Figure S1 *[Related to Figure 1]*

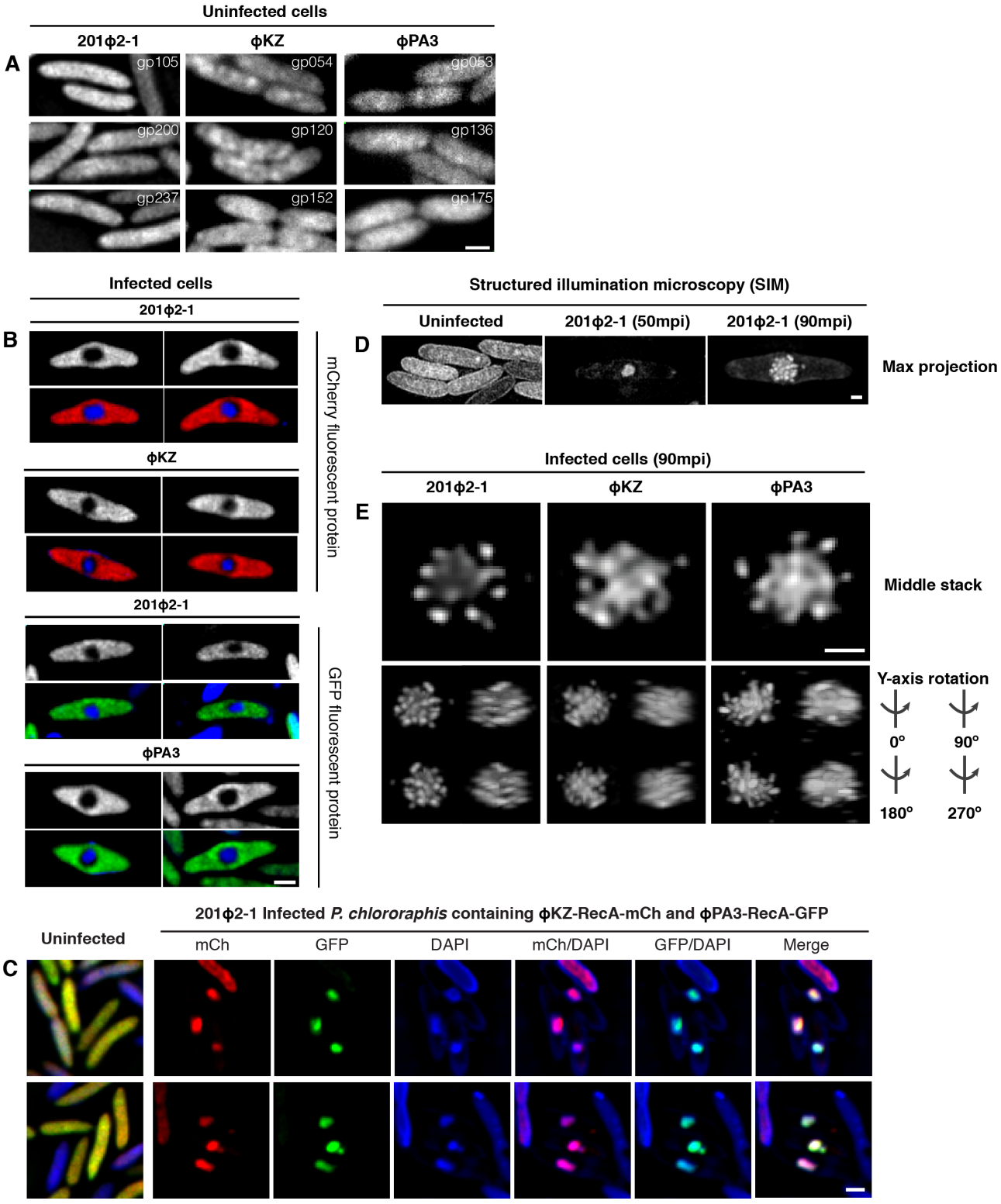


Figure S1: (A) The compartment homologs (gp105 of 201Φ2-1, gp054 of ΦKZ, and gp053 of ΦPA3), major capsid homologs (gp200 of 201Φ2-1, gp120 of ΦKZ, and gp136 of ΦPA3), and RecA-related proteins (gp237 of 201Φ2-1, gp152 of ΦKZ, and gp175 of ΦPA3) do not assemble any specific structure in

the absence of phage infection. The cells were grown on agarose pads and were induced by arabinose to express fluorescent protein fusions. Cover slips were put on right before the microcopy and images were then collected. Scale bar equals 1 micron. Arabinose concentrations are indicated in the related Figure 1. **(B)** The fluorescent proteins (GFP and mCherry) are excluded from the phage nucleus during the phage infection. The cells were grown on agarose pads and were induced by arabinose to express fluorescent proteins. High titer phage lysates were added to infect their corresponding hosts (*P. chlororaphis and P. aeruginosa*). Cover slips were put on the pad before microcopy and images were then collected at 60 mpi. Phage nucleoid is stained blue by DAPI. Scale bar equals 1 micron. Arabinose concentrations are indicated in the related Figure 1. **(C)** Fluorescent images showing co-localization of ΦKZ-gp152-mCherry (red) and ΦPA3-gp175-GFP (green) in the 201Φ2-1 nucleus (stained blue by DAPI). Examples of uninfected cells are shown. The *P. chlororaphis* cells were grown on an agarose pad for 3 hours at 30°C and were induced by 0.1% arabinose. In the absence of infection, ΦKZ-gp152-mCherry (red) and ΦPA3-gp175-GFP (green) are uniformly distributed. Upon infection at 60 mpi, both ΦKZ-gp152-mCherry (Red) and ΦPA3-gp175-GFP (Green) localize together with 201Φ2-1 DNA (blue). Scale bar equals 1 micron. **(D-E)** Structured illumination microscopy (SIM) images showing the maximum projection of cells from uninfected and infected *P. chlororaphis* cells at 50 and 90 mpi. **(E)** SIM images of *P. chlororaphis* and *P. aeruginosa* infected with corresponding phages (201Φ2-1, ΦKZ, and ΦPA3) at 90 mpi. Degree of rotation of the phage nucleus around Y-axis is indicated on the right for each subset. The *Pseudomonas* cells were grown on an agarose pad and the infection was started when the high-titer phage lysates were added. At the indicated time point, the cells were fixed and DNA was stained by DAPI (gray). Scale bar equals 0.5 micron. Related to Figure 1.

Figure S2 *[Related to Figure 2]*

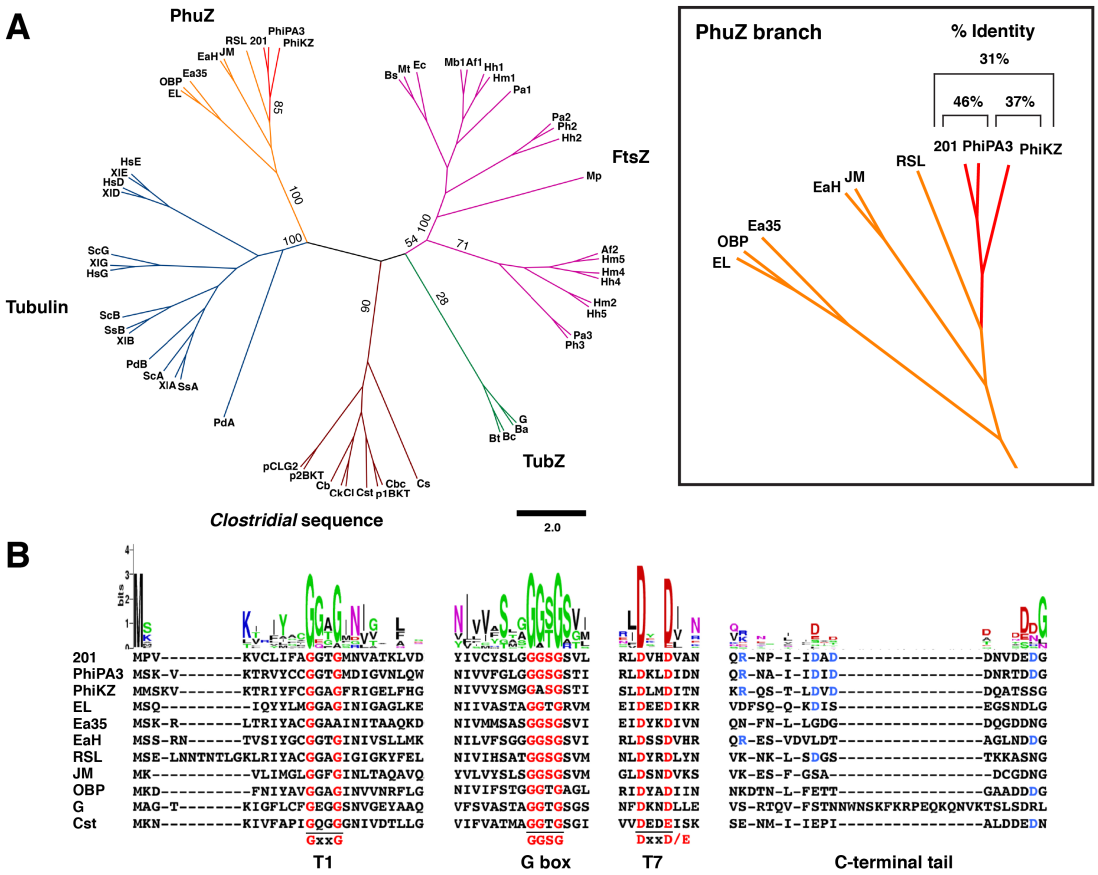


Figure S2: The PhuZ proteins from 201Φ2-1, ΦKZ, and ΦPA3 show relatively high conservation in essential domains. Related to Figure 2.

A) Phylogenetic tree showing the relationships between major families of eukaryotic tubulin and prokaryotic tubulin-like proteins (PhuZ, FtsZ, TubZ, and *Clostridial* proteins). Selected bootstrap values are shown at major points. Amino acid identity of $PhuZ_{201}$, $PhuZ_{\Phi KZ}$ and $PhuZ_{\Phi PA3}$ is shown in the inset.

B) Sequence alignment of conserved tubulin domains across the bacteriophage tubulins showing conserved residues of the T1 loop, signature motif (G box), and the T7 catalytic loop which is involved in GTP hydrolysis. The C-terminal tail shows high divergence among phage tubulins, but arginine and aspartic residues in this domain are most conserved in 201Φ2-1, ΦKZ, and ΦPA3 (shown in blue).

Figure S3 *[Related to Figures 2 and 3]*

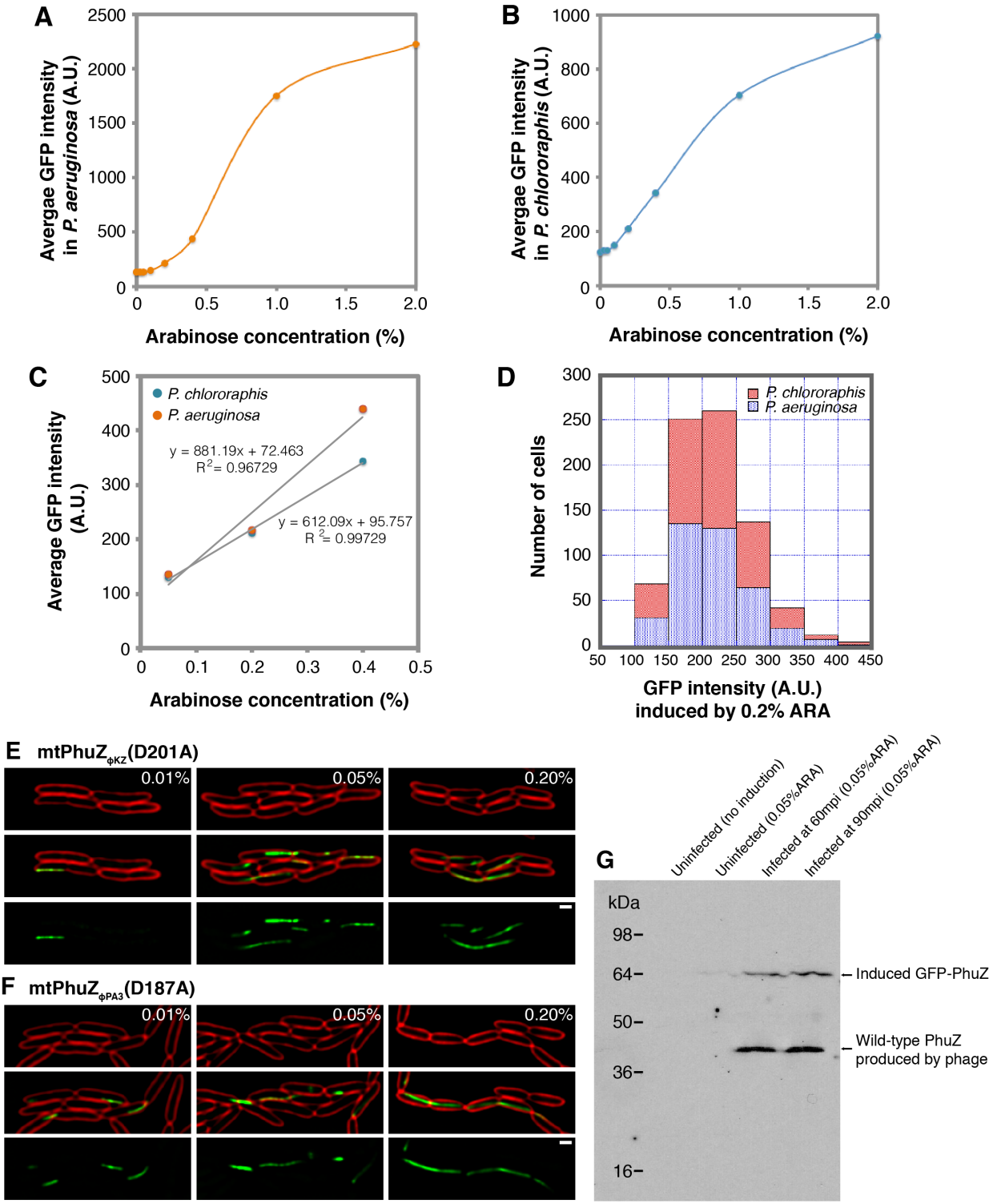
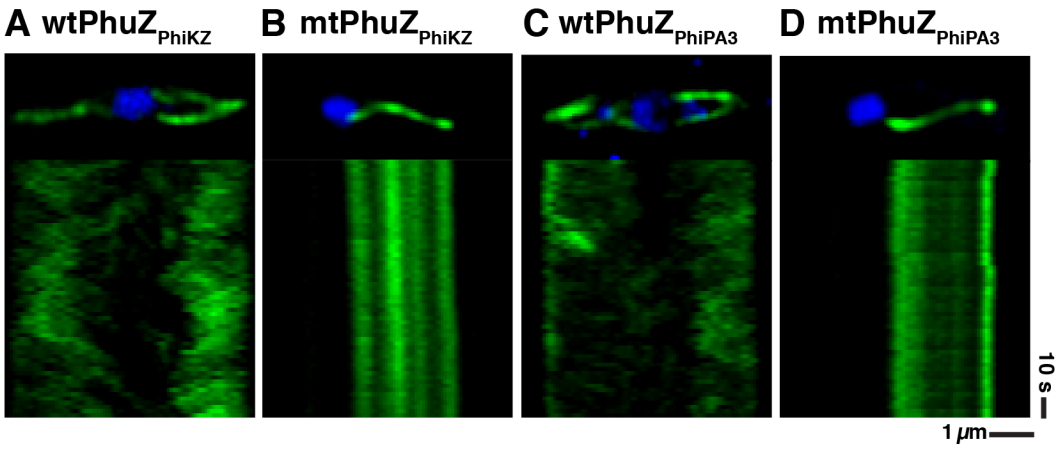


Figure S3: (A-D) Line graphs presenting average intensity of GFP induced at various concentrations of arabinose from the arabinose promoter in pHERD30T in *P. aeruginosa* **(A)** and *P. chlororaphis* **(B)**. The

average intensity of GFP expressed in the *P. aeruginosa* and *P. chlororaphis* cells linearly increased (R^2 close to 0.99) in the range of 0.05% to 0.4% arabinose **(C)**, and the proteins are uniformly expressed throughout the cell population, with less than a 2 fold variation from the mean for 99% of cells in the population **(D)**. Data represented as mean were collected from the induced cells at indicated arabinose concentration from at least 3 different fields with at least 100 cells per field. Related to Figure 2. **(E-F)** Additional fluorescence data on the second GTPase mutant GFP-$PhuZ_{\Phi KZ}$D201A **(E)** and GFP-$PhuZ_{\Phi PA3}$D187A **(F)**. Cells were grown on agarose pads and the fusion proteins were induced at the indicated arabinose concentrations. Cell membranes were stained red by FM 4-64. All scale bars equal 1 micron. Related to Figure 2. **(G)** Western blot analysis of total $PhuZ_{\Phi PA3}$ in *P. aeruginosa* cells. The *P. aeruginosa* cells containing GFP-$PhuZ_{\Phi PA3}$ under an arabinose promoter in pHERD30T were grown on agarose pads in 60 mm-petri dishes supplemented with either 0.2% glucose for the no induction control or 0.05% arabinose for induction and the cells were harvested at 4 hours. For the infected cells, a high-titer lysate of phage ΦPA3 was added to the cells and they were later collected at 60 and 90 mpi. SDS-PAGE and western blotting were carried out and both GFP-PhuZ and untagged PhuZ detected using anti-$PhuZ_{201}$ antibodies, which cross react with $PhuZ_{\Phi PA3}$. GFP-$PhuZ_{\Phi PA3}$ induced from a plasmid with 0.05% was expressed at lower levels than the wild-type protein and represents less than 1/3 of the total PhuZ protein produced. GFP-PhuZ expressed from the plasmid was detected at higher levels in cells infected with phage than in uninfected cells. Related to Figure 3.

Figure S4 *[Related to Figure 3]*



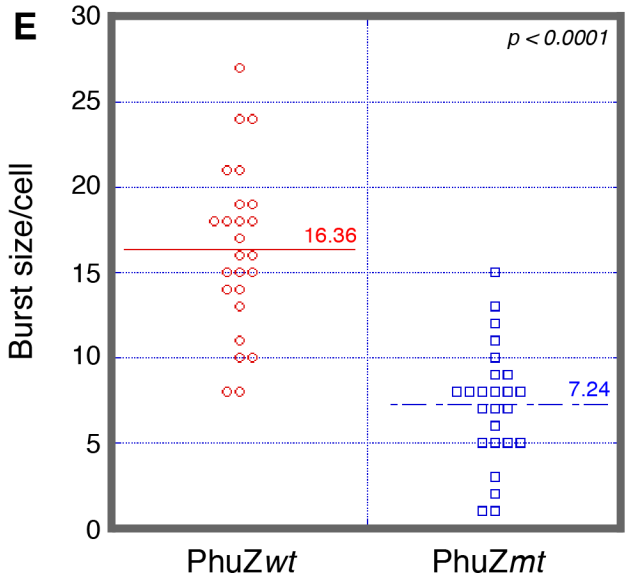


Figure S4: (A-D) Kymographs showing dynamically unstable filaments of wild-type GFP-PhuZ$_{\Phi KZ}$ **(A)** and wild-type GFP-PhuZ$_{\Phi PA3}$ **(C)** and completely static filaments of mutant GFP-PhuZ$_{\Phi KZ}$ **(B)** and mutant GFP-PhuZ$_{\Phi PA3}$ **(D)**. The kymographs from time-lapse microscopy in which images were collected every 2 seconds are plotted showing the GFP fluorescence intensity of the filaments (as imaged in the top row) for a total of 2 minutes. The phage nucleus was stained by DAPI (blue). **(E)** Phage burst size analysis from a single-cell infection assay (Kraemer, *et.al.* 2012). As reported in Kraemer, JA. et al., 2012, the graph shows that the average number of phage particles produced by the cells expressing wild-type PhuZ was 16.36 phage per cell (n=25), which was significantly higher *($p < 0.0001$)* than the average burst size of 7.24 of cells expressing mutant PhuZ (n = 25). Related to Figure 3.

Table S1: List of plasmids and strains used in this study. Related to Experimental procedures

Insert	**Backbone**	**Host**	***Plasmid***	***Strain***
GFP-201-PhuZ (gp059)	pHERD30T	PC	pME028	ME041
GFP-ΦKZ-PhuZ (gp039)	pHERD30T	PA01	pVC029	VC072
GFP-ΦPA3-PhuZ (gp028)	pHERD30T	PA01	pME056	ME090
GFP-ΦKZ-PhuZ (gp039) -D201A	pHERD30T	PA01	pVC033	VC242
GFP-ΦKZ-PhuZ (gp039) -D204A	pHERD30T	PA01	pVC034	VC243
GFP-ΦPA3-PhuZ (gp028) -D187A	pHERD30T	PA01	pVC031	VC245
GFP-ΦPA3-PhuZ (gp028) -D190A	pHERD30T	PA01	pVC032	VC247
GFP-201-compartment (gp105)	pHERD30T	PC	pJC001	JC002
mCh-ΦKZ-compartment (gp054)	pHERD30T	PA01	pMAC011	MAC023
GFP-ΦPA3-compartment (gp053)	pHERD30T	PA01	pVC077	VC414
201-major capsid-GFP (gp200)	pHERD30T	PC	pVC007	VC125
ΦKZ-major capsid-mCh (gp120)	pHERD30T	PA01	pMAC039	MAC043
ΦPA3-major capsid-GFP (gp136)	pHERD30T	PA01	pVC087	VC417
201-RecA-GFP (gp237)	pHERD30T	PC	pVC008	VC127
ΦKZ-RecA-mCh (gp152)	pHERD30T	PA01	pMAC016	MAC021
ΦPA3-RecA-GFP (gp175)	pHERD30T	PA01	pVC089	VC421
ΦPA3-RecA-GFP-ΦKZ-RecA-mCh	pHERD30T	PC	pMAC032	MAC036

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmid Constructions and Bacterial Transformation

The stop codons at the end of the *gfp* were deleted from pME27 (Kraemer et al., 2012) and this new plasmid, pME54, was used to clone in the phuZ genes from the two different *P. aeruginosa* phage. ΦKZ *gp39* was amplified from the phage ΦKZ genome and ΦPA3 *gp28* was amplified from the phage ΦPA3 genome. Each PCR products (gp28 and gp39) and pME54 were digested with HindIII and ligated together. The phage genes encoding the compartment protein (*gp105* from 201Φ2-1, *gp054* from ΦKZ, and *gp053* from ΦPA3), major capsid protein (*gp200* from 201Φ2-1, *gp120* from ΦKZ, and *gp136* from ΦPA3), and RecA-like protein (*gp237* from 201Φ2-1, *gp152* from ΦKZ, and *gp175* from ΦPA3) were PCR-amplified from high-titer phage lysates and were constructed into pHERD30T containing either GFP or mCherry fluorescent proteins by Gibson Assembly® (New England Biolabs), as previously described in Chaikeeratisak et al., 2017.

These resulting recombinant plasmids were transformed into *E.coli* DH5A plated on selective media. DNA sequencing was conducted to confirm the correctness of constructs and they were later electroporated into *Pseudomonas* competent cells (*P. chlororaphis* strain 200-B and *P. aeruginosa* strain PA01) to create strains, as listed in Supplemental table 1. All bacterial cultures were grown at 30°C.

Sequence Analysis and Phylogenetic Tree Construction

The PhuZ sequence from bacteriophage 201Φ2-1 (YP_001956784.1) was used to initiate an iterative blast search against NCBI database. All obtained protein sequences were aligned using TCoffee and ClustalW, and the phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) version 5.2.2., as described by Tamura, K. et al., 2013. A bootstrap consensus tree of 100 trees is shown in Figure S2A with the selected bootstrap numbers on selected branches. Similar grouping of Phage tubulin/FtsZ (PhuZ) was obtained regardless of the alignment methods used.

The tree includes the following sequences indicated by their initial names with accession numbers: 201 (YP_001956784.1); ΦPA3 (AEH03455.1); ΦKZ (NP_803605.1); EL (YP_418049.1); Ea35 (YP_009005002.1); EaH (YP_009010087.1); RSL (BAQ02539.1); JM (YP_006383382.1); OBP (YP_004957954.1); G (YP_009015441.1); Ba (NP_052741); Bc (ZP_00236418); Bt (CAD30186); Cs (YP_003936284.1); Cbc (EDS76598.1); Cst (YP_398619.1); Cl (YP_003845715.1); Ck (YP_001393972.1); Cb (EDT76720.1); p1BKT (YP_004385787.1); p2BKT (YP_004397219.1); pCLG2 (YP_003034138.1); Ec (NP_308126); Bs (NP_389412); Mt (NP_216666); Mp (P75464); Af1 (NP_069371); Af2 (NP_070043); Pa1 (Q9V2S0); Pa2 (Q9UZ61); Pa3 (NP_126497); Ph2 (O58491); Ph3 (O59060); Mb1 (ZP_00562770); Hh1 (NP_279457); Hh2 (NP_279324); Hh4 (NP_279378); Hh5 (NP_395771); Hm1 (YP_135405); Hm2 (YP_138195); Hm4 (AAV48016); Hm5 (YP_137684); ScA (NP_013625); ScB (NP_116616); ScG (NP_013313); SsA (P02550); SsB (P02554); PdA (AAO12155); PdB (AAO12159); XlA (P08537); XlB (AAA49977); XlD (AAL27450); XlE (AAN77278); XlG (AAA49720); HsD (Q9UJT1); HsE (Q9UJT0); HsG (NP_001061).

Amino acid sequence analysis of FtsZ-like protein sequences of bacteriophage was further performed in order to investigate tubulin-conserved domains; T1 loop, G box, T7 loop and C-terminal domain. The sequence conservation and relative frequency of each amino acid were represented on the top of the sequence alignment in Figure S2B by WebLogo as described by Crooks, GE. et al., 2004.

SUPPLEMENTAL REFERENCES

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution *30*, 2725–2729.
Crooks, G.E., Hon, G., Chandonia, J.-M., and Brenner, S.E. (2004). WebLogo: A Sequence Logo Generator. Genome Research *14*, 1188–1190.