Supplemental Materials and methods

Quantitative Polymerase Chain Reaction (qPCR)

We carried out qPCR to estimate the copy number of the 16S ribosomal RNA gene and colony forming unit (CFU) for *Staphylococcus aureus* and *Staphylococcus epidermidis* in the extracted DNA sample. For qPCR, THUNDERBIRD SYBR qPCR Mix (Toyobo Co., Ltd., Osaka, Japan) was used with LightCycler 480 System (F. Hoffmann-La Rochre, Ltd.). The primer sequences were as follows: 321Fmod (5′-ACT GAG AYA CGG YCC A-3′) and 524R (5′-CTG CTG GCA CGD AGT TAG CC-3′) for 16S rRNA gene (Ogai et al., 2018); nucF (5′-GCG ATT GAT GGT GAT ACG GTT-3′) and nucR (5′-AGC CAA GCC TTG ACG AAC TAA AGC-3′) for *S. aureus* (Brakstad, Aasbakk, & Maeland, 1992); SepF (5′- CAG TTA ATC GGT ATG AGA GC -3′) and SepR (5′-CTG TAG AGT GAC AGT TTG GT-3′) for *S. epidermidis* (Iorio et al., 2011). A solution with known concentration (10^7 CFU) of each bacterial suspension (*S. aureus* ATCC 6538P or *S. epidermidis* ATCC 14990) was processed for DNA extraction, and the extracted DNA solution was then serially diluted by 1:10 down to 10^0 CFU (eight series in total) and used as standards.

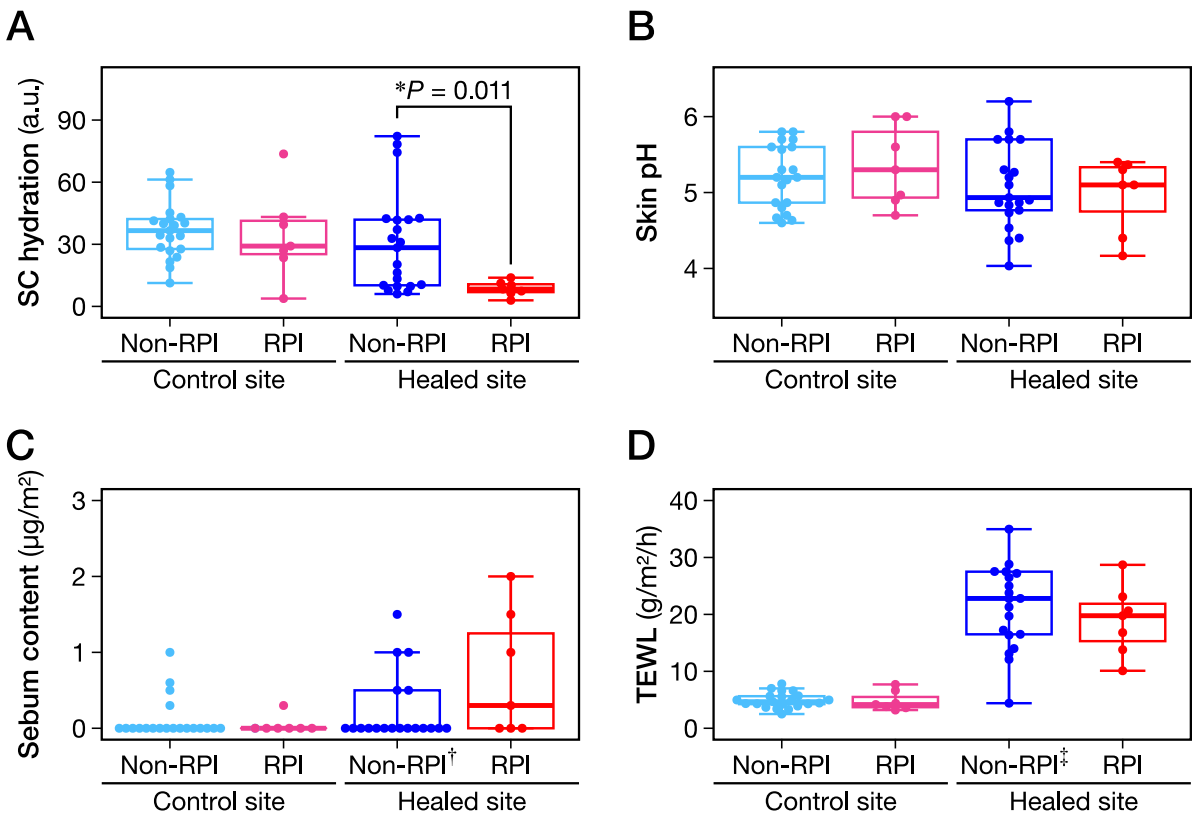
References

Brakstad, O. G., Aasbakk, K., & Maeland, J. A. (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J Clin Microbiol, 30*(7), 1654-1660.

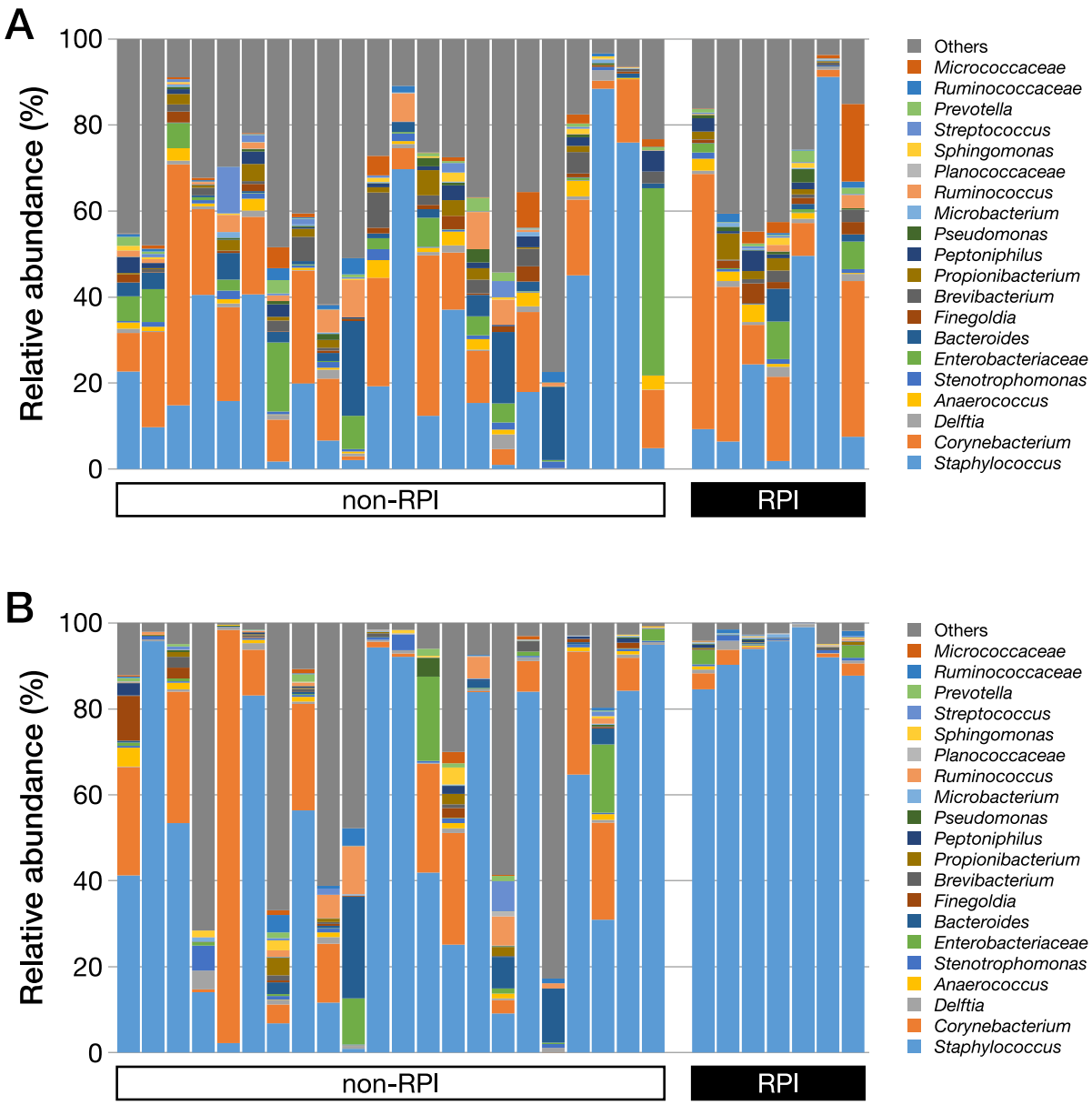
Iorio, N. L., Azevedo, M. B., Frazao, V. H., Barcellos, A. G., Barros, E. M., Pereira, E. M., et al. (2011). Methicillin-resistant *Staphylococcus epidermidis* carrying biofilm formation genes: detection of clinical isolates by multiplex PCR. *Int Microbiol, 14*(1), 13-17.

Ogai, K., Nagase, S., Mukai, K., Iuchi, T., Mori, Y., Matsue, M., et al. (2018). A comparison of techniques for collecting skin microbiome samples: swabbing versus tape-stripping. *Front Microbiol, 9*, 2362.

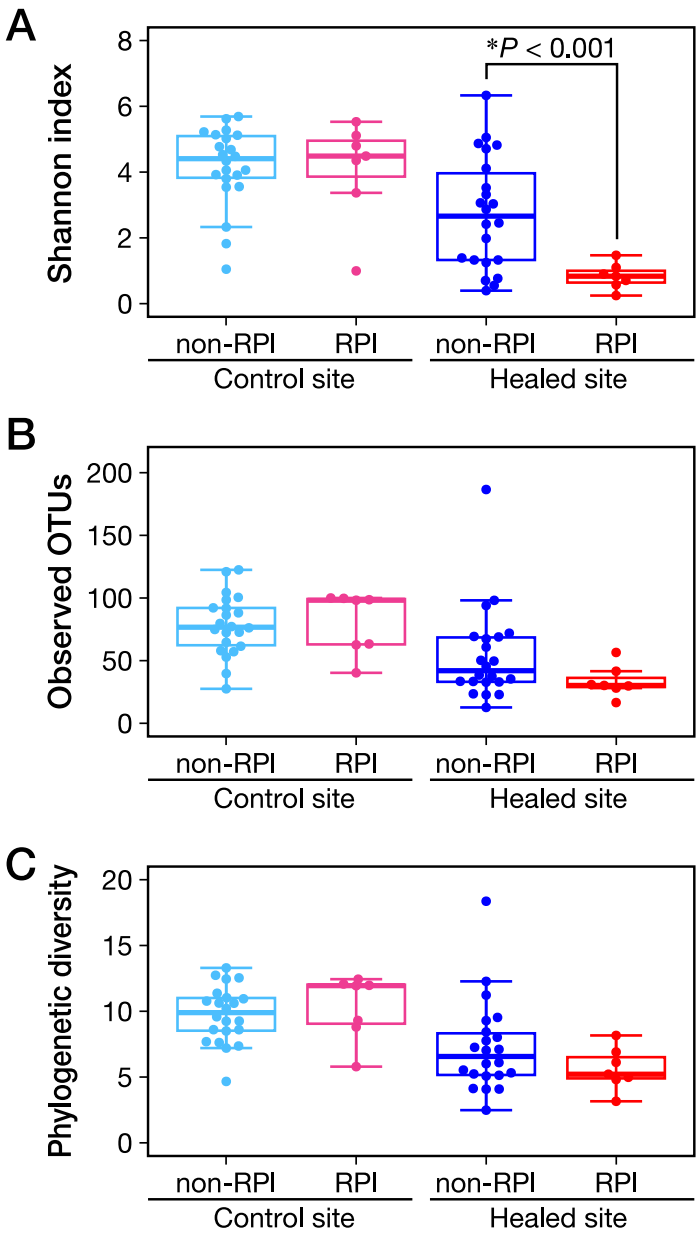
Supplemental Figures



Supplemental Figure 1. Characteristics of skin physiology.
Stratum corneum (SC) hydration (A), pH (B), sebum content (C), and transepidermal water loss (TEWL) (C) are presented as box plots, grouped by sites and recurrence.
*$P < 0.05$ in Mann–Whitney U test.
†Two data points (24.0 and 30.1) are not displayed.
‡Two data points (62.4 and 116) are not displayed.
RPI, recurrent pressure injury.



Supplemental Figure 2. Compositions of the top 20 skin bacteria on the control site (A) and on the healed site (B). RPI, recurrent pressure injury.

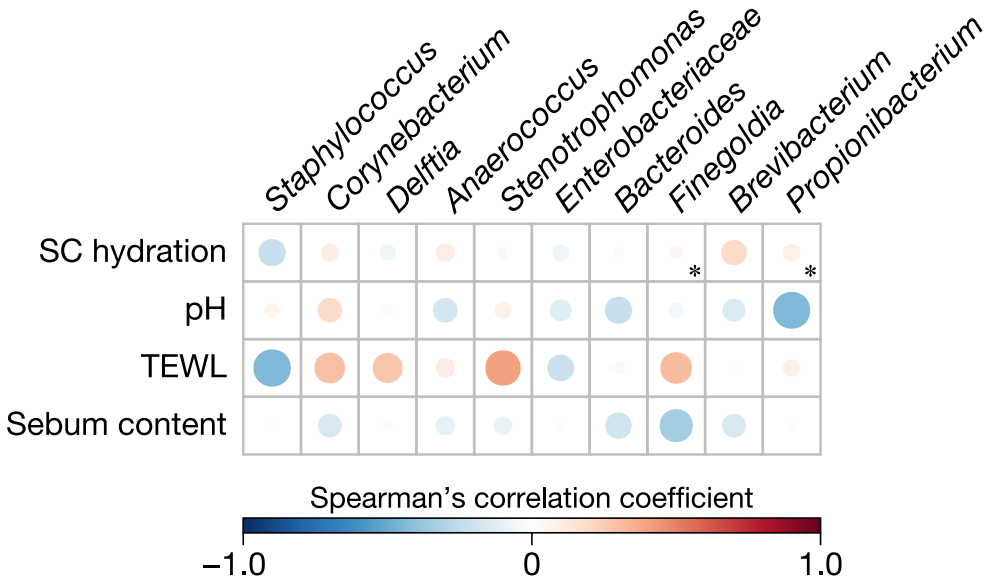


Supplemental Figure 3. Alpha diversity.

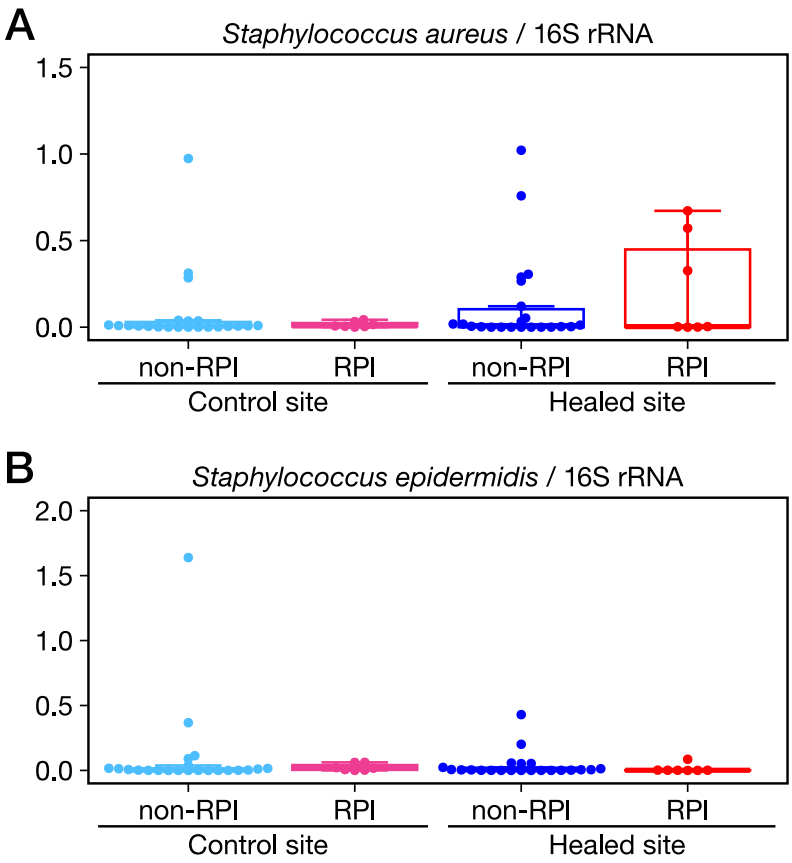
The Shannon index (A), the number of observed OTUs (B), and phylogenetic diversity (C) are shown.

$^{*}P < 0.001$ in Mann–Whitney *U* test.

RPI, recurrent pressure injury; OTU, operational taxonomic unit.



Supplemental Figure 4. Correlation matrix between skin physiology and microbiome. Spearman's correlation coefficient was expressed by color and the size of each circle.
$^{*}P < 0.05$.
SC, stratum corneum; TEWL, transepidermal water loss.



Supplemental Figure 5. Relative amounts of *Staphylococcus aureus* and *S. epidermidis.*
Relative amounts of *Staphylococcus aureus* (A) and *S. epidermidis* (B) on the skin. Normalization was achieved by simply dividing the results of quantitative PCR for either *S. aureus* or *S. epidermidis* by the total copy number of the 16S rRNA gene. RPI, recurrent pressure injury.