SUPPLEMENTAL INFORMATION

Novel Phenol Soluble Modulin Derivatives in Community-Associated Methicillin-Resistant *Staphylococcus aureus* Identified Through Imaging Mass Spectrometry

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*Running title: Novel PSM derivatives in CA-MRSA

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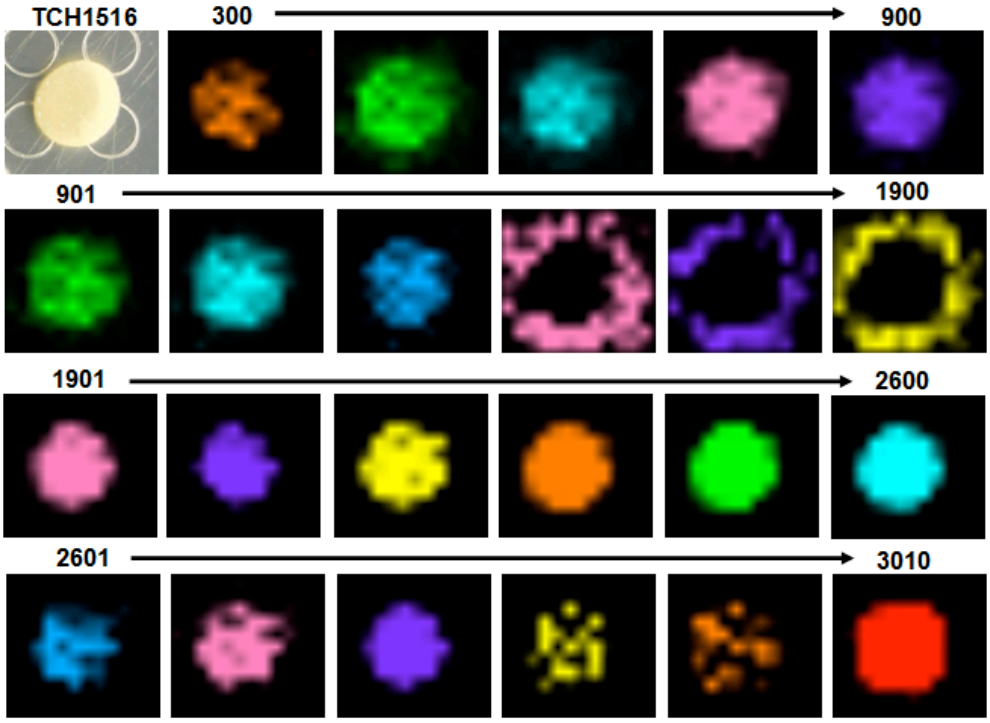
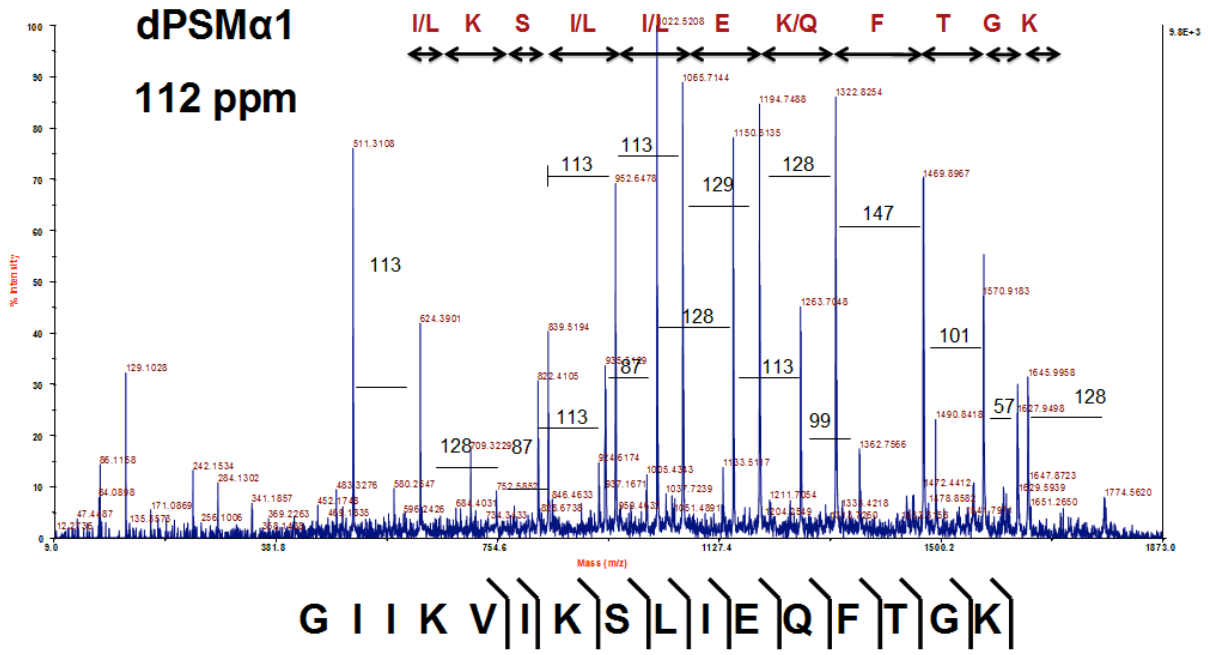
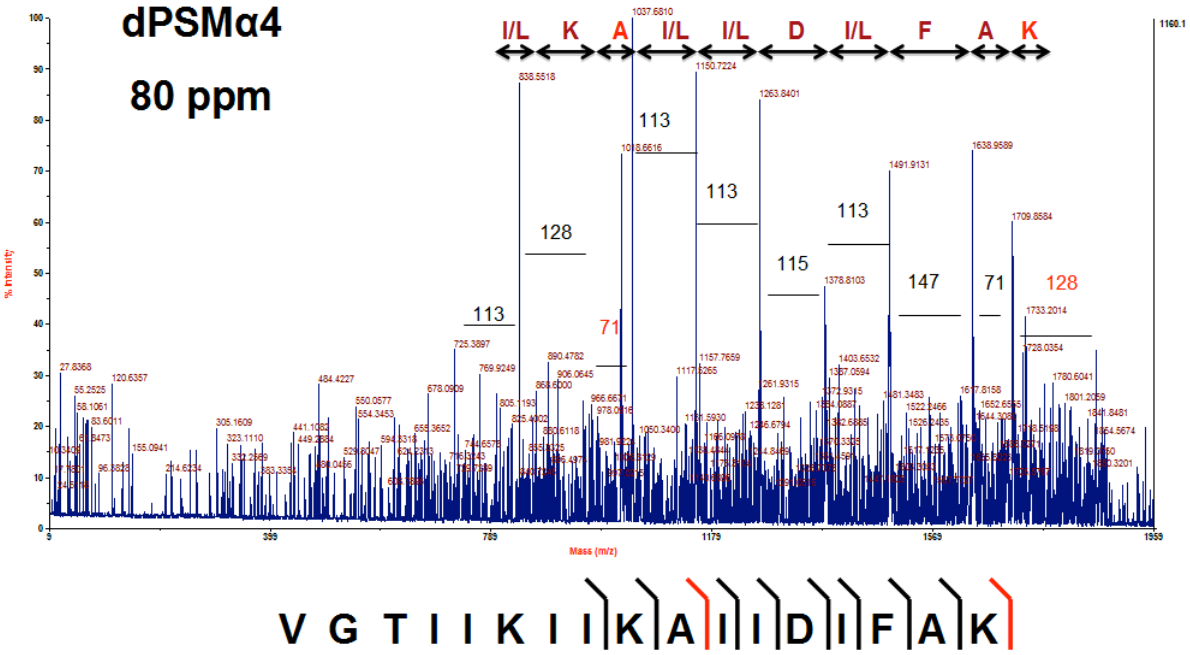


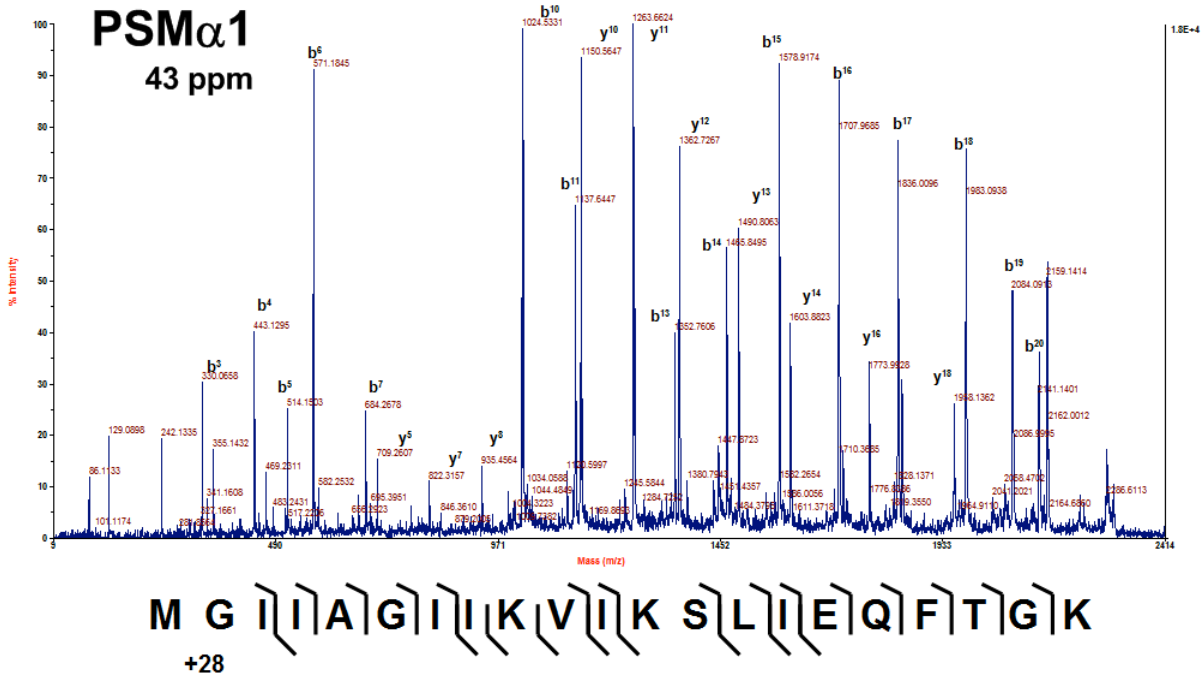
Supplemental Figure S1. IMS molecular fingerprint of CA-MRSA TCH1516. The metabolic output of CA-MRSA TCH1516 is displayed. Several ions in the small molecule range were observed in addition to ions in the range of low molecular weight peptides. False coloring indicates the spatial resolution of the observed ions and the numbers indicate the mass ranges.



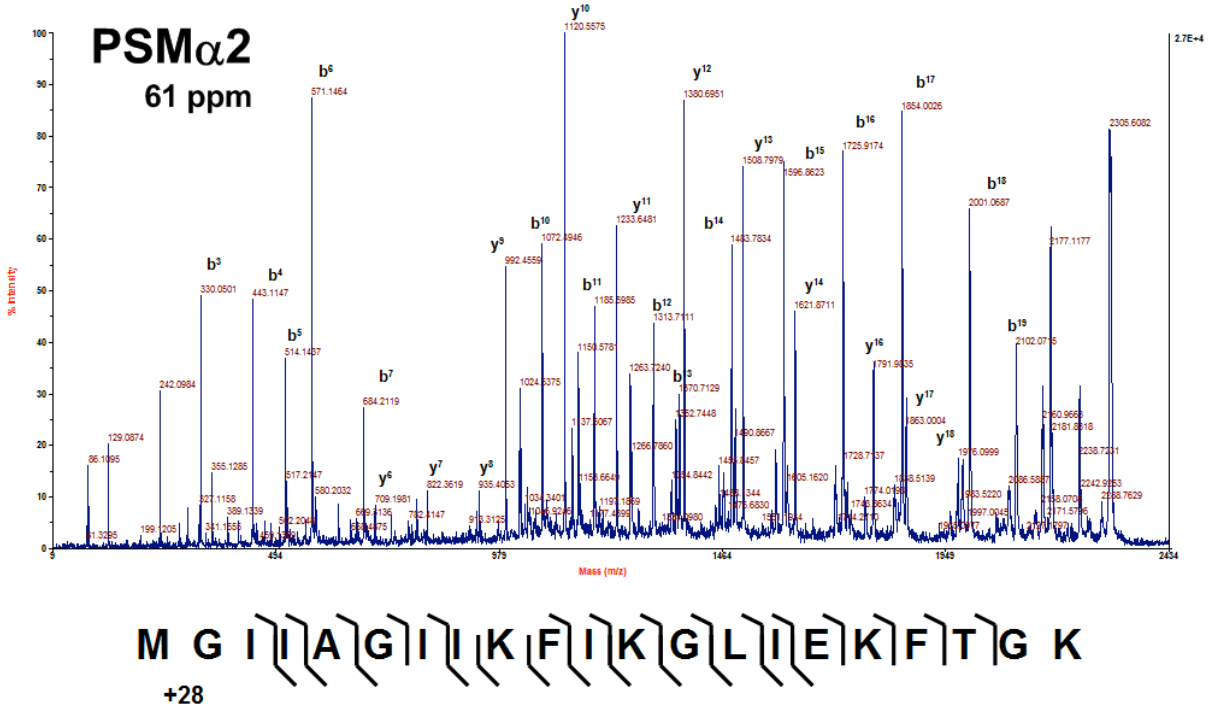
Supplemental Figure S2. TOF/TOF spectrum of dPSMα1. The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Fragment masses were pieced together to generate a peptide sequence tag that contained eleven consecutive ions. The peptide sequence tag was identified as PSMα1 by BLAST analysis and confirmed to be a truncated derivative by intact mass with an error of 112 ppm.



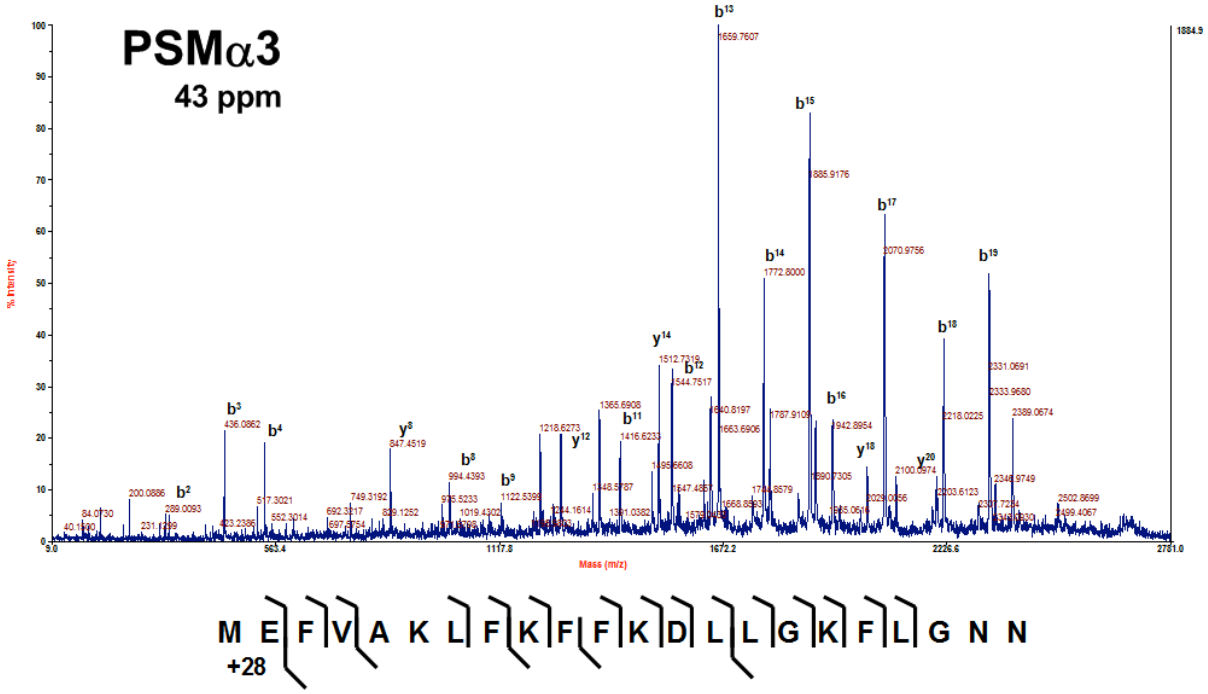
Supplemental Figure S3. TOF/TOF spectrum of dPSMα4. The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Fragments masses were pieced together to generate a peptide sequence tag that contained eight consecutive ions of high confidence and two ions that were at the noise level but fit the pattern of the peptide sequence (indicated in red). The peptide sequence tag was identified as PSMα4 by BLAST analysis and confirmed to be a truncated derivative by intact mass with an error 80 ppm.



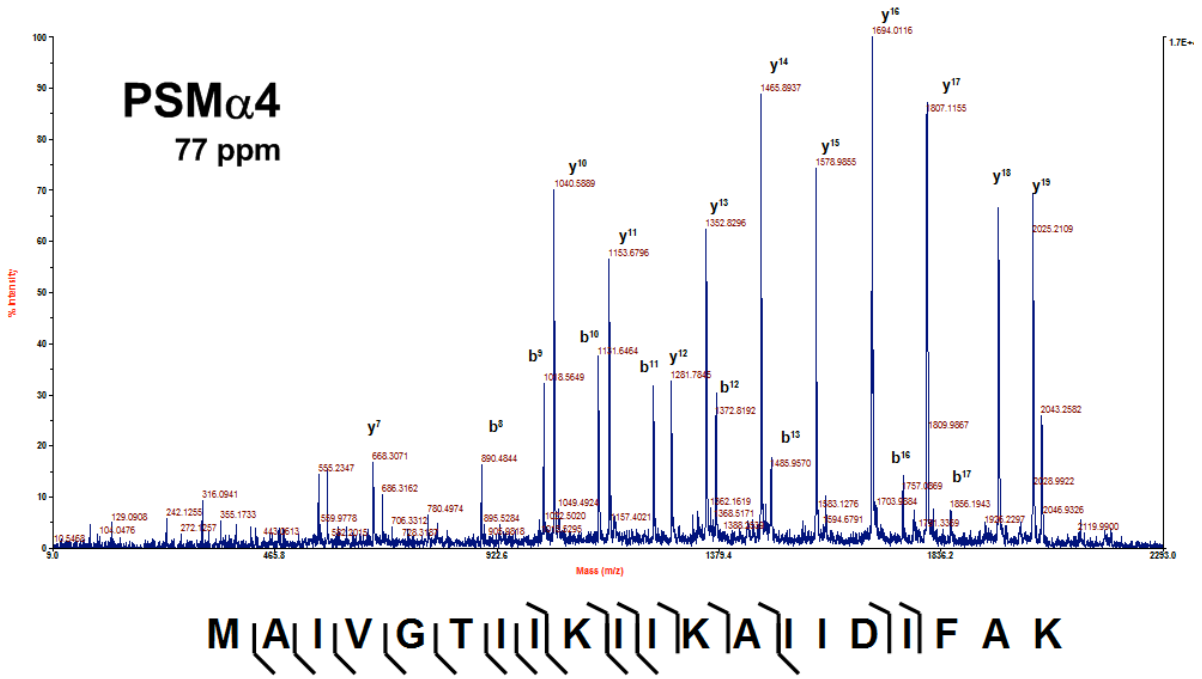
Supplemental Figure S4. TOF/TOF spectrum of PSMα1. The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b or y. The generated peptide sequence tag was identified as PSMα1. Peptide coverage and mass error are also displayed (+28 formylation).



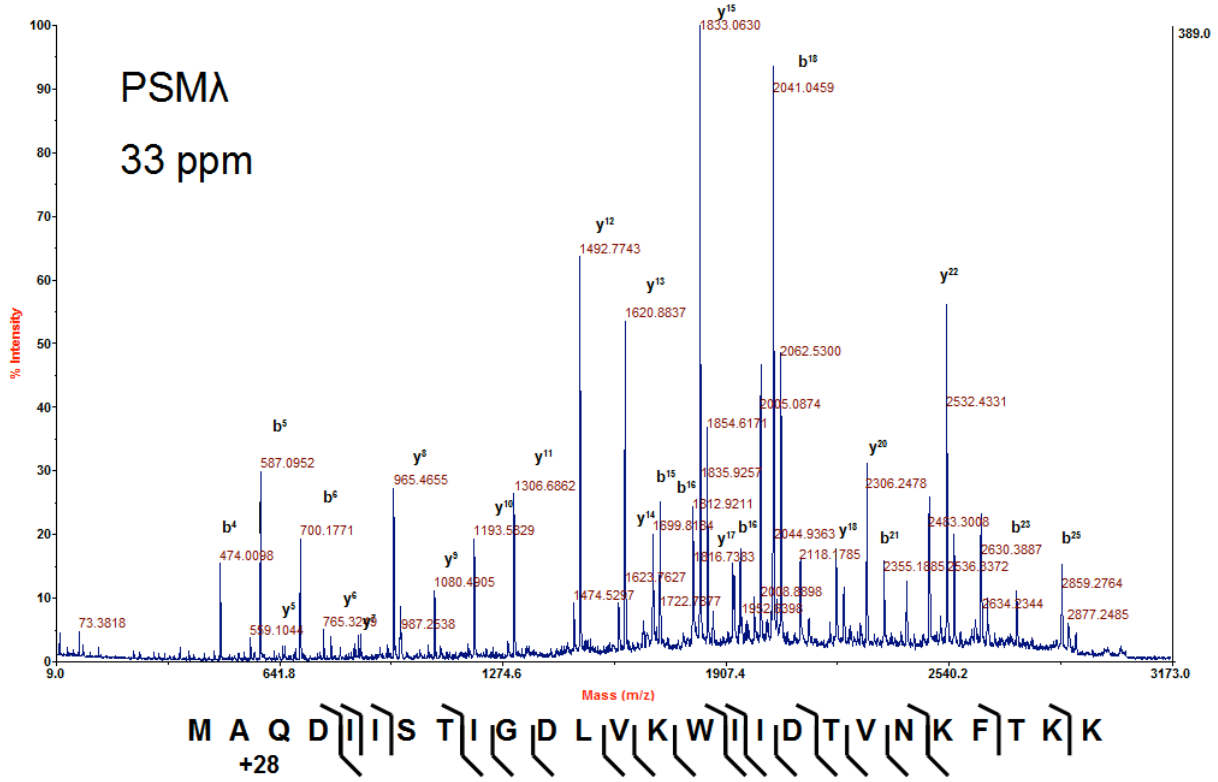
Supplemental Figure S5. TOF/TOF spectrum of PSMα2. The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b or y. The generated peptide sequence tag was identified as PSMα2. Peptide coverage and mass error are also displayed (+28 formylation).



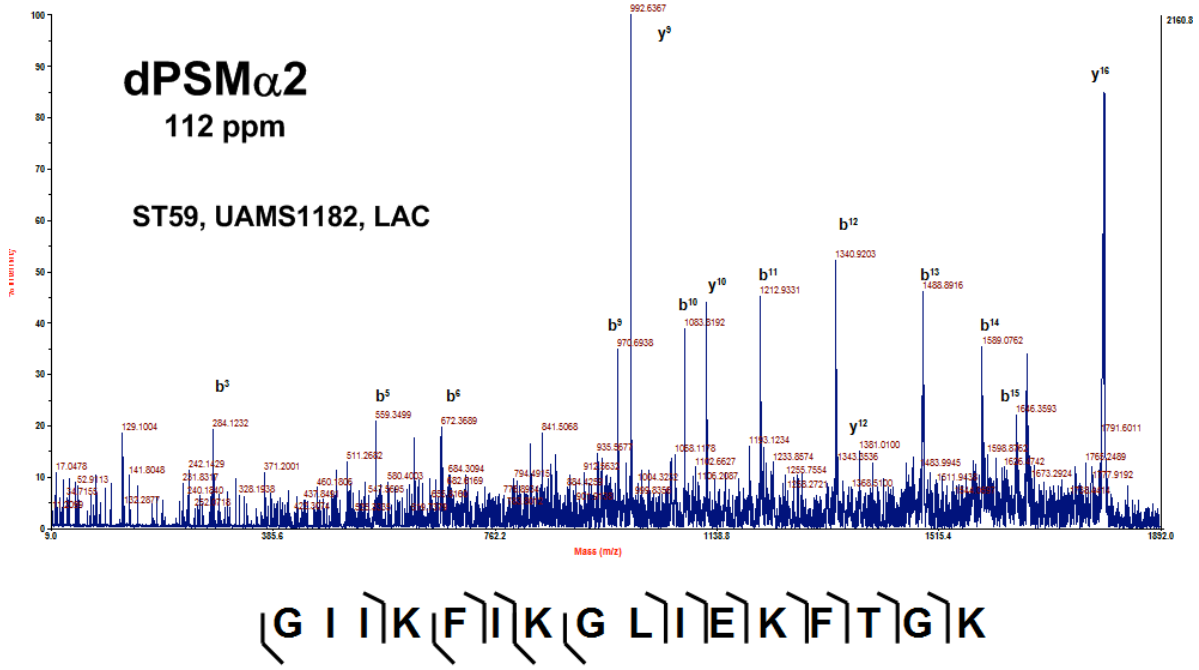
Supplemental Figure S6. TOF/TOF spectrum of PSMα3. The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b or y. The generated peptide sequence tag was identified as PSMα3. Peptide coverage and mass error are also displayed (+28 formylation).



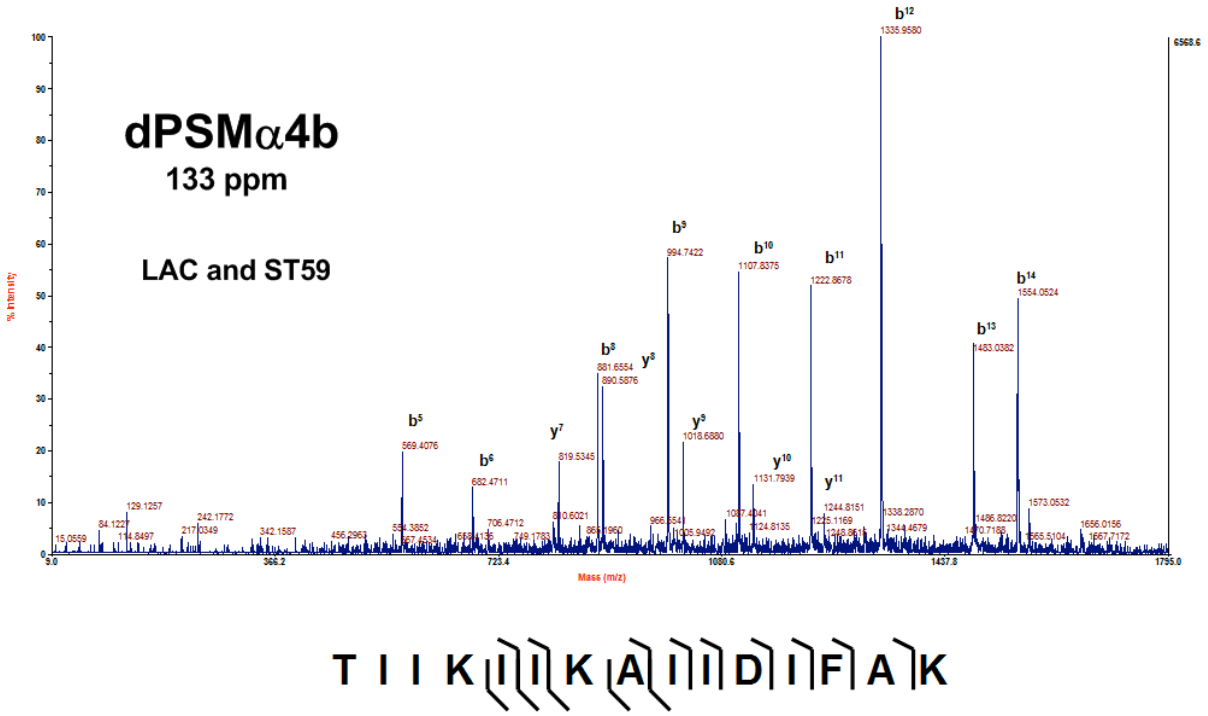
Supplemental Figure S7. TOF/TOF spectrum of PSMα4. The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b or y. The generated peptide sequence tag was identified as PSMα4. Peptide coverage and mass error are also displayed.



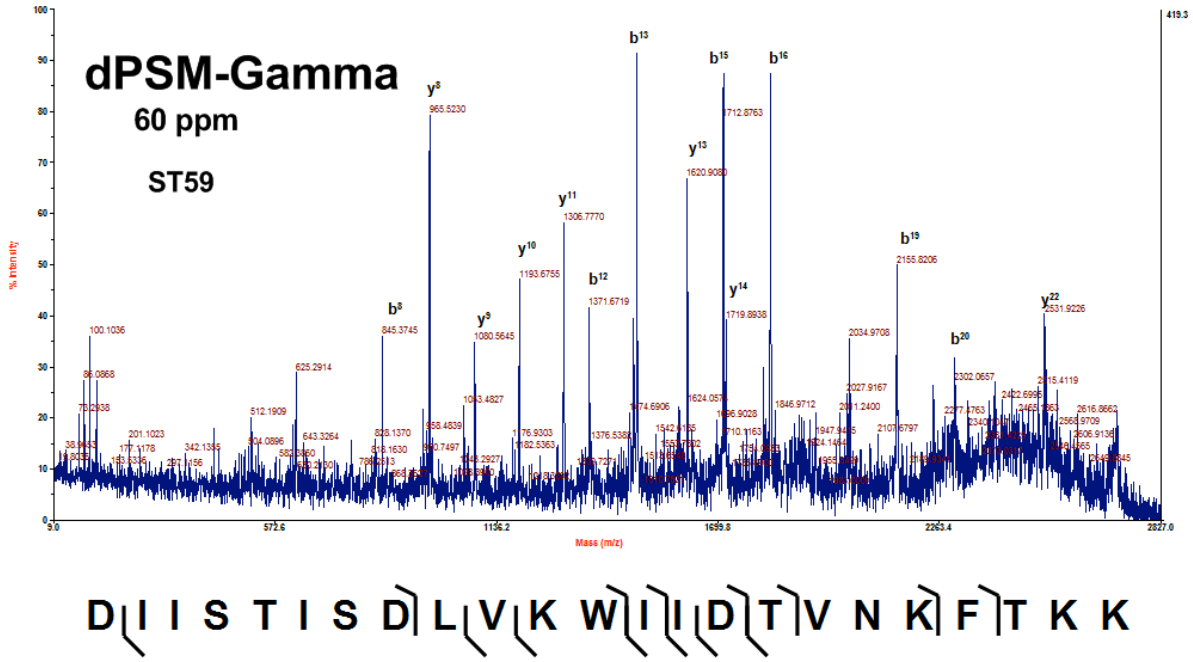
Supplemental Figure S8. TOF/TOF spectrum of PSMλ. The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b or y. The generated peptide sequence tag was identified as PSMλ. Peptide coverage and mass error are also displayed (+28 formylation).



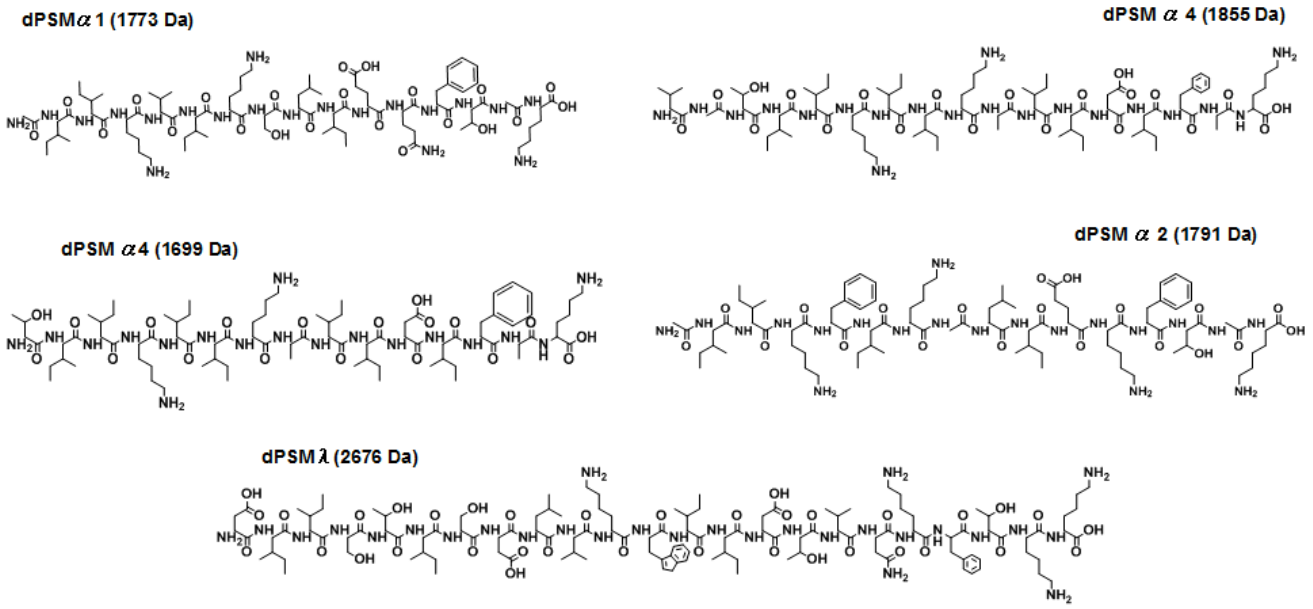
Supplemental Figure S9. TOF/TOF spectrum of dPSMα2. The parent ion (1791 Da) was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b or y. The generated peptide sequence tag was identified as a truncated derivative originating from PSMα2. Peptide coverage and mass error are also displayed. The peptide dPSMα2 was detected in *S. aureus* LAC, UAMS1182, and ST59.



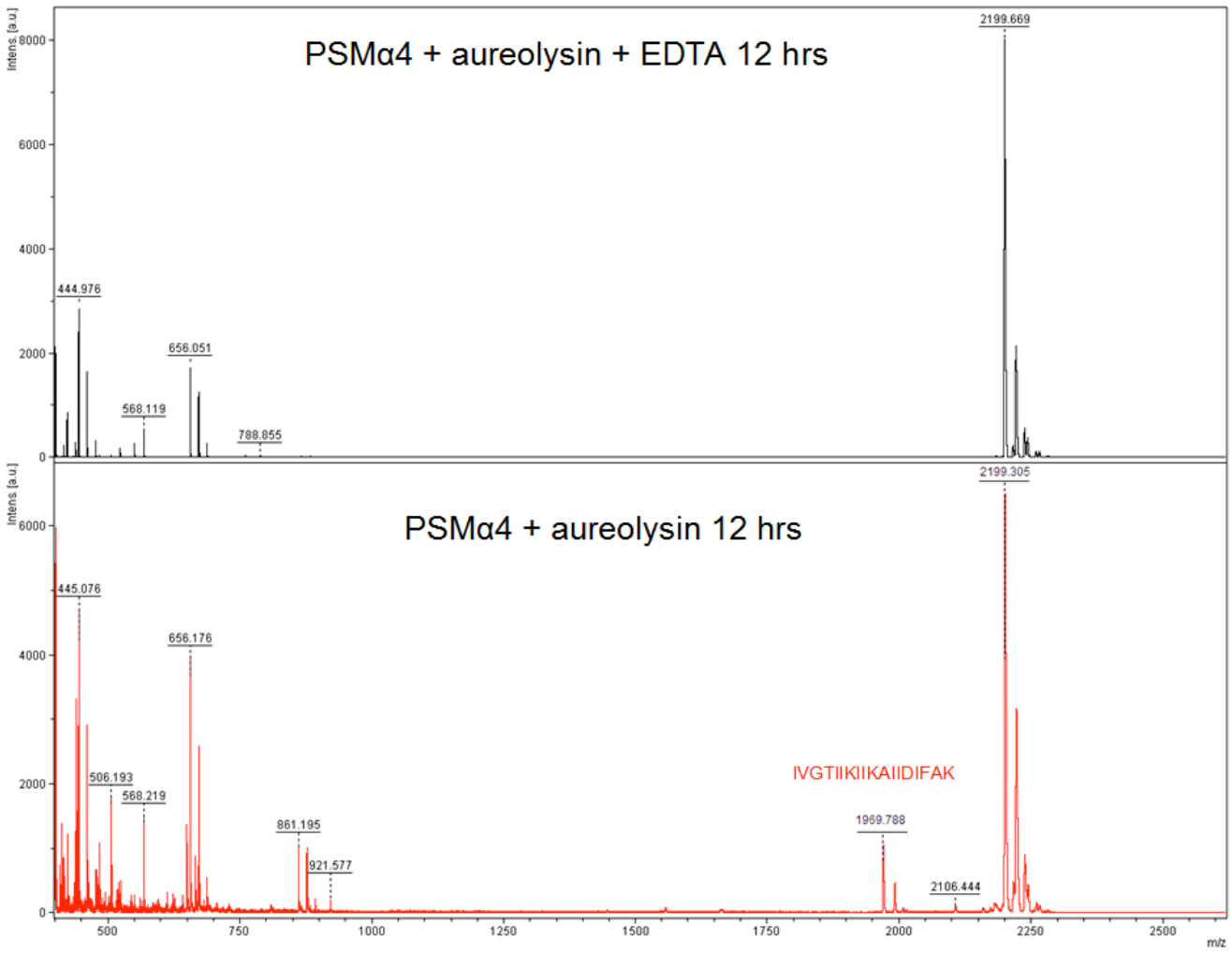
Supplemental Figure S10. TOF/TOF spectrum of dPSMα4b. The parent ion (1699 Da) was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b or y. The generated peptide sequence tag was identified as a truncated derivative originating from PSMα4. Peptide coverage and mass error are also displayed. The peptide dPSMα4 was detected in *S. aureus* LAC and ST59.



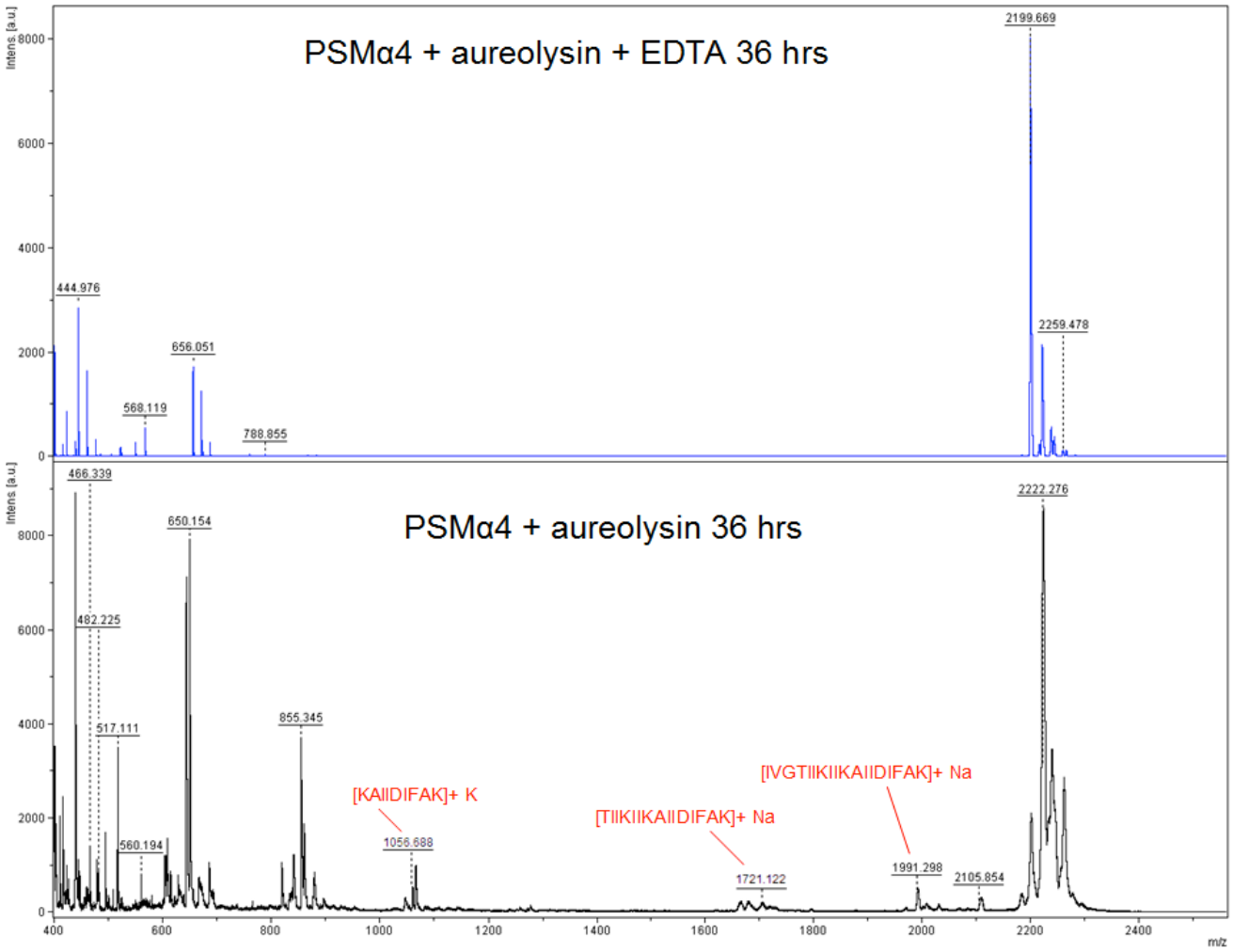
Supplemental Figure S11. TOF/TOF spectrum of dPSMλ. The parent ion (2676 Da) was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b or y. The generated peptide sequence tag was identified as a truncated derivative originating from PSMλ. Peptide coverage and mass error are also displayed. The peptide dPSMλ was detected in *S. aureus* ST59.



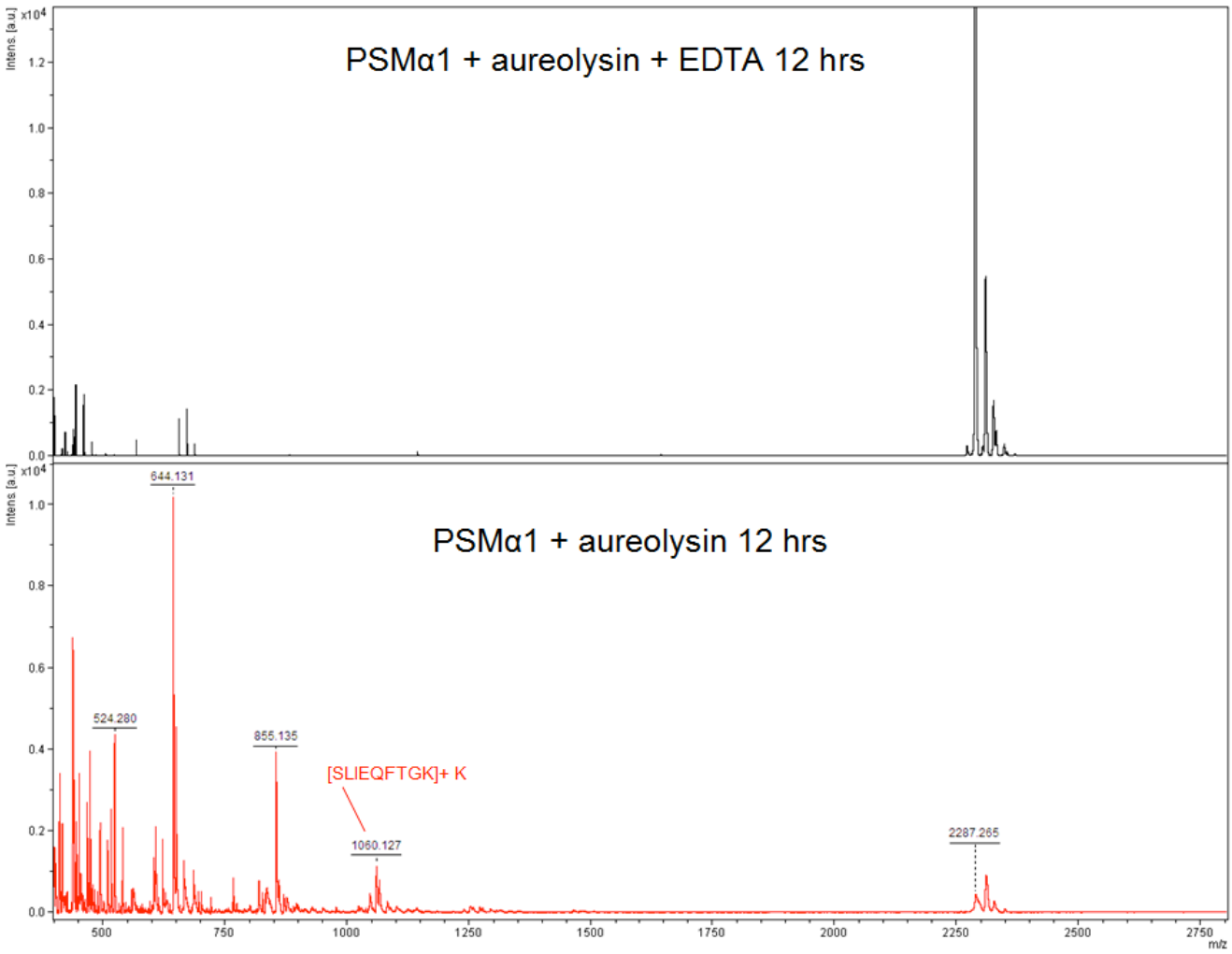
Supplemental Figure S12. Structures of additional MRSA PSM derivatives identified by MALDI-MS. Mass spectrometry survey of *S. aureus* strains shows PSMs derivatives are biosynthesized globally among the strains tested. The structures for the peptides amenable to collision-induced dissociation are shown above and are provided in the Supplemental Information.



Supplemental Figure S13. Aureolysin effects on PSMα4 (12 h). Recombinant aureolysin was incubated with the peptide PSMα4 in 20 mM Tris-HCl pH 7.8 containing 5 mM $CaCl_2$ and incubated for 12 h. The reaction was then assessed by MALDI-MS and cleavage products were observed (red) that were absent in the control sample. The control sample (black) consisted of the same reaction conditions except substituting EDTA for $CaCl_2$ in the reaction mixture. The MALDI-MS spectrum of PSMα4 alone had an identical profile to the control sample. Within the cleavage reaction one peptide was identified based on intact mass.



Supplemental Figure S14. Aureolysin effects on PSMα4 (36 h). Recombinant aureolysin was incubated with the peptide PSMα4 in 20 mM Tris-HCl pH 7.8 containing 5 mM $CaCl_2$ and incubated for 36 h. The reaction was then assessed by MALDI-MS and cleavage products were observed (red) that were absent in the control sample. The control sample (blue) consisted of the same reaction conditions except substituting EDTA for $CaCl_2$ in the reaction mixture. The MALDI-MS spectrum of PSMα4 alone had an identical profile to the control sample. Within the cleavage reaction three peptides were identified based on intact mass. Potassium (+38) and Sodium (+22) indicate salt adducts of the observed ions.



Supplemental Figure S15. Recombinant aureolysin incubated with PSMα1 for 12hrs. Recombinant aureolysin was incubated with the peptide PSMα1 in 20 mM Tris-HCl pH 7.8 containing 5 mM $CaCl_2$ and allowed to sit for 12hr. The reaction was then assessed by MALDI-MS and cleavage products were observed (red) that were absent in the control sample. The control sample (black) consisted of the same above described reaction conditions except substituting EDTA for $CaCl_2$ in the reaction mixture. The MALDI-MS spectrum of PSMα1 alone had an identical profile to the control sample. Within the cleavage reaction one peptide was identified based on intact mass (K and Na indicate salt adducts of the observed ions).

Gene cluster	Length [bp]	*S. aureus* USA300 FPR3757 (% identity)	*S. aureus* USA300 TCH959 (% identity)
PSMα	9645	100 (9645 bp)	99 (9645 bp)
δ -toxin	3129	100 (3129 bp)	99 (3190 bp)

PSMα gene cluster analysis from *Staphylococcus aureus* USA300 (including corresponding gene clusters in 2 other USA300 strains)

Gene	Protein	Size [aa]	Predicted function	*S. aureus* USA300 TCH959 homolog (% identity, % similarity)	*S. aureus* USA300 FPR3757 homolog (% identity, % similarity)
USA300HOU_0452	YP_001574357	400	cobalamin biosynthesis protein	ZP_04864961.1 (99/100)	YP_493137
USA300HOU_0453	YP_001574358	47	hypothetical protein	N/A (100/100)	N/A (100/100)
USA300HOU_0454	YP_001574359	42	hypothetical protein	N/A (100/100)	N/A (100/100)
N/A	N/A	20	PSMα 4	N/A (100/100)	N/A (100/100)
N/A	N/A	22	PSMα 3	N/A (100/100)	N/A (100/100)
USA300HOU_0455	YP_001574360	21	PSMα 2	N/A (100/100)	N/A (100/100)
USA300HOU_0456	YP_001574361	21	PSMα 1	N/A (100/100)	N/A (100/100)
USA300HOU_0457	YP_001574362	494	NADH dehydrogenase	ZP_04864963.1 (98/99)	YP_493138 (100/100)
USA300HOU_0458	YP_001574363	901	NADH dehydrogenase	ZP_04864964.1 (99/99)	YP_493139 (100/100)
USA300HOU_0459	YP_001574364	120	hypothetical protein	ZP_04864965.1 (99/99)	YP_493140 (100/100)
USA300HOU_0460	YP_001574365	138	hypothetical protein	ZP_04864966.1 (100/100)	YP_493141 (100/100)
USA300HOU_0461	YP_001574366	224	PAP2 superfamily phosphatase	ZP_04864967.1 (100/100)	YP_493142 (100/100)
USA300HOU_0462	YP_001574367	244	carboxyesterase	ZP_04864968.1 (100/100)	YP_493143 (100/100)

δ -toxin gene cluster analysis from *Staphylococcus aureus* USA300 (including corresponding gene clusters in 2 other USA300 strains)

Gene	Protein	Size [aa]	Predicted function	*S. aureus* USA300 TCH959 homolog (% identity, % similarity)	*S. aureus* USA300 FPR3757 homolog (% identity, % similarity)
USA300HOU_2031	YP_001575907	44	delta-hemolysin	N/A (100/100)	YP_494639 (100/100)
USA300HOU_2032	YP_001575908	189	agrB	ZP_04863968 (99/100)	YP_494640 (100/100)
USA300HOU_2033	YP_001575909	46	agrD	ZP_04863969 (100/100)	YP_494641 (100/100)
USA300HOU_2034	YP_001575910	430	agrC	ZP_04863970 (99/99)	YP_494642 (100/100)
USA300HOU_2035	YP_001575911	238	agrA	ZP_04863971 (99/100)	YP_494643 (100/100)

Table 1 | Sequence similarity of target PSM gene clusters from *Staphylococcus aureus* USA300 TCH1516 with other S.aureus strains (FPR3757, TCH959)

Supplemental Table S1. CA-MRSA USA300 TCH1516 genome comparison. A survey of the genes adjacent to the PSMs and δ–toxin show no gene with significant homology to known proteases.

A

dPSM1	1262	1361	1489	1602	1716	1773	1844	1957	2070	2127
LAC wild-type			xxx			xx			xx	
LAC delta-aureolysin			xxx			xx	xx	xx	xx	
LAC recomplimented			xxx			xx		xx	xx	
dPSM2	**1232**	**1379**	**1507**	**1621**	**1734**	**1791**	**1862**	**1975**	**2088**	**2145**
LAC wild-type						xx		xx		
LAC delta-aureolysin						xx		xx		
LAC recomplimented						xx		xx	xx	
dPSM3	**1217**	**1364**	**1511**	**1639**	**1786**	**1900**	**2028**	**2099**	**2198**	**2345**	**2474**
LAC wild-type	xx		xx	xx		xx				xx	
LAC delta-aureolysin		xx	xx			xx			xx	xx	
LAC recomplimented		xx	xx			xx		xx	xx	xx	xx
dPSM4	**1243**	**1371**	**1484**	**1598**	**1699**	**1756**	**1855**	**1968**	**2039**	**2170**	**2198**
LAC wild-type		xx	xx	xx	xx	xx	xxx			xx	
LAC delta-aureolysin			xx	xx	xx	xx					
LAC recomplimented			xx	xx	xx	xx	xxx				
dPSMλ	**1192**	**1305**	**1491**	**1619**	**1719**	**1832**	**1947**	**2004**	**2117**	**2218**	**2305**	**2418**	**2531**	**2646**	**2774**
LAC wild-type		xx		xx				xx		xx			xx	xx	xx
LAC delta-aureolysin		xx		xx		xx		xx					xx	xx	xx
LAC recomplimented		xx		xx		xx		xx		xx		xx	xx	xx	xx

B

dPSM1	1262	1361	1489	1602	1716	1773	1844	1957	2070	2127
ST59						xx				
UAMS1182						xx				
UAMS1182 delta-aureolysin						xx				
dPSM2	**1232**	**1379**	**1507**	**1621**	**1734**	**1791**	**1862**	**1975**	**2088**	**2145**
ST59						xx				
UAMS1182						xx				
UAMS1182 delta-aureolysin										
dPSM3	**1217**	**1364**	**1511**	**1639**	**1786**	**1900**	**2028**	**2099**	**2198**	**2345**	**2474**
ST59											
UAMS1182											
UAMS1182 delta-aureolysin											
dPSM4	**1243**	**1371**	**1484**	**1598**	**1699**	**1756**	**1855**	**1968**	**2039**
ST59				xx	xx		xx		
UAMS1182				xx	xx	xx			
UAMS1182 delta-aureolysin				xx	xx	xx			
dPSMλ	**1192**	**1305**	**1491**	**1619**	**1719**	**1832**	**1947**	**2004**	**2117**	**2218**	**2305**	**2418**	**2531**	**2646**	**2774**
ST59											xx			xx	
UAMS1182										xx					
UAMS1182 delta-aureolysin										xx					

Supplemental Table S2. Masses observed by MALDI-MS of multiple MRSA strains. **A.** Mass lists of *S. aureus* LAC, isogenic Δ*aur* mutant, and the Δ*aur* mutant complemented with pAur. The list contained the sequences of N-terminal truncations of each PSM. Supernatants from multiple *S. aureus* strains were fractionated by HPLC then examined by MALDI-MS in duplicate (xx) or triplicate (xxx). All ions that had a mass corresponding to a potential PSM truncated derivatives were isolated within the mass spectrometer and subjected to tandem mass spectrometry (MS/MS). The majority of the ions were not amenable to MS/MS by collision induced dissociation. The ions that were amenable to MS/MS are reported within this study. **B.** MALDI-MS of *S. aureus* ST59, UAMS1182, and UAMS1182 Δ*aur*.