Supplementary Figures

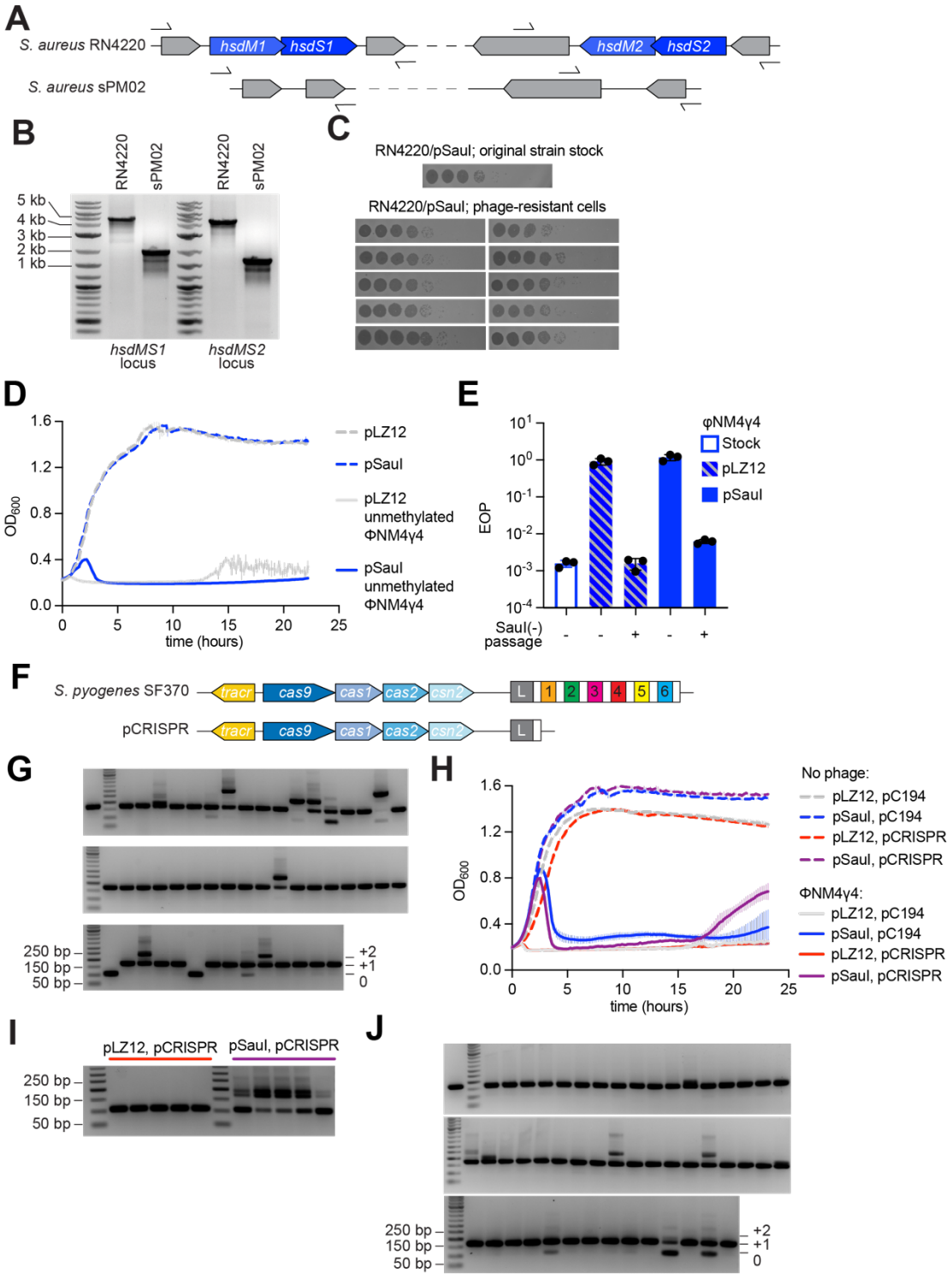

Figure S1. Construction of *S. aureus* sPM02 lacking the SaulI restriction-modification system (related to Figure 1). (**A**) The two *saul-hsdMS* operons present in *S. aureus* RN4220 were removed (in-frame deletion) to generate strain sPM02. Arrows; primers used to check for the presence of the deletion. (**B**) Agarose gel

electrophoresis of PCR products obtained after amplification of the *hsdMS1* and *hsdMS2* loci using template DNA from RN4220 and sPM02 strains, and the primers shown in (**A**). (**C**) Plaque assays using bacterial lawns seeded with cells from 10 single colonies recovered from phage-resistant cells at the end of the growth curve shown in Fig. 1B, in which staphylococci harboring pSauI were infected with unmethylated ΦNM4γ4. 10-fold dilutions of unmethylated phage were spotted on the lawns. A lawn of the original stock strain carrying pSauI was used as control. (**D**) Growth of staphylococci expressing SauI or carrying a vector control in the presence or absence of ΦNM4γ4 infection, measured as the OD_{600} of the cultures over time. MOI ~250. Mean of three biological replicates ± SD are reported. (**E**) EOP of a phage stock, or of phages obtained at the end of the growth curve shown in (**D**), amplified or not through the non-methylating strain sPM02, after plating on lawns of staphylococci expressing SauI, relative to PFUs obtained with cells carrying a vector control. Mean of three biological replicates ± SD are reported. (**F**) Schematic of the *S. pyogenes* type II-A CRISPR-*cas* locus. Grey rectangle, leader sequence ('L"); white rectangle, repeat; colored, numbered rectangles, spacers. It was cloned into the staphylococcal vector pC194 without spacers, just a single repeat. (**G**) Agarose gel electrophoresis of PCR products obtained after the amplification of the CRISPR array from 50 individual colonies recovered from one of the pSauI/pCRISPR cultures infected with ΦNM4γ4 at the end of experiment in Fig. 1D. (**H**) Growth of staphylococci harboring different plasmid combinations, in the presence or absence of ΦNM4γ4 infection, measured as the OD_{600} of the cultures over time. MOI ~10. Mean of three biological replicates ± SD are reported. (**I**) Agarose gel electrophoresis of PCR products obtained after amplification of the CRISPR array using DNA obtained from the cultures shown in (**H**). (**J**) Same as (**G**) after the amplification of the CRISPR array from 50 individual colonies recovered from one of the pSauI/pCRISPR cultures infected with ΦNM4γ4 at the end of the experiment shown in (**H**).

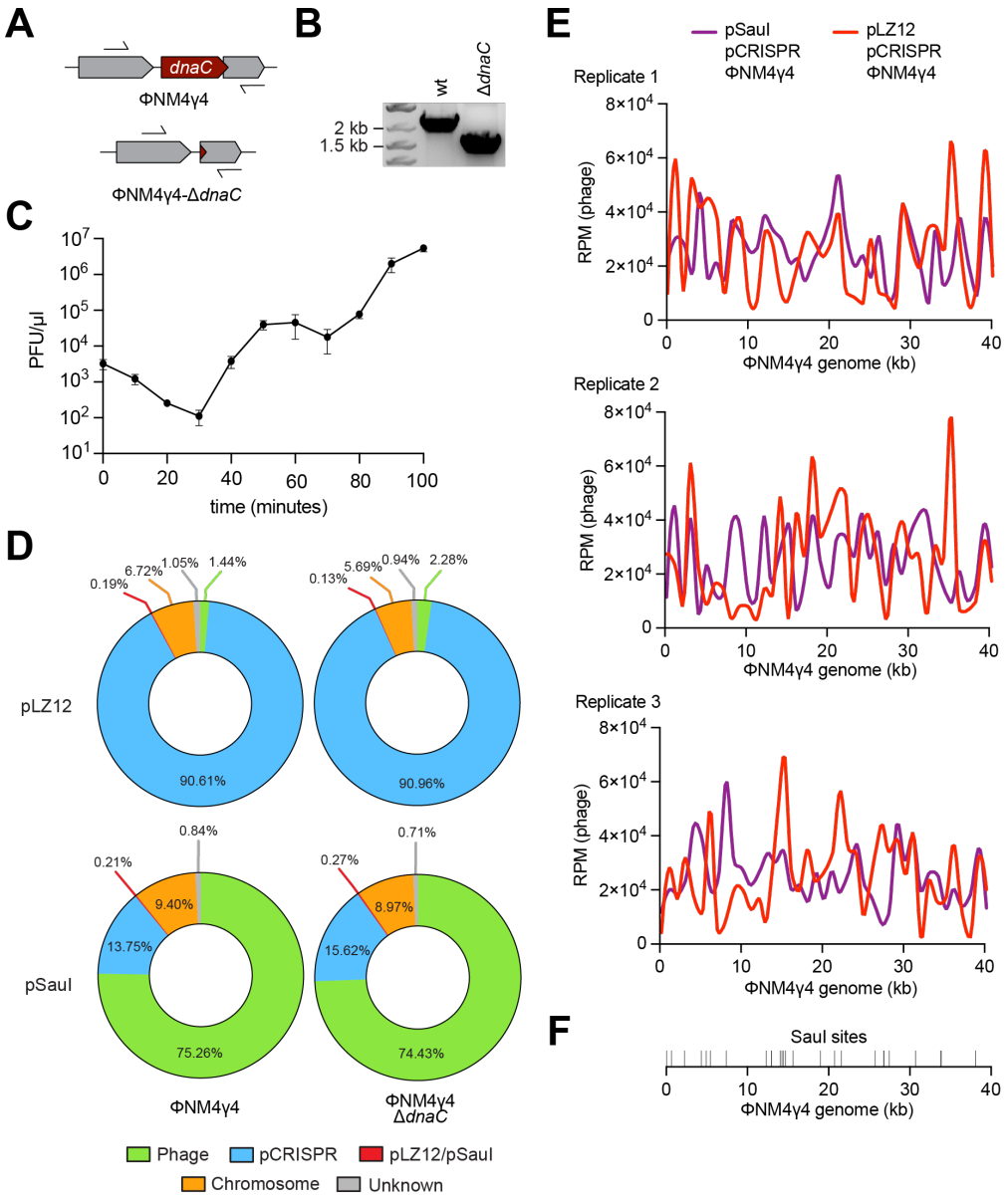

Figure S2. Spacer acquisition from the ΦNM4γ4's genome in the presence of SaulI restriction (related to Figure 2). (**A**) The *dnaC* gene of ΦNM4γ4 was removed (in-frame deletion) to generate ΦNM4γ4-Δ*dnaC*. Arrows; primers used to check for the presence of the deletion. (**B**) Agarose gel electrophoresis of PCR products obtained after amplification of the *dnaC* locus using template DNA from ΦNM4γ4 and ΦNM4γ4-Δ*dnaC*, and the primers shown in (**A**). (**C**) Quantification of the number of phage in the supernatant after 10-minute intervals following infection of *S. aureus* RN4220 with ΦNM4γ4 at MOI ~0.1. Mean of three biological replicates ± SD are reported. (**D**) Quantification, using NGS reads, of spacers derived from the DNA substrates indicated with different colors, relative to total new spacers, acquired 30 minutes after infection of *S. aureus* harboring pCRISPR and expressing SaulI or carrying a vector control, pLZ12, with ΦNM4γ4 or ΦNM4γ4-Δ*dnaC* phages. MOI ~250. Mean of three biological replicates

are reported. (**E**) Distribution of spacer abundance (measured as RPM of phage-matching reads) obtained in Fig. 2B across the ΦNM4γ4 genome. Maps for three independent replicates are shown. (**F**) Schematic representation of the ΦNM4γ4 genome showing its 26 SauI sites (grey lines).

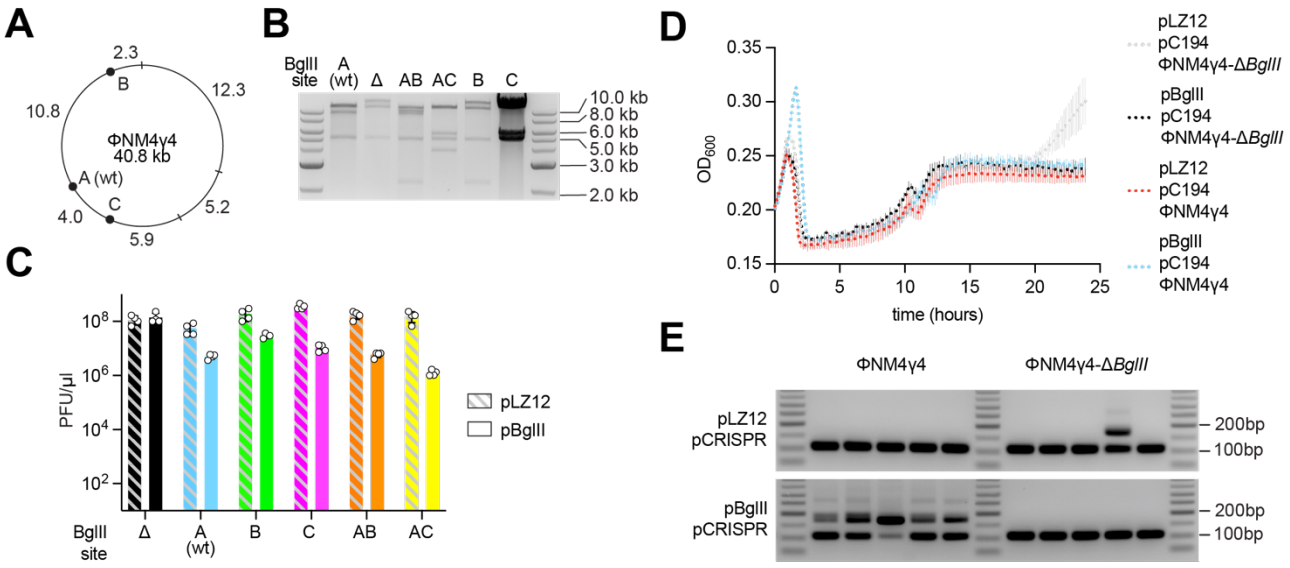

Figure S3. BglII restriction of ΦNM4γ4 promotes spacer acquisition (related to Figure 3). (**A**) Map of the ΦNM4γ4 genome showing the different BglII sites analyzed in this study (black circles, "A", "B", "C"). The three BssHII sites used for restriction mapping are shown as well (black lines). (**B**) Agarose gel electrophoresis of restriction fragments of different ΦNM4γ4 phages, after digestion with BssHII and BglII. The sizes of molecular weight markers are shown. (**C**) Enumeration of PFU generated by different ΦNM4γ4 phage stocks on lawns of staphylococci expressing BglII or carrying an empty vector control. Mean of four biological replicates ± SD are reported. (**D**) Growth of staphylococci expressing BglII or carrying a vector control after infection with ΦNM4γ4 or ΦNM4γ4-Δ*BglII* phages, measured as the OD_{600} of the cultures over time. MOI ~10. Mean of five biological replicates ± SD are reported. (**E**) Agarose gel electrophoresis of PCR products obtained after amplification of the CRISPR array using DNA obtained from the cultures used in Fig. 3B.

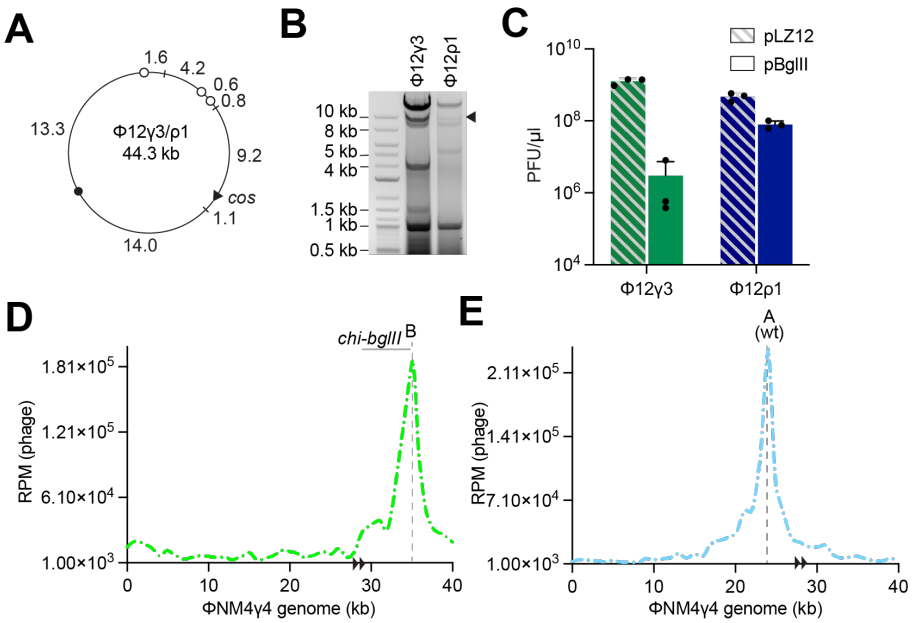

Figure S4. Analysis of BglII restriction of ΦNM4γ4 and Φ12ρ1 (related to Figure 4). (**A**) Map of the Φ12γ3 and Φ12ρ1 genomes showing the BglII present in both phages (black circles), as well as those removed from Φ12γ3 to generate Φ12ρ1 (white circles). The three BsrBI sites used for restriction mapping (black lines), as well as the *cos* site (black arrowhead), are also shown. (**B**) Agarose gel electrophoresis of restriction fragments of Φ12γ3 and Φ12ρ1 phage DNA, after digestion with BsrBI and BglII. The sizes of molecular weight markers are shown. The black arrowhead marks a restriction fragment produced after the annealing of the *cos* site. (**C**) Enumeration of PFU generated by Φ12γ3 or Φ12ρ1 on lawns of staphylococci expressing BglII or carrying an empty vector control. Mean of three biological replicates ± SD are reported. (**D**) Distribution of spacer abundance (measured as RPM of phage-matching reads) across the ΦNM4γ4-B genome, using NGS data collected after infection of *addA*^{n} mutant staphylococci cells carrying pCRISPR and pBglII. (**E**) Same as (**D**) using data from ΦNM4γ4-A(wt) infection experiments.

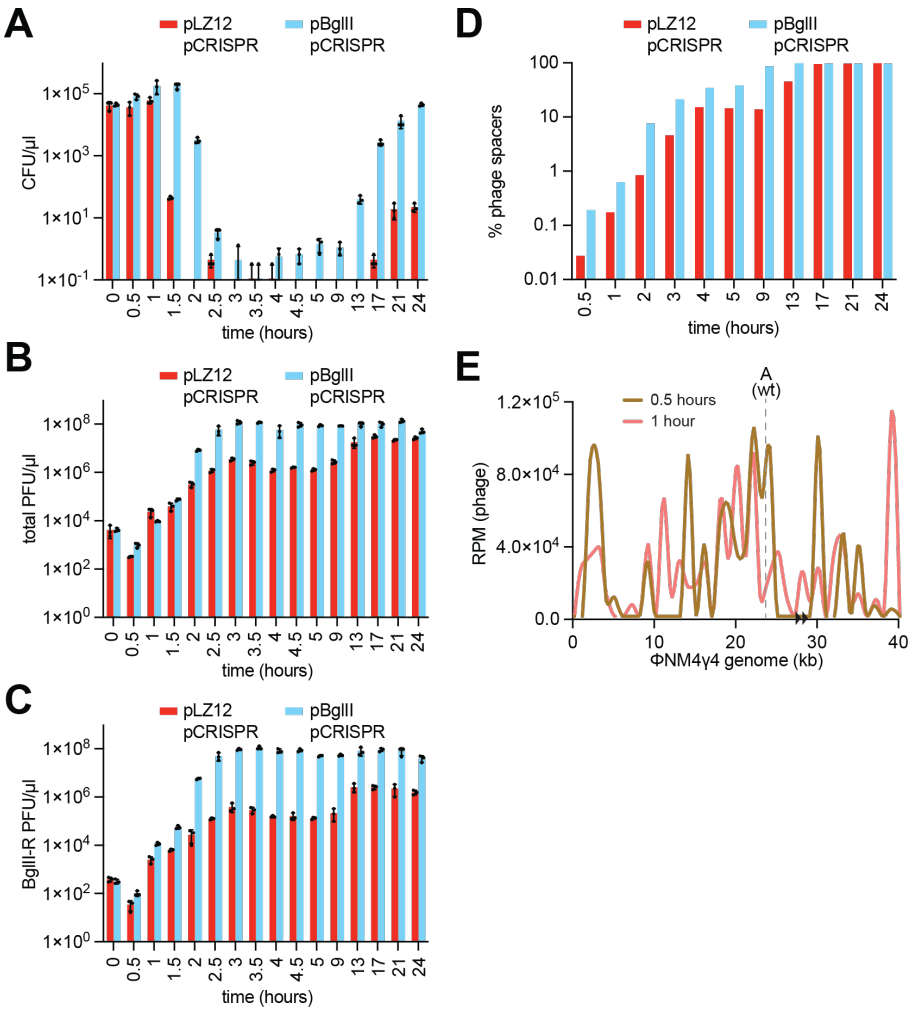

Figure S5. Dynamics of ΦNM4γ4 restriction, methylation, and spacer acquisition (related to Figure 5). (**A**) Enumeration of CFUs present at different times following infection of *S. aureus* cells harboring pCRISPR and expressing BglII or carrying a vector control with ΦNM4γ4 (MOI ~1). Mean of three technical replicates ± SD are reported. (**B**) Enumeration of total PFUs present in the cultures of the experiment described in (**A**), after plaquing on the CRISPR(-), RM(-) strain RN4220. Mean of three technical replicates ± SD are reported. (**C**) Same as (**B**) but plaquing on staphylococci expressing BglII to enumerate PFUs resistant to restriction. Mean of three technical replicates ± SD are reported. (**D**) Quantification of phage-derived spacers, relative to total new spacers, acquired at the different time points of the experiment shown in (**A**). (**E**) Distribution of spacer abundance (measured as RPM of phage-matching reads) obtained after NGS of the CRISPR locus present in the cultures used in (**A**) across the ΦNM4γ4 genome, using data from the 0.5- and 1-hour time points.

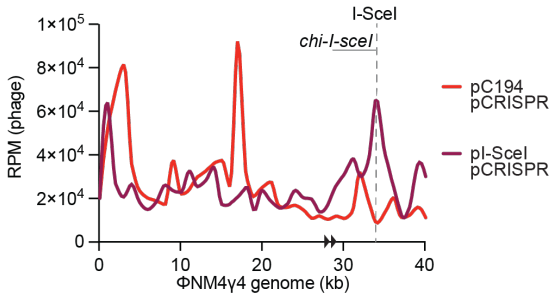

Figure S6. SceI restriction of ΦNM4γ4 promotes spacer acquisition at the cleavage site (related to Figure 6). Distribution of spacer abundance (measured as RPM of phage-matching reads) across the ΦNM4γ4-I-SceI genome, using NGS data collected after infection of staphylococcal cells carrying pCRISPR and pI-SceI or the control vector pC194.