SUPPLEMENTAL INFORMATION

Phenol Soluble Modulin Variants of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Captured Using Mass Spectrometry-Based Molecular Networking

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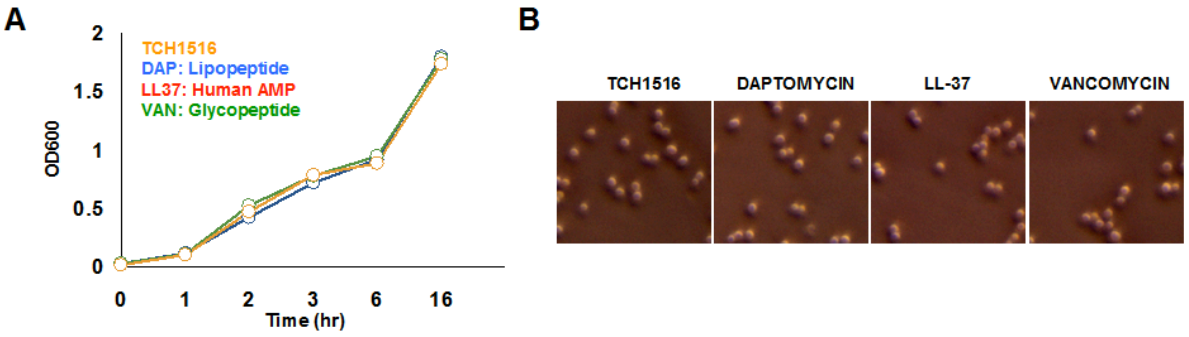
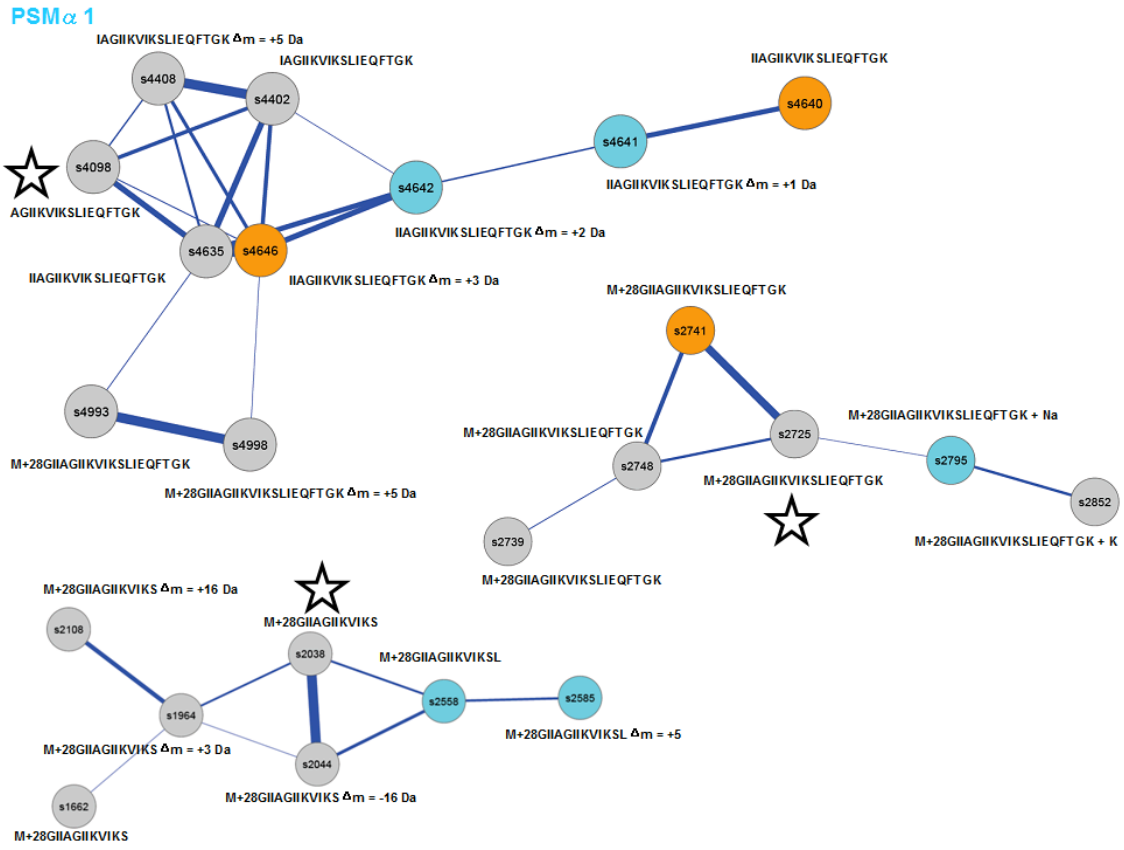
Content

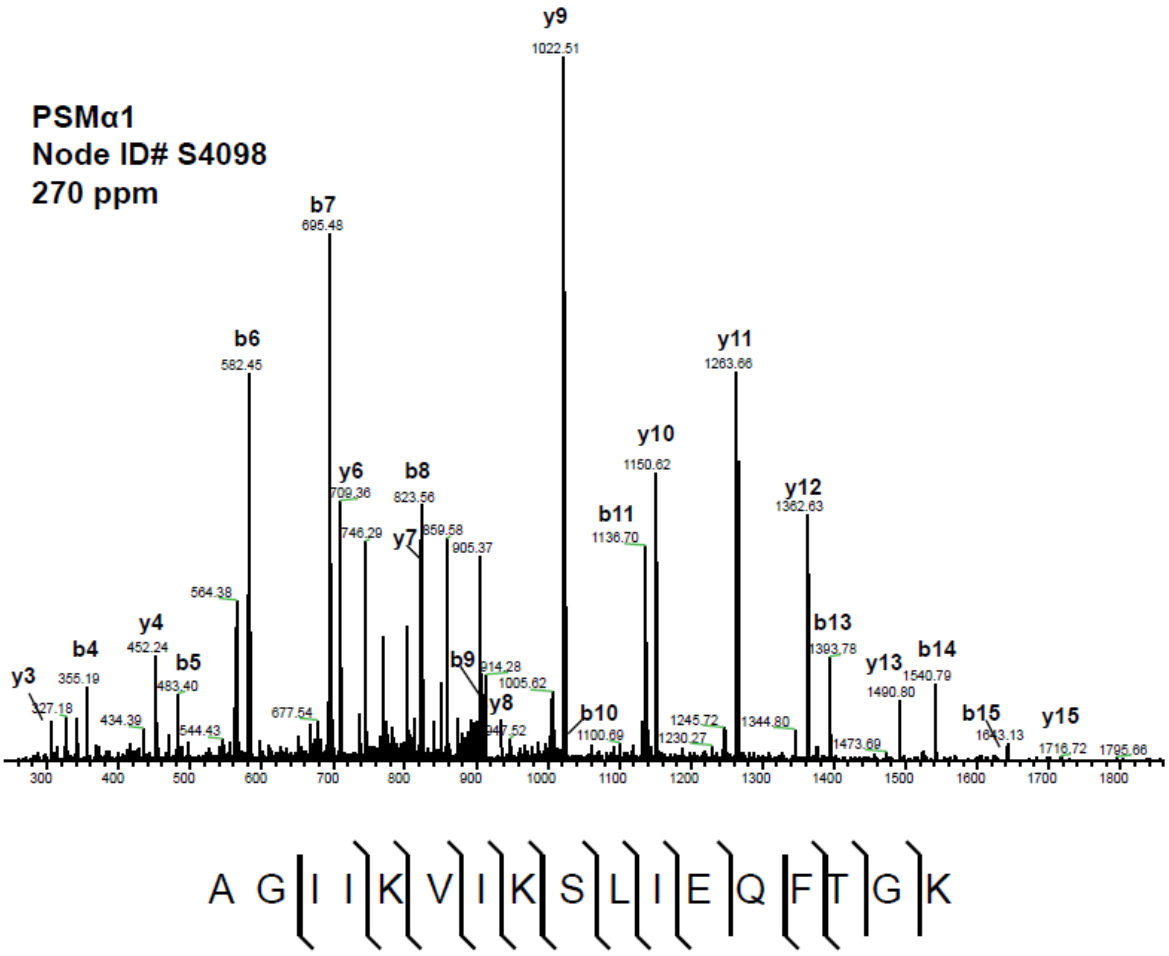
Figures **Page**



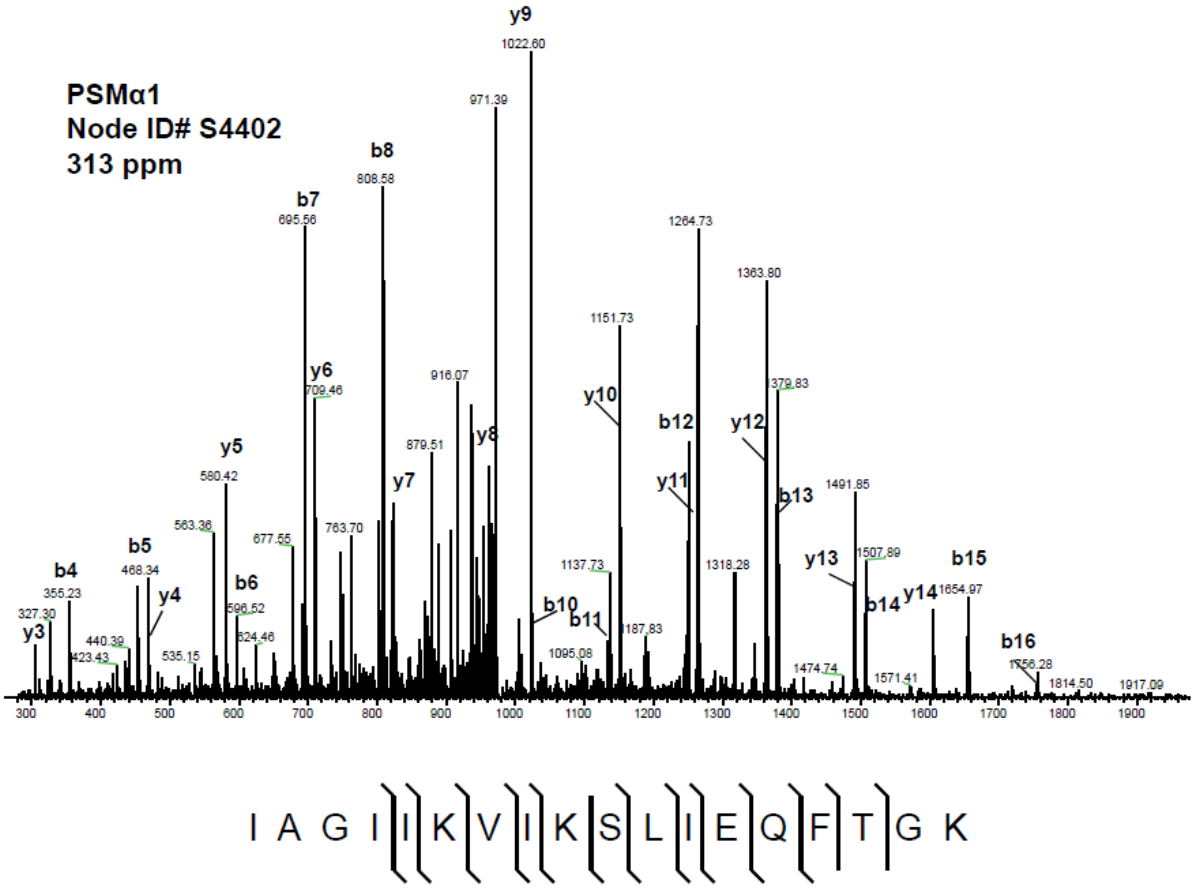
Supplemental Figure S1. **Effects of antibiotics treatment on MRSA growth and morphology** A. Growth curve of CA-MRSA strain TCH1516 under sub-MIC treatment monitored over 16 h. B. CA-MRSA strain TCH1516 cell morphology assessed by light microscopy.



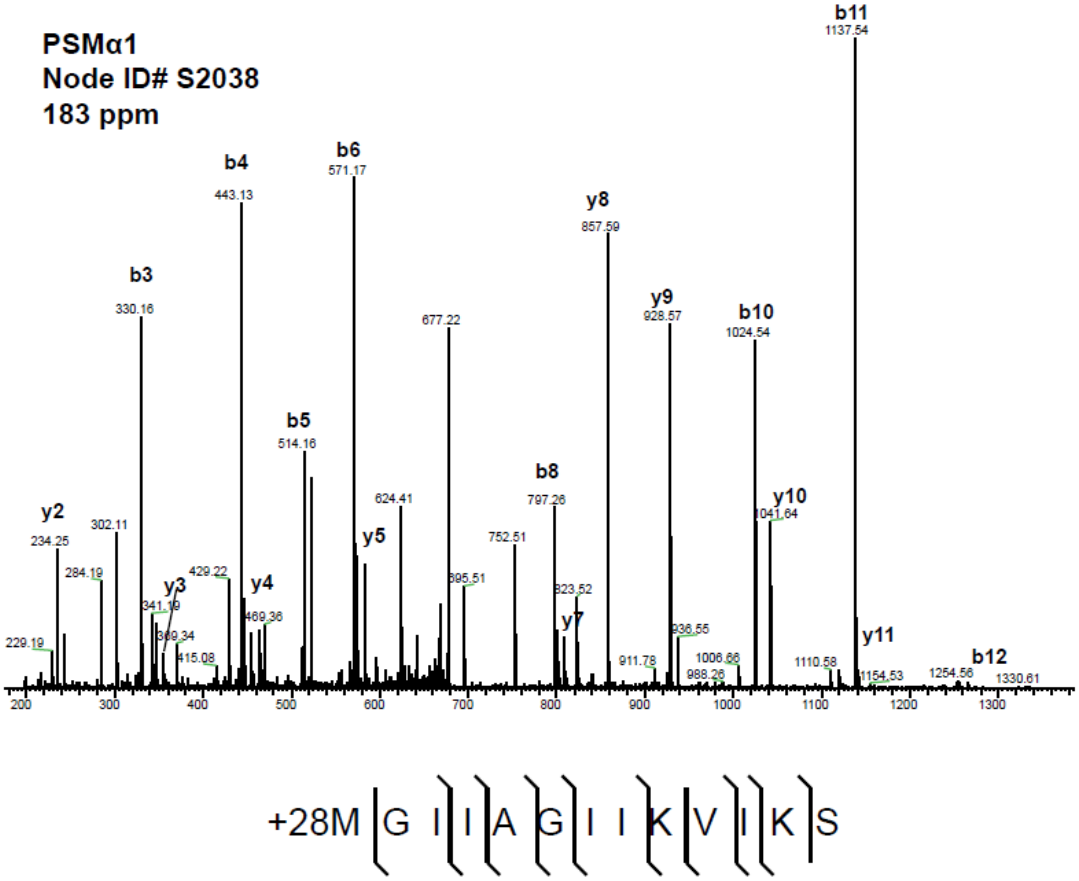
Supplemental Figure S2. **PSMα1 associated node clusters captured in the generated CA-MRSA molecular network +/- daptomycin treatment.** Nodes indicated by a star were sequenced and contained reasonable mass accuracy for theoretical PSMs. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Common salt adducts (e.g. sodium and potassium) are also indicated. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



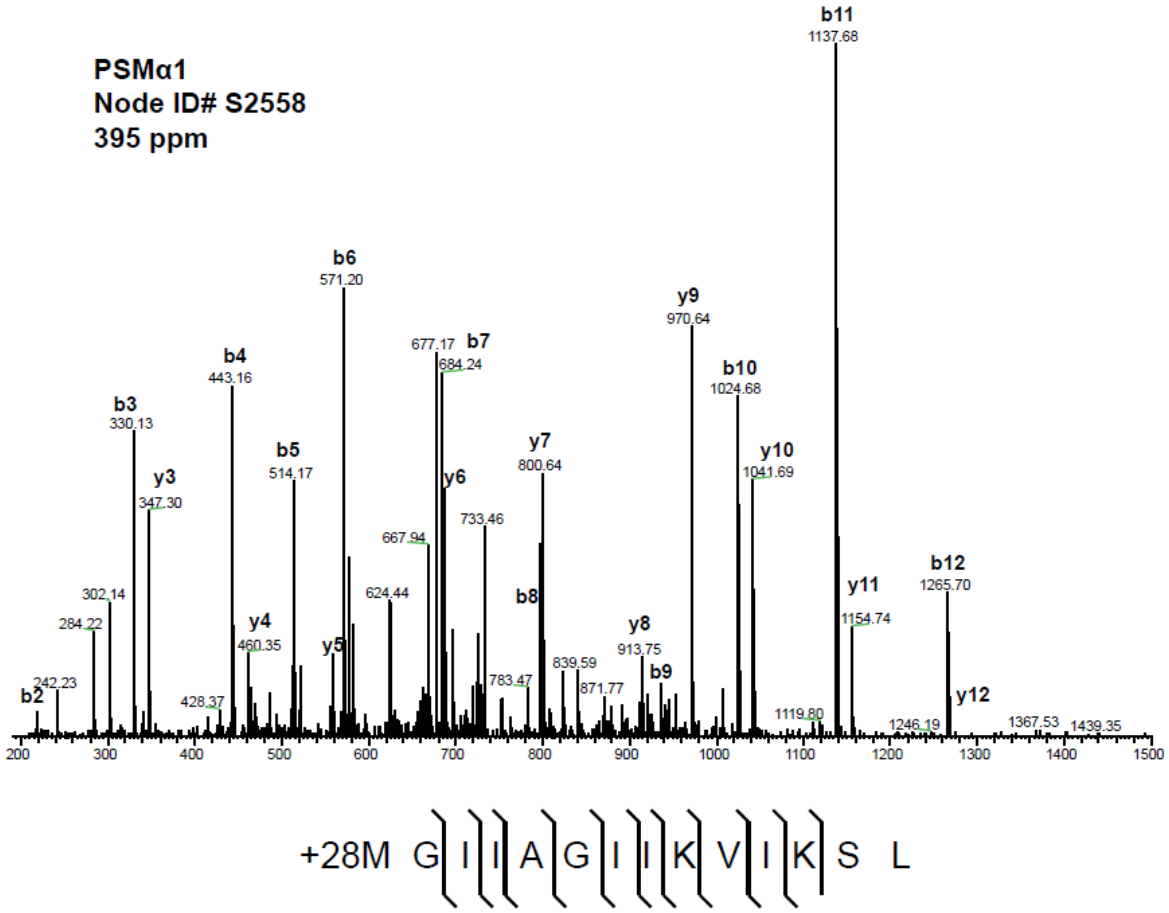
Supplemental Figure S3. **Tandem mass spectrum of the dPSMα1 17-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



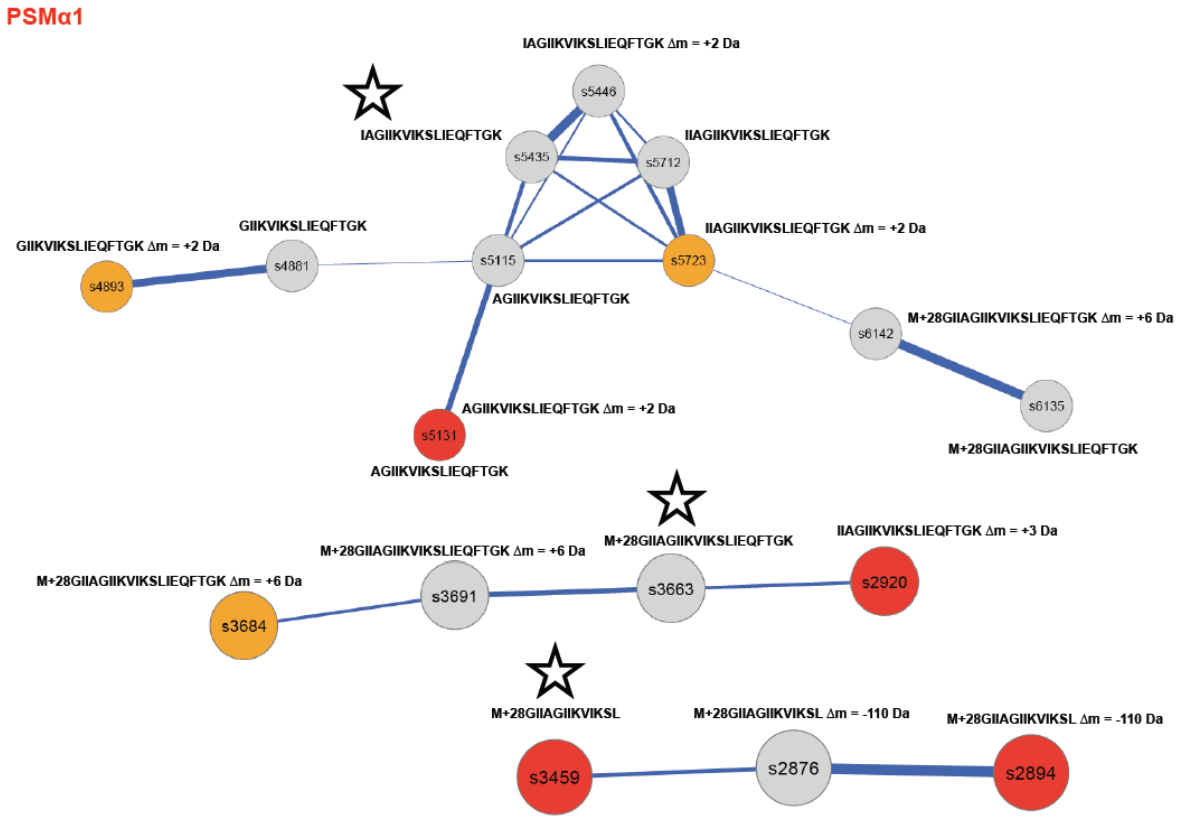
Supplemental Figure S4. **Tandem mass spectrum of the dPSMα1 18-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



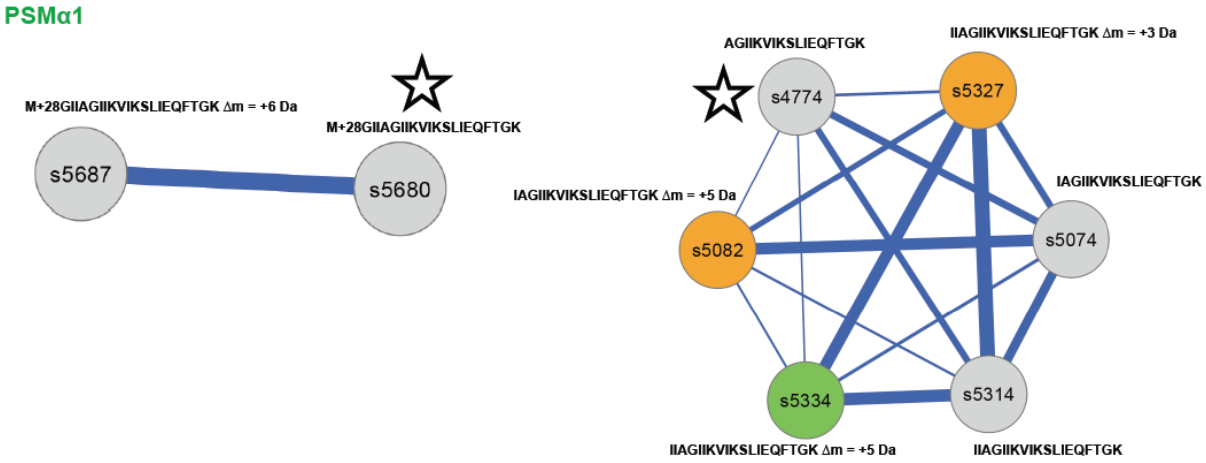
Supplemental Figure S5. **Tandem mass spectrum of the N-terminal formylated dPSMα1 13-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



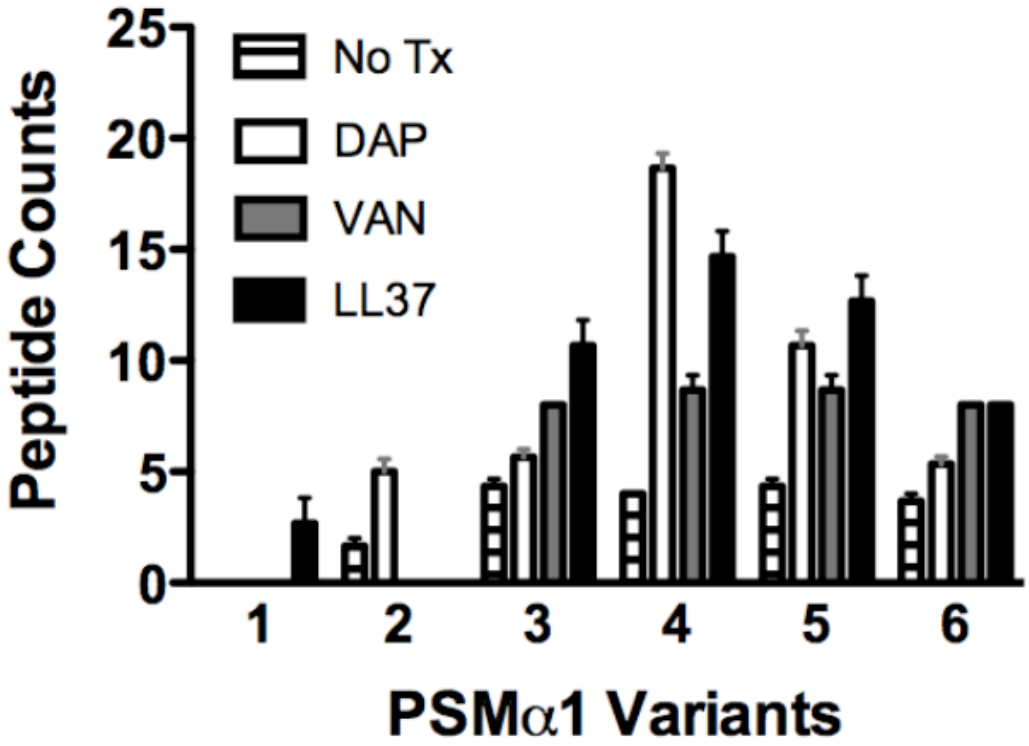
Supplemental Figure S6. **Tandem mass spectrum of the N-terminal formylated dPSMα1 14-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



Supplemental Figure S7. **PSMα1 associated node clusters captured in the generated CA-MRSA molecular network under +/- LL-37 treatment.** Nodes that have a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



Supplemental Figure S8. **PSMα1 associated node clusters captured in the generated CA-MRSA molecular network +/- vancomycin treatment.** Nodes indicated by a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



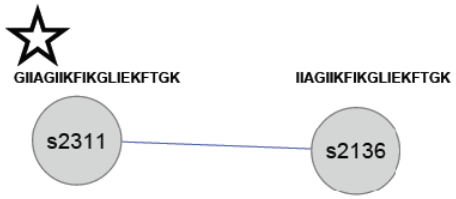
Supplemental Figure S9. **Spectral counts for PSMα1 derivatives**.

PSMα1 variants:

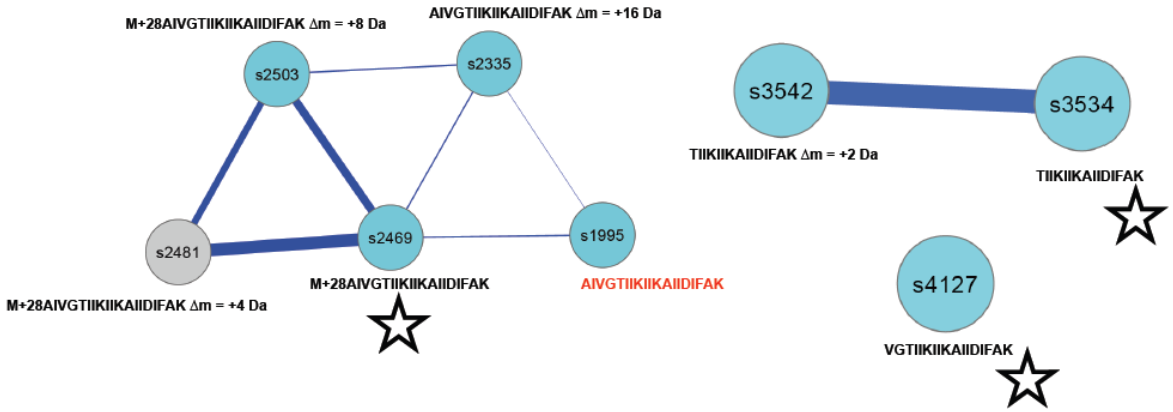
(1) +28MGIIAGIIKVIKSL
(2) +28MGIIAGIIKVIKS
(3) IIAGIIKVIKSLIEQFTGK
(4) IAGIIKVIKSLIEQFTGK
(5) AGIIKVIKSLIEQFTGK
(6) GIIKVIKSLIEQFTGK

Peptide 1 was not detected in the CA-MRSA alone, daptomycin and vancomycin treatments. Peptide 2 was not detected in the vancomycin and LL-37 treatments.

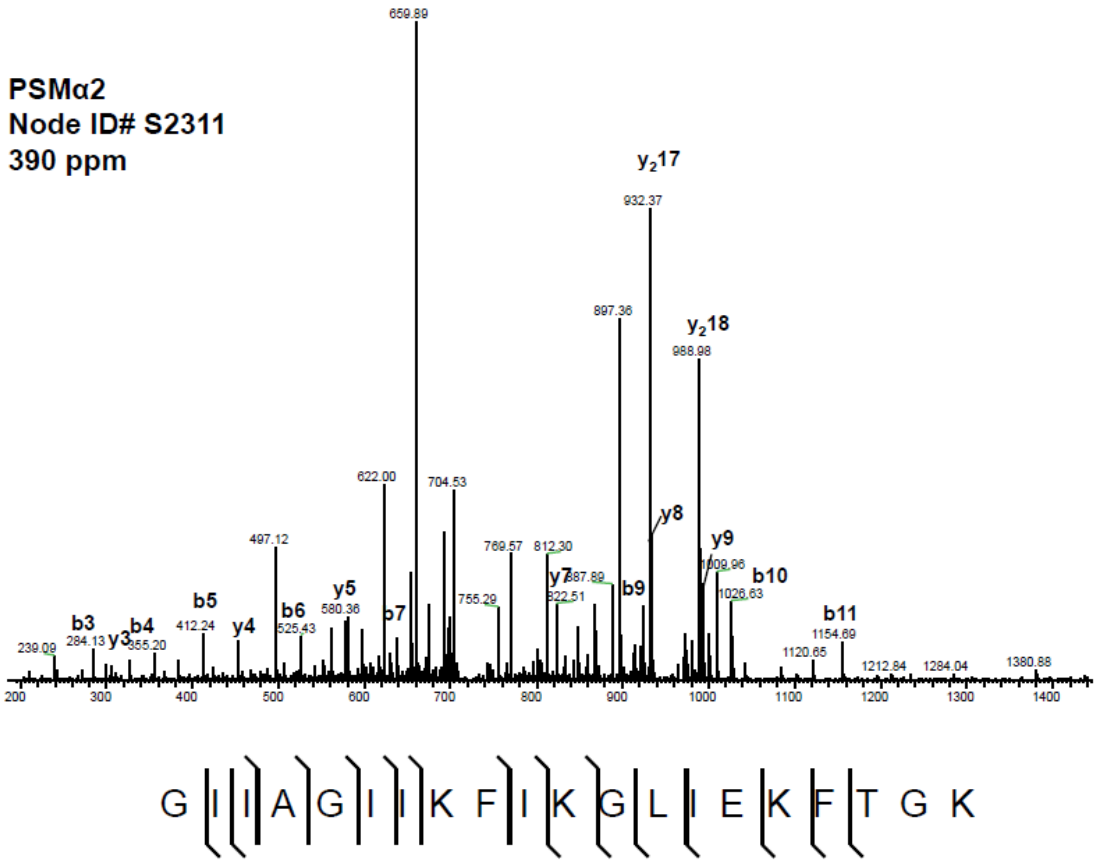
PSMα2



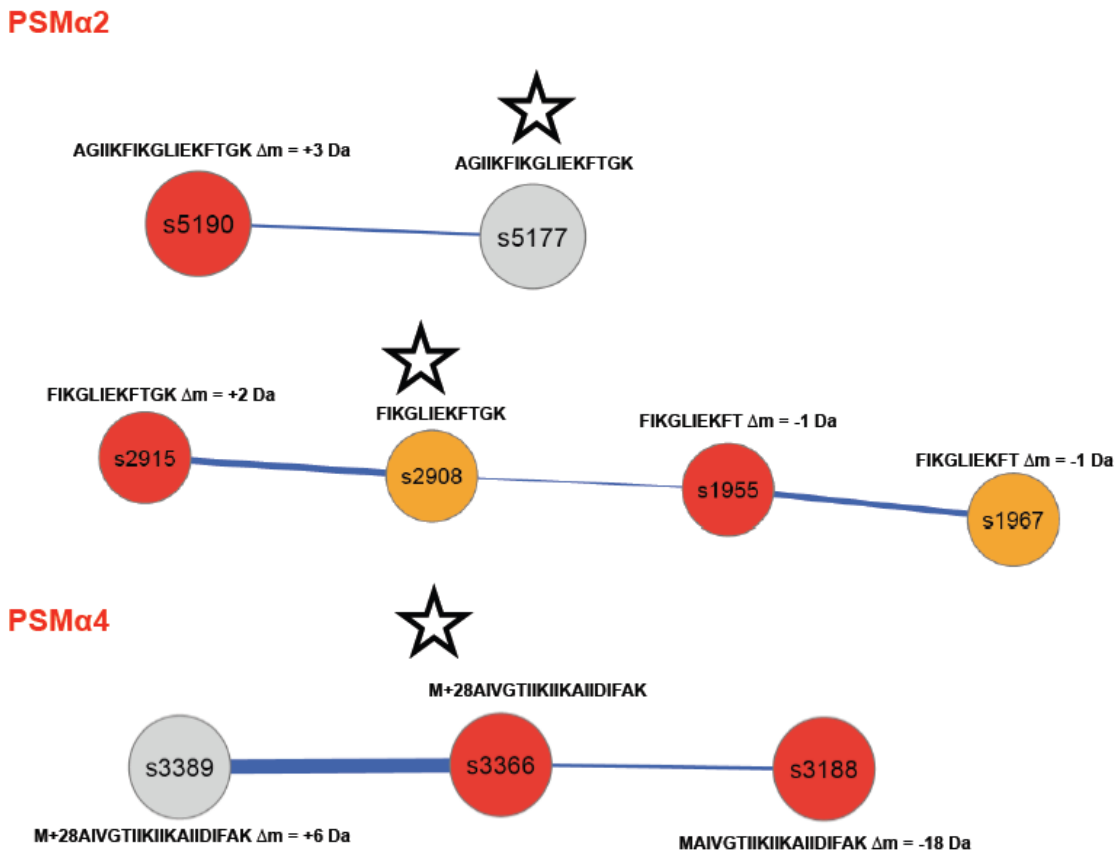
PSMα4



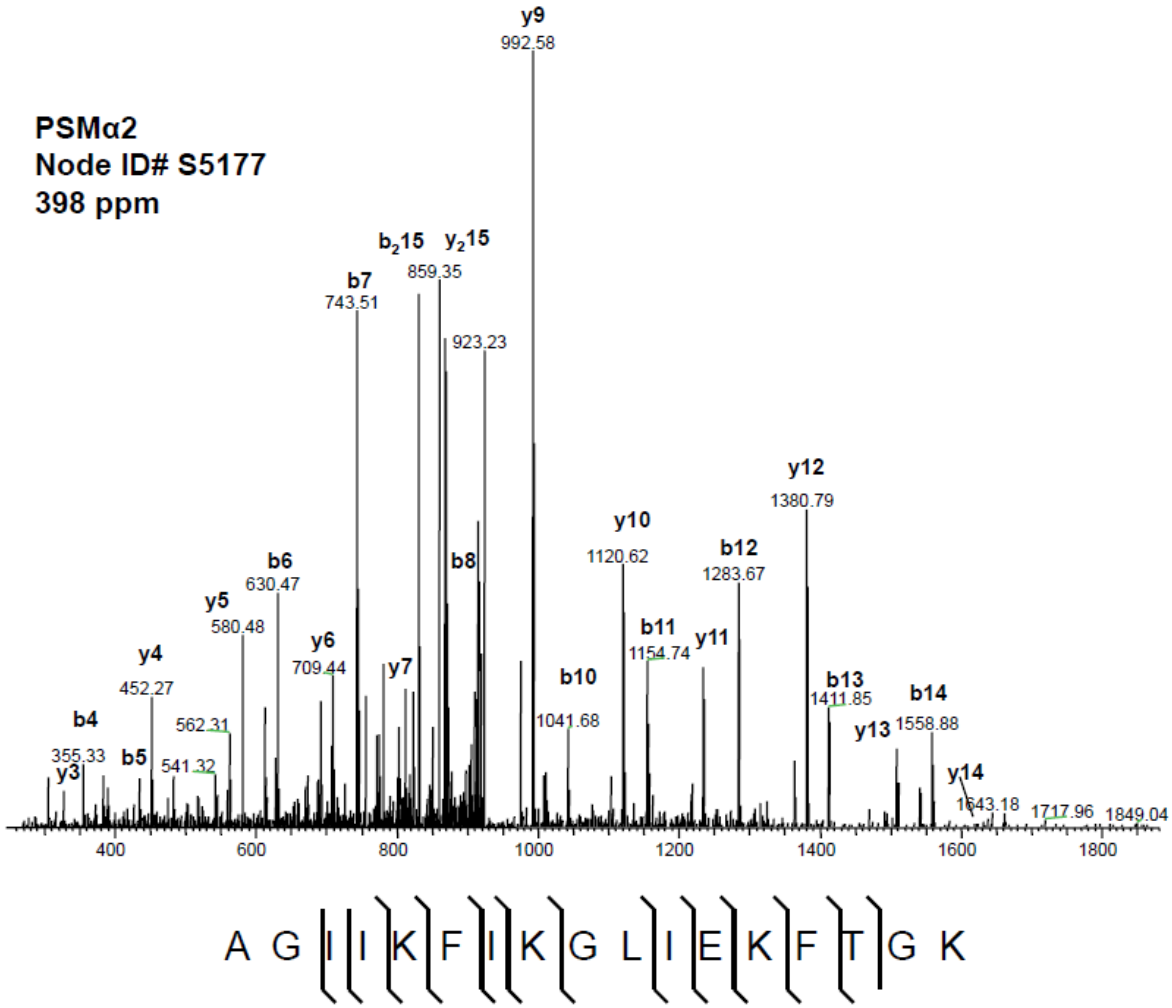
Supplemental Figure S10. **PSMα2 and PSMα4 associated node clusters captured in the generated CA-MRSA molecular network +/- daptomycin treatment.** Nodes indicated by a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications. The sequence colored red indicates the node could not be validated due to poor spectra.



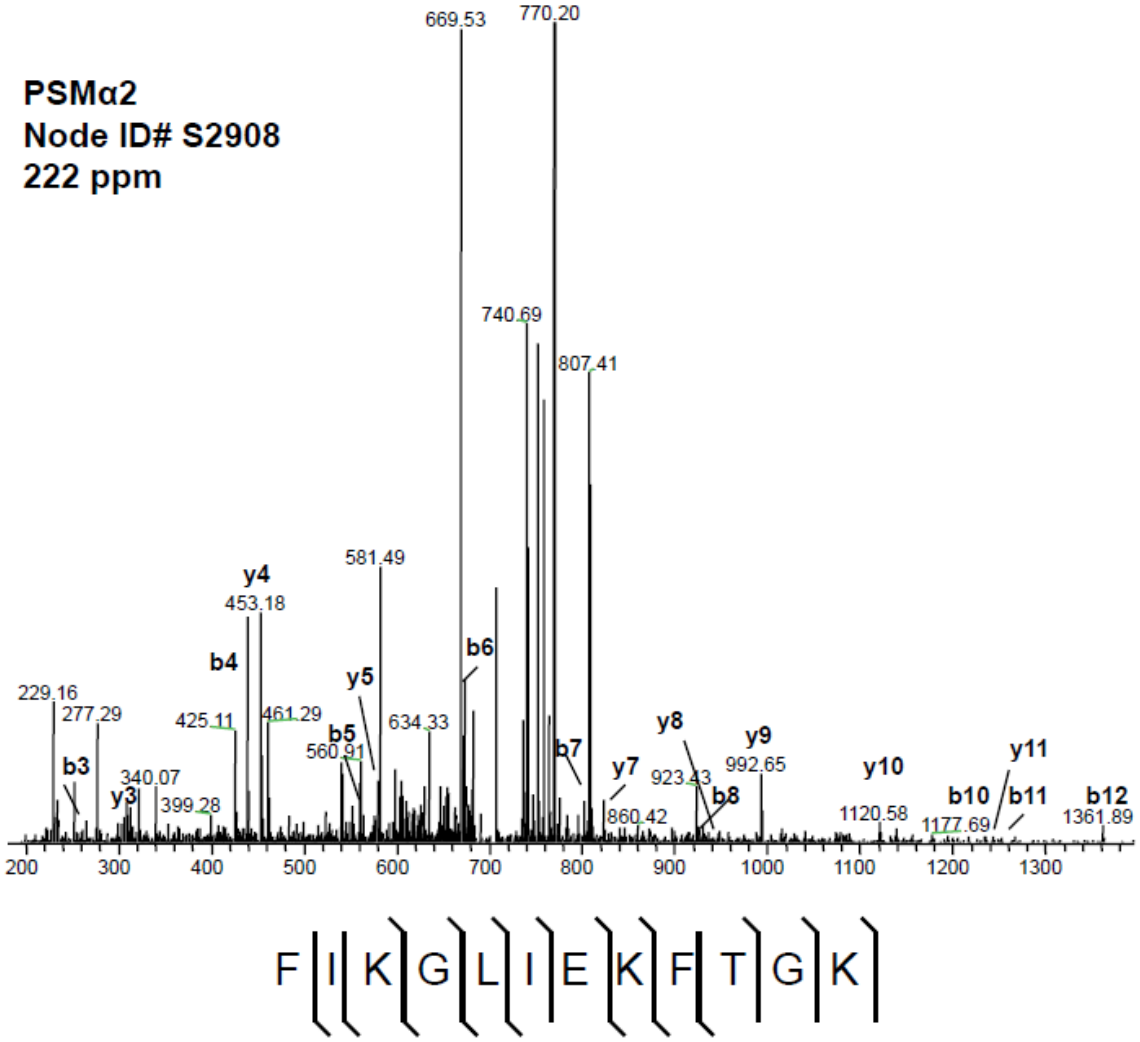
Supplemental Figure S11. **Tandem mass spectrum of the dPSMα2 20-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



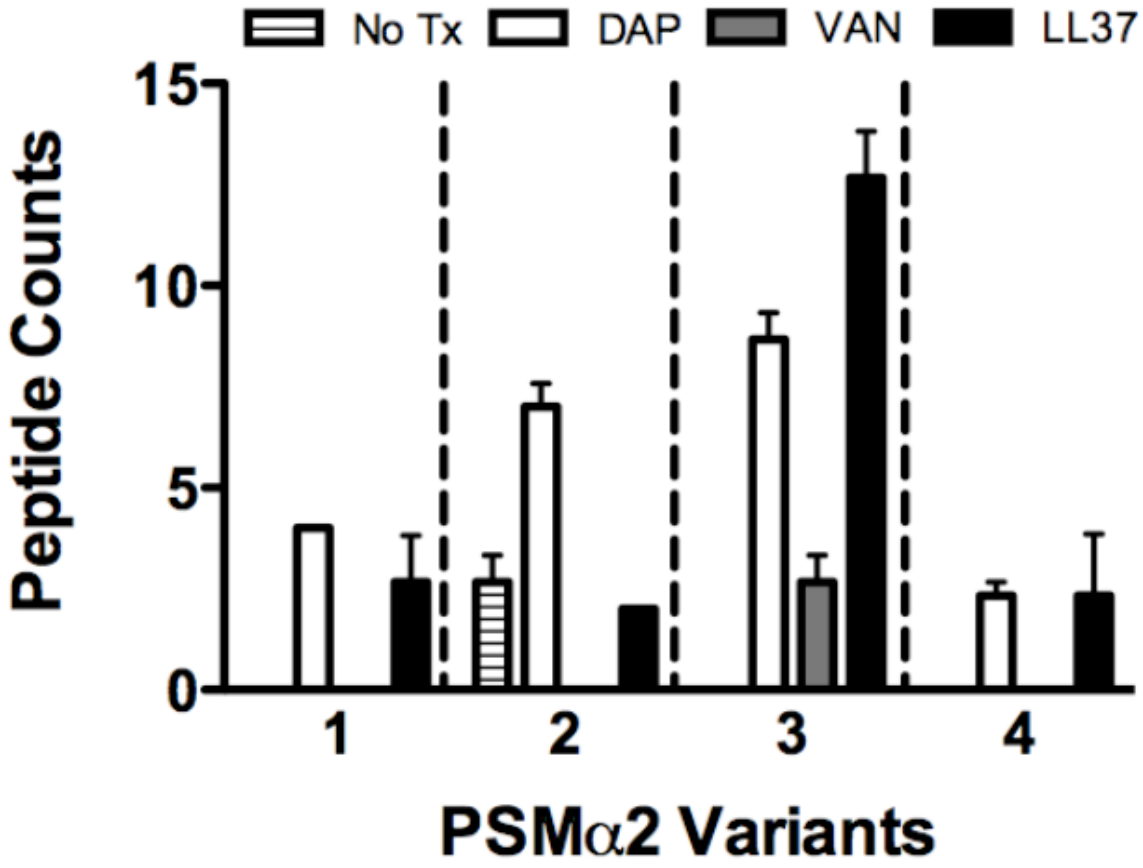
Supplemental Figure S12. **PSMα2 and PSMα4 associated node clusters captured in the generated CA-MRSA molecular network under +/- LL-37 treatment.** Nodes that have a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



Supplemental Figure S13. **Tandem mass spectrum of the dPSMα2 17-mer peptide.** 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 (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



Supplemental Figure S14. **Tandem mass spectrum of the dPSMα2 12-mer peptide.** 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 (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.

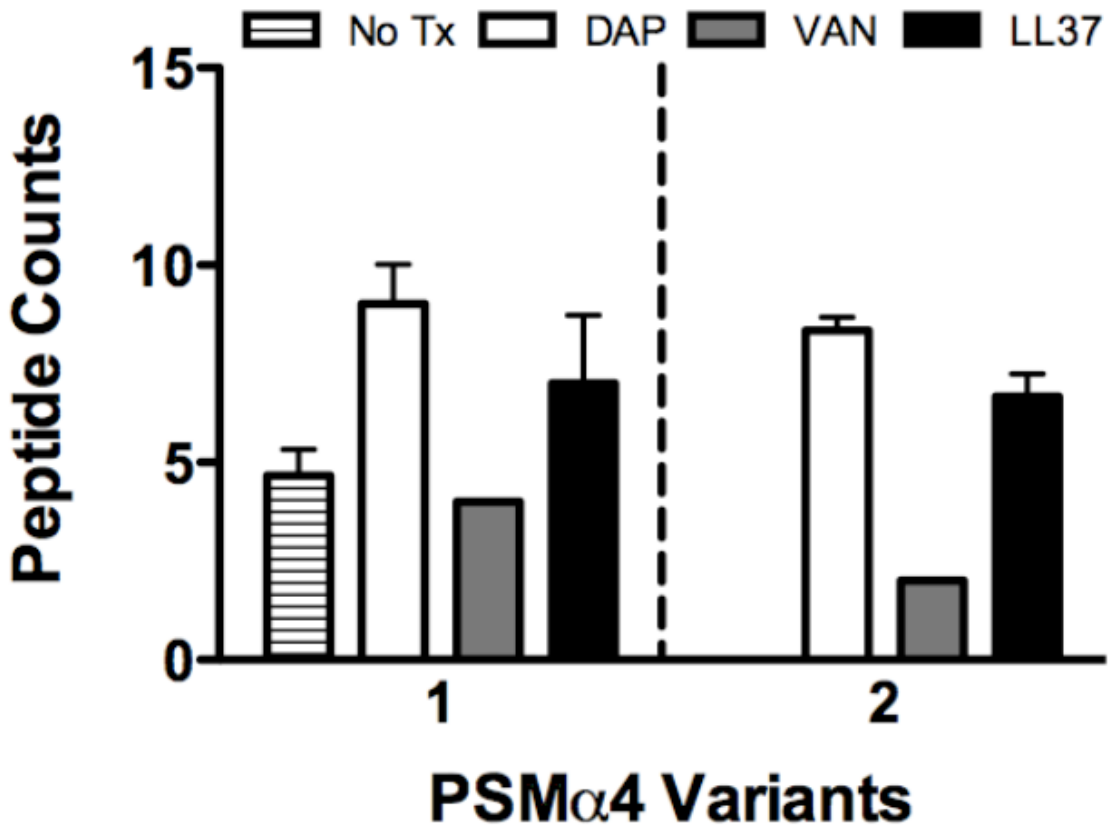


Supplemental Fig. S15. **Spectral counts for PSMα2 derivatives**.

PSMα2 variants:

(1) GIIAGIIKFIKGLIEKFTGK
(2) IIAGIIKFIKGLIEKFTGK
(3) AGIIKFIKGLIEKFTGK
(4) FIKGLIEKFTGK

Peptide 1 was not detected in the CA-MRSA alone and daptomycin treatment. Peptide 2 was not detected in the vancomycin. Peptide 3 was not detected in the CA-MRSA alone sample. Peptide 4 was not detected in the CA-MRSA alone and vancomycin treatment. Dashed lines are used as borders between individual experiments performed on each peptide.

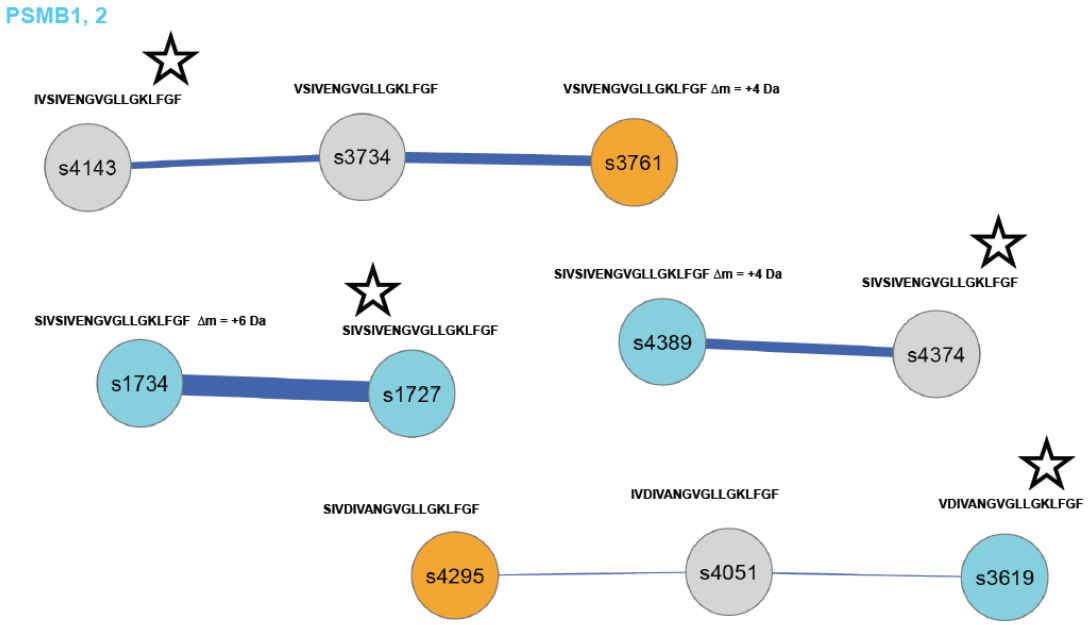


Supplemental Fig. S16. **Spectral counts for PSMα4 derivatives**.

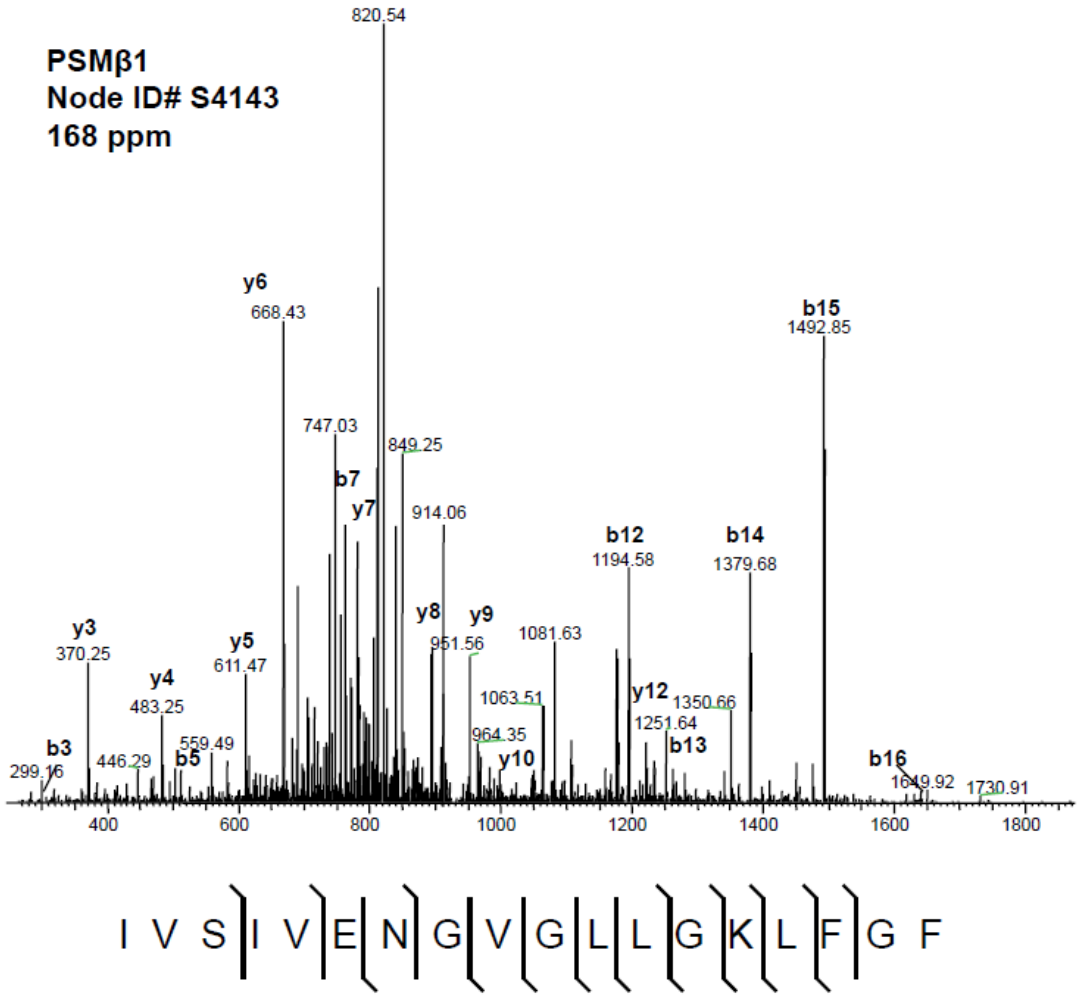
PSMα4 variants:

(1) VGTIIKIIKAIIDIFAK
(2) TIIKIIKAIIDIFAK

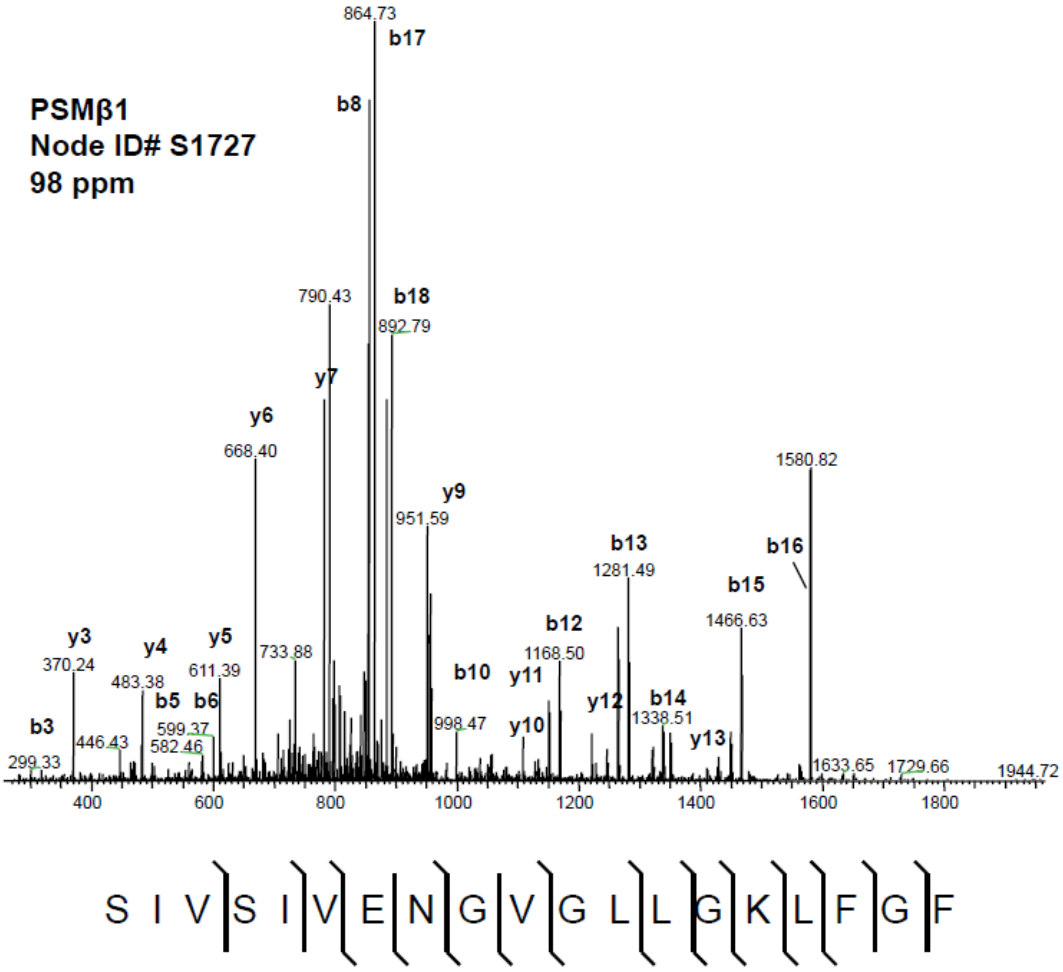
Peptide 2 was not detected in the CA-MRSA alone sample. Dashed lines are used as borders between individual experiments performed on each peptide.



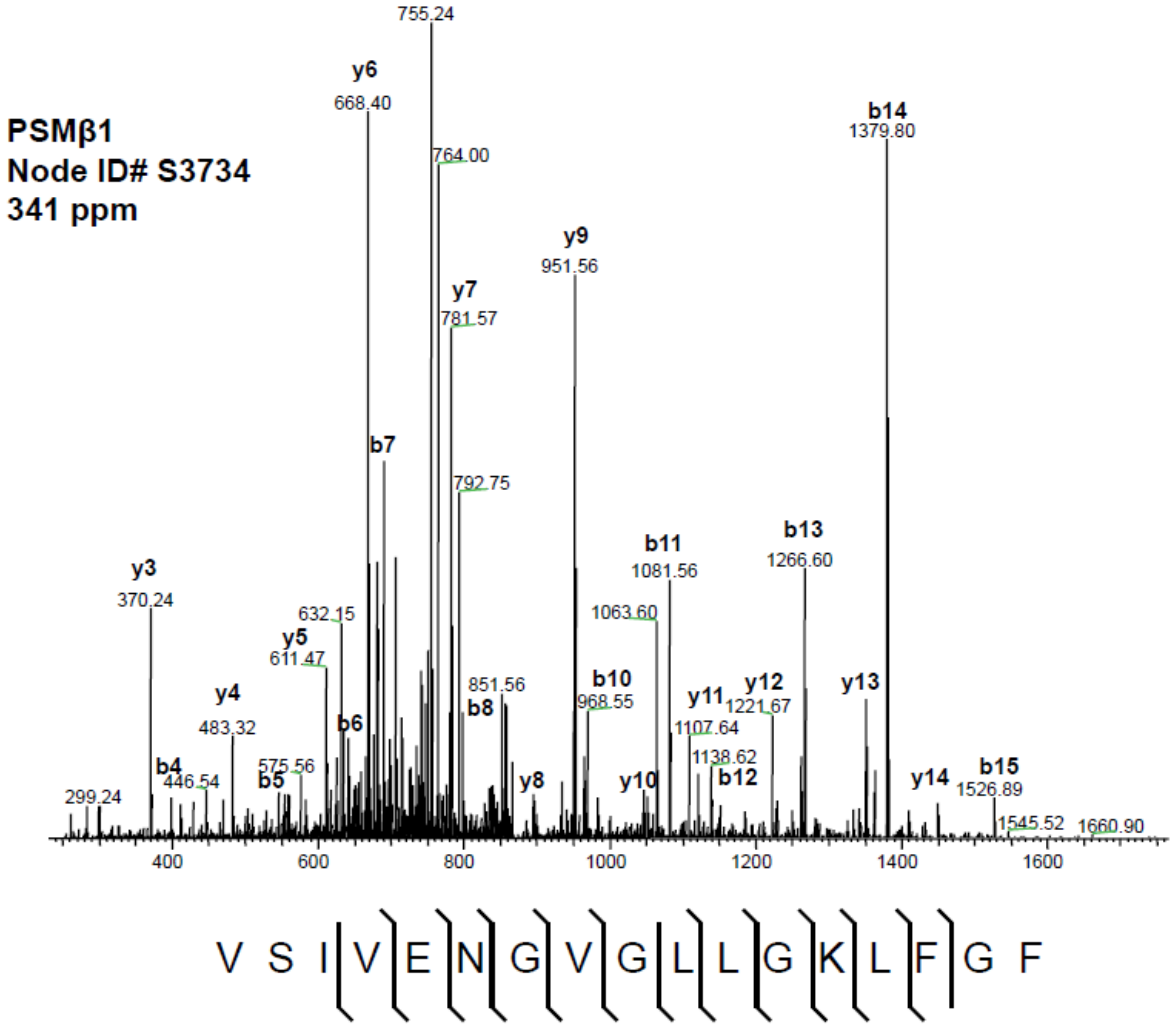
Supplemental Figure S17. **PSMβ1 and PSMβ2 associated node clusters captured in the generated CA-MRSA molecular network under +/- daptomycin treatment.** Nodes that have a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



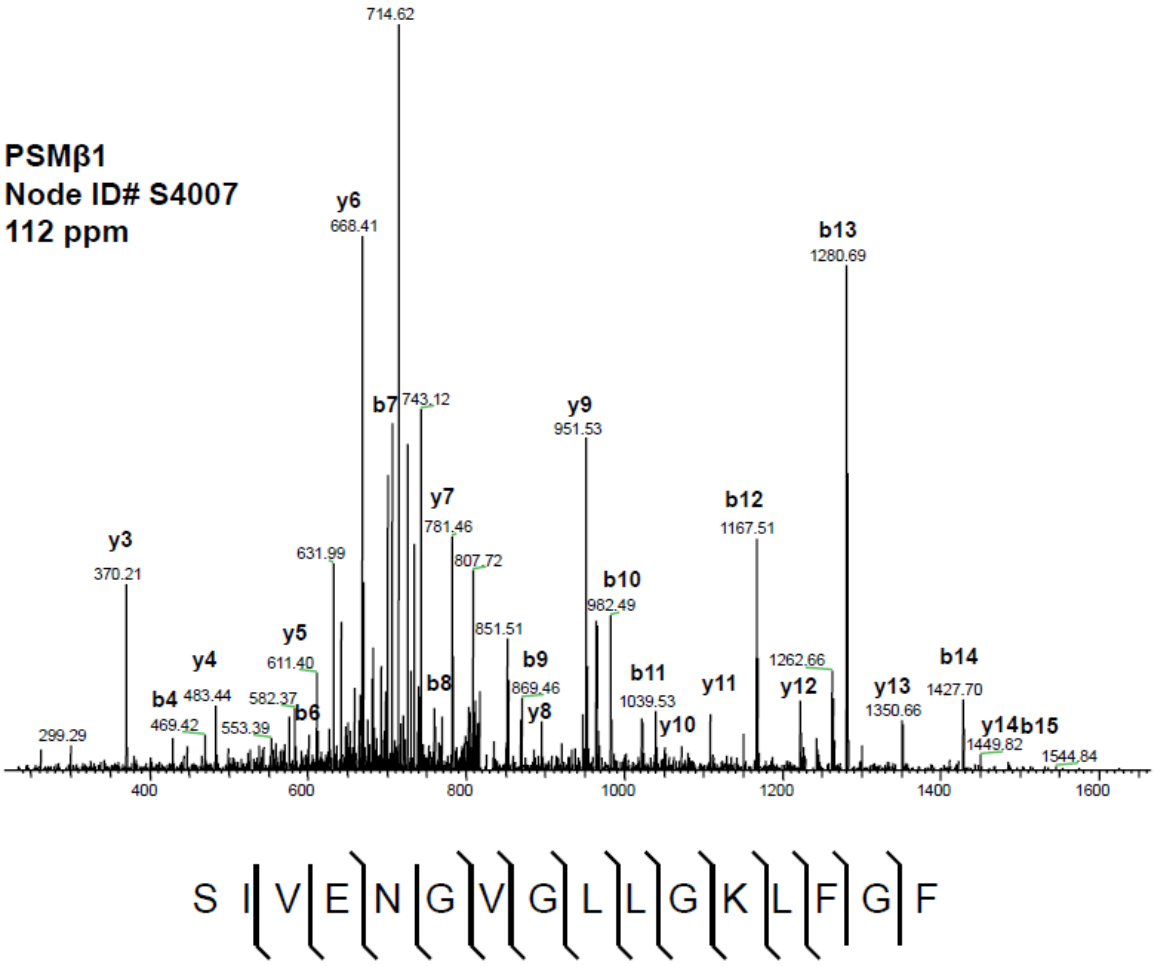
Supplemental Figure S18. **Tandem mass spectrum of the dPSMβ1 18-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



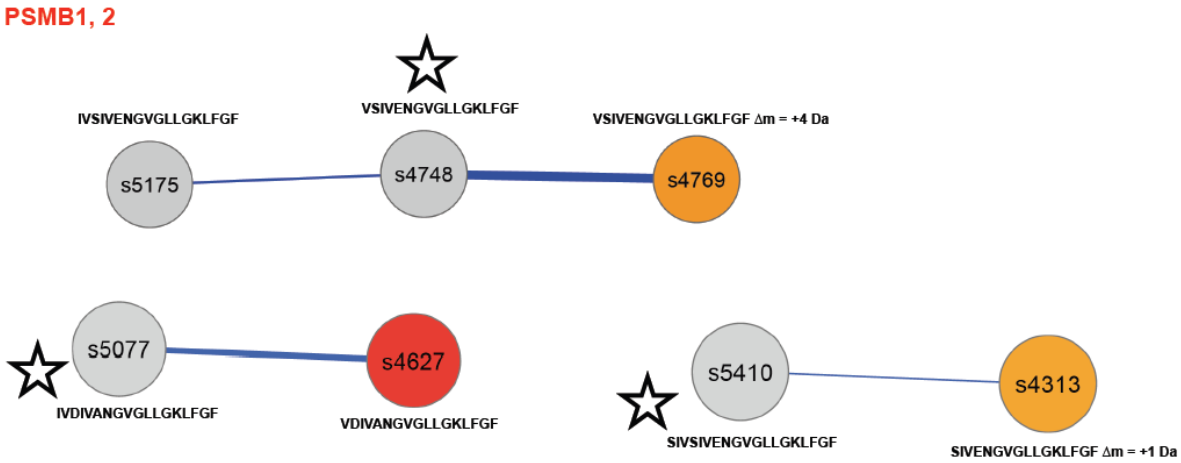
Supplemental Figure S19. **Tandem mass spectrum of the dPSMβ1 19-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



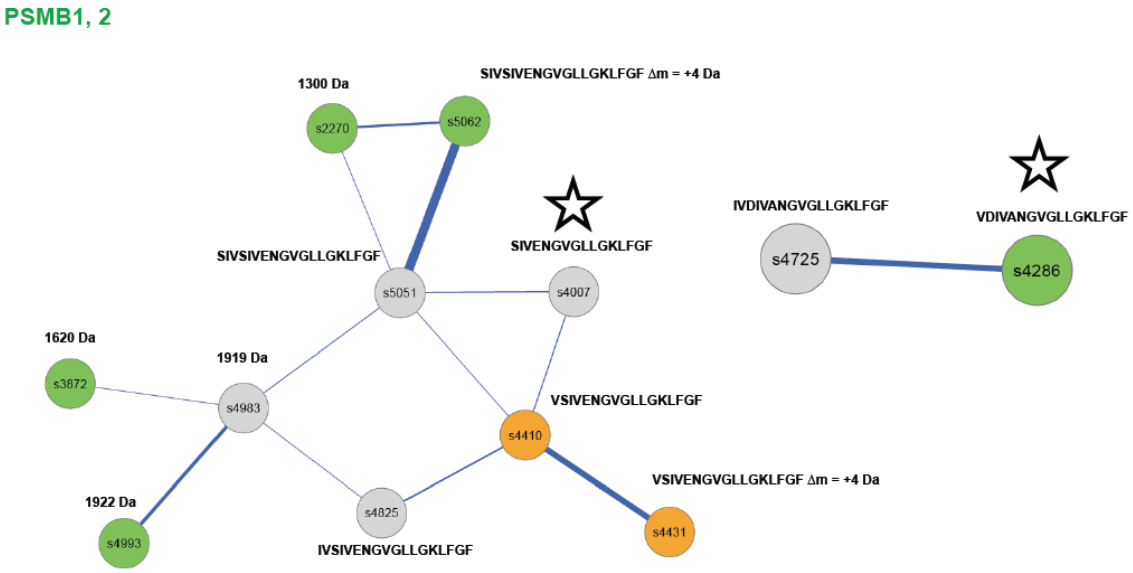
Supplemental Figure S20. **Tandem mass spectrum of the dPSMβ1 17-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



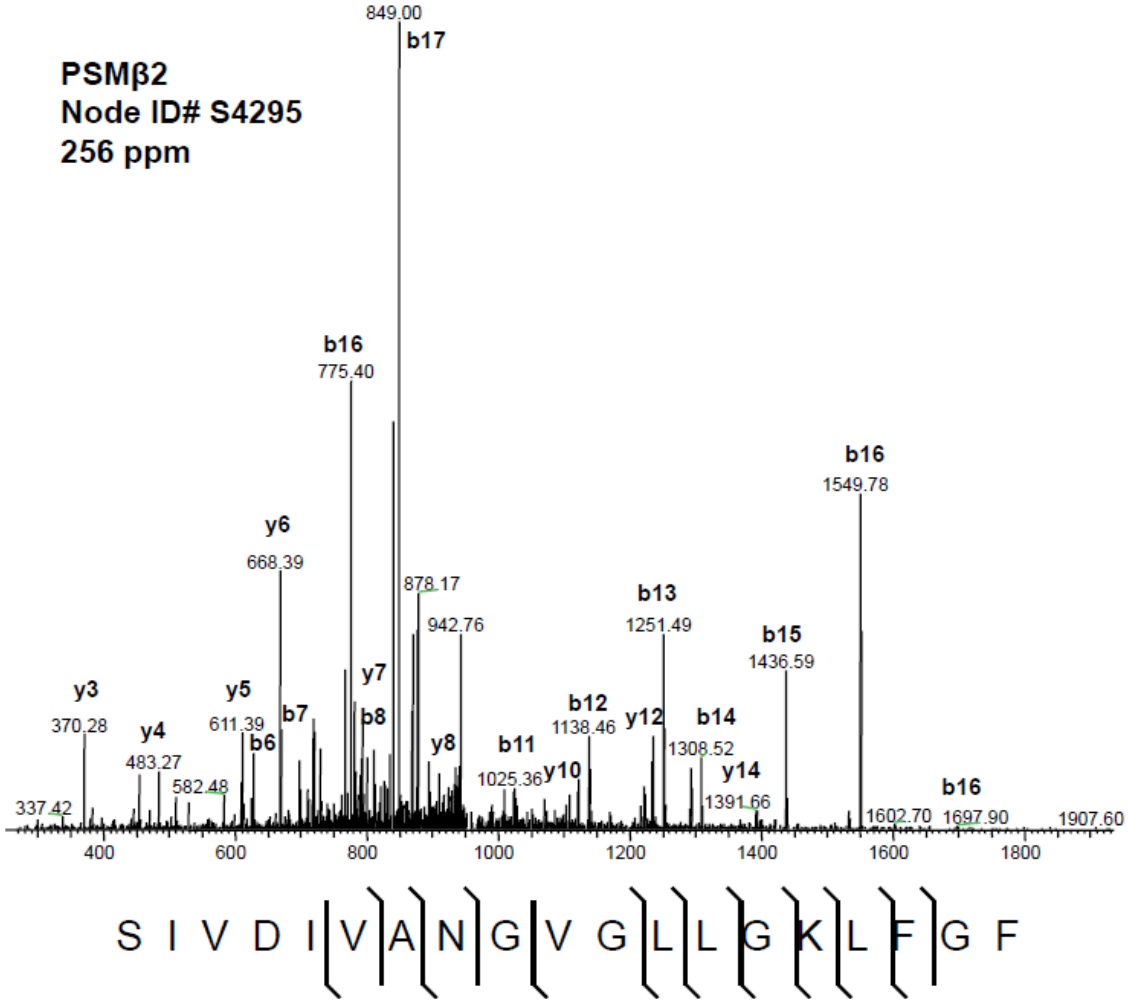
Supplemental Figure S21. **Tandem mass spectrum of the dPSMβ1 16-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



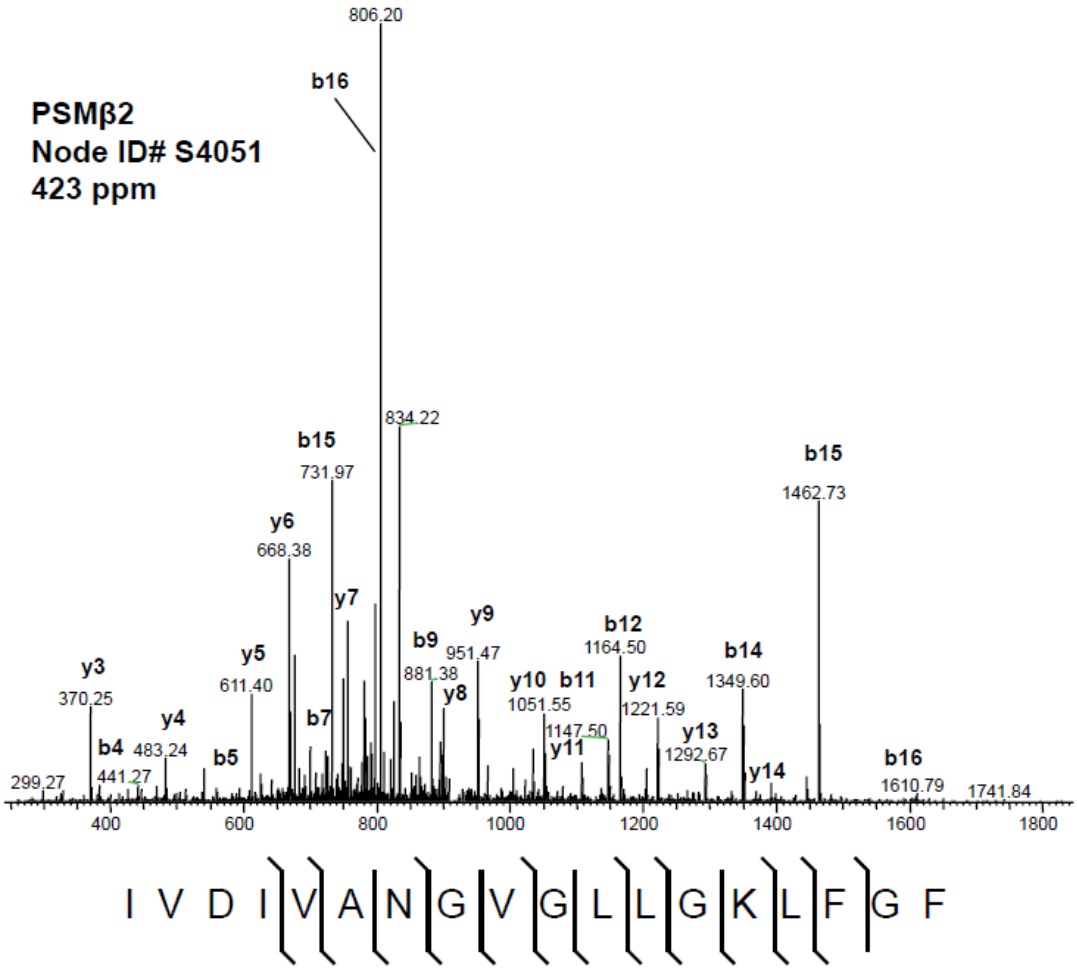
Supplemental Figure S22. **PSMβ1 and PSMβ2 associated node clusters captured in the generated CA-MRSA molecular network +/- LL-37 treatment.** Nodes indicated by a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



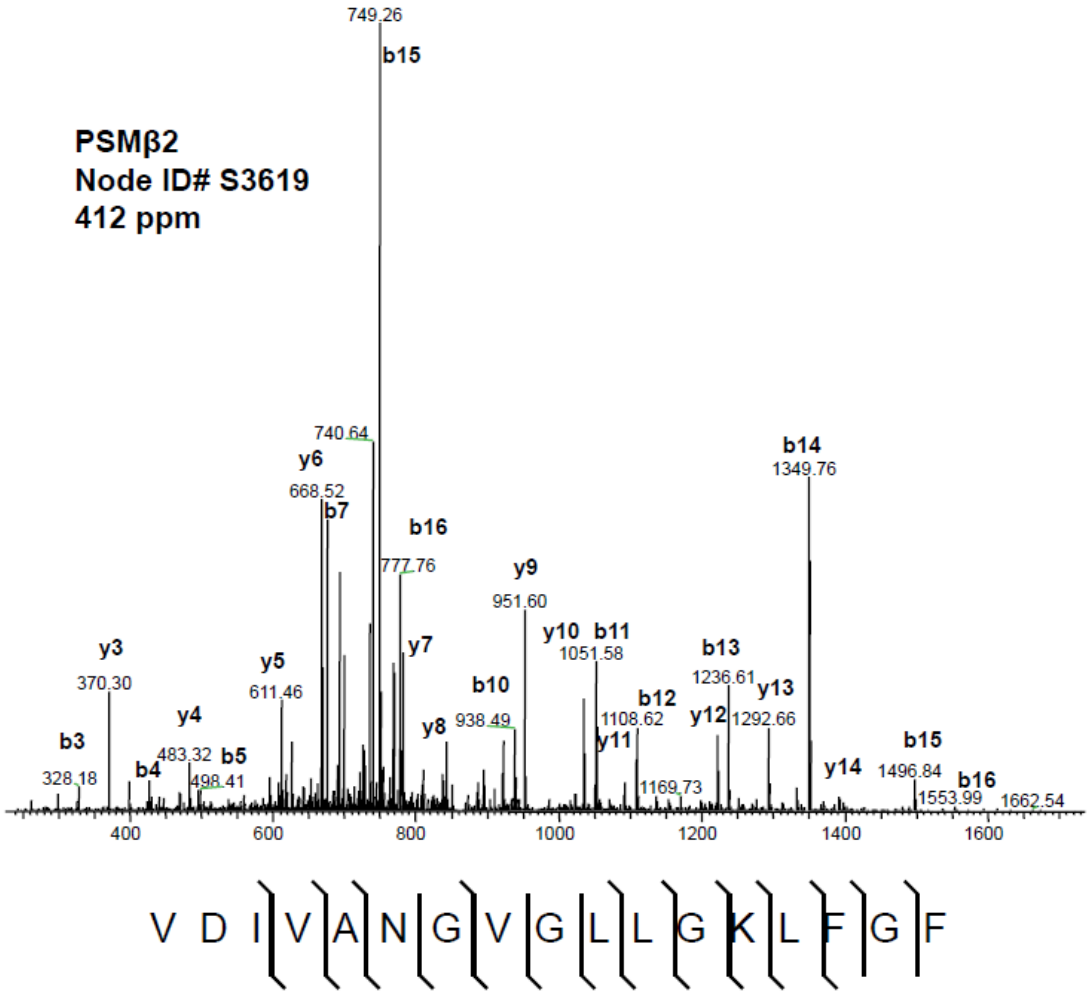
Supplemental Figure S23. **PSMβ1 and PSMβ2 associated node clusters captured in the generated CA-MRSA molecular network +/- vancomycin treatment.** Nodes indicated by a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



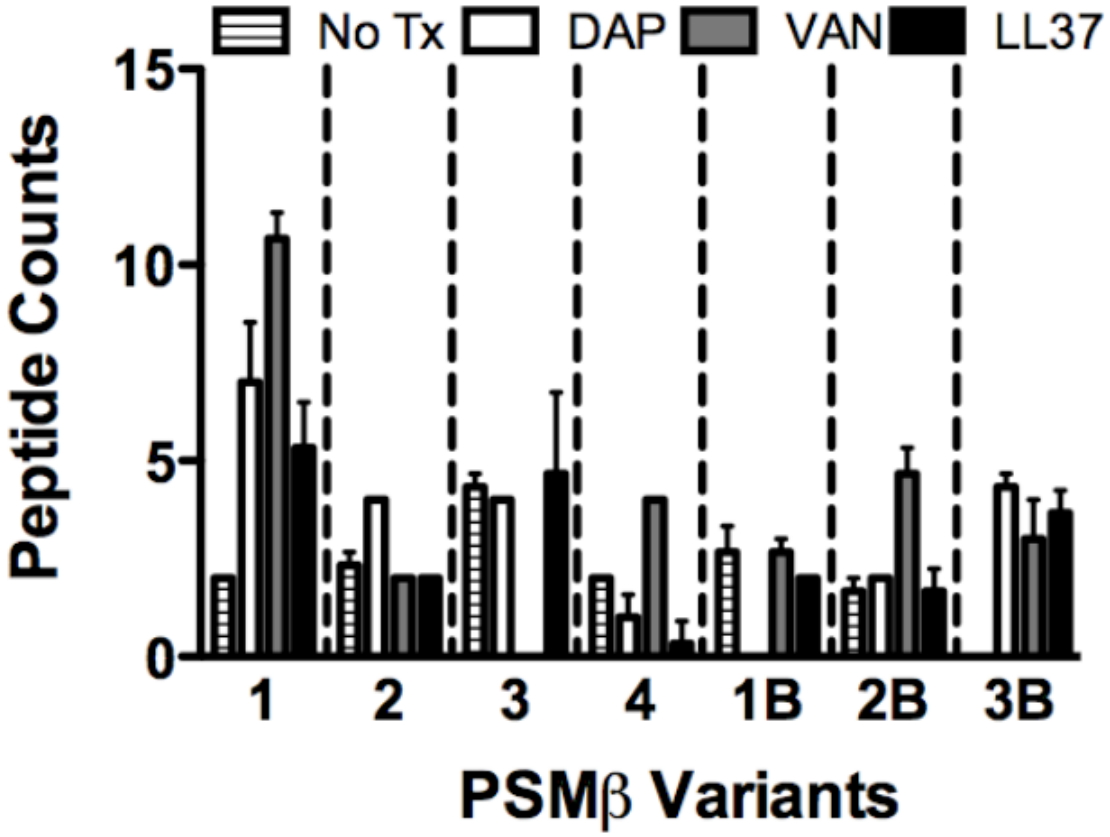
Supplemental Figure S24. **Tandem mass spectrum of the dPSMβ2 19-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



Supplemental Figure S25. **Tandem mass spectrum of the dPSMβ2 18-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



Supplemental Figure S26. **Tandem mass spectrum of the dPSMβ2 17-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



Supplemental Fig. S27. **Spectral counts for PSMβ1 and PSMβ2 derivatives**.

PSMβ1 variants:

(1) SIVSIVENGVGLLGKLFGF
(2) IVSIVENGVGLLGKLFGF;
(3) VSIVENGVGLLGKLFGF
(4) SIVENGVGLLGKLFGF

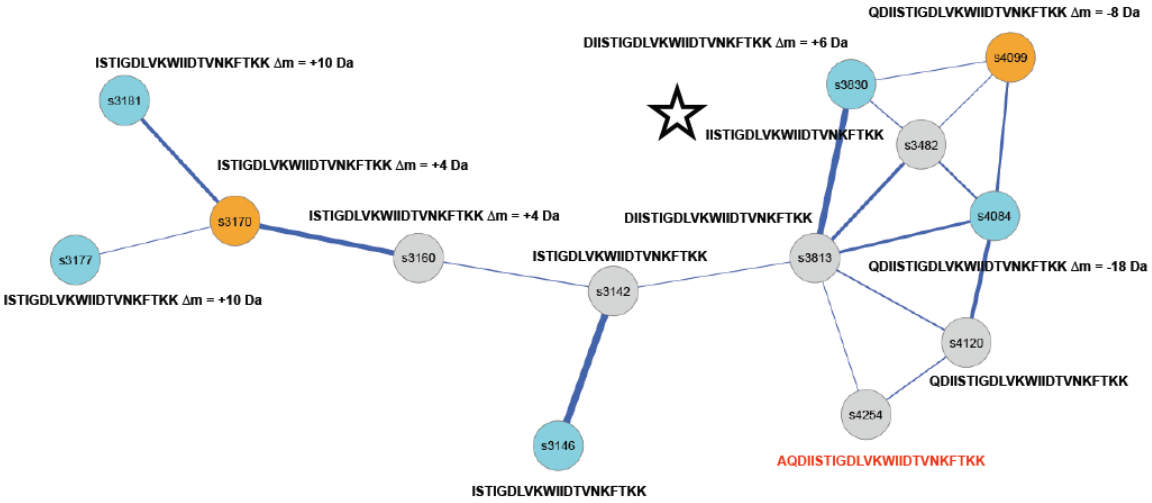
Peptide 3 was not detected in the vancomycin treated sample.

PSMβ2 variants:

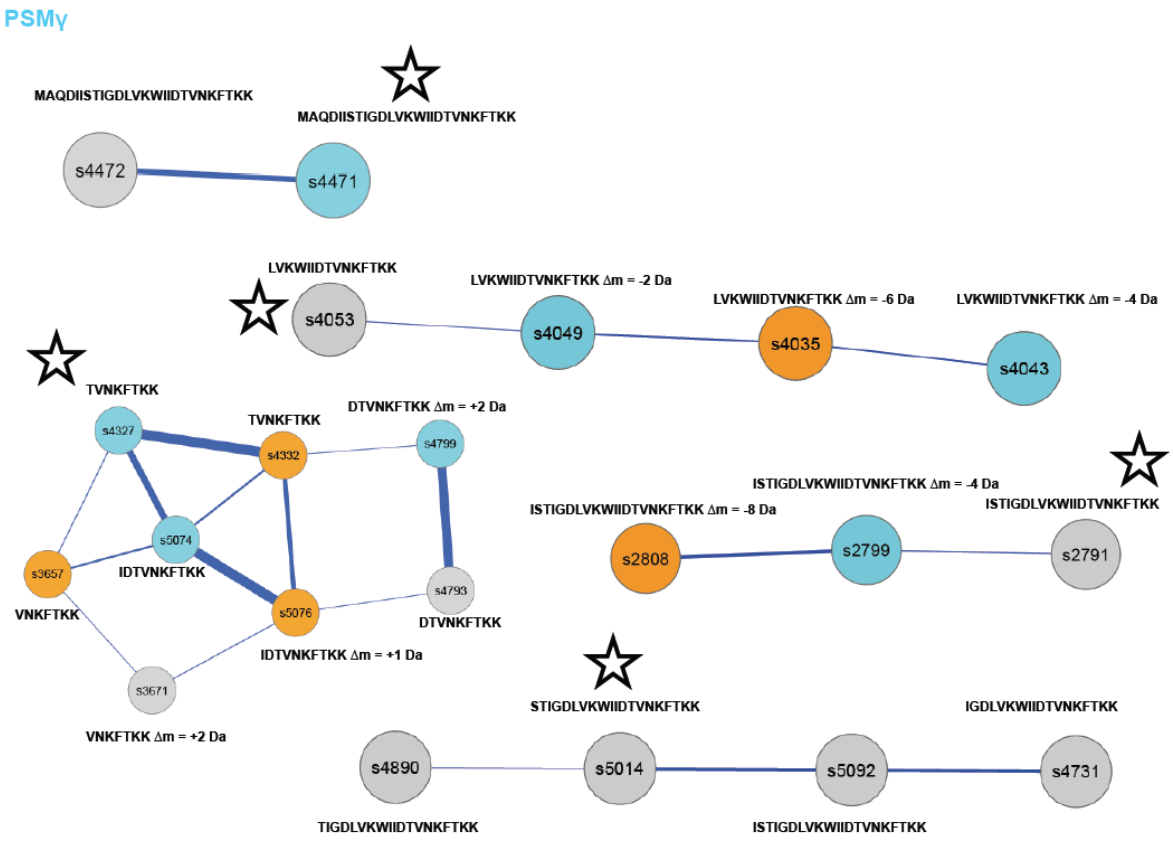
(1B) SIVDIVANGVGLLGKLFGF
(2B) IVDIVANGVGLLGKLFGF
(3B) VDIVANGVGLLGKLFGF

Peptide 1B was not detected in the CA-MRSA alone sample. Dashed lines are used as borders between individual experiments performed on each peptide.

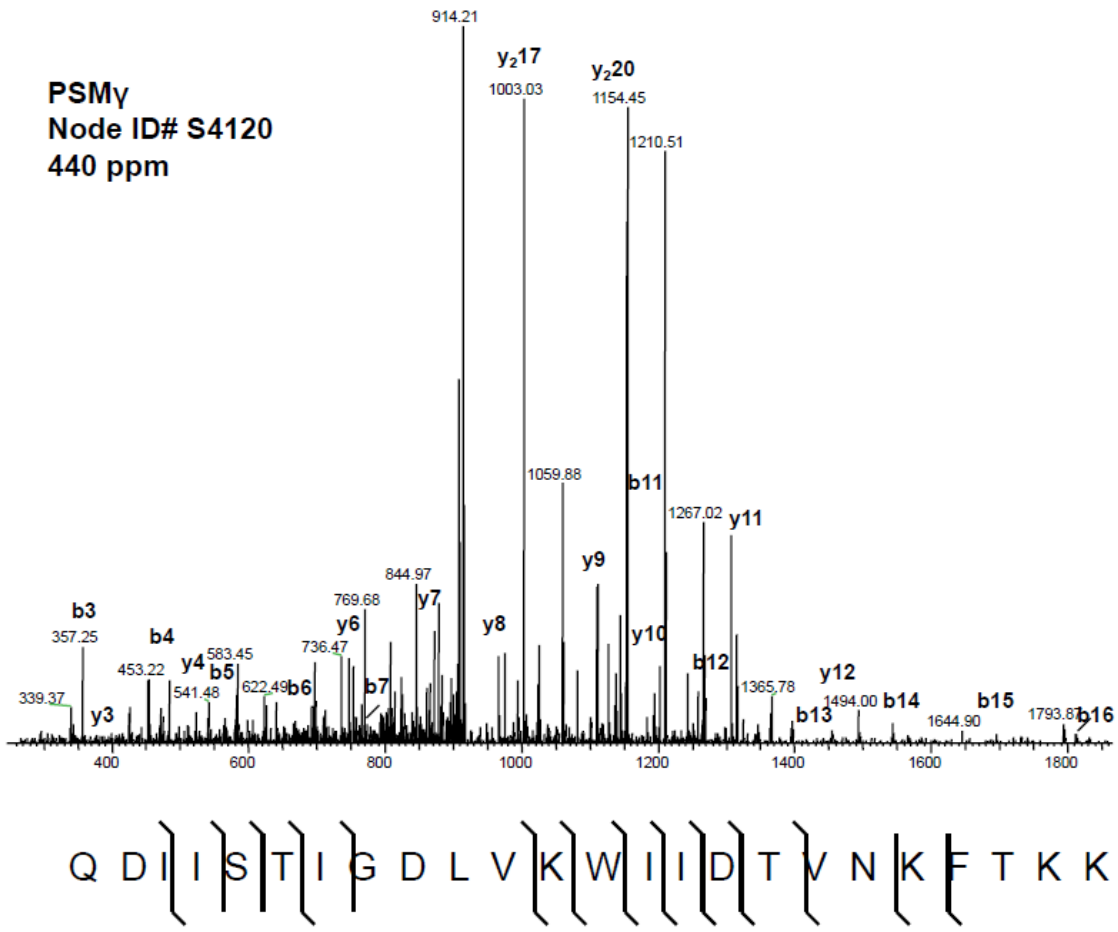
PSMγ



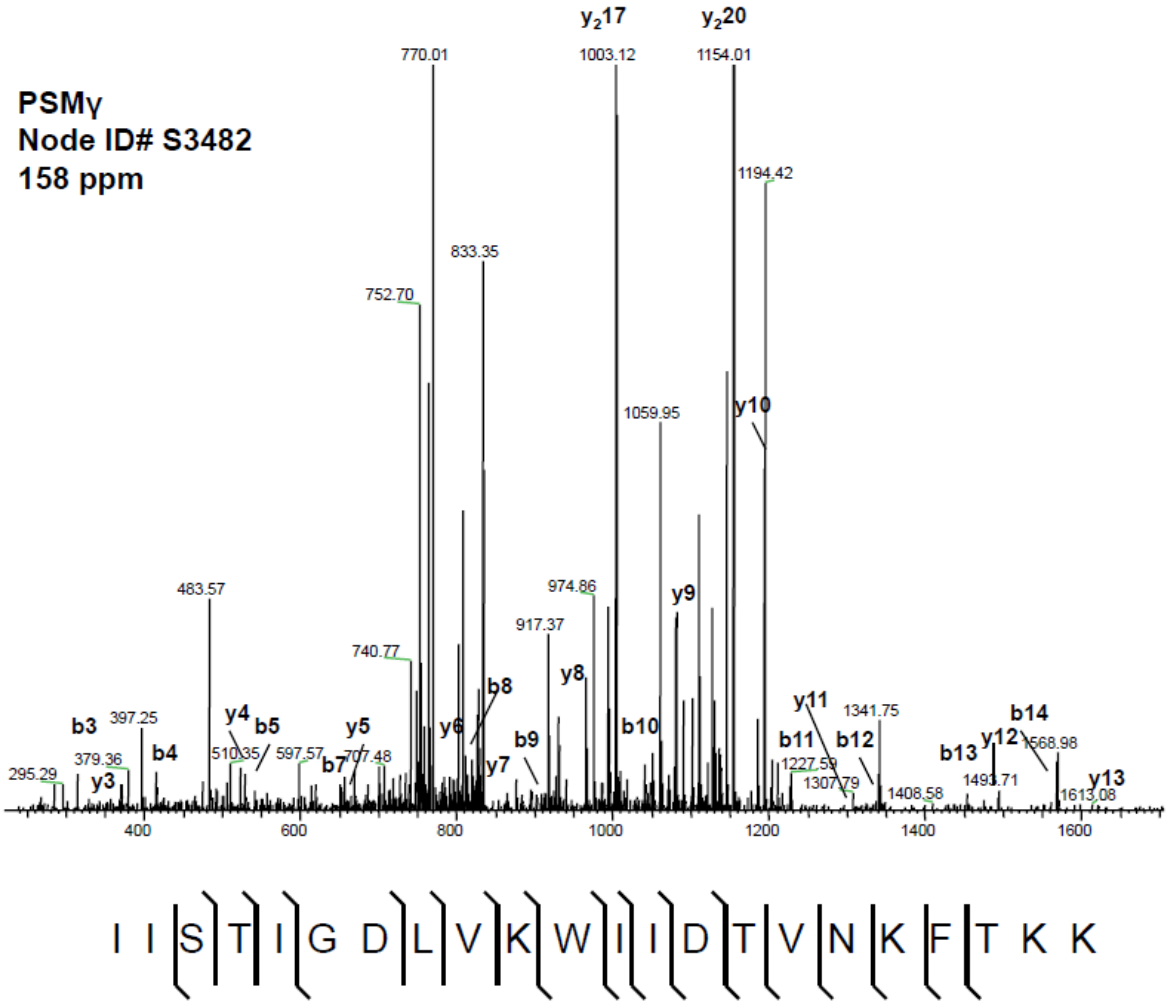
Supplemental Figure S28. **PSMγ associated node clusters captured in the generated CA-MRSA molecular network under +/- daptomycin treatment.** Nodes that have a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications. Colored in red is a sequence that could not be verified due to insufficient b and y-ion coverage.



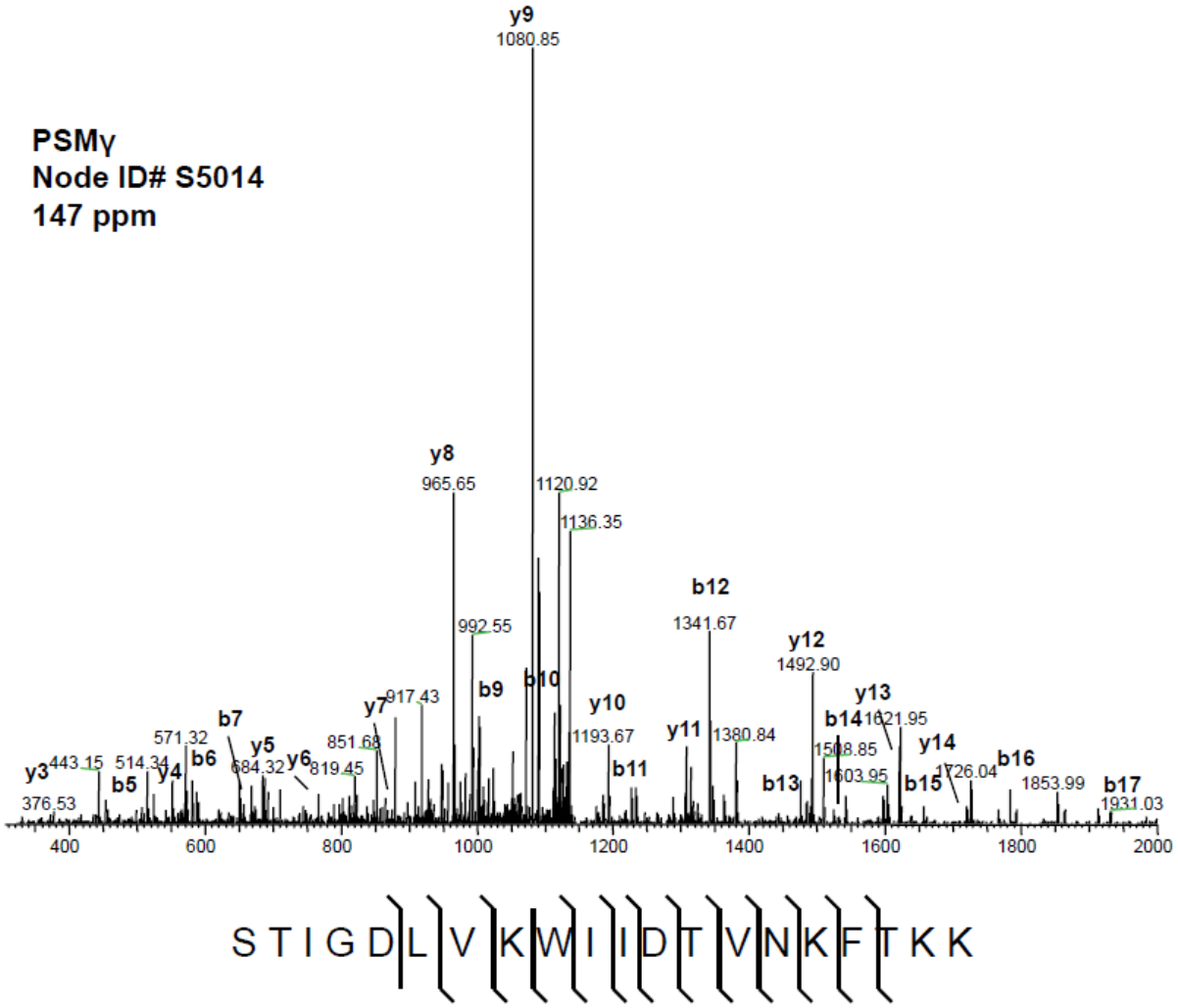
Supplemental Figure S29. **PSMγ associated node clusters captured in the generated CA-MRSA molecular network under +/- daptomycin treatment.** Nodes that have a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



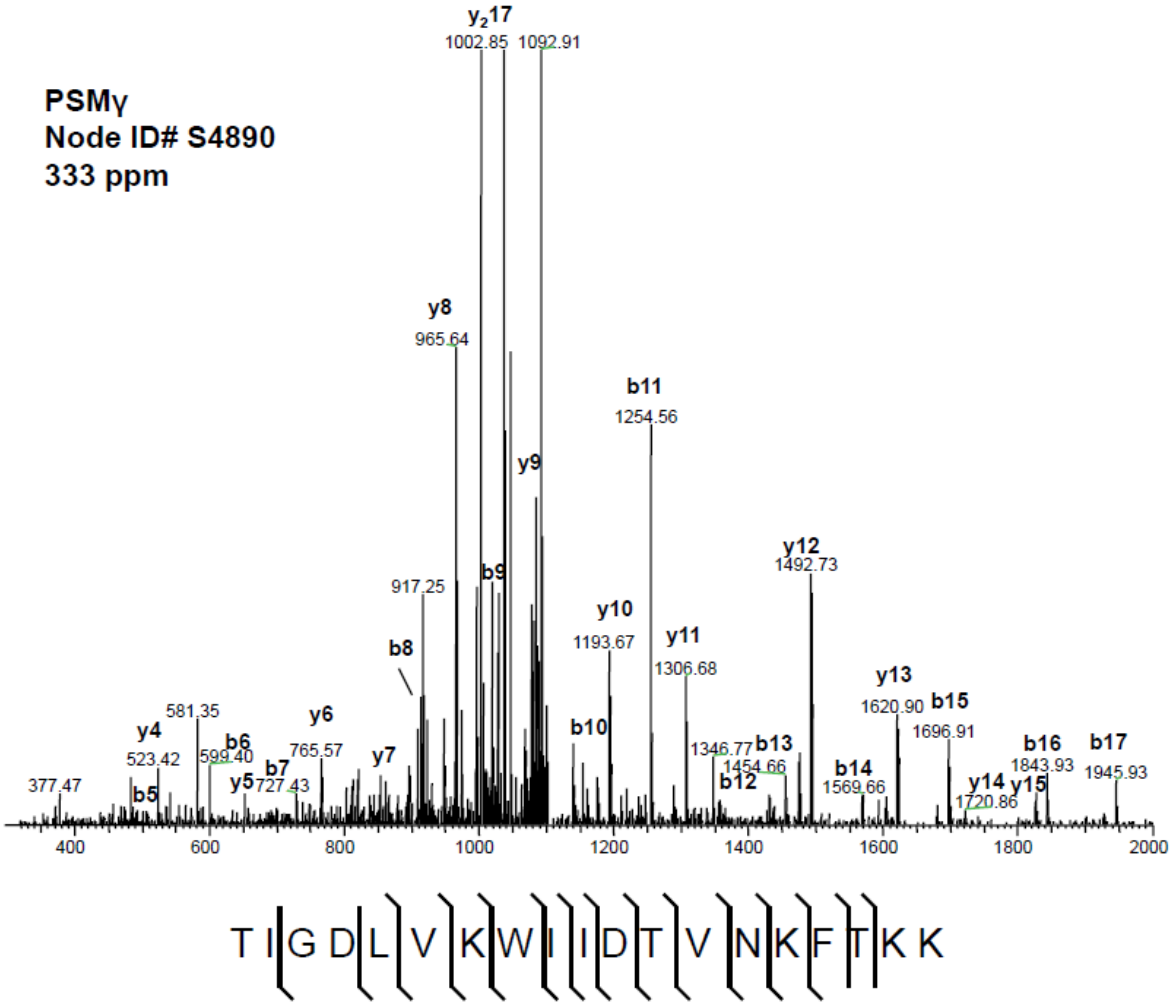
Supplemental Figure S30. **Tandem mass spectrum of the dPSMγ 23-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



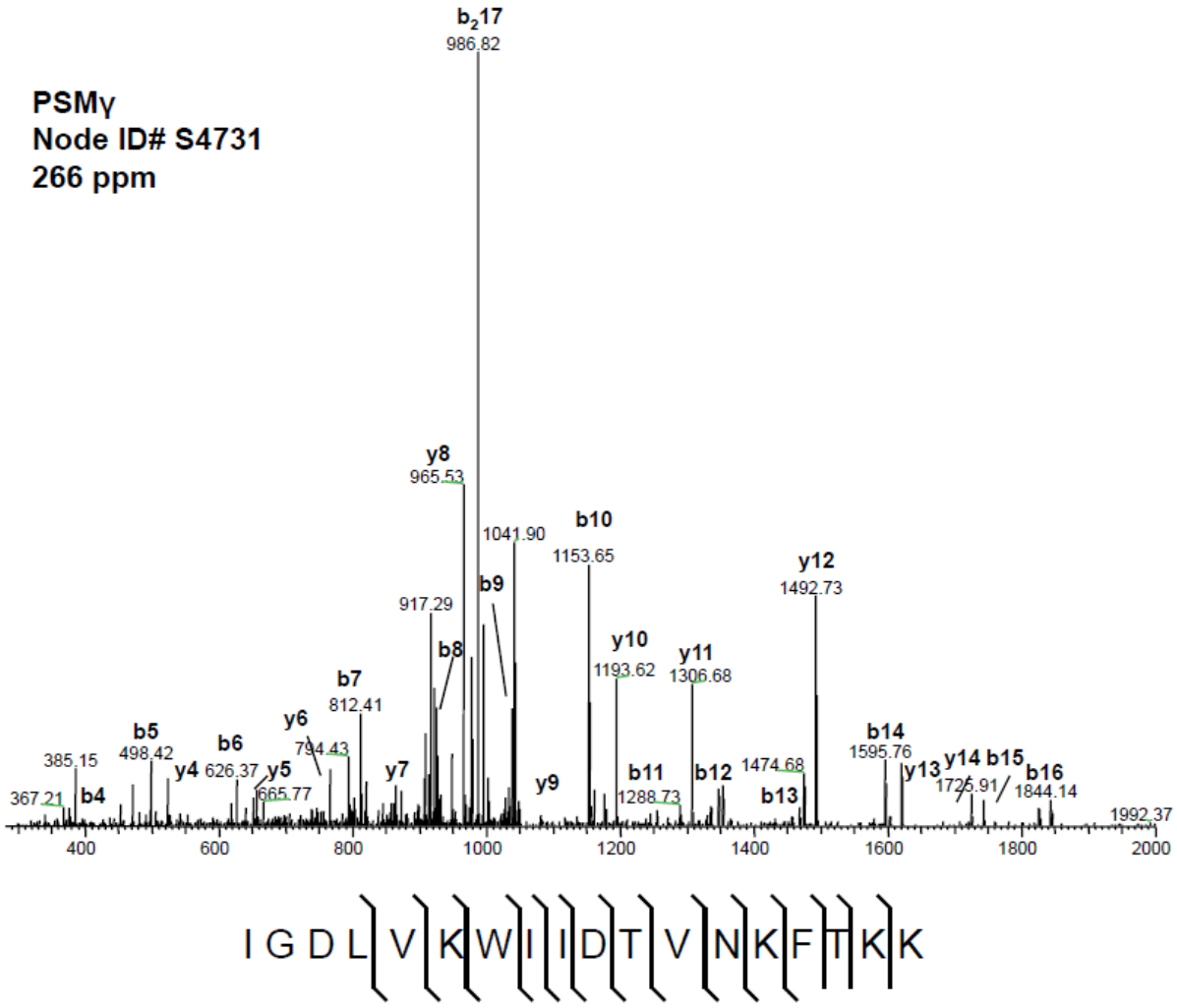
Supplemental Figure S31. **Tandem mass spectrum of the dPSMγ 22-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



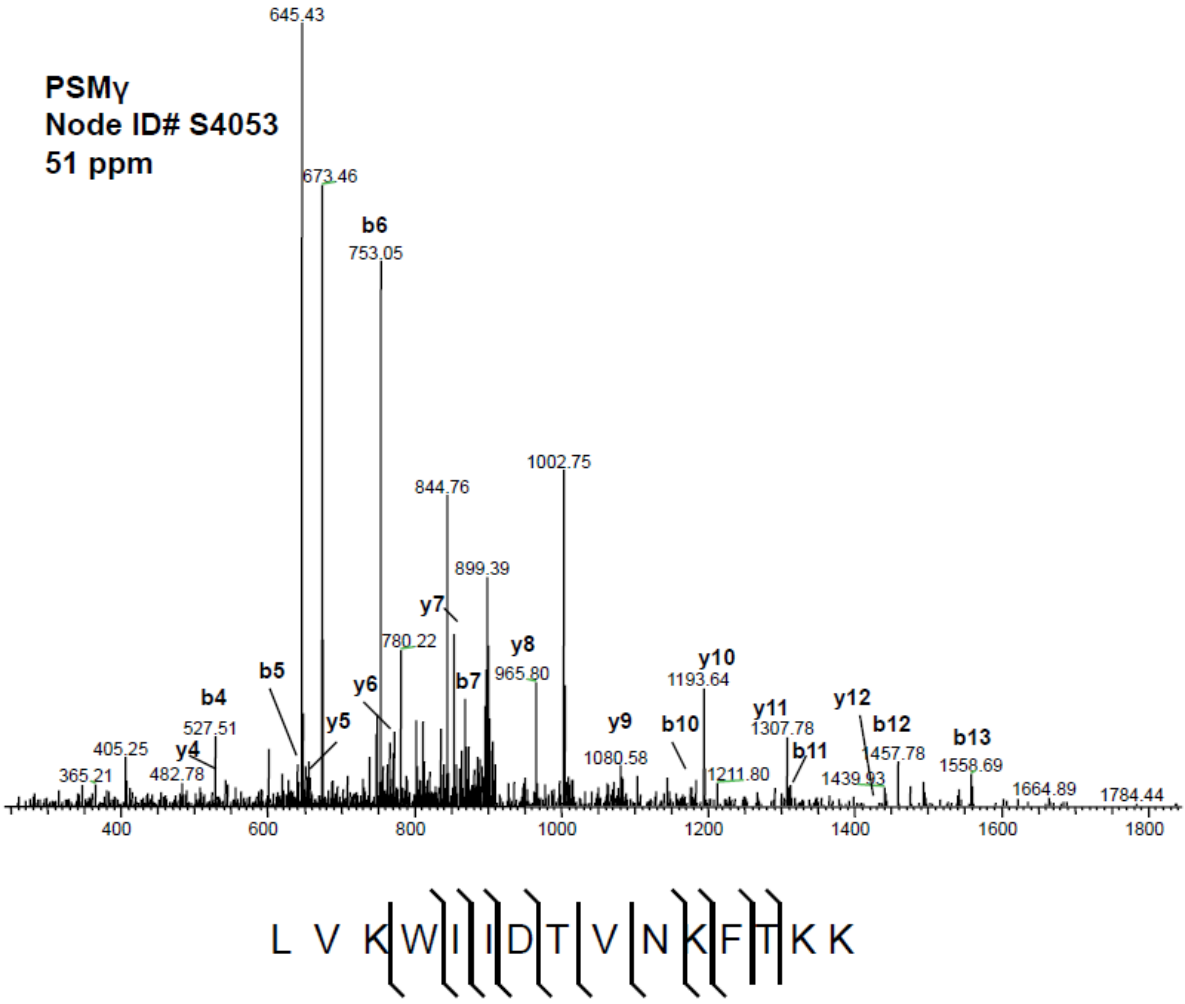
Supplemental Figure S32. **Tandem mass spectrum of the dPSMγ 20-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



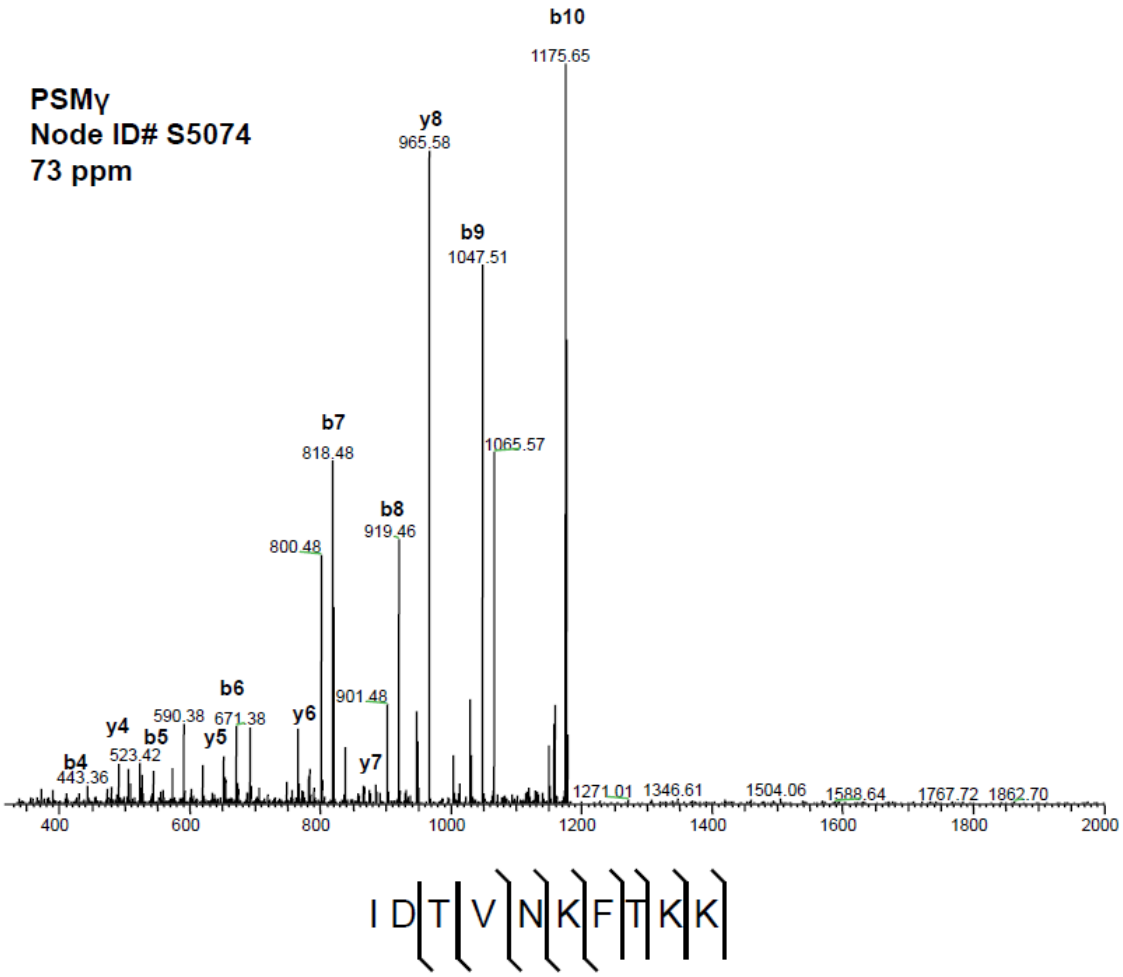
Supplemental Figure S33. **Tandem mass spectrum of the dPSMγ 19-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



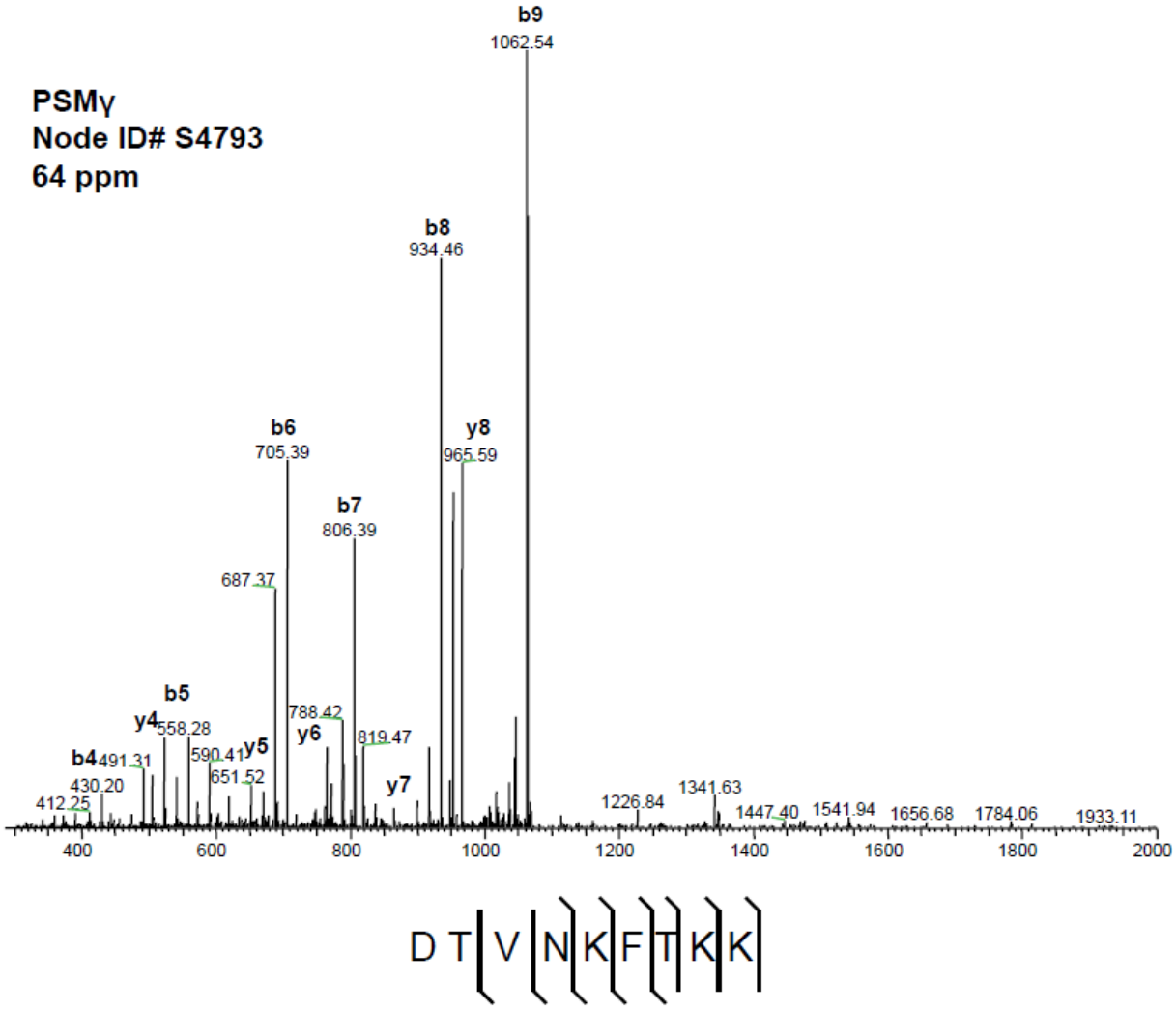
Supplemental Figure S34. **Tandem mass spectrum of the dPSMγ 18-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



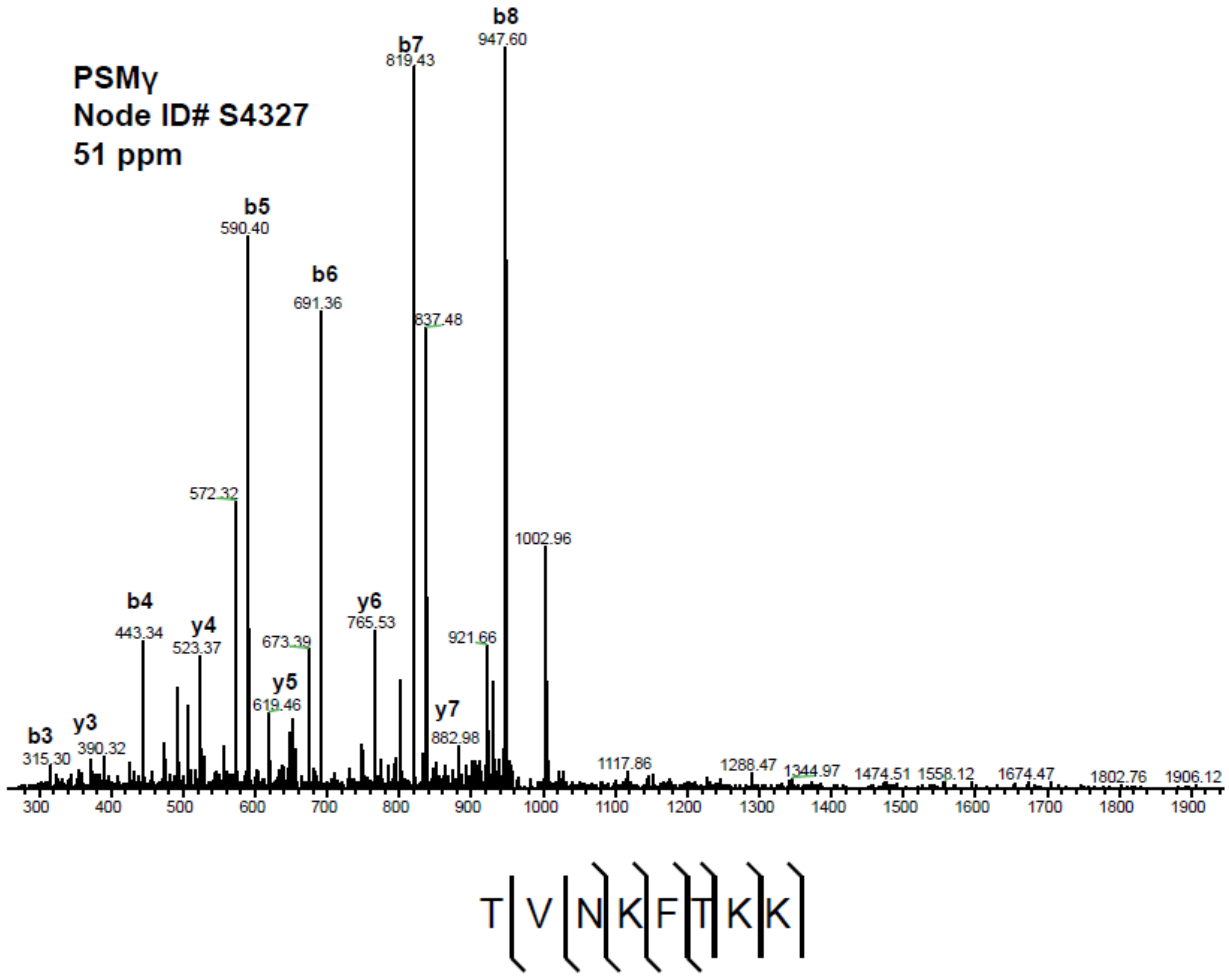
Supplemental Figure S35. **Tandem mass spectrum of the dPSMγ 15-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



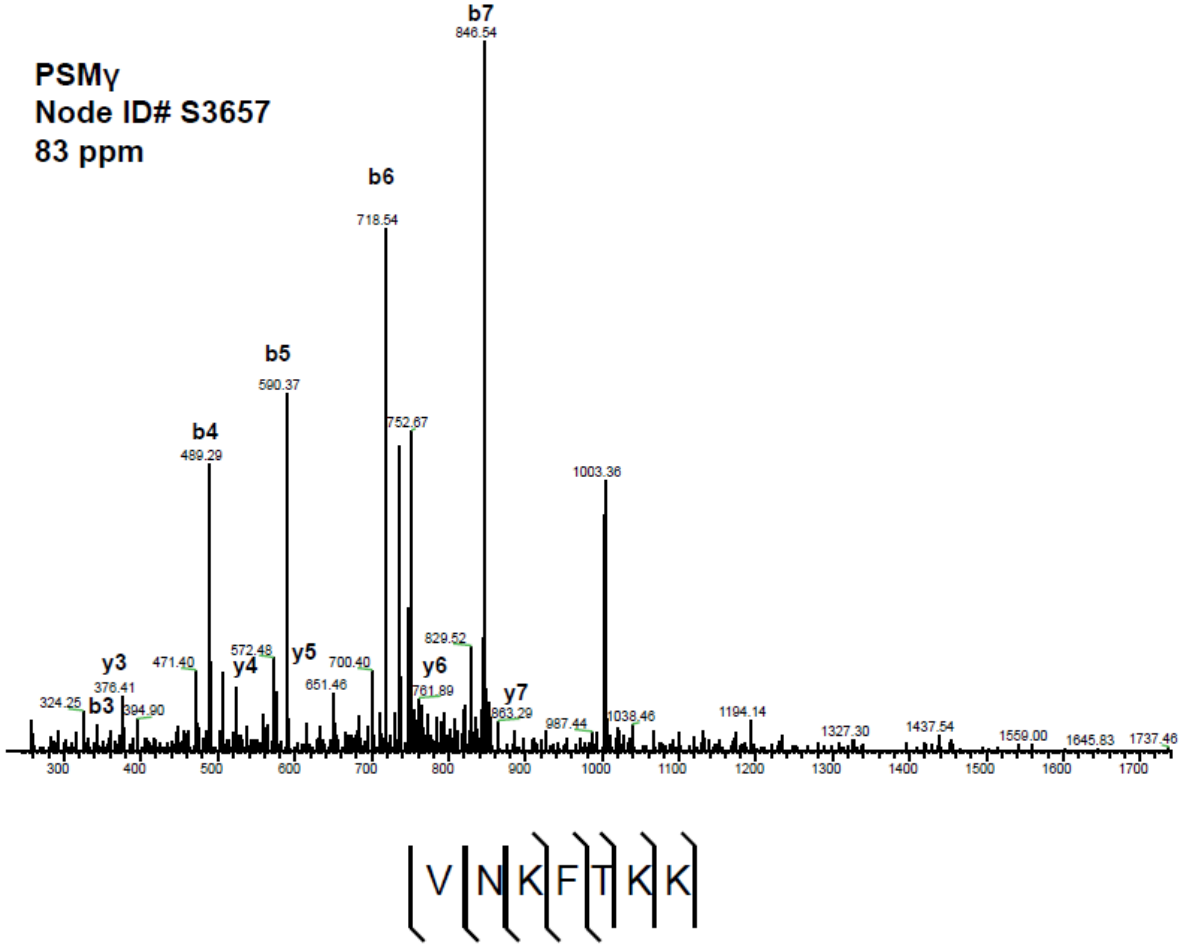
Supplemental Figure S36. **Tandem mass spectrum of the dPSMγ 10-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



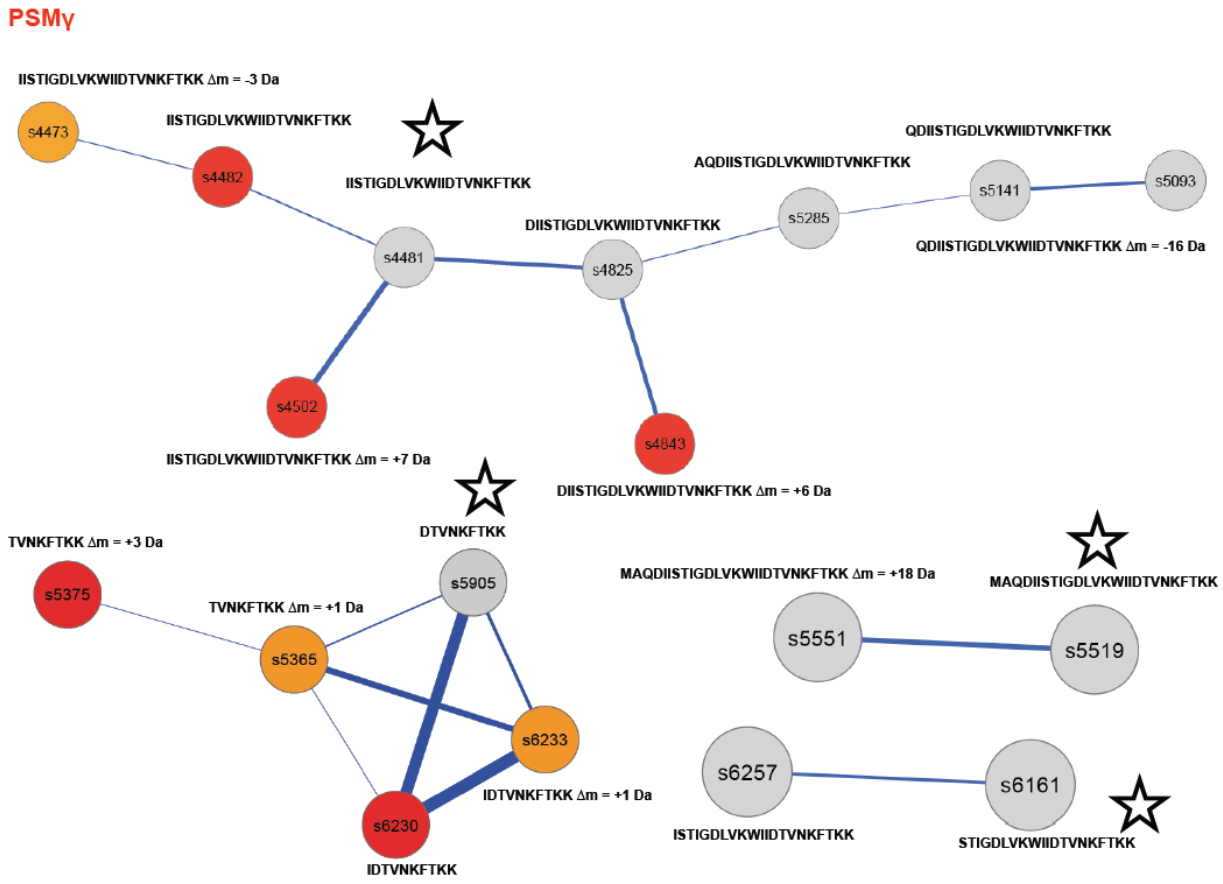
Supplemental Figure S37. **Tandem mass spectrum of the dPSMγ 9-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



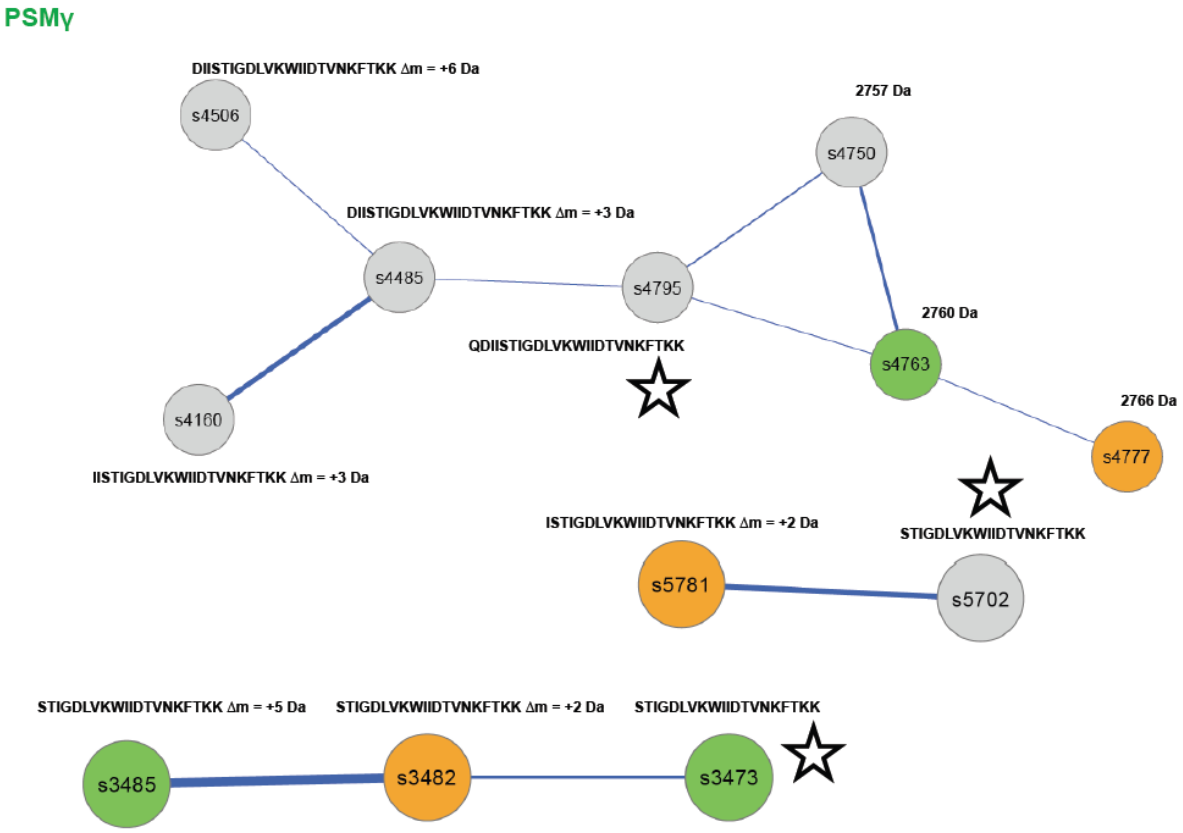
Supplemental Figure S38. **Tandem mass spectrum of the dPSMγ 8-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



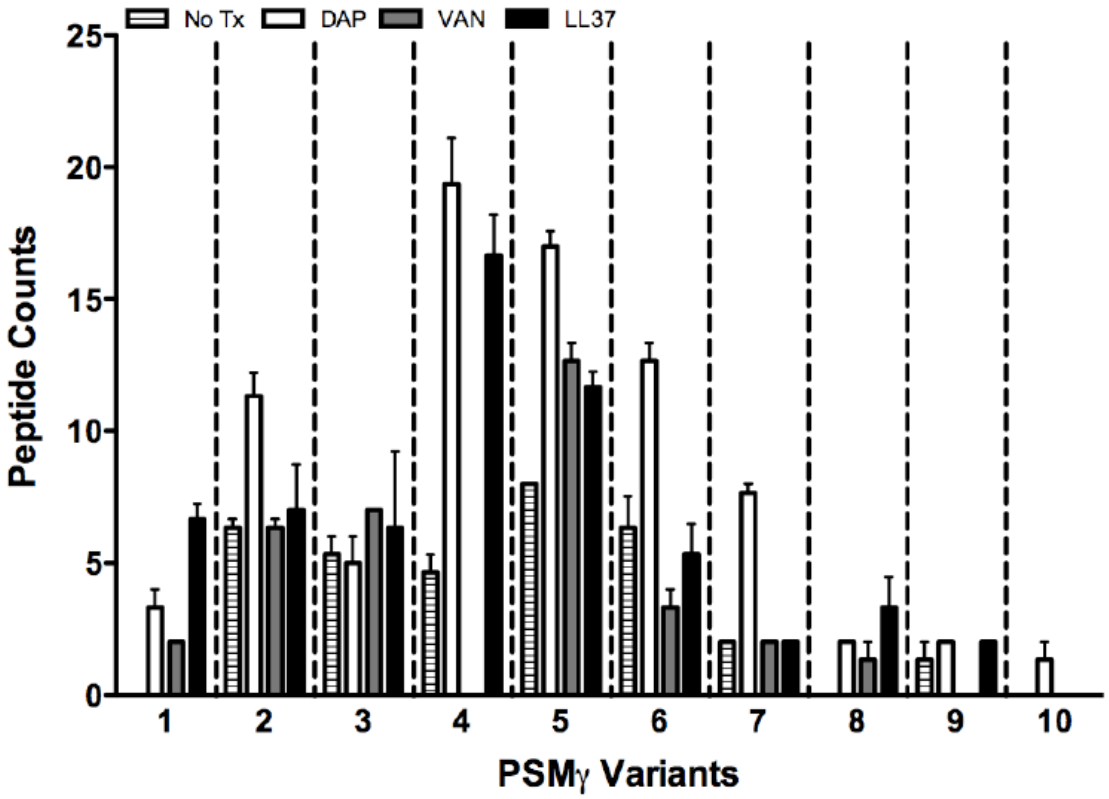
Supplemental Figure S39. **Tandem mass spectrum of the dPSMγ 7-mer peptide.** The parent ion was isolated within the mass spectrometer and fragmented using collision induced dissociation. Ions corresponding to fragmentations at the peptide bond are indicated as b (N-terminal coverage) or y (C-terminal coverage) and are labeled. Peptide sequence coverage, node ID, and mass accuracy (ppm error) are also displayed.



Supplemental Figure S40. **PSMγ associated node clusters captured in the generated CA-MRSA molecular network +/- LL-37 treatment.** Nodes indicated by a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the supplemental materials. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.



Supplemental Fig. S41. **PSMγ associated node clusters captured in the generated CA-MRSA molecular network +/- vancomycin treatment.** Nodes indicated by a star were sequenced and contained reasonable mass accuracy. The sequence of the identified node was then used as a point of propagation to identify adjacent nodes in the cluster. Each node is labeled with its corresponding amino acid sequence and mass offsets (Δm) relative to theoretical mass values. Sequences that are not labeled with mass offsets had accurate masses and were of high confidence based on sequencing. Mass spectrometry based peptide sequencing of all novel derivatives reported were performed and are provided within the Supplemental. For previously reported PSMs and derivatives refer to reference (18) for sequence verifications.

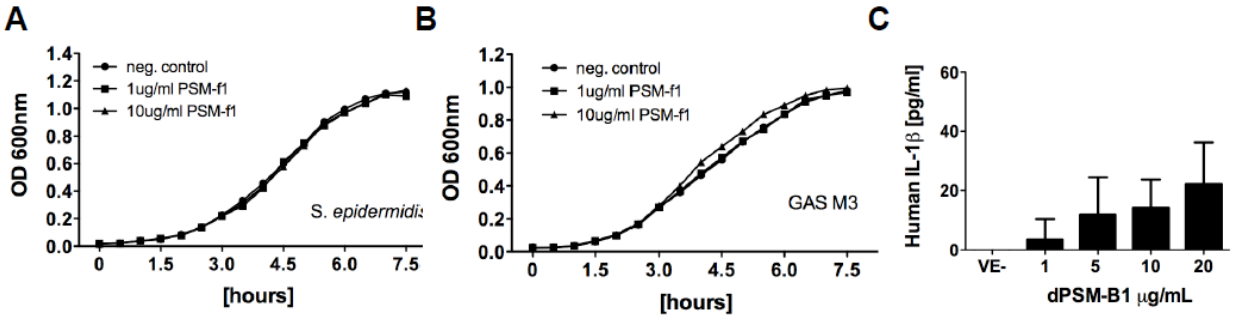


Supplemental Fig. S42. **Spectral counts for PSMγ derivatives**.

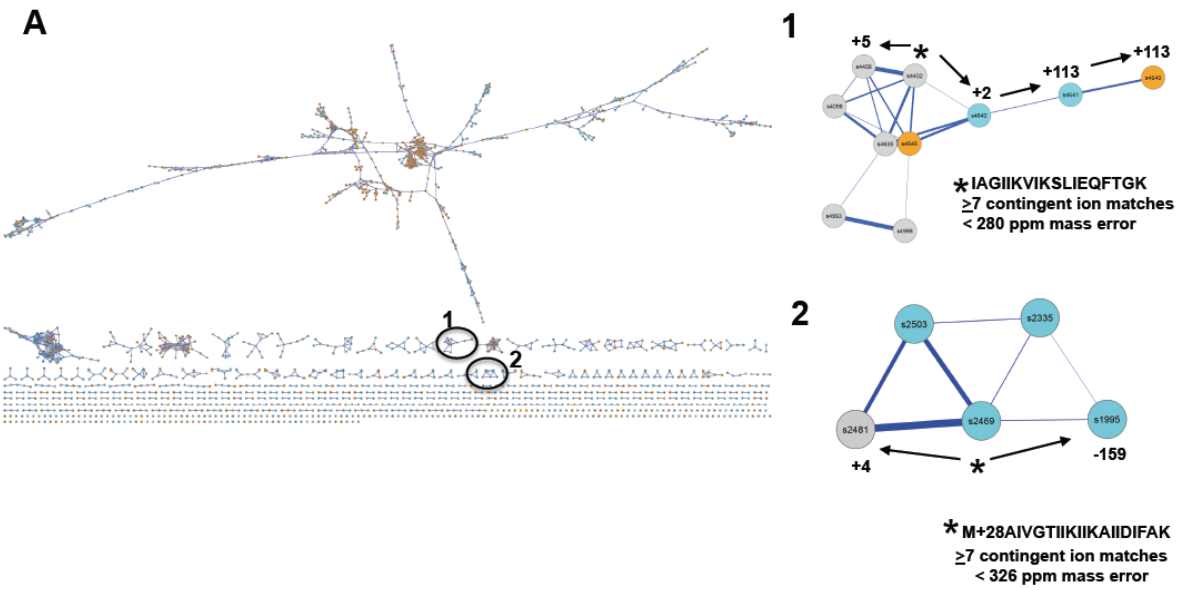
PSMγ variants:

(1) QDIISTIGDLVKWIIDTVNKFTKK
(2) DIISTIGDLVKWIIDTVNKFTKK
(3) IISTIGDLVKWIIDTVNKFTKK
(4) ISTIGDLVKWIIDTVNKFTKK
(5) STIGDLVKWIIDTVNKFTKK
(6) TIGDLVKWIIDTVNKFTKK
(7) IGDLVKWIIDTVNKFTKK
(8) LVKWIIDTVNKFTKK
(9) IDTVNKFTKK
(10) DTVNKFTKK

Peptide 1 was not detected in the CA-MRSA alone sample. Peptide 4 was not detected in the vancomycin. Peptide 8 was not detected in the CA-MRSA alone sample. Peptide 9 was not detected in the vancomycin. Peptide 10 was not detected in the CA-MRSA alone sample and the vancomycin treatment sample. Dashed lines are used as borders between individual experiments performed on each peptide.



Supplemental Fig. S43. **Growth curves and IL-1β release stimulated by dPSMβ1.** A. Growth curve of *Staphylococcus epidermidis* under dPSMα1 treatment monitored over 8 hrs. B. Growth curve of group A *Streptococcus* under dPSMα1 treatment monitored over 8 hrs. C. IL-1β release from THP-1 cells under dPSMβ1 treatment at 20 μg/ml, 10 μg/ml, 5 μg/ml or 1 μg/ml.



Supplemental Fig. S44. **Representative complete molecular network map.** A. The complete molecular network resulting from ion clusters of the CA-MRSA USA300 strain TCH1516 untreated and treated with daptomycin is shown. Circled inserts 1 and 2 are shown as adjacent enlarged figures. Cluster 1 contained a node of accurate theoretical mass for the dPSMα1 N-terminal truncated 18-mer peptide. The spectrum was sequenced (Supplemental Fig. 3) and contained the correct amino acid tag consistent with the observed mass. The dPSMα1 18-mer node, indicated by an asterisk, was used as a point of propagation to identify the adjacent nodes in the cluster. Neighboring nodes contained mass shifts corresponding to the amino acid isoleucine were also captured, consistent with the addition of an N-terminal isoleucine found in the primary sequence of PSMα1. Analysis of all nodes in the different conditions containing dPSMs is provided in Supplemental Information. Cluster 2 contained a node matching the theoretical mass of full-length formylated PSMγ. Sequencing was performed and validated the node identification. The validated node indicated with an asterisk was used as a point of propagation to adjacent nodes. A mass offset of minus 159 Da was observed for an adjacent node that corresponds to N-terminal truncation of the formylated initiator methione.