SUPPORTING ONLINE MATERIAL

Table S1. Mean fitness effect of each mutation on each host. Means are estimates from a REML mixed model where assay replicate and qPCR replicate were included as random effects to account for these sources of experimental variance.

	E.coli					*Salmonella*				
Mutant	Mean	Std Err Mean	DF	Test Statistic	Prob > |t|	Mean	Std Err Mean	DF	Test Statistic	Prob > |t|
P1	-0.612	0.026	7	-23.77	5.93E-08	-0.727	0.036	6	-20.17	9.63E-07
P10	0.426	0.063	7	6.76	2.62E-04	0.037	0.057	6	0.65	5.37E-01
P11	-0.133	0.090	7	-1.49	0.18	-0.529	0.045	7	-11.80	7.12E-06
P12	-0.794	0.026	7	-30.94	9.51E-09	-0.882	0.034	7	-25.68	3.47E-08
P14	-0.288	0.052	7	-5.53	8.74E-04	-0.599	0.048	7	-12.40	5.10E-06
P15	0.060	0.054	7	1.11	3.03E-01	-0.535	0.049	7	-10.84	1.26E-05
P16	-0.060	0.050	7	-1.20	2.68E-01	-0.203	0.057	7	-3.54	9.47E-03
P17	-0.046	0.041	7	-1.13	2.98E-01	-0.642	0.037	7	-17.41	5.07E-07
P18	-0.021	0.034	7	-0.63	5.49E-01	-0.697	0.041	7	-16.84	6.38E-07
P19	-0.372	0.044	7	-8.48	6.28E-05	-0.740	0.023	7	-32.08	7.39E-09
P23	-0.116	0.044	7	-2.63	3.38E-02	-0.473	0.043	6	-10.96	3.43E-05
P25	-0.164	0.022	7	-7.48	1.40E-04	-0.626	0.030	7	-21.04	1.38E-07
P26	-0.332	0.040	7	-8.24	7.54E-05	-0.513	0.035	7	-14.87	1.49E-06
P27	-0.242	0.044	7	-5.52	8.83E-04	-0.642	0.036	7	-18.01	4.03E-07
P29	-0.143	0.091	7	-1.57	1.61E-01	-0.431	0.063	7	-6.80	2.53E-04
P3	-0.301	0.086	7	-3.51	9.80E-03	-0.590	0.058	7	-10.16	1.93E-05
P30	0.020	0.020	7	0.99	3.57E-01	-0.361	0.032	7	-11.35	9.25E-06
P32	-0.475	0.051	7	-9.27	3.52E-05	-0.696	0.074	7	-9.38	3.26E-05
P33	-0.532	0.047	7	-11.42	8.84E-06	-0.618	0.029	7	-21.46	1.20E-07
P35	-0.080	0.046	7	-1.72	1.30E-01	-0.636	0.067	7	-9.42	3.17E-05
P36	-0.379	0.048	7	-7.90	9.87E-05	-0.679	0.032	7	-21.12	1.34E-07
P37	-0.633	0.059	7	-10.75	1.33E-05	-0.466	0.049	7	-9.54	2.92E-05
P39	-0.954	0.015	7	-61.59	7.81E-11	-0.971	0.022	7	-43.67	8.62E-10
P41	-0.451	0.049	7	-9.27	3.52E-05	-0.392	0.063	7	-6.19	4.49E-04
P42	-0.551	0.030	7	-18.20	3.74E-07	-0.683	0.074	7	-9.26	3.55E-05
P43	-0.529	0.057	7	-9.32	3.40E-05	-0.664	0.057	7	-11.66	7.69E-06
P45	-0.995	0.005	7	-191.26	2.82E-14	-0.983	0.011	7	-88.84	6.03E-12
P47	-0.533	0.022	7	-24.33	5.05E-08	-0.550	0.052	7	-10.57	1.49E-05
P48	-0.555	0.035	7	-15.97	9.17E-07	-0.373	0.052	7	-7.12	1.90E-04
P49	-0.356	0.094	7	-3.80	6.69E-03	-0.287	0.066	7	-4.37	3.28E-03
P5	-0.483	0.114	7	-4.22	3.92E-03	-0.848	0.055	7	-15.29	1.23E-06
P52	-0.771	0.034	7	-22.64	8.32E-08	-1		6	-	0
P54	-0.487	0.027	7	-18.03	3.99E-07	-0.471	0.057	7	-8.23	7.59E-05
P6	0.043	0.072	7	0.60	5.70E-01	-0.482	0.119	7	-4.03	4.97E-03
P7	0.072	0.034	7	2.08	7.56E-02	-0.855	0.051	7	-16.67	6.82E-07
P9	0.338	0.049	7	6.88	2.36E-04	-0.110	0.049	7	-2.25	5.95E-02

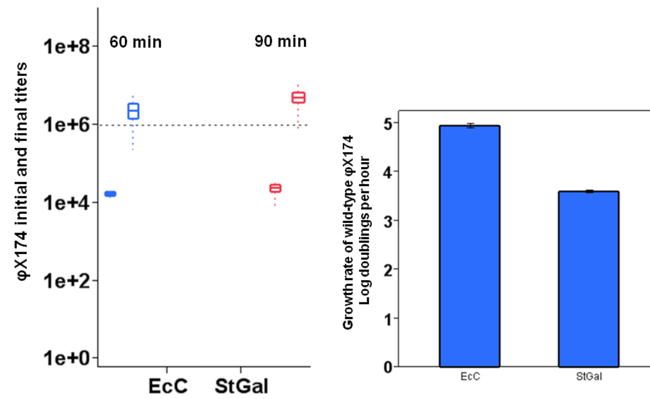

Figure S1. A. Incubation times on each host were determined based on the time it took the wild-type ϕX174 to reach at least 10^6 pfu, after incubating host cultures with ~10^4 pfu. B. From the outset, the wild-type ϕX174 showed higher growth rate on EcC than on StGal.

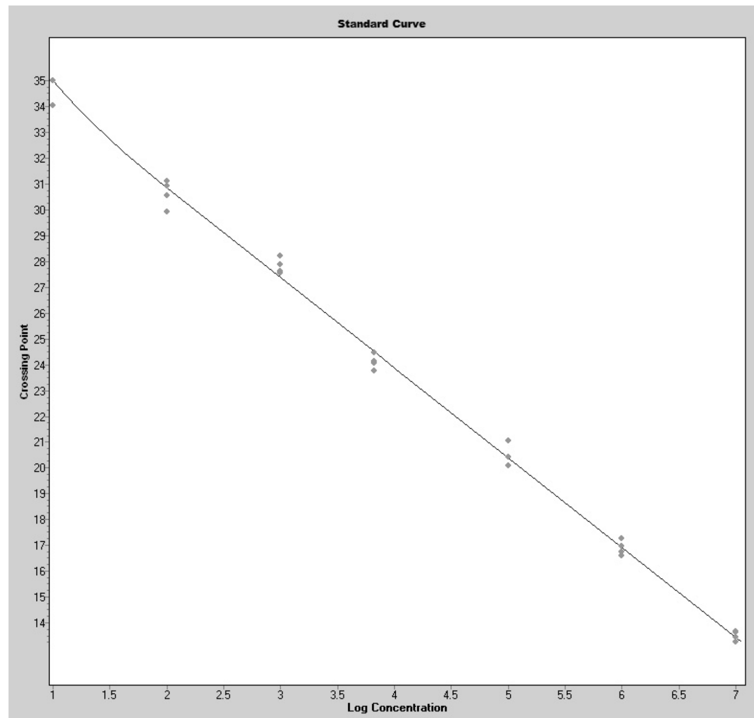

Figure S2. qPCR standard curve. Replicate 10-fold dilutions of the wild-type ϕX174 were amplified using ϕX174-specific qPCR, and their respective crossing points (CPs) calibrated to numbers of pfu obtained by plating the same dilutions on a lawn of EcC. One intermediate dilution was aliquoted and stored at -80°c, and later included as an external control in all qPRC reactions. This allowed an independent estimation of viral titers based on the original calibration curve. This curve had error =0.0242, showing that the qPCR reaction had an efficiency of 1.94 copies per strand (maximum is 2 copies per DNA strand).

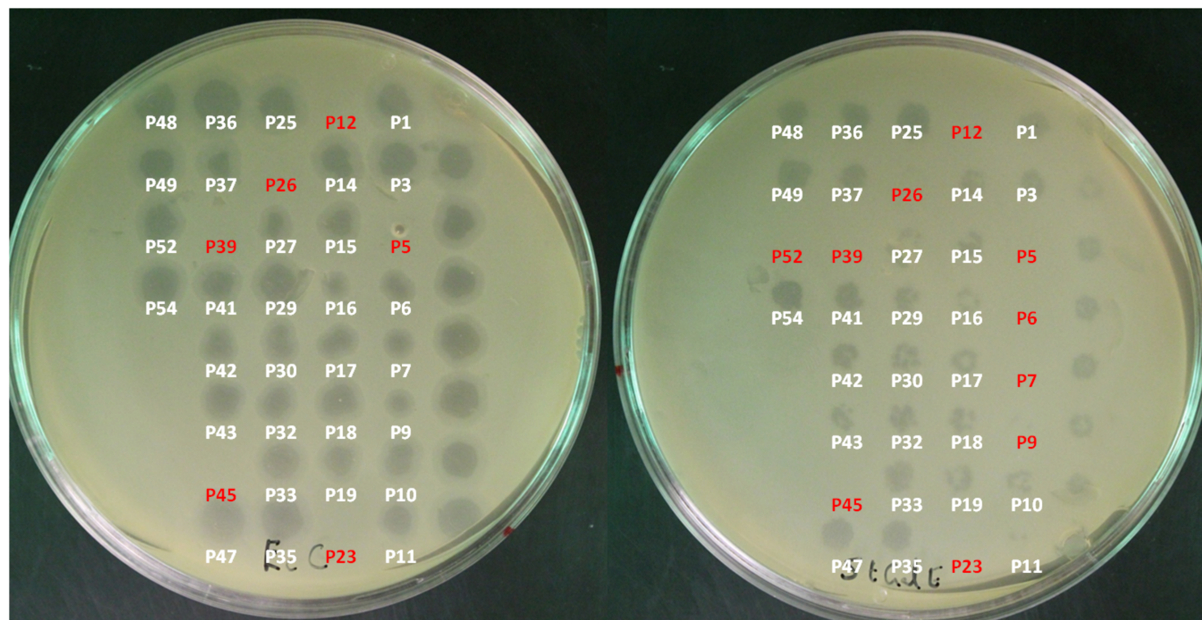

Figure S3. Lethality on solid medium. We tested whether any of the 36 mutants showed a lethal phenotype when plated directly on a solid bacterial lawn. All 36 mutants were plated on a lawn of either EcC (left) or StGal (right). The absence of clear lysis plaques indicates that a given mutation is lethal on that host. Six out of 36 (17%) and 10 out of 36 (28%) mutants failed to form visible lysis plaques on EcC or StGal, respectively. With the possible exception of phage mutants P23 and P26, mutants with lethal phenotypes when plated on a bacterial lawn generally corresponded to those with the lowest estimated *s*-values when measured with qPCR (Figure 1). Mutants are labeled by their IDs (see Table 1) and lethal mutations are labeled in red. The column on the far right in each Petri dish contains repeated spots of the wild-type φX174.

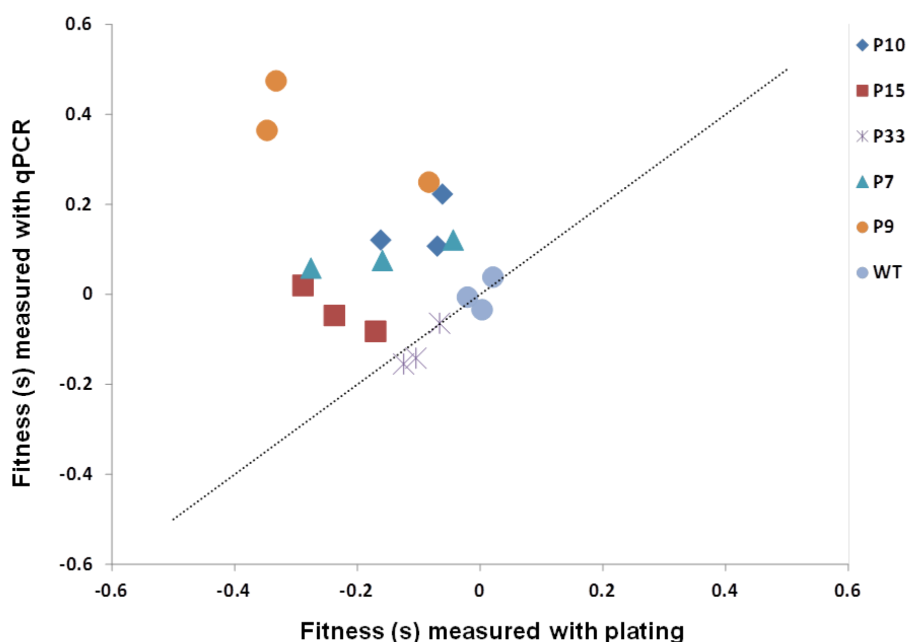

Figure S4. Measuring fitness. We assayed the densities of a subset of mutants relative to the wild-type in triplicate, both in liquid using qPCR and in solid medium by counting plaque forming units (pfu) on a bacterial lawn. Growth rates of 5 mutant and the wild-type φX174 were calculated as the ratio of final to initial titers, measured either using φX174-specific qPCR (y axis) or counting of plaque-forming units (pfus) on a bacterial lawn. This comparison of both assay methods revealed measures of fitness were higher when using qPCR relative to plating. Given that qPCR was calibrated using plating of serial-diluted wild-type φX174 (Figure S2), the two methods give congruent fitness values for the wild-type. However, for some (but not necessarily all) mutants, it is possible that a fraction of viruses estimated using qPCR is unviable (or undetectable) when grown on solid medium. Such mutants would yield inflated fitness values when densities are measured with qPCR relative to plating, which would only reveal virions that are viable in solid environments. Each data point is an independent replicate. *S*-values were calculated as described for the main experiment, as the hourly growth rate relative to the growth rate of the wild-type phage (see methods). The dotted diagonal line is illustrative of an expected correlation of 1.