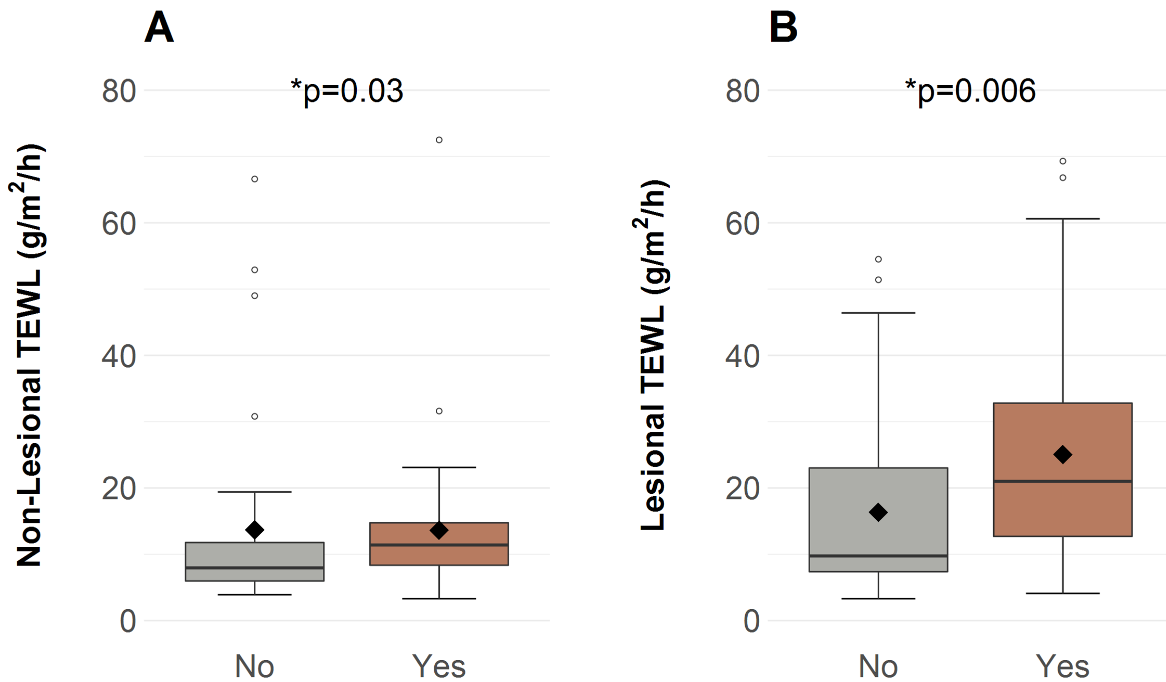
1 **Appendix S1**

2 **Biofilm propensity of *Staphylococcus aureus sk*in isolates is associated with**
3 **increased atopic dermatitis severity and barrier dysfunction in the MPAACH**
4 **pediatric cohort**

5 T. Gonzalez et al.

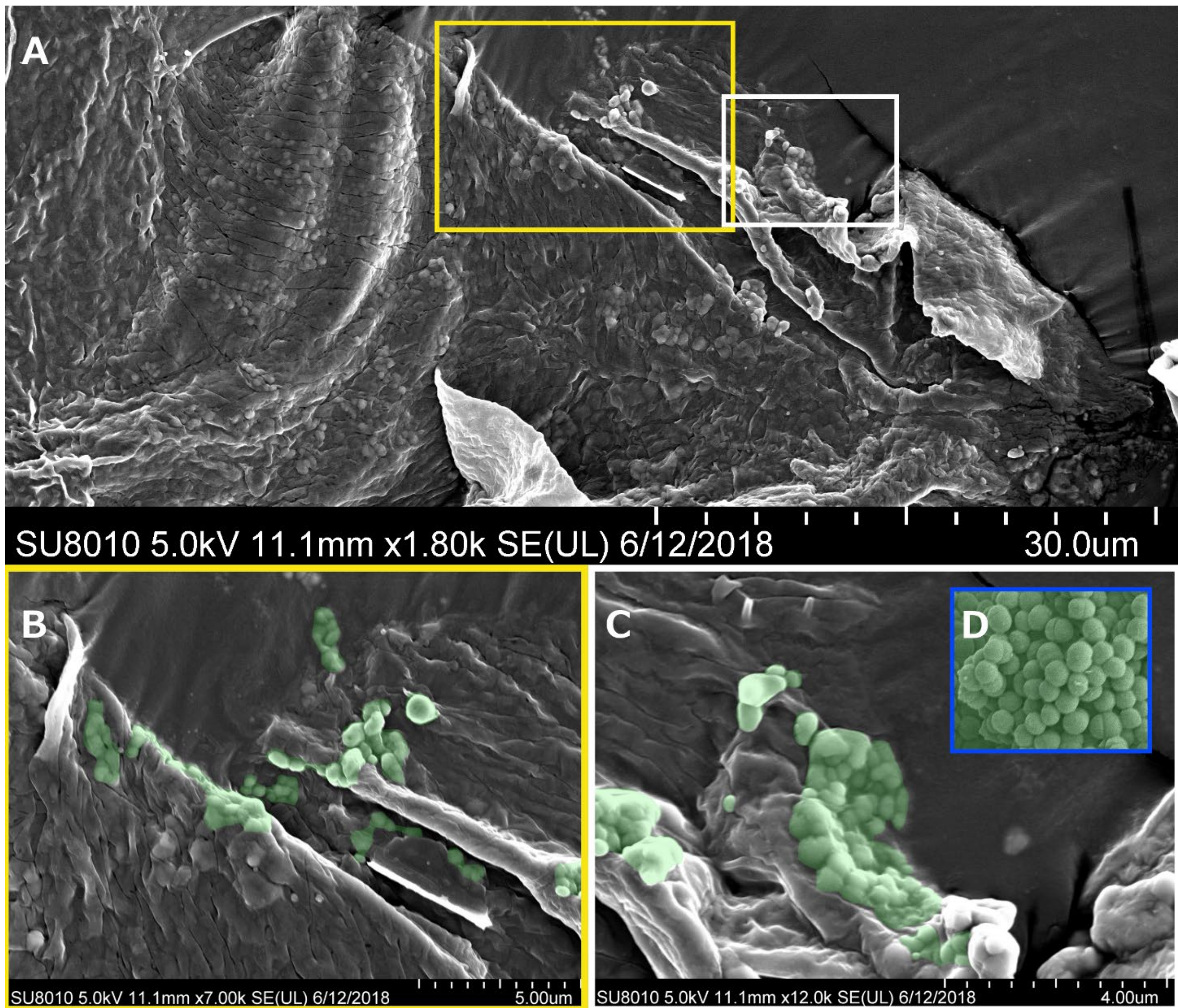
6

7 ## Supplemental Figures



8
9
10 **Supplementary Fig 1. Relative *S. aureus* biofilm propensity among co-colonized subjects**
11 **is associated with increased TEWL in both lesional and non-lesional AD skin.** Biofilm
12 propensity of *S. aureus* relative to the propensity of *S. epidermidis* from the same MPAACH
13 subject was assessed (only co-colonized subjects were used in this analysis). If multiple isolates
14 of *S. aureus* or *S. epidermidis* were sampled from the same subject, the isolate with the maximal
15 biofilm propensity was used in the analysis. Higher relative *S. aureus* biofilm propensity was
16 associated with: A) increased non-lesional TEWL, and B) increased lesional TEWL.
17

18



19

20 **Supplementary Figure 2: SEM of biofilms on keratinocytes collected from healthy adult.**
21 Tegaderm tape was used to collect keratinocytes from healthy adult skin and SEM was used to
22 identify biofilms on keratinocyte surfaces. Clusters of cocci-shaped organisms can be seen at A)
23 1.80k, B) 7.00k (yellow box), and C) 12.0k magnification (white box). An SEM image of an in-vitro
24 staphylococcal biofilm is shown in panel D (blue box) for comparison.
25

26 **Supplementary Table 1.** Associations of bacterial colonization with clinical outcomes and
27 expression of *FLG* or alarmin genes.
28

Bacterial Presence Group	CoNS without *S. epidermidis*	*S. epidermidis & CoNS*	*S. aureus & CoNS*	*S. aureus, S. epidermidis & CoNS*	p-value*
No.	80	210	31	75	
Black Race (%)	59.5	59.1	74.2	66.7	0.31
Male (%)	40	53	58	53	0.16
Age (y), median (IQR)	2.37 (1.84 – 2.46)	2.18 (1.60 – 2.44)	2.34 (1.94 – 2.46)	2.29 (1.82 – 2.45)	0.07
SCORAD	19.0 (13.1 – 27.6)	18.4 (11.0 – 28.3)	16.9 (12.0 – 40.0)	19.7 (12.2 – 34.5)	0.24
Non-Lesional TEWL (g m^{-2} h^{-1})	10.4 (7.2 – 13.4)	9.3 (7.0 – 13.4)	10.0 (8.2 – 13.8)	9.3 (6.6 – 14.3)	0.87
Lesional TEWL (g m^{-2} h^{-1})	10.9 (8.9 – 17.3)	11.5 (8.1 – 19.4)	12.0 (8.2 – 19.4)	13.8 (8.5– 27.3)	0.35
Maximal *FLG* expression, lesional (x 10,000)	12.0 (5.0 – 31.8)	9.8 (2.4 – 28.6)	5.2 (0.6 – 19.1)	5.9 (0.9 – 17.4)	***0.007***
Maximal *FLG* expression, non-lesional (x 10,000)	17.5 (6.9 – 42.9)	20.1 (5.1 – 44.0)	10.4 (2.6 – 20.9)	10.7 (3.7 – 28.2)	***0.038***
Maximal *S100A8* expression, lesional (x 10,000)	1.8 (0.4 – 3.6)	1.6 (0.5 – 4.1)	1.2 (0.5 – 3.4)	1.1 (0.2 – 2.4)	0.21
Maximal *S100A9* expression, lesional (x 10,000)	2.7 (0.7 – 6.1)	2.6 (0.9 – 6.7)	2.5 (1.0 – 5.7)	2.3 (0.6 – 6.1)	0.87
Maximal *S100A8* expression, non-lesional (x 10,000)	1.2 (0.3 – 3.7)	1.6 (0.5 – 5.0)	0.6 (0.3 – 3.2)	0.9 (0.3 – 2.0)	0.11
Maximal *S100A9* expression, non-lesional (x 10,000)	2.4 (0.5 – 8.5)	2.4 (0.6 – 7.5)	2.0 (0.5 – 7.5)	1.8 (0.5 – 5.2)	0.90

29 **P values were obtained by chisq test or Kruskal-Wallis rank sum test.*

30

31 # Supplemental Methods

32 ## Contact Plate Sampling

33 Contact plates made from sheep blood agar (Hardy Diagnostics, Santa Maria, CA) were
34 placed on both non-lesional and lesional sites of MPAACH children for 30 seconds and then
35 incubated for 48 hours at 37º C. Lesional sites were determined initially and subsequent non-
36 lesional sample sites were determined based on the need of certain body sites for collection of
37 other biospecimens. Each morphologically distinct colony was further isolated on individual sheep
38 blood agar plates (Remel, San Diego, CA) and incubated for another 24 hours. *S. aureus* and *S.*
39 *epidermidis* colonies were identified by culturing on Mannitol Salt agar (MSA) (BD, Sparks MD).
40 *S. aureus* isolates were confirmed by coagulase testing using the StaphAurex Latex Agglutination
41 Kit (Thermo Scientific, Waltham, MA). Bacterial genomic DNA isolated from colonies that appear
42 pink on MSA were further characterized by polymerase chain reaction (PCR) to screen for *S.*
43 *epidermidis*. A PCR screen to confirm the identity of *S. epidermidis* colonies was created using
44 established primers against a 705 bp chromosomal DNA fragment specific to *S. epidermidis* [1]:
45 SE705 (F: 5'- ATCAAAAAGTTGGCGAACCTTTTCA – 3' R: 5'-
46 CAAAAGAGCGTGGAGAAAAGTATCA -3'); and primers targeting the gene for the *S. epidermidis*
47 surface protein, Aap (F: 5' – TGCGACAAATTTAACGAGATA – 3', R: 5' –
48 CCACTTGCGTATGTACCACTA – 3'). PCR products were assessed on a 1% agarose gel.
49

50 ## Biofilm Crystal Violet Assay

51 Single MPAACH-isolated colonies were obtained from glycerol stocks streaked on
52 5% sheep blood agar and were inoculated in 5mL of tryptic soy broth supplemented with 0.5%
53 glucose (TSB-G). The colonies were cultured overnight with shaking at 37° C, followed by 1:10
54 dilution into TSB-G; cell growth was tracked by measuring optical density (OD) at 600 nm. For
55 biofilm assays, each culture was diluted to a final OD of 0.03 and added in triplicate to a plate that

56 was incubated statically at 37° C for 18 hours. Mono-species biofilms were grown from 200 µL of
57 culture and a 100 µL:100µL mixture was used to grow mixed biofilms. Crystal violet assays were
58 conducted essentially as described [2] with addition of 0.1% crystal violet. Absorbance was
59 measured at 570 nm using a BioTek Synergy H1 Hybrid spectrophotometer. *S. aureus* strain
60 SA35556 and *S. epidermidis* strain RP62a (American Type Culture Collection, Manassas, VA)
61 were used as positive controls, and negative controls were wells inoculated with TSB-G alone.
62

63 **Scanning Electron Microscopy**

64 To visualize biofilms on skin, a tape strip sampling technique was developed similar to that
65 described by Masako et al. [3, 4]. The design of this experiment was implemented after enrollment
66 began and required an additional step of tape sampling; we received consent for the additional
67 sampling for 26 subjects, who were included in this analysis. A 1x1 in square of Tegaderm Film
68 (3M, St. Paul MN) was placed on the subject's skin in both non-lesional and lesional areas for 30
69 minutes. A carbon adhesive tab (Electron Microscopy Sciences, Hatfield, PA) was placed on the
70 tape while still on the skin, followed by a scanning electron microscopy (SEM) aluminum mount
71 (Electron Microscopy Sciences, Hatfield PA) to hold the Tegaderm flat on the surface of the mount
72 after removal from the skin. The adhesive side of the Tegaderm film was coated with gold
73 palladium and visualized using a Hitachi SU8010 Ultra-High Resolution Scanning Electron
74 Microscope (Hitachi, Tokyo) at 5.0 kV.
75

76 **Tape Strip Sampling**

77 Corneocyte and keratinocyte sampling was conducted by tape stripping, using adhesive
78 SmartSolve Strips pre-cut into eleven 1x1 inch strips, as described [5]. Skin cells of lesional and
79 non-lesional sites were sampled by placing the tape strip on the skin, gently massaging the tape
80 for 15-20 seconds, removing the tape, and storing it in ice-cold in BL buffer supplemented with
81 2% thio-glycerol (Promega, Madison, WI). This process was repeated to provide a total of 11 tape

82 strips per sampled skin site. Tubes were vortexed for 10-25 seconds and incubated at 42° C for
83 30 min. Collected skin tapes were flash frozen and stored at -80° C. The first 3 tapes from each
84 lesional or non-lesional site were stored for microbiome analysis, and keratinocyte DNA and RNA
85 were extracted from tapes 4-7 and 8-11, respectively. Only the RNA extracted from tape 8 & 9
86 were used in this study; all other tapes are utilized in other current and ongoing studies.
87

88 ***S100A8*, *S100A9*, and *FLG* expression data**

89 RNA extraction was first done with phenol:chloroform for the removal of the tape residue, and the
90 aqueous phase was used for the ReliaPrep™ RNA Cell Miniprep (PROMEGA) extraction.
91 Complementary DNA was made with SuperScript IV VILO (ThermoFisher) and PCRs reactions
92 were carried out with the following taqman gene expression assays: 18S (Hs03003631_g1), *FLG*
93 (Hs00856927_g1), *S100A8* (Hs00374264_g1) and *S100A9* (Hs00610058_m1). The PCR cycling
94 conditions were 95° C for 10 minutes followed by 50 cycles of 95° C for 10 seconds and 60° C for
95 30 seconds. *S100A8*, *S100A9*, and maximum level of FLG expression between Tape 8 and Tape
96 9 were normalized to 18S. To maximize confidence in the gene expression data, the maximum
97 value reported from either tape strip 8 or 9 from each subject was used to measure *S100A8*,
98 *S100A9*, and *FLG* expression.
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