

### **Supplementary Material**

**Supplementary Table S1.** Strains and plasmids used in this study.

<b>Strains, Phages, or Plasmids</b>	<b>Genotype or Relevant Characteristics</b>	<b>Reference/Source</b>
<i>E. coli</i>		
BW25113(pKD46)	F-, $\Delta(araD-araB)567$ , $\Delta lacZ4787(::rrnB-3)$ , $\lambda$ , <i>rph-1</i> , $\Delta(rhaD-rhaB)568$ , <i>hsdR514</i> , pKD46	The Coli Genetic Stock Center, Yale University (CGSC)
MG1655	<i>lacI<sup>ts</sup>tonA::Tn10</i>	Lab stock
WA921	F-, <i>thr-1</i> , <i>leuB6</i> (Am), <i>fhuA21</i> , <i>lacY1</i> or $\Delta(cod-lacI)6$ , <i>glnX44</i> (AS), $\lambda$ , <i>metB1</i> , <i>thiE1?</i> , <i>hsdS3</i>	CGSC
W3110	F-, $\lambda$ , <i>IN(rrnD-rrnE)1</i> , <i>rph-1</i>	CGSC
WA2379	F-, <i>leuB6</i> (Am), <i>fhuA21</i> , <i>lacY1</i> or $\Delta(cod-lacI)6$ , <i>glnX44</i> (AS), $\lambda$ , <i>metB1</i> , <i>thiE1?</i> , <i>deoB20</i>	CGSC
WA960	F-, <i>leuB6</i> (Am), <i>fhuA21</i> , <i>lacY1</i> or $\Delta(cod-lacI)6$ , <i>glnX44</i> (AS), $\lambda$ , <i>metB1</i> , <i>thiE1?</i> , [ <i>r+B,m+B</i> ]	CGSC
5-alpha competent	Used for its high efficiency transformation	New England Biolabs
<b>Phages</b>		
P1CMclr100	<i>Clr100</i> ts; Chloramphenicol resistant	CGSC
P1 $\Delta$ <i>darB</i>	In-frame deletion of <i>darB</i> in P1CMclr100	Piya et al., 2017
P1 $\Delta$ <i>pmgA</i>	In-frame deletion of <i>pmgA</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgB</i>	In-frame deletion of <i>pmgB</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgC</i>	In-frame deletion of <i>pmgC</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgG</i>	In-frame deletion of <i>pmgG</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgR</i>	In-frame deletion of <i>pmgR</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgF</i>	In-frame deletion of <i>pmgF</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgL</i>	In-frame deletion of <i>pmgL</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgM</i>	In-frame deletion of <i>pmgM</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgN</i>	In-frame deletion of <i>pmgN</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgO</i>	In-frame deletion of <i>pmgO</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgP</i>	In-frame deletion of <i>pmgP</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgQ</i>	In-frame deletion of <i>pmgQ</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgS</i>	In-frame deletion of <i>pmgS</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgT</i>	In-frame deletion of <i>pmgT</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgU</i>	In-frame deletion of <i>pmgU</i> in P1CMclr100	This Work
P1 $\Delta$ <i>pmgV</i>	In-frame deletion of <i>pmgV</i> in P1CMclr100	This Work
P1 $\Delta$ <i>upfA</i>	In-frame deletion of <i>upfA</i> in P1CMclr100	This Work
P1 $\Delta$ <i>upfB</i>	In-frame deletion of <i>upfB</i> in P1CMclr100	This Work
P1 $\Delta$ <i>upfC</i>	In-frame deletion of <i>upfC</i> in P1CMclr100	This Work
P1 $\Delta$ <i>upfM</i>	In-frame deletion of <i>upfM</i> in P1CMclr100	This Work

P1 $\Delta$ <i>upfN</i>	In-frame deletion of <i>upfN</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>upfO</i>	In-frame deletion of <i>upfO</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>pdca</i>	In-frame deletion of <i>pdca</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>pdcb</i>	In-frame deletion of <i>pdcb</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>prt</i>	In-frame deletion of <i>prt</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>pro</i>	In-frame deletion of <i>pro</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>uhr</i>	In-frame deletion of <i>uhr</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>upl</i>	In-frame deletion of <i>upl</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>dbn</i>	In-frame deletion of <i>dbn</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>ppp</i>	In-frame deletion of <i>ppp</i> in P1CM <i>clr</i> 100	This Work
P1 $\Delta$ <i>pap</i>	In-frame deletion of <i>pap</i> in P1CM <i>clr</i> 100	This Work
<b>Plasmids</b>		
pBAD24	Empty cloning vector	Piya et al., 2017
pBAD24g	pBAD24 with gentamicin marker replacing ampicillin marker	This Work
ppmgA	P1 <i>pmgA</i> cloned into pBAD24g	This Work
ppmgB	P1 <i>pmgB</i> cloned into pBAD24g	This Work
ppmgC	P1 <i>pmgC</i> cloned into pBAD24g	This Work
ppmgG	P1 <i>pmgG</i> cloned into pBAD24g	This Work
ppmgR	P1 <i>pmgR</i> cloned into pBAD24g	This Work
pdarB $\Delta$ 1-4	P1 <i>darB</i> missing the first 4 codons cloned into pBAD24	This Work
pdarB $\Delta$ 1-9	P1 <i>darB</i> missing the first 9 codons cloned into pBAD24	This Work
pdarB $\Delta$ 1-14	P1 <i>darB</i> missing the first 14 codons cloned into pBAD24	This Work
pdarB $\Delta$ 1-19	P1 <i>darB</i> missing the first 19 codons cloned into pBAD24	This Work
pdarB $\Delta$ 6-30	P1 <i>darB</i> missing codons 6-30 cloned into pBAD24	This Work
pdarB $\Delta$ 11-30	P1 <i>darB</i> missing codons 11-30 cloned into pBAD24	This Work
pdarB $\Delta$ 16-30	P1 <i>darB</i> missing codons 16-30 cloned into pBAD24	This Work
pdarB $\Delta$ 21-30	P1 <i>darB</i> missing codons 21-30 cloned into pBAD24	
pFTSKi-tetR-mCherry	Template for PCR amplification of <i>tetR-mCherry</i>	(Shao et al., 2017) / Zeng Lab
pBAD24_N30	The first 30 amino acids of <i>darB</i> cloned into pBAD24	This Work

pBAD24_N9	The first 9 amino acids of <i>darB</i> cloned into pBAD24	This Work
pBAD24_N30-tetR-mCherry	mCherry fused to the first 30 amino acids of P1 <i>darB</i> cloned into pBAD24	This Work
pBAD24_N9-tetR-mCherry	mCherry fused to the first 9 amino acids of P1 <i>darB</i> cloned into pBAD24	
pBAD24_tetR-mCherry	pBAD24 expressing tetR-mCherry alone	
pMB838 cassette	source of gentamicin resistance	Addgene / Lab stock

**Supplementary Table S2.** Primers and synthetic DNA fragments

Forward 5'-3'	Reverse 3'-5'	Use
tcaattgatgatccgttgatgaat gactacgcgagagttgattgatgg ccgtgtaggctggagctgcttc	ctgtctttagtcggtgtgttt tactcatagcaccacgtcctgt gtgataatgggaattagccatg gtcc	Knockout <i>pmgA</i> from P1 genome
gcttcccttctgggcttagtttt tctcttgaattaaggagcaagga tggtaggctggagctgcttc	ggaaaaacgcataaggattcagt gagcctttcagcgtcctgaatc ggataatgggaattagccatg gtcc	Knockout <i>pmgB</i> from P1 genome
gtagaatcggttaacacaccagat tctacgaggtttcaatgacaccac gagtgtaggctggagctgcttc	aagcaacattaagccccatatac agccccctcactcaacatgga tgagaaatgggaattagccatg gtcc	Knockout <i>pmgC</i> from P1 genome
ggaactccggccttaacttgaat ggctcctatagcttatggggttta cagtgtaggctggagctgcttc	agtgttattgtgccccatataa aatccttttactggaacgccc aacaatatgggaattagccatg gtcc	Knockout <i>pmgG</i> from P1 genome
atgtacactcatcagctttttat tagagcaatctacaaggtgcacta tggtaggctggagctgcttc	ttgattggtttgtgctgaagt gatcatttcaaagttccgtatt agcttgatgggaattagccatg gtcc	Knockout <i>pmgR</i> from P1 genome
tttttgtgcttaaatcatgtcaa tatagcgaaatttgagcatatta tggtaggctggagctgcttc	gataattccgggagagcgggttg ggtttttcatgcttttctgcg cagagaatgggaattagccatg gtcc	Knockout <i>pmgF</i> from P1 genome
aaatgtatcattctgcccttaagt aggttcttcacgaggaaacaaaat tggtaggctggagctgcttc	aaactctcctcagtaaagcgc aacataatcagtcacaaatcctgc cggtcgatgggaattagccatg gtcc	Knockout <i>pmgL</i> from P1 genome
gcaaaacagcgtaaataatggcatg cgaccggcaggatttgactgatta tggtaggctggagctgcttc	agcgcacatgcactaattaatt tattatttaagcagcatacaa ccacttatgggaattagccatg gtcc	Knockout <i>pmgM</i> from P1 genome
catgtgcgctcttgtgtagtgc actttaacatcgggagaataatcg tggtaggctggagctgcttc	agctgcaaattcagcctccagg cgagcattcattcagcgatta cagggtatgggaattagccatg gtcc	Knockout <i>pmgN</i> from P1 genome

gctgaattgcagctgagaatgaa cataccacccagggcgactaggac tggtaggctggagctgcttc	ctgcctctaaattattcttgg gaaacattgaattgcactgctc ctacttatgggaattagccatg gtcc	Knockout <i>pmgO</i> from P1 genome
gcgagtgccgattatagcggta atggggccatcgactggcttaacc gcgtgtaggctggagctgcttc	gtgggtgctatcatgtattctt cagccattcttaagagtcac tgcggaatgggaattagccatg gtcc	Knockout <i>pmgP</i> from P1 genome
ggaaacctgacattgatgaaagt aaaggtgatggtgcagcatgaaga tggtaggctggagctgcttc	ttaaaaatatggaattattag agcaatattattctgattctcg ctcaaatgggaattagccatg gtcc	Knockout <i>pmgQ</i> from P1 genome
atacttaggaaaaatgaccgaagc acaagctaatacggaaacttgaaa tggtaggctggagctgcttc	caccagcattaaaagtgaact gtaactatcagcgaacgtaaat agtccatgggaattagccatg gtcc	Knockout <i>pmgS</i> from P1 genome
tctctatcacttttggcggcatcg tcgcatggttaagggggatgca tggtaggctggagctgcttc	aatgtccgcatagtgtccagcc tcgtaaccagggttagcact ccagctatgggaattagccatg gtcc	Knockout <i>pmgT</i> from P1 genome
ttattacgccagatcatcataaac aagccgagaaaagtgaactagaaa gggtgtaggctggagctgcttc	agggtttgatgtgatctgcaa ctctcatactcaccctcgctt gtatcgatgggaattagccatg gtcc	Knockout <i>pmgU</i> from P1 genome
ttgccgttggtgatgctggcgat acaagcgagggtgaagtatgagag tggtaggctggagctgcttc	ttctgatgtgctggtgggtac ctgtagttcagcttctgtggc atttaaatgggaattagccatg gtcc	Knockout <i>pmgV</i> from P1 genome
gttaatgattacaaccgagctatt agcggtaactaaaagggatttta tggtaggctggagctgcttc	ccaacgtagccggttgaaggg agtcgtattattctacgcttc gatgagatgggaattagccatg gtcc	Knockout <i>upfA</i> from P1 genome
gagacggcctagttcaggttaagt aggagatatcatgctggaaaaaga ctgttaggctggagctgcttc	tgaagaaaaattatcaatgaag tccttgttactgtgccgctt gtttaaattgggaattagccatg gtcc	Knockout <i>upfB</i> from P1 genome
aatctaagttaaaataacgaaaat cagagcaaatcattggtgatgacg tggtaggctggagctgcttc	cgtctccggtgcaacggtaga gccgcatgaaacaggatcacca acgcatatgggaattagccatg gtcc	Knockout <i>upfC</i> from P1 genome
gatgaggataatagccagaatctg gctaataacaggcgcatctaaaaa tggtaggctggagctgcttc	tgccgtaagctcacgttaacga cttctttcaccgaatccaact atataaatgggaattagccatg gtcc	Knockout <i>upfM</i> from P1 genome
aaaccaacccttatatagttgga ttcggtgaaagaaagtcgttaacg tggtaggctggagctgcttc	acaaagttagcacttgcaaga gggccattttctaaatatttg atgttatgggaattagccatg gtcc	Knockout <i>upfN</i> from P1 genome
gaaacatcacatatttagaaaaat ggccctcttgaagtcataact tggtaggctggagctgcttc	tcccatccagaataattgagta acgactattttaaccagcaa agtaacatgggaattagccatg gtcc	Knockout <i>upfO</i> from P1 genome

tctgtcgccgacacgttacgtaga ttgatgggtctgcggagtagatt aagtgtaggctggagctgcttc	acatcacacctttaactactga ttgggctttatctgctgcccgg cattctatgggaattagccatg gtcc	Knockout <i>pdca</i> from P1 genome
gaatgccgggcagcagataaagcc caatcagtgattaaaggtgtgatg tgggtaggctggagctgcttc	taaagaatatagcaatacattag agcaattttatcaactcga cgaatgatgggaattagccatg gtcc	Knockout <i>pdcb</i> from P1 genome
aaattccgttcaaacacgatgtga attattctaattaaggtgcaatct tgggtaggctggagctgcttc	tcagtgaccgttttcaaaacat cagtcattatcgttccctctt taaagaatgggaattagccatg gtcc	Knockout <i>prt</i> from P1 genome
aaatccttgaatcggtctttaag agggaaacgataatgactgatgtt tgggtaggctggagctgcttc	tttgggtgtatttaaacggat tgattgaattattaaacgtgat gatgctatgggaattagccatg gtcc	Knockout <i>pro</i> from P1 genome
accattcagccatcgccctcaat gggcatttggtagctgcgaga tgggtaggctggagctgcttc	atagtaatgttttccagtgt taaacaatgttaaccctgaa tatcaaatgggaattagccatg gtcc	Knockout <i>uhr</i> from P1 genome
ttcaccgcaacggaaagagcattc ctggtggacctgtagattgggata tgggtaggctggagctgcttc	tgggaatgcacaggctgatgt gccgcaactacagtagtgcgcg ctttgatgggaattagccatg gtcc	Knockout <i>upl</i> from P1 genome
cggtttgataactatgccggtgct gactggcaagaggattattaatgc aagtgtaggctggagctgcttc	ggcttttctgttatgacgggtt caatttttatccgttaccgcg cgacggatgggaattagccatg gtcc	Knockout <i>dbn</i> from P1 genome
ggtattccgcagatgactcttaaa gaatggctgaagaatacatgatag cagtgtaggctggagctgcttc	ccagagcgaagaactaaagcaa tcttcatgctgcaccatcacct ttcactatgggaattagccatg gtcc	Knockout <i>ppp</i> from P1 genome
ggtggagtgcgcccaccagcattt tttctccaatgaggagggcatt tgggtaggctggagctgcttc	gttttaaaaatcaagatttatt agagcaattattgtgatgaag aagcgcatgggaattagccatg gtcc	Knockout <i>pap</i> from P1 genome
atcggacgtcatgttacgcagcag caacgatg	atcggagctcttaggtggcgg acttgggtc	PCR amplify gentamicin resistance cassette from pMB838
atcggagctcctgtcagaccaagt ttactcatatatactttagattg	atcggagctcactcttctttt tcaatattattgaag	PCR amplify pBAD24 backbone
ggggatcctctagaataaaggagg tcagtacatggccaataataacga aattgatcc	aaaacagccaagctttcatagc accacgtcctg	clone <i>pmgA</i> into pBAD24g
ggggatcctctagaataaaggagg gatgctaagtctttacccctttt ccc	aaacagccaagctttcagcgtc ctgaatcgg	clone <i>pmgB</i> into pBAD24g
ggggatcctctagaataaaggagg gatgctaataacaccacgacaatt actc	aaacagccaagctttcacttca acatggatgagaaaag	clone <i>pmgC</i> into pBAD24g
ggggatcctctagaataaaggagg gatgctaattggctcctatagctta tggg	aaacagccaagcttttactgga acgcccga	clone <i>pmgG</i> into pBAD24g

ggggatcctctagaataaaggagg gatgctaattgtggccattccgacg	aaacagccaagctttcaaagtt ccgtattagcttg	clone <i>pmgR</i> into pBAD24g
atcgtctagagtaataaaggaggt atcgagatgtctatgggggtgtt cgctg	atcgaagctttatgcgtattg ttggatgacggc	Truncate the N terminal region of DarB by amino acids 4
atcgtctagagtaataaaggaggt atcgagatgcgctgttcaagtgc agcg	atcgaagctttatgcgtattg ttggatgacggc	Truncate the N terminal region of DarB by amino acids 9
atcgtctagagtaataaaggaggt atcgagatgagcgaaatattgaaa tacattagggc	atcgaagctttatgcgtattg ttggatgacggc	Truncate the N terminal region of DarB by amino acids 14
atcgtctagagtaataaaggaggt atcgagatgtacattagggcaata acatctcaccg	atcgaagctttatgcgtattg ttggatgacggc	Truncate the N terminal region of DarB by amino acids 19
attaaatacggcgtggaaaagg	agatagctgttcatatcgatacct	Internal deletion of amino acids 6-30 of DarB
attaaatacggcgtggaaaagg	gcgaaacacccccataga	Internal deletion of amino acids 11-30 of DarB
attaaatacggcgtggaaaagg	gctgacactgaacagcg	Internal deletion of amino acids 16-30 of DarB
attaaatacggcgtggaaaagg	gtatttcaatatttcgctgacacttg	Internal deletion of amino acids 21-30 of DarB
actctctactgtttctccataccc g	tgtatcaggctgaaaatcttct ctcatc	PCR amplify synthetic DNA fragments designed to introduce N- terminal DarB residues into pBAD24
atcgagaattcgtgtctagattag ataaaagtaaagtattaacagcg	atcgaggtaccttactgtaca gctcgtccatgc	Clone mCherry to pBAD24_ <i>darBN30</i>
Synthetic DNA Fragments 5'-3'		
gacgcttttatcgcaactctctactgtttctccatacccggtttttt gggctagcaggaggttaatacacccatgaacaagctatctatgggggtgt ttcgctgttcaagtgtcagcgaaatattgaaatacattagggcaataa catctcaccgagcgccggaattcaccatggtacccggggatcctctag agtcgacctgcaggcatgcaagctggctgttttgccgatgagagaa gatttcagcctgatacagattaaatc		Synthetic DNA fragment to introduce 30 N- terminal DarB residues into pBAD24

**Supplementary Table S3.** EOPs of phage P1 single-gene knockouts. Phage mutants were induced from lysogens in the restrictionless WA921 and plated to *E. coli* strains expressing *EcoA*, *EcoB* and *EcoK*. The restriction phenotype was compared to the parental P1 as described previously in Piya *et al.* 2017. Six phage mutants did not produce viable P1 phages upon induction, denoted as “PFU/mL <10<sup>2</sup>”. All other knockouts produced infectious virions and showed a restriction phenotype similar to that of the parental P1 (see Supplementary Figure S1).

Gene Knockout	Plating Phenotype
<i>pacA</i>	PFU/mL < 10 <sup>2</sup>
<i>pmgA</i>	PFU/mL < 10 <sup>2</sup>
<i>pmgB</i>	PFU/mL <10 <sup>2</sup>
<i>pmgC</i>	PFU/mL <10 <sup>2</sup>
<i>pmgG</i>	PFU/mL <10 <sup>2</sup>
<i>pmgR</i>	PFU/mL <10 <sup>2</sup>
<i>upfA</i>	normal restriction
<i>upfB</i>	normal restriction
<i>upfC</i>	normal restriction
<i>uhr</i>	normal restriction
<i>upl</i>	normal restriction
<i>dbn</i>	normal restriction
<i>pmgL</i>	normal restriction
<i>pmgM</i>	normal restriction
<i>pmgN</i>	normal restriction
<i>pmgO</i>	normal restriction
<i>pmgP</i>	normal restriction
<i>ppp</i>	normal restriction
<i>pmgQ</i>	normal restriction
<i>pmgS</i>	normal restriction
<i>pap</i>	normal restriction
<i>pmgT</i>	normal restriction
<i>pmgU</i>	normal restriction
<i>pmgV</i>	normal restriction
<i>upfM</i>	normal restriction
<i>upfN</i>	normal restriction
<i>upfO</i>	normal restriction

<i>pdca</i>	normal restriction
<i>pdcb</i>	normal restriction



**Supplementary Table S4.** Results of HHPred searches for P1 proteins identified as virion-associated by LC-MS/MS analysis or essential by genetic knockouts. Proteins Gp7, Gp25 and Gp16 did not return high-quality matches in HHPred and their functions remain unknown.

	<b>P1 protein</b>	<b>Match accession</b>	<b>Probability (%)</b>	<b>Function</b>	<b>PMID</b>
<b>Proteins identified by LC-MS/MS</b>					
	<b>gp23</b>	<b>5VF3_L</b>	<b>100</b>	<b>Bacteriophage T4 isometric capsid</b>	<b>28893988</b>
	<b>Prt</b>	<b>3JA7_B</b>	<b>100</b>	<b>Portal protein gp20; VIRAL PROTEIN; 3.63A {Enterobacteria phage T4}</b>	<b>26144253</b>
	<b>Pro</b>	<b>5JBL_A</b>	<b>99.44</b>	<b>Prohead core protein protease; protease pentamer, phage T4, prohead, HYDROLASE; 1.943A {Enterobacteria phage T4}</b>	<b>27667692</b>
	<b>DdrB</b>	<b>ND</b>		<b>Antirestriction component</b>	<b>28509398</b>
	<b>DarA</b>	<b>ND</b>		<b>Antirestriction component</b>	<b>28509398</b>
	<b>DarB</b>	<b>ND</b>		<b>Antirestriction component</b>	<b>28509398</b>
	<b>Hdf</b>	<b>ND</b>		<b>Antirestriction component</b>	<b>28509398</b>

	DdrA	ND		Antirestriction component	28509398
	Ulx	ND		Antirestriction component	28509398
	gp22	3J2M_Y	100	Tail sheath protein Gp18; bacteriophage T4, phage tail terminator protein, phage sheath protein, VIRAL PROTEIN; 15.0A {E	23434847
	Tub	5IV5_IB	99.94	Tail tube protein gp19; T4, baseplate-tail tube complex, pre-attachment, bacteriophage, bacterial virus, hexagonal, memb	27193680
	gp6	1WTH_D	99.06	Baseplate structural protein Gp27; Triple-stranded beta-helix, OB fold, pseudohexamer, T4 tail lysozyme, HUB, gp5-gp27,	15701513
	gp7	None >50%			
	gp25	None >50%			

	Sit	5OHU_A	98.92	Soluble lytic murein transglycosylase; Lytic Transglycosylase, LYASE; HET: PO4; 2.2A {Pseudomonas aeruginosa}	29632171
	BpIA	5HX2_D	100	Baseplate wedge protein gp6; T4, baseplate, complex, VIRAL PROTEIN; 3.8A	26929357
	S	5YVQ_A	99.45	Tail fiber protein S; bacteriophage, VIRAL PROTEIN; HET: GOL; 2.103A {Escherichia phage Mu}	31209305
	R	6HHK_C	98.72	Gp105; bacteriophage baseplate protein, VIRAL PROTEIN; HET: MSE; 2.38A {Listeria phage A511}	30606715
	gp24	4HUD_C	94.58	Tail connector protein Gp15; Bacteriophage T4, phage tail terminator protein, gp15, VIRAL PROTEIN; 2.7001A {Enterobacter	23434847
	gp16	None >50%			

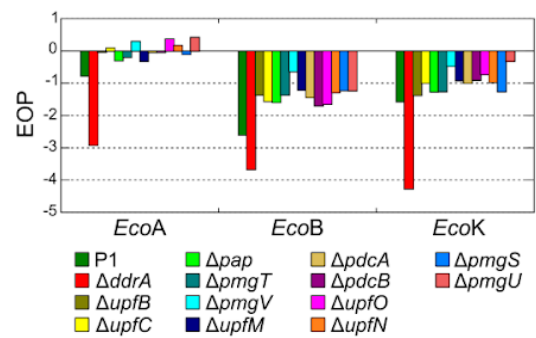
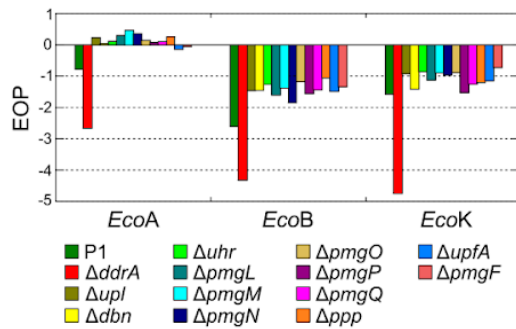
	BplB	5IV5_IB	99.79	Tail tube protein gp19; T4, baseplate-tail tube complex, pre-attachment, bacteriophage, bacterial virus, hexagonal, memb	27193680
	PmgC	7BOX_N	87.4	T7 gp11 tail adaptor	32266588
	PmgG	5IV5_r	97.3	T4 tail tube assembly protein gp48; baseplate-tail tube junction	27193680
Additional proteins identified as essential					
	PmgA	5IW9_B	99.4%	T4 gp25 inner baseplate wedge, initiator of sheath polymerization	27193680
	PmgB	None >50%		Tape measure chaperone?	
	PmgR	None >50%			
Other proteins with bioinformatically assigned structural roles					

	<b>UpfC</b>	<b>4KU0_D</b>	<b>99.4%</b>	<b>T4 gp5.4 baseplate needle structure</b>	
	<b>gp5</b>	<b>1WTH_A</b>	<b>99.67%</b>	<b>T4 gp5 tail needle</b>	
	<b>gp26</b>	<b>5HX2_F</b>	<b>98.9%</b>	<b>T4 gp53 wegde component</b>	

**Supplementary Table S5.** Baseplate components of coliphage T4 compared to baseplate components identified in phage P1. Functions of P1 proteins were predicted using HHPred at default settings. Detection of the P1 protein by LC-MS/MS of purified P1 virions is also shown (see Table 1). Nine P1 proteins with orthologs to T4 baseplate components were identified, most of these are in the baseplate core. P1 proteins with no matches in HHPred are denoted with dashes (--). T4 proteins and their functions are adapted from Taylor et al. 2016 (PMID 7193680).

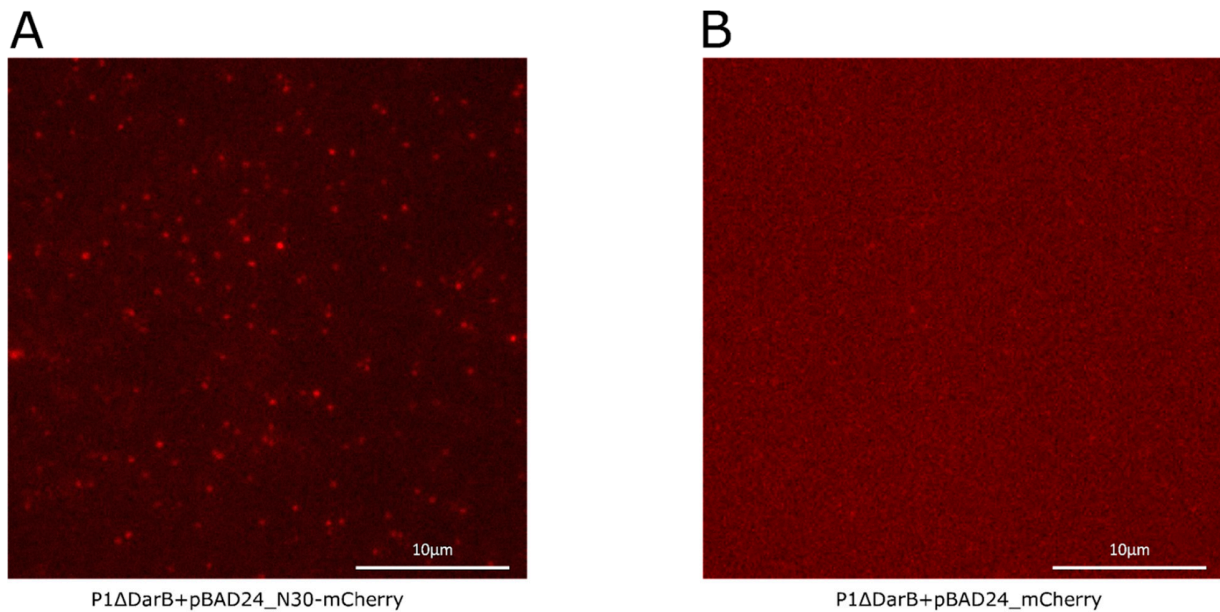
T4 protein		T4 function	P1 ortholog	HHPred result		Detected in LC-MS/MS
				Structure/ domain	% Probability	
<b>Baseplate core proteins</b>						
	gp5.4	Spike tip	UpfC	4KU0_D	99.4%	No
	gp5	Hub, needle, tail lysozyme	gp5	1WTH_A	99.67%	No
	gp6	Wedge	BplA	5HX2_D	100	Yes
	gp7	Wedge	--	--	--	--
	gp25	Wedge	PmgA	5IW9_B	99.4%	No
	gp27	Hub	gp6	1WTH_D	99.06	Yes
	gp48	Baseplate-tail tube junction	PmgG	5IV5_r	97.3	Yes
	gp53	Wedge	gp26	5HX2_F	98.9%	No
<b>Baseplate other components</b>						

	gp8	Wedge	R	6HHK_C	98.72	Yes
	gp29	Hub, tape measure	Sit	5OHU_A	98.92	Yes
	gp54	Baseplate-tail tube junction	--	--	--	--
<b>Baseplate tail fiber attachment</b>						
	gp9	Wedge, LTF attachment site	--	--	--	--
	gp10	Wedge, STF attachment	--	--	--	--
	gp11	Wedge, STF binding interface	--	--	--	--
	gp12	Baseplate outer rim, STF	--	--	--	--

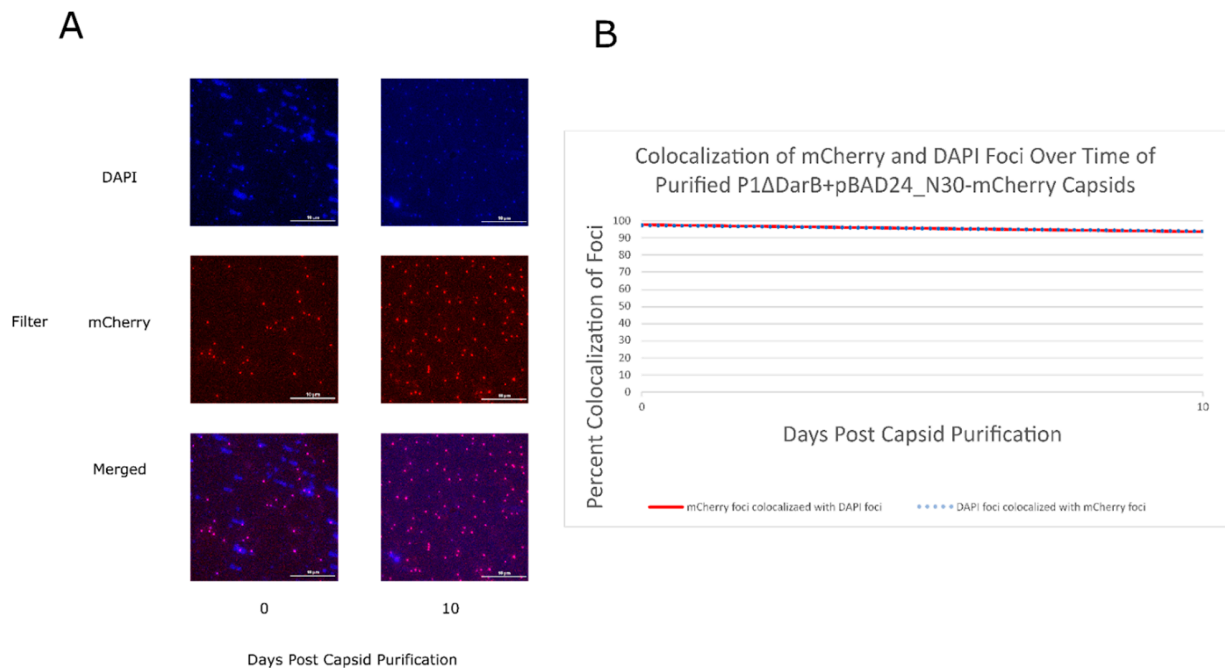


**Supplementary Figure S1.** P1 genes of unknown function are not required for antirestriction. Phage P1 or mutants were lysogenized into restriction-modification deficient *E. coli* strain WA921. The lysogens were thermally induced and tested for plating efficiency in *E. coli* strains with type I EcoA, EcoB or EcoK R-M systems. The EOP of parental P1, with functional antirestriction system is  $\sim 10^{-1}$ ,  $\sim 10^{-3}$  or  $10^{-2}$  in strains with type I EcoA, EcoB or EcoK systems respectively, whereas the EOP of P1 $\Delta ddrA$ , with disrupted antirestriction system, is  $\sim 10^{-3}$ ,  $\sim 10^{-4}$  or  $\sim 10^{-4}$  -  $10^{-5}$  in strains with type I EcoA, EcoB or EcoK systems respectively. The EOP of all other P1 mutants generated in this study appeared normal compared to the EOP of P1 $\Delta ddrA$ , suggesting that these genes do not play any roles in protecting P1 DNA from the type I R-M systems tested in this study.





**Supplementary Figure S2.** P1Δ*darB*+pBAD24\_N30-mCherry and P1Δ*darB*+pBAD24\_mCherry virions were purified by side-by-side by CsCl isopycnic centrifugation and applied to slides for observation by fluorescence microscopy. (A) Fluorescence imaging of P1Δ*darB*+pBAD24\_N30-mCherry shows fluorescent foci using the mCherry filter. (B) Fluorescence imaging of P1Δ*darB*+pBAD24\_mCherry shows the absence of fluorescent foci using the same filter. Camera signal gain was set automatically for image capture, therefore the image background is amplified in (B), which appears dark under manual observation.



**Supplementary Figure S3.** P1 $\Delta$ *darB* capsids purified by cesium chloride isopycnic centrifugation. (A) The simultaneous induction of the pBAD24\_N30-mCherry expression vector and the P1 $\Delta$ *darB* lysogen results in the localization of mCherry to the P1 capsid. Fluorescence imaging of cesium chloride isopycnic centrifugation P1 $\Delta$ *darB*+pBAD24\_N30-mCherry purified heads on days 0 and 10 are shown using a DAPI filter, mCherry filter, and the two filters merged. (B) The line graph illustrates the colocalization of DAPI and mCherry foci over the 10 days post cesium chloride isopycnic centrifugation of the P1 $\Delta$ *darB*+pBAD24\_N30-mCherry heads. The red line indicates the percent colocalization of mCherry foci and DAPI foci. Here, colocalization of foci occurs when an mCherry foci is observed first, then, secondly, the filter is switched to DAPI and the presence of a DAPI foci overlaps with the initial mCherry foci. The blue dotted line indicates the percent colocalization of DAPI foci and mCherry foci. Here, colocalization of foci occurs when a DAPI foci is observed first, then, secondly, the filter is switched to mCherry and the presence of an mCherry foci overlaps with the initial DAPI foci.