Legends of Supplementary Figures

Figure S1. Nucleoside analysis of phage S6 DNA using liquid chromatography–mass spectrometry (LC-MS). (a) Ultraviolet (UV) chromatograms of the nucleoside samples. Phage S6 DNA (1 μg) was digested to release nucleosides using DNA Degradase Plus (Zymo Research, Irvine, CA, USA), according to the manufacturer's instructions. After incubation for 2 h, 175 μL of 0.1% formic acid was added to the 25-μL reaction volume. 2′-Deoxyadenosine monohydrate, 2′-deoxycytidine, 2′-deoxyguanosine monohydrate, thymidine, and 2′-deoxyuridine (Sigma-Aldrich Co., St Louis, MO, USA) were used as the nucleoside standards. The nucleoside samples were analyzed using an electrospray ionization (ESI)-LC-MS instrument (Exactive, Thermo Fisher Scientific, Waltham, MA, USA), which was combined with a UV detector (Nanospace SI-2, Shiseido, Tokyo, Japan). The UV absorbance was measured at 254 nm. The top and bottom columns show the UV chromatograms of standard nucleosides and the nucleosides of phage S6 DNA, respectively. The peaks were numbered and subjected to mass spectrometric analysis. (b) Analysis of nucleosides by mass spectrometry. The peaks shown in (a) were analyzed using ESI-MS. According to the nucleoside standard data (peaks 1–5), peaks 6–9 were identified as deoxycytidine, deoxyguanosine, deoxyuridine, and deoxyadenosine, respectively. No peak corresponding to thymidine was detected in the

bottom UV chromatogram shown in (a). Thus, thymine was considered to have been replaced by uracil in the genomic DNA of phage S6.

Figure S2. Infectivity of phages in *Staphylococcus* spp. (a) Siphophage 80. (b) Siphophage ϕMR25. (c) Podophage S13′. (d) Myophage S25-3. (e) Giant myophage S6. The phage infectivity was examined in *S. felis*, *S. arlettae*, *S. kloosii*, *S. pettenkoferi*, *S. schleiferi*, *S. sciuri*, *S. pseudintermedius*, *S. aureus*, and *S. epidermidis* (Supplementary Table S1). Each graph shows a summary of the phage host range. In the graphs, the thick gray, black, and white bars indicate the plaque-forming capacity, lysis-from-without activity, and the absence of both activities, respectively. Phages S6 and S25-3 exhibited infectivity in non-*aureus* staphylococci, whereas phages 80, ϕMR25, and S13′ did not.

Figure S3. Phage S6 cultivation. (a) Lytic activity in liquid media. *S. aureus* strain SA27 (ca. 1.0×10^7 cells) was incubated with the phage at an initial multiplicity of infection of 10^{-4} in 150-μL tryptic soy broth per well in a 96-well flat-bottomed microtiter plate, at 30°C or 37°C. Bacterial growth was monitored every 15 min by measuring the optical density at 600 nm using a HiTS incubation plate reader (Scinics Corporation, Tokyo, Japan). The black and white circles indicate the 30°C and 37°C incubations, respectively.

(b) Plaque formation. Phage S6 was cultured overnight with *S. aureus* strain SA27 on tryptic soy broth-based double-layered agar at 30°C or 37°C. The bacterial lawn was stained with 2% 2,3,5,-triphenyltetrazolium chloride. Plaque formation at 30°C and 37°C are shown on the left and right, respectively. The scale bars represent 1 cm. The plaque sizes at 30°C and 37°C incubations were 0.91 ± 0.30 cm and 0.22 ± 0.08 cm, respectively (mean ± standard deviation; n = 30). Based on these observations, we cultured phage S6 at 30°C during phage amplification, which is different from the typical culture temperature of 37°C for staphylococcal phages.

Figure S4. Intraspecies phage transduction in *S. aureus*. Efficiency of transduction from *S. aureus* strain RN4220 harboring pCU1 into strain RN4220 via staphylococcal phages. The graph shows the transduction efficiencies with phages S6, 80, ϕMR25, S25-3, and S13′. The means and standard deviations are shown in the graph (mean ± standard deviation; n = 6). Phages ϕMR25 and S6 exhibited significantly higher transduction efficiencies than phage 80 (ANOVA, $P < 0.05$). A lack of transduction activity is indicated by “ND” in the graph.