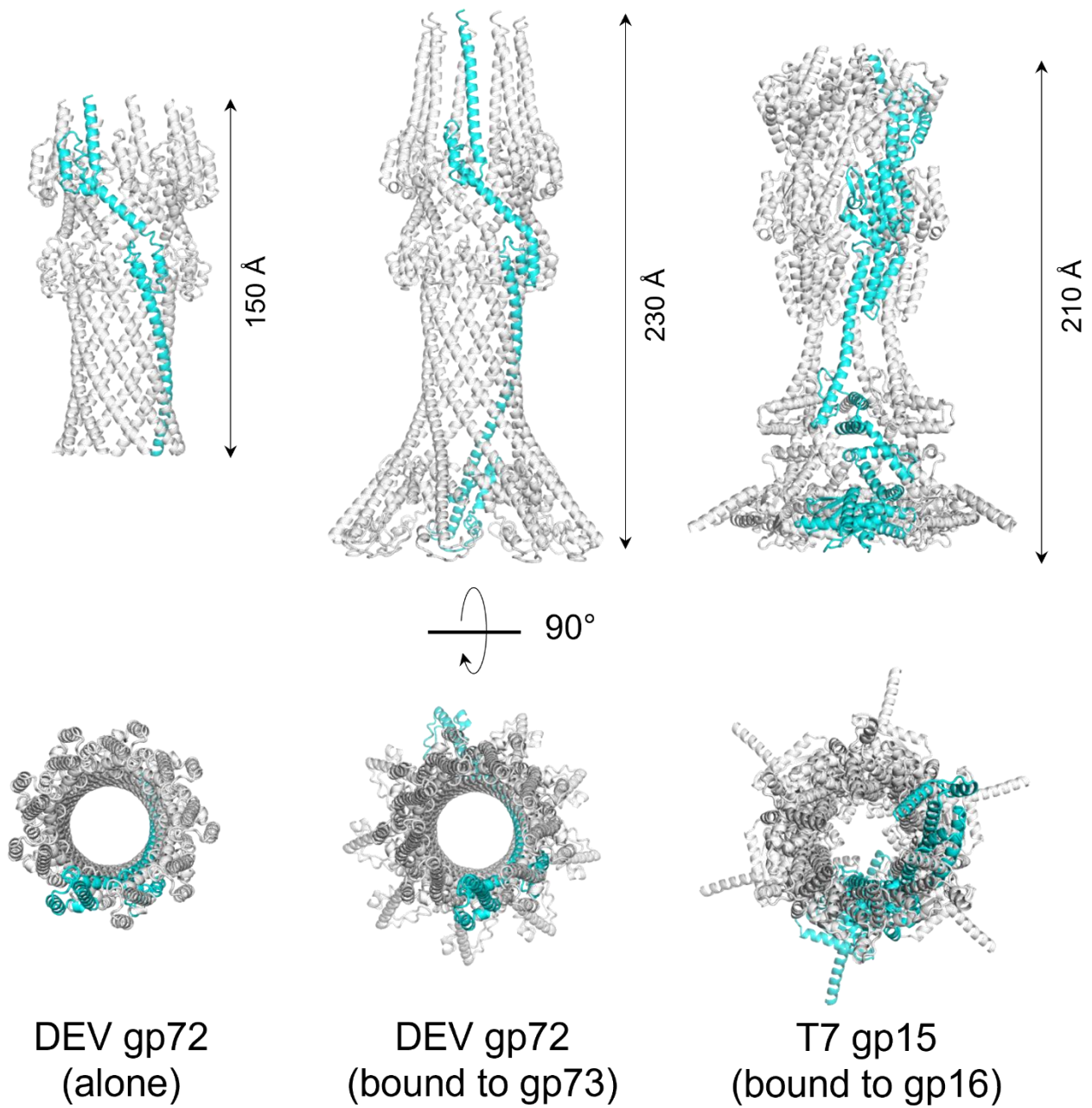
Supplemental Figure 7. Symmetry mismatch between DEV long tail fiber gp53 and the HT-adaptor gp83. A cut-out view from inside the capsid of the DEV tail shows a glimpse of the asymmetric C1 map. Gp53 exists in 15 copies (the protomer is circled in red), generating a symmetry mismatch with 12 copies of gp83 (numbered in red).



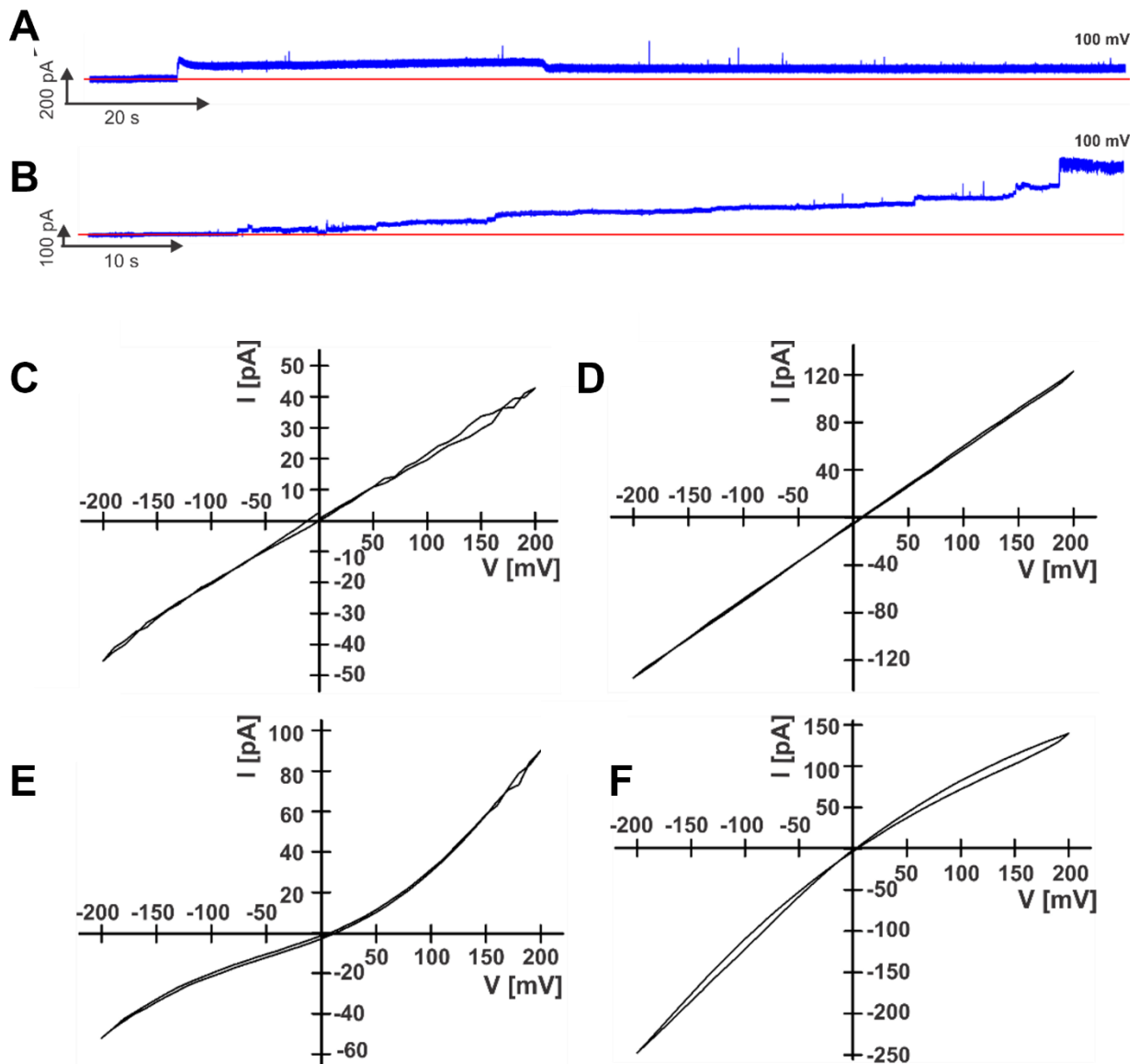
Supplemental Figure 8. DEV gp53 mutagenesis. (A) Induction with rhamnose (rha) of cr-RNA53 and cas genes cloned in pCas3-01 and pCas3-09 prevents DEV growth on PAO1. Serial dilutions (x10) of the indicated phages were replicated on the indicated strains with (+) or without (-) rhamnose in the medium. (B) PCR analysis of gp53 locus. Plaques formed by phages able to grow on PAO1 only in the presence of pD53 plasmid were analyzed by PCR with the oligonucleotides 3806-3809. Five plaques derived from each of three replicate mutagenesis experiments, indicated as R1, R2, and R3, were analyzed. DEV, PCR performed on purified DEV DNA.



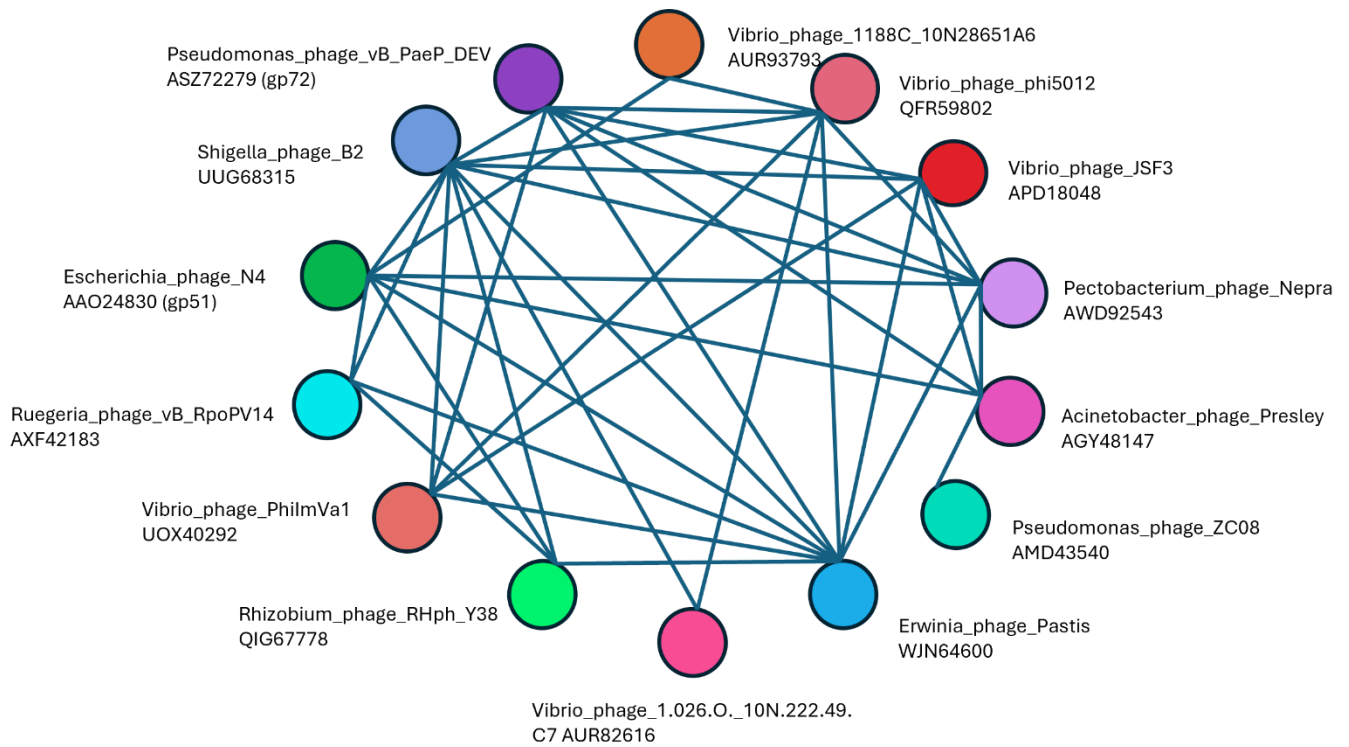
Supplemental Figure 9. DEV ejection protein gp72. (A) (left) SDS-PAGE analysis of purified his-gp73 solubilized from membranes; (middle) gp72 expressed under native conditions; (right) gel filtration fractions containing the gp72:gp73 complex. (B) Representative 2D classes of recombinant gp72. (C) 3D reconstruction of gp72 in the post-ejection conformation. The density is calculated at 3.65 Å resolution and is contoured at 3.5σ .



Supplemental Figure 10. Structural comparison of DEV gp72 (left and middle) and phage T7 gp17 periplasmic tunnel (PT). The central lumen of either tube-like structure measures 25-30 Å in diameter and is partially gated in T7. In all panels, only one protomer is colored in cyan, and all others are gray.



Supplemental Figure 11. Lipid bilayer experiments with purified gp73. Current traces with multiple insertions when 2.6 μg (A) or 1.9 μg (B) of protein is present in the cuvette. All experiments were performed at 100 mV applied potential in diphytanoyl phosphatidylcholine (DPhPC) membranes bathed in 1 M KCl, 10 mM HEPES, pH7.4 electrolyte. The protein samples were added to the grounded *cis* side of the cuvette which had 100 μm SU-8 aperture. (C-F) Current-voltage curves of purified gp73. Each panel shows current-voltage curves of one gp73 pore inserted in the membrane at the voltage range of -200 to 200 mV. All experiments were performed in diphytanoyl phosphatidylcholine (DPhPC) membranes bathed in 1 M KCl, 10 mM HEPES, pH 7.4 electrolyte. The protein samples were added to the grounded *cis* side of the cuvette which had 100 μm SU-8 aperture. At 100 mV pores had current amplitude of 21 pA (C), 60 pA (D), 31 pA (E), and 72 pA (F), respectively.



Supplemental Figure 12. Network diagram showing the significant predictions of gp72 homology based on comparisons of predicted tertiary structure. The disks are gp72-putative proteins for which AlphaFold predicted the structure. The links show structures with $\geq 90\%$ probability of being homologs (according to Foldseek ¹).

Supplementary Table S1. DEV proteins identified by mass spectrometry.

Name ^a	Description	Length (AAs) ^b	MW [kDa] ^c	Coverage [%]	Unique Peptides	SeqEst score	N4 homologous protein; virion presence ^d
gp27		184	21.7	24	5	12.04	NH
gp44		838	94.1	31	21	17.75	gp33; NI
gp50		170	18.7	29	6	34.07	NH
gp53	Long Tail fiber	1090	117.9	23	19	42.70	NH
gp54		223	24.9	30	6	10.79	NH
gp56	Short Tail fiber	429	46.3	12	5	5.61	NH
gp60	Putative DNA primase	724	82.4	21	15	8.65	gp43; NI
gp71	vRNAP	3398	369.5	31	86	235.76	gp50
gp72	Ejection protein	521	57.1	35	14	7.95	NH
gp75	Tail tube	321	35.2	41	12	52.45	gp54
gp76		221	25.0	47	5	9.77	gp55; NI
gp77	Major coat protein	399	44.0	67	21	833.17	gp56; Y
gp80	Portal protein	726	81.6	50	32	164.74	gp59; Y
gp83	Head-tail adaptor	244	27.8	29	7	23.00	gp67; Y

^a Proteins identified by ≥ 5 unique peptides with a SeqEst score ≥ 5 .
In bold, proteins identified also in the *Litunavirus* LIT1 virions ².

DEV gp55, gp73, gp74, and gp78 orthologues of LIT1 virion proteins were identified by 1, 2, 8, and 7 unique peptides, respectively, and SeqEst score of 0. The orthologue of LIT1 gp52 was not detected.

^b AAs, amino acids.

^c MW, molecular weight.

^d The gp names in this column refer to *E. coli* phage N4 nomenclature. NH, No Homologous protein in N4; NI, the homologous protein exists, but it was Not Identified in N4 virions ³; Y, Yes, the homologous protein was identified in N4 virions ³.

Supplementary Table S2. Bacteria, phages, and plasmids

<i>Bacteria</i>		
Name	Relevant feature	Reference
PAO1	Standard lab strain	4
PADR6	Frame shift mutation in <i>wzy</i>	5
PAER5b	Nonsense mutation in <i>wapH</i>	5
PAER6b	Frame shift mutation in <i>galU</i>	5
PAER10b	Nonsense mutation in <i>algC</i>	5
<i>Bacteriophages</i>		
Name	Relevant features ^a	Reference
DEV		6
DEV Δ53	Deletion 29656-32966; it eliminates gp53	This work
<i>Plasmids</i>		
Name	Relevant features ^a	Reference
pCas3cRh	It expresses the components of Type I-C CRISPR-Cas system	7
pCas3-01	pCas3cRh derivative carrying DEV 31196-31229 region; it expresses cr-RNA53 targeting gp53	This work
pCas3-09	pCas3-01 derivative carrying the two gp53 flanking regions with coordinates 29171-29655 and 32967-33450	This work
pD53	pGM2148 derivative with 9 silent point mutations in the 31198-31230 region targeted by cr-RNA53	This work
pGM931	Shuttle vector carrying <i>araC-araBp</i> region	8
pGM2148	pGM931 derivative carrying the gp53-encoding 29656-32949 region	This work
gp72_pET 30b(+)	untagged gp72 cloned in pET30b(+) between NdeI and XhoI	This work
gp73_pET 22b(+)	gp73 cloned in pET22b(+) between NdeI and XhoI comprising C-terminal 6x his tag	This work

^a Coordinates refer to Genbank MF490238.1

Supplementary Table S3. Oligonucleotides used in this study

Name	Sequence^a	Coordinates^b
3697	GAAACGTGCCCTGCATGGAGTTGAACAGCACTACCTGAGG	31195-31229
3698	GCGACCTCAGGTAGTGCTGTTCAACTCCATGCAGGGCACG	31229-31195
3804	CTCTCTACTGTTTCTCCATGGTACACGGTACACTGCACGCTC	32966-32949
3805	GACTCTAGAGGATCCCCGGGTACTTATTTCCACCGTCCTACTG CG	29656-29677
3806	CCCTCATGGTTTCATGGAC	33450-33432
3807	TTATTCGATGGCCAACAAAGG	32967-32987
3808	CTTTGTTGGCCATCGAATAATGCAATTCATCCTCAAGCCTG	32987-32967 29654-29635
3809	GTGAGGTCAGCTGTCTCC	29171-29188
3840	CGGGGATTCCCTTAAGGTACCCTCATGGTTTCATGGAC	33450-33432
3841	GTTATGCAGCGGAAAGTAGTGAGGTCAGCTGTCTCC	29171-29188
3859	GAAGAGGACAACCTTGTGCACCGACAGCATGAAGC	31230-31198
3955	AATTCGATGCAAGGGACGAAGGCGTACAACGATG	31212-31179
3969	CGTCACCAGCAGCAGTCTG	54401-54419
3970	TTCGGAGTCGAGTTGGATACTTC	56827-56805
Gp72-f	CGCTATGGCATATGAACGAAGAACACGCTATTC	
Gp72-r	CGCTATCTCGAGTTACTCTTCATCCTCCTCTTC	
Gp73-f	CGCTATGGCATATGGCCTATCCGTACAGTGATATG	
Gp73-r	CGCTATCTCGAGGCGAATCCGGTTCCGTTCC	

^aDEV sequences are in italics. Silent mutations in 3859 and 3955 are in yellow.

^bCoordinates refer to GenBank MF490238.1

Supplementary Table S4. Residues involved in the intermolecular interactions between gp72-gp73.

Gp72	Residue position (Chain)	Gp73	Residue position (Chain)	Type of interaction	Non-bonded contacts (<4Å)
Gln44	J	Phe64	A	H bond	56
Gly25	J	Ala94	B	H bond	27
Gly25	J	Gln98	B	H bond	
Gly25	K	Ala94	C	H bond	32
Asn50	K	Gln73	C	H bond	
Gln44	K	Phe64	I	H bond	56
Gln44	L	Phe64	B	H bond	38
Gly25	L	Ala94	D	H bond	31
Gly25	M	Ala94	E	H bond	29
Gly25	M	Gln98	E	H bond	
Gln44	M	Phe64	F	H bond	55
Asn50	N	Gln73	F	H bond	25
Gly25	N	Gln98	F	H bond	
	N		G		40
Gly25	O	Ala94	G	H bond	28
Gln44	O	Phe64	H	H bond	56
Gly25	P	Ala94	H	H bond	26
Asn50	P	Gln73	H	H bond	
	P		C		62
Gly25	Q	Ala94	I	H bond	29
Gly25	Q	Gln98	I	H bond	
Asn50	Q	Gln73	I	H bond	
Gln44	Q	Phe64	D	H bond	47
Gly25	R	Gln98	A	H bond	29
Asn50	R	Gln73	A	H bond	
Gln44	R	Phe64	E	H bond	57

Intermolecular interactions between gp72 (chains J-R) and gp73 (chains A-I). The total number of non-bonded contacts (<4Å) between the chains of the gp72 and gp73 are shown in the last column.

Supplementary Table S5. Summary of lipid bilayer experiments with purified DEV ejection proteins at 100 mV in DPhPC membranes.

Sample	Total membranes	Membranes with insertions	Comments
Buffer	6	0	The buffer does not produce any insertion-like events.
gp73	88	17 (a total of 33 insertions)	Insertions observed in the range of 0.3 – 2.6 μg protein; observed pores were heterogeneous.
gp72	7	0	24 μg of protein was tested, which was 10 times greater than gp73.
gp72:gp73	19	1	No insertions, one membrane had 10-20 pA fluctuations of the baseline when 10 μg of protein sample was in the cuvette.

Supplementary Table S6. Names and accession numbers for all *Schitoviridae* genome sequences considered in this study. Accession numbers for gp71/gp50, gp72/gp51 and gp73/gp52 homologs are provided for each genome.

Phage	Genome sequence Accession	GP71 candidate	GP72 candidate	GP73 candidate	Clade*	Exemplar**
Escherichia_phage_vB_EcoM_PD205	GCA_024830925.1	UVK80525.1	UVK80524.1	UVK80523.1	1	
Escherichia_phage_KKP_3715	GCA_030875165.1	WLW40941.1	WLW40940.1	WLW40939.1	1	
Escherichia_phage_vB_EcoP_SP5M	GCA_013426605.1	QLF80667.1	QLF80676.1	QLF80705.1	1	
Escherichia_phage_vB_EcoP-ZQ2	GCA_019095225.1	QWY13177.1	QWY13176.1	QWY13175.1	1	
Escherichia_phage_PD38	GCA_003575305.1	AXY81341.1	AXY81342.1	AXY81343.1	1	
Shigella_virus_Moo19	GCA_020882845.1	UEN68863.1	UEN68864.1	UEN68865.1	1	
Escherichia_phage_PGN829.1	GCA_003575565.1	AXY82599.1	AXY82598.1	AXY82596.1	1	
Escherichia_phage_HK2	GCA_038023635.1	WYA83849.1	WYA83848.1	WYA83847.1	1	
Escherichia_phage_EC1-UPM	GCA_002617245.1	AGC31565.1	AGC31566.1	AGC31567.1	1	
Escherichia_phage_vB_EcoS_Uz-1	GCA_025085925.1	UWJ04322.1	UWJ04319.1	UWJ04318.1	1	
Escherichia_phage_UE-S5a	GCA_036584225.1	WVP99857.1	WVP99856.1	WVP99855.1	1	
Escherichia_phage_ECBP1	GCA_000900235.1	AFR52009.1	AFR52010.1	AFR52011.1	1	
Escherichia_phage_IME11	GCA_000903115.1	AFV29065.1	AFV29064.1	AFV29063.1	1	
Escherichia_phage_N4	GCA_000867865.1	AAO24831.2	AAO24830.1	AAO24829.1	1	Yes
Escherichia_phage_phi_G17	GCA_003307555.1	AWY03381.1	AWY03380.1	AWY03379.1	1	
Escherichia_phage_AlfredRasser	GCA_020892825.1	QXV75793.1	QXV75792.1	QXV75791.1	1	
Escherichia_phage_Bp4	GCA_000922735.2	AJB43741.1	AJB43738.1	AHN83378.1	1	
Escherichia_phage_OLB145	GCA_003723055.1	AYR04234.1	AYR04235.1	AYR04236.1	1	
Escherichia_phage_E20	GCA_027183915.1	WAQ79258.1	WAQ79267.1	WAQ79292.1	1	
Escherichia_phage_vB_Eco_SPSP	GCA_920105895.1	CAH0462296.1	CAH0462297.1	CAH0462298.1	1	
Escherichia_phage_St11Ph5	GCA_002956185.1	ATS92525.1	ATS92526.1	ATS92527.1	1	
Escherichia_phage_U1G	GCA_020475385.1	QXV71896.1	QXV71897.1	QXV71899.1	1	
Escherichia_phage_VEC25	GCA_016103715.1	QPN96319.1	QPN96320.1	QPN96322.1	1	

Escherichia_phage_vB_Eco_F22	GCA_0276179 65.1	WBF79664. 1	WBF79673. 1	WBF79699. 1	1	
Escherichia_phage_vB_EcoP_3HA 13	GCA_0131120 55.1	QDF14948. 1	QDF14949. 1	QDF14950. 1	1	
Escherichia_phage_vB_EcoP_PhA PEC7	GCA_0009242 15.1	AHV82680. 1	AHV82681. 1	AHV82682. 1	1	
Escherichia_phage_PMBT57	GCA_0029583 65.1	AUV59054. 1	AUV59055. 1	AUV59056. 1	1	
Escherichia_phage_vB_EcoP_PhA PEC5	GCA_0009225 15.1	AGV99338. 1	AGV99339. 1	AGV99340. 1	1	
Escherichia_phage_vB_Eco_AL25	GCA_9433232 95.1	CAH64218 70.1	CAH64218 71.1	CAH64218 72.1	1	
Escherichia_phage_vB_Eco_Jura	GCA_9052196 75.1	CAE64101 47.1	CAE64101 62.1	CAE64101 78.1	1	
Klebsiella_phage_VLCpiP4b	GCA_0250854 55.1	UVX31104. 1	UVX31105. 1	UVX31106. 1	1	
Klebsiella_phage_KP8	GCA_0029974 55.1	AVJ48977.2	AVJ48978.1	AVJ48979.2	1	
Klebsiella_phage_VLCpiP4a	GCA_0250854 25.1	UVX30959. 1	UVX30958. 1	UVX30957. 1	1	
Achromobacter_phage_JWAalpha	GCA_0009147 75.1	AHC94021. 1	AHC94022. 1	AHC94023. 1	1	
Achromobacter_phage_vB_AxyP_ 19-32_Axy04	GCA_0069643 25.1	QDH83729 .1	QDH83737 .1	QDH83760 .1	1	
Achromobacter_phage_JWDelta	GCA_0026041 85.1	AHC56581. 1	AHC56582. 1	AHC56583. 1	1	
Achromobacter_phage_phiAxp-3	GCA_0022110 55.1	ALA45523. 1	ALA45524. 1	ALA45525. 1	1	
Achromobacter_phage_vB_AxyP_ 19-32_Axy10	GCA_0069643 85.1	QDH83913 .1	QDH83920 .1	QDH83946 .1	1	
Achromobacter_phage_vB_AxyP_ 19-32_Axy13	GCA_0069644 45.1	QDH84159 .1	QDH84166 .1	QDH84191 .1	1	
Achromobacter_phage_vB_AxyP_ 19-32_Axy24	GCA_0069646 25.1	QDH84720 .1	QDH84728 .1	QDH84752 .1	1	
Achromobacter_phage_vB_AxyP_ 19-32_Axy11	GCA_0069644 05.1	QDH83996 .1	QDH84004 .1	QDH84030 .1	1	
Achromobacter_phage_vB_AxyP_ 19-32_Axy12	GCA_0069644 25.1	QDH84077 .1	QDH84084 .1	QDH84109 .1	1	
Achromobacter_phage_vB_AxyP_ 19-32_Axy22	GCA_0069645 85.1	QDH84592 .1	QDH84599 .1	QDH84626 .1	1	
Pseudomonas_phage_inbricus	GCA_0029571 55.1	ATW58114. 1	ATW58115. 1	ATW58116. 1	1	
Pseudomonas_phage_Zuri	GCA_0083754 35.2	QEM41168. 1	QEM41169. 1	QEM41170. 1	1	
Erwinia_phage_vB_EamP_Rexella	GCA_0027576 95.1	ANJ65299. 1	ANJ65300. 1	ANJ65301. 1	1	
Erwinia_phage_vB_EamP_Frozen	GCA_0022110 75.1	ANJ65200. 1	ANJ65201. 1	ANJ65202. 1	1	
Erwinia_phage_vB_EamP_Gutmei ster	GCA_0027577 15.1	ANJ65375. 1	ANJ65376. 1	ANJ65377. 2	1	
Erwinia_phage_Ea9-2	GCA_0009172 95.1	AHI60130. 1	AHI60131. 1	AHI60132. 1	1	
Erwinia_phage_Kuerle	GCA_0278864 55.1	WBQ32526 .1	WBQ32527 .1	WBQ32528 .1	1	

Erwinia_phage_Fifi067	GCA_0268982 95.1	WAK44948. 1	WAK44947. 1	WAK44946. 1	1	
Stenotrophomonas_phage_Piffle	GCA_0204843 05.1	QYW01922 .1	QYW01923 .1	QYW01924 .1	1	
Stenotrophomonas_phage_Paxi	GCA_0204841 55.1	QYW01833 .1	QYW01834 .1	QYW01835 .1	1	
Xanthomonas_virus_PB119	GCA_0329181 95.1	UZV39846. 1	UZV39847. 1	UZV39848. 1	1	
Xanthomonas_phage_RiverRider	GCA_0030141 95.2	AVO23151. 1	AVO23152. 1	AVO23153. 1	1	
Stenotrophomonas_phage_Pokke n	GCA_0082153 05.1	QEG09290. 1	QEG09291. 1	QEG09292. 1	1	
Podoviridae_sp._ctda_1	GCA_0036575 85.1	AXH72064. 1	AXH72079. 1	AXH72022. 1	1	
Stenotrophomonas_phage_Philip pe	GCA_0204842 75.1	QYW02270 .1	QYW02271 .1	QYW02272 .1	1	
Stenotrophomonas_phage_C121	GCA_0222136 15.1	UKL14807. 1	UKL14808. 1	UKL14809. 1	1	
Alcaligenes_phage_vB_Af_QDWS 595	GCA_0205231 95.2	UCR75508. 1	UCR75507. 1	UCR75506. 1	1	
Delftia_phage_RG-2014	GCA_0020378 15.1	AIU44322.1	AIU44323.1	AIU44324.1	1	
Burkholderia_phage_vB_BpP_HN 02	GCA_0364294 25.1	WVK89986. 1	WVK89988. 1	WVK89989. 1	1	
Burkholderia_phage_vB_BpP_HN 01	GCA_0232398 75.1	UNI71499. 1	UNI71500. 1	UNI71501. 1	1	
Sinorhizobium_phage_ort11	GCA_0086056 85.1	QEP29865. 1	QEP29866. 1	QEP29867. 1	2	
Rhizobium_phage_RHph_I1_6	GCA_0168356 25.1	QIG76594. 1	QIG76595. 1	QIG76596. 1	2	
Rhizobium_phage_RHph_Y2_6	GCA_0168353 75.1	QIG68811. 1	QIG68812. 1	QIG68813. 1	2	
Rhizobium_phage_RHEph16	GCA_0204920 45.1	QXV74378. 1	QXV74379. 1	QXV74380. 1	2	
Rhizobium_phage_RHph_X3_15	GCA_0204920 15.1	QWY83756 .1	QWY83757 .1	QWY83758 .1	2	
Rhizobium_phage_RHEph24	GCA_0204920 85.1	QXV74841. 1	QXV74842. 1	QXV74843. 1	2	
Rhizobium_phage_RHph_N38	GCA_0168358 45.1	QIG70535. 1	QIG70536. 1	QIG70537. 1	2	
Rhizobium_phage_RHEph22	GCA_0204920 75.1	QXV74747. 1	QXV74748. 1	QXV74749. 1	2	
Rhizobium_phage_RHph_X2_28B	GCA_0204920 05.1	QWY83520 .1	QWY83521 .1	QWY83522 .1	2	
Rhizobium_phage_RHph_Y38	GCA_0168361 55.1	QIG67777. 1	QIG67778. 1	QIG67779. 1	2	Yes
Agrobacterium_phage_OLIVR2	GCA_0179035 05.1	QIW87388. 1	QIW87389. 1	QIW87390. 1	2	
Agrobacterium_phage_OLIVR3	GCA_0179035 15.1	QIW87495. 1	QIW87496. 1	QIW87497. 1	2	
Agrobacterium_phage_OLIVR1	GCA_0179034 85.1	QIW87281. 1	QIW87282. 1	QIW87283. 1	2	
Pseudomonas_phage_ZC08	GCA_0026055 25.1	AMD43541. 1	AMD43540. 1	AMD43539. 1	3	Yes

Pseudomonas_phage_ZC03	GCA_0026055 05.1	AMD43402. 1	AMD43403. 1	AMD43404. 1	3	
Roseovarius_Plymouth_podovirus_1	GCA_0087255 35.1	CBX87992. 1	CBX87993. 1	CBX87994. 1	4	
Dinoroseobacter_phage_DS-1410Ws-06	GCA_0033291 45.1	ANJ20714. 1	ANJ20715. 1	ANJ20716. 1	4	
Roseobacter_phage_RD-1410Ws-07	GCA_0033291 65.1	ANJ20865. 1	ANJ20866. 1	ANJ20867. 1	4	
Dinoroseobacter_phage_DFL12phi1	GCA_0009230 15.1	AHX00983. 1	AHX00982. 1	AHX00981. 1	4	
Ruegeria_phage_vB_RpoP-V21	GCA_0033086 75.1	AWY09017. 1	AWY09018. 1	AWY09019. 1	4	
Ruegeria_phage_vB_RpoP-V13	GCA_0033087 35.1	AWY09411. 1	AWY09412. 1	AWY09413. 1	4	
Ruegeria_phage_vB_RpoP-V14	GCA_0033693 25.1	AXF42182. 1	AXF42183. 1	AXF42184. 1	4	Yes
Roseovarius_sp._217_phage_1	GCA_0033291 85.1	CBW47056 .1	CBW47057 .1	CBW47058 .1	4	
Dinoroseobacter_phage_vB_DshP-R7L	GCA_0204896 65.1	UAT28905. 1	UAT28906. 1	UAT28907. 1	4	
Ruegeria_phage_vB_RpoP-V17	GCA_0033087 75.1	AWY09578. 1	AWY09579. 1	AWY09580. 1	4	
Silicibacter_phage_DSS3phi2	GCA_0008829 35.1	ACL81328. 1	ACL81329. 1	ACL81330. 1	4	
Ruegeria_phage_vB_RpoP-V12	GCA_0033086 15.1	AWY08849. 1	AWY08850. 1	AWY08851. 1	4	
Dinoroseobacter_phage_vBDshP R2C	GCA_0026045 25.1	AID16877. 1	AID16878. 1	AID16879. 1	4	
Roseobacter_phage_RD-1410W1-01	GCA_0033292 05.1	ANJ20798. 1	ANJ20799. 1	ANJ20800. 1	4	
Sulfitobacter_phage_phiCB2047-B	GCA_0009059 95.1	AGH07392. 1	AGH07390. 1	AGH07389. 1	4	
Erwinia_phage_Pastis	GCA_0333111 55.1	WJN64599. 1	WJN64600. 1	WJN64601. 1	5	Yes
Erwinia_phage_vB_EamP-S6	GCA_0009018 55.1	AEJ81598.1	AEJ81599.1	AEJ81600.1	5	
Salmonella_phage_vB_SenP_UTK 0002	GCA_0288976 15.1	WDR22564 .1	WDR22565 .1	WDR22566 .1	6	
Escherichia_phage_Pollock	GCA_0010421 95.1	AIX12418.1	AIX12419.1	AIX12420.1	6	
Pseudomonas_phage_MY01	GCA_0351350 45.1	WQY99713 .1	WQY99714 .1	WQY99715 .1	6	
Salmonella_phage_FSL_SP-076	GCA_0009095 55.1	AGF88373. 1	AGF88374. 1	AGF88375. 1	6	
Klebsiella_phage_KpCHEMY26	GCA_0079985 55.1	QEA03295. 1	QEA03303. 1	QEA03328. 1	6	
Enterobacter_phage_EcP1	GCA_0009006 55.1	ADU79201. 1	ADU79202. 1	ADU79203. 1	6	
Salmonella_phage_FSL_SP-058	GCA_0009088 95.1	AGF88174. 1	AGF88175. 1	AGF88176. 1	6	
Shigella_phage_B2	GCA_0246061 55.1	UUG68314. 1	UUG68315. 1	UUG68316. 1	6	Yes
Salmonella_phage_vB_SenP_UTK 0001	GCA_0288976 05.1	WDR22471 .1	WDR22472 .1	WDR22473 .1	6	

Salmonella_phage_SP154	GCA_0302219 85.1	WIC41624. 1	WIC41625. 1	WIC41626. 1	6	
Salmonella_phage_vB_SalP_TR2	GCA_0173479 05.1	QSJ04060. 1	QSJ04059. 1	QSJ04058. 1	6	
Pseudomonas_phage_phCDa	GCA_0033414 35.1	AXC36451. 1	AXC36452. 1	AXC36453. 1	7	
Pseudomonas_phage_98PfluR60 PP	GCA_0031433 95.1	AWH15475 .1	AWH15476 .1	AWH15477 .1	7	
Pseudomonas_phage_Littlefix	GCA_0029584 75.1	AUV61826. 1	AUV61827. 1	AUV61828. 1	7	
Pseudomonas_phage_vB_PaeS_T UMS_P6	GCA_0212165 75.1	UGL60893. 1	UGL60892. 1	UGL60891. 1	7	
Pseudomonas_phage_vB_PaeP_Y L2	GCA_0380230 55.1	WMI40382. 1	WMI40381. 1	WMI40380. 1	7	
Pseudomonas_phage_phiPA1-3	GCA_0304395 85.1	WJY90848. 1	WJY90849. 1	WJY90850. 1	7	
Pseudomonas_phage_vB_Pae575 P-3	GCA_0026111 45.1	ANT44349. 1	ANT44350. 1	ANT44351. 1	7	
Pseudomonas_phage_vB_Pae139 6P-5	GCA_0026111 65.1	ANT44439. 1	ANT44440. 1	ANT44441. 1	7	
Pseudomonas_phage_vB_PaeP_P YO2	GCA_0029559 35.1	ASZ72120. 1	ASZ72121. 1	ASZ72122. 1	7	
Pseudomonas_phage_vB_PaeS_T UMS_P81	GCA_0212165 85.1	UGL60979. 1	UGL60978. 1	UGL60977. 1	7	
Pseudomonas_phage_vB_PaeP_4 029	GCA_0257673 25.1	UYE96391. 1	UYE96483. 1	UYE96482. 1	7	
Pseudomonas_phage_YH30	GCA_0015513 85.1	AKC04779. 1	AKC04777. 1	AKC04776. 1	7	
Pseudomonas_phage_LP14	GCA_0033073 95.1	AWY02724. 1	AWY02723. 1	AWY02722. 1	7	
Pseudomonas_phage_PAP02	GCA_0225164 25.1	QKE55114. 1	QKE55115. 1	QKE55116. 1	7	
Pseudomonas_phage_Pa2	GCA_0010409 95.1	AIZ94913.1	AIZ94914.1	AIZ94915.1	7	
Pseudomonas_phage_phi176	GCA_0027564 15.1	AIZ95005.1	AIZ95006.1	AIZ95007.1	7	
Pseudomonas_phage_PASB7	GCA_0348576 65.1	WNV46167 .1	WNV46168 .1	WNV46169 .1	7	
Pseudomonas_phage_PEV2	GCA_0017453 75.1	ANJ63774. 1	ANJ63775. 1	ANJ63776. 1	7	
Pseudomonas_phage_vB_Pae- PA14	GCA_0302946 85.1	QWT71827 .1	QWT71828 .1	QWT71829 .1	7	
Pseudomonas_phage_KPP21	GCA_0015015 35.1	BAR94673. 1	BAR94561. 1	BAR94562. 1	7	
Pseudomonas_phage_PaVOB	GCA_0299483 65.1	WGN90647 .1	WGN90650 .1	WGN90676 .1	7	
Pseudomonas_phage_vB_PaeP_Y L1	GCA_0380230 45.1	WMI40290. 1	WMI40291. 1	WMI40292. 1	7	
Pseudomonas_phage_VB_PaeP_V L1	GCA_0213546 65.1	UGV19870. 1	UGV19871. 1	UGV19873. 1	7	
Pseudomonas_phage_PWJ	GCA_0316783 05.1	WMX17950 .1	WMX17951 .1	WMX17952 .1	7	
Pseudomonas_phage_Ka4	GCA_0364949 45.1	WVM05154 .1	WVM05155 .1	WVM05156 .1	7	

Pseudomonas_phage_LIT1	GCA_0008846 15.1	CAZ66327. 1	CAZ66328. 1	CAZ66329. 1	7	
Pseudomonas_phage_vB_PaeP_T UMS_P121	GCA_0209066 75.1	UEP18653. 1	UEP18652. 1	UEP18651. 1	7	
Pseudomonas_phage_vB_PaeP_4 034	GCA_0257673 65.1	UYE96669. 1	UYE96670. 1	UYE96671. 1	7	
Pseudomonas_phage_vB_PaeP_F MD5	GCA_0371983 75.1	WWY65178 .1	WWY65177 .1	WWY65176 .1	7	
Pseudomonas_phage_vB_Pae_A M.P2	GCA_0161176 75.1	QKE55967. 1	QKE55968. 1	QKE55969. 1	7	
Pseudomonas_phage_LUZ7	GCA_0008862 35.1	CAZ66214. 1	CAZ66215. 1	CAZ66216. 1	7	
Pseudomonas_phage_vB_PaeP_P S28	GCA_0303785 35.1	WBM84633 .1	WBM84725 .1	WBM84724 .1	7	
Pseudomonas_phage_PA26	GCA_0026172 65.1	AFO70568. 1	AFO70569. 1	AFO70570. 1	7	
Pseudomonas_phage_vB_PaeP_D EV	GCA_0029559 55.1	ASZ72278. 1	ASZ72279. 1	ASZ72280. 1	7	Yes
Pseudomonas_phage_CMS1	GCA_0228183 75.1	UNY40777. 1	UNY40778. 1	UNY40779. 1	7	
Pseudomonas_phage_vB_PaeP_ MAG4	GCA_0017443 75.1	AKH49516. 1	AKH49517. 1	AKH49518. 1	7	
Pseudomonas_phage_vB_PaeP_T UMS_P10	GCA_0226945 65.1	UNI71975. 1	UNI71974. 1	UNI71973. 1	7	
Pseudomonas_phage_PJNP053	GCA_0368521 85.1	WVX91144. 1	WVX91145. 1	WVX91146. 1	7	
Pseudomonas_phage_PA15	GCA_0222137 05.1	WJZ49002. 1	WJZ49003. 1	WJZ49004. 1	7	
Pseudomonas_phage_vB_PaeP_4 032	GCA_0257673 55.1	UYE96582. 1	UYE96484. 1	UYE96485. 1	7	
Pseudomonas_phage_YH6	GCA_0010414 35.1	AIX13227.1	AIX13228.1	AIX13229.1	7	
Pseudomonas_phage_Ka3	GCA_0351350 25.1	WQZ52371 .1	WQZ52372 .1	WQZ52373 .1	7	
Pseudomonas_phage_vB_PaeP_C 2-10_Ab09	GCA_0009182 75.1	CDN96879. 1	CDN96880. 1	CDN96881. 1	7	
Pseudomonas_phage_DL64	GCA_0015023 75.1	AKF13985. 1	AKF13984. 1	AKF13983. 1	7	
Pseudomonas_phage_vB_Pae_H MKU_23	GCA_0356808 35.1	WRN92285 .1	WRN92284 .1	WRN92283 .1	7	
Pseudomonas_phage_RWG	GCA_0027563 95.1	AIZ94822.1	AIZ94823.1	AIZ94824.1	7	
Pseudomonas_phage_vB_PaeP_F BPa1	GCA_0248308 25.1	UVN14444. 1	UVN14445. 1	UVN14446. 1	7	
Pseudomonas_phage_L15	GCA_0324604 75.1	WNV49457 .1	WNV49456 .1	WNV49455 .1	7	
Pseudomonas_phage_vB_Pae_TU MS_P11	GCA_0345134 85.1	WQA18395 .1	WQA18394 .1	WQA18393 .1	7	
Pseudomonas_phage_vB_PaP_H N01	GCA_0364188 05.1	WVH07442 .1	WVH07441 .1	WVH07440 .1	7	
Klebsiella_phage_VB_KpP_HS106	GCA_0268983 45.1	WAK45185. 1	WAK45186. 1	WAK45187. 1	8	
Klebsiella_phage_vB_KpP_FBKp2 7	GCA_0168114 75.1	QQV91599. 1	QQV91616. 1	QQV91642. 1	8	

Klebsiella_phage_vB_KpnP_P184	GCA_0173478 15.2	QSJ03719. 1	QSJ03718. 1	QSJ03717. 1	8	
Klebsiella_phage_pKP-BM327-1.1	GCA_0262731 75.1	UZN24491. 1	UZN24492. 1	UZN24493. 1	8	
Kosakonia_phage_Kc283	GCA_0210293 85.1	QYN79871. 1	QYN79872. 1	QYN79873. 1	8	
Pectobacterium_phage_vB_PatP_CB3	GCA_0029899 35.1	ARB11906. 1	ARB11907. 1	ARB11908. 1	8	
Pectobacterium_phage_Horatius	GCA_0157671 85.1	QPL11026. 1	QPL11027. 1	QPL11028. 1	8	
Pectobacterium_phage_Possum	GCA_0157671 95.1	QPL10924. 1	QPL10925. 1	QPL10926. 1	8	
Pectobacterium_phage_vB_PatP_CB1	GCA_0029898 95.1	ARB11804. 1	ARB11805. 1	ARB11806. 1	8	
Pectobacterium_phage_Neptra	GCA_0030943 15.1	AWD92544 .1	AWD92543 .1	AWD92542 .1	8	Yes
Pectobacterium_phage_vB_PatP_CB4	GCA_0029899 55.1	AQT27923. 1	AQT27924. 1	AQT27925. 1	8	
Acinetobacter_phage_Presley	GCA_0009173 35.1	AGY48146. 1	AGY48147. 1	AGY48148. 1	9	Yes
Acinetobacter_phage_nACB1	GCA_0275745 35.1	WAW11592 .1	WAW11599 .1	WAW11633 .1	9	
Vibrio_phage_1.025.O._10N.222.46.B6	GCA_0039264 95.1	AUR82509. 1	AUR82508. 1	AUR82507. 1	10	
Vibrio_phage_vB_VspP_pVa5	GCA_0026150 85.1	APC46019. 1	APC46042. 1	APC46051. 1	10	
Vibrio_phage_1.152.O._10N.222.46.E1	GCA_0039285 75.1	AUR90932. 1	AUR90931. 1	AUR90930. 1	10	
Vibrio_phage_2.130.O._10N.222.46.C2	GCA_0039313 75.1	AUS02400. 1	AUS02399. 1	AUS02398. 1	10	
Vibrio_phage_1.026.O._10N.222.49.C7	GCA_0039265 15.1	AUR82617. 1	AUR82616. 1	AUR82615. 1	10	Yes
Vibrio_phage_1.150.O._10N.222.46.A6	GCA_0039285 35.1	AUR90759. 1	AUR90758. 1	AUR90757. 1	10	
Vibrio_phage_VBP47	GCA_0009044 75.1	AGH57069. 1	AGH57070. 1	AGH57071. 1	10	
Vibrio_phage_VBP32	GCA_0009069 55.1	AGH57223. 1	AGH57222. 1	AGH57221. 1	10	
Vibrio_phage_phi50-12	GCA_0093883 65.1	QFR59803. 1	QFR59802. 1	QFR59801. 1	11	Yes
Vibrio_phage_BUCT194	GCA_0204755 25.1	UAW01149 .1	UAW01150 .1	UAW01151 .1	12	
Vibrio_phage_PhilmVa-1	GCA_0292697 15.1	UOX40291. 1	UOX40292. 1	UOX40293. 1	12	Yes
Vibrio_phage_pVco-5	GCA_0026207 25.1	ARM71100. 1	ARM71101. 1	ARM71102. 1	13	
Vibrio_phage_12VC501	GCA_0257877 95.1	UYF10881. 1	UYF10880. 1	UYF10879. 1	13	
Vibrio_phage_VCO139	GCA_0026031 65.1	AGI61882. 1	AGK85679. 1	AGI61883. 1	13	
Vibrio_phage_JSf3	GCA_0026156 85.1	APD18049. 1	APD18048. 1	APD18047. 1	13	Yes
Vibrio_phage_JA-1	GCA_0009087 55.1	AGI61805. 1	AGI61806. 1	AGI61807. 1	13	

Vibrio_phage_1.188.C._10N.286. 51.A6	GCA_0039292 15.1	AUR93794. 1	AUR93793. 1	AUR93792. 1	14	Yes
Vibrio_phage_1.188.B._10N.286. 51.A6	GCA_0039291 95.1	AUR93708. 1	AUR93707. 1	AUR93706. 1	14	
Vibrio_phage_1.169.O._10N.261. 52.B1	GCA_0039288 35.1	AUR92093. 1	AUR92094. 1	AUR92095. 1	14	
Vibrio_phage_1.188.A._10N.286. 51.A6	GCA_0039291 75.1	AUR93622. 1	AUR93621. 1	AUR93620. 1	14	
Vibrio_phage_1.261.O._10N.286. 51.A7	GCA_0039305 55.1	AUR99055. 1	AUR99054. 1	AUR99053. 1	14	
Vibrio_phage_1.224.A._10N.261. 48.B1	GCA_0039298 75.1	AUR96421. 1	AUR96420. 1	AUR96420. 1	14	

* Refers to the clade to which the gp71/gp50 homolog was assigned.

** Sequences used as “exemplars” are indicated.

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