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Supplementary Materials for

Quorum sensing between bacterial species on the skin protects against epidermal injury in atopic dermatitis

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Other Supplementary Material for this manuscript includes the following:

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Materials and Methods

PSMα3 protein expression

A *S. aureus* PSMα3 affinity purified rabbit polyclonal antibody (Rb10904) was developed (LifeTein) for this study and used at a 1:1000 dilution for immunoblotting and 1:100 dilution for immunofluorescence. For immunoblotting, sterile-filtered *S. aureus* supernatant was heated for 7min at 95°C with 1x Laemmli sample buffer (Bio-Rad) containing 1% β-mercaptoethanol prior to running on 16% Tricine pre-cast gels (Novex), transferred to 0.22μm PVDF membranes (Bio-Rad) using a Trans-blot Turbo Transfer System (Bio-Rad), blocked for 1h at RT in 1x Odyssey blocking solution containing 0.1% Tween-20 (LI-COR), and stained overnight at 4°C with the primary antibody above. Odyssey (LI-COR) secondary goat anti-rabbit 488nm antibody was applied to membranes for 1h at RT on an orbital shaker after 3x PBST (PBS with 0.1% Tween-20) washes. 3x additional PBST washes were applied before analysis on an infrared imager (LI-COR). For immunoflourescence, AD lesional skin 4mm punch biopsies were incubated for 1h in 4% PFA followed by overnight 15% and 30% sucrose dehydration steps. Biopsies frozen in OCT were sectioned (10μm) followed by an additional 20min 4% PFA fixation, 3x 5min PBS washes, followed by a blocking/permeabilization step for 1h at room temperature (PBS in 3% Triton X-100, 2% BSA, and 2% FBS). The PSMα3 antibody was applied overnight at 4°C in antibody solution (PBS with 0.03% Triton X-100, 2% BSA, and 2% FBS) followed by 3x 10min washes in blocking buffer, application of secondary donkey anti-rabbit Alexa Fluor 488 (1:1000 dilution) antibody for 1h at room temperature in the dark in blocking buffer, and an additional 3x washes as above. Tissue sections were mounted with ProLong Gold Antifade with DAPI (Life Technologies) and a cover slide. Fluorescent signal was measured using an Olympus BX51 fluorescent microscope.

Generation of RP62A competent cells and transformation

Electro-competent RP62A cells were prepared as previously described (*44, 45*). Briefly, an overnight culture of *S. epidermidis* RP62A was diluted to an OD600nm of 0.5 in pre-warmed Brain Heart Infusion (BHI) broth, incubated for an additional 30 min at 37°C with shaking, transferred to centrifuge tubes and then chilled on ice for 10min. Cells were harvested by centrifugation (10min, 4000RPM, 4°C), washed serially with 1 volume, 1/10 volume and then 1/25 volume of cold autoclaved water followed by re-pelleting at 4°C after each wash. After the final wash, cells were re-suspended in 1/200 volume of cold 10% sterile glycerol and aliquoted at 50μL into tubes for storage at -80°C. Transformation of *S. epidermidis* RP62A was carried out as previously described (*54*). Briefly, frozen competent cells were thawed on ice for 5min and then at RT for 5min. Thawed cells were briefly centrifuged (1min, 5000g, RT) and the pellet was re-suspended in 50μL of 10% glycerol supplemented with 500mM sucrose. After addition of DNA, cells were transferred to a 1mm cuvette and pulsed on a Micropulser (Bio-Rad) at 2.1kV with a time constant of 1.1msec. Immediately after electroporation, cells were re-suspended in 1mL of BHI broth supplemented with 500mM sucrose, shaken for 1hr at 30°C and then plated on BHI agar with 10μg/mL chloramphenicol (Cm) at 30°C.

Allelic replacement of *S. epidermidis* RP62A autoinducing peptide

The allelic replacement plasmid pMAD (*46*) was used to selectively generate an in-frame deletion of the autoinducing peptide (AIP) coding sequence of *agrD* in *S. epidermidis* RP62A. Briefly, approximately 1000bp fragments upstream and downstream of the AIP sequence of RP62A were amplified by PCR and joined by gene splicing by overlap extension or 'SOEing'. The sewn fragments and pMAD vector were digested with BamHI and SalI, ligated together by T4 DNA ligase (New England Biolabs) and subsequently used to chemically transform the *S. epidermidis* clonal complex 10 plasmid artificial

modification *E. coli* strain, DC10B-CC10. Transformants were plated on LB with 100μg/mL Amp and 30μg/mL Cm at 37°C. Correct transformants were validated by restriction digest and sequencing. The verified construct was annotated as pMAD:: ΔAIP. Electro-competent RP62A was then transformed with ~5μg of pMAD:: ΔAIP derived from DC10B-CC10 and then plated on BHI agar with 10μg/mL Cm and 50μL of 40 mg/mL 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) at 30°C. A single blue colony was selected and grown in BHI with 10μg/mL Cm overnight at 30°C. The overnight culture was then diluted 1:100 (for final volume of 100mL) into fresh, pre-warmed, BHI without antibiotics and incubated for 24hrs at 43°C. The dilution and growth at 43°C was repeated an additional time to promote the single crossover event by selecting for light blue colonies grown on BHI agar supplemented with 10μg/mL Cm and 50μL of 40mg/mL X-Gal at 43°C. A light blue colony was selected and incubated in BHI without antibiotics overnight at 30°C to promote the double crossover event. Dilutions of this overnight were plated on BHI agar supplemented with 50μL of 40mg/mL X-Gal and incubated overnight at 37°C. White colonies were selected and patched on BHI agar supplemented with either 10μg/mL Cm or 50μL of 40 mg/mL X-Gal. Colonies that failed to grow in the presence of Cm and remained white in the presence of X-Gal were selected and screened for deletion of the AIP coding sequence by sequencing. The verified mutant strain was annotated as *S. epidermidis* RP62A ΔAIP.

Genome sequencing and assembly

Coagulase-negative staphylococci (CoNS) genomic DNA was isolated using the DNeasy UltraClean Microbial Kit (Qiagen). The libraries were sequenced using the MiSeq platform (Illumina) for two cycles, generating 2x250bp paired-end reads. Adapters were removed using cutadapt (version 1.9.1) (http://cutadapt.readthedocs.io/en/stable/). Low-quality sequences (quality score <30) were removed

using the Trim Galore (version 1.9.1) (https://www.bio informatics.babraham.ac.uk/projects/trim _galore/) with default parameters. Sequences mapping to the human genome were removed from the quality-trimmed dataset using the Bowtie2 (version 2.2.8) (*47*) with the following parameters (-D 20 -R 3 -N 1 -L 20 --very-sensitive-local) and the human reference genome hg19 (UCSC Genome Browser). The filtered reads were de novo assembled using SPAdes (version 3.8.0) (*48*) with k-mer length ranging from 33 to 127. The genomes were annotated with rapid annotation of microbial genomes using subsystems technology (RAST) with default parameters (*49*). Amino acid sequences from annotated CDS (coding DNA sequence) were aligned to bacterial *agr* proteins obtained from Uniprot database (downloaded in October 2017). *Agr* genes from the assembled genome were identified following three criteria: i) sequence identity > 60%; ii) e-value < e100; and iii) the *agr* locus organization, an operon of four genes, *agr*BDCA *(50)*.

RNA isolation and quantitative real-time PCR

All RNA was isolated using the Purelink RNA isolation kit according to manufacturer's instructions (Thermo Fisher Scientific). For NHEKs, 350µL RNA lysis buffer (with 1% β-mercaptoethanol) was added directly to cells. For mouse tissue, 0.5cm^2 full-thickness skin was bead beat (2x30sec, 2.0mm zirconia bead) in 750µL of RNA lysis buffer with 5min on ice in between. Tissue was than centrifuged (10min, 13,000 RPM, 4°C), followed by adding 350µL of clear lysate to 70% EtOH and column-based isolation of RNA. For *S. aureus* RNA isolation, 1e^8 CFU bacteria was incubated with a 2:1 ratio of RNAprotect (Qiagen) for 10min prior to centrifugation (10min, 13,000RPM, RT), re-suspension in 750µL of RNA lysis buffer, and beading beating (2x 1min 6.5 speed) using lysis matrix B tubes and a Fastprep-24 (MP Biomedicals). Samples were than centrifuged again and 350µL of clear lysate was added to 70% EtOH as above. Isolation of human skin swab RNA was performed similarly to bacteria

RNA isolation except skins swabs were vortexed for 30s followed by incubation of 250µL with RNAprotect reagent. After RNA isolation, samples were quantified with a Nanodrop (ThermoFisher Scientific), and 500ng of RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad). qPCR reactions were run on a CFX96 Real-Time Detection System (Bio-Rad). For mammalian cells, gene-specific primers and TaqMan probes (Thermo Fisher Scientific) were used, with *GAPDH* as a housekeeping gene. For bacterial RNA, Sybr Green 2x master mix (Bimake) was used along with specific primers as indicated in table S2.

RNA-seq

RNA was submitted to the University of California, San Diego (UCSD) genomic core. TruSeq mRNA Library Prep Kit (Illumina) was used for library preparation followed by high-throughput sequencing on a HiSeq 2500 sequencer (Illumina). Data were analyzed using Partek Flow and Partek Genomics Suite software using default settings, and gene ontology analysis was performed using the PANTHER classification system (http://pantherdb.org).

Histology

Full-thickness murine skin (0.5cm^2) was collected, fixed in paraformaldehyde (4%), and washed in PBS prior to overnight incubation with 15% and 30% sucrose prior to freezing tissue in OCT mounting medium with dry ice. Cryostat cut sections (10µm) were mounted onto Superfrost Plus glass slides (Fisher Scientific) and stained with hematoxylin and eosin (H&E). Sections were incubated for 5min intervals in an EtOH gradient of 75%-100% prior to xylene incubation and mounting with permount on glass slide. Pictures were taken on an Olympus BX51 fluorescent microscope at a 200x magnification.

Epidermal thickness determined using Image J Software by measuring 5 sites per section for each mouse per condition (n=6).

Cytokine level determination

Conditioned medium from NHEKs (25μL) were used to quantify protein concentration of various cytokines. Magnetic bead-based milliplex assay kits (Millipore) for the human cytokines IL-6 and TNFα were used according to manufacturer instructions on a Magpix 200 (Luminex) system. Human IL-1α was quantified by ELISA (R&D Systems).

***S. aureus agr* multiplex PCR**

S. aureus (SA) genotyping of the *agr* locus was performed as previously (*51*). Briefly, gDNA from SA USA300 LAC or 11 SA AD isolates was harvested using the Purelink microbiome DNA purification kit (Thermofisher). 1μL of bacterial gDNA was used for PCR amplification in a C1000 Touch Thermocycler (Bio-rad) using a Q5 high fidelity polymerase (NEB) and the primers and settings as referenced above followed by running samples on a 1.5% agarose gel and visualization using transillumination under UV for determination of amplified *agr* type I-IV products of unique sizes.

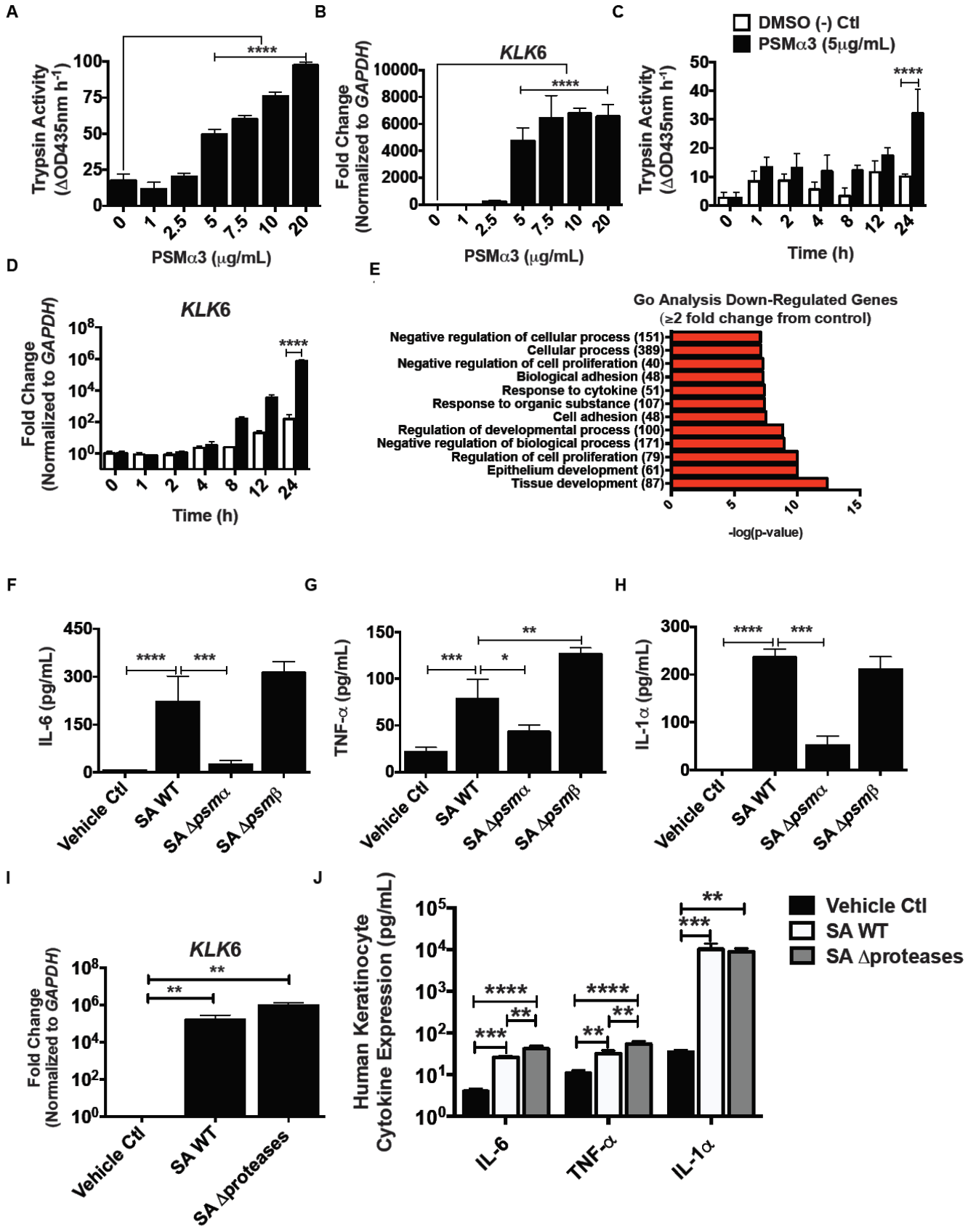

A
Trypsin Activity (ΔOD435nm h-1)
PSMα3 (μg/mL)
B
KLK6
Fold Change (Normalized to GAPDH)
PSMα3 (μg/mL)
C
DMSO (-) Ctl
PSMα3 (5μg/mL)
Trypsin Activity (ΔOD435nm h-1)
Time (h)
D
KLK6
Fold Change (Normalized to GAPDH)
Time (h)
E
Go Analysis Down-Regulated Genes
(≥2 fold change from control)
Negative regulation of cellular process (151)
Cellular process (389)
Negative regulation of cell proliferation (40)
Biological adhesion (48)
Response to cytokine (51)
Response to organic substance (107)
Cell adhesion (48)
Regulation of developmental process (100)
Negative regulation of biological process (171)
Regulation of cell proliferation (79)
Epithelium development (61)
Tissue development (87)
-log(p-value)
F
IL-6 (pg/mL)
Vehicle Ctl
SA WT
SA Δpsmα
SA Δpsmβ
G
TNF-α (pg/mL)
H
IL-1α (pg/mL)
I
KLK6
Fold Change (Normalized to GAPDH)
SA Δproteases
J
Human Keratinocyte Cytokine Expression (pg/mL)
Vehicle Ctl
SA WT
SA Δproteases
IL-6
TNF-α
IL-1α

Fig. S1. *S. aureus* PSMα changes essential barrier gene and cytokine expression in human keratinocytes. (A-D) Human keratinocytes treated with synthetic PSMα3 were assessed for changes in trypsin activity and *KLK*6 transcript expression normalized to the housekeeping gene *GAPDH* in both a dose and time dependent manner (n=4). **(E)** GO-term analysis of genes down-regulated ≥2 fold from the control in human keratinocytes treated with PSMα3 (5μg-mL) for 24h. **(F-H)** Changes in human keratinocyte cytokine protein expression of IL-6, TNF-α, or IL-1α treated with 5% supernatant from an overnight culture ($1e^9$ CFU) of *S. aureus* (SA) USA300 LAC wild-type (WT) or corresponding SA phenol-soluble modulin alpha (Δ*psm*α) and SA phenol-soluble modulin beta (Δ*psm*β) knockout strain supernatants for 24h. **(I, J)** Similarly to "F-H" NHEKs were treated for 24h with 5% sterile-filtered supernatant from an overnight culture ($1e^9$ CFU) of SA USA300 LAC WT (AH1263) and corresponding SA protease (Δproteases) knockout supernatant followed by analysis of *KLK*6 expression normalized to *GAPDH* and cytokine protease expression of IL-6, TNF-α, or IL-1α (n=4). Error bars are standard error of the mean (SEM) and representative of 3 independent experiments. One-way ANOVA was used to determine statistical significance: $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***, $p<0.0001$ ****.

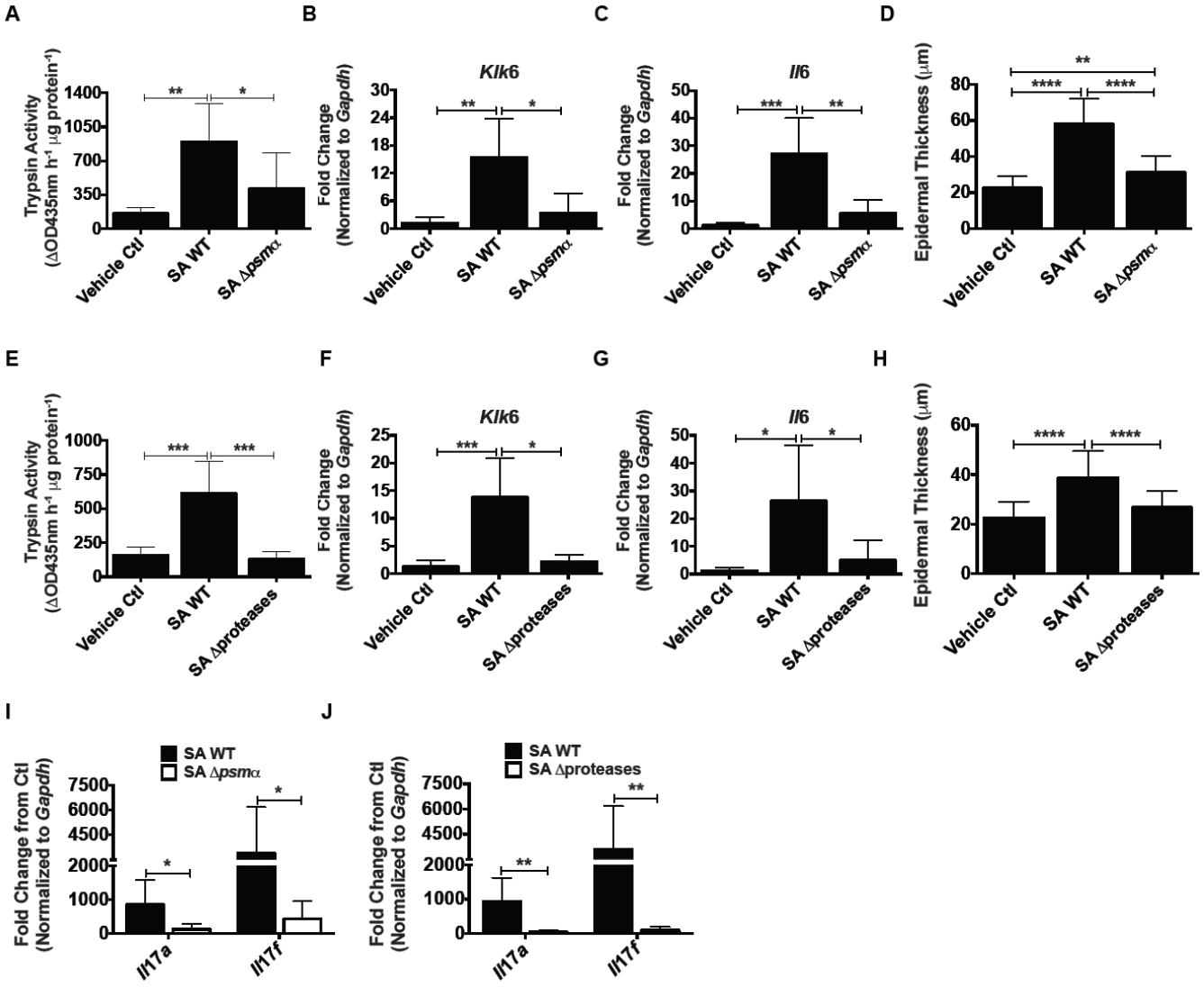


Fig. S2. *S. aureus* PSMα and proteases are responsible for barrier damage and induction of inflammation on murine skin. *S. aureus* (SA) USA300 LAC wild-type (WT) and corresponding SA phenol-soluble modulin alpha (Δ*psmα*) knockout, or SA USA300 LAC WT (AH1263) and corresponding SA protease (Δproteases) knockout strains were applied ($1e^7$ CFU) to male murine back skin for 72h (n=6) and changes in **(A,E)** trypsin activity, **(B,F)** *Klk6*, **(C,G)** *Il6*, **(D,H)** epidermal thickness, and **(I,J)** *Il17a/f* mRNA expression normalized to the housekeeping gene *Gapdh* were measured. Bacterial growth medium only was used as a vehicle control. Data are representative of 3 independent experiments. Error bars are standard error of the mean (SEM). One-way ANOVA was used to determine statistical significance: $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***, $p<0.0001$ ****.

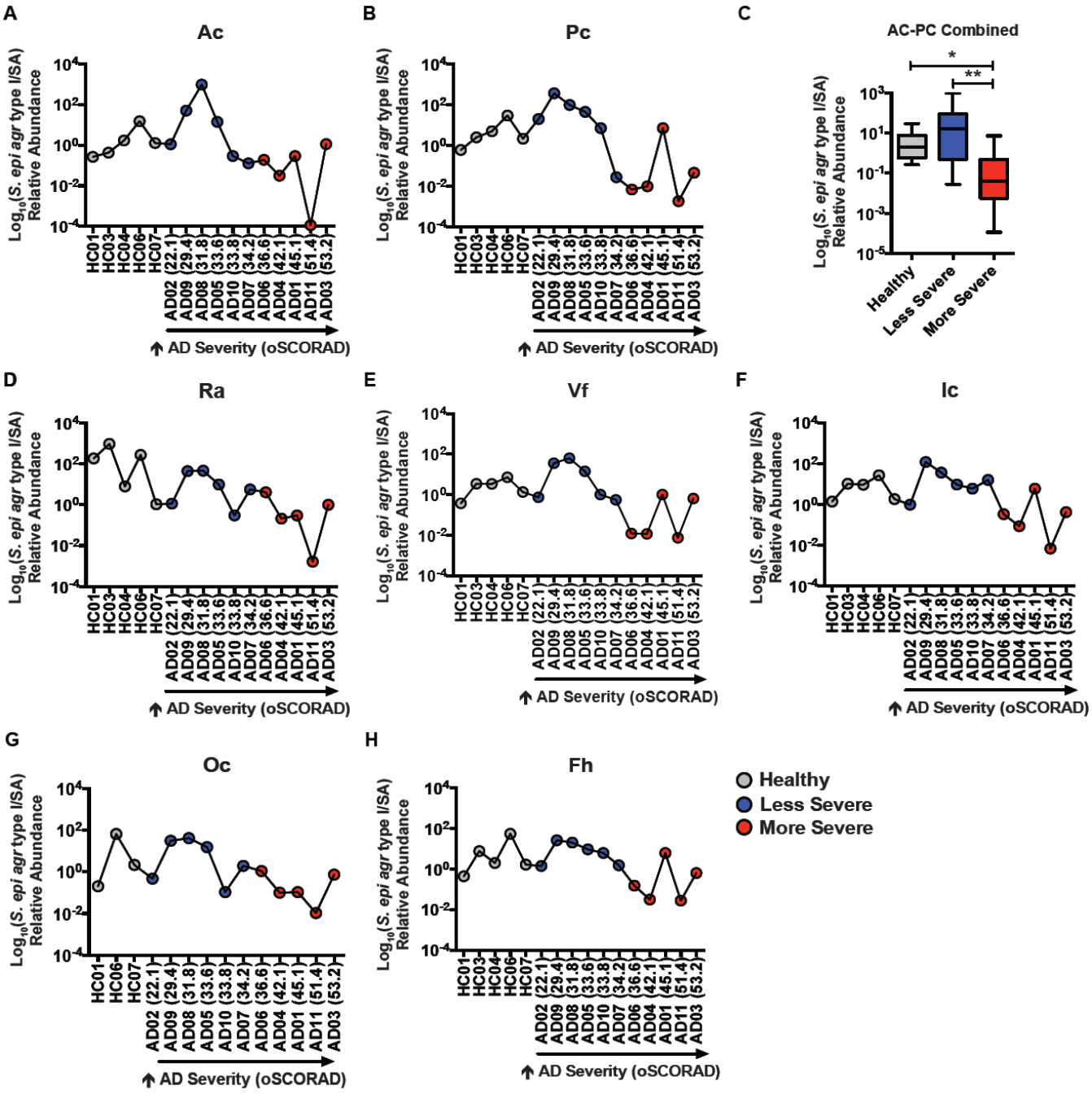


Fig. S3. Effect of AD skin disease severity on *S. epidermidis agr* type I to *S. aureus* relative abundance by body site. Analysis of relative abundance ratio of *S. epidermidis* (*S. epi*) *agr* type I to *S. aureus* (SA) based upon bilateral assessment of 7 individual body sites in 5 health subjects and 11 atopic dermatitis subjects. The ratio of *S. epidermidis* (*S. epi*) *agr* type I to *S. aureus* (SA) relative abundance per patient were arranged from 'less severe' and 'more severe' disease severity depending on objective SCORAD (oSCORAD) per each body site. **(A, B)** Frequent AD effected sites including the antecubital

crease (Ac) and popliteal crease (Pc) were analyzed along with **(C)** quantification of these combined sites. **(D-H)** Analysis of relative abundance at all other body sites including the retroauricular crease (Ra), volar forearm (Vf), inguinal crease (Ic), occiput (Oc), and forehead (Fh). An (nonparametric) unpaired Kruskal–Wallis test was used in (C) to test significance: $p<0.05$ *, $p<0.01$ **.

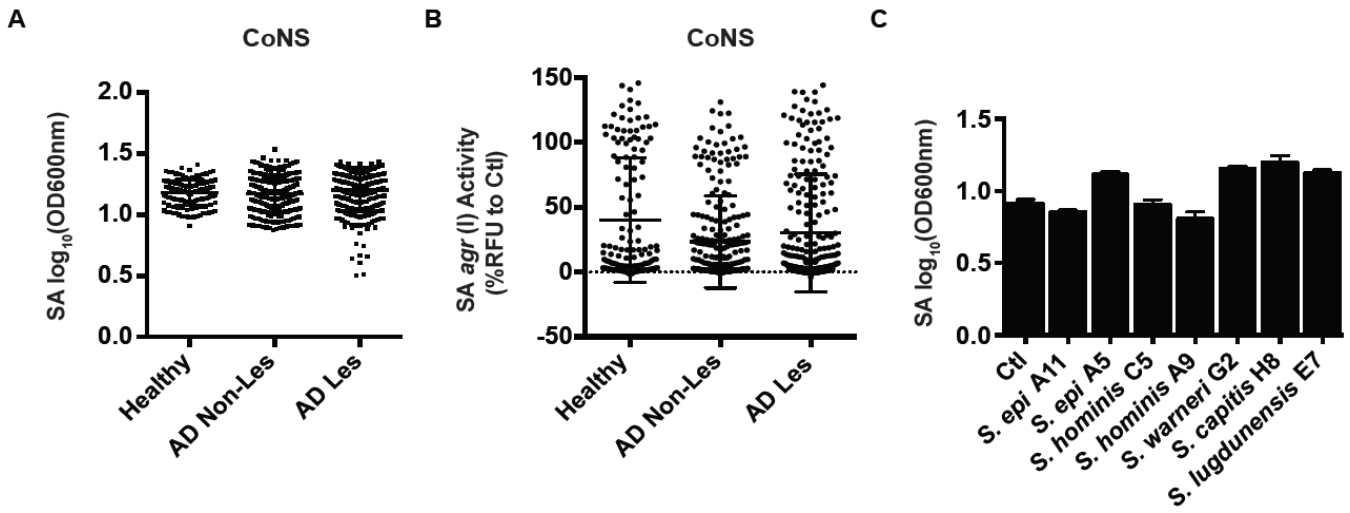


Fig. S4. Clinical CoNS isolates inhibit *S. aureus agr* activity without affecting growth. (A,B) Bacterial density (OD600nm) and fluorescent (*agr* activity) reading of *S. aureus* (SA) USA300 LAC *agr* type I P3-YFP reporter strain grown overnight with 25% sterile-filtered supernatant from CoNS strains grown overnight isolated from healthy controls (n=144 colonies), or AD non-lesional (Non-Les) and lesional (Les) skin swabs (n=288 colonies each). **(C)** Effect of select clinical CoNS isolate overnight supernatant (25%) on SA reporter bacterial density (n=4) (~$1e^9$ CFU for all SA grown with CoNS supernatant). Data in (C) are representative of 3 independent experiments. Error bars are standard error of the mean (SEM).

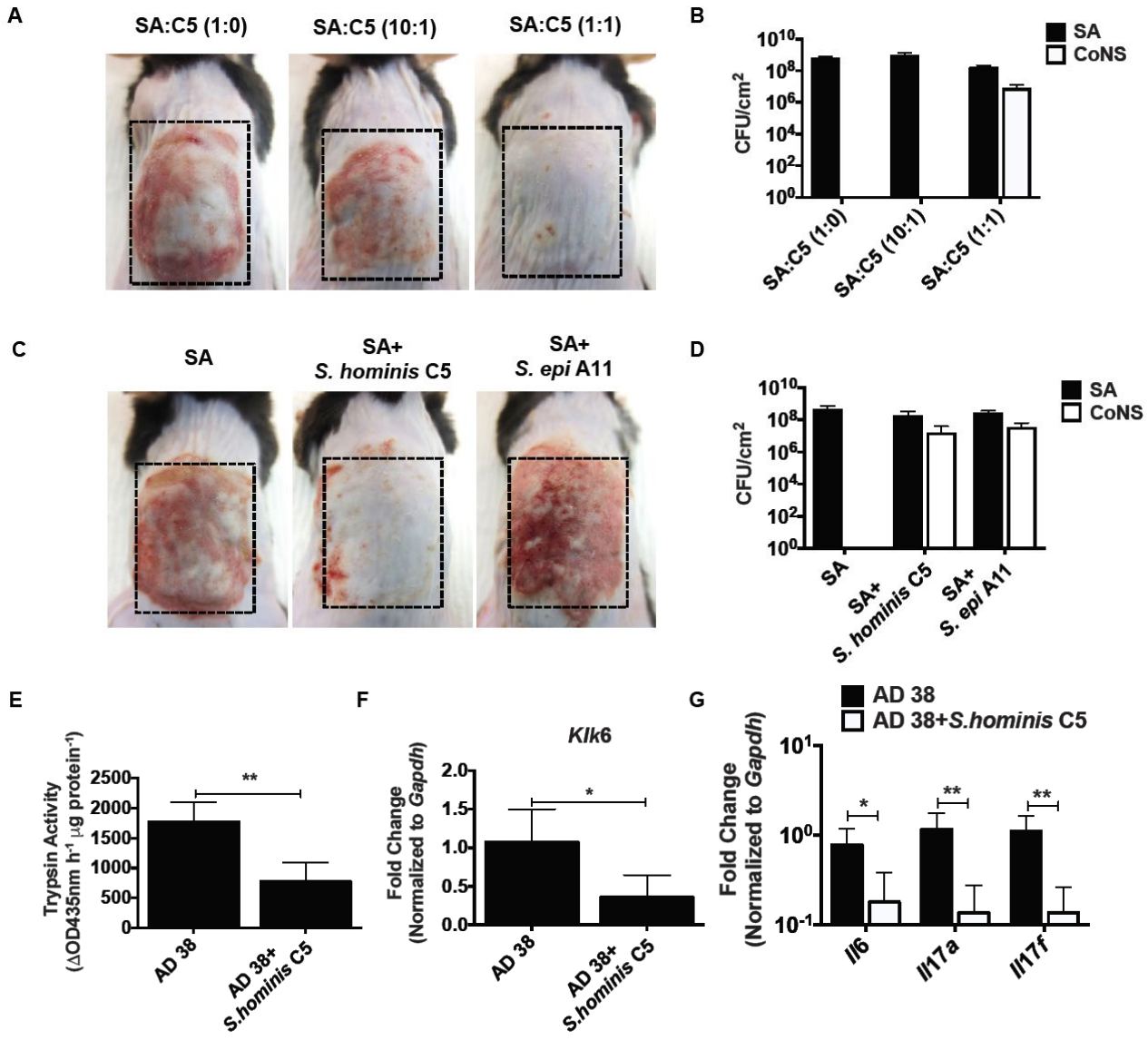
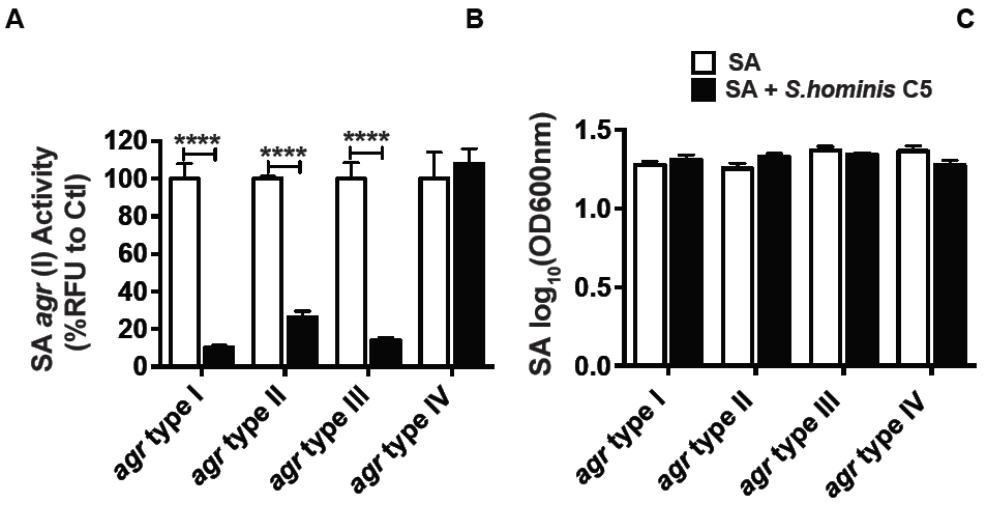


Fig. S5. *S. hominis* C5 live bacteria specifically inhibit *S. aureus*-mediated barrier damage and inflammation. (A, B) *S. aureus* (SA) USA300 LAC pAmi P3-Lux (1e^7 CFU) with or without *S. hominis* C5 co-colonized on mouse dorsal skin at different ratios for 72 hrs (n=5). **(C, D)** SA (1e^7 CFU) incubated at a 1:1 ratio with or without *S. hominis* C5 or a non-inhibitory strain of *S. epidermidis* (A11) for 72h (n=5) (Dashed lines indicate treatment area on murine dorsal skin for all experiments). **(E-G)** Application of a clinical AD SA isolate (AD 38) (1e^7 CFU) with or without *S. hominis* C5 at a 1:1 ratio on mouse dorsal skin for 72h followed by analysis of trypsin activity, *Klk*6, and cytokine (*Il*6, *Il17a*, and *Il17f*) mRNA expression normalized to *Gapdh* (n=5). Data representative of 2 independent experiments. Error bars represent standard error of the mean (SEM). Student's t-tests were used to determine statistical significance: $p<0.05$ *, $p<0.01$ **.



C

AD *S. aureus* Isolate	*agr* type (I-IV)
USA300 LAC	I
AD 2	I
AD 5	II
AD 6	I
AD 7	I
AD 14	I
AD 22	I
AD 25	I
AD 28	I
AD 29	I
AD 30	I
AD 38	I

D

Agr Type for SA Strain on AD Subjects	#
type I	27
type II	19
type III	9
type IV	6

E

AD SA Predominant Subjects	Clade	*agr* type (I-IV)
AD01	F1	IV
AD03	A2	I
AD04	E17	II
AD06	E13	I
AD11	E17	II

Fig. S6. *S. hominis* C5 inhibits *S. aureus agr* types I to III but not type IV. (A) *S. aureus* (SA) *agr* types I-IV P3-YFP reporter strains were cultured for 18h (~1e^9 CFU) with or without *S. hominis* C5 supernatant (25%) (n=3). **(B)** Measurement of growth by OD600nm from "A". **(C)** 11 AD clinical SA isolate *agr* types as well as SA USA300 LAC control. **(D,E)** Analysis of *agr* type of all 61 unique SA strains detected on AD patients from metagenomic dataset and analysis of *agr* type of dominant SA clones found on 5 AD patients. Data are representative of 2 independent experiments. Error bars are standard error of the mean (SEM). One-way ANOVA was used to determine statistical significance indicated by: $p<0.0001$ ****.

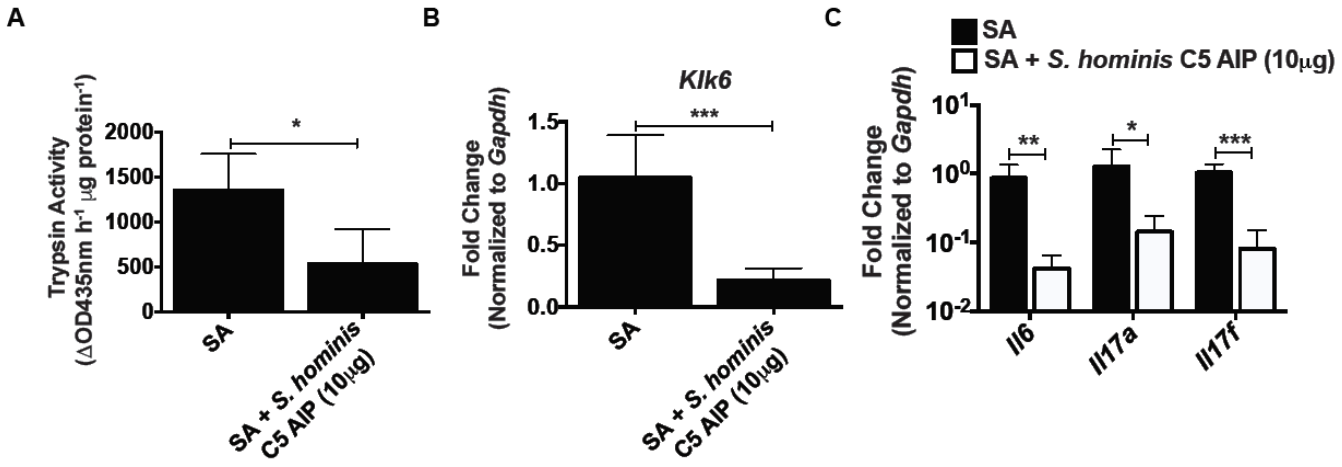


Fig. S7. *S. hominis* C5 autoinducing peptide inhibits *S. aureus*-driven inflammation and barrier damage. (A-C) Analysis of trypsin activity, *Klk*6, and cytokine (*Il*6, *Il17a*, and *Il17f*) mRNA expression normalized to *Gapdh* for SA USA300 LAC pAmi P3-Lux ($1e^7$ CFU) with or without *S. hominis* C5 synthetic autoinducing peptide (AIP) (10μg) for 48h (n=5). Data are representative of 2 independent experiments. Error bars are standard error of the mean (SEM). One-way ANOVA was used to determine statistical significance indicated by: $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***.

Table S1. Bacteria strains and plasmids used.

Bacteria and Plasmids		
Bacteria		
Strain Name	Source	Reference
S. epidermidis RP62A WT (*agr* type I)	Gallo (UCSD) Cheung (Darmouth)	This Study
S. epidermidis RP62A ΔAIP (#47)	Gallo (UCSD) Cheung (Darmouth)	This Study
S. epidermidis 1457 (*agr* type II*)*	Gallo (UCSD)	This Study
S. epidermidis 8247 (*agr* type III*)*	Horswill (UC Denver)	(*43*)
S. epidermidis A5	Gallo (UCSD)	This Study
S. epidermidis A11	This Study	This Study
S. aureus USA300 LAC WT	Otto (NIH/NIAID)	(*52*)
S. aureus USA300 Δ*psm*α	Otto (NIH/NIAID)	(*52*)
S. aureus USA300 Δ*psm*β	Otto (NIH/NIAID)	(*52*)
S. aureus USA300 LAC WT (AH1263)	Horswill (UC Denver)	(*24*)
S. aureus USA300 LAC Δprotease (AH1919), Erm^R, Lcm^R	Horswill (UC Denver)	(*24*)
S. aureus USA300 LAC *agr* type I pAmi P3-Lux (AH2759), Cm^R	Horswill (UC Denver)	(*53, 54*)
S. aureus USA300 LAC *agr* type I P3-YFP (AH1677), Cm^R	Horswill (UC Denver)	(*55*)
S. aureus 502a *agr* type II P3-YFP (AH430), Cm^R	Horswill (UC Denver)	(*55*)
S. aureus MW2 *agr* type III P3-YFP (AH1747), Cm^R	Horswill (UC Denver)	(*55*)
S. aureus MN TG *agr* type IV P3-YFP (AH1872), Cm^R	Horswill (UC Denver)	(*55*)
S. aureus AD 2	Gallo (UCSD)	This Study
S. aureus AD 5	Gallo (UCSD)	This Study
S. aureus AD 6	Gallo (UCSD)	This Study
S. aureus AD 7	Gallo (UCSD)	This Study
S. aureus AD 14	Gallo (UCSD)	This Study
S. aureus AD 22	Gallo (UCSD)	This Study
S. aureus AD 25	Gallo (UCSD)	This Study
S. aureus AD 28	Gallo (UCSD)	This Study
S. aureus AD 29	Gallo (UCSD)	This Study
S. aureus AD 30	Gallo (UCSD)	This Study
S. aureus AD 38	Gallo (UCSD)	This Study
S. hominis C5	Gallo (UCSD)	(*29*)
S. hominis A9	Gallo (UCSD)	(*29*)
S. epidermidis A5	Gallo (UCSD)	This Study
S. epidermidis A11	Gallo (UCSD)	This Study
S. warneri G25	Gallo (UCSD)	This Study
S. capitis H8	Gallo (UCSD)	This Study

S. lugdunensis E7	Gallo (UCSD)	This Study
DC10B-CC10	Cheung (Dartmouth)	(*44*)
Plasmids		
Strain Name	Source	Reference
pMAD (Amp^R in *E. coli*, Erm^R in *Staphylococci*)	Cheung (Dartmouth)	(*44-46*)
pMAD:: ΔAIP	Cheung (Dartmouth)	This Study

Table S2. Primers used for quantitative polymerase chain reaction and allelic replacement.

Primers	Description
TaqMan Murine *Gapdh* primers	Mm99999915_g1 (Applied Biosystems)
TaqMan Murine *Klk*6 primers	Mm00478322_m1 (Applied Biosystems)
TaqMan Murine *Il*6 primers	Mm00446190_m1 (Applied Biosystems)
TaqMan Murine *Il*17*a* primers	Mm00439618_m1 (Applied Biosystems)
TaqMan Murine *Il*17*f* primers	Mm00521423_m1 (Applied Biosystems)
TaqMan Human *KLK*6 primers	Hs00160519_m1 (Applied Biosystems)
TaqMan Human *GAPDH* primers	Hs02786624_g1 (Applied Biosystems)
*S. aureus psm*α primers (*56*)	Forward: TAAGCTTAATCGAACAATTC Reverse: CCCCTTCAAATAAGATGTTCATATC
S. aureus 16S rRNA primers (*56*)	Forward: TGATCCTGGCTCAGGATG Reverse: TTCGCTCGACTTGCATGTA
*agr*D-Up (This Study; *S. epidermidis* RP62A ΔAIP Construction)	Forward-Bam: AGCTA**GGATC**CGTTGGGATGGCTCAACAAC TCACTAATAATAT Reverse: AGTTCTTCTGGTACTTCTGGTTCGTCTCCTGC TACAGTACCAATAAATTCCAAGATTG
*agr*D-Down (This Study; *S. epidermidis* RP62A ΔAIP Construction)	*Forward:* CAATCTTGGAATTTATTGGTACTGTAGCAGG AGACGAACCAGAAGTACCAGAAGAACT *Reverse-Sal:* AGCTA**GTCGA**CTTCCTCATTATCGATGAATG CGATATTGATGAG
*agr*D Seq (This Study; *S. epidermidis* RP62A ΔAIP Construction)	*Forward:* GTATAATGACACTTGAGGAGAGTAGTAAA *Reverse:* CCATATCTTACACACTTTCTAGGGTTATA