

Table S1: Individual absorbance values and percent PrestoBlue reduction* of 1-day, 4-day, and 7-day uninfected explants.

Explant	570 nm	600 nm	PrestoBlue reduction (%)*
1-day explants			
A1	1.5	0.334	73.73
A2	1.552	0.386	74.697
A3	1.475	0.487	66.216
B1	1.499	0.497	67.207
B2	1.498	0.501	66.99
B3	1.548	0.542	68.233
C1	1.494	0.422	69.877
C2	1.499	0.427	69.969
C3	1.529	0.521	67.972
4-day explants			
A4	1.331	0.404	61.713
A5	1.439	0.464	65.639
A6	1.388	0.399	65.266
B4	1.248	0.371	58.163
B5	1.532	0.51	69.268
B6	1.22	0.489	51.78
C4	1.314	0.401	60.883
C5	1.341	0.416	61.808
C6	1.105	0.337	51.164
7-day explants			
A7	1.271	0.952	35.792
A8	1.302	0.946	37.843
A9	1.223	0.872	36.229
B7	1.287	0.884	39.444
B8	1.207	0.981	30.966
B9	1.245	0.976	33.372
C7	1.214	0.819	37.812
C8	1.271	1	33.882
C9	1.251	0.89	37.11

*Percent dye reduction refers to chemical reduction of PrestoBlue, a resazurin-based chemical reagent

Table S2: Fold accumulation¹ of porcine-origin proteins in media from biofilm-infected relative to biofilm-free explants.

Uniport identification	Description	Fold (log ₂) accumulation ²
F1S585	Uncharacterized protein	11.63 ± 0.1
I3LQJ0	Uncharacterized protein	11.63 ± 2.07
I3L5Z3	Uncharacterized protein	11.24 ± 0.99
F1SM78	Uncharacterized protein	11.15 ± 0.61
MYL9	Myosin regulatory light polypeptide 9	10.78 ± 0.88
F1SB42	Uncharacterized protein	10.6 ± 0.15
F1RNX2	Programmed cell death protein 5	10.41 ± 0.01
F1SSF7	Uncharacterized protein	10.27 ± 2.27
D0G7F7	Tropomyosin 4	9.82 ± 2.94
I3LTH8	Uncharacterized protein	9.69 ± 2.53
F1RQR4	Uncharacterized protein	9.55 ± 1.93
F1STR9	Uncharacterized protein	9.35 ± 2.03
F1S3M9	Uncharacterized protein	8.81 ± 0.32
F1RKG9	Uncharacterized protein	8.79 ± 2.66
LIMA1	LIM domain and actin-binding protein 1	8.38 ± 1.92
K7GNZ3	Uncharacterized protein	7.64 ± 2.33
K9IWG9	Tensin-1	7.54 ± 1.84
Q2XVP5	Microtubule-associated protein RP/EB family member 1	7.52 ± 2.05
F1SGD7	Uncharacterized protein	7.51 ± 3.75
B3F0B7	Cellular retinoic acid binding protein	7.36 ± 3.25
F1SG00	Uncharacterized protein	7.27 ± 0.96
A9ED96	CARG-binding factor A	6.53 ± 3.12
F1SC51	Uncharacterized protein	6.46 ± 1.6
COF1	Cofilin-1	6.46 ± 3.41
I3LU55	Uncharacterized protein	6.29 ± 2.34
A3EX84	Lectin galactoside-binding soluble 3	6.01 ± 2.72
D0G778	Macrophage migration inhibitory factor	5.85 ± 2.01
B5L0Y3	Calpastatin	4.56 ± 1.86
F1RS37	Uncharacterized protein	4.41 ± 2.1
F1SH92	Inter-alpha-trypsin inhibitor heavy chain H4	4.08 ± 0.51
Q19KE2	Fatty acid binding protein 5	4.02 ± 1.42
CAH3	Carbonic anhydrase 3	3.95 ± 0.73
F1RII7	Hemoglobin subunit beta	3.71 ± 0.33
I3L5D5	Uncharacterized protein	3.64 ± 0.15
I3LUC8	Uncharacterized protein	3.62 ± 0.64
F1SJS8	Uncharacterized protein	3.57 ± 0.7
I3LQS0	Uncharacterized protein	3.4 ± 0.86
I3LD72	Uncharacterized protein	3.35 ± 1.05
Q5W7K7	Interleukin 6	3.27 ± 2.31
F1RLQ2	Prelamin-A/C	3.26 ± 0.55
F2Z5B6	Tropomyosin alpha-1 chain	3.17 ± 0.62
F1S827	Uncharacterized protein	3.16 ± 0.75
F1SQW8	Uncharacterized protein	2.95 ± 0.64
F1SQS2	Uncharacterized protein	2.95 ± 1.16
B6DX83	V-crk sarcoma virus CT10 oncogene-like protein isoform b	2.52 ± 0.55
F1RJ93	Uncharacterized protein	2.15 ± 1.12
VIME	Vimentin	1.18 ± 0.53
F1SMV6	Uncharacterized protein	0.68 ± 0.24
F1S021	Uncharacterized protein	-1.33 ± 0.45
CFAD	Complement factor D	-1.59 ± 0.72
F1S285	Uncharacterized protein	-1.6 ± 0.61
I3LRH7	Uncharacterized protein	-1.64 ± 0.59
F1SRS2	Uncharacterized protein	-1.78 ± 0.56
A7U5U2	Ubiquitin B	-1.8 ± 0.03
I3LEE6	Uncharacterized protein	-1.84 ± 0.26

G8FUN5	Y box binding protein 1	-1.95 ± 0.22
A8D737	T-cadherin	-2.24 ± 1.18
E2I6L6	Insulin-like growth factor-binding protein 5	-2.69 ± 1.25
Q00P28	Beta-2-microglobulin protein	-2.74 ± 0.69
F1SS26	Uncharacterized protein	-2.9 ± 0.89
F1S9V5	Uncharacterized protein	-2.98 ± 0.69
F1S895	Uncharacterized protein	-2.99 ± 1.81
K7GKR9	Uncharacterized protein	-2.99 ± 0.33
I3LLI8	Uncharacterized protein	-3 ± 1.44
G8ENL4	FUS	-3.5 ± 0.74
MOES	Moesin	-3.63 ± 1.1
F1RYI8	Uncharacterized protein	-3.78 ± 0.17
I3LJX2	Uncharacterized protein	-3.82 ± 0.31
I3LS72	Uncharacterized protein	-3.86 ± 1.01
I3LC64	Uncharacterized protein	-3.89 ± 1.26
I3LLD8	Uncharacterized protein	-4.99 ± 0.23
F1S4Y8	Uncharacterized protein	-5.18 ± 0.44
RNAS4	Ribonuclease 4	-6.11 ± 1.9
I3LI44	Uncharacterized protein	-10.53 ± 1.42
I3LPZ3	Uncharacterized protein	-11.22 ± 1.17
F1RT61	Uncharacterized protein	-11.93 ± 0.1
L8B0W9	IgG heavy chain	-15.19 ± 5.05

¹Comparisons were made using t-test (n=6, $P < 0.05$) and changes in protein content were considered significant at a false discovery rate of less than 1%

²"-" sign preceding a number indicates fold decrease in a protein from biofilm-infected relative to biofilm-free explants

Table S3: Abundance¹ of biofilm-origin proteins in media from *S. aureus*-infected explants.

UNIPROT identification	Description	Average abundance (log ₂) ²
N6T2M0	Chaperone dnaK	6.44 ± 3.24
H4DA51	Aspartate-semialdehyde dehydrogenase	7.09 ± 2.55
N5GCT2	Enolase	7.28 ± 3.71
H3YUB5	Triacylglycerol lipase	7.83 ± 3.92
N5WBC0	Succinyl-CoA ligase [ADP-forming] subunit beta	8.21 ± 0.84
N5HZJ4	Alanine dehydrogenase 1	8.65 ± 1.14
N4ZRD9	Transaldolase	8.89 ± 0.2
G8V0S2	Heme uptake protein IsdB	8.95 ± 0.93
N5W0V3	GNAT family acetyltransferase	9.14 ± 0.3
G8RED4	Virulence-associated cell-wall-anchored protein SasG	9.34 ± 1.66
N6RJM9	50S ribosomal protein L9	9.52 ± 1.51
N5E603	Nitrogen fixation protein NifU	9.53 ± 0.16
D7RHB1	SpA IgG-binding domain protein	9.74 ± 1.05
N5IN79	Uncharacterized protein	10.02 ± 0.5
Q9L4P0	Transketolase, putative	10.09 ± 0.87
C8MLC3	Glucosamine-6-phosphate isomerase	10.18 ± 0.27
Q7WRR9	FmtB-like protein	10.19 ± 0.74
N5PDC8	2'-5' RNA ligase	10.28 ± 1.04
N4Z3Q4	Uncharacterized protein	10.39 ± 1.21
H4HHJ2	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C	10.75 ± 0.9
H4H4R5	Putative uncharacterized protein	10.77 ± 0.77
ADH	Alcohol dehydrogenase	10.83 ± 1.56
E7MUZ4	Beta-channel forming cytolysin	10.89 ± 0.53
H3ZWG4	30S ribosomal protein S8	11.05 ± 0.34
K0LN55	60 kDa chaperonin	11.08 ± 0.22
C8LF75	Virulence factor esxA	11.1 ± 1.33
H1THJ5	Elastin-binding protein EbpS	11.18 ± 0.31
N5HT19	Elongation factor Ts	11.2 ± 0.54
D2FA31	Nickel ABC transporter, periplasmic nickel-binding protein	11.22 ± 0.39
N5C0S7	Membrane protein	11.27 ± 0.46
H0AFU2	Putative uncharacterized protein	11.32 ± 0.71
N5Y0M3	ATP-dependent Clp protease proteolytic subunit	11.35 ± 0.4
N5W6U5	Pyruvate kinase	11.52 ± 0.78
H3YQ62	PF09954 family protein	11.56 ± 0.15
H3RZY6	Putative uncharacterized protein	11.57 ± 0.53
F3T8G3	Phosphoglycerate kinase	11.6 ± 0.72
H4C116	Lipase	11.63 ± 0.25
N6EWC2	Seryl-tRNA synthetase	11.67 ± 0.14
N5V6P8	50S ribosomal protein L29	11.73 ± 0.19
N6Q9Q5	L-lactate dehydrogenase 2	11.75 ± 0.09
N5XFM1	50S ribosomal protein L11	11.75 ± 0.55
N6IZH4	50S ribosomal protein L17	12.02 ± 0.33
LTAS	Lipoteichoic acid synthase	12.36 ± 0.56
N5Y3G8	Peptidyl-prolyl cis-trans isomerase B (Cyclophilin B)	12.43 ± 0.88
N6QHD8	General stress response protein CsbD	12.49 ± 0.75
N5G4G5	Succinyl-CoA ligase [ADP-forming] subunit alpha	12.55 ± 0.29
H4AWF5	30S ribosomal protein S13	12.57 ± 0.11
H4EKE4	Iron-regulated ABC transporter siderophore-binding protein SirA	12.58 ± 0.1
H4BX29	30S ribosomal protein S7	12.74 ± 0.48
F3TLA6	1-pyrroline-5-carboxylate dehydrogenase	12.9 ± 0.58
N5X4S3	50S ribosomal protein L5	12.99 ± 0.37
FTSZ	Cell division protein FtsZ	13.01 ± 0.81
N4Z0Y4	Transcription elongation factor greA	13.33 ± 0.49
H0CTB1	Putative uncharacterized protein	13.39 ± 0.27
N6AC37	30S ribosomal protein S10	13.43 ± 0.12
RL31B	50S ribosomal protein L31 type B	13.45 ± 0.18
N6IKU5	Thioredoxin	13.47 ± 0.51
N6PXS9	Uncharacterized protein	13.58 ± 0.58
N5EN69	50S ribosomal protein L21	13.68 ± 0.47
N5JJD4	50S ribosomal protein L10	13.72 ± 0.15
R9D9I9	PTS system, glucose-specific enzyme II, A component	13.76 ± 0.49
D2N540	Manganese ABC transporter substrate-binding lipoprotein (Adhesin B) (S	13.82 ± 1.35
H1TCG7	N-acetylmuramoyl-L-alanine amidase Sle1	13.87 ± 0.32

N1N515	N-acetylmuramoyl-L-alanine amidase, family 4	13.96 ± 0.43
I0JAT6	LPXTG surface protein	14.15 ± 0.61
H3X8A1	Putative uncharacterized protein	14.16 ± 0.34
N6RCZ1	Lipoprotein	14.43 ± 0.45
N5YZX5	Stage V sporulation protein G	14.46 ± 0.11
FIB	Fibrinogen-binding protein	14.47 ± 0.57
H4A334	Glyceraldehyde-3-phosphate dehydrogenase, type I	14.52 ± 0.66
N5ENW5	Elongation factor G	14.57 ± 0.05
H3YFB8	Putative transglycosylase IsaA	14.83 ± 0.44
F0D2G6	Stress response protein	14.87 ± 0.08
N6HVU9	Complement inhibitor SCIN	14.91 ± 0.12
N4Y4Z6	Ribosome-recycling factor	15.01 ± 0.1
L7C3X1	Alkaline shock protein 23	15.05 ± 0.3
Q7A060	SsaA protein	15.1 ± 0.39
N5ADM6	Translation initiation factor IF-1	15.18 ± 0.29
N6LPZ1	Serine-aspartate repeat-containing protein E	15.23 ± 0.17
N6SJN3	UPF0342 protein	15.26 ± 0.26
H4B1P1	Cold shock protein CspA	15.48 ± 0.8
H4E902	Putative exported protein	15.53 ± 0.31
RL7	50S ribosomal protein L7/L12	15.54 ± 0.38
I3F8X4	DNA-binding protein HU	15.54 ± 0.45
H3X3W1	Trigger factor	15.75 ± 0.3
C8KRJ1	Putative uncharacterized protein	15.88 ± 0.09
H4E5E4	Glycerophosphoryl diester phosphodiesterase	16.05 ± 0.47
N5WXZ3	Immunoglobulin G-binding protein A	16.06 ± 0.29
N5F0W7	Thermonuclease	16.48 ± 0.43
RS6	30S ribosomal protein S6	16.56 ± 0.23
D6T6U9	Putative uncharacterized protein	16.62 ± 0.41
N6FXZ1	30S ribosomal protein S1	16.65 ± 0.18
D4U986	Elongation factor Tu	16.78 ± 0.19
N4Z8G9	Uncharacterized protein	17.32 ± 0.29
N5QIW4	Fructose-bisphosphate aldolase class 1	17.33 ± 0.5
L7C4C8	Bifunctional autolysin	17.46 ± 0.11

¹Protein abundance is calculated from sum of all unique normalized-peptide- ion abundances representing a protein in a sample.

²The average protein abundance (± SE) was calculated from three biological replicates.

Table S4: Likely role of some *S. aureus* biofilm-origin soluble proteins on dermal explants.

UNIPROT identification	Description	Average abundance (log ₂) ¹	Likely role in dermal tissue
N5HZJ4	Alanine dehydrogenase 1	8.65 ± 1.14	This enzyme catalyzes alanine to pyruvate, NADH and ammonium ion (1) and could contribute to increase in pH in dermal explants. Alanine is essential amino acid for <i>S. aureus</i> growth (2).
G8V0S2	Heme uptake protein IsdB	8.95 ± 0.93	Hemoglobin is present in dermal explants. <i>S. aureus</i> can use IsdB to acquire iron in dermal tissue
D7RHB1	SpA IgG-binding domain protein	9.74 ± 1.05	Very low amounts of IgG were found in media from infected explants. This strongly suggests that SpA binds and traps IgG in tissue matrix.
C8MLC3	Glucosamine-6-phosphate isomerase	10.18 ± 0.27	This enzyme converts glucosamine-6-phosphate to fructose-6-phosphate and urea (3). Hyaluronic acid as an extracellular matrix protein is rich in glucosamine (4). The production of ammonia by this enzyme could contribute to high pH seen in dermal tissue
E7MUZ4	Beta-channel forming cytolysin	10.89 ± 0.53	Possible involvement in killing of cells in dermal tissue
H1THJ5	Elastin-binding protein EbpS	11.18 ± 0.31	Elastin is abundant in dermis (4). <i>S. aureus</i> can bind elastin and other extracellular matrix proteins (5).
N5Y0M3	ATP-dependent Clp protease proteolytic subunit	11.35 ± 0.4	This enzyme uses ATP to cleave damaged proteins within bacterial cells and is highly expressed by <i>S. aureus</i> biofilms (6)
N6Q9Q5	L-lactate dehydrogenase 2	11.75 ± 0.09	Converts pyruvate to lactate and vice versa (4). The bacterial LDH can contribute to higher activity of LDH activity seen in spent media from dermal tissue
F3TLA6	1-pyrroline-5-carboxylate dehydrogenase	12.9 ± 0.58	This enzyme catabolizes proline for energy generation (7). <i>S. aureus</i> likely consumes proline as an energy source. Proline is a major constituent of collagen found in abundance in dermis (4) and is essential amino acid for <i>S. aureus</i> growth (2).
N1N515	N-acetylmuramoyl-L-alanine amidase, family 4	13.96 ± 0.43	Induces autolysis (8). Possible role in explant biofilm autolysis
I0JAT6	LPXTG surface protein	14.15 ± 0.61	Surface proteins with LPXTG sequence help <i>S. aureus</i> bind extracellular cell matrix proteins (9). <i>S. aureus</i> binds tightly to dermal tissue
FIB	Fibrinogen-binding protein	14.47 ± 0.57	<i>S. aureus</i> could use this protein to bind fibrinogen in dermal tissue
L7C3X1	Alkaline shock protein	15.05 ± 0.3	This protein is highly expressed under alkaline conditions (10). <i>S. aureus</i> -infected dermal tissue had higher pH
Q7A060	SsaA protein	15.1 ± 0.39	This protein is an endopeptidase (11) and may have a role in dermal biofilm bacterial lysis
N5F0W7	Thermonuclease	16.48 ± 0.43	This enzyme degrades DNA (12) and might contribute to DNA degradation in dermal cells
L7C4C8	Bifunctional autolysin	17.46 ± 0.11	This protein hydrolyzes peptidoglycan (13). In addition to its role in cell division, this enzyme could lyse bacterial cells in biofilms. Bacterial cell lysis was observed in dermal biofilms.

¹Protein abundance was calculated from the sum of all unique normalized-peptide- ion abundances of a protein in a sample, and the average protein abundance (± S.E.) was calculated from three different biological replicates.

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