Supplementary Methods

The Schrödinger Suite 2024 was used for protein preparation, homology modeling and molecular dynamics (MD) simulations [1].

Protein Preparation and Homology Modeling

The crystal structure of the carboxy-terminal domain of the bacteriophage T7 tail fiber Gp17 (residues 371-553) was used for the MD simulations. The Protein Data Bank (www.rcsb.org) was used to retrieve the 3D crystal structure at 1.9 Å resolution [2]. This structure served as a template for the knowledge-based homology modeling of mutant T7evo-YP (131-5) using the BioLuminate interface [1,3,4]**.** The knowledge-based method is the default, and it constructs insertions and closes gaps using segments from known structures [1,5].

Molecular Dynamics (MD) simulations

MD simulation was performed using the DESMOND module in the Schrödinger LLC software[1,6]. The crystal structure 4A0T was used for the MD simulation. Each protein was immersed in an orthorhombic box of the SPC solvent model through the system builder panel. The solvated system was neutralized with counterions and a physiological NaCl concentration of 0.15 M. The OPLS4 force field was utilized [7]. The simulation was 500 ns long, using the NPT ensemble class at 300 K and 1.01325 bar. Finally, to do a comprehensive examination, trajectories of simulated systems were generated. Using the Desmond module of the Schrödinger program, the following metrics were measured at various points along the trajectory: root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF). RMSD displays the variation in a protein atom's structure during molecular simulation. A low RMSD indicates a more stable system in a simulation result. The Root Mean Square Fluctuation (RMSF) is useful for characterizing local changes along the protein chain.

Supplementary Figure legends

Supplementary Fig. 1 | Schematic of the continuous evolution system setup and fluidic configuration

a,b, Schematics of the continuous evolution systems. Growth medium is continuously supplied from media reservoirs to chemostat vessels, where bacterial cultures are maintained in exponential growth by continuous dilution and mixing. Culture from the chemostats is then transferred to a lagoon vessel, where phage infection and evolution occur under continuous-flow conditions. Effluent from the lagoon is directed to a waste reservoir to maintain steady-state dynamics, and sampling ports enable culture collection throughout the evolution process. **a, Positive selection system.** A single chemostat containing *Y. pestis* was used to impose positive selection for phage adaptation to the new host. Red arrows indicate the flow of *Y. pestis* culture, and green arrows indicate the flow of phage-infected culture to the waste reservoir. **b, Combined positive and negative selection system.** Separate media reservoirs and chemostats were used for *Y. pestis* and *E. coli* Δ*trxA* cultures. Effluents from both chemostats were combined in the lagoon vessel, where phage evolution occurred under simultaneous positive selection for *Y. pestis* infectivity and negative selection against variants retaining infectivity toward the original host. Red and black arrows indicate the flow of *Y. pestis* and *E. coli* cultures, respectively, and green arrows indicate the flow of phage-infected culture to the waste reservoir.

Supplementary Fig. 2 | Deletions within class I genes in evolved T7 mutants

a, Genomic organization of bacteriophage T7 highlighting functional gene classes, including class I genes involved in host–phage interactions. The region spanning genes 0.3–1 is shown in detail. **b,** Schematic representation of genomic deletions in eight evolved phage mutants relative to the parental T7 genome. All mutants harbor deletions within the class I gene region. Colored regions indicate retained genomic segments, whereas gray dashed boxes denote deleted regions. Segments derived from the positive-selection process are shown in turquoise, whereas those derived from the combined positive and negative selection process are shown in purple. Red lines within gene 1 indicate the N823H substitution in mutant 193-4 and the A827V substitution in mutant 190-4.

Supplementary Fig. 3 | Deletions within class II genes in a subset of evolved T7 mutants

a, Genomic organization of bacteriophage T7 highlighting functional gene classes, including class II genes associated with DNA replication. The region spanning genes 4.1–5 is shown in detail. **b,** Schematic representation of genomic deletions in evolved phage mutants relative to the parental T7 genome. Deletions within the class II region were detected in a subset of mutants. Colored regions indicate retained genomic segments, whereas dashed boxes denote deleted regions. Segments derived from the positive-selection process are shown in turquoise, whereas those derived from the combined positive and negative selection process are shown in purple. A red line within gene 5 in mutant 193-4 indicates the A650V substitution.

Supplementary Fig. 4 | Structural localization of substitutions in the T7evo-Yp tail fiber

a, Schematic representation of bacteriophage T7. **b,** Structural model of the trimeric T7 Gp17 tail fiber generated using AlphaFold2, highlighting the positions of amino acid substitutions identified in the T7evo-Yp mutant. Substitutions within the distal tip domain (G521R and N537I), as well as additional substitutions located in the hinge (N215D) and N-terminal (T118M) regions, are indicated. The N537I substitution site is shown in orange using CPK representation, whereas the remaining substitution sites are shown in yellow. The top-ranked AlphaFold2 prediction of the assembled tail fiber structure is colored according to the AlphaFold confidence score, ranging from blue (high confidence) to red (low confidence).

Supplementary References

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