Table S1: Related to Figure 1

Table S1: Summary Statistics for Clinical Variables at the Baseline Visit (n=46)

Variable	Min	Median	Max	Mean	SD
Duration of Diabetes (years)	0	12	45	14	9.5
Duration of Ulcer (days)	1	20	187	32.3	37.7
Age (years)	28	53	78	53	9.5
Toe/Brachial Pressure Index	0.39	0.87	1.36	0.86	0.26
Ankle/Brachial Pressure Index	0.6	1.0	1.2	0.96	0.16
HgbA1c (%)	5.4	7.6	15.3	8.17	2.05
WBC (mm3)	4900	8200	13200	8293	1949
C Reactive Protein (mg/L)	0.5	1.2	40.4	3.12	6.51
Glucose	78	190	460	195.38	83.27

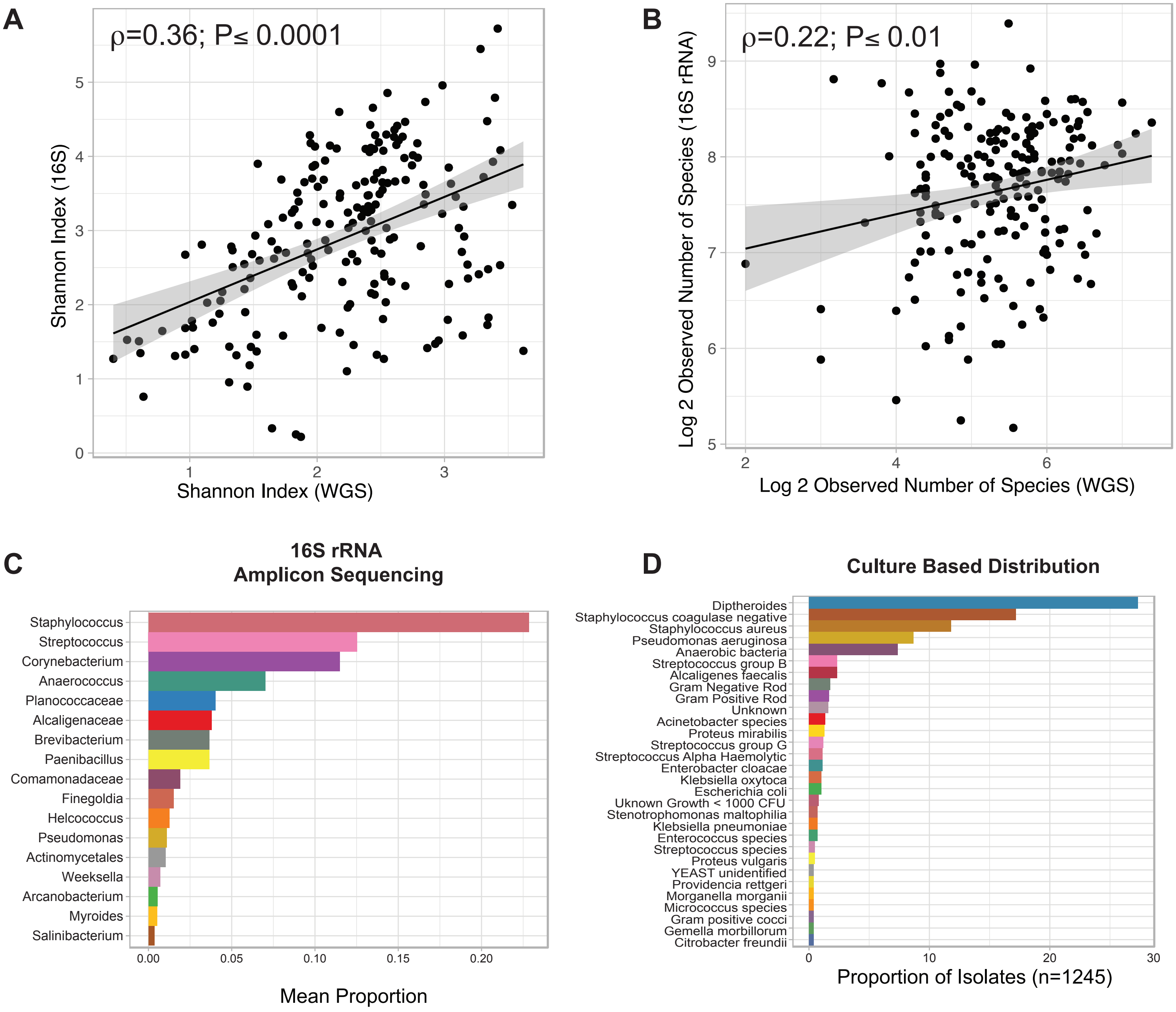

Figure S1; Related to Figure 1: Alpha diversity metrics of the DFU microbiome for 16S rRNA gene sequencing versus shotgun metage-nome sequencing. A) Shannon Index and B) Observed Number of Species. Most abundant taxa Identified in diabetic foot ulcers. C) Taxa identified by 16S rRNA gene sequencing of the V1-V3 hypervariable region from the same samples and DNA extracts as this study. Mean proportion calculated as mean proportion in all samples >1%. D) Isolates cultured in the laboratory from DFU. Proportion indicates total proportion of all isolates (n=1245 from a total of 384 samples).

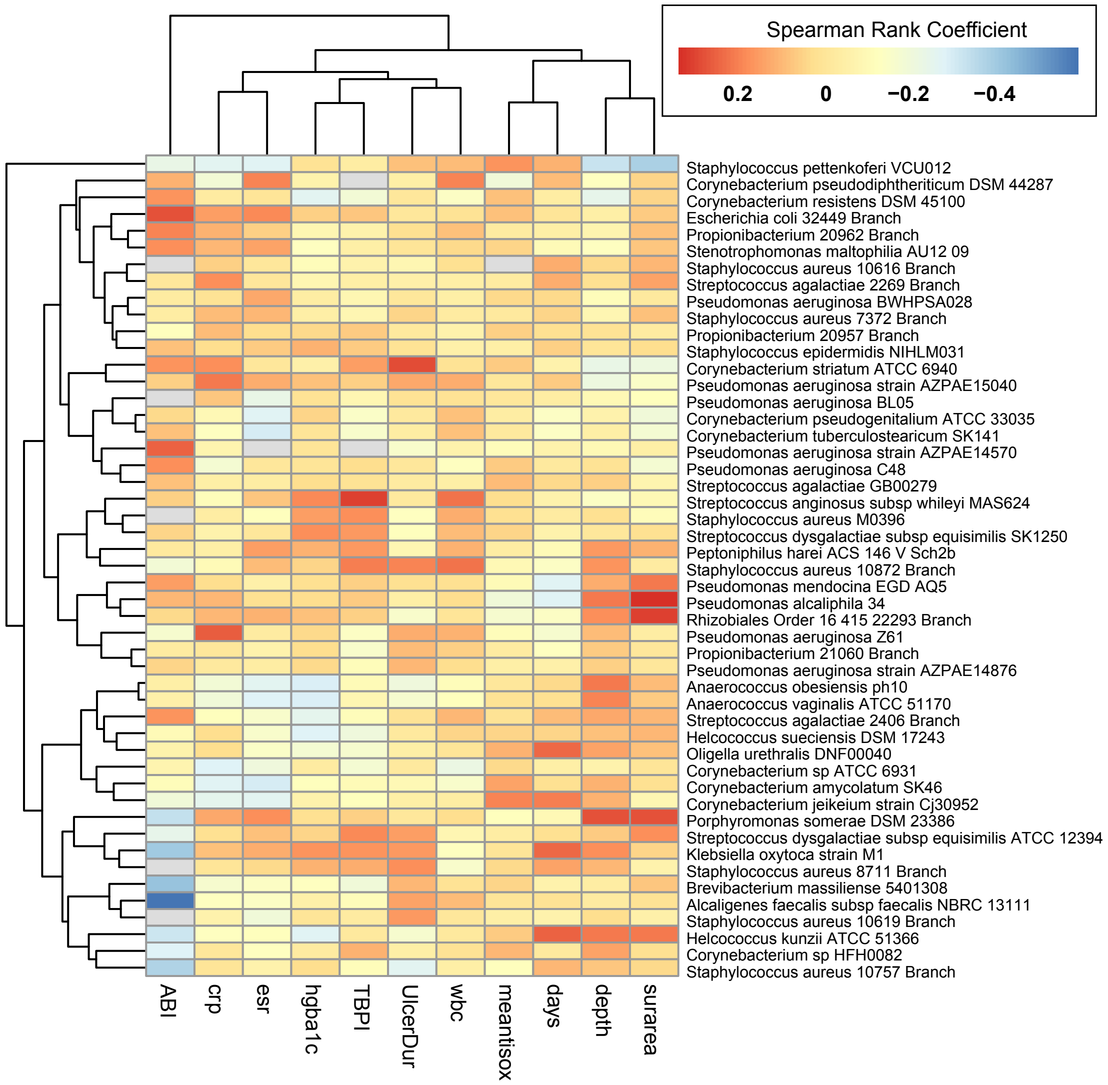

Figure S2; Related to Figure 2: Association of top taxa with clinical factors. Shown is a correlation heatmap and hierarchical clustering of strain-level classification of taxa (>0.5% abundance in all samples) with all measured clinical co-variates. Color corres-ponds to Spearman rank coefficient (red and blue indicating positive and negative correlation, respectively).

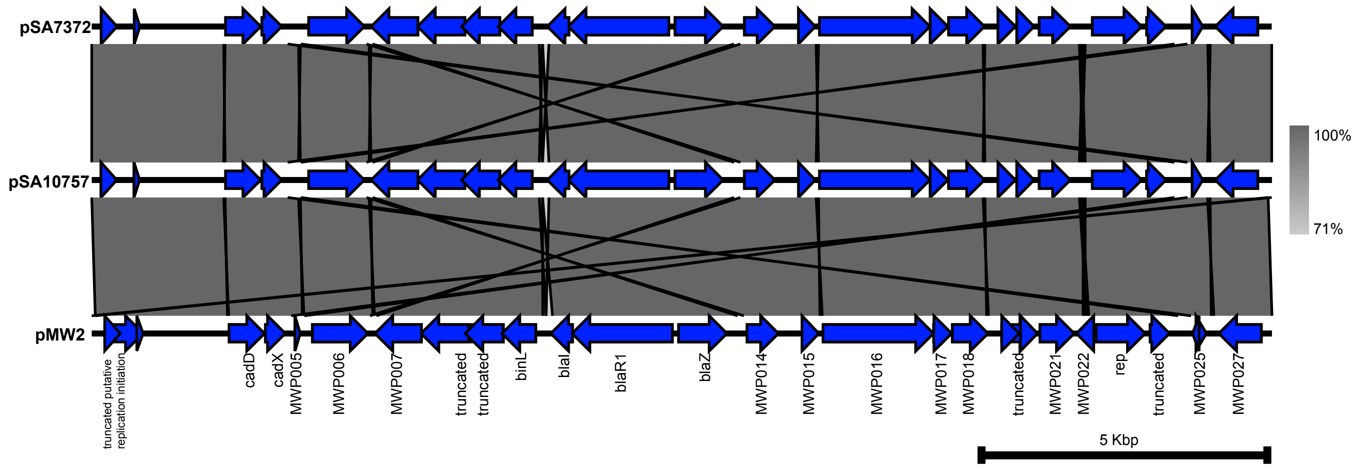

Figure S3; Related to Figure 4: Plasmids of SA7372, SA10757, and S. aureus MW2. Gray bars denote blastn identity between each plasmid sequence.

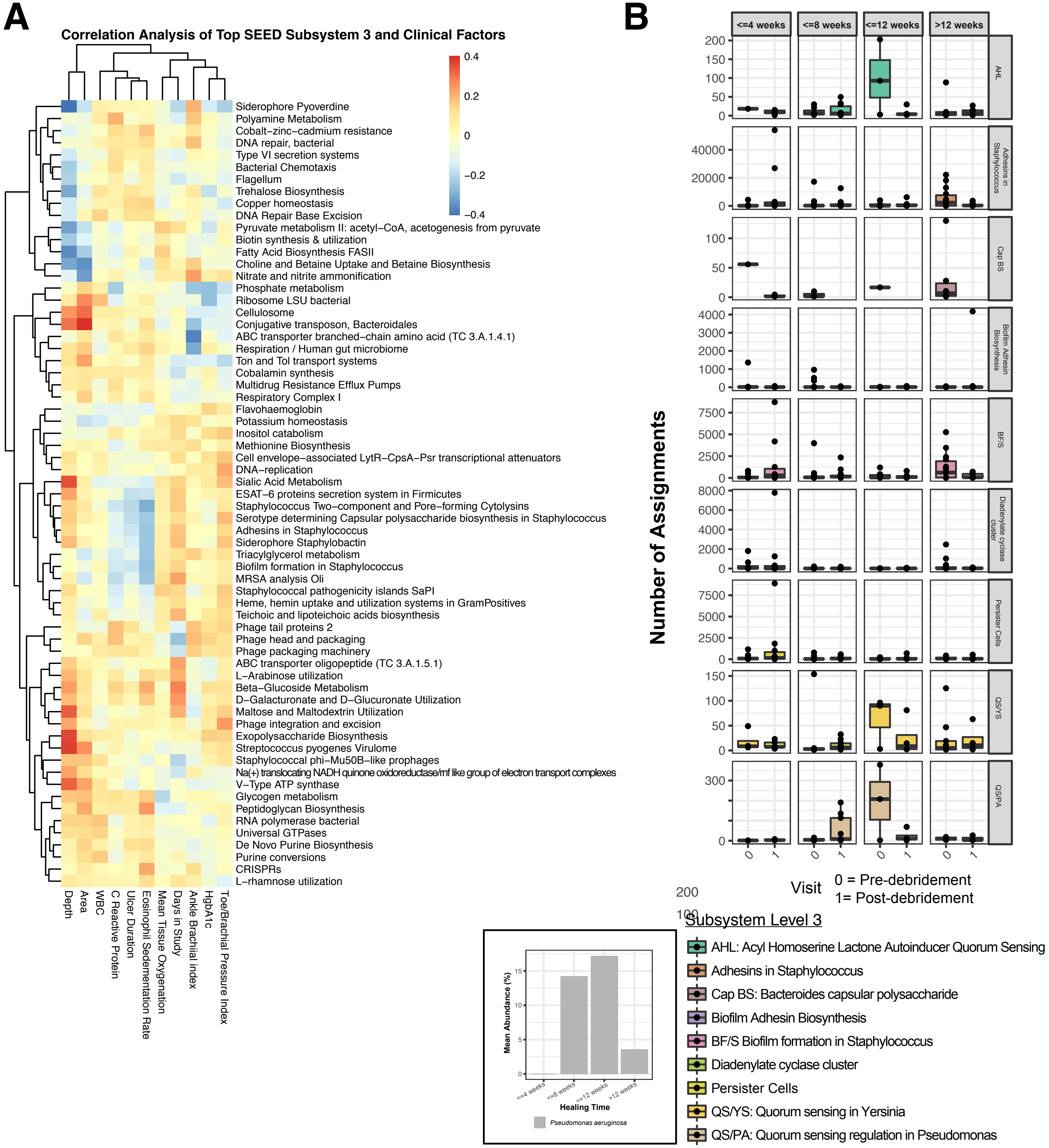

Figure S4; Related to Figure 5: Functional SEED subsystems level 3 associated with all clinical factors and outcomes. A) Shown is a correlat-ionheatmap and hierarchical clustering of SEED subsystem level 3 annotations with all measured clinical co-variates. Color corresponds to Spearman rank coefficient (red and blue indicating positive and negative correlation, respectively). B) Functional annotation in response to debridement. Number of read assignments to SEED subsystem level 3 annotat-ions, normalized by total read depth per sample, with biofilm-specific terms (y-axis). Samples are stratified by pre- or post-debridement visits, indicated by 0 or 1, respectively, on the x-axis. Each plot represents a single term. Inset shows mean abundance (%; y-axis) of *Pseudomonas aeruginosa* by healing time (x-axis).

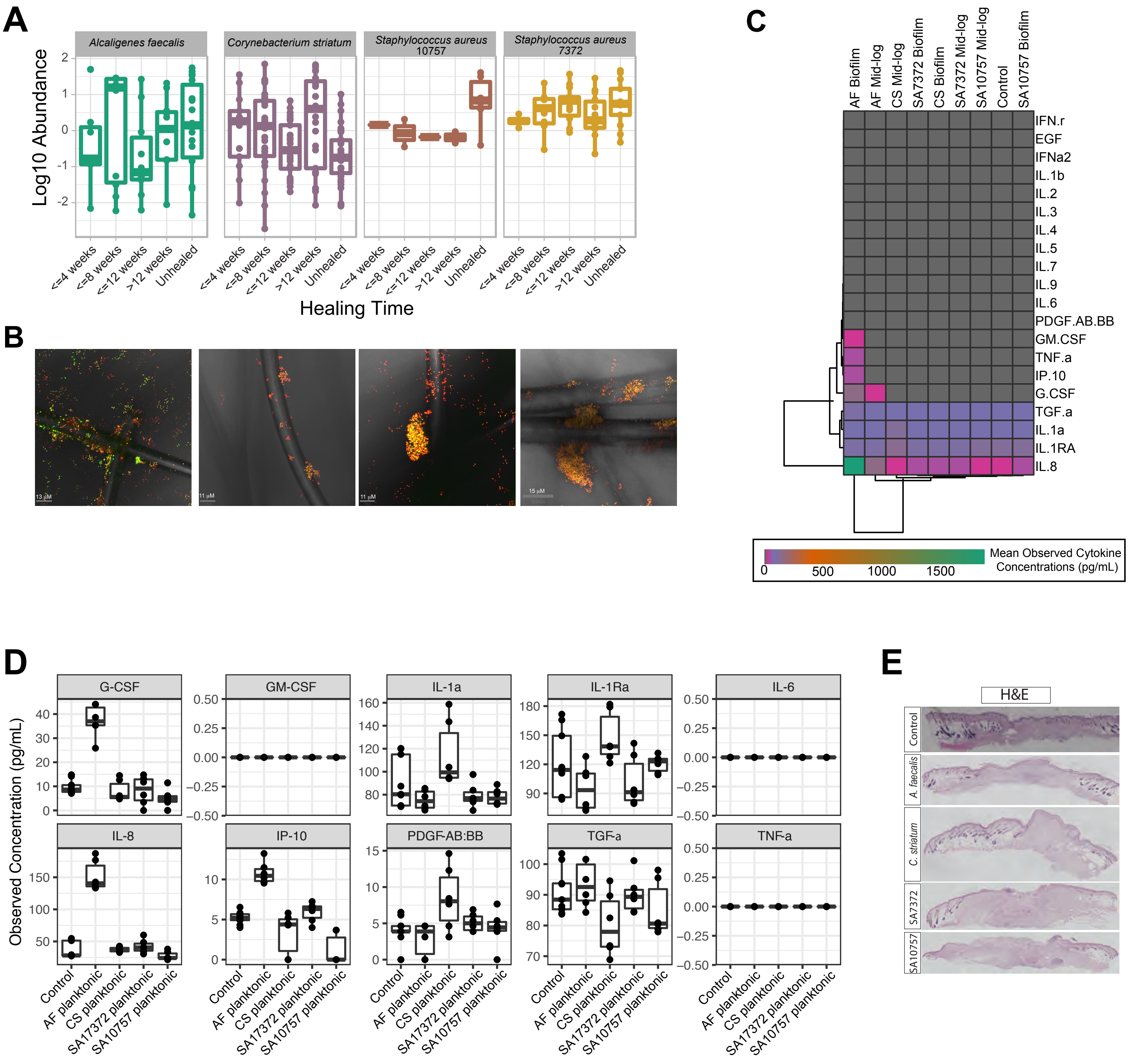

Figure S5; Related to Figure 6: Biofilm formation by wound isolates. A) Mean proportion of each species targeted for in vitro and in vivo B) Representative image of biofilm formation by each isolated species and stained with the LIVE BacLight Bacterial Gram Stain Kit. C) Heatmap and hierarchal clustering of all conditions displaying the mean concentration of cytokines measured (pg/mL). B) Observed concentration of secreted cytokines (pg/mL) after exposure to planktonic cultures. D) Detection of cytokines from primary keratinocytes exposed for 8 hours to conditioned media from mature biofilms or mid-log phase planktonic cultures of *A. faecalis* (AF), *C. striatum* (CS), *S. aureus* 7372 (SA7372), or *S. aureus* 10757 (SA10757). Each condition was repeated with three biological replicates and two or three technical replicates of each (n=6-9). E) Hemotoxylin and eosin (H&E) staining of cross-sectioned mouse wounds at day 28 post-wounding and infection.

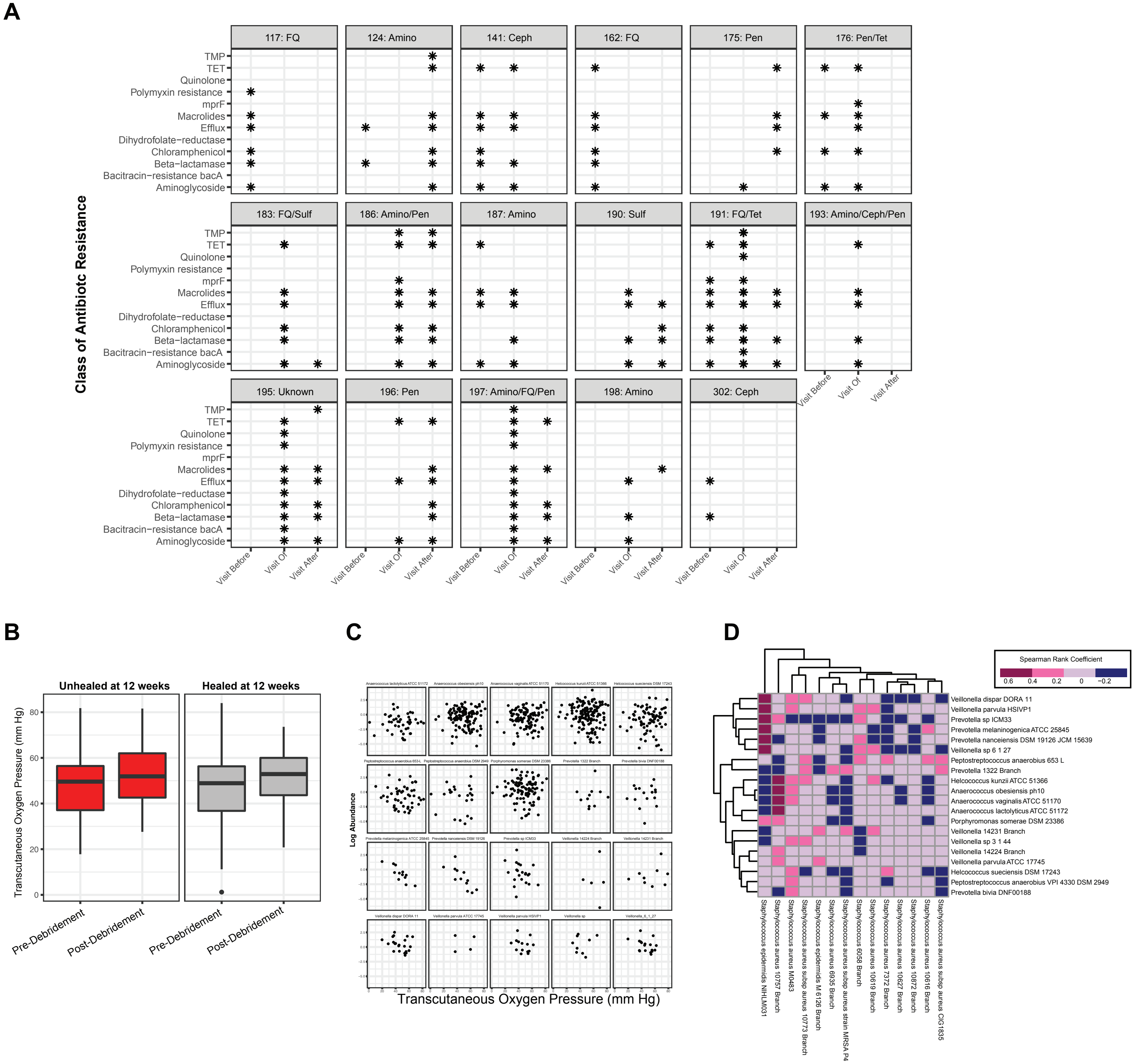

Figure S6; Related to Figure 7: A) Antibiotic resistance patterns and tissue oxygenation. Each box represents a single patient labelled by the class or classes of antibiotic administered. The visit before, visit of, and visit after antibiotics are given is along the x-axis and each class of antibiotic resistance detected is plotted along the y-axis and represented as a (*) to indicate positive detection. B) Mean tissue oxygenation measured as transcutaneous oxygen pressure (mm Hg) pre- and post-debridement. C) Transcutaneous oxygen pressure (mm Hg) of each sample (x-axis) and abundance (%) of individual anaerobic strains (y-axis). D) Strain-level association of S. aureus with anaerobes. Shown is a correlation heatmap and hierarchical clustering of unique S. aureus strains detected with unique anaerobic strains detected. Color corresponds to Spearman rank coefficient (burgundy and blue indicating positive and negative correlation, respectively).