

Supplementary Information

An antimicrobial daptide from human skin commensal *Staphylococcus hominis* protects against skin pathogens

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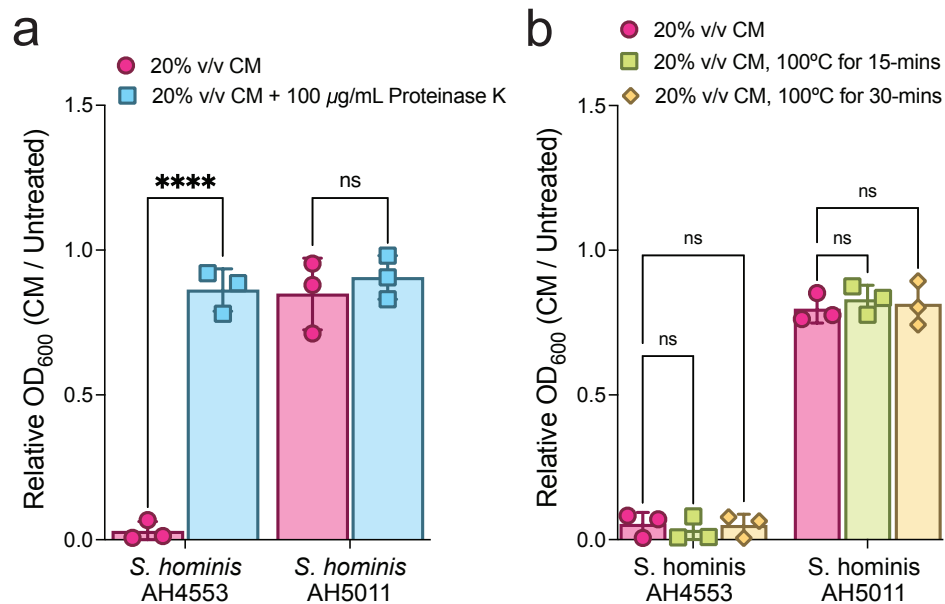
Supplementary Table 1: List of bacterial strains and plasmids used in this study

Supplementary Table 2: List of primers used in this study

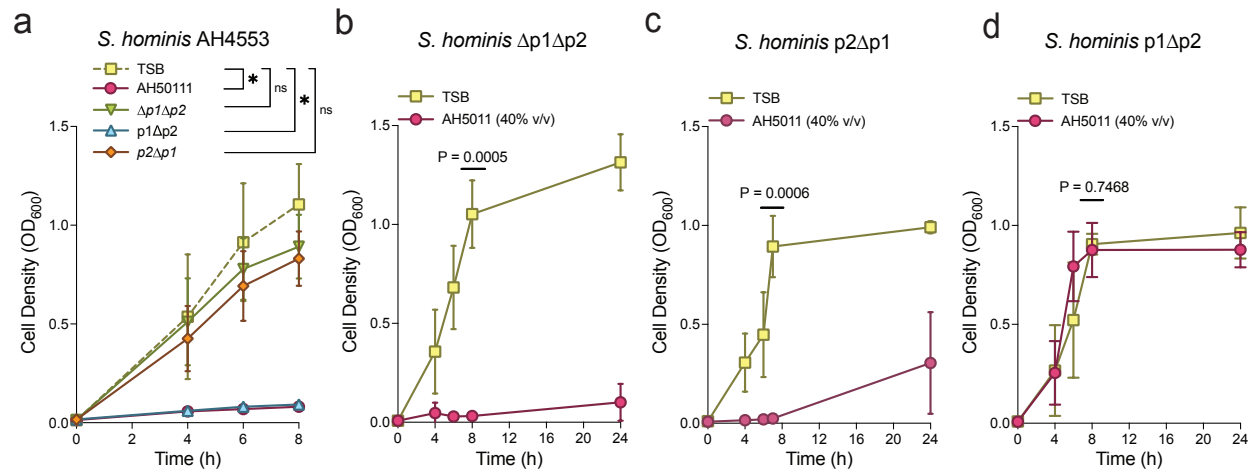
Supplementary Table 3: Table of publicly available staphylococcal daptide BGC assemblies

Supplementary Table 4: ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of AH5011 hominidin in methanol- d_3

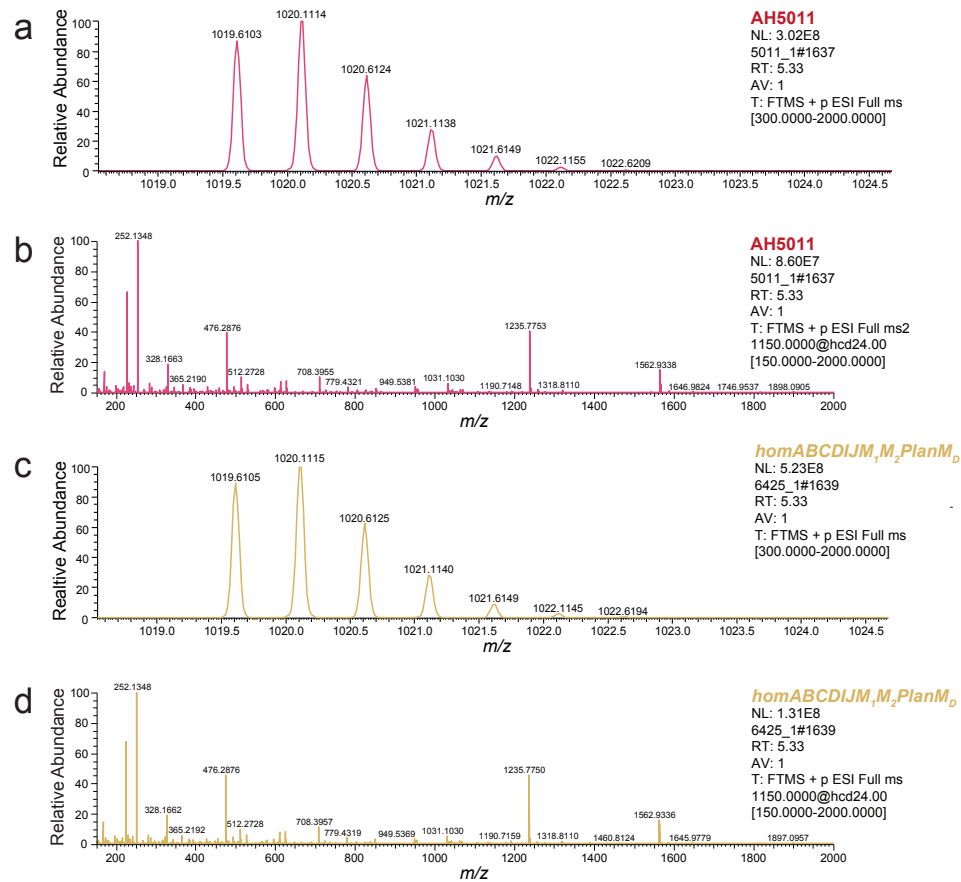
Supplementary Table 5: Locus tags for the *S. hominis* AH5011 BGC



Supplementary Figure 1. Effects of heat and protease treatment on AH5011 activity. **a** *S. hominis* strains were incubated with Proteinase K-treated, or untreated 20% v/v CM from AH5011 for 8 h. Data represent relative growth compared to the tryptic soy broth growth control at 8 h post-incubation and the mean of $n = 3$ independent replicates. Error bars show standard deviation. Statistical significance in comparison to growth in 20% v/v untreated conditioned media was determined by a two-tailed unpaired t -test. Exact p values from left to right are as follows: **a** <0.0001 , 0.5331 . **b** *S. hominis* strains were incubated with heat treated, or untreated 20% v/v CM for 8 h. Data represent relative growth compared to the tryptic soy broth growth control at 8 h post-incubation and the mean of $n = 3$ independent replicates. Error bars show standard deviation. Statistical significance in comparison to growth in 20% v/v untreated conditioned media was determined using one-way ANOVA followed by Dunnett's multiple comparison test. Exact p values from top to bottom are as follows: **b** (AH4553) 0.9934 , 0.8381 , (AH5011) 0.8927 , 0.6585 . Source data are provided as a Source Data file.

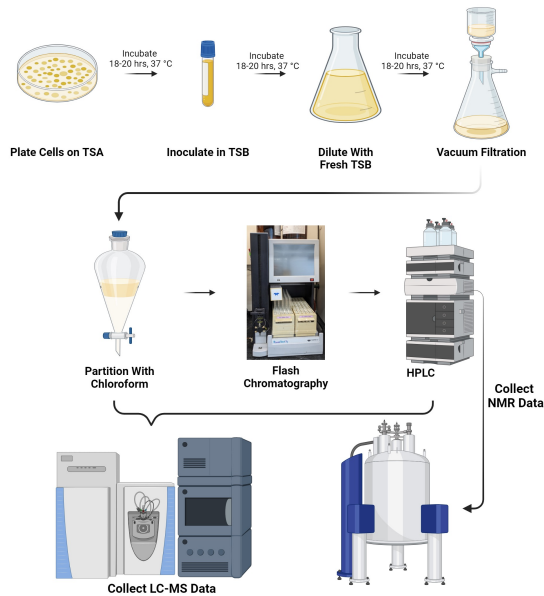


Supplementary Figure 2. Isolation of plasmid-cured mutants. **a** *S. hominis* AH4553 was incubated with 40% v/v CM from AH5011, or plasmid-cured mutants for 8 h. Data represent the mean of $n = 3$ biological replicates and error bars show standard deviation. Statistical differences were compared to TSB control group at 8 h using one-way ANOVA followed by Dunnett's multiple comparison test. Exact p values from left to right are as follows: **a** 0.0283, 0.5076, 0.0277, 0.3184. **b, c, d** Plasmid-cured $\Delta p1\Delta p2$ (**b**), $p2\Delta p1$ (**c**), and $p1\Delta p2$ (**d**) mutants were incubated in either 40% v/v CM from wild-type AH5011 or TSB growth medium for 24 h. Data represent the mean of $n = 3$ biological replicates and error bars show standard deviation. Statistical differences were compared to growth in TSB at 8 h using two-tailed unpaired t -test (**b, c, d**). Source data are provided as a Source Data file.

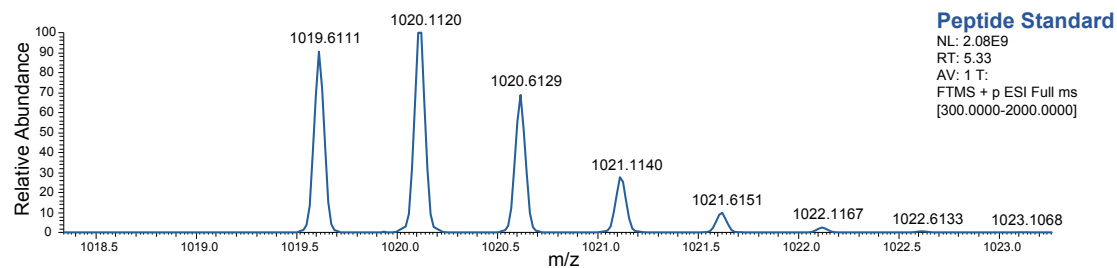


Supplementary Figure 3. Mass spectrometric analysis of conditioned media from AH5011 and heterologously expressed *homABCDIJM₁M₂PlanM_D*. **a, c** Mass spectra zoomed in on the daptide $[M+2H]^{2+}$ m/z (calculated at 1020.1103) from the conditioned media of *S. hominis* AH5011 (**a**) and the *S. aureus* RN4220 expressing *homABCDIJM₁M₂PlanM_D* (**c**). **b, d** All ion fragmentation (AIF) spectra at retention time 5.33 (where the putative daptide is observed) for both AH5011 (**b**) and the expression mutant (**d**).

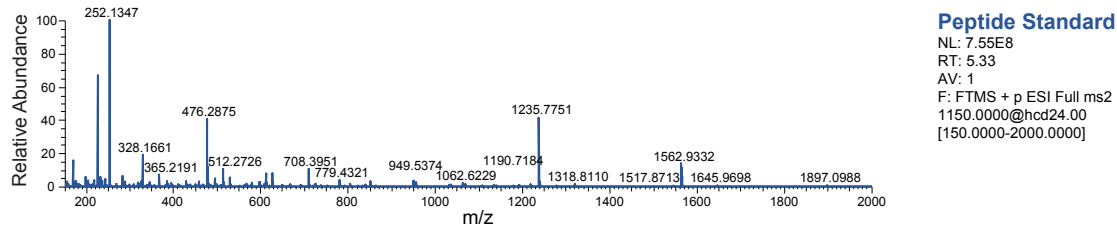
a



b



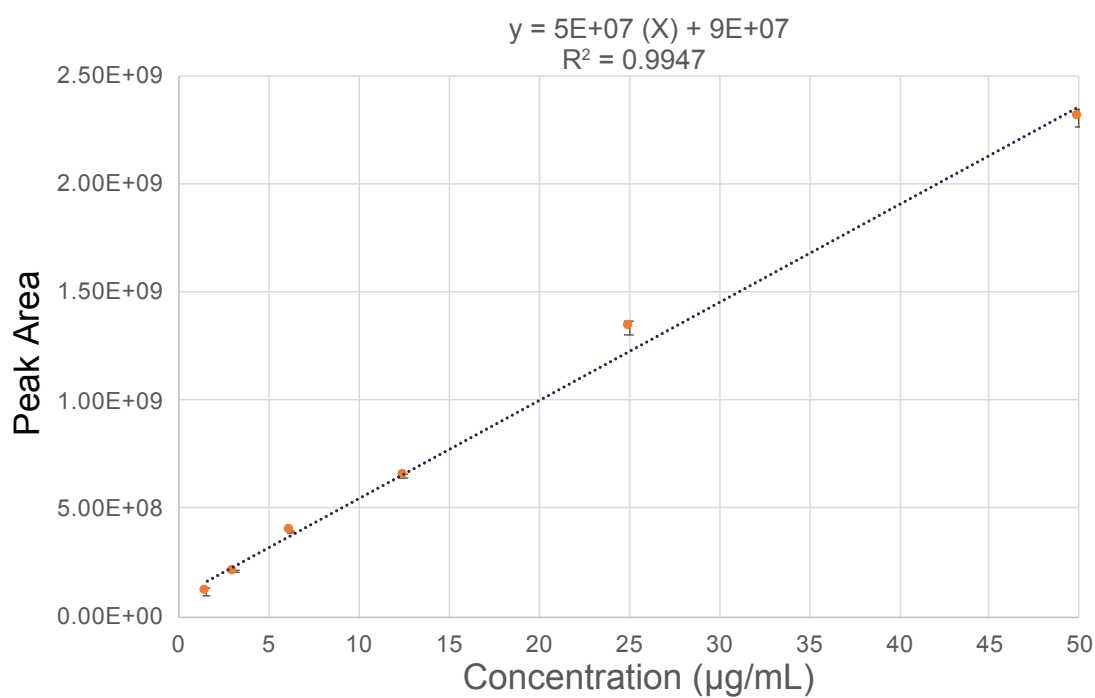
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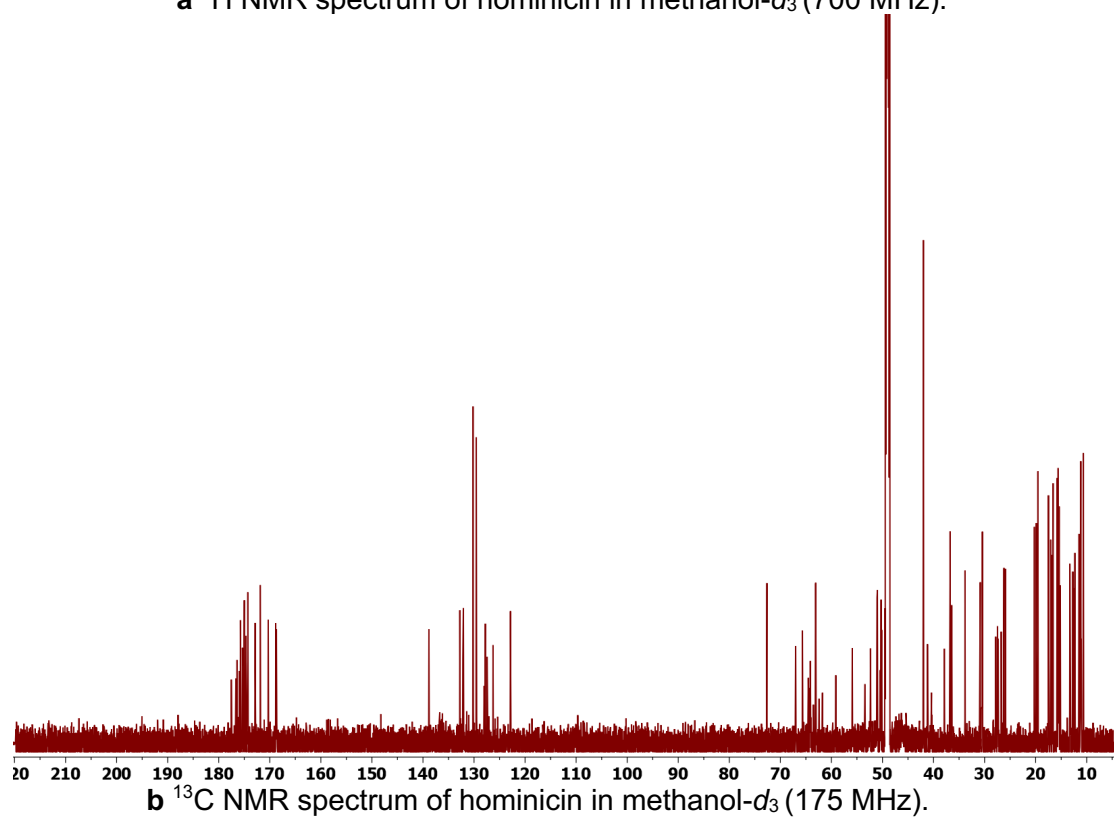
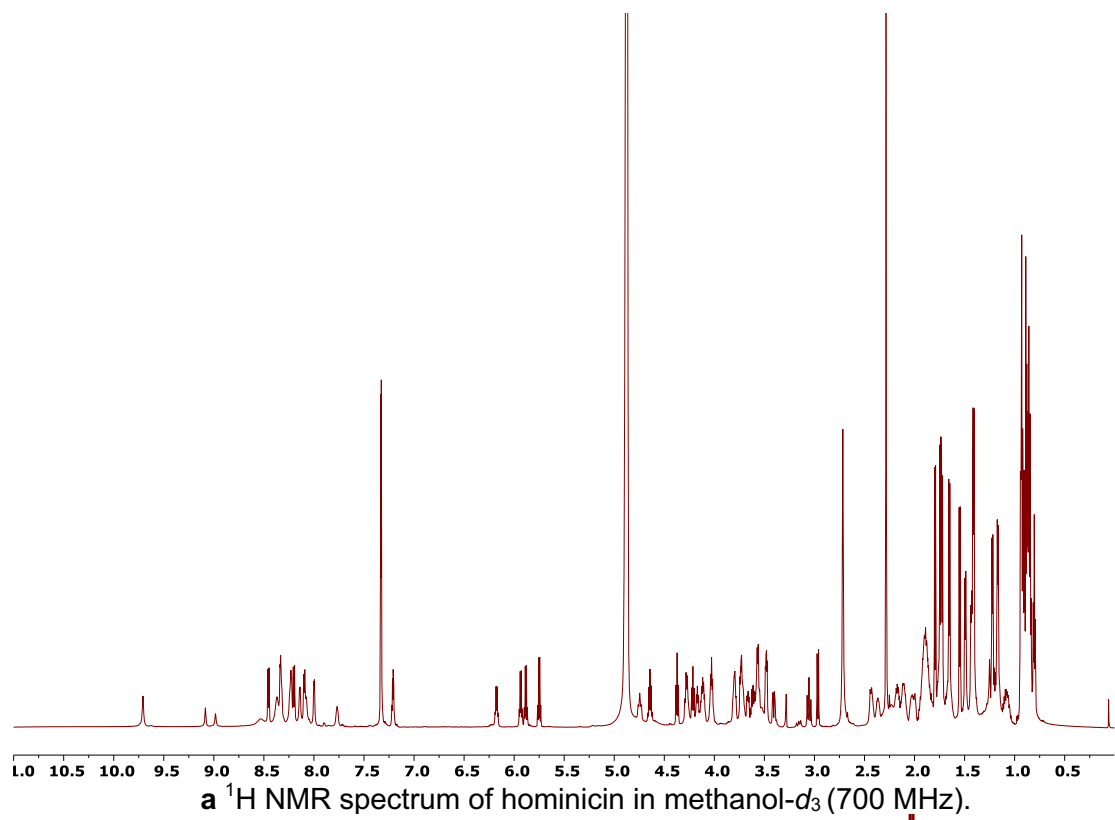
d

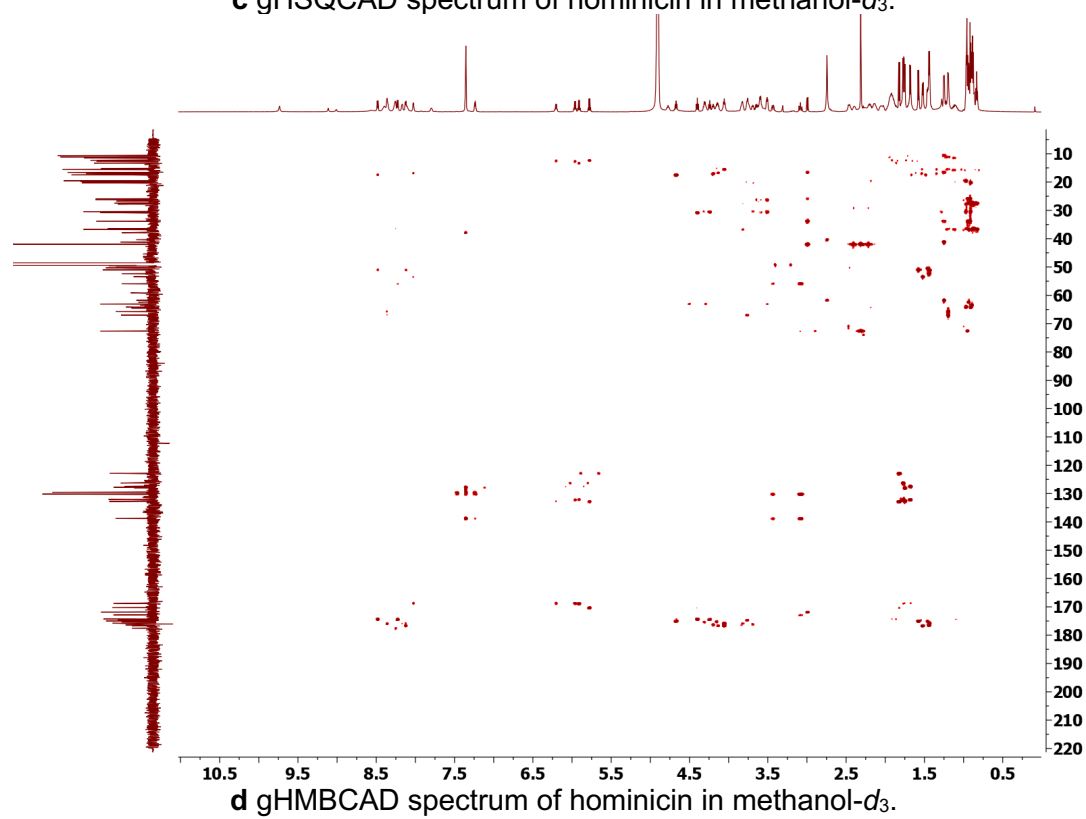
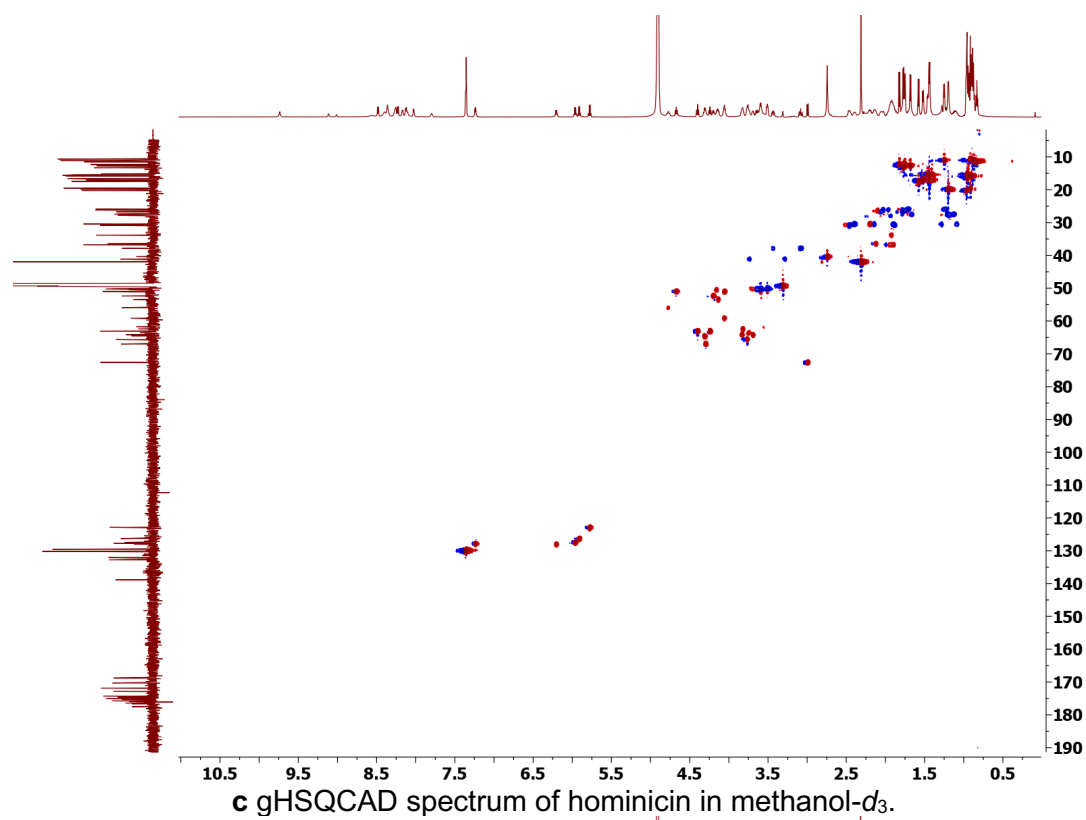
Ion Type	m/z	Δ ppm	AA Sequence
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y ₁₉	1814.0553	1.55	Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-Thr-Glu-Ile-Dhb-Ala-Ala-Val-Ile-Ala-Dmp
y ₁₇	1645.9652	2.92	Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-Thr-Glu-Ile-Dhb-Ala-Ala-Val-Ile-Ala-Dmp
y ₁₆	1562.9301	2.69	Pro-Phe-Dhb-Pro-Ala-Ile-Thr-Glu-Ile-Dhb-Ala-Ala-Val-Ile-Ala-Dmp
y ₁₃	1235.7748	4.05	Pro-Ala-Ile-Thr-Glu-Ile-Dhb-Ala-Ala-Val-Ile-Ala-Dmp
y ₁₀	954.6023	4.30	Thr-Glu-Ile-Dhb-Ala-Ala-Val-Ile-Ala-Dmp
y ₆	528.3881	2.47	Ala-Ala-Val-Ile-Ala-Dmp
y ₅	457.3499	0.44	Ala-Val-Ile-Ala-Dmp
y ₄	386.3136	2.59	Val-Ile-Ala-Dmp
y ₃	287.2445	1.05	Ile-Ala-Dmp
y ₂	174.1594	4.02	Ala-Dmp
b ₂	225.1599	0.89	DmIle-Dhb
b ₃	322.2131	1.87	DmIle-Dhb-Pro
b ₄	393.2503	1.79	DmIle-Dhb-Pro-Ala
b ₅	476.2876	1.89	DmIle-Dhb-Pro-Ala-Dhb
b ₆	573.3394	0.18	DmIle-Dhb-Pro-Ala-Dhb-Pro
b ₇	720.4079	2.23	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe
b ₈	803.4480	3.74	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb
b ₁₀	971.5370	2.17	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala
b ₁₁	1084.6148	3.88	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-

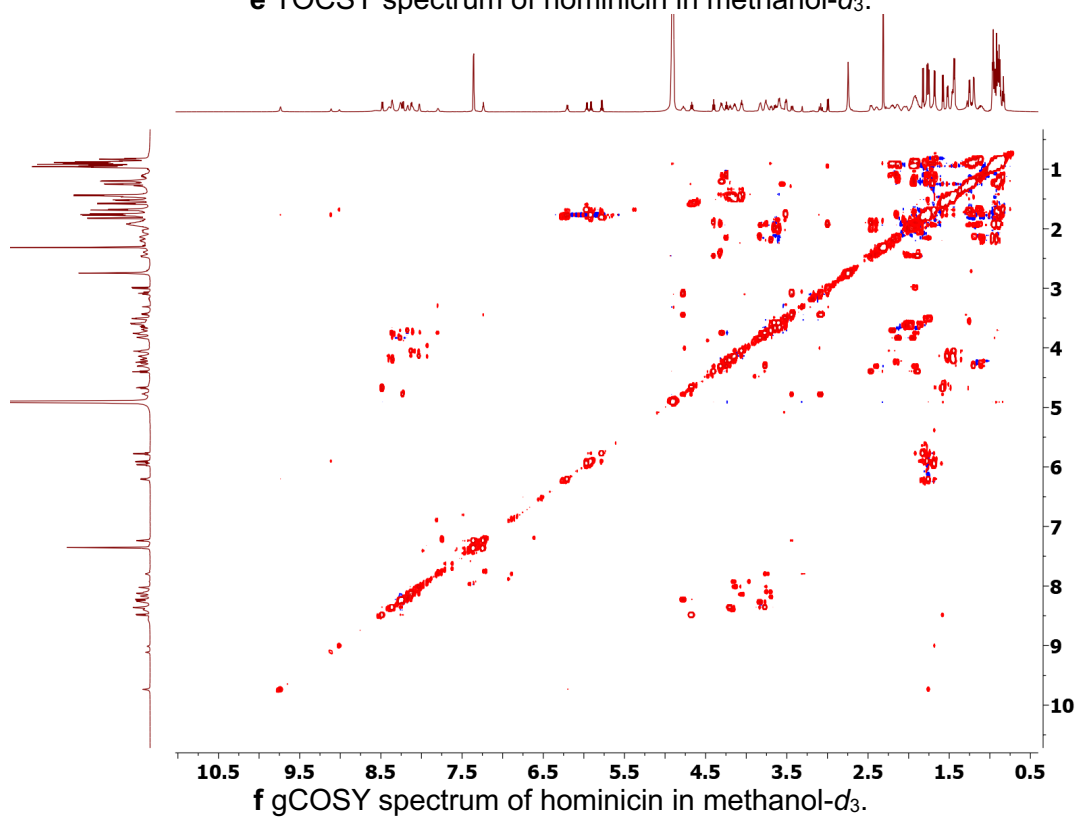
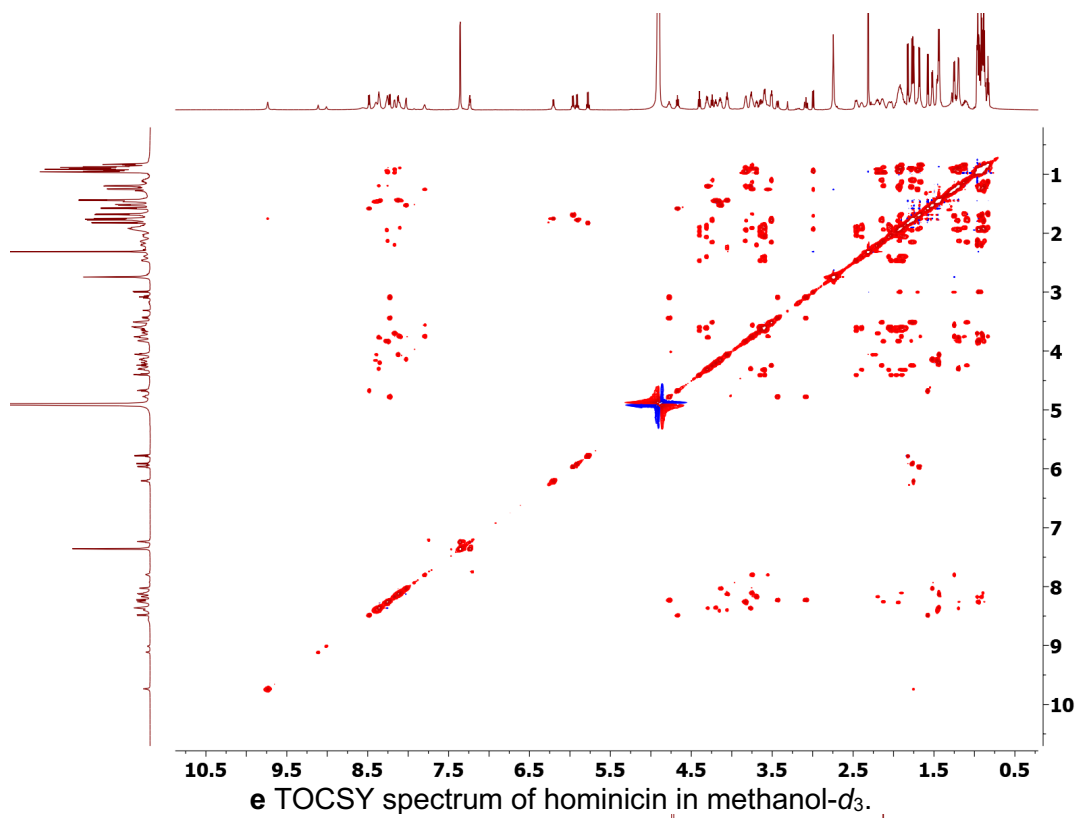
Supplementary Figure 4. Purification of *S. hominis* AH5011 hominidin. **a** Purification scheme to isolate hominidin from conditioned media. **b** Mass spectrum of the purified hominidin. **c** All ion fragmentation (AIF) spectrum for the peak eluting at 5.33 min in the purified hominidin sample. **d** Table of MS-MS ion assignments for fragments formed from the precursor ion detected in conditioned media with m/z 1020.110.

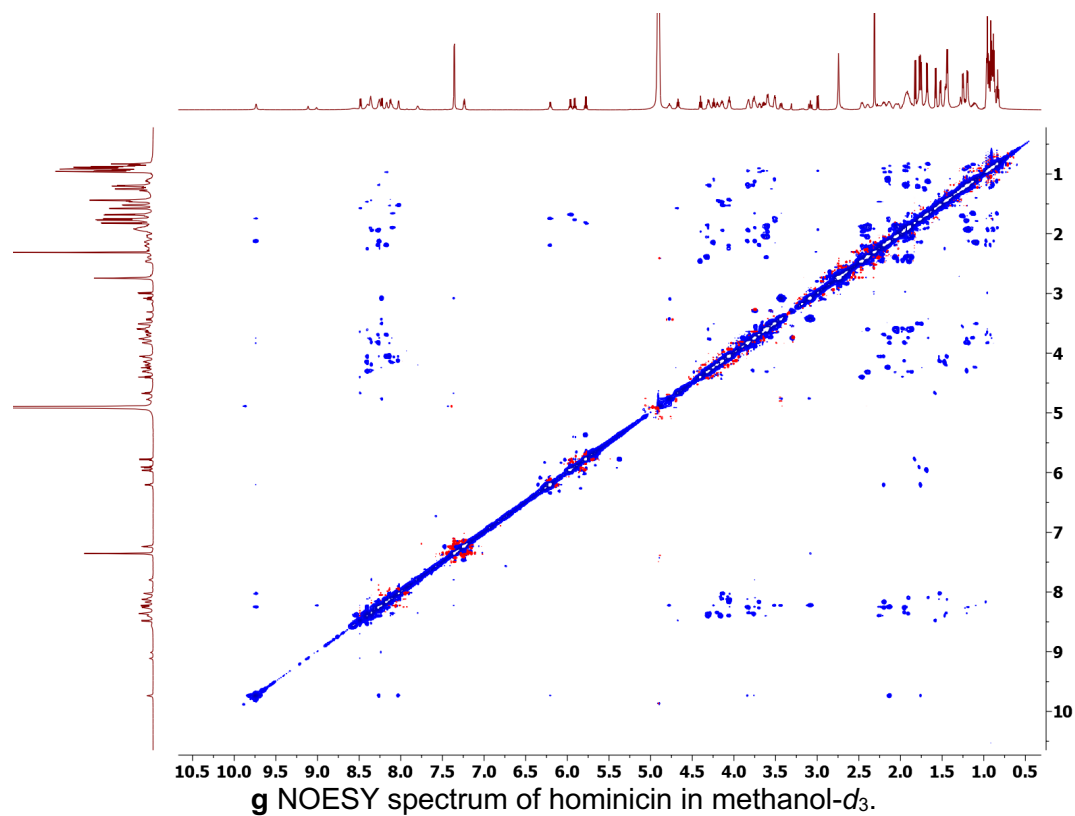


Supplementary Figure 5. Standard curve for the [M+2H]²⁺ ion of homininic. LC-MS peak area of the identified peptide [M+2H]²⁺. The quantitation of homininic in the 20x AH5011 extract prepared by *n*-butanol extraction is 817.0 ± 59 µg/mL. Data represents the mean of $n = 3$ injections and error bars show standard deviation.

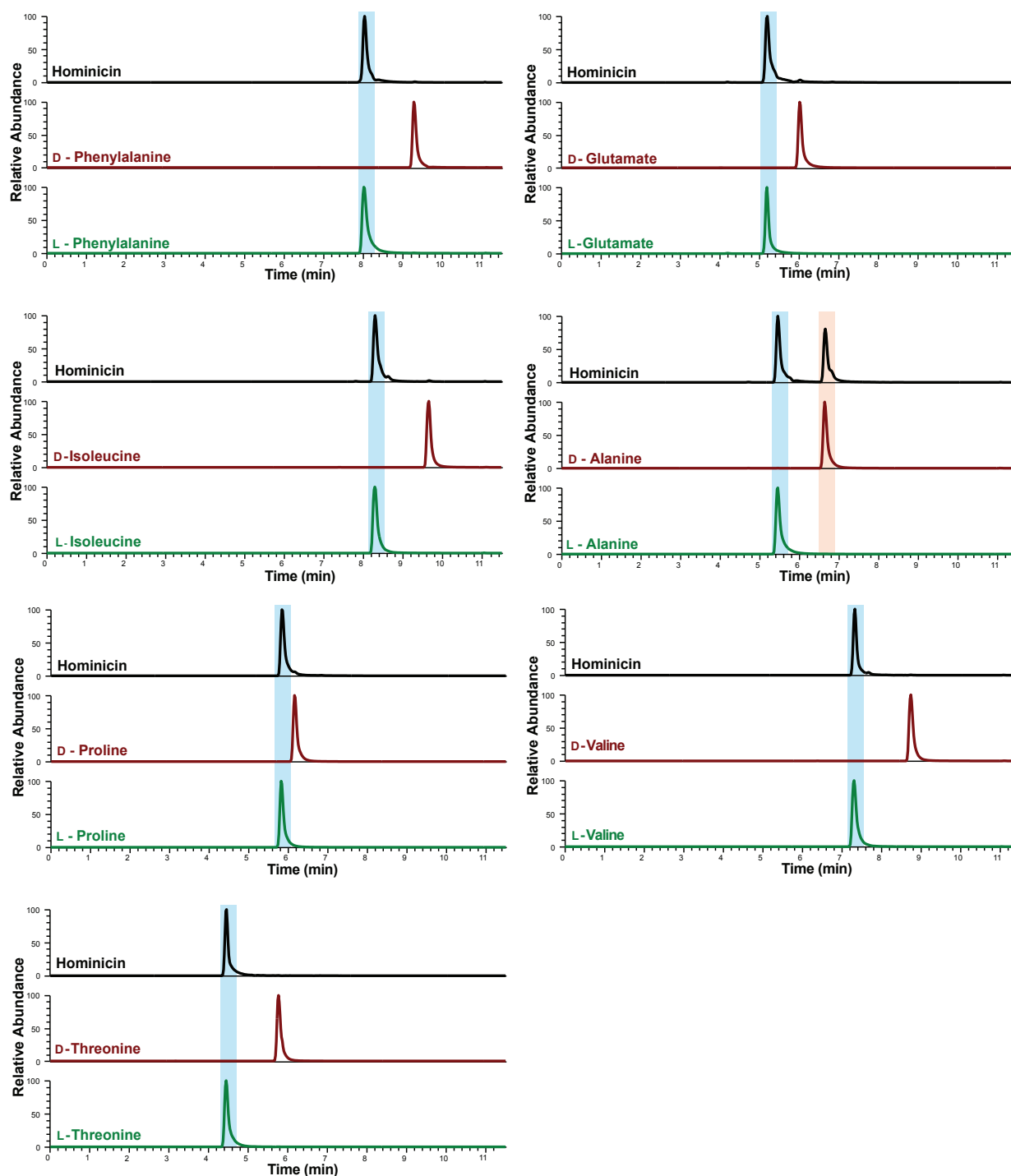






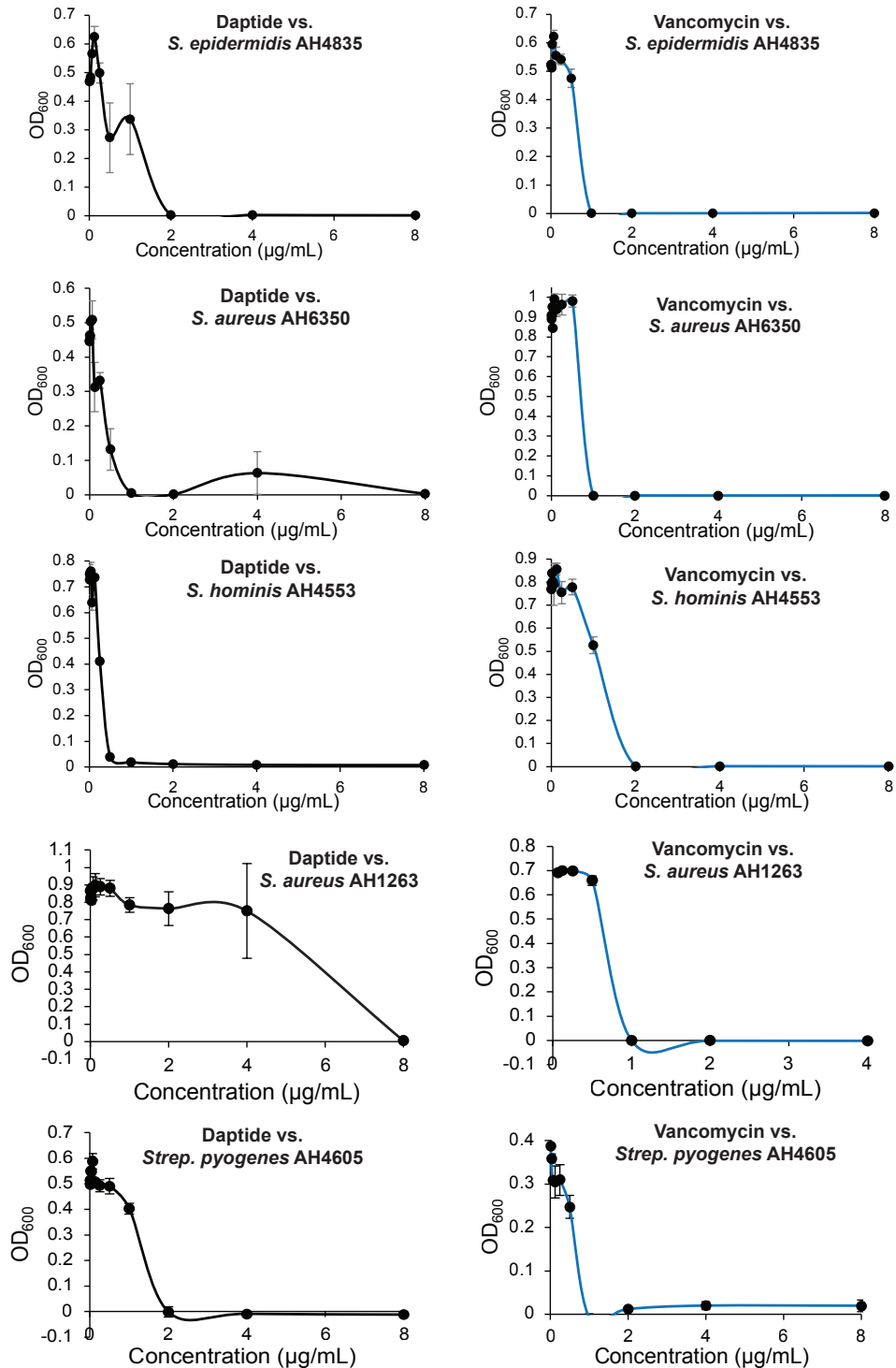


Supplementary Figure 6. NMR spectra of *S. hominis* AH5011 homininic. **a** ^1H NMR spectrum of homininic in methanol- d_3 (700 MHz). **b** ^{13}C NMR spectrum of homininic in methanol- d_3 (175 MHz). **c** gHSQCAD spectrum of homininic in methanol- d_3 . **d** gHMBCAD spectrum of homininic in methanol- d_3 . **e** TOCSY spectrum of homininic in methanol- d_3 . **f** gCOSY spectrum of homininic in methanol- d_3 . **g** NOESY spectrum of homininic in methanol- d_3 .

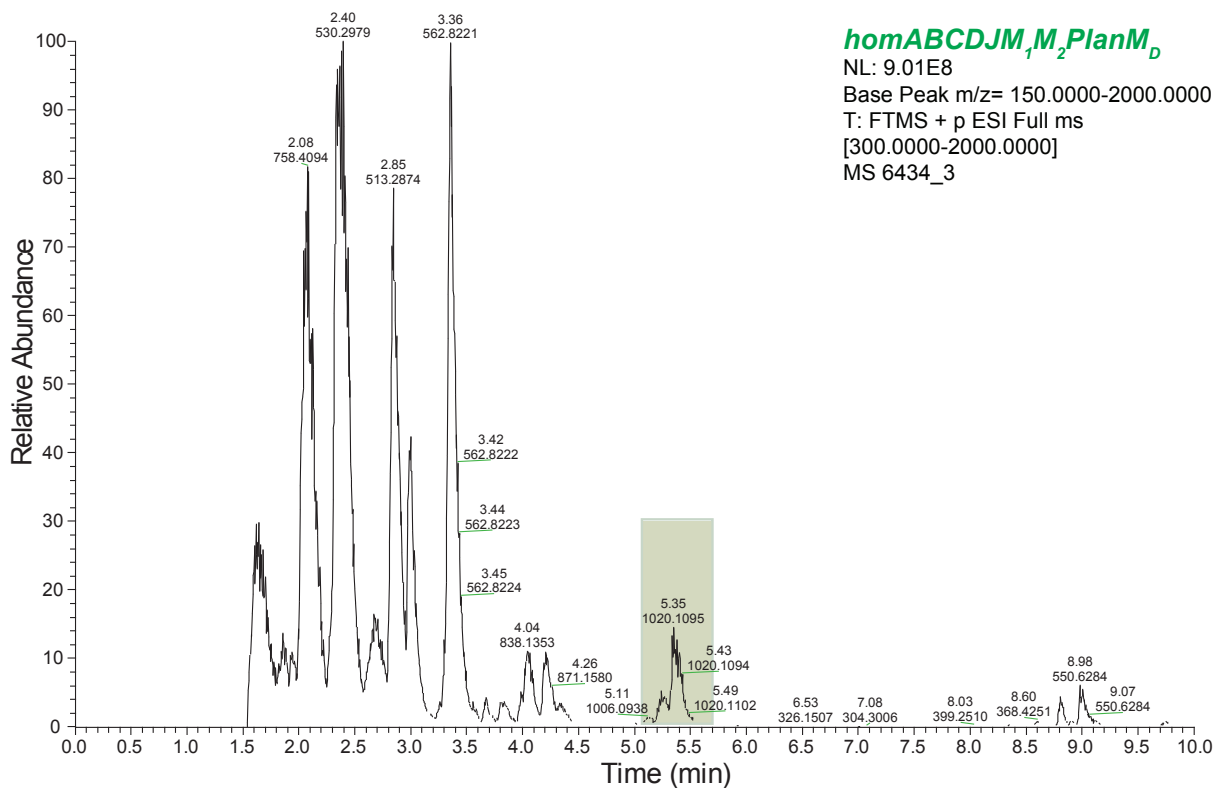


Supplementary Figure 7. Extracted ion chromatograms (XICs) of Marfey's derivatives for seven amino acid standards (phenylalanine, glutamate, isoleucine, alanine, proline, valine, and threonine) and homininic. All amino acids in the homininic sample were confirmed to be the L-enantiomers, with the exception of alanine, which exhibited both D- and L-enantiomers in a ratio of 1.2:1 L:D. The retention time (RT) and mass to charge ratio (m/z) values, respectively, for each amino acid are as follows: phenylalanine (8.03 min, 418.1357), glutamate (5.20 min, 400.1096), isoleucine (8.30 min, 384.1512), proline (5.84 min, 368.1200), valine (7.33 min,

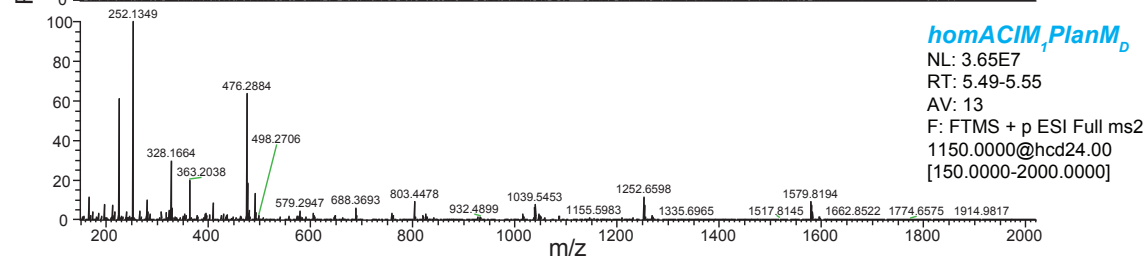
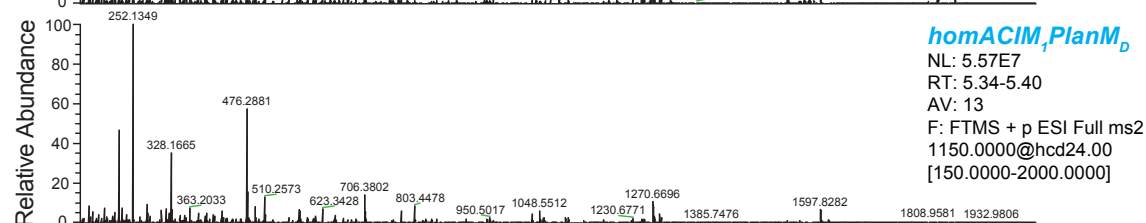
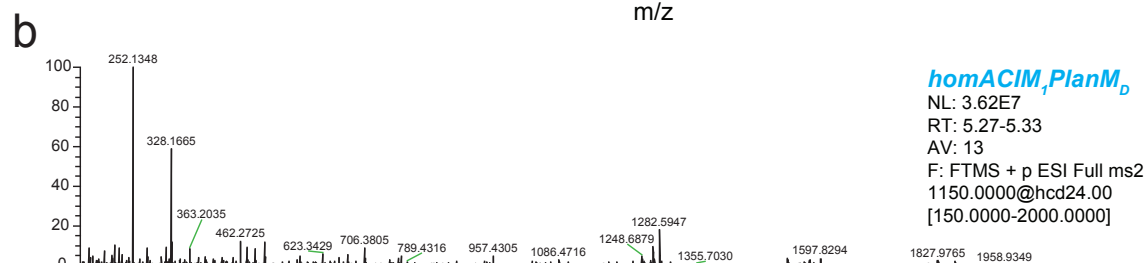
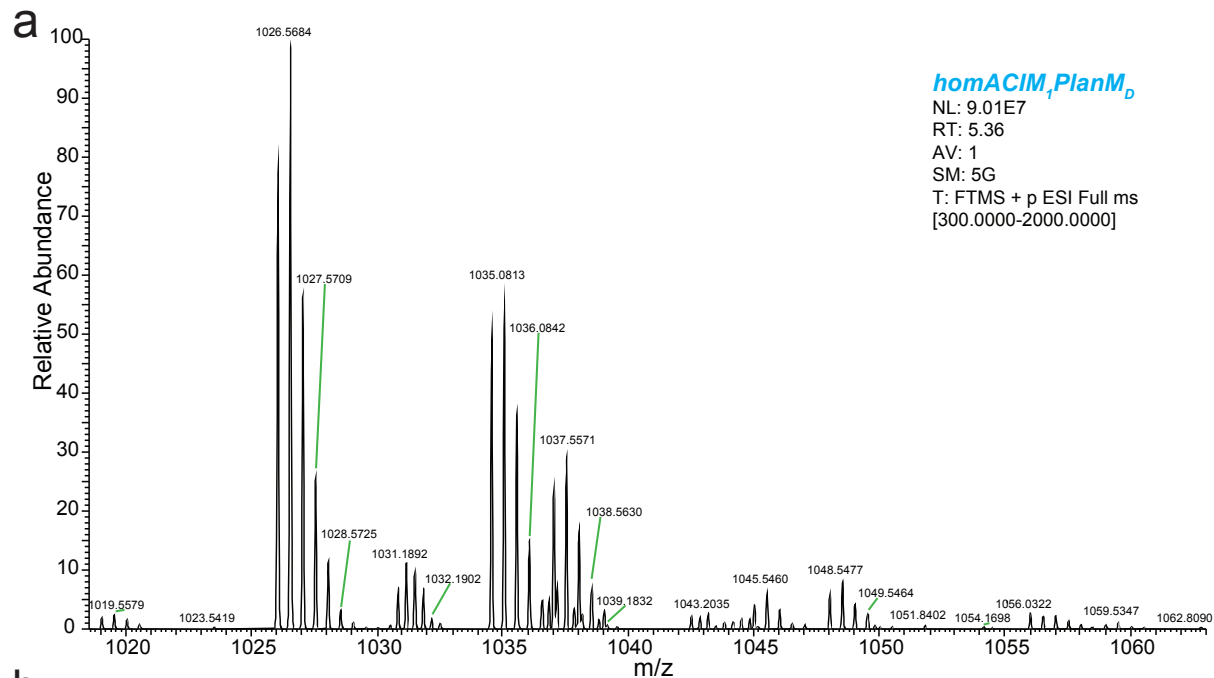
370.1358), threonine (4.46 min, 372.1148), and alanine (L: 5.46 min, 342.1043; D: 6.66 min, 342.1043).



Supplementary Figure 8. Minimum inhibitory concentration (MIC) curve. Minimum inhibitory concentration (MIC) curves for AH5011 hominycin and vancomycin against all tested strains. The starting concentration was 8 µg/mL, serially diluted down to 0.002 µg/mL. Error bars indicate the standard deviation calculated from three replicates.



Supplementary Figure 9. Mass spectrometric analysis of conditioned media from heterologously expressed *homABCDJM₁M₂PlanM_D*. Base peak chromatogram of the CM from *S. aureus* RN4220 expressing *homABCDJM₁M₂PlanM_D* with the peak representing the $[M+2H]^{2+}$ ion with m/z 1020.1095 for the daptide boxed in green.

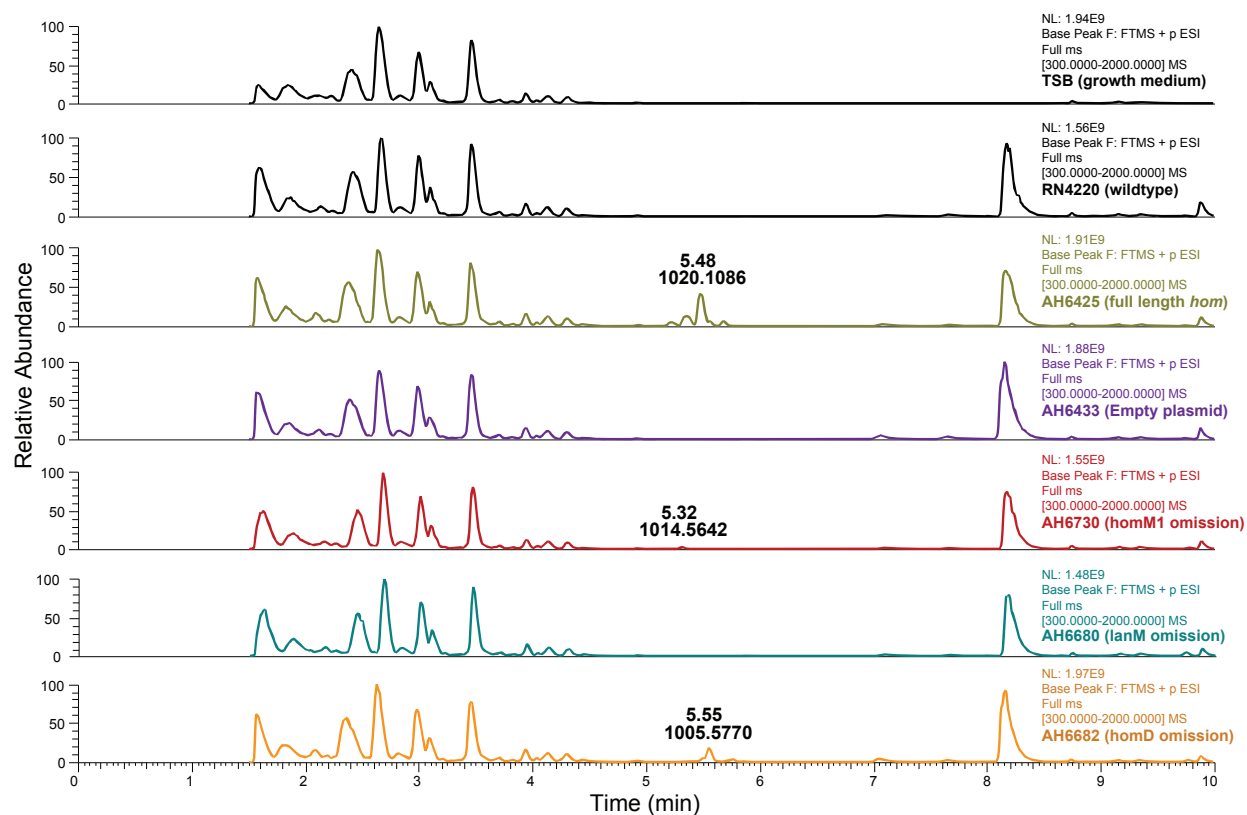


C

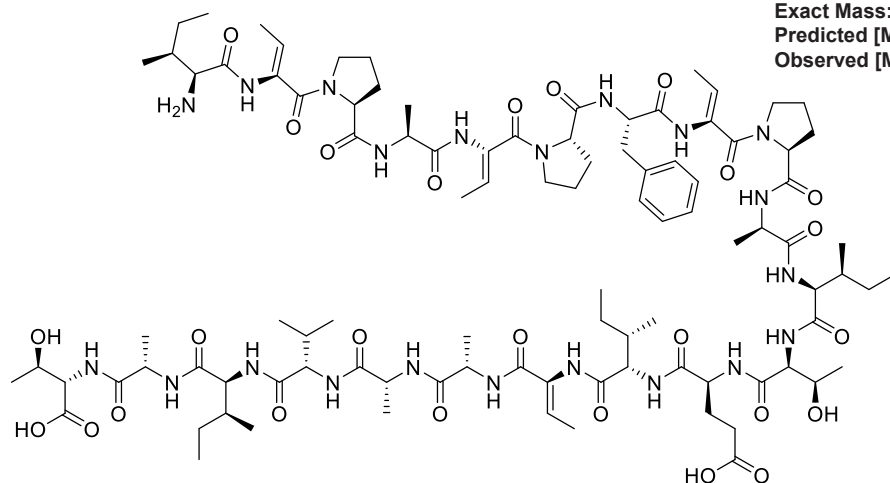
Ion Type	m/z	Δ ppm	AA Sequence
y ₄	403.2566	3.72	Val-Ile-Ala-Thr
y ₃	304.1872	1.65	Ile-Ala-Thr
y ₂	191.1031	2.62	Ala-Thr
b ₂	225.1602	1.78	DmIle-Dhb
b ₃	322.2133	2.49	DmIle-Dhb-Pro
b ₄	393.2504	2.04	DmIle-Dhb-Pro
b ₅	476.2882	3.15	DmIle-Dhb-Pro-Ala
b ₇	720.4111	4.45	DmIle-Dhb-Pro-Ala-Dhb
b ₈	803.4482	3.99	DmIle-Dhb-Pro-Ala-Dhb-Pro
b ₉	900.5012	3.78	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe

Supplementary Figure 10. Mass spectrometric analysis of conditioned media from heterologously expressed *homACIM₁PlanM_D*. **a** Mass spectrum showing various peptide detected in the CM from *S. aureus* RN4220 expressing *homACIM₁PlanM_D*. **b** All ion fragmentation spectrum showing fragments of the peptide detected in the CM from *S. aureus* RN4220 expressing *homACIM₁PlanM_D*. **c** Table of fragment ion assignments.

a



b

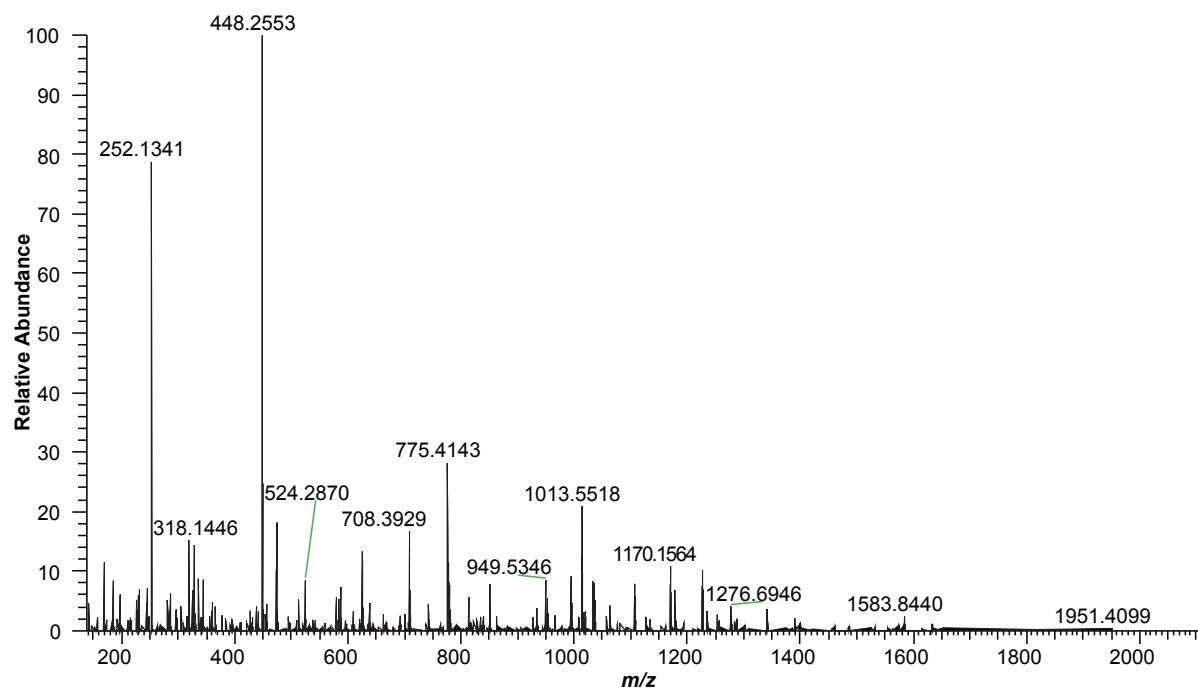


AH6730 (homM1 omission)

Exact Mass: 2026.1139

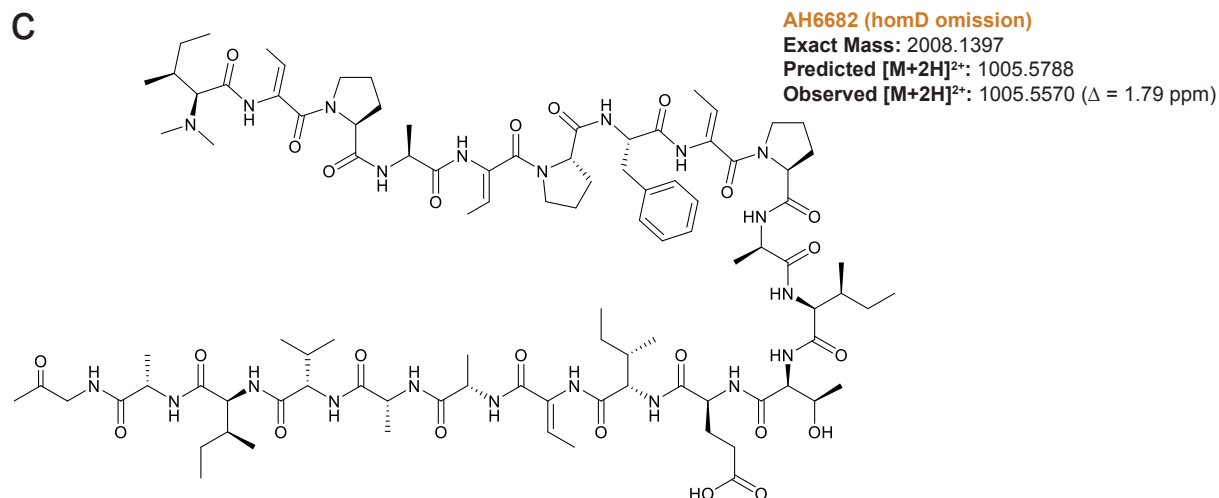
Predicted $[M+2H]^{2+}$: 1014.5659Observed $[M+2H]^{2+}$: 1014.5652 ($\Delta = 0.69$ ppm)

20250724_04_AH6730_03 #2279-3624 RT: 4.63-5.62 AV: 4 NL: 7.32E4
 F: FTMS + p ESI d Full ms2 1014.5583@hcd24.00 [139.3333-2090.0000]

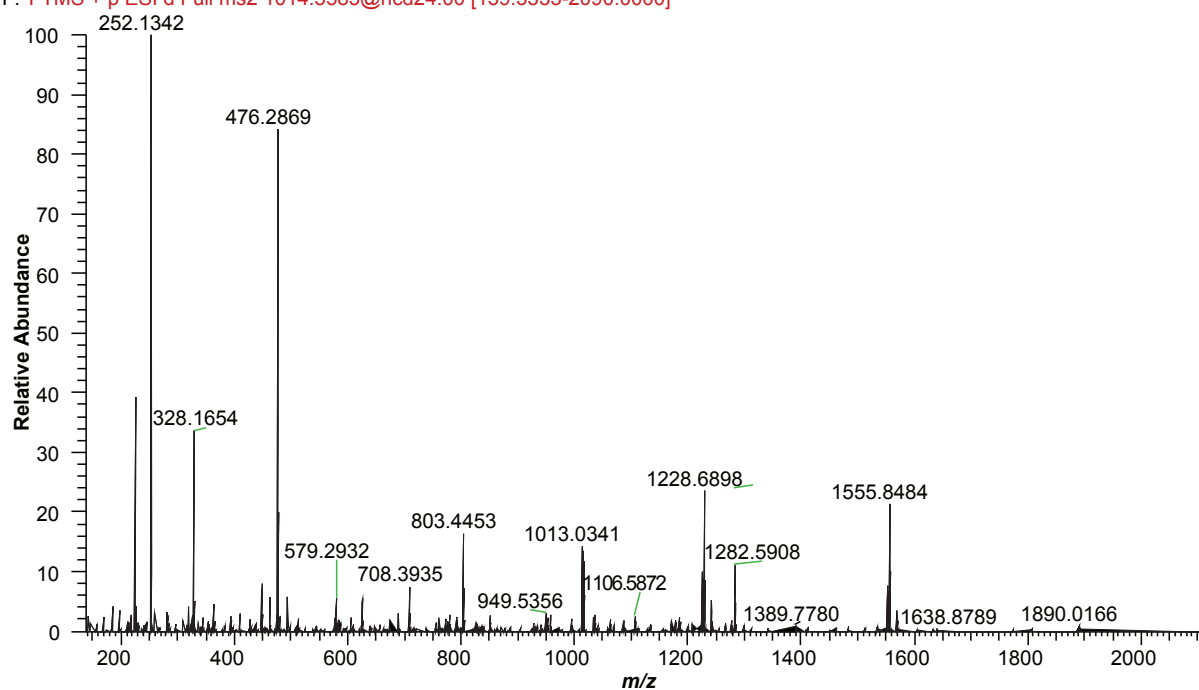


Ion Type	m/z	Δ ppm	AA Sequence
y ₇	628.3639	4.14	Dhb-Ala-Ala-Val-Ile-Ala-Thr
y ₃	304.1866	0.33	Ile-Ala-Thr
y ₂	191.1024	1.05	Ala-Thr
b ₂	197.1283	1.02	Ile-Dhb
b ₃	294.181	0.68	Ile-Dhb-Pro
b ₄	365.2188	1.37	Ile-Dhb-Pro-Ala
b ₅	448.2554	0.00	Ile-Dhb-Pro-Ala-Dhb
b ₇	692.3782	2.32	Ile-Dhb-Pro-Ala-Dhb-Pro-Phe
b ₈	775.4134	0.39	Ile-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb
b ₁₀	943.5013	2.44	Ile-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala
b ₁₁	1056.5867	0.95	Ile-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile
b ₁₂	1157.6341	1.13	Ile-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-Thr
b ₁₃	1286.6733	3.66	Ile-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-Thr-Glu
b ₁₄	1399.7592	2.01	Ile-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-Thr-Glu-Ile
b ₁₅	1482.7944	3.17	Ile-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-Thr-Glu-Ile-Thr

C

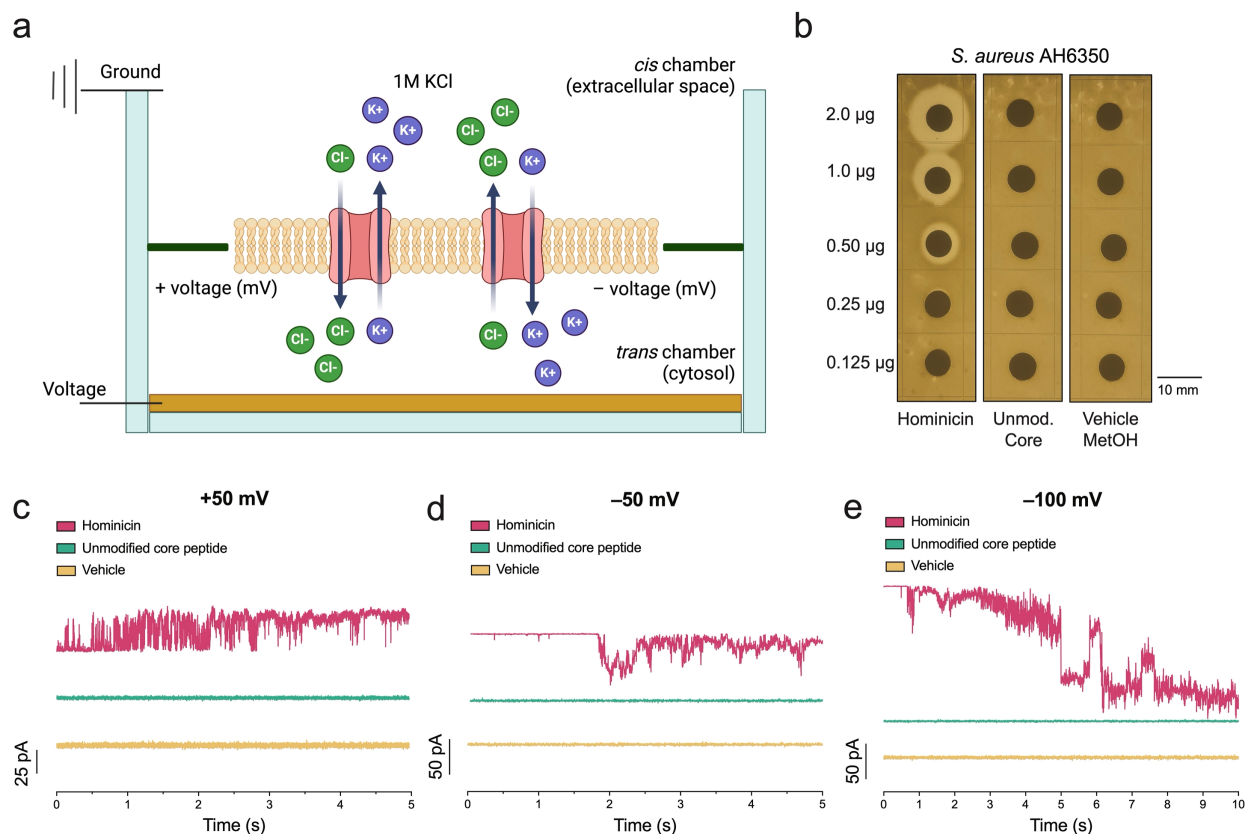


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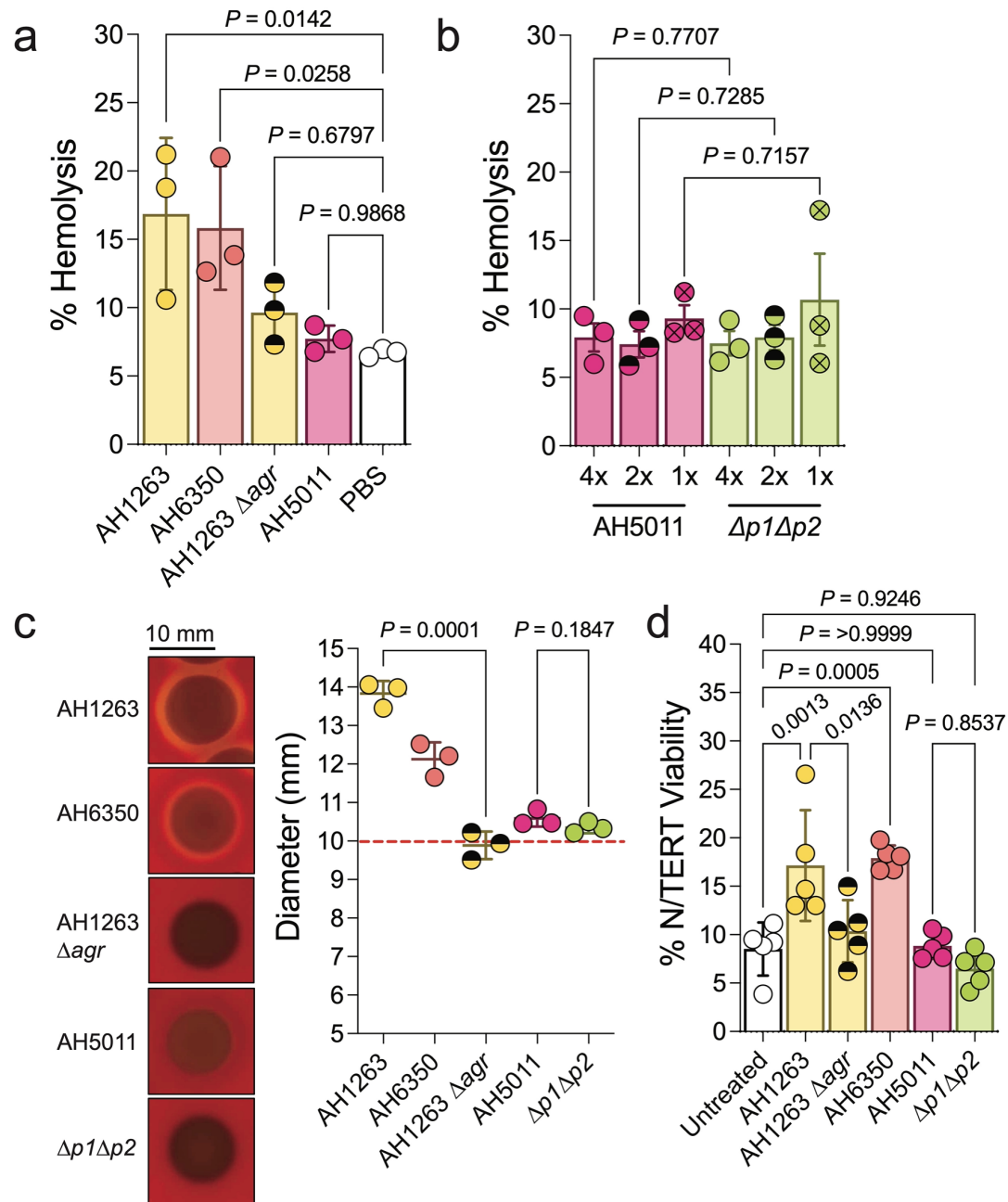


Ion Type	m/z	Δ ppm	AA Sequence
y ₁₃	1206.7067	2.16	Pro-Ala-Ile-Thr-Glu-Ile-Dhb-Ala-Ala-Val-Ile-Ala-OxD*
y ₇	582.3614	0.69	Dhb-Ala-Ala-Val-Ile-Ala-OxD
y ₆	499.3242	0.61	Ala-Ala-Val-Ile-Ala-OxD
y ₅	428.2876	2.11	Ala-Val-Ile-Ala-OxD
y ₄	357.2502	1.68	Val-Ile-Ala-OxD
y ₃	258.181	0.78	Ile-Ala-OxD
b ₂	225.1597	1.78	DmIle-OxD
b ₃	322.2122	0.94	DmIle-Dhb-Pro
b ₄	393.2498	0.51	DmIle-Dhb-Pro-Ala
b ₅	476.287	0.63	DmIle-Dhb-Pro-Ala-Dhb
b ₆	573.3421	4.54	DmIle-Dhb-Pro-Ala-Dhb-Pro
b ₇	720.41	2.92	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe
b ₈	803.4449	0.13	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb
b ₁₀	971.5311	3.92	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala
b ₁₁	1084.6165	2.31	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile
b ₁₃	1314.7052	3.12	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-Thr-Glu
b ₁₅	1510.8315	0.73	DmIle-Dhb-Pro-Ala-Dhb-Pro-Phe-Dhb-Pro-Ala-Ile-Thr-Glu-Dhb-Ala

Supplementary Figure 11. Mass spectrometric analysis of conditioned media from heterologously expressed *homABCDIJM₂PlanM_D*, *homABCIJM₁M₂PlanM_D*, and *homABCDIJM₁M₂P*. **a** Base peak chromatograms of conditioned media from *S. aureus* wild type and mutant strains and TSB control. **b** Peptide structure, MS-MS spectrum, and table of fragment ion assignments obtained from fragmentation of the $[M+2H]^{2+}$ ion with m/z 1014.5652 detected in the spent media of strain AH6730 expressing *homABCDIJM₂PlanM_D*. **c** Peptide structure, MS-MS spectrum, and assignment of fragment ions obtained from fragmentation of the $[M+2H]^{2+}$ ion with m/z 1005.5570 detected in the spent media of strain AH6682 expressing *homABCIJM₁M₂PlanM_D*.

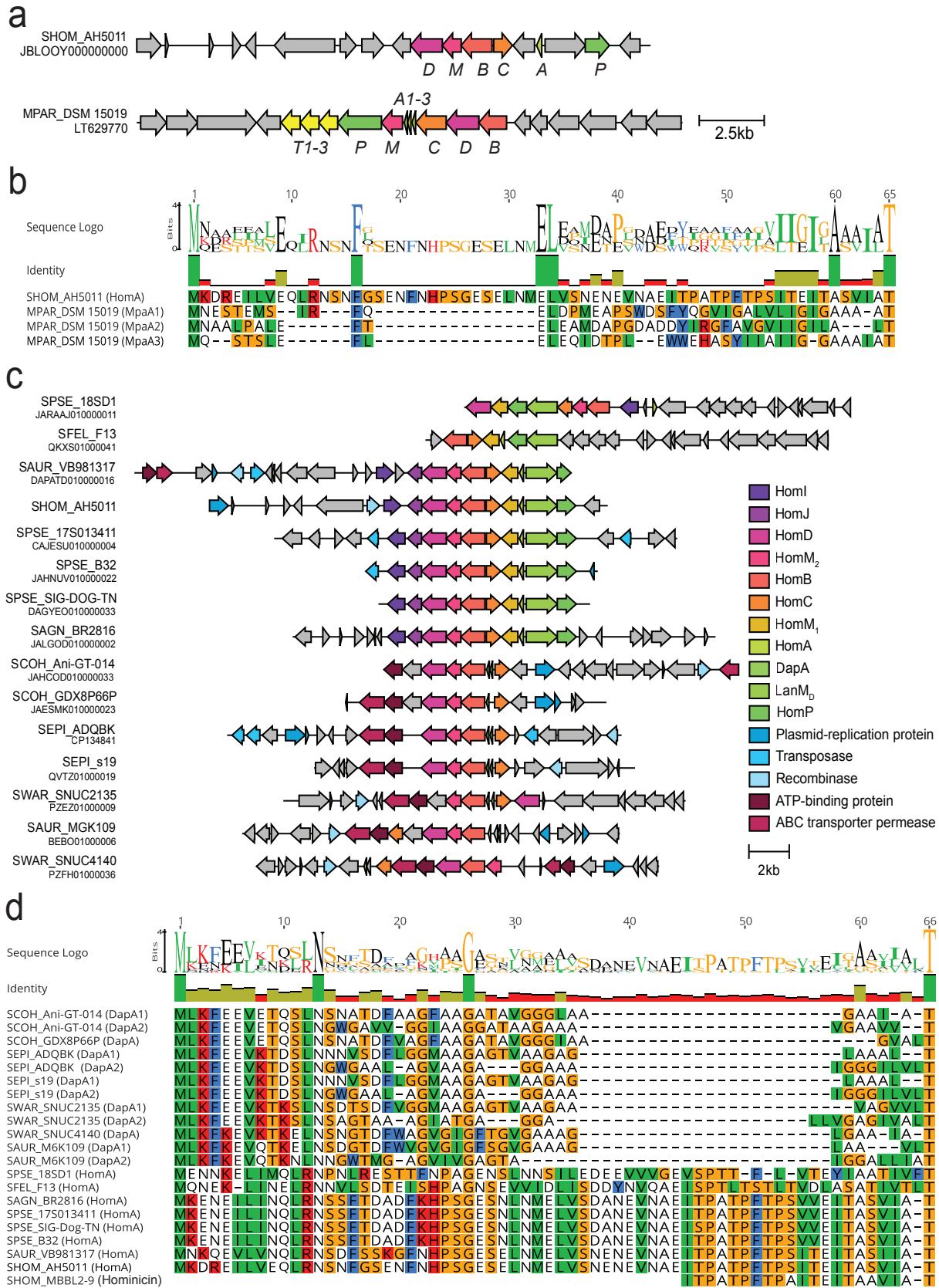


Supplementary Figure 12. Single-channel properties of membrane channels formed by homininicin. **a** Schematic of planar lipid bilayer setup and the electrophysiological methods used in this study. Image was generated in BioRender. **b** Disk diffusion assay with the purified homininicin, synthetic unmodified core peptide, and methanol (vehicle) on a bacterial lawn of *S. aureus* strain AH6350. **c, d, e** Current traces of homininicin, unmodified core peptide, and methanol vehicle at the indicated holding potential of +50 mV (**c**), -50 mV (**d**), and -100 mV (**e**). Source data are provided as a Source Data file.



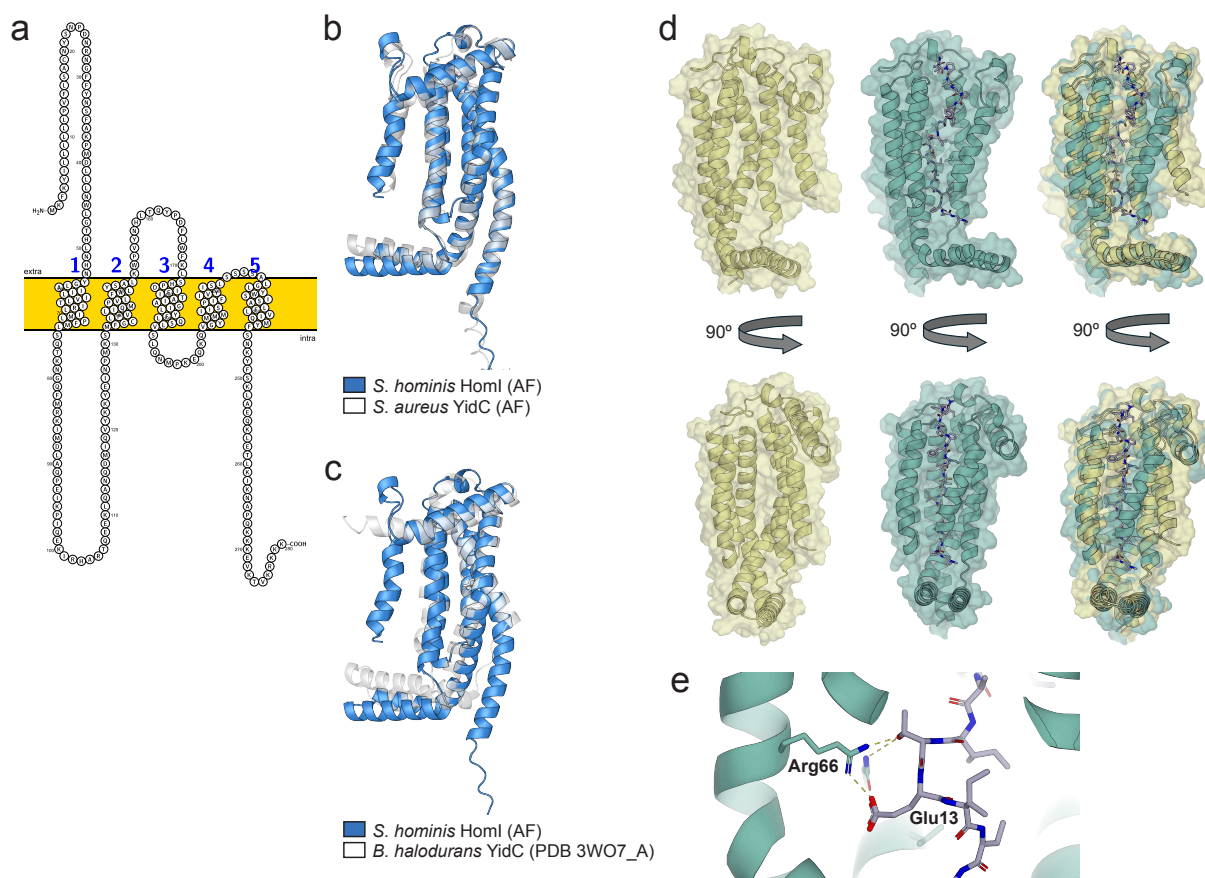
Supplementary Figure 13. Hemolytic activity in mammalian erythrocytes and cytotoxicity in human keratinocytes. **a, b** Whole human blood was prepared separately in a heparin tube from three healthy donor. Erythrocytes were incubated for 4 h with 1×10^7 CFU of AH1263, Δagr mutant, AH6350, AH5011, PBS, or water alone (**a**), or with concentrated conditioned media from AH5011 or $\Delta p1\Delta p2$ prepared by *n*-butanol extraction (**b**). Absorbance was measured at 543 nm and normalized to water, which serves as a total lysis control. Data represents the mean of $n = 3$ biological replicates and error bars show standard deviation. Statistical differences were compared to PBS control group using one-way ANOVA followed by Dunnett's multiple comparison test (**a**). Statistical differences between extract treatments were determined using a two-tailed unpaired *t*-test (**b**). **c** Representative images of hemolysis from *S. aureus* and *S. hominis* strains

on 5% sheep blood agar, with measurements of hemolysis. Red dashed line indicates the limit of detection. Data represents the mean of $n = 3$ biological replicates, with error bars showing standard deviation. Statistical differences between strains were determined using a two-tailed unpaired t -test. **d** Immortalized human N/TERT-2G keratinocytes were incubated for 4 h with 1×10^7 CFU of AH1263, Δagr mutant, AH6350, AH5011, or PBS alone. The release of lactate dehydrogenase (LDH) was measured as an indicator of cytotoxicity and normalized to total lysis. Data represents the mean of $n = 5$ biological replicates and error bars show standard deviation. Statistical differences between treatment groups were determined using a one-way ANOVA analysis followed by Bonferroni's multiple comparison test. Source data are provided in the Source Data file.

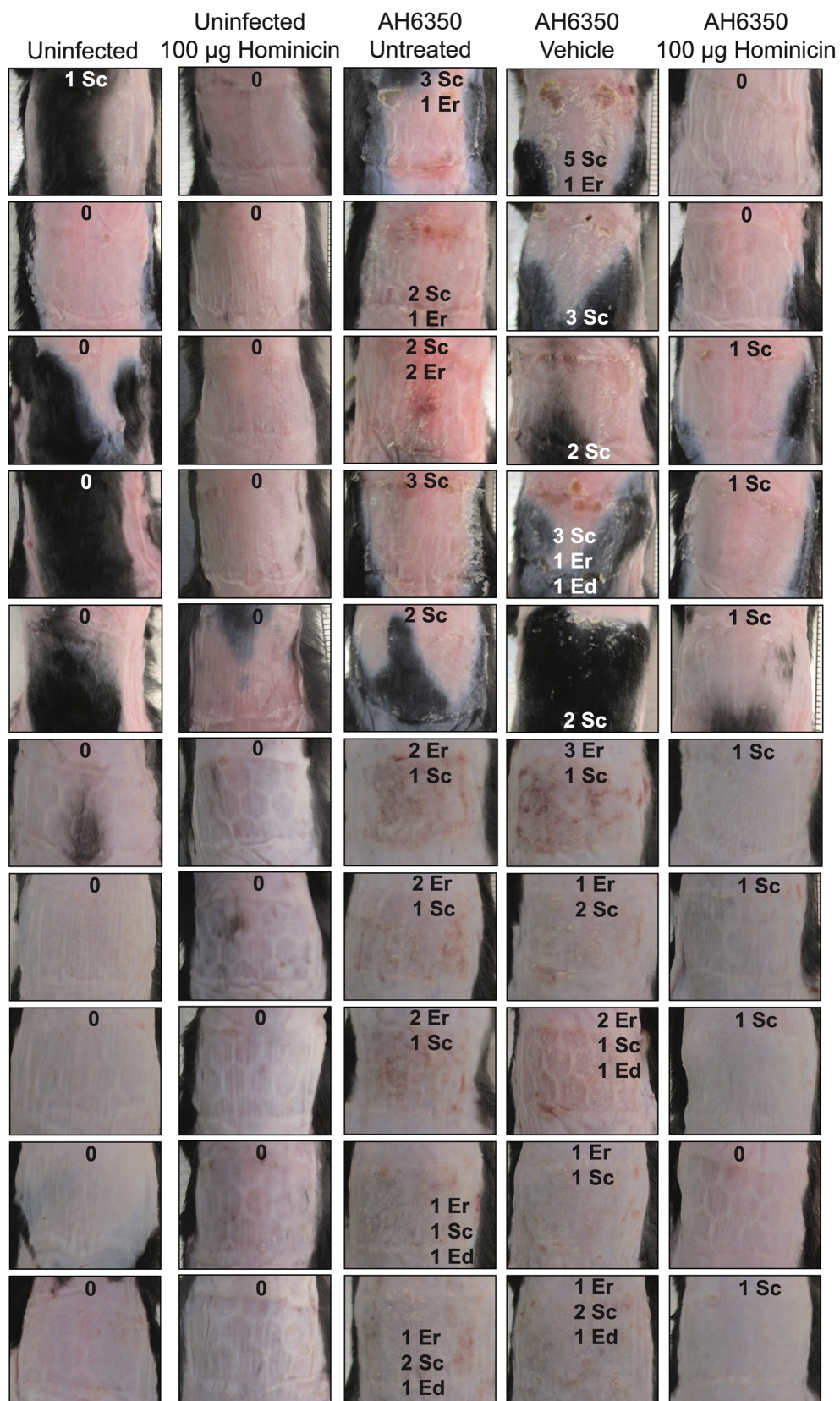


Supplementary Figure 14. Identification of *hom*-like gene clusters in *Staphylococcus*.

a Gene arrangement of *hom* BGC from *S. hominis* AH5011 and *mpa* BGC from *M. paraoxydans* DSM 15019 with accompanying genome accession numbers. The labeled genes correspond to the precursor peptide (*A*), a protein of unknown function fused to the RiPP recognition element (*B*), decarboxylase (*C*), aminotransferase (*D*), methyltransferase (*M*), peptidase (*P*), and predicted transporters (*T*). **b** Amino acid alignment of the precursor daptide sequences between HomA, MpaA1, MpaA2, and MpaA3. **c** Genomic visualization of staphylococcal daptide BGCs. Each colored gene within the BGC represents a protein cluster with $\geq 30\%$ identity and gray genes belong to a lone cluster. Species are labeled as follows: *S. agnetis* (SAGN), *S. aureus* (SAUR), *S. cohnii* (SCOH), *S. epidermidis* (SEPI), *S. felis* (SFEL), *S. hominis* (SHOM), *S. pseudintermedius* (SPSE), and *S. warneri* (SWAR). Strain names and genome accession numbers are labeled. **d** Peptide alignment of HomA homologs from staphylococcal BGCs and the reported hominidin from SHOM_MBBL2-9. The sequence logo is displayed, with colors indicating residue side-chain properties. His, Lys, Arg (red); Phe, Trp, Tyr (blue); Leu, Ile, Met, Val (green); Gly, Pro, Ser, Thr (orange). The mean pairwise percent identity is shown, denoting 100% identity (green), $30\% \leq \text{identity} < 100\%$ (yellow), and $< 30\%$ identity (red).

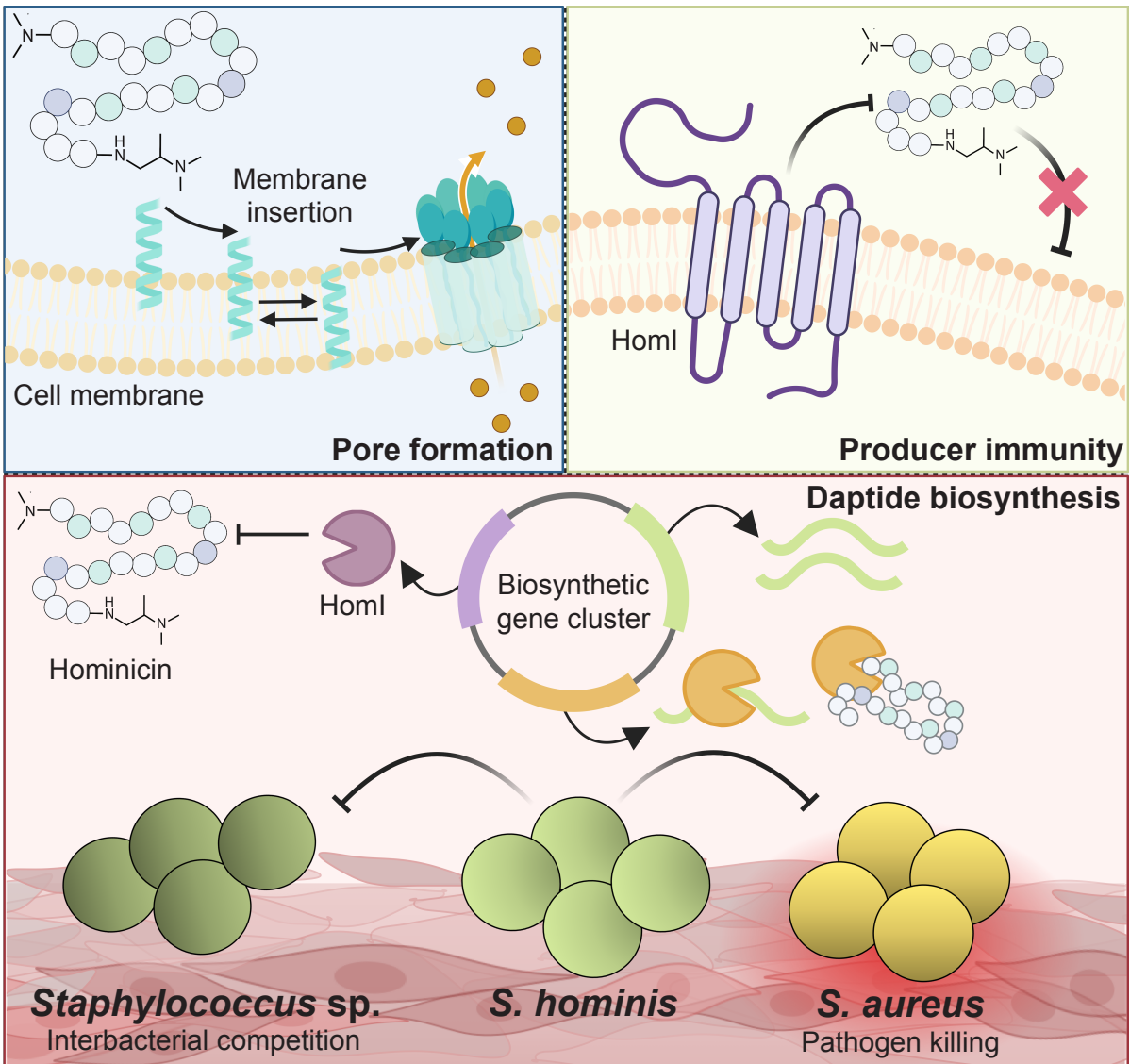


Supplementary Figure 15. Structural homology between HomI and YidC. **a** Predicted domain topology of HomI protein with the transmembrane helices labeled. **b** The AlphaFold 3 (AF) predicted three-dimensional structures of *S. hominis* HomI (blue) and *S. aureus* YidC (white) were superimposed utilizing PyMOL's 'align' command, which performs an iterative, least-squares fitting of C α atoms. This alignment produced a root mean square deviation (RMSD) of 1.41 Å over 1572 atoms, indicating high structural similarity. **c** The HomI model (AF) and a crystal structure of *Bacillus halodurans* YidC (PDB 3WO7_A) (white), resolved at a resolution of 2.4 Å, were superimposed utilizing PyMOL's 'align' command. This alignment produced an RMSD of 1.76 Å over 1011 atoms, indicating high structural similarity. **d** The HomI-hominicin complex model depicted in Fig. 6 is presented with alternative perspectives. The models are presented in a side-profile view (top) and a front-facing view (bottom). Hominicin is represented in purple as sticks. HomI modeled without homininicin is represented in yellow. HomI modeled in complex with homininicin is represented in teal. **e** The predicted interaction between homininicin (purple) and HomI (teal) reveals an interaction between the homininicin Glu13 and HomI Arg66 residues. Polar interactions within hydrogen bonding distance between these residues are indicated by yellow dashed lines.



10 mm

Supplementary Figure 16. AH5011 hominidin reduces *S. aureus*-induced inflammation and epicutaneous injury. After 72 h of epicutaneous exposure to phosphate-buffered saline (uninfected) or *S. aureus*, with or without hominidin treatment, the gross morphology of the skin was scored by a blinded observer. The severity of skin inflammation was quantified using a total disease score, which is the sum of the individual grades for erythema (Er), edema (Ed) [each graded as 0, 1, 2, 3] and scaling/erosion (Sc) [scaling graded as 0, 1, 2, 3 while erosion graded as 4, 5, 6, 7]. Source data are provided in the Source Data file.



Supplementary Figure 17. A working model for the hominycin daptide. The *hom* BGC from *S. hominis* strain AH5011 encodes the precursor peptide, immunity factor, and post-translationally modifying enzymes (*bottom*). Hominycin displays antimicrobial activity against the genus *Staphylococcus*. To exert its mechanism of action, hominycin partitions into the hydrophobic core of the bacterial membrane and self-assembles into ion-conducting transmembrane pores (*top left*). The formation of peptidic channels consequently impairs cell membrane integrity by dissipating the bacterial transmembrane potential. HomI functions as the cognate resistance factor that protects from hominycin-induced membrane damage (*top right*). Image was generated in BioRender.

Supplementary Table 1. List of bacterial strains and plasmids used in this study.

Strains	Description or Genotype	Reference
<i>Staphylococcus hominis</i>		
AH5011	Non-lesional atopic dermatitis skin isolate D11	1
AH6424	AH5011 $\Delta p1\Delta p2$	This study
AH6471	AH5011 p1 $\Delta p2$	This study
AH6472	AH5011 p2 $\Delta p1$	This study
AH4553	American Type Culture Collection strain DM 122	ATCC 27844
<i>Staphylococcus aureus</i>		
RN4220	Restriction-deficient cloning host	2
AH1178	USA100 Newman	3
AH1263	USA300 LAC	4
AH1292	AH1263 $\Delta agr::tetM$	5
AH6350	Lesional atopic dermatitis skin isolate	This study
AH6423	AH1178 + pCM28- <i>homI</i>	This study
AH6425	RN4220 + pCM28- <i>homABCDIJM₁M₂PlanM_D</i>	This study
AH6433	RN4220 + pCM28	This study
AH6434	RN4220 + pCM28- <i>homABCDJM₁M₂PlanM_D</i>	This study
AH6447	RN4220 + pCM28- <i>homACIM₁PlanM_D</i>	This study
AH6448	AH1178 + pCM28	This study
AH6680	RN4220 + pCM28- <i>homABCDIJM₁M₂P</i>	This study
AH6682	RN4220 + pCM28- <i>homABCIJM₁M₂PlanM_D</i>	This study
AH6730	RN4220 + pCM28- <i>homABCDIJM₂PlanM_D</i>	This study
<i>Staphylococcus epidermidis</i>		
AH4835	Human skin isolate A9	Gallo (UCSD)
<i>Streptococcus pyogenes</i>		
AH4605	MGAS315	6
Plasmids		
pCM28	CamR, AmpR	7
pAN1	pCM28- <i>homABCDIJM₁M₂PlanM_D</i>	This study
pAN2	pCM28- <i>homABCDJM₁M₂PlanM_D</i>	This study
pAN3	pCM28- <i>homACIM₁PlanM_D</i>	This study
pAN4	pCM28- <i>homABCDIJM₂PlanM_D</i>	This study
pAN5	pCM28- <i>homI</i>	This study
pAN6	pCM28- <i>homABCIJM₁M₂PlanM_D</i>	This study
pAN7	pCM28- <i>homABCDIJM₁M₂P</i>	This study

UCSD, University of California, San Diego

Supplementary Table 2. List of primers used in this study.

Gene(s)	Source	Sequence (5' → 3')
<i>homA</i>	<i>S. hominis</i> AH5011	Fwd: CAATTATGTAGCAATTACAGAAGCTG
		Rev: CAGGGTAAACACCATGAGAAATAAT
<i>mupA</i>	<i>S. hominis</i> AH5011	Fwd: GTGATACTCTAGGAGGCTG
		Rev: GGTGTTCCCAATATCTATTT
<i>homBGC_p1</i>	<i>S. hominis</i> AH5011	Fwd: CAGCTATGACCATGATTACGATTGATACGTTATAAACCTATCG
		Rev: AAATATTTGAATATATTCAAAAAAATGTAAAATAATTATACTC
<i>homBGC_p2</i>	<i>S. hominis</i> AH5011	Fwd: TTTTGAATATATTCAAATATTTCTATAATTGACAAATTCTTG
		Rev: TCCAGTATAACAAAAAATAATGGAGGAATTGTTG
<i>homBGC_p3</i>	<i>S. hominis</i> AH5011	Fwd: TTATTTTTTGT TATACTGGATATTATATTATGAGATTGG
		Rev: TTACAATATAATTAATTGTACTATAAATATGTTATATTATATAAAAGG
<i>homBGC_p4</i>	<i>S. hominis</i> AH5011	Fwd: AGTACAATTAATTATATTGTAAATCAACACAAGTTG
		Rev: CCTGCAGGTCGACTCTAGAGCTTAATTTAAATCATATATTAATTAAGATTC
<i>homBGC_p5</i>	<i>S. hominis</i> AH5011	Fwd: GGAAACAGCTATGACCATGATTACGCGTATGATTATACTTTGCAAG
		Rev: GGAGGAATTGTTGAAAATATTATTTTTCTTTCTCTTTACAG
<i>homBGC_p6</i>	<i>S. hominis</i> AH5011	Fwd: AAATAATATTTTCAACAATTCCTCCATTATTTTTTG
		Rev: TTACAATATAATTAATTGTACTATAAATATGTTATATTATATAAAAGG
<i>homBGC_p7</i>	<i>S. hominis</i> AH5011	Fwd: CAGCTATGACCATGATTACGTCAAATATTTCTATAATTGACAAATTCTTG
		Rev: TCCAGTATAACAAAAAATAATGGAGGAATTGTTG
<i>homBGC_p8</i>	<i>S. hominis</i> AH5011	Fwd: CAGCTATGACCATGATTACGATTGATACGTTATAAACCTATCG
		Rev: AATATGGAGATATTCAATGAAAAAATATTAGCGC
<i>homBGC_p9</i>	<i>S. hominis</i> AH5011	Fwd: TCATTGAATATCTCCATATTTCTTAATAGTTCTATTCAATTTTATATTCCG
		Rev: TCCAGTATAACAAAAAATAATGGAGGAATTGTTG
<i>homBGC_p10</i>	<i>S. hominis</i> AH5011	Fwd: TTATTTTTTGT TATACTGGATATTATATTATGAGATTGG
		Rev: ATAATAATTATTTAGTTCCATGATAACCTCC
<i>homBGC_p11</i>	<i>S. hominis</i> AH5011	Fwd: CATGGAACATAATAATTATTATAAGGAGTTAAAAAATGGATG
		Rev: CCTGCAGGTCGACTCTAGAGCTTAATTTAAATCATATATTAATTAAGATTC
<i>homBGC_p14</i>	<i>S. hominis</i> AH5011	Fwd: ATATAACCATAGATCTTTACATCAATCAATTATGTAG
		Rev: CCTGCAGGTCGACTCTAGAGCTTAATTTAAATCATATATTAATTAAGATTC
<i>homBGC_p15</i>	<i>S. hominis</i> AH5011	Fwd: TTATTTTTTGT TATACTGGATATTATATTATGAGATTGG
		Rev: TTGATGTAAAGATCTATGGTTATATTATAGTGTAACCATAG
<i>homI</i>	<i>S. hominis</i> AH5011	Fwd: CTGAGGATCCATTGATACGTTATAAACCTATC
		Rev: GCGCCTGCAGCTTCTCTTCTAAAATATTATTTTT

Supplementary Table 3. Table of publicly available staphylococcal daptide BGC assemblies.

Strain	Species	Accession	Contig of Interest	BioProject	Reference
BR2816	<i>S. agnetis</i>	JALGOD010000000	JALGOD010000002	PRJNA809943	⁸
VB981317	<i>S. aureus</i>	DAPATD010000000	DAPATD010000016	PRJNA514245	N/A
M6K109	<i>S. aureus</i>	BEBO01000000	BEBO01000006	PRJDB5246	⁹
Ani-GT-014	<i>S. cohnii</i>	JAHCOD000000000	JAHCOD010000033	PRJNA609060	¹⁰
GDX8P66P	<i>S. cohnii</i>	JAESMK010000000	JAESMK010000023	PRJNA693170	N/A
ADQBK	<i>S. epidermidis</i>	NZ_CP134841	CP134841	PRJNA546603	¹¹
s19	<i>S. epidermidis</i>	QVTZ01000000	QVTZ01000019	PRJNA224116	N/A
F13	<i>S. felis</i>	QKXS01000000	QKXS01000041	PRJNA475620	¹²
AH5011	<i>S. hominis</i>	JBLOOY000000000	JBLOOY010000003	PRJNA1222585	This study
B32	<i>S. pseudintermedius</i>	JAHNUV000000000	JAHNUV010000022	PRJNA737324	N/A
18SD1	<i>S. pseudintermedius</i>	JARAAJ000000000	JARAAJ010000011	PRJNA934922	¹³
SIG-Dog-TN	<i>S. pseudintermedius</i>	DAGYEO010000000	DAGYEO010000033	PRJNA514245	N/A
17S01341-1	<i>S. pseudintermedius</i>	CAJESU010000000	CAJESU010000004	PRJEB39511	N/A
SNUC 4140	<i>S. warneri</i>	PZFH01000000	PZFH01000036	PRJNA342349	¹⁴
SNUC 2135	<i>S. warneri</i>	PZEZ01000000	PZEZ01000009	PRJNA342349	¹⁴

N/A, not applicable

Supplementary Table 4. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of AH5011 homininicin in methanol- d_3 .

	Pos.	δ_c	Type	δ_H (mult, J in Hz)
Dmle¹	α	72.6	CH	2.99 (d, 9.7)
	β	33.8	CH	1.92 (m)
	γ	25.9	CH_2	1.71 (m), 1.25(m)
	γ'	16.6	CH_3	0.95 (d, 6.7)
	δ	10.7	CH_3	0.92 (t, 7.5)
	NCH_3	42.0	CH_3	2.31 (s)
	NCH_3	42.0	CH_3	2.31 (s)
	C=O	171.9	C	
Dhb²	NH			-
	α	132.8	C	
	β	122.9	CH	5.78 (q, 7.2)
	γ	12.3	CH_3	1.82 (d, 7.1)
	C=O	170.3	C	
Pro³	α	63.1	CH	4.40 (t, 8.4)
	β	30.8	CH_2	2.46 (m), 1.87 (m)
	γ	26.2	CH_2	2.02 (m), 1.96 (m)
	δ	50.3	CH_2	3.60 (m)
	C=O	174.3	C	
Ala⁴	NH			8.49 (br s)
	α	51.0	CH	4.67 (p, 7.5)
	β	17.5	CH_3	1.58 (d, 7.4)
	C=O	175.0	C	
Dhb⁵	NH			9.11 (br s)
	α	132.1	C	
	β	126.3	CH	5.91 (q, 7.2)
	γ	13.3	CH_3	1.77 (d, 7.3)
	C=O	168.8	C	
Pro⁶	α	63.1	CH	4.24 (t, 8.5)
	β	30.5	CH_2	2.14 (m), 1.10 (m)
	γ	26.6	CH_2	2.05 (m), 1.79 (m)
	δ	50.1	CH_2	3.51 (dd, 8.7, 4.6)
	C=O	174.4	C	

Supplementary Table 4. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of AH5011 homininicin in methanol- d_3 (cont).

	Pos.	δ_{C}	Type	δ_{H} (mult, J in Hz)
Phe⁷	NH			8.23 (d, 9.1)
	α	55.9	CH	4.77 (ddd, 13.0, 9.0, 4.0)
	β	37.9	CH ₂	3.44 (dd, 13.0, 4.0), 3.08 (t, 13.0)
	1	138.8	C	
	2/6	130.2	CH	7.36 (br s)
	3/5	129.2	CH	7.36 (br s)
	4	127.8	CH	7.24 (m)
	C=O	172.9	C	
Dhb⁸	NH			9.01 (br s)
	α	132.2	C	
	β	127.4	CH	5.96 (q, 7.1)
	γ	12.7	CH ₃	1.68 (d, 7.2)
	C=O	168.7	C	
Pro⁹	α	64.5	CH	4.31 (m)
	β	30.4	CH ₂	2.40 (m), 1.90 (m)
	γ	26.7	CH ₂	2.05 (m), 1.79 (m)
	δ	50.3	CH ₂	3.60 (m)
	C=O	175.4	C	
Ala¹⁰	NH			8.40 (br s)
	α	50.5	CH	4.15 (m)
	β	15.1	CH ₃	1.46 (d, 7.2)
	C=O	175.2	C	
Ile¹¹	NH			-
	α	62.4	CH	3.81 (m)
	β	36.7	CH	1.94 (m)
	γ	27.5	CH ₂	1.66 (m), 1.13 (m)
	γ'	15.8	CH ₃	0.90 (m)
	δ	11.5	CH ₃	0.83 (t, 7.3)
	C=O	175.9		
Thr¹²	NH			8.36 (br s)
	α	65.7	CH	3.76 (dd, 8.0, 4.4)
	β	67.0	CH	4.29 (m)
	γ	19.9	CH ₃	1.20, (d, 6.3)
	C=O	174.7	C	

Supplementary Table 4. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of AH5011 homininicin in methanol- d_3 (cont).

	Pos.	δ_{C}	Type	δ_{H} (mult, J in Hz)
Glu¹³	NH			-
	α	63.5	CH	3.74, m
	β	-	CH_2	-
	γ	-	CH_2	-
	δ	-	C	-
	C=O	177.5	C	
Ile¹⁴	NH			8.26 (br s)
	α	64.1	CH	3.82 (m)
	β	36.4	CH	2.13 (m)
	γ	27.8	CH_2	1.92 (m), 1.23 (m)
	γ'	15.5	CH_3	0.95 (m)
	δ	11.2	CH_3	0.87 (m)
	C=O	176.0	C	
Dhb¹⁵	NH			9.74 (br s)
	α	132.7	C	
	β	128.0	CH	6.20 (q, 7.0)
	γ	12.5	CH_3	1.75 (d, 7.3)
	C=O	168.7	C	
Ala¹⁶	NH			8.03
	α	53.4	CH	4.13 (m)
	β	16.8	CH_3	1.52 (d, 7.4)
	C=O	176.6	C	
Ala¹⁷	NH			8.12
	α	51.1	CH	4.06 (p, 7.1)
	β	15.4	CH_3	1.44 (d, 7.1)
	C=O	175.8	C	
Val¹⁸	NH			8.17
	α	64.3	CH	3.69 (dd, 9.4, 5.6)
	β	30.4	CH	2.20 (m)
	γ	20.3	CH_3	0.97 (d, 6.5)
	γ'	19.6	CH_3	0.91 (d, 6.6)
	C=O	176.1	C	

Supplementary Table 4. ^1H (700 MHz) and ^{13}C (175 MHz) NMR spectroscopic data of AH5011 homininicin in methanol- d_3 (cont).

	Pos.	δ_{C}	Type	δ_{H} (mult, J in Hz)
Ile¹⁹	NH			8.26 (br s)
	α	64.1	CH	3.82 (m)
	β	36.4	CH	2.12 (m)
	γ	27.8	CH_2	1.92 (m), 1.23 (m)
	γ'	15.5	CH_3	0.96 (d, 6.7)
	δ	11.2	CH_3	0.87 (t, 7.7)
	C=O	176.4	C	
Ala²⁰	NH			8.34 (br s)
	α	52.4	CH	4.20 (q, 6.8)
	β	17.0	CH_3	1.44 (d, 7.3)
	C=O	176.3	C	
Dmp²¹	NH			7.80 (br s)
	α	41.2	CH_2	3.74 (m), 3.30 (m)
	β	61.8	CH	3.56 (m)
	γ	11.1	CH_3	1.25 (d, 6.7)
	NCH_3	40.4	CH_3	2.74 (s)
	NCH_3	40.4	CH_3	2.74 (s)

Chemical shifts (δ) are reported in ppm. Assignments are provided for each position with corresponding carbon and proton chemical shifts, multiplicities, and coupling constant (J) values.

Supplementary Table 5. Locus tags for the *S. hominis* AH5011 BGC

Gene Name	Function	Pfam ID	Locus tags
HomI	Immunity protein	N/A	ACM66P_10905
HomJ	Oxidoreductase	PF03358	ACM66P_10910
HomD	Aminotransferase	PF00202	ACM66P_10915
HomM ₂	Methyltransferase	PF13649	ACM66P_10920
HomB	DUF-RRE fusion protein	N/A	ACM66P_10925
HomC	Oxidative decarboxylase	PF13561	ACM66P_10930
HomM ₁	Methyltransferase	PF13649	ACM66P_10935
HomA	Precursor peptide	N/A	ACM66P_10940
LanM _D	Lanthipeptide dehydratase	PF13575	ACM66P_10945
HomP	Peptidase	PF00082	ACM66P_10950

N/A, not applicable

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