

Supplementary Materials

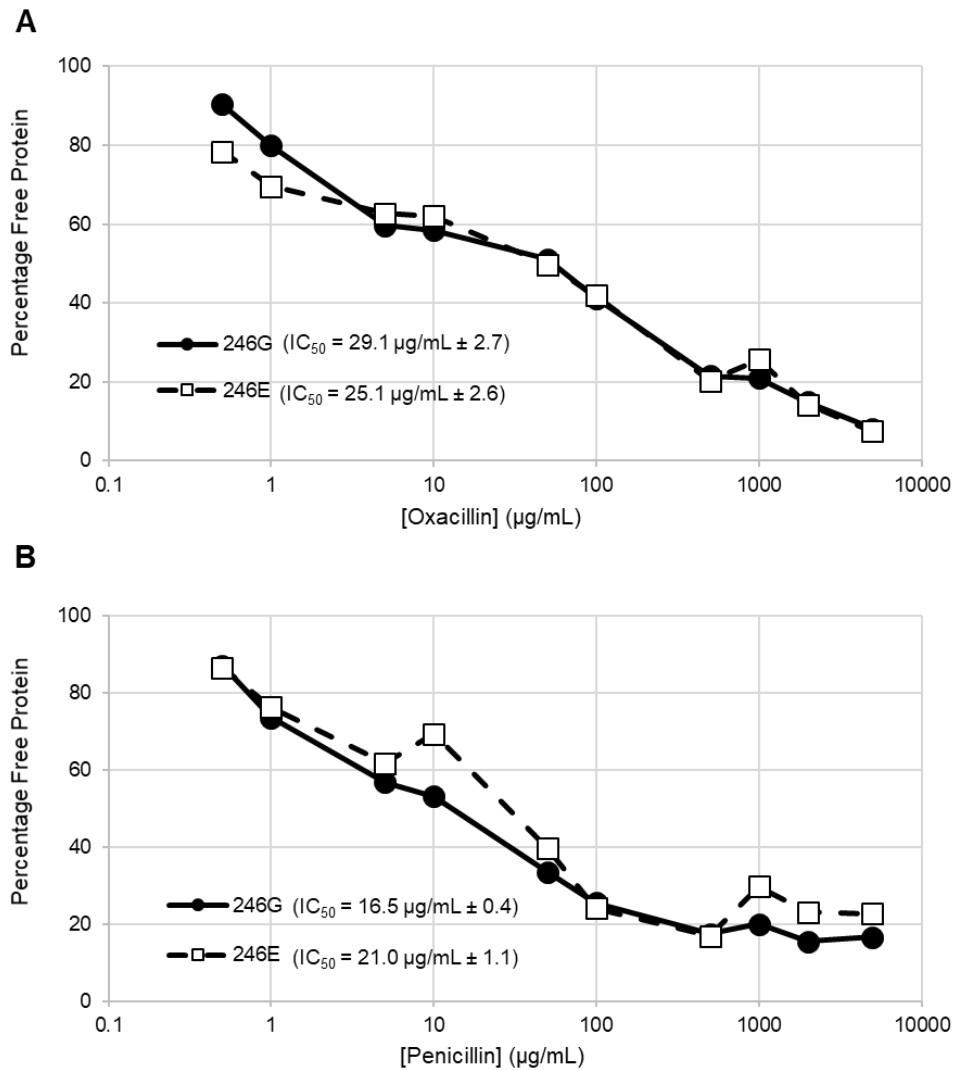


Figure S1. ‘Cold’ oxacillin (**A**) and penicillin (**B**) competition of Bocillin-FL binding to purified PBP2a variants (246G and 246E variants).

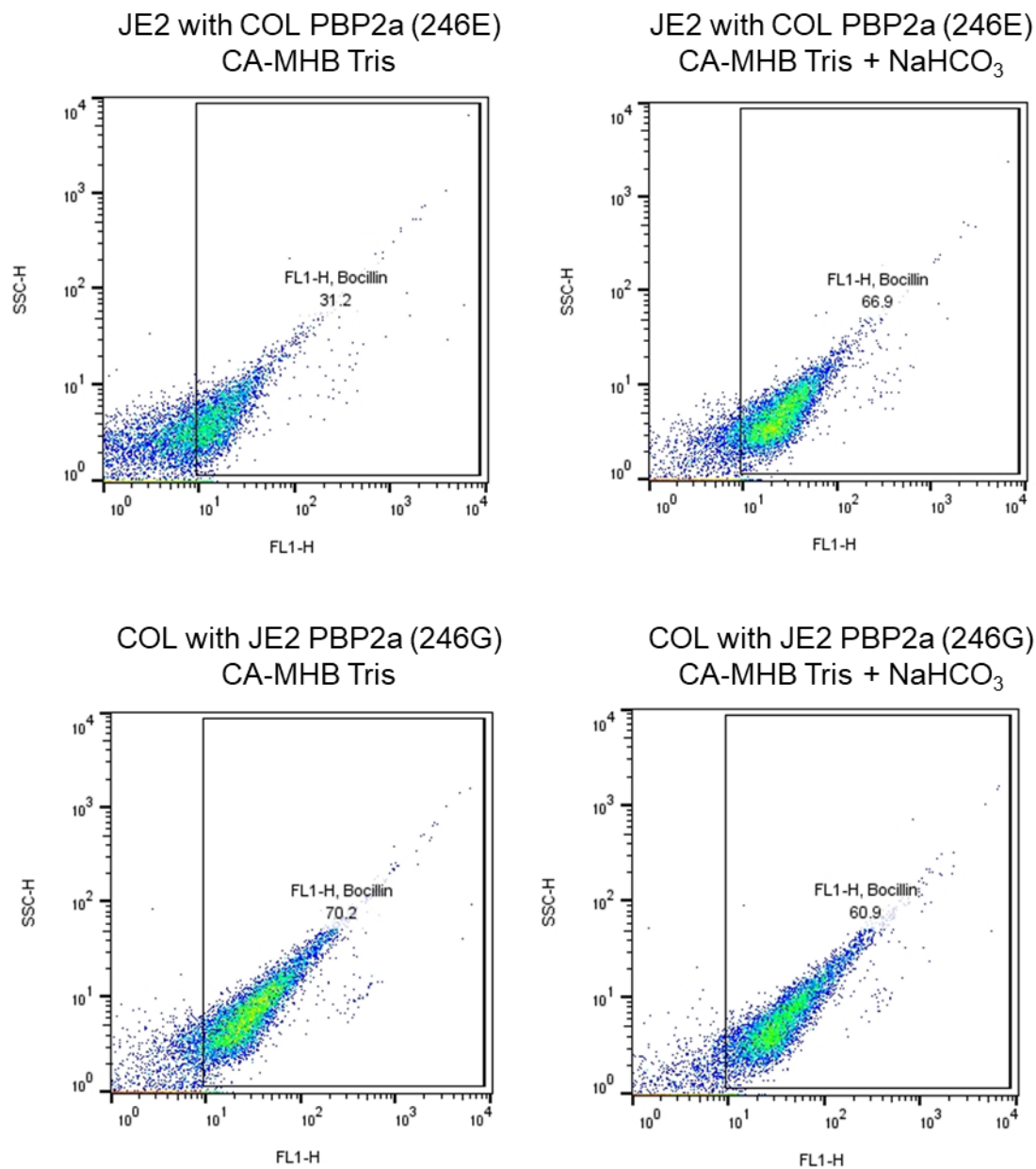


Figure S2. Representative dot plot images of JE2 and COL PBP2a swap strains grown in CA-MHB Tris \pm NaHCO_3 .

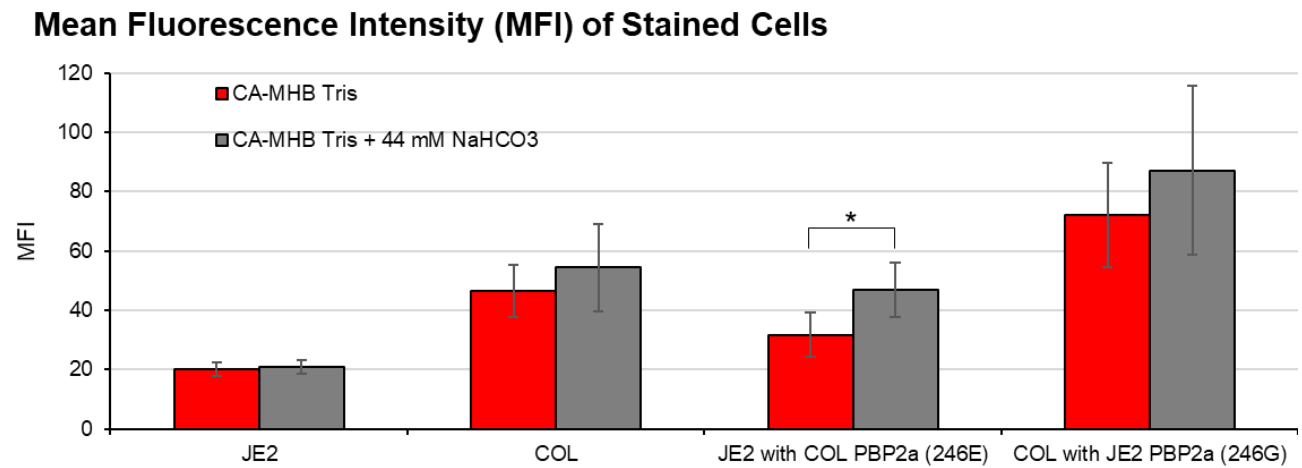


Figure S3. Mean fluorescence intensity of JE2 and COL whole cells stained with Bocillin-FL, following growth in CA-MHB Tris media, with and without NaHCO₃. Statistics were calculated by a Student's *t*-test (**P* < 0.05).

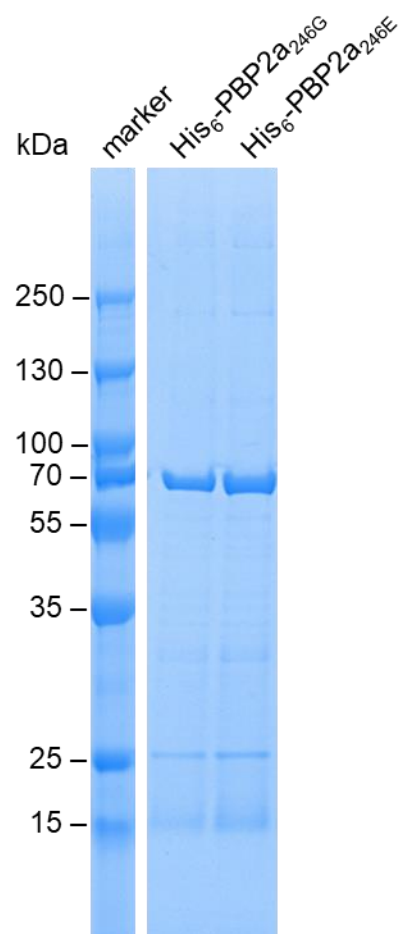


Figure S4. SDS-PAGE of purified PBP2a His₆-tagged proteins.

Table S1. Oxacillin minimum inhibitory concentrations (MICs) for JE2, COL, and their *mecA* swap mutant MRSA strains. MIC determinations were performed in the presence of 2% NaCl.

Strain	Oxacillin MIC (µg/mL)	
	Ca-MHB Tris	
	Ca-MHB Tris	44 mM NaHCO ₃
JE2	64	1
COL	512	512
JE2 with COL PBP2a (246E)	64	2
COL with JE2 PBP2a (246G)	512	512

Table S2. Oligonucleotides used for *mecA* sequencing, construction of His-tagged *mecA*_{246E} and *mecA*_{246G} plasmids, and construction of JE2 and COL *mecA* mutant variants.

Primer designation	Name	Sequence (5' to 3')	Application
None	<i>mecA</i> F0	ACCGAAGAAGTCGTGTCAGA	PCR amplification (for sequencing)
None	<i>mecA</i> R2	ACGTTGTAACCACCCCAAGA	PCR amplification (for sequencing)
None	<i>mecA</i> F1	ATTAACGTGGAGACGAGCACT	Sequencing
None	<i>mecA</i> F2	CGGTAACATTGATCGCAACG	Sequencing
None	<i>mecA</i> R3	TCGTCAACGATTGTGACACG	Sequencing
None	<i>mecA</i> R4	CGAAGGTATCATCTTGTACCCA	Sequencing
A	fwd_ <i>mecA</i> -Cterm	GATATACATATGGCTATGAAAAAGATAAAAATTGTTCCA	PBP2a protein purification
B	rev_ <i>mecA</i> -Cterm	GTGGTGGTGGTGCTCTTCATCTATATCGTATTTTTTATT	PBP2a protein purification
C	fwd_ <i>mecA</i> -Nterm	GGCAGCCATATGGCTATGAAAAAGATAAAAATTGTTCCA	PBP2a protein purification
D	rev_ <i>mecA</i> -Nterm	GTGGTGGTGGTGCTCTTATTCATCTATATCGTATTTTTT	PBP2a protein purification
E	fwd_pET21b/pET28b	GAGCACCACCACCACCAC	PBP2a protein purification
F	rev_pET21b	AGCCATATGTATATCTCCTTC	PBP2a protein purification
G	rev_pET28b	AGCCATATGGCTGCCGCG	PBP2a protein purification

None	731	AAGAGAAGGATCCATCGTTACGGATTGCTTCACTGTT	Cloning
None	732	AAGAGAAGGATCCTTAACTGTTTTAGTTAACTCATGT	Cloning
None	733	AACCAAATCTTATGTGACATAATATTT	PCR verification
None	734	GTAATCTGGAAGTTGTTGAGCAGA	PCR verification
None	745 (<i>mecA</i> 246G fwd)	TTC CTA GAG GAT AGT TAC GAC TTT CTG	Mutagenesis AA246
None	746 (<i>mecA</i> 246E fwd)	TTT CTA GAG GAT AGT TAC GAC TTT CTG	Mutagenesis AA246
None	747 (<i>mecA</i> 246 rev)	AAG CGA CTT CAC ATC TAT TAG GTT A	Mutagenesis AA246

Table S3. JE2 MICs ($\mu\text{g/mL}$)
for Penicillin G and Bocillin-FL
(Penicillin V conjugated to FL).

Antibiotics	Ca-MHB
PEN G	2
Bocillin -FL	2