# Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

**eAppendix.** Supplemental Appendix

## Statistics and data analysis

All data analysis was conducted using the statistical software package R v. 3.2.3 [1]. Microbiome data was handled using the add-on package 'phyloseq' v. 1.16.2 [2] and visualized with 'ggplot2' v. 2.1.0 [3]. In the barplot, species belonging to the genera *Staphylococcus* and *Propionibacterium* were analyzed on species level, all other species were merged to genus level, by agglomerating counts within each genus. Counts were normalized to percentages per sample and the ten species with the highest sum of percentage were kept. Microbiome types were defined using vegdist (from the add-on package 'vegan', v. 2.3-2) [4] and hierarchal clustering with default settings, the number of optimal groups was decided by manual inspection.

Differences between groups were assessed with barplots and Principal Coordinates Analysis (PCoA) plots using Bray-Curtis distances and tested with a permutational multivariate analysis of variance (PERMANOVA), 'adonis' from the package 'vegan'. Differential abundances were visualized with violin plots of proportional abundances on a log scale using a pseudocount of $10^{-6}$ and tested using linear models (log-relative abundances using half the lowest nonzero count as pseudocount). All DA analyses were adjusted for anatomical location (axilla vs groin), and only taxa present in at least 25% of samples with a mean relative abundance of 0.001 were tested. This was conducted at species level, using the species defined by the BION pipeline, as well as at genus level, by agglomerating counts within each genus. False discovery rate was controlled using the Benjamini-Hochberg approach [5], and only the top 15

most significant taxa in each comparison were considered (all $p < 0.05$ and $q < 0.20$).

Distributions of observed number of species and Shannon index between sample groups were visualized with boxplots and tested using Kruskal-Wallis tests (between the 3 groups) or Wilcoxon tests (pairwise comparisons) after bootstrap rarefaction to equal library size (5000 reads), repeated 100 times with the median value used. Statistical analyses were performed for associations between the five microbiome types in the three groups and the variables BMI, Sartorius, Hurley Stage, smoking, age and gender using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables. A p-value below 0.05 was considered statistically significant.

**HS Severity**

An experienced dermatologist assessed patients during physical examination using Hurley staging and Sartorius score. Both systems rely on physical findings such as involvement of specific anatomical sites, number of nodules, sinus tracts and scars [6]. The Hurley system is a classification of three levels (mild (I), moderate (II) and severe (III)) [7]. The Sartorius system allows calculation of a numerical score for each body area involved and points are awarded for each skin manifestation with no upper limit [8]. The diameter for all nodules were measured and all patients reported the duration of the lesion (how long time the patients has noticed the presence of the lesion)

**Primer design:**

The 16S rDNA gene was targeted for amplification, using a modified version of the published universal prokaryotic primers 341F/806R, targeting the V3-V4 hyper-variable regions (pmid 15696537). The forward primer had three additional nucleotides attached in the 5' end (ACTCCTAYGGGRBGCASCAG, 341F3) and the reverse primer had five additional nucleotides attached in the 5' end (AGCGTGGACTACNNGGGTATCTAAT, 806R5).

The 18S rDNA gene was selected as target gene to ensure an as broad spectrum of eukaryotic species (parasites and fungi) to be amplified from as few primer sets as possible, when assuming that the 18S rDNA sequence would be the most inter-species conserved gene. 18S rDNA sequences were aligned for all species in each of 56 parasite genus' and a consensus sequence was generated. The consensus sequences for each genus were used for phylogenetic analysis in order to group the parasites according to their 18S rDNA sequence similarity rather than taxonomy. Six distinct groups appeared, and the consensus sequence from all species within each group was aligned and primers were designed, to amplify the species within each group. Additionally, a consensus sequence from human 18S rDNA was compared to the alignment in order to design primers that would avoid human 18S rDNA. Three different primer sets were chosen, G3F1/G3R1 (GCCAGCAGCCGCGGTAATTC / ACATTCTTGGCAAATGCTTTCGCAG), G4F3/G4R3 (CAGCCGCGGTAATTCCAGCTC / GGTGGTGCCCTTCCGTCAAT) and G6F1/G6R1 (TGGAGGGCAAGTCTGGTGCC / ACGGTATCTGATCGTCTTCGATCCC). G3 and G6 primers are targeting the hyper-variable regions V3-V4 of the 18S rDNA gene, and G4 is targeting V3-V5. Each primer set was aligned using BLAST to the NCBI database, using

NCBI's Primer-Blast, with standard settings (excluding predicted Refseq transcripts and uncultured / environmental samples) to test for unintended amplification.

**Library preparation**:

Purified genomic DNA from each sample was initially amplified using the 16S and 18S primers. The 16S and 18S rDNA was amplified in a 25µl volume, using the REDExtract-N-Amp PCR ReadyMix (Sigma-Aldrich, St Louis, MO, USA) with 0.4µM of each primer and 2µl template. The 16S PCR was run with an initial denaturation at 95°C for 2 min, 20 cycles of 95°C for 30 sec, 60°C for 1 min, and 72°C for 30 sec, and a final elongation at 72°C for 7 min. The 18S PCR setup was run with an nitial denaturation at 95°C for 3 min, 20 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 30 sec, and a final elongation at 72°C for 4 min. These PCR runs are referred to at PCR1 or amplification PCR. The products from PCR1 were prepared for sequencing by a second PCR (PCR2 or adaptor PCR), using the same PCR protocol as described above. PCR2 attached an adaptor A, an index i5, and a forward sequencing primer site (FSP) in the 5' end of the amplicons and an adaptor B, an index i7, and a reverse sequencing primer site (RSP) to the 3' end of the amplicons. DNA was quantified using the Quant-IT™ dsDNA High Sensitive Assay Kit (Thermo Fisher Scientific), and PCR2 products were pooled in equimolar amounts between samples. Undesirable DNA amplicons were removed from the pooled amplicon library (PAL) by Agencourt AMPure XP bead (Beckman Coulter) purification in a two-step process. Firstly, DNA fragments below 300nt were removed by a PAL AMPure beads 10:24 ratio, following the manufactures protocol and eluted in 40µL TE buffer (AM1). Secondly, large DNA fragments

above 1kbp were removed by AM1 to AMPure beads 10:16 ratio, as previously described. The resulting AMPure beads purified PAL (bPAL) was diluted to its final concentration of 11.5pM DNA in a 0.001 N NaOH, used for sequencing on the Illumina MiSeq desktop sequencer (Illumina Inc., San Diego, CA 29122, USA). The library was sequenced with the 500-cycle MiSeq Reagent Kit V2 in a 2x250nt setup (Illumina Inc., San Diego, CA 29122, USA).
The sequencing was performed at Statens Serum Institut (SSI).

**Bioinformatics**

BION (http://box.com/bion), a newly developed analytical semi-commercial open-source package for 16S rRNA and other reference gene analysis, classifying mostly to species was used. The pipeline accepts raw sequence and includes steps for de-multiplexing, primer-extraction, sampling, sequence- and quality based trimming and filtering, de-replication, clustering, chimera-checking, reference data similarities and taxonomic mapping and formatting. Non-overlapping paired reads are allowed for analysis, and BION is often accurate to the species level.

**BION steps used**

The data were processed by following automated steps:
1. Pair extraction. A given primer set was used to extract the pairs from the raw reads with both mates present.
2. Individual read trimming. A minimum quality of 99% was required for at least 14 of 15 bases for forward reads and 28 out of 30 for reverse reads. A minimum length of 50 was also required.

3. Pair joining. Read pairs were fused into one if there were overlaps of at least 18 bases with at least 90% similarity. Both overlapping and non-overlapping pairs are used in the following.

4. Dereplication. Identical pairs were converted to one while keeping track of the original read count.

5. Chimera filtering. Sequences were pre-clustered by 99% and a rather strict minimum chimera score of 25 was chosen. The logic moves an artificial break point through each query sequence to see if the left and right fragments have best matches against different reference sequences.

6. Clustering. Non-chimeric sequences were sorted by overall quality and clustered by 99% stringency.

7. Reference matching. A k-mer based approach was used that can take low quality into account and accommodate sequences with annotated break points and read counts. A k-mer length of 8 was used, with a step size of 4. Query sequences were compared against amplicon extracts from Silva version 123, with improved taxonomy for eukaryotes.

8. Taxonomy profiling. Query similarity values for cluster center sequences were projected onto the taxonomy and total counts for cluster center- plus member-reads were computed. The projection achieves higher accuracy through an initial noise-cancellation step, where overall dominant taxa are pre-selected for close calls.

9. Taxonomy tables. From individual profile files, tables were written for phylum, class, order, family, genus, species and sequence (strain) levels.

Reference List


(1) *R: A Language and Enviroment for Statistical Computing* [ 2015.

(2) McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;8:e61217.

(3) Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag, 2009.

(4) *Vegan: Community Ecology Package* [ 2015.

(5) Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological)* 1995;57:289-300.

(6) Zouboulis CC, Del M, V, Mrowietz U, Prens EP, Tzellos T, Jemec GB. Hidradenitis Suppurativa/Acne Inversa: Criteria for Diagnosis, Severity Assessment, Classification and Disease Evaluation. *Dermatology* 2015;231:184-190.

(7) Zouboulis CC, Desai N, Emtestam L et al. European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol* 2015;29:619-644.

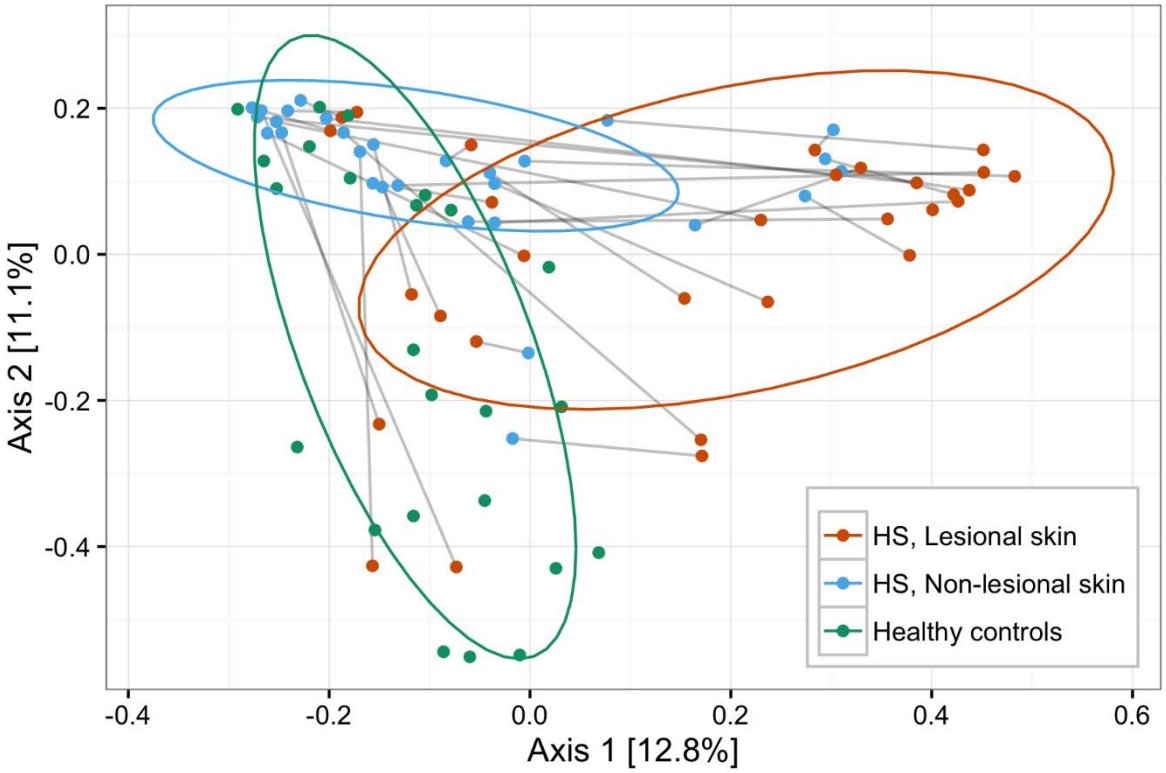
(8) Sartorius K, Emtestam L, Jemec GB, Lapins J. Objective scoring of hidradenitis suppurativa reflecting the role of tobacco smoking and obesity. *Br J Dermatol* 2009;161:831-839.

**eTable.** Distribution of Microbiome Types and Association With Location and Medication

| Microbiome type | N | Location of Lesion | | Medication | |
|---|---|---|---|---|---|
| HS patients (n=30, lesional) | | Groin | Axilla | Resorcinol (n) | Finacea (n) |
| I | 7 | 3 | 4 | 4 | 1 |
| II | 7 | 1 | 6 | 0 | 2 |
| III | 3 | 1 | 2 | 1 | 0 |
| IV | 13 | 10 | 3 | 1 | 1 |
| V | 0 | 0 | 0 | 0 | 0 |
| HS patients (n=29, non-lesional) | | | | | |
| I | 6 | 3 | 3 | 4 | 1 |
| II | 18 | 6 | 12 | 2 | 3 |
| III | 0 | 0 | 0 | 0 | 0 |
| IV | 5 | 5 | 0 | 0 | 0 |
| V | 0 | 0 | 0 | 0 | 0 |
| Healthy controls (n=24) | | | | | |
| I | 8 | - | 8 | - | - |
| II | 6 | - | 6 | - | - |
| III | 7 | - | 7 | - | - |
| IV | 0 | - | 0 | - | - |
| V | 3 | - | 3 | - | - |

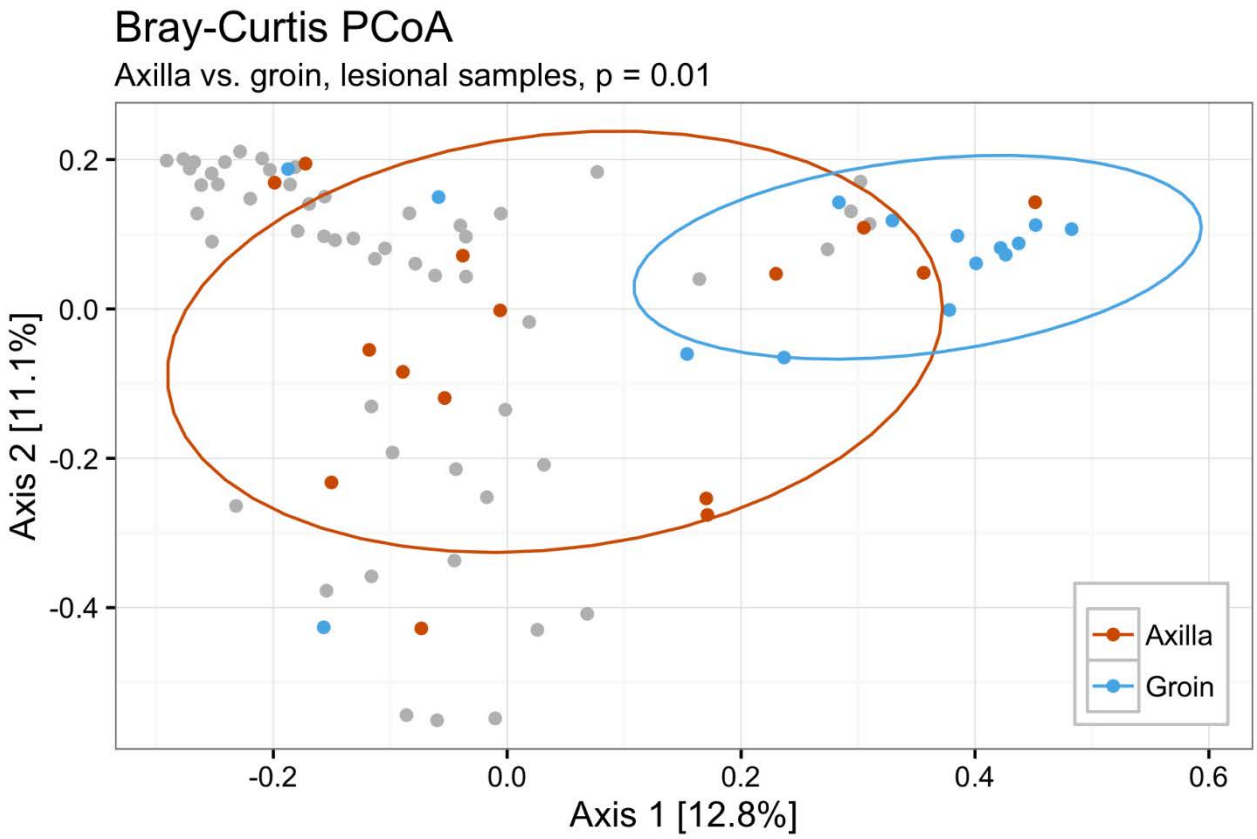
Association between the medication used (Resorcinol or Finacea), location of lesion and the corresponding microbiome types. N =Number of participants, HS = Hidradenitis Suppurativa.

**eFigure 1.** PCoA Plot Illustrating Differences Between the 3 Groups



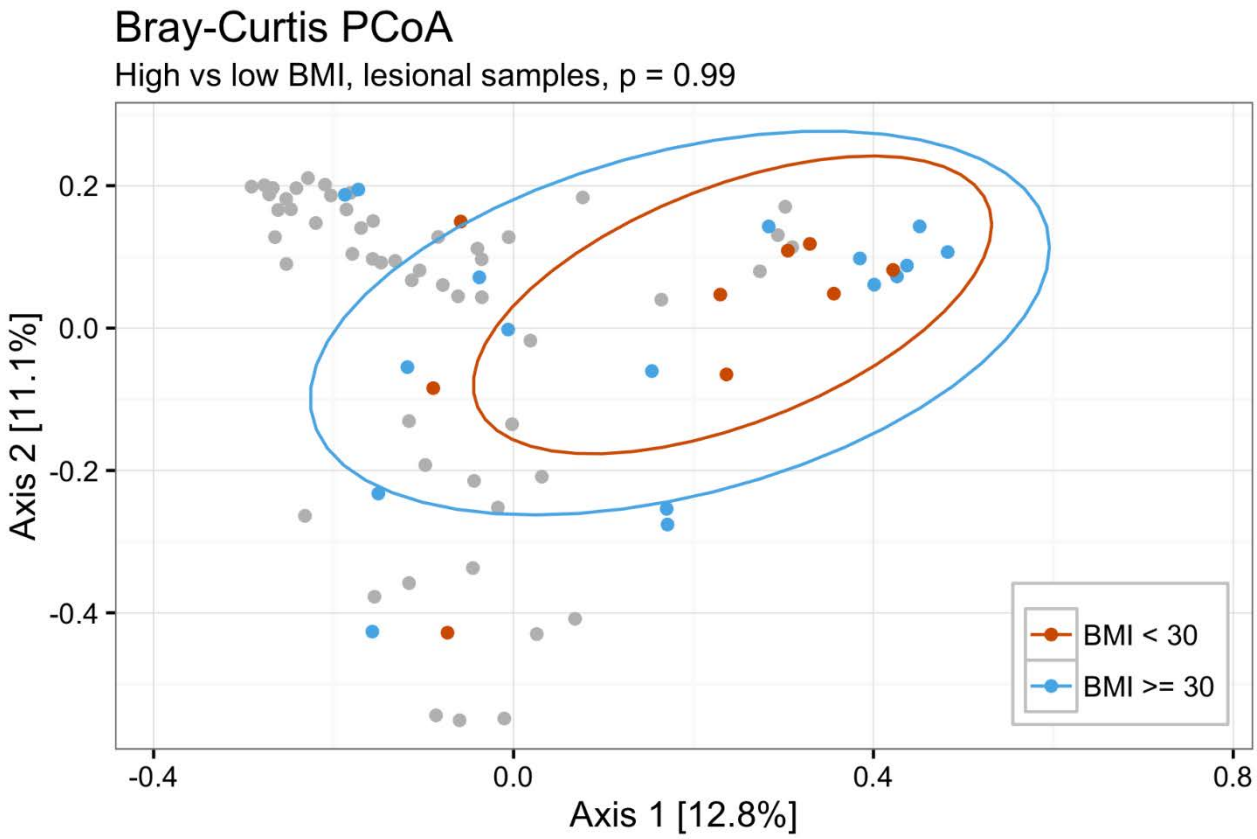

PCoA plot illustrating differences between the three groups (lesional, non-lesional and healthy controls). Each lesional sample is connected with its corresponding non-lesional sample. Ellipses delineate the 75% prediction areas of samples from each group.

**eFigure 2A.** Bray-Curtis PCoA, HS Lesional Skin, Axilla vs Groin



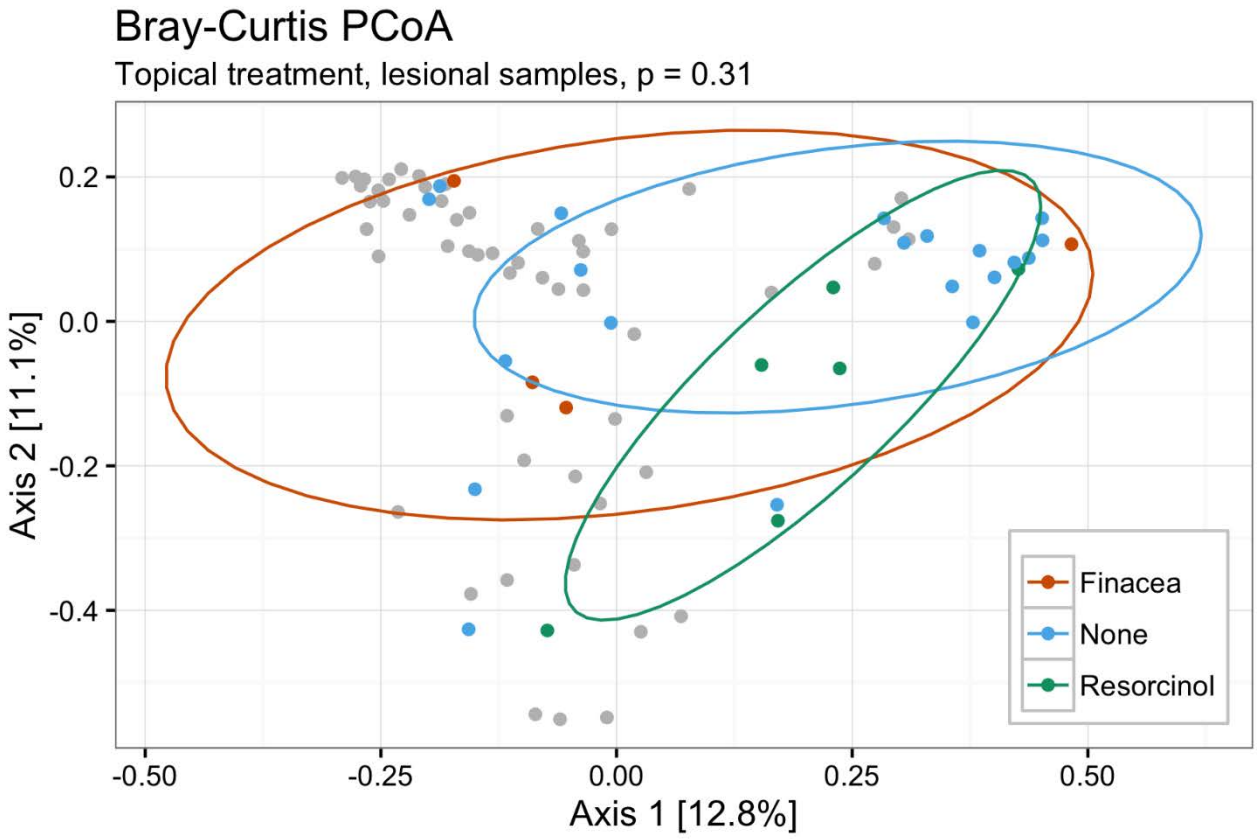

PCoA plot showing the difference between lesional samples originating from the axilla and the groin. P-value corresponds to Adonis PERMANOVA test. Ellipses delineate the 75% prediction areas of samples from each group.

**eFigure 2B.** Bray-Curtis PCoA, HS Lesional Skin, High vs Low BMI



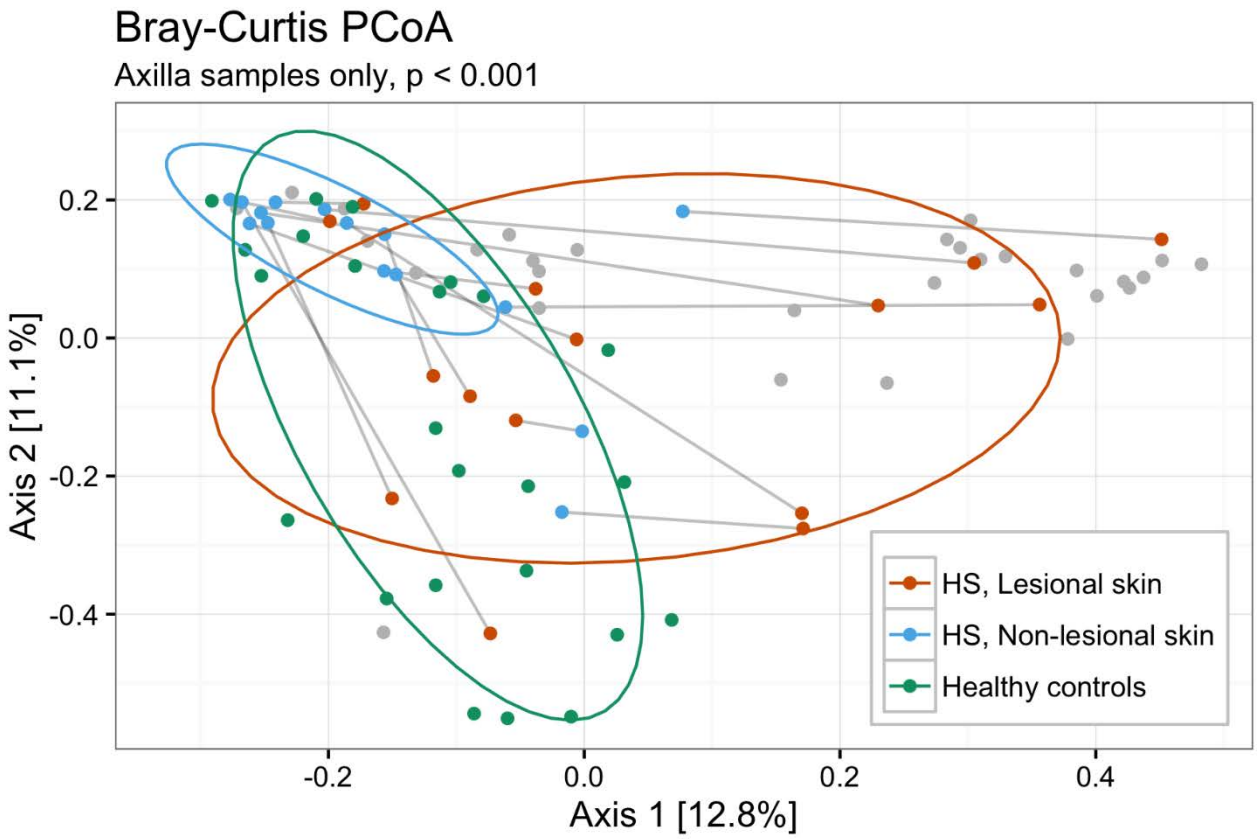

PCoA plot showing the difference between lesional samples originating from patients with BMI above versus below 30. P-value corresponds to Adonis PERMANOVA test. Ellipses delineate the 75% prediction areas of samples from each group.

**eFigure 2C.** Bray-Curtis PCoA, HS Lesional Skin, Topical Treatment vs No Treatment



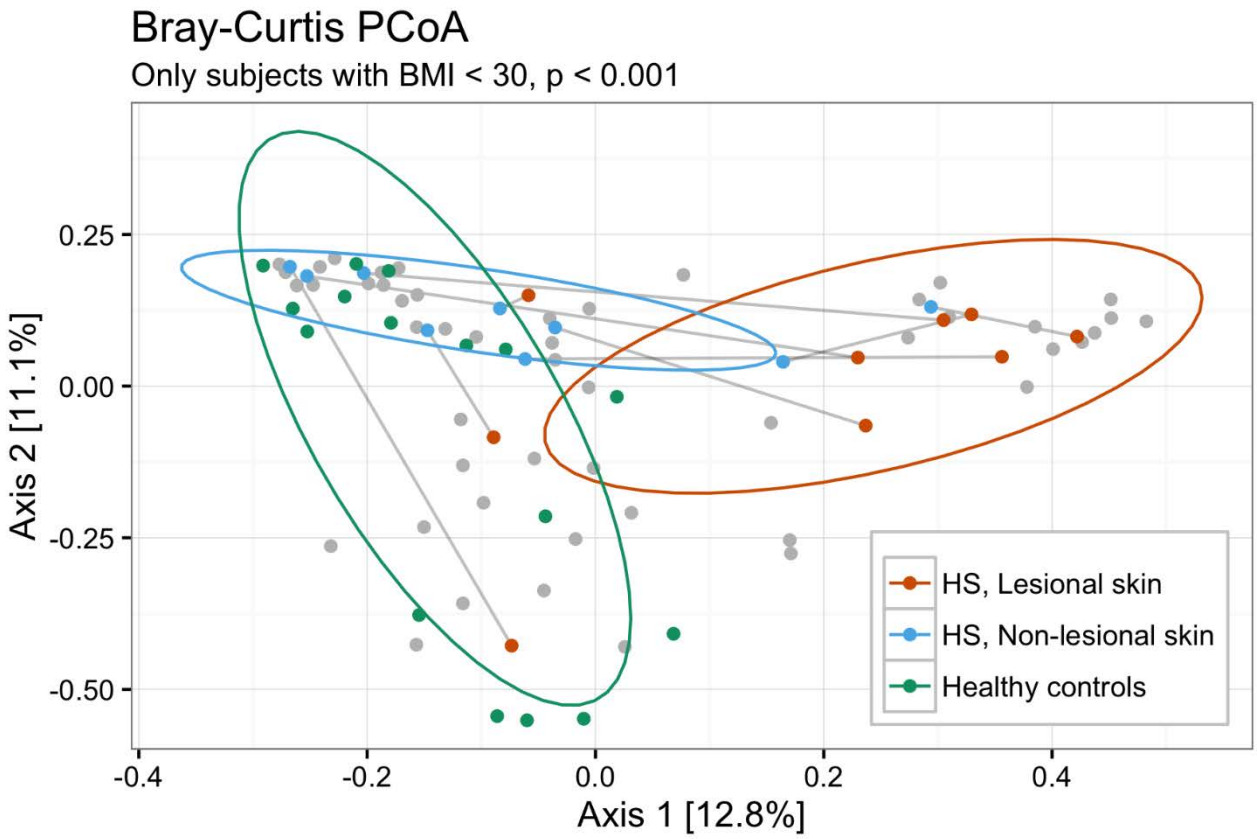

PCoA plot showing the difference between lesional samples originating from patients receiving topical treatment versus no treatment. P-value corresponds to Adonis PERMANOVA test. Ellipses delineate the 75% prediction areas of samples from each group.

**eFigure 3A.** Bray-Curtis PCoA, HS Lesional Skin, Axilla Samples Only



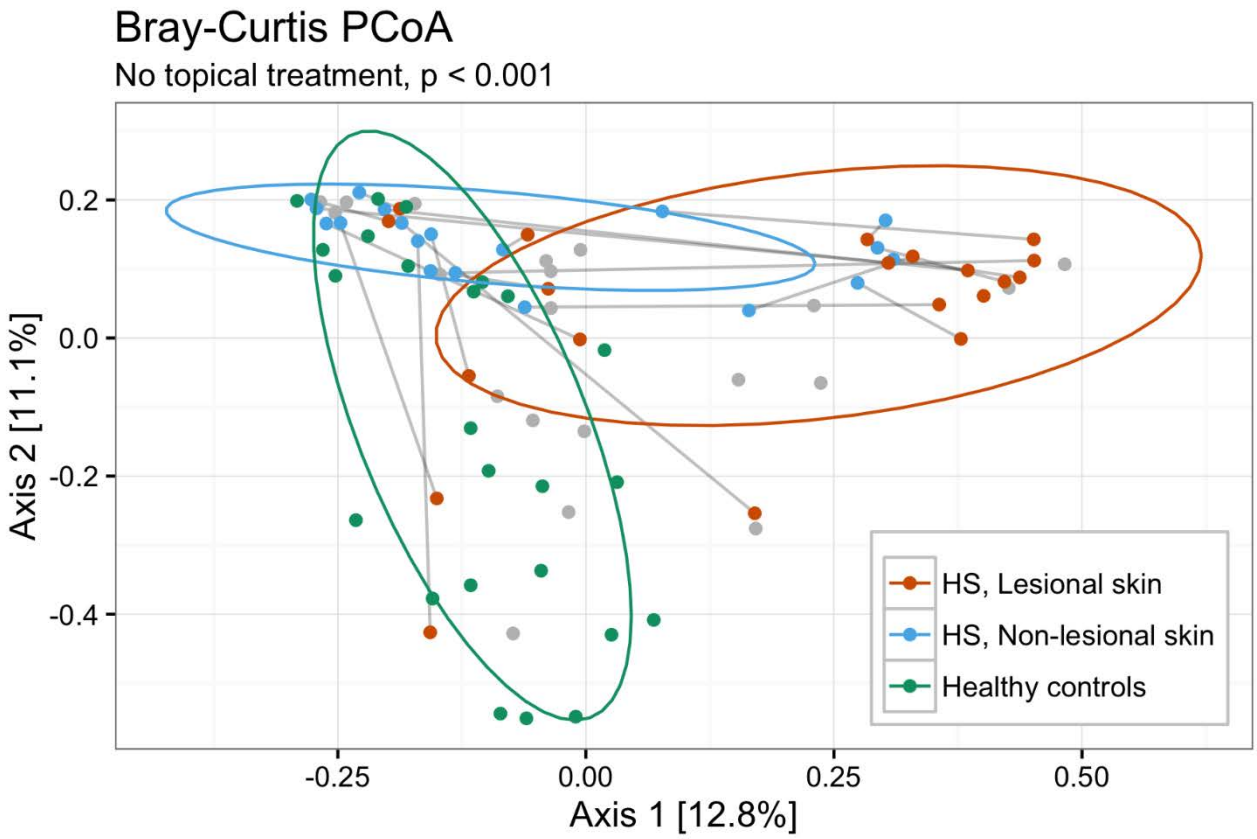

PCoA plot showing the difference between sample groups, stratified to only samples originating from the axilla. P-value corresponds to Adonis PERMANOVA test. Ellipses delineate the 75% prediction areas of samples from each group.

**eFigure 3B.** Bray-Curtis PCoA, HS Lesional Skin, Low BMI Only




PCoA plot showing the difference between sample groups, stratified to only samples originating from participants with BMI below 30. P-value corresponds to Adonis PERMANOVA test. Ellipses delineate the 75% prediction areas of samples from each group.

**eFigure 3C.** Bray-Curtis PCoA, HS Lesional Skin, No Topical Treatment




PCoA plot showing the difference between sample groups, stratified to only samples originating from participants not receiving any topical treatment. P-value corresponds to Adonis PERMANOVA test. Ellipses delineate the 75% prediction areas of samples from each group.