Supplemental Information

Figure S1. Host ranges of T7 and T3 phages, related to Figure 2. (**A)** Host ranges of T7 and T3 phages. Each bacterial overnight culture and LB soft agar were mixed, and poured onto LB plates. 2.5 μL of 10-fold serially diluted T7 and T3 phages were spotted onto the bacterial lawns and incubated at 37°C. T3 phage did not plaque efficiently on *E. coli* BW25113 and MG1655, whereas T7 phage plaqued efficiently on all tested *E. coli* strains. (**B**) Adsorption assay. Bacteria and T3 phage were mixed at an MOI ~0.5 and incubated for 10 min. Growth of adsorbed progeny was stopped by the addition of chloroform. After centrifugation, supernatants were serially diluted and mixed with *E. coli* BL21 and LB soft agar, and poured onto LB plates. After incubation at 37°C, phage plaques were counted, and adsorption efficiencies were calculated. The data are presented as the mean of three independent experiments, and the error bars represent the SD. Small error bars are obscured by bar charts.

Figure S2. Plaque formation assays with natural, reconstructed wild-type, and synthetic phages, related to Figure 3. Bacterial lawns were spotted with 2.5 μL of 10-fold serially diluted phages and incubated at 30 or 37°C. Synthetic phages showed tail-fiber- or tail-component-dependent host ranges and the ability to cross between species. T7 and $T7_{WT}$, T3 and $T3_{WT}$, and K11 and $K11_{WT}$ phages are the same at the genetic level; however, $T7_{WT}$, $T3_{WT}$, and $K11_{WT}$ phages were created by capturing the genomes in yeast and then rebooting these phage genomes in bacteria. Efficiency of plating (EOP) results are shown in Figure 3.

Figure S3. Plaque formation assays with $T7_{K11(gp11\text{-}12\text{-}17)}$ and $K11_{T7(gp11\text{-}12\text{-}17)}$, related to Figure 5. 2.5 μL of 10-fold serially diluted phages were spotted onto bacterial lawns and incubated at 37°C. $K11_{T7(gp11\text{-}12\text{-}17)}$ adopted the host range of $T7_{WT}$ while $T7_{K11(gp11\text{-}12\text{-}17)}$ adopted the host range of K11, thus demonstrating that tail component swapping can lead to acquisition of novel host ranges.

Figure S4. Antimicrobial susceptibilities of *E. coli* Nissle 1917, *Klebsiella* sp. 390, and *Y. pseudotuberculosis* IP2666, related to Figure 6. (**A**) Five microliters of each overnight cultures (>10^9 CFU/ml) for each bacteria were streaked on LB plates with or without antibiotics. Plates were incubated at 30°C for 24 h. *Klebsiella* sp. 390 was naturally resistant to 25 μg/ml carbenicillin and *Y. ptb* IP2666 was naturally resistant to 1 μg/ml triclosan, while *E. coli* Nissle 1917 was sensitive to both. (**B**) Diluted log-phase cultures were plated onto LB with or without antibiotics. After incubation at 30°C for 18-24 h, colonies were enumerated. These concentrations of antimicrobials completely killed susceptible strains but did not visibly affect the growth of resistant strains.

Figure S5. Antimicrobial susceptibilities of *E. coli* Nissle 1917, *E. coli* ECOR16, and *Y. pseudotuberculosis* IP2666, related to Figure S6. (**A**) Five microliters of each overnight cultures (>10^9 CFU/ml) for each bacteria were streaked on LB plates with or without antibiotics. Plates were incubated at 30°C for 24 h. *E. coli* ECOR16 was naturally resistant to 50 μg/ml streptomycin and *Y. ptb* IP2666 was naturally resistant to 1 μg/ml triclosan, while *E. coli* Nissle 1917 was sensitive to both. (**B**) Diluted log-phase cultures were plated onto LB with or without antibiotics. After incubation at 30°C for 18-24 h, colonies were enumerated.

Figure S6. Microbial population editing assay with synthetic T3-based cocktail, related to Figure 6. A synthetic microbial community composed of *E. coli* Nissle 1917, *E. coli* ECOR16, and *Y. ptb* IP2666 was treated with individual synthetic phages ($T3_{WT}$ and $T3_{R(gp17)}$) and the pairwise combination of these phages. After adding ~10^7 PFU/ml of each phage, the resulting samples were incubated at 30°C with shaking for 1 h. At each time point, bacteria were collected, washed in saline, serially diluted, and plated onto selective plates for viable cell counts after a 24 h incubation at 30°C. The data are presented as the mean of three independent experiments and the total numbers of cells (CFU/ml) are shown. The sizes of the pie charts reflect the total number of cells. Note that the chart does not allow the display of fractions smaller than ~1%. The detailed data are shown in Table S4B.

Table S1, related to Figure 1. Results of plaque formation assays on *E. cloni* 10G and one-time phage propagation assays in *E. cloni* 10G for natural phages.

Table S2, related to Figure 2. Sequence of codon-optimized and fully synthesized gene *17* of phage 13a.

Table S3, related to Figure 4 and 5. (**A**) Detailed data for the *Y. ptb* IP2666 killing assay (Figure 4C). (**B**) Detailed data for the *Klebsiella* sp. 390 killing assay (Figure 5C).

Table S4, related to Figure 6 and S6. (**A**) Detailed data for microbial population editing with engineered phages from Figure 6. (**B**) Detailed data for microbial population editing with engineered phages from Figure S6.

Table S5, related to Experimental Procedures. Description of synthetic phages created in this study.

Table S6, related to Experimental Procedures. Oligonucleotide primers used in this study.

Figure S1

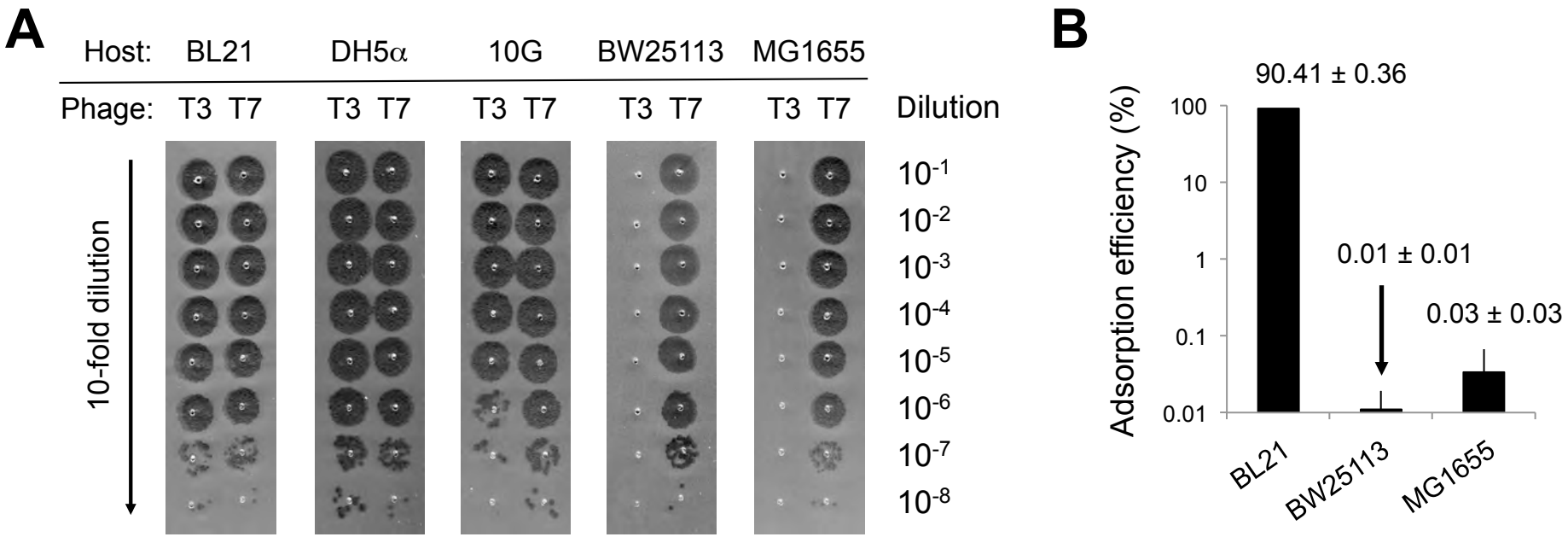

A
Host: BL21 DH5α 10G BW25113 MG1655
Phage: T3 T7 T3 T7 T3 T7 T3 T7 T3 T7
Dilution
10-fold dilution
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
B
90.41 ± 0.36
0.01 ± 0.01
0.03 ± 0.03
Adsorption efficiency (%)
100
10
1
0.1
0.01
BL21
BW25113
MG1655

Figure S2

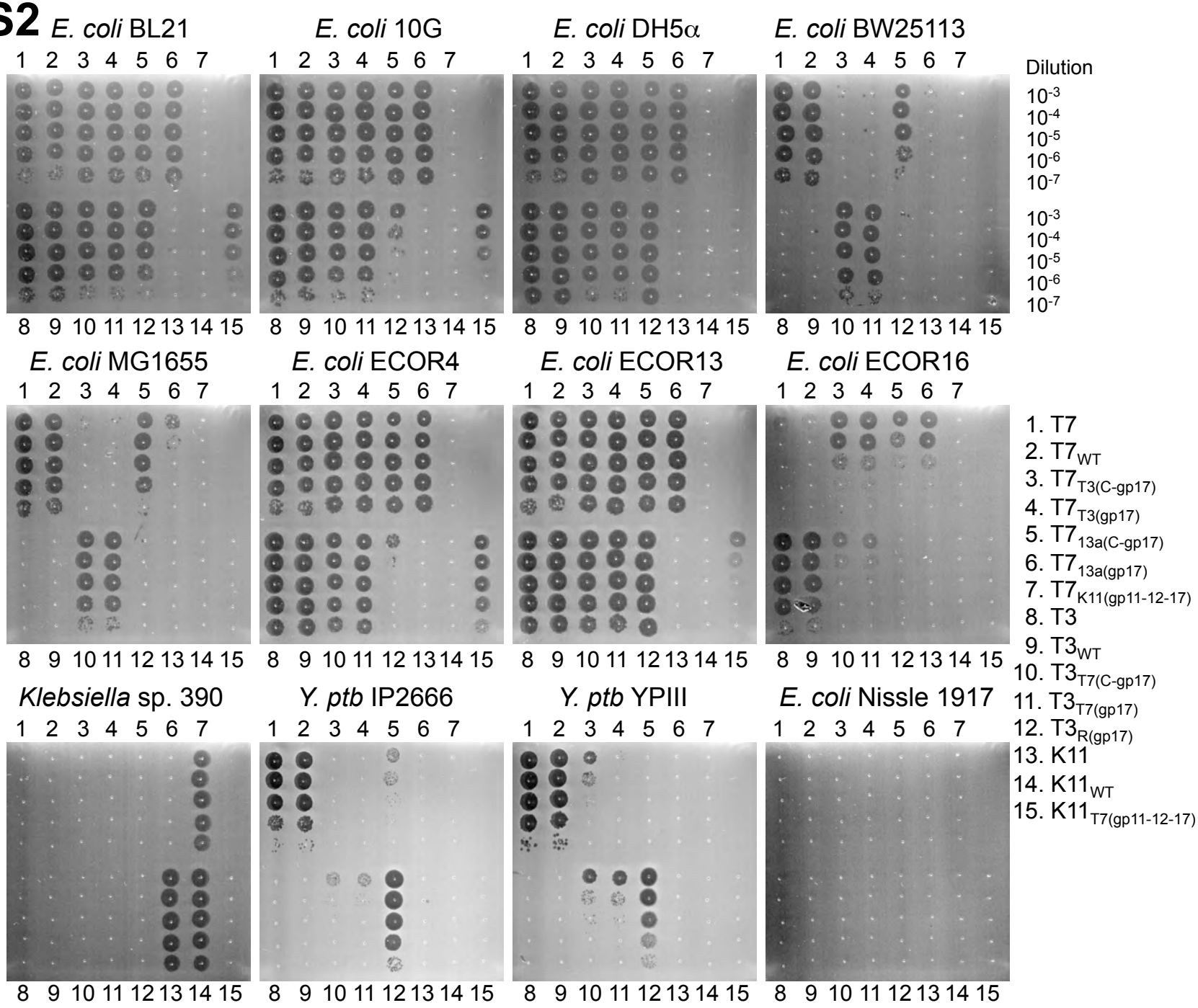

E. coli BL21
E. coli 10G
E. coli DH5α
E. coli BW25113
E. coli MG1655
E. coli ECOR4
E. coli ECOR13
E. coli ECOR16
Klebsiella sp. 390
Y. ptb IP2666
Y. ptb YPIII
E. coli Nissle 1917
1 2 3 4 5 6 7
8 9 10 11 12 13 14 15
Dilution
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
1. T7
2. $T7_{WT}$
3. $T7_{T3(C\text{-}gp17)}$
4. $T7_{T3(gp17)}$
5. $T7_{13a(C\text{-}gp17)}$
6. $T7_{13a(gp17)}$
7. $T7_{K11(gp11\text{-}12\text{-}17)}$
8. T3
9. $T3_{WT}$
10. $T3_{T7(C\text{-}gp17)}$
11. $T3_{T7(gp17)}$
12. $T3_{R(gp17)}$
13. K11
14. $K11_{WT}$
15. $K11_{T7(gp11\text{-}12\text{-}17)}$

Figure S3

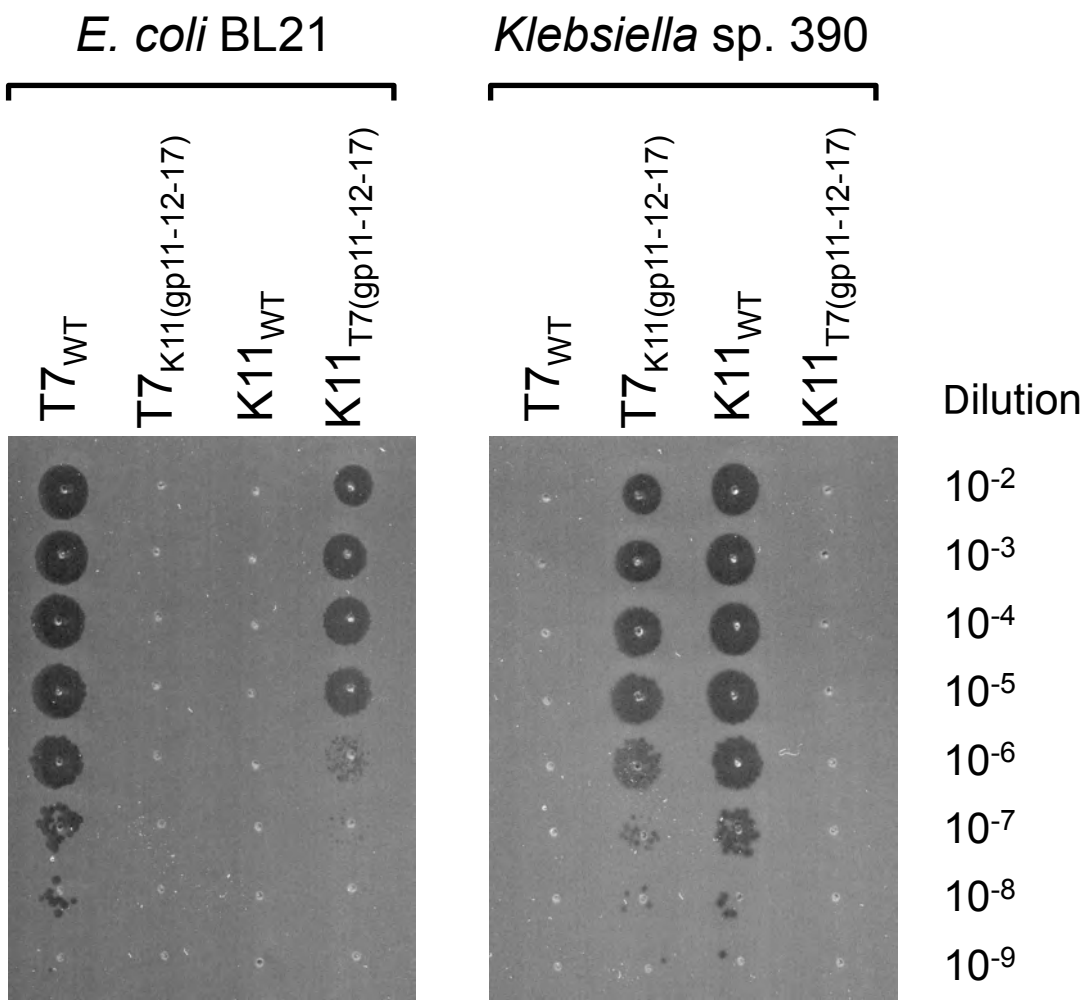
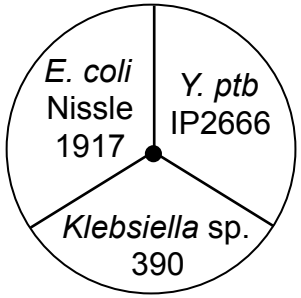

E. coli BL21
Klebsiella sp. 390
$T7_{WT}$
$T7_{K11(gp11\text{-}12\text{-}17)}$
$K11_{WT}$
$K11_{T7(gp11\text{-}12\text{-}17)}$
$T7_{WT}$
$T7_{K11(gp11\text{-}12\text{-}17)}$
$K11_{WT}$
$K11_{T7(gp11\text{-}12\text{-}17)}$
Dilution
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}

Figure S4

A


E. coli
Nissle
1917
Y. ptb
IP2666
Klebsiella sp.
390

B

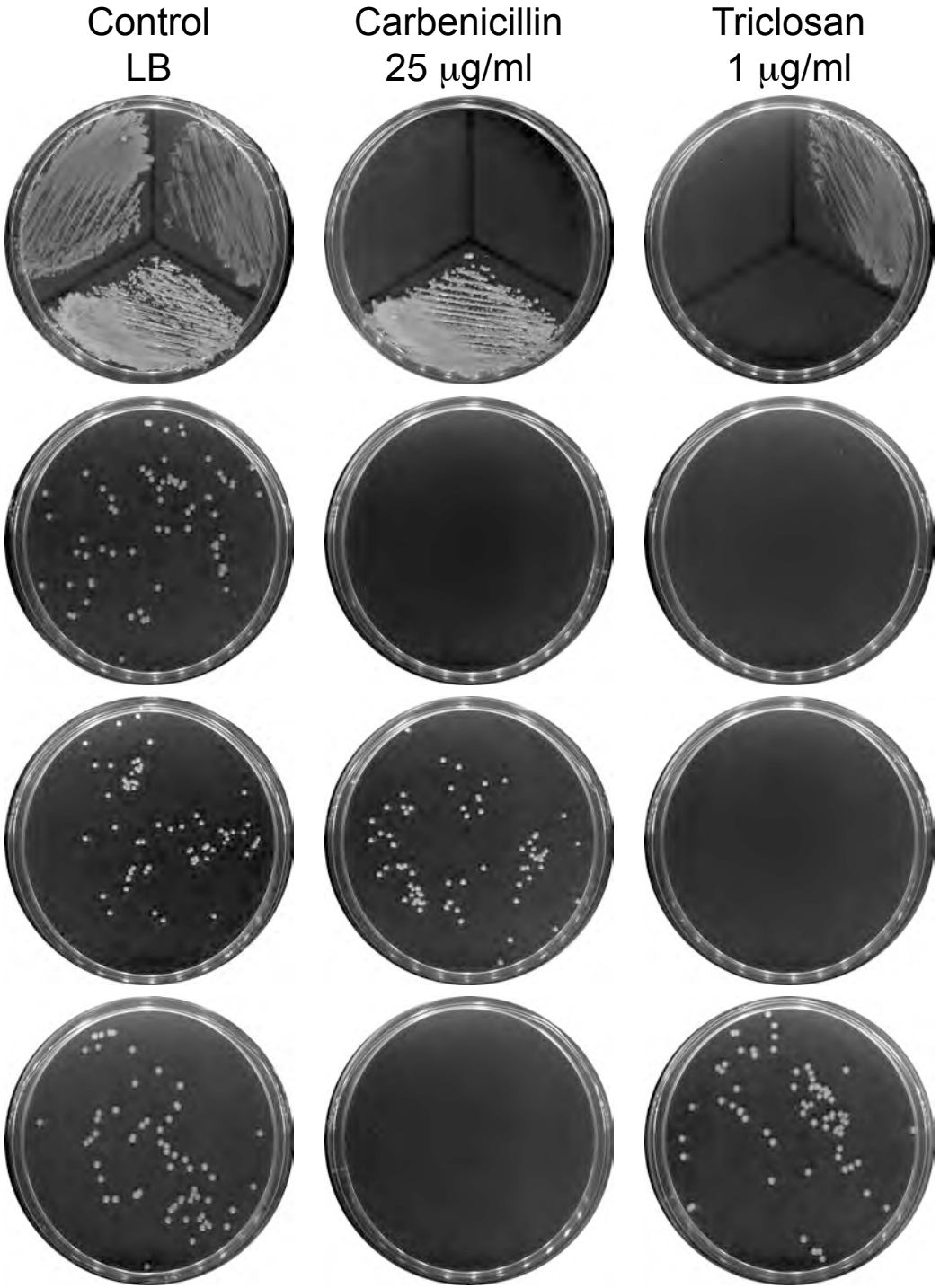

Control
LB
Carbenicillin
25 μg/ml
Triclosan
1 μg/ml
E. coli Nissle 1917
Klebsiella sp. 390
Y. ptb IP2666

Figure S5

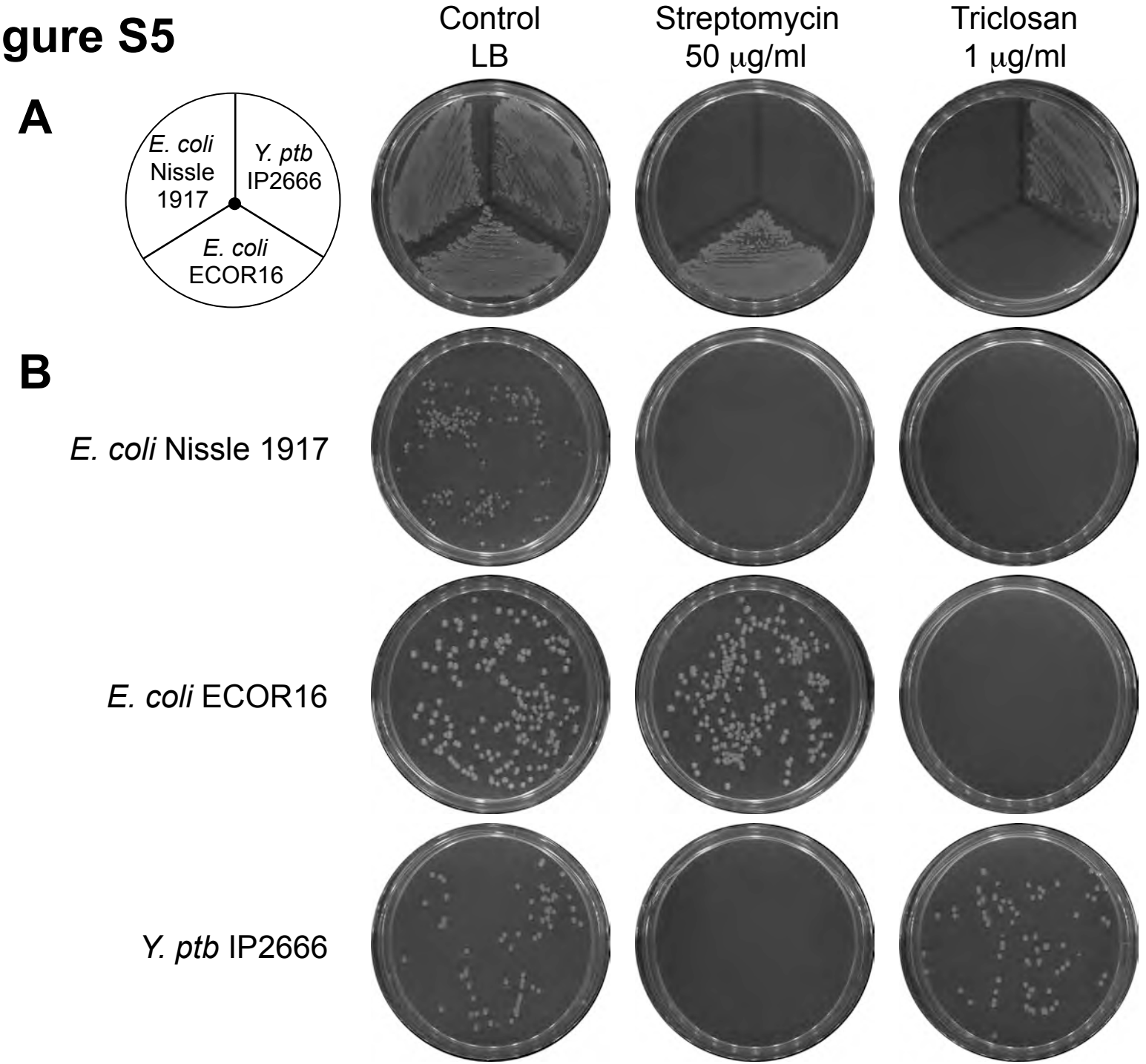

A
E. coli Nissle 1917
Y. ptb IP2666
E. coli ECOR16
Control LB
Streptomycin 50 μg/ml
Triclosan 1 μg/ml
B
E. coli Nissle 1917
E. coli ECOR16
Y. ptb IP2666

Figure S6

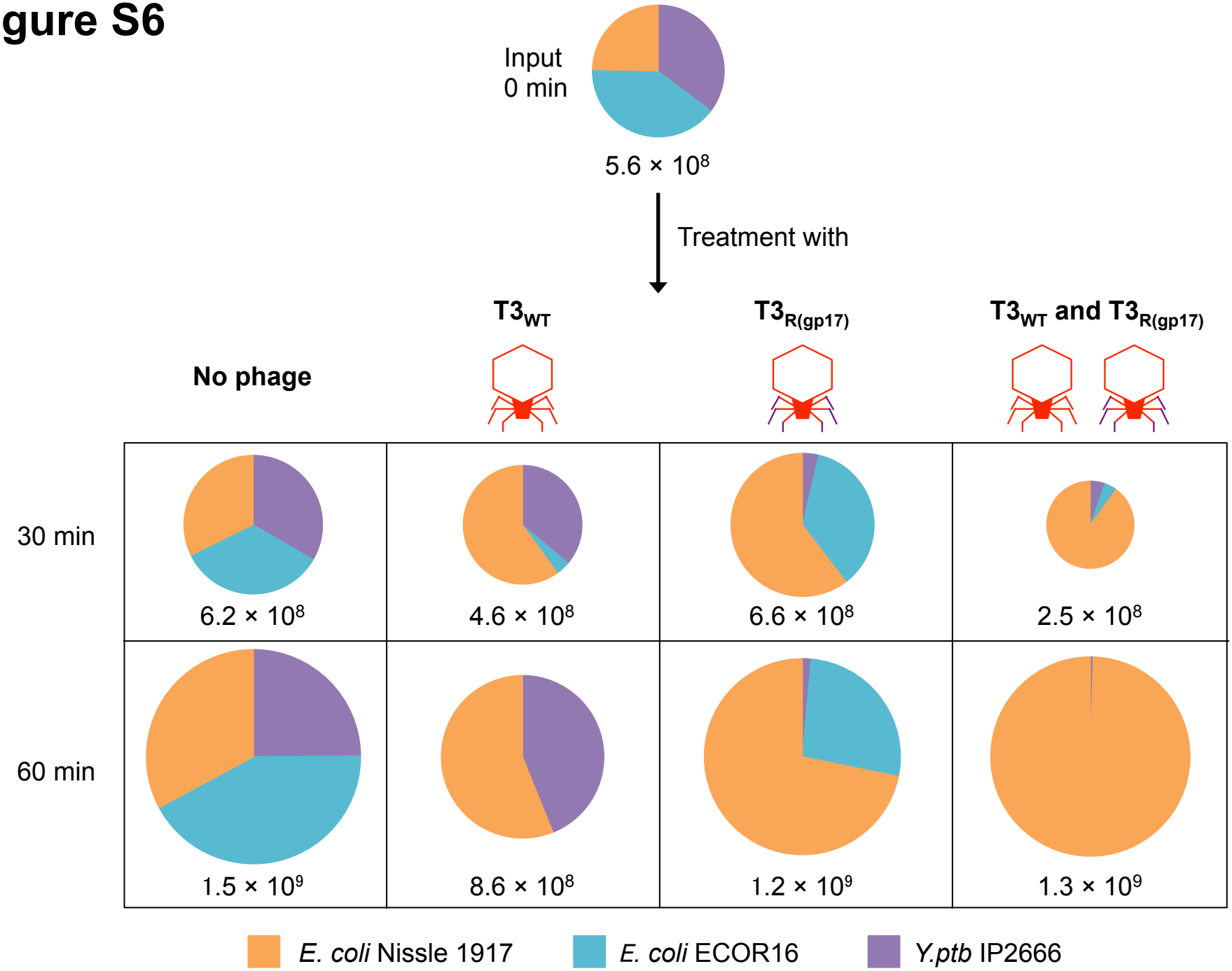

Input
0 min
5.6 × 10^8
Treatment with
No phage
T3WT
T3R(gp17)
T3WT and T3R(gp17)
30 min
6.2 × 10^8
4.6 × 10^8
6.6 × 10^8
2.5 × 10^8
60 min
1.5 × 10^9
8.6 × 10^8
1.2 × 10^9
1.3 × 10^9
E. coli Nissle 1917
E. coli ECOR16
Y.ptb IP2666

Table S1

One-time phage propagation assay.

Phage	Plaque formation on *E. cloni* 10G	Propagation in *E. cloni* 10G	Host bacteria
T7	Yes	Yes	*E. coli* BL21
T3	Yes	Yes	*E. coli* BL21
K1E	No	Yes	*E. coli* IJ1668
K1F	No	Yes	*E. coli* IJ1668
K1-5	No	Yes	*E. coli* IJ1668
SP6	No	Yes	*S. typhimurium* IJ612
LUZ19	No	Yes	*P. aeruginosa* PAO1
gh-1	No	Yes	*P. putida* C1S
K11	No	Yes	*Klebsiella* sp. 390

Table S2

Sequence of codon-optimized 13a gene *17*.

ATGGCGAATGTGATTAAGACCGTTCTGACGTATCAGTTAGATGGATCCAATAGCGATTTTAATATTCCATTTGAAT
ACCTGGCGCGCAAATTTGTCGCCGTGACGCTGATTGGGGTTGATCGCAAGGTATTAACCATTAACACCGACTAT
CGCTTTGCTACGCGCACGACCATCTCTCTCACTAAGGCGTGGGGACCGGCGGATGGTTATACTACCATCGAGT
TGCGCCGCGTTACGTCGACAACTGACCGCCTCGTGGACTTTACCGACGGTAGCATTCTGCGTGCGTACGATC
TTAACGTGGCCCAGATTCAGACAATCCACGTCGCAGAAGAAGCACGTGACCTGACTGCCGACACCATTGGCG
TAAACAATGACGGCCATCTGGATGCGCGCGGTCGCCGTATTGTCAATTTGGCGAACGCCGTTGATGATCGTGA
TGCGGTGCCGCTCGGCCAACTCAAAACTATGAATCAGAACAGTTGGCAGGCTCGCAACGAGGCACTGCAATT
CCGCAATGAAGCCGAAACTTTTCGCAATCAGGCGGAAGGTTTTAAAAACGAGAGCGGTACTAACGCCACTAAC
ACGAAACAGTGGCGCGACGAGACAAAAGGCTTCCGCGACGAAGCGGAACAATTTAAGAACACCGCGGGTCA
GTATGATACATCCGCGGGTAACAGCGCGAGCGCTGCCCATCAGAGCGAAGTAAACGCAGAAAACAGTGCGAC
CGCGTCCGCTAATAGCGCCCACCTCGCCGAGCAACAGGCCGATCGCGCGGAGCGTGAAGCTGATAAACTCG
GCAACTTTAATGGTCTTGCTGGTGCGATCGACAAGGTCGACGGCACAAACGTGTATTGGAAAGGTAACATCCA
TGCAAACGGTCGCCTCTACATCACGACCAATGGCTTCGACTGCGGTCAATATCAGCAGTTCTTTGGTGGCGAT
ACGAATCGCTACAGCGTGATGGAATGGGGTGACGATAACGGGTGGCTGATGTATGTGCAGCGCCGTGAATGG
ACCACCGCAATCGGCGGCAACATTCAGTTGGTTGTCAACGGTCAGATCATCACCCAGGGCGGTGCCATGACG
GGGCAACTGAAACTTCAGAATGGTCACGTTCTGCAATTGGAATCCGCCAGTGACAAAGCGCATTATATTTTATC
AAAGGATGGCAACCGTAATAATTGGTATATTGGTCGTGGATCGGATAACAATAACGACTGCACGTTTCACTCCTA
CGTTCACGGTACCACGTTAACCTTGAAACAGGATTATGCAGTGGTCAACAAACACTTTCATGTGGGACAGGCG
GTCGTGTCAACTGATGGCAATATCCAGGGCACCAAATGGGGTGGTAAATGGCTGGATGTTTATTTAAATGATAC
ATACGTTAAGAAAACGATGGCTTGGACACAAGTGTGGGCTGCGGACTCGGGTAAATACCTCCCGGGTGGGAG
TCAAACTGATACTCTGCCGCAGGATCTGCGTTTCCGCAACATCTGGATTCGTACGCGTAACAACTATTGGAACT
TTTTTCGCACGGGCCCGGATGGGATCTATTTCCTGTCGGCTGAAGGAGGTTGGCTGAAATTTCAGATCCACTC
AAACGGTCGCGTCTTCAAAAACATCTCTGATCGCGACGCACCCCCGACCGCAATCGCTGTGGAAGACGTTTAA

Table S3

A

	$\log_{10}$ (mean ± SD CFU/ml)		
Time after treatment	Control (no phage)	$T3_{WT}$	$T3_{R(gp17)}$
0 min	8.312 ± 0.052	8.312 ± 0.052	8.312 ± 0.052
30 min	8.503 ± 0.131	8.412 ± 0.055	7.799 ± 0.048
60 min	8.585 ± 0.046	8.556 ± 0.020	6.369 ± 0.096
90 min	8.732 ± 0.072	8.685 ± 0.046	3.301 ± 0
120 min	8.727 ± 0.116	8.767 ± 0.120	3.301 ± 0
150 min	9.016 ± 0.100	8.903 ± 0.246	3.301 ± 0
180 min	9.219 ± 0.051	9.263 ± 0.084	3.301 ± 0

B

	$\log_{10}$ (mean ± SD CFU/ml)			
Time after treatment	Control (no phage)	$K11_{WT}$	$T7_{WT}$	$T7_{K11(gp11\text{-}12\text{-}17)}$
0 min	8.579 ± 0.019	8.579 ± 0.019	8.579 ± 0.019	8.579 ± 0.019
15 min	8.796 ± 0.070	7.560 ± 0.197	8.715 ± 0.035	7.952 ± 0.269
30 min	8.869 ± 0.188	5.400 ± 0.288	8.900 ± 0.142	6.354 ± 0.195
45 min	9.117 ± 0.086	3.301 ± 0	9.053 ± 0.037	5.053 ± 0.518
60 min	9.199 ± 0.145	3.301 ± 0	9.253 ± 0.127	5.036 ± 0.419
75 min	9.365 ± 0.048	3.301 ± 0	9.350 ± 0.069	5.026 ± 0.037

Table S4

A

Treatment	Time after treatment	log_{10} (mean ± SD CFU/ml) *E. coli* Nissle 1917	*Klebsiella* sp. 390	*Y. ptb* IP2666
No phage	0 min	8.300 ± 0.036	8.307 ± 0.086	8.290 ± 0.155
	30 min	8.327 ± 0.037	8.407 ± 0.084	8.312 ± 0.125
	60 min	8.598 ± 0.148	8.761 ± 0.123	8.524 ± 0.180
$T7_{WT}$	30 min	8.355 ± 0.018	8.390 ± 0.043	6.341 ± 0.032
	60 min	8.768 ± 0.114	8.560 ± 0.400	3.301 ± 0
$T3_{WT}$	30 min	8.327 ± 0.037	8.379 ± 0.030	8.331 ± 0.100
	60 min	8.861 ± 0.059	8.935 ± 0.046	8.709 ± 0.192
$K11_{WT}$	30 min	8.368 ± 0.018	5.386 ± 0.154	8.327 ± 0.037
	60 min	8.935 ± 0.046	3.301 ± 0	8.820 ± 0.059
$T7_{K11gp(11\text{-}12\text{-}17)}$	30 min	8.379 ± 0.030	6.740 ± 0.230	8.361 ± 0.080
	60 min	8.861 ± 0.059	5.560 ± 0.197	8.324 ± 0.643
$T3_{R(gp17)}$	30 min	8.367 ± 0.034	8.436 ± 0.015	7.587 ± 0.163
	60 min	8.984 ± 0.115	8.994 ± 0.072	6.327 ± 0.460
T7 and $T7_{K11(gp11\text{-}12\text{-}17)}$	30 min	8.414 ± 0.027	6.968 ± 0.046	6.588 ± 0.223
	60 min	8.987 ± 0.063	5.683 ± 0.350	3.301 ± 0

B

Treatment	Time after treatment	log_{10} (mean ± SD CFU/ml) *E. coli* Nissle 1917	*E. coli* ECOR16	*Y. ptb* IP2666
No phage	0 min	8.127 ± 0.127	8.354 ± 0.037	8.295 ± 0.072
	30 min	8.183 ± 0.346	8.339 ± 0.059	8.326 ± 0.059
	60 min	8.657 ± 0.177	8.793 ± 0.084	8.529 ± 0.187
$T3_{WT}$	30 min	8.422 ± 0.131	7.249 ± 0.073	8.195 ± 0.155
	60 min	8.574 ± 0.354	5.534 ± 0.111	8.545 ± 0.169
$T3_{R(gp17)}$	30 min	8.604 ± 0.031	8.379 ± 0.030	7.355 ± 0.153
	60 min	8.917 ± 0.203	8.486 ± 0.209	6.869 ± 0.630
$T3_{WT}$ and $T3_{R(gp17)}$	30 min	8.219 ± 0.622	7.008 ± 0.500	7.271 ± 0.022
	60 min	9.106 ± 0.138	4.271 ± 0.022	6.781 ± 0.098

Table S5

Synthetic phages.

Phage	Genotype	Description
$T7_{WT}$	T7 wild-type	Synthesized from PCR products
$T3_{WT}$	T3 wild-type	Synthesized from PCR products
$K1E_{WT}$	K1E wild-type	Synthesized from PCR products
$K1F_{WT}$	K1F wild-type	Synthesized from PCR products
$K1\text{-}5_{WT}$	K1-5 wild-type	Synthesized from PCR products
$SP6_{WT}$	SP6 wild-type	Synthesized from PCR products
$gh\text{-}1_{WT}$	gh-1 wild-type	Synthesized from PCR products
$K11_{WT}$	K11 wild-type	Synthesized from PCR products
$T7_{T3(C\text{-}gp17)}$	$T7_{WT}$ gene *17* (1-447)-T3 gene *17* (448-1677)	T7 with T7-T3 hybrid tail fiber
$T7_{T3(gp17)}$	$T7_{WT}$ Δgene *17*, T3 gene *17*	T7 with T3 tail fiber
$T7_{13a(C\text{-}gp17)}$	$T7_{WT}$ gene *17* (1-450)-13a gene *17* (451-1677)	T7 with T7-13a hybrid tail fiber
$T7_{13a(gp17)}$	$T7_{WT}$ Δgene *17*, 13a gene *17*	T7 with 13a tail fiber
$T7_{K11(gp11\text{-}12\text{-}17)}$	$T7_{WT}$ Δgene (*11 12 17*), K11 gene (*11 12 17*)	T7 with K11 tail
$T3_{T7(C\text{-}gp17)}$	$T3_{WT}$ gene *17*(1-447)-T7 gene *17*(448-1662)	T3 with T3-T7 hybrid tail fiber
$T3_{T7(gp17)}$	$T3_{WT}$ Δgene *17*, T7 gene *17*	T3 with T7 tail fiber
$T3_{R(gp17)}$	$T3_{WT}$ Δgene *17*, R gene *17*	T3 with R tail fiber
$K11_{T7(gp11\text{-}12\text{-}17)}$	$K11_{WT}$ Δgene (*11 12 17*), T7 gene (*11-12 17*)	K11 with T7 tail