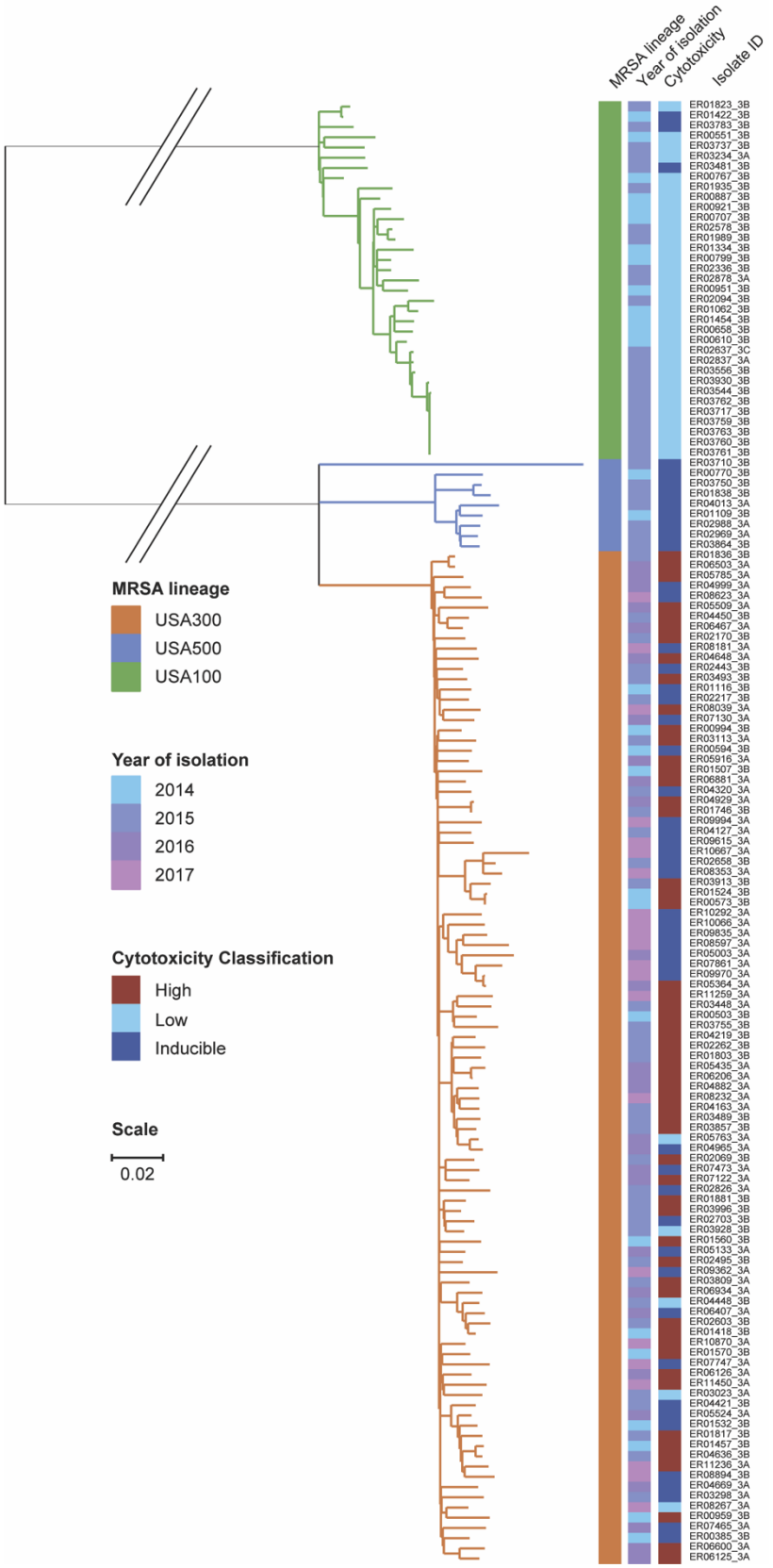
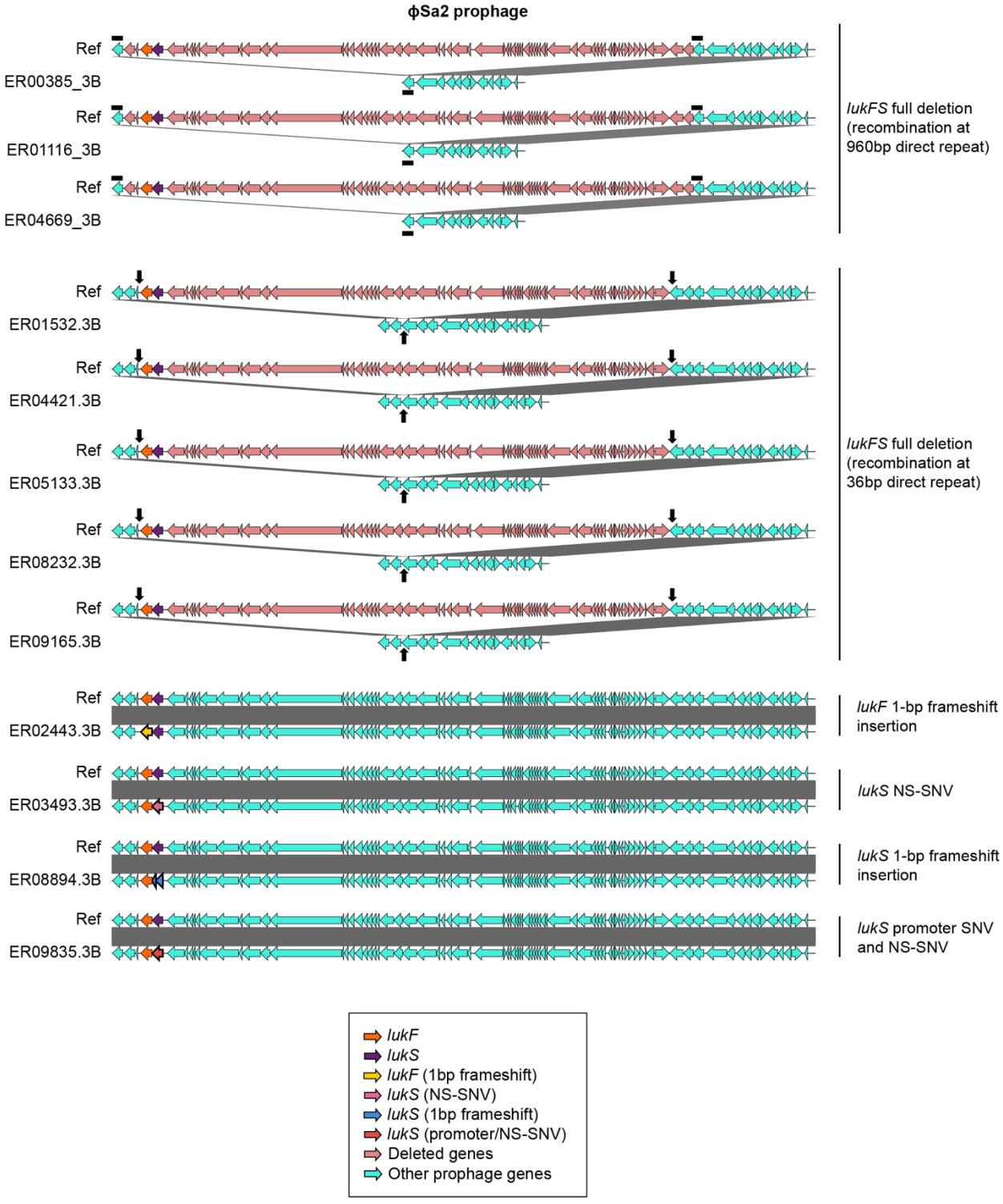
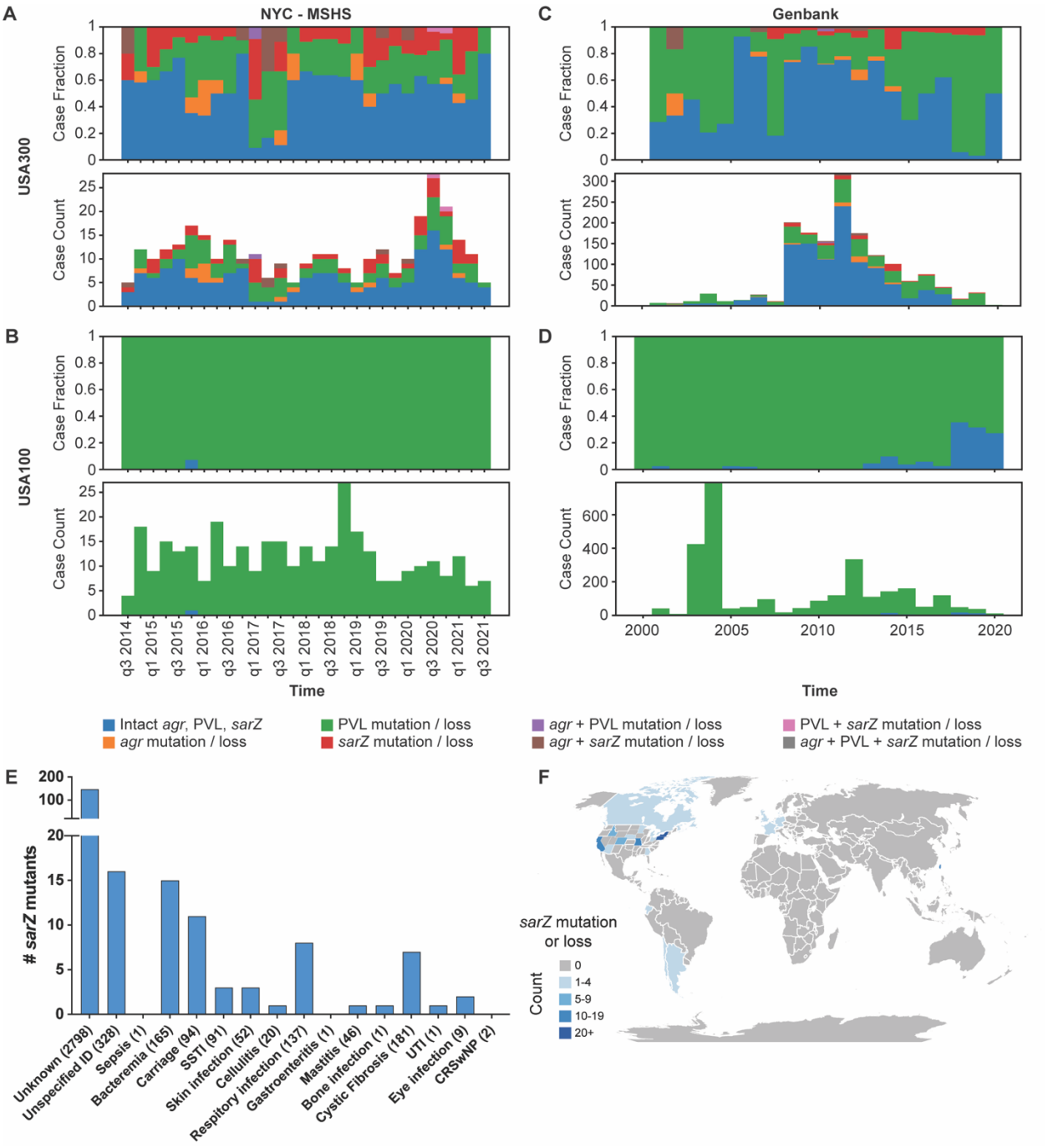
SUPPLEMENTAL INFORMATION



Supplementary Figure 1. Phylogenetic tree of all BSI isolates, related to Figure 1. Phylogenetic tree of all BSI isolates used in this study with USA group, year and cytotoxicity classification shown on the right-hand side.

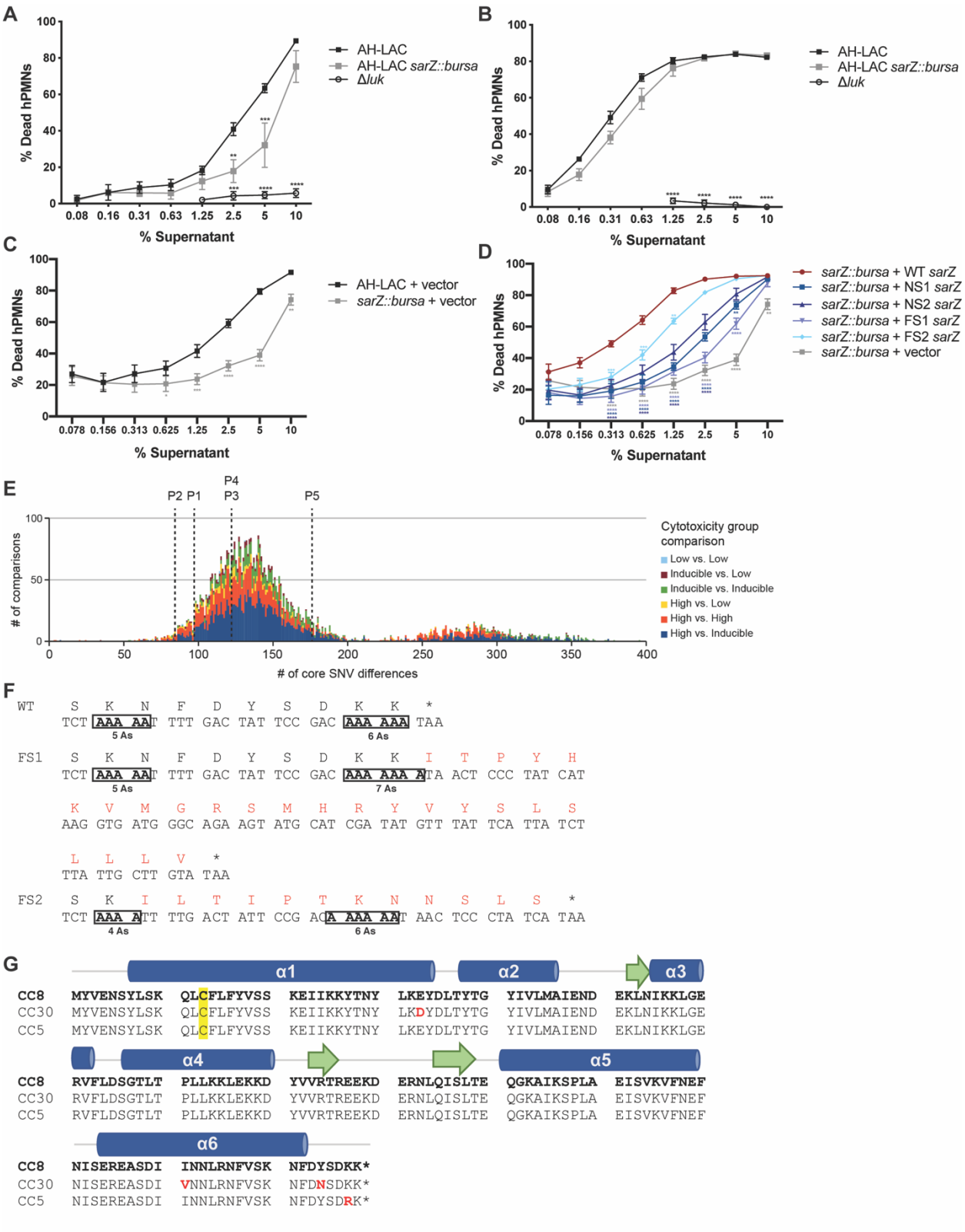

ϕSa2 prophage
Ref
ER00385_3B
Ref
ER01116_3B
Ref
ER04669_3B
lukFS full deletion (recombination at 960bp direct repeat)
Ref
ER01532.3B
Ref
ER04421.3B
Ref
ER05133.3B
Ref
ER08232.3B
Ref
ER09165.3B
lukFS full deletion (recombination at 36bp direct repeat)
Ref
ER02443.3B
lukF 1-bp frameshift insertion
Ref
ER03493.3B
lukS NS-SNV
Ref
ER08894.3B
lukS 1-bp frameshift insertion
Ref
ER09835.3B
lukS promoter SNV and NS-SNV
lukF
lukS
lukF (1bp frameshift)
lukS (NS-SNV)
lukS (1bp frameshift)
lukS (promoter/NS-SNV)
Deleted genes
Other prophage genes

Supplementary Figure 2. Changes found in the PVL phage, related to Figure 2. Pairwise genome comparisons of isolates with complete deletion or mutation of one or both genes in the *lukSF-PV* locus. Each isolate is compared to the PVL phage of *Staphylococcus aureus* USA300_FPR3757 (NC_007793.1) using Easyfig[S1]. Gray blocks indicate regions of alignment between the genomes determined by BLASTn. Arrows indicate predicted genes and colored according to the key shown below.



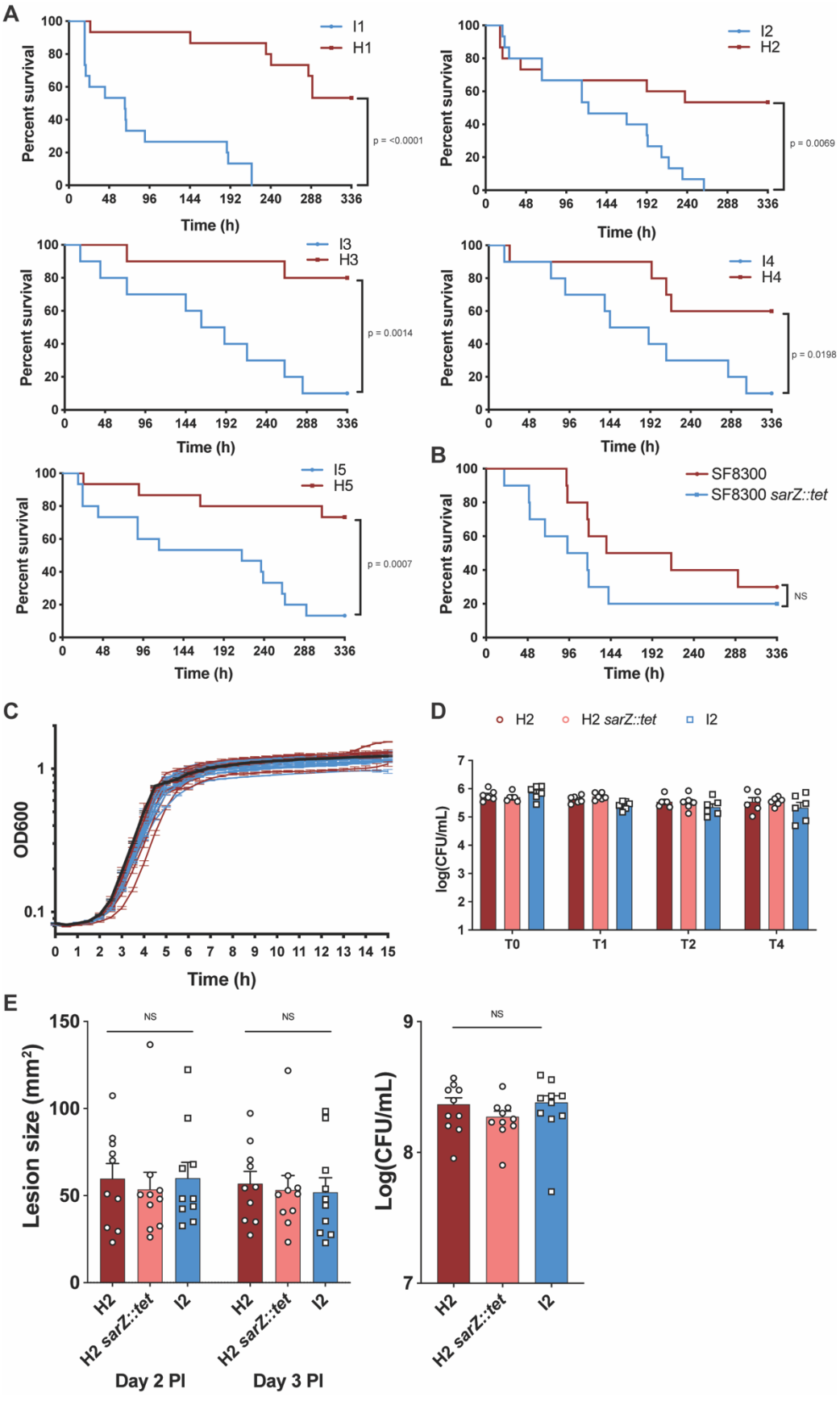
Supplementary Figure 3. Frequency of *sarZ*, *agr*, and *lukSF-PV* mutations and gene loss identified in inducible cytotoxicity isolates among MRSA genomes from human isolates sampled in NYC and worldwide, related to Figure 2. The quarterly frequency of *sarZ*, *agr* and PVL inducible cytotoxicity mutations in **(A)** USA300 and **(B)** USA100 MRSA bacteremia genomes from the MSHS in NYC between the third quarter (q3) of 2014 and q3 of 2021, are shown together with the annual frequency of mutations

in global **(C)** USA300 and **(D)** USA100 MRSA genomes from Genbank between 2000 and 2020. Each panel includes the fraction (top) and number (bottom) of genomes with a specific combination of mutations and/or gene loss (legend at bottom). **(E)** Number of USA300 genomes with a disease annotated in the Biosample data and a *sarZ* mutation identical to one observed in our dataset. X-axis labels indicate the type of disease with the total number of USA300 isolates in brackets. **(F)** Same as E, but now showing the distribution across countries.

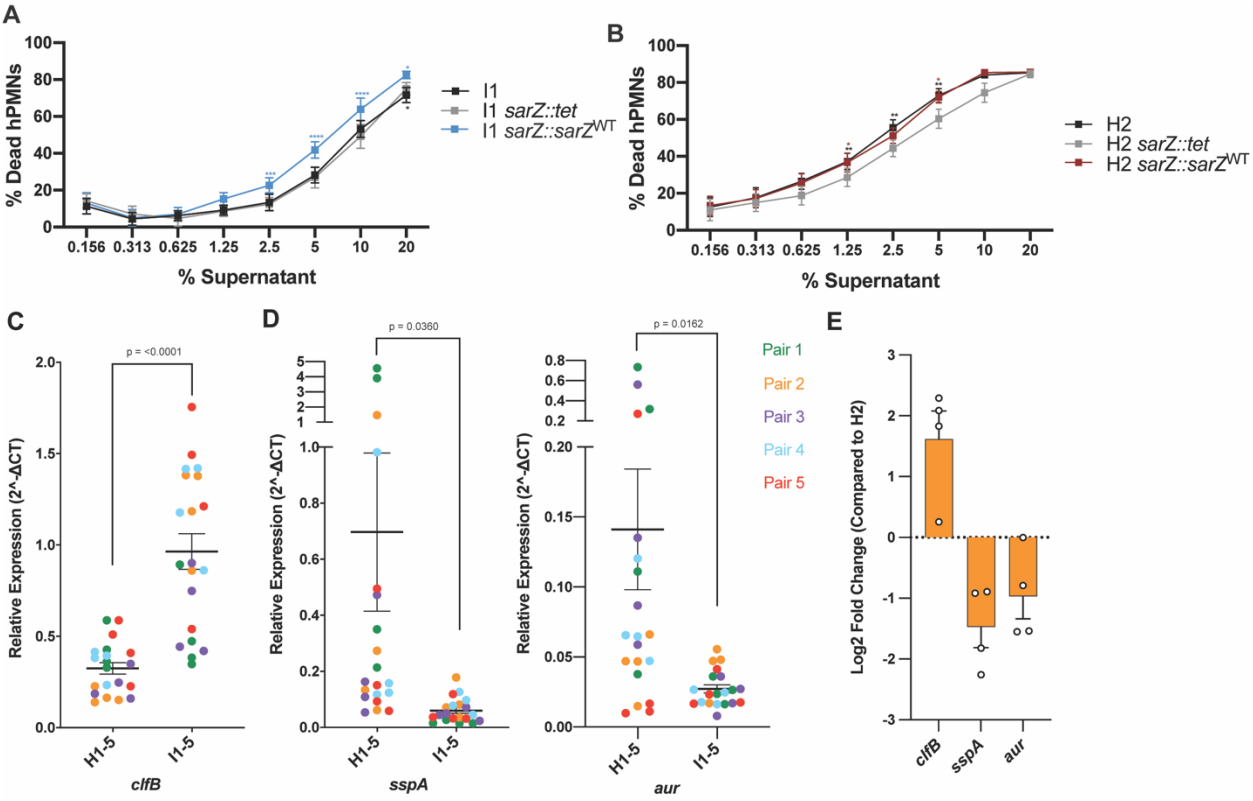


Supplementary Figure 4. Examining naturally occurring *sarZ* mutations, related to Figure 3. Panels **A-D** are cytotoxicity titration curves, showing each concentration of

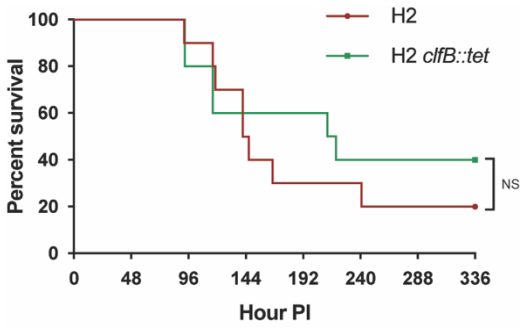
USA300 supernatant tested for cytotoxicity to hPMNs. Representative concentrations are depicted in Fig.3 for simplicity. **(A)** AH-LAC, AH-LAC *sarZ::bursa* and AH-LAC ∆*luk* strains grown in TSB, n = 4 donors. Statistical significance comparing AH-LAC to mutants. **(B)** AH-LAC, AH-LAC *sarZ::bursa* and AH-LAC ∆*luk* strains grown in YCP, n = 4 donors. Statistical significance comparing AH-LAC to mutants. **(C)** AH-LAC + vector and AH-LAC *sarZ::bursa* + vector strains grown in TSB, n = 8 donors. Statistical significance comparing AH-LAC + vector to mutant. **(D)** AH-LAC *sarZ::bursa* strains complemented with *sarZ* alleles grown in TSB, n = 8 donors. Statistical significance comparing AH-LAC *sarZ::bursa* + $sarZ^{WT}$ to other strains. Comparisons for all panels were done using a 2-way ANOVA, Sidak's multiple comparisons test. Error bars indicate SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, color indicates the sample the P-value corresponds to. **(E)** Histogram showing relatedness of pairs. All-versus-all pairwise core-genome SNV distances between all USA300 isolates used in this study. Color indicates the phenotype of pairs at that distance (key on right). Vertical dotted lines indicate where each of the five experimental pairs (P1-P5) falls in the distribution. Pairs were chosen to minimize total pairwise difference between inducible and non-inducible strains. **(F)** Nucleotide and amino acid sequences of *sarZ* alleles starting at S139. WT = wild type USA300 *sarZ* allele found in AH-LAC. The indel/frameshift mutant alleles found in USA300 BSI isolates are aligned below with amino acid differences highlight in red. The two polyA stretches are shown in boxes with the number of adenines within the stretch written underneath. For FS1, there is an insertion into the second polyA stretch. For FS2, there is a deletion in the first polyA stretch. **(G)** Amino acid sequence of *sarZ* alleles from three different clonal complexes. CC8 = USA300 *sarZ* allele found in NCBI reference sequence FPR3757 (NC_007793.1). CC30 = USA200 *sarZ* allele found in MRSA252 (GenBank: BX571856.1). CC5 = USA100 *sarZ* allele found in NCBI reference sequence (NZ_CP021105.1). The major structural features of SarZ are shown above, where alpha helices are blue, beta sheets are represented by green arrows, and the critical cysteine (Cys13) is highlighted in yellow.


A
Percent survival
Time (h)
I1
H1
p = <0.0001
I2
H2
p = 0.0069
I3
H3
p = 0.0014
I4
H4
p = 0.0198
I5
H5
p = 0.0007
B
SF8300
SF8300 sarZ::tet
NS
C
OD600
Time (h)
D
H2
H2 sarZ::tet
I2
log(CFU/mL)
T0
T1
T2
T4
E
Lesion size (mm²)
NS
H2
H2 sarZ::tet
I2
Day 2 PI
Day 3 PI
Log(CFU/mL)

Supplementary Figure 5. Increased virulence of *sarZ* mutants specific to BSI, related to Figure 4. (A) Survival of mice infected i.v. ($5x10^7$ CFU) with USA300 BSI isolates. Inducible USA300 (I1-I5) that have mutations in *sarZ* are shown in blue. Closely related high cytotoxicity USA300 (H1-5) that have a WT *sarZ* locus are shown in red (n = 10-15). Pooled data shown in Fig. 5A. **(B)** Survival of mice infected i.v. ($5x10^7$ CFU) with USA300 strain SF8300 or SF8300 *sarZ::tet.* **(C)** Growth curves of clinical isolates used in Figure 5A in TSB media. High cytotoxicity isolates (H1-5) are in red, inducible isolates (I1-5) are in blue, AH-LAC control is in black. **(D)** Whole blood survival of clinical isolates that have differing phenotypes in bloodstream infections. Human venous whole blood was infected at ~10^6 CFU/mL. At each time point (T = 0, 1, 2 and 4h post-infection), CFU were enumerated. **(E)** Abscess size and CFU counts of mice with skin infections using USA300 clinical isolates that have differing phenotypes in bloodstream infections. Mice were infected subcutaneously ($1x10^7$ CFU). Lesion size was measured Day 2 and 3 post-infection (PI). Lesions were harvested and CFU enumerated on Day 3 PI. Statistical significance using a 2-way ANOVA with Sidak's multiple comparisons test. Error bars indicate SEM. NS not significant. Statistical analysis for survival curves done with the Log-rank (Mantel-Cox) test.



Supplementary Figure 6. Confirmation of strains and results for RNA-seq, related to Figure 5. (A) I1, and **(B)** H2, *sarZ::tet* mutant and WT *sarZ* complemented strains grown to stationary phase in TSB, n = 10 donors. Statistical significance comparing *sarZ::tet* strains to parental and WT *sarZ* complement. **(C-D)** qRT-PCR of USA300 BSI isolates grown 3h in TSB (n=4). High cytotoxicity isolates with a WT *sarZ* are pooled (H1-5), as are inducible isolates with a mutated *sarZ* (I1-5). Each dot represents an independent sample, color coded by the isolate. Panel **C** shows the results from *clfB* and panel **D** shows the proteases *sspA* and *aur*. Statistical significance by an unpaired two-tailed t test with Welch's correction. Shapiro-Wilk normality test used to determine use of Welch's correction. **(E)** qRT-PCR of USA300 BSI isolates grown in 50% human serum (n=4). Log2 Fold Change of inducible isolate I2 compared to closely related high cytotoxic isolate H2.



Supplementary Figure 7. ClfB does not contribute to lethality of a SarZ WT Strain, related to Figure 6. Survival of mice infected i.v. ($7.5x10^7$ CFU) with USA300 isolate H2 or H2 *clfB::tet* (n=10). Statistical analysis for survival curve done with the Log-rank (Mantel-Cox) test.

Supplementary Table 1. Demographics and clinical characteristics of USA300 cases, related to Figure 1.

Variable	Full Cohort N = 97
Sex	N (%)
Male	66 (68.0)
Female	31 (32.0)
Race/Ethnicity	
White, non-Hispanic	29 (30.5)
Black, non-Hispanic	35 (36.9)
Hispanic	21 (22.1)
Other	10 (10.5)
Unknown	5
Age at Time of Infection	
<18 Years	3 (3.1)
18-64 Years	60 (61.9)
≥ 65 Years	34 (35.0)
History of Injection Drug Use	
Yes	13 (13.8)
No	81 (86.2)
Unknown	3
HIV Diagnosis	
Yes	11 (11.7)
No	83 (88.3)
Unknown	3
Admission Source	
Home[a]	69 (73.4)
NH/Rehab/LTACH/Other Hospital	25 (26.6)
Unknown	3
Prior Hospital Admission (90 Days)	
Yes	50 (53.2)
No	44 (46.8)
Unknown	3
Hospital or Community Onset [b]	
HO	25 (26.6)
HACO	46 (48.9)
CO	23 (24.5)
Unknown	3
Frequent Healthcare Interaction	
Hemodialysis	11 (13.4)
Infusion Center[c]	14 (17.1)
None	57 (69.5)
Unknown	15
Presence of Invasive Device [d]	
Yes	63 (67.0)
No	31 (33.0)
Unknown	3

Invasive Procedures [e]	
Yes	**34 (36.2)**
No	**60 (63.8)**
Unknown	**3**
Wound Present, N [f]	**N (%)**
Yes	**54 (57.4)**
No	**40 (42.6)**
Unknown	**3**
Charlson Comorbidity Index (CCI)	**N (%)**
0-5	**45 (47.9)**
> 5	**49 (52.1)**
Unknown	**3**
Comorbidities [g]	
Myocardial Infarction	**8 (8.5)**
Congestive Heart Failure	**20 (21.3)**
Peripheral Vascular Disease	**11 (11.7)**
Cerebrovascular Disease	**4 (4.3)**
Dementia	**7 (7.4)**
Chronic Pulmonary Disease	**9 (9.6)**
Peptic Ulcer Disease	**2 (2.1)**
Diabetes (no complications)	**15 (16.0)**
Diabetes with Organ Damage	**18 (19.1)**
Para or Hemiplegia	**7 (7.4)**
Moderate/Severe Renal Disease	**26 (27.7)**
Solid Tumor	**8 (8.5)**
Leukemia	**7 (7.4)**
Lymphoma/Multiple Myeloma	**8 (8.5)**
Moderate/Severe Liver Disease	**13 (13.8)**
Metastatic Solid Tumor	**5 (5.3)**
History of Transplant [h]	
Yes	**11 (11.7)**
No	**83 (88.3)**
Unknown	**3**
History of MRSA Colonization [i]	
Yes	**43 (45.7)**
No	**51 (54.3)**
Unknown	**3**
Presumed Source of MRSA BSI	
Skin Source	**43 (45.7)**
Pneumonia	**11 (11.7)**
Vascular Access	**24 (25.5)**
Other	**7 (7.4)**
Source Truly Unknown	**9 (9.6)**
Unknown	**3**
ICU Admission Prior to BSI	
Yes	**9 (22.0)**
No	**32 (78.0)**
No ICU Admission	**53**
Unknown	**3**

Abbreviations: HIV, human immunodeficiency virus; NH, nursing home; LTACH, long-term acute care hospital; HO, hospital-onset; HACO, hospital-associated community-onset; CO, community-onset; BSI, blood stream infection; MRSA, methicillin-resistant *Staphylococcus aureus*; ICU, intensive care unit

[a] "Admission from home": included non-medical residences such as home, group homes, assisted living facilities, and homeless shelters.
[b] "Hospital-onset": included positive MRSA culture > 3 days after admission.
"Hospital-associated community-onset": includes a positive MRSA culture $\leq$ 3 days after admission with presence of at least one risk factor including presence of an invasive device, frequent healthcare interaction, hemodialysis, hospital admission within the past 3 months, or residence at a LTACH, NH, or rehabilitation center.
"Community-onset": includes a positive MRSA culture $\leq$ 3 days after admission without presence of risk factors.
[c] "Infusion center": outpatient centers for chemotherapy, intravenous fluids, intravenous immunomodulators, and blood products.
[d] "Presence of an invasive device": included pacemaker, implantable cardioverter defibrillator (ICD), left ventricular assist device (LVAD), vascular access (excluding peripheral intravenous catheters), orthopedic hardware, nephrostomy, suprapubic catheter, ileal conduit, foley catheter, arteriovenous graft placement (AVG), percutaneous endoscopic gastrostomy (PEG) tube, or ostomy.
[e] "Invasive procedures": Included any invasive procedures or surgery occurring within one month prior to first positive blood culture for MRSA, excluding electroencephalogram (EEG), electrocardiogram (EKG), and transthoracic echocardiogram (TTE).
[f] "Wound Present": Presence of a chronic skin wound overlying the sacrum, limb, abdomen, or other body part.
[g] "Comorbidities": As defined by the Charleston Comorbidity Index (CCI) refer to standard definitions for CCI.[17]
[h] "History of transplant": Included solid organ and bone marrow transplant.
[i] "History of MRSA colonization": Any positive culture from urine, sputum, tissue, or nares with MRSA prior to the positive MRSA blood culture or a documented history of prior MRSA infection or colonization

Supplementary Table 3. Bacterial strains used, related to STAR Methods.

VJT#	Strain Name	Description	Reference
15.77	AH-LAC	Erm^S USA300 SSTI	[S2]
20.06	FPR3757	USA300 SSTI	[S3]
44.45	SF8300	USA300 SSTI	[S4]
47.15	AH-LAC Δ*luk*	AH-LAC carrying Δ*lukAB hlgABC::tet lukED::kan PVL::spec*	[S5]
66.73	AH-LAC *sarZ::bursa*	AH-LAC carrying the *bursa aurealis* transposon in the *sarZ* gene	This study
70.63	AH-LAC + vector	AH-LAC carrying empty vector pOS1	This study
70.65	AH-LAC *sarZ::bursa* + vector	AH-LAC *sarZ::bursa* carrying empty vector pOS1	This study
70.67	AH-LAC *sarZ::bursa* + $sarZ^{WT}$	AH-LAC *sarZ::bursa* carrying *sarZ* WT allele (from AH-LAC) under its native promoter on pOS1	This study
70.69	AH-LAC *sarZ::bursa* + $sarZ^{NS1}$	AH-LAC *sarZ::bursa* carrying *sarZ* NS1 allele (from ER00385) under its native promoter on pOS1	This study
70.71	AH-LAC *sarZ::bursa* + $sarZ^{NS2}$	AH-LAC *sarZ::bursa* carrying *sarZ* NS2 allele (from ER08597) under its native promoter on pOS1	This study
70.73	AH-LAC *sarZ::bursa* + $sarZ^{FS1}$	AH-LAC *sarZ::bursa* carrying *sarZ* FS1 allele (from ER00594) under its native promoter on pOS1	This study
70.75	AH-LAC *sarZ::bursa* + $sarZ^{FS2}$	AH-LAC *sarZ::bursa* carrying *sarZ* FS2 allele (from ER09970) under its native promoter on pOS1	This study
83.33	I1 *sarZ::tet*	ER00594 carrying *sarZ::tet*	This study
83.42	I1 *sarZ::*$sarZ^{WT}$	ER00594 *sarZ::tet* complemented with *sarZ* WT allele (from AH-LAC)	This study
83.39	H2 *sarZ::tet*	ER00573 carrying *sarZ::tet*	This study
83.48	H2 *sarZ::*$sarZ^{WT}$	ER00573 *sarZ::tet* complemented with *sarZ* WT allele (from AH-LAC)	This study
83.50	H2 *sarZ::tet* + vector	ER00573 *sarZ::tet* carrying empty vector pOS1	This study
83.52	H2 *sarZ::tet* + $sarZ^{WT}$	ER00573 *sarZ::tet* carrying *sarZ* WT allele (from AH-LAC) under its native promoter on pOS1	This study
83.54	H2 *sarZ::tet* + $sarZ^{FS1}$	ER00573 *sarZ::tet* carrying *sarZ* FS1 allele (from ER00594) under its native promoter on pOS1	This study
85.62	SF8300 *sarZ::tet*	SF8300 carrying *sarZ::tet*	This study
78.86	I2 *clfB::tet*	ER02658 carrying *clfB::tet*	This study
85.67	I2 *clfB::clfB*	ER02658 *clfB::tet* complemented with *clfB*	This study
85.64	H2 clfB::tet	ER00573 carrying *clfB::tet*	This study
75.29	BL21 pET15b-*sarZ*	*E. coli* BL21 carrying the expression plasmid pET15b-$sarZ^{WT}$	This study
84.14	BL21 pET15b-$sarZ^{NS1}$	*E. coli* BL21 carrying the expression plasmid pET15b-$sarZ^{NS1}$	This study
84.15	BL21 pET15b-$sarZ^{NS2}$	*E. coli* BL21 carrying the expression plasmid pET15b-$sarZ^{NS2}$	This study

Supplementary Table 4. List of primers and oligos, related to STAR Methods.

Primer Name	Sequence (5'-3')
sarZ-XmaI	TCCCCCCGGGTATAGAAATTTGTATTACAAAGCCATTAT
sarZ-NheI	GATCAGCTAGCTATGTAACTCCTTTTTGCTTATCTATTC
Up_sarZ_pIMAY_F	CTAAAGGGAACAAAAGCTGGGTACCTGAAGGCACTAAACTGCTTCAC
Up_sarZ_tet-R	TACCAATATTTGTTATTTTACAATCCCAATCACTCCTTGTTAAAATAAACAATAT
Down_sarZ_tet_F	TTTCTTGCTTCATTGATACCTTTTGCTCCCTATCATAAGGTGATGGG
Down_sarZ_pIMAY_R	TGGATCCCCCGGGCTGCAGGAATTCCCATGCGTCATTTATACCAAAAAG
tetM_F	GATTGTAAAATAACAAATATTGGTACATG
tetM_R	CAAAAGGTATCAATGAAGCAAGAAATATTG
pIMAY_F	GGTACCCAGCTTTTGTTCCCTTTAGTGAGG
pIMAY_R	GAATTCCTGCAGCCCGGGG
clfB_UP_pIMAY_F	CTCTAGAACTAGTGGATCCCCCGGGGCAAGTATTATTTCCAATCAGTAAC
clfB_UP_tet_R	TACCAATATTTGTTATTTTACAATCAAATATTACTCCATTTCAATTTCTAGATTAG
clfB_DOWN_pIMAY_R	GCTGGGTACCGGGCCCCCCCTCGAGCTTCAAATTGATAAGGAACATTTATGC
clfB_DOWN_tet_F	TTTCTTGCTTCATTGATACCTTTTGATACTTTTTTAGGCCGAATACATTTG
pOS1_1_F	TCAGGGGATAACGCAGGAAAG
pOS1_1_R	ATGAGCAAAGGAGAAGAACTTTTCA
pOS1_2_F	TGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGC
pOS1_2_R	CTGCAGCCAAGCTAGCTTGT
sarZ_NdeI	CCCCCATATGTATGTAGAAAACAGCTATCTTAGCAAAC
sarZ_STOP_BamHI	CCCGGATCCTTATTTTTTGTCGGAATAGTCAAAATTTTTAG
rpoB_F	GAACATGCAACGTCAAGCAG
rpoB_R	AATAGCCGCACCAGAATCAC
rpoB_probe	TACAGGTATGGAACACGTTGCAGCA
clfB_F	CACAAACAGTGCGAATGTAGATAG
clfB_R	CGGTTGAGGTGTTTCATTTGTT
cflB_probe	AGCAATACCACTACAACAGAGCCAGC
sspA_F	ATCGTCACCAAATCACAGATACA
sspA_R	ACAACTACACCGGAAGCAATAA
sspA_probe	ACGAATGGTCATTATGCACCCGTAACT
aur_F	GGTCGCACATTCACAAGTTTATC
aur_R	CGCCTGACTGGTCCTTATATTC
aur_probe	CGGTGTGACACAAGAGACAGCGAA
pSsp_F	AATTCATAGTACGTTCAGTT
pSsp_R	CTAAAAACCTCCAAAAAATT
pSsp_R_biotin	CTAAAAACCTCCAAAAAATT
pClfB_F	TATACAATGTAAAATGAATAA
pClfB_R	AAATATTACTCCATTTCAATT
pClfB_ R_biotin	AAATATTACTCCATTTCAATT

pPurA_F	AAAAGTTTTTCCGTACAATA
pPurA_ R_biotin	ACATGTGAGCACCTCCAAGT
pPurA_biotin	ACATGTGAGCACCTCCAAGT

SUPPLEMENTAL REFERENCES

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2. Boles, B.R., et al. (2010). *Identification of genes involved in polysaccharide-independent Staphylococcus aureus biofilm formation.* PLoS One. **5**(4): p. e10146.
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4. Diep, B.A., et al. (2008). *Contribution of Panton-Valentine leukocidin in community-associated methicillin-resistant Staphylococcus aureus pathogenesis.* PLoS One. **3**(9): p. e3198.
5. Blake, K.J., et al. (2018). *Staphylococcus aureus produces pain through pore-forming toxins and neuronal TRPV1 that is silenced by QX-314.* Nat Commun. **9**(1): p. 37.