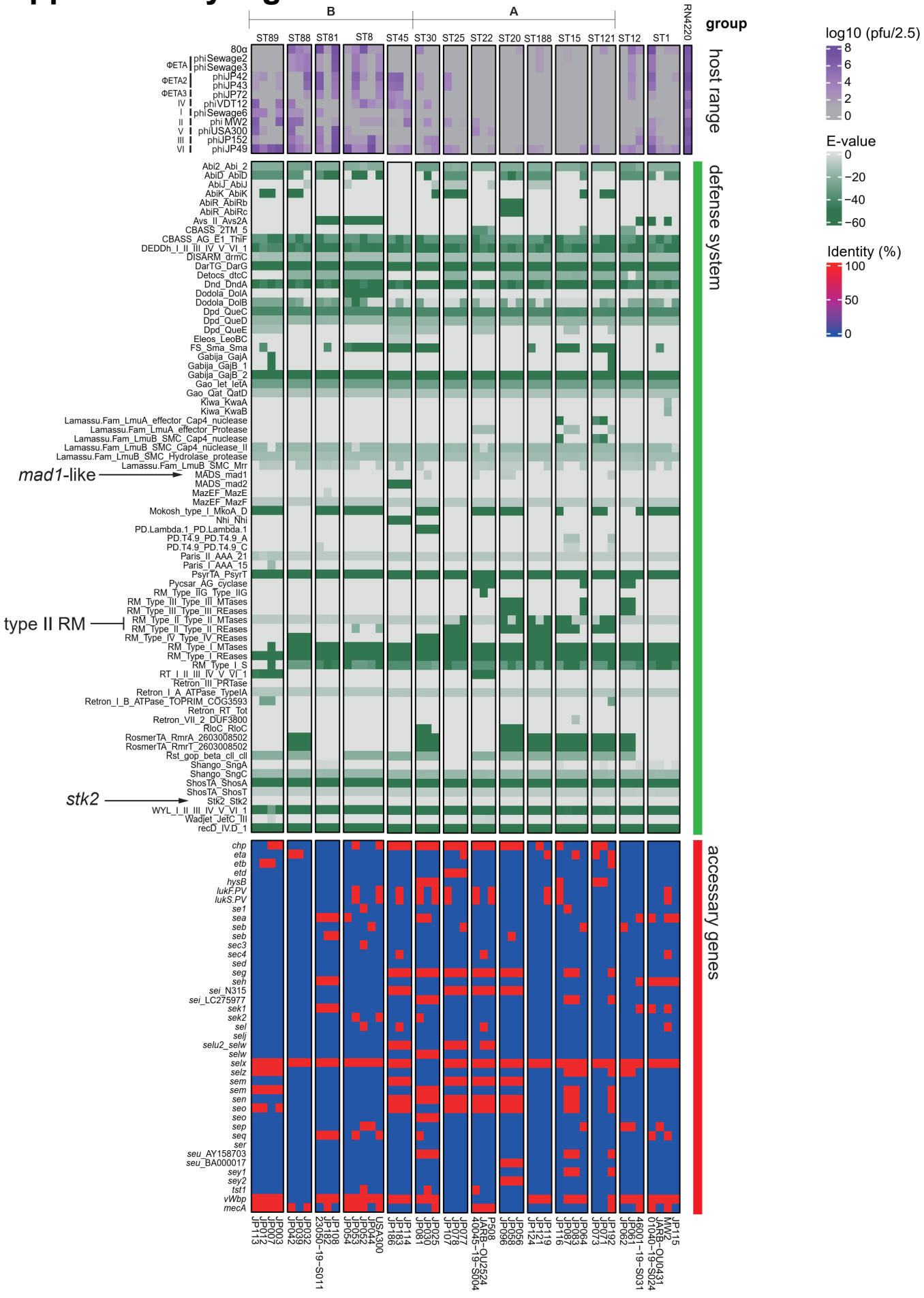
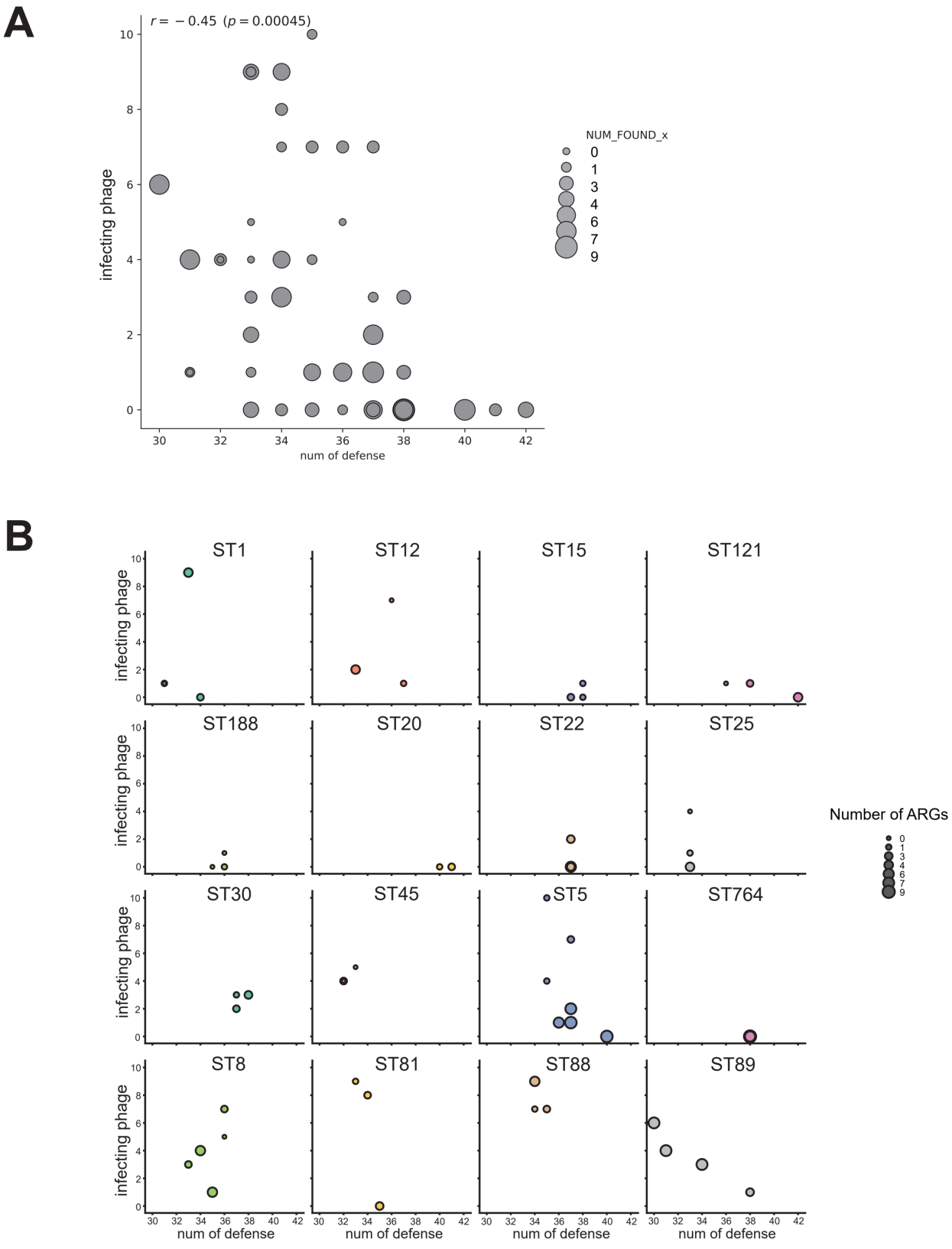
Supplementary Fig. 1.



Supplementary Fig. 1. Distribution of phage defense systems, virulence factors, antimicrobial resistance genes, and temperate phage host range among clinical *S. aureus* isolates

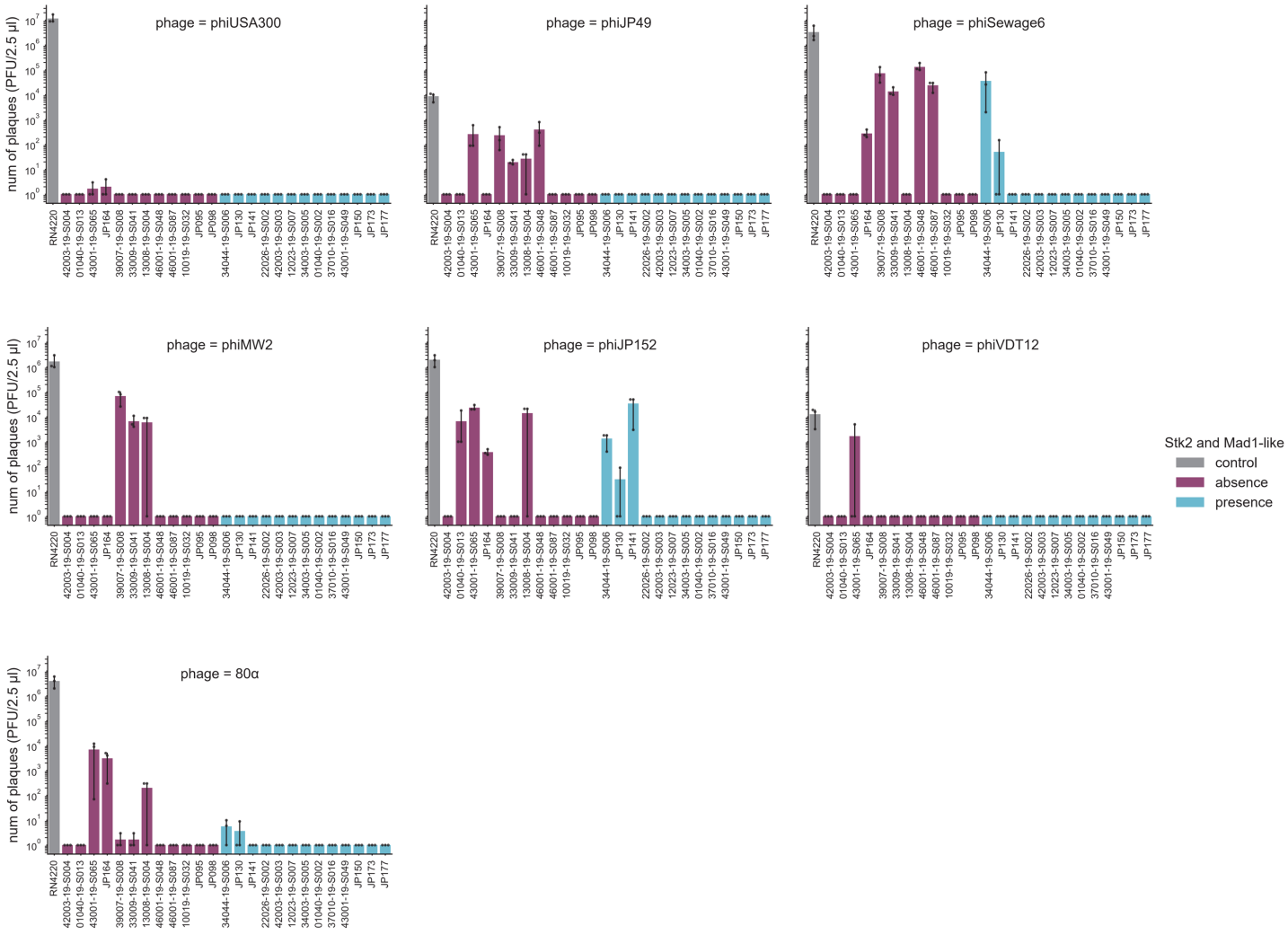
Heatmap summarizing the characteristics of 58 clinical *S. aureus* isolates representing 16 sequence types (STs) collected in Japan. Columns represent individual isolates grouped by ST. The upper panel shows the infectivity of representative temperate phages grouped as in Fig. 1 (ΦETA, ΦETA2, ΦETA3, and I–VI), expressed as log10 PFU/2.5 µL. A and B denote host-range groups: A, generally susceptible to fewer than five phages, and B, generally susceptible to five or more phages. The middle panel shows the predicted phage defense systems, with color intensity reflecting the log10-transformed E-value. The lower panel indicates the presence or absence of accessory genes, including virulence factor and antimicrobial resistance genes.

Supplementary Fig. 2.



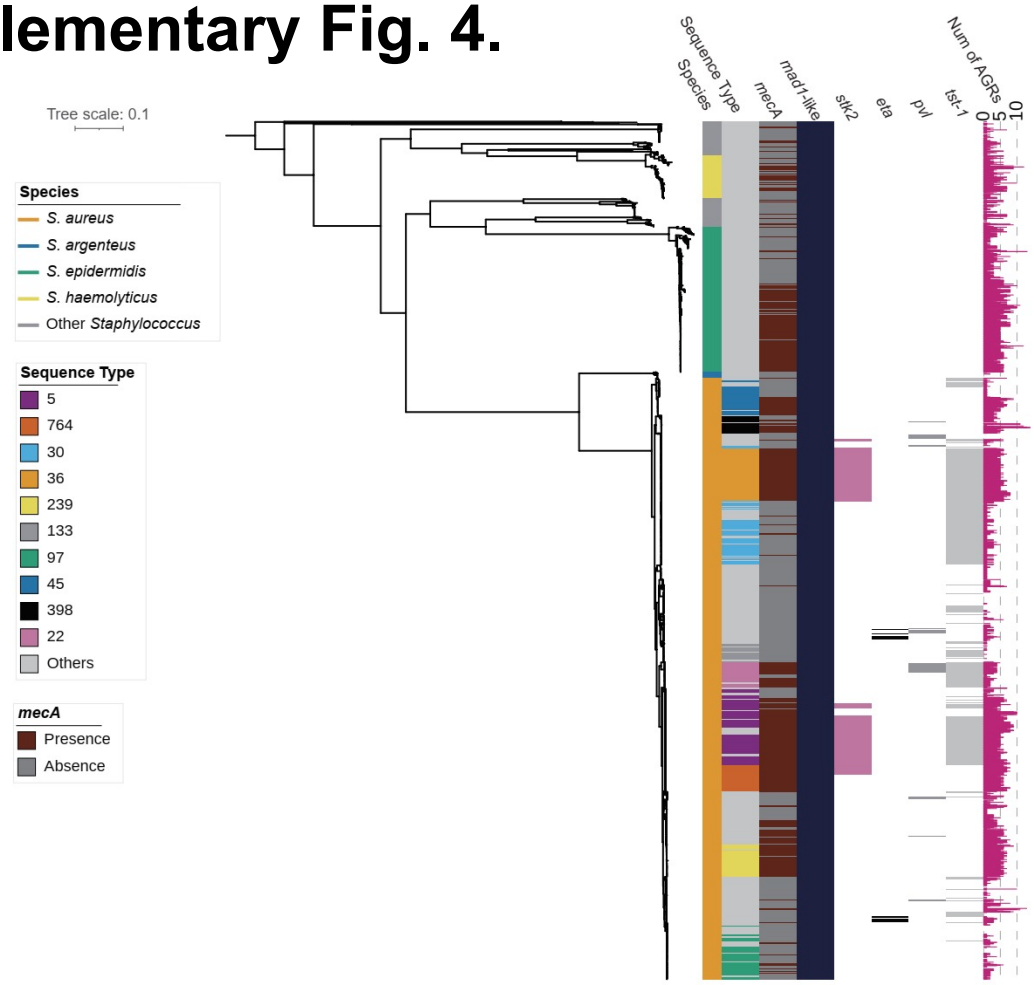
Supplementary Fig. 2. Relationship between the number of phage defense systems and susceptibility to phage infection
(A, B) Bubble plots depicting the relationship between the number of phage defense systems and the number of infecting phages per strain, shown overall (A) and stratified by sequence type (ST; B). Each circle represents a single strain used in the host-range assay; the x-axis indicates the number of phage defense systems identified in each genome, the y-axis indicates the number of infecting phages, and circle size reflects the number of antibiotic resistance genes (ARGs) detected in each strain. A weak negative correlation was observed between the number of phage defense systems and the number of infecting phages (R = −0.45).

Supplementary Fig. 3.

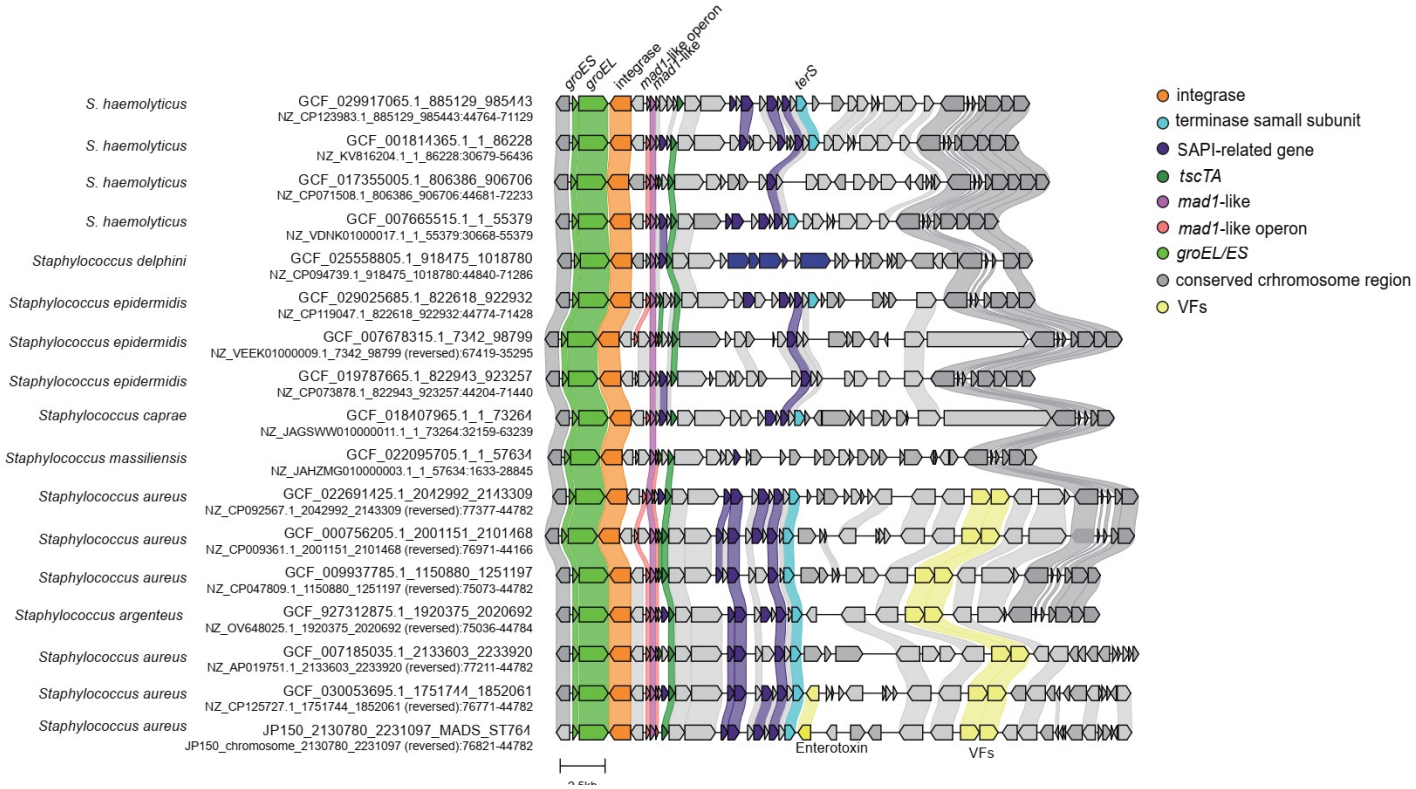


Supplementary Fig. 3. Relationship between the phage defense system and phage susceptibility in CC5 strains used in Fig. 2B
A spot assay using a broader panel of CC5 strains was performed to investigate the relationship between *stk2* and *mad1*-like systems and susceptibility to representative non-ETA temperate phages, as shown in Fig. 2A. A total of 25 CC5 strains were tested: 12 strains lacking both *stk2* and *mad1*-like systems (cyan) and 13 strains possessing both systems (light blue). For each phage, the x-axis shows the strain name, and the y-axis shows the phage infectivity as the number of plaques (PFU/2.5 µL).

Supplementary Fig. 4.



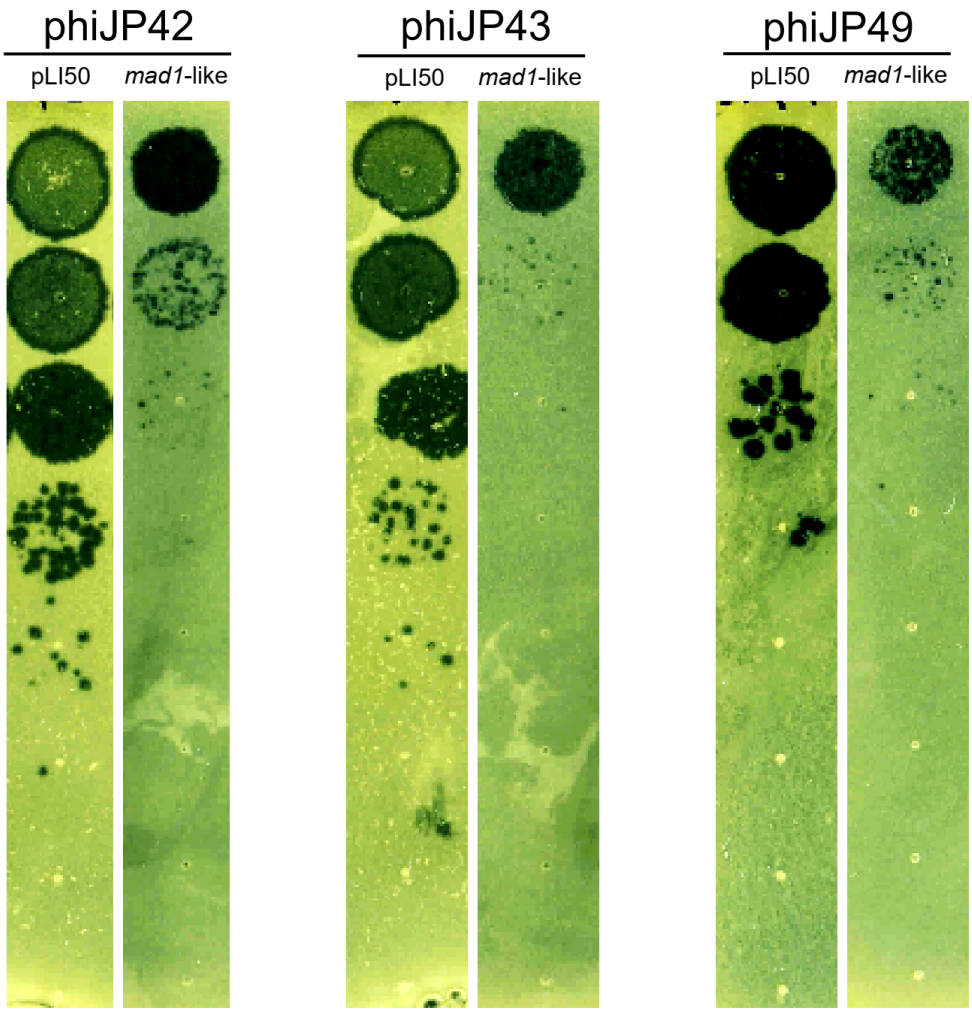
B



Supplementary Fig. 4. Genomic landscape of strains harboring the *mad1*-like operon

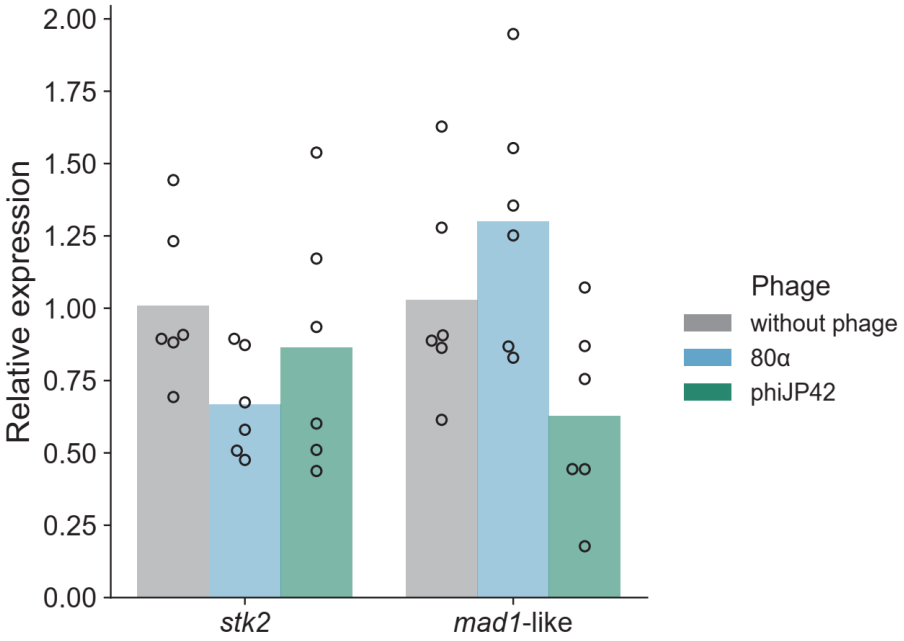
(A) Phylogenetic tree constructed from SNPs identified in 418 core genes across 3,144 genomes. Metadata displayed alongside each strain include the strain name, ST, and the presence of *mecA* and several VFGs (*eta*, *pvl*, and *tst-1*). The bar chart on the right shows the number of ARGs per strain. **(B)** Representative genomic contexts of the *mad1*-like operon across *Staphylococcaceae* strains. The *mad1*-like operon was typically positioned adjacent to integrase genes (orange) and inserted near the conserved *groES–groEL* region (shown in green arrows), although the surrounding operon structures varied among lineages. Genes associated with SaPIs, such as *terS*, are shown in blue, and VFGs in yellow.

Supplementary Fig. 5.



Supplementary Fig. 5. Plaque morpholpgies corresponding to the "S" phenotype shown in Fig. 5B
Spot assay images showing plaque morphology of phages phiJP42, phiJP43, and phiJP49 diluted in a 10-fold serial dilution on *S. aureus* RN4220 harboring pLI50, pLI50/*mad1*-like, or pLI50/*stk2*. Expression of the *mad1*-like operon resulted in reduced plaque size for phiJP42, phiJP43, and phiJP49 compared with the empty vector control (RN4220-pLI50), corresponding to the "S" phenotype in Fig. 5B.

Supplementary Fig. 6.



Supplementary Fig. 6. Relative expression of *stk2* and *mad1*-like genes 30 min after phage exposure
Relative expression levels of *stk2* and *mad1*-like were measured by quantitative real-time PCR under the no-phage condition and 30 min after exposure to phages 80α or phiJP42. Expression levels were normalized to *gyrB* and calculated using the $2^{-\Delta\Delta Ct}$ method, with the no-phage condition used as the calibrator. Bars represent the mean of two independent biological replicates; each performed in technical triplicate. Mann–Whitney *U* test with Holm correction: *stk2*, phiJP42, $P = 0.39$; 80α, $P = 0.18$; *mad1*-like, phiJP42, $P = 0.05$; 80α, $P = 0.48$.