PNAS
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Supplementary Information for:

Sequential evolution of virulence and resistance during clonal spread of community-acquired methicillin-resistant *Staphylococcus aureus*

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www.pnas.org/cgi/doi/10.1073/pnas.1814265116

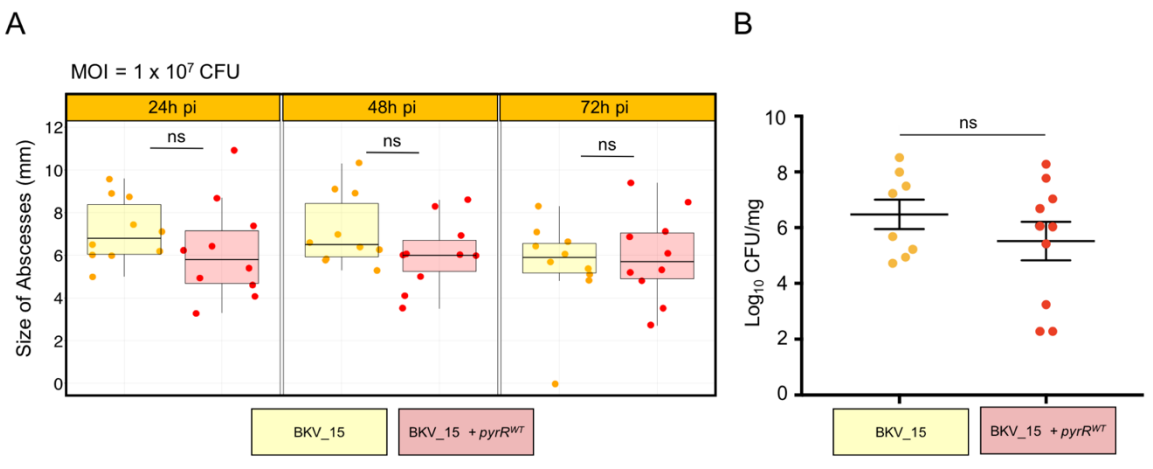

Fig. S1. Supplementary Figure 1: *pyrR* mutation associated with the USA300-BKV clone does not enhance virulence in a murine skin infection model. USA300-BKV + *pyrR* WT and USA300-BKV-isolate were compared in a murine abscess model of infection. (**A**) Abscess size at 24h, 48h, and 72h following subcutaneous infection with ~1x10^7 CFU of the indicated strain ((n=10) 5 mice/group with two abscesses/mouse). Statistical analyses were performed with a two-tailed unpaired *t* test. (**B**) Viable counts of the indicated strain of bacteria were enumerated at nine-days post infection. Statistical analyses were performed with a two-tailed unpaired *t* test. BKV: Brooklyn variant; USA300-BKV (BKV_15; the same strain used in Fig. 2).

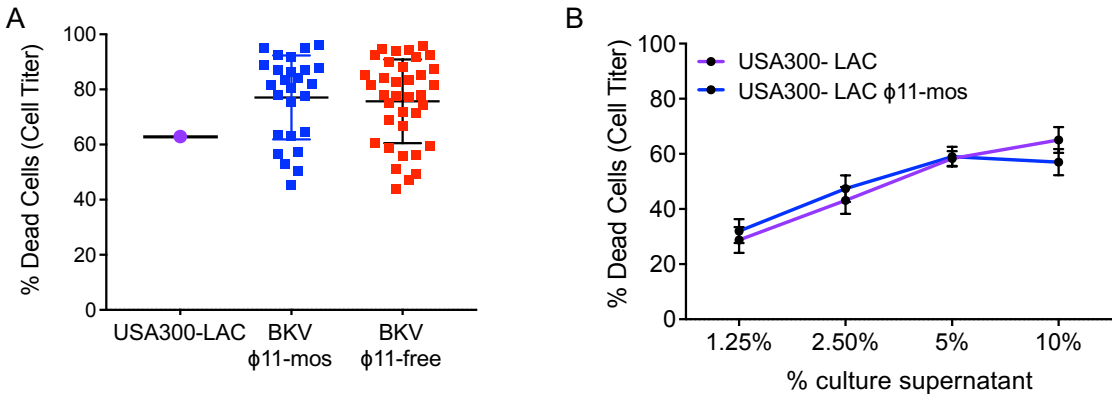

Fig. S2. Supplementary Figure 2: Mosaic ϕ11 does not affect cytotoxic killing of neutrophils by *S. aureus*. (A) Intoxication of primary human neutrophils with 5% culture filtrates from the indicated *S. aureus* strains and controls (USA300 USA300-LAC wild-type and clinical isolates with our without mosaic ϕ11). Each isolate was tested on PMNs obtained from n=5 donors over 3 individual experiments. Cell viability of PMN cells measured with the metabolic dye CellTiter. (B) Cytotoxicity of USA300-LAC with or without mosaic ϕ11, as descried in A. Symbols: USA300 wild-type (LAC), USA300-LAC + chromosomally inserted mosaic ϕ11 (USA300-LAC + ϕ11-mos), and USA300-BKV variants that lack mosaic ϕ11 (ϕ11-free).

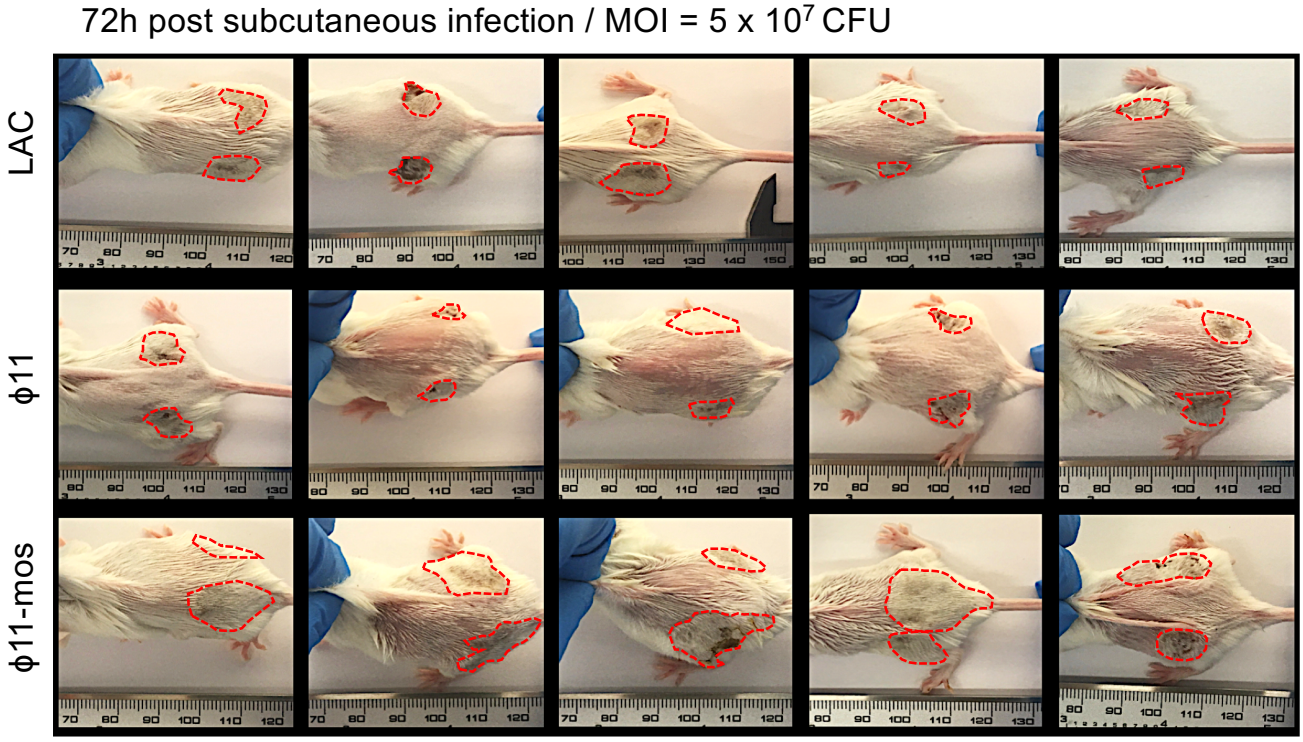

Fig. S3: Effects of the mosaic portion of ϕ11 are enhanced at high inoculum for lesion size but not dermonecrosis. Supplementary data to **Fig. 4**. USA300-LAC lysogens of mosaic ϕ11 (produced by induction of USA300-BKV_28) and prototypical ϕ11 (produced by induction of RN451) were compared in a murine abscess model of infection. Representative panel of murine skin abscesses at 3-days infection post subcutaneous-infection at ~$5x10^7$ CFU of the indicated strain ((n=15) 5 mice/group with two abscesses/mouse). No difference in the rate of dermonecrosis was noted after daily measurement for 9-days.

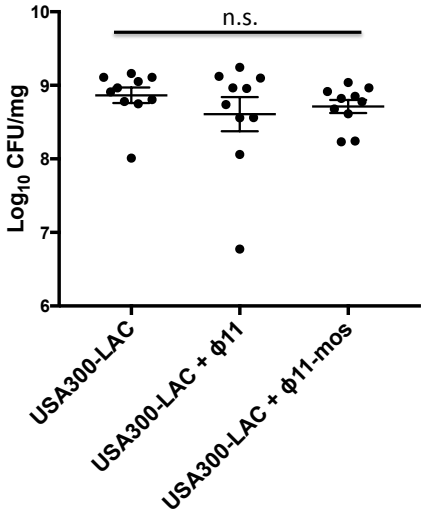

Fig. S4: The mosaic portion of ϕ11 does not affect bacterial CFU. Viable counts of the indicated strain of bacteria from Fig. S3 enumerated at 9-days post subcutaneous-infection with ~5x10^7 CFU ((n=15) 5 mice/group with two abscesses/mouse). Statistical analysis was performed using one-way analysis of variance (ANOVA) with *post hoc* Holm-Sidak multiple-comparison test.

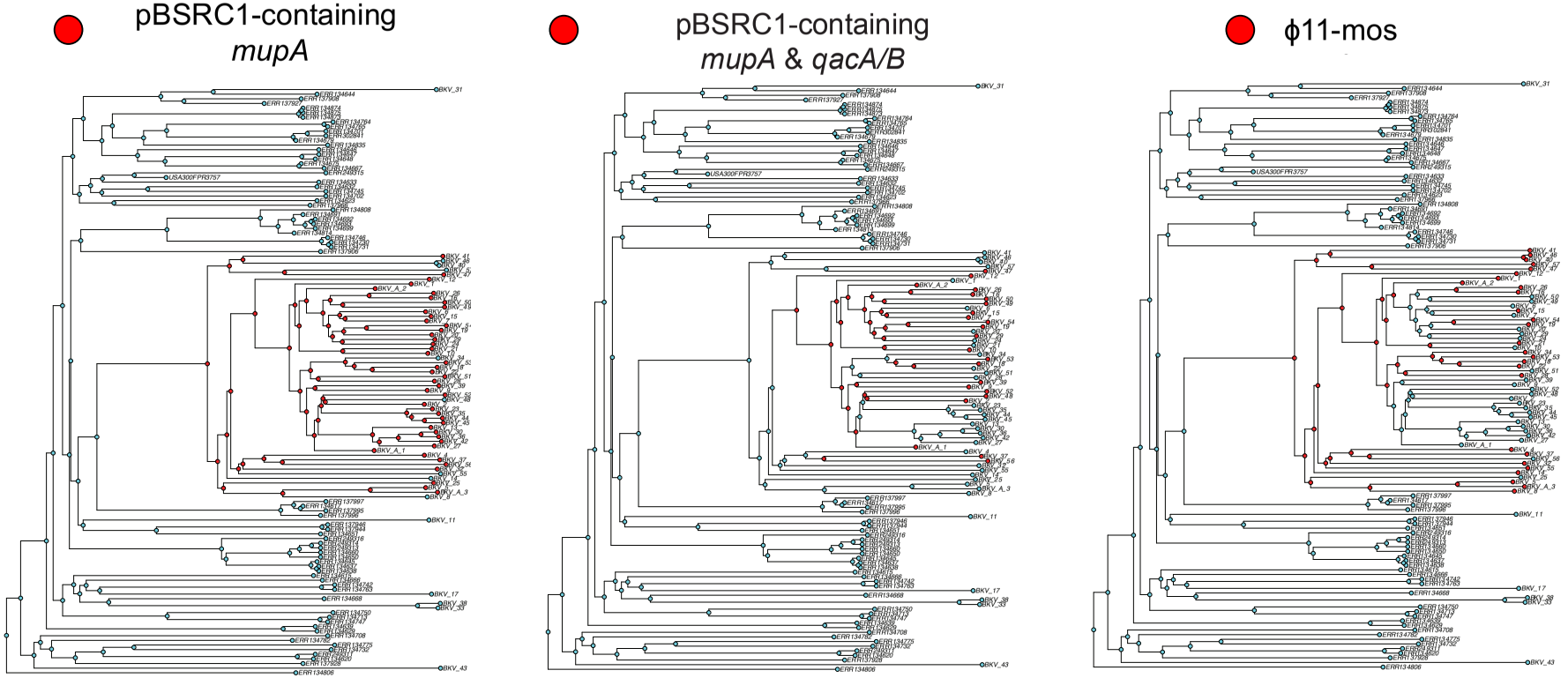

Fig. S5. Maximum parsimony based ancestral state reconstruction indicating presence or absence of mosaic ϕ11, mupirocin resistance, and chlorhexidine resistance genes. Phylogenetic ancestral state reconstruction on the timed trees supports an evolutionary scenario for the diversification of the USA300-BKV clone involving sequential acquisition of mosaic ϕ11, mupirocin, and finally chlorhexidine resistance. Ancestral state reconstructions were performed using the phangorn R package (version 2.4.0) with the accelerated transformation function; all states and transformations were given equal weight.

Tables S1 (separate file). Proportion of *S. aureus* clinically infected* patients in the pediatric ward during May 1, 2015 - December 31, 2016.

S. aureus type	High risk ZIP code (n= 895)	Low risk ZIP code (n= 4368)	Infection rate** high risk zip code	Infection rate** low risk zip code	Odd ratio	Coefficient interval	*p* value
MRSA	75 (8.0%)	22 (0.5%)	80.2	4.9	15.7	7.3, 18.9	<.0001
MSSA	8 (0.8%)	43 (0.9%)	8.1	9.3	8.6	0.3, 1.3	0.25

*based on positive clinical cultures
**per 1,000 pediatric patients

Proportion of *S. aureus* clinically colonized patients on admission* in the pediatric ward during May 1, 2015 - December 31, 2016

S. aureus type	High risk ZIP code (n= 127)	Low risk ZIP code (n= 324)	Odd ratio	Coefficient interval	*p* value
MRSA	10 (7%)	11 (3%)	2.5	1.0,6.09	0.04
MSSA	29 (19%)	57 (14%)	1.4	0.9,2.3	0.15

*based on active surveillance cultures

Table S2. Nonsynonymous single-nucleotide polymorphisms common to all isolates of the USA300-BKV clone.

Gene name	*Locus tag*	*Mutation nature*	*Annotated function*	*Protein family*
pyrR	SAUSA300_1091	nSNP	Pyrimidine and Amino acid metabolism	Metabolism
aroD	SAUSA300_0787	nSNP	Amino acid metabolism	Metabolism
arcB	SAUSA300_2569	nSNP	Amino acid metabolism	Metabolism
metK	SAUSA300_1730	nSNP	Amino acid metabolism	Metabolism
fadA	SAUSA300_0225	nSNP	Amino acid metabolism	Metabolism
licR	SAUSA300_0333	nSNP	Sugar metabolism	Metabolism
melR	SAUSA300_2326	nSNP	Sugar metabolism	Metabolism
glvC	SAUSA300_2270	nSNP	Sugar metabolism	Metabolism
setC	SAUSA300_0659	nSNP	Sugar metabolism	Metabolism
thiD	SAUSA300_0562	nSNP	Vitamin metabolism	Metabolism
cdr	SAUSA300_0873	nSNP	Disulfide metabolism	Metabolism
ddh	SAUSA300_2463	nSNP	Pyruvate metabolism	Metabolism
pyc	SAUSA300_1014	nSNP	Pyruvate metabolism	Metabolism
bltR	SAUSA300_2445	nSNP	Sugar metabolism	Drug efflux
lyrA	SAUSA300_2282	nSNP	Lysostaphin resistance	Virulence
splF	SAUSA300_1753	Stop-gain	Serine protease	Virulence
SAUSA300_0773	SAUSA300_0773	nSNP	Staphylocoagulase	Virulence
cap5F	SAUSA300_0157	nSNP	Capsular biosynthesis protein	Cell wall
icaB	SAUSA300_2601	nSNP	intercellular adhesion protein B	Cell wall
SAUSA300_0090	SAUSA300_0090	nSNP	Hypothetical protein	Hypothetical protein
SAUSA300_0011	SAUSA300_0011	nSNP	Hypothetical protein	Hypothetical protein

Dataset S1 (excel file). Clinical data and strain characteristics of isolates from high-risk zip code-associated patients and control patients.

Datset S2 (excel file). List of nucleotide polymorphisms identify among the 60 high-risk zip code patient isolates.

Table S3. Primers

Name	Sequence (5'-3')	Reference
pyrR-1	CGCAATAGACATCGACACAGATA	This study
pyrR-2	CGATCAACCAAAGCAGCTAAAC	This study
carA-1	ACCATATACAACTACTGCCGAAG	Kriegestkorte, 2014
carA-2	CACATTCTACAACTTCAGGATTACC	Kriegestkorte, 2014
pyrB-1	ACGTCAACTACCAAACTTTG	Kriegestkorte, 2014
pyrB-2	AACCCTAGCTTAAGTTCTGC	Kriegestkorte, 2014
VJT278	TGAGATGTTGGGTTAAGTCCCGCA	Alonzo, 2012
VJT279	CGGTTTCGCTGCCCTTTGTATTGT	Alonzo, 2012