

Supplementary Files in Excel format

File: "Table S1.xlsx": Read counts with taxonomic annotation per ASV per sample for the first sequencing batch of 50 samples

File: "Table S2.xlsx": Read counts with taxonomic annotation per ASV per sample for the second sequencing batch of 39 samples

Supplementary tables in Word format

| Centre | Bacteria | Mean PSA (min – max) | Median PSA | N* |
|--------|-------------------|-------------------------|------------|----|
| All | Neither | 4.75 (0.3-17.6) | 3.40 | 22 |
| All | Microaerophilic | 6.51 (2.43-12.0) | 6.34 | 6 |
| All | Obligate anaerobe | 9.25 (1.12-26.0) | 6.75 | 12 |

Table S3. Summary result of PSA vs type of bacteria for Barts and Newham patients. *N=40, Of the 18 patients having anaerobes, 1 had both Obligate anaerobe and Microaerophilic bacteria and actual number of individual patients with either OA &/or MA is 17 out of 39 (43.5%). "Neither": samples with no microaerophilic or obligate anaerobe species detected

| | No of Cases | TUR of Prostate | Cancer interventions | Continued surveillance | PSA median | PSA Mean (range) |
|----------------------------------|-------------|-----------------|----------------------|------------------------|------------|--------------------|
| Anaerobe negative PC negative | 11 | 1 | 0 | 10 (91%) | 0.75 | 2.89 (0.3-17.6) |
| Anaerobe positive PC negative | 7 | 2 | 0 | 5 (71%) | 4.54 | 5.39 (1.1-13.6) |
| Anaerobe negative PC positive | 11 | 0 | 4 | 7 (64%) | 6.70 | 6.61 (1.3-12) |
| Anaerobe positive PC positive | 10 | 1 | 7 | 2 (20%) | 7.36 | 10.49 (2.4-26) |

**Table S4. Long term follow-up of pooled analysis of anaerobe* versus Prostate Cancer and PSA.
*Anaerobe positive status includes both obligate anaerobes and microaerophilic**

| | OA positive | OA negative | OA threshold |
|---------------------------|----------------|--------------|--------------------|
| Culturomics cohort (N=39) | 30.8% (n=12) | 69.2% (n=27) | - |
| PSA (mean ng/mL) | 9.25 (p=0.132) | 5.04 | - |
| PROVENT series (N=89) | 29.2% (n=26) | 70.8% (n=63) | ≥10% of all genera |
| PSA (mean ng/mL) | 7.65 (p=0.747) | 7.38 | ≥10% of all genera |
| PROVENT series (N=89) | 25.8% (n=23) | 74.2% (n=66) | ≥15% of all genera |
| PSA (mean ng/mL) | 8.13 (p=0.291) | 7.23 | ≥15% of all genera |
| PROVENT series (N=89) | 19.1% (n=17) | 80.9% (n=72) | ≥20% of all genera |
| PSA (mean ug/L) | 9.30 (p=0.007) | 7.03 | ≥20% of all genera |

Table S5. Sample frequency and mean PSA for OA positive and OA negative groups. Culturomics samples were stratified based on the presence of one or more OAs. The nine OA genera used to stratify PROVENT samples are: *Finegoldia*, *Fusobacterium*, *Prevotella*, *Peptoniphilus_A*, *Peptoniphilus_B*, *Peptoniphilus_C*, *Peptostreptococcus*, *Veillonella* and *Veillonella_A*. OA threshold: Pooled relative abundance threshold for a PROVENT sample to be considered as OA positive. The displayed p-value is from Welch's two-sample t-test

| | Number of Positive Patients | Median Relative Abundance (+/- of those >0%) | Percentage ≥10%/Number Relative Abundance |
|---------------------------|-----------------------------|--|---|
| <i>Ochrobactrum_A</i> | n = 14 | 0.084% 0.016-0.803% | n = 0/14 (0%) |
| <i>Fusobacterium</i> | n = 10 | 0.199% 0.006-41.25% | n = 2/10 (20%) |
| <i>Peptostreptococcus</i> | n = 9 | 0.718% 0.009 – 3.703% | n = 0/9 (0%) |
| <i>Peptoniphilus_A</i> | n = 43 | 0.950% 0.002-69.83% | n = 1/43 (2.3%) |
| <i>Prevotella</i> | n = 44 | 5.597% 0.007-62.73% | n = 17/44 (38.6%) |

Table S6. Relative abundance of 5 bacteria of interest in PROVENT series (n=89)

Supplementary Figures

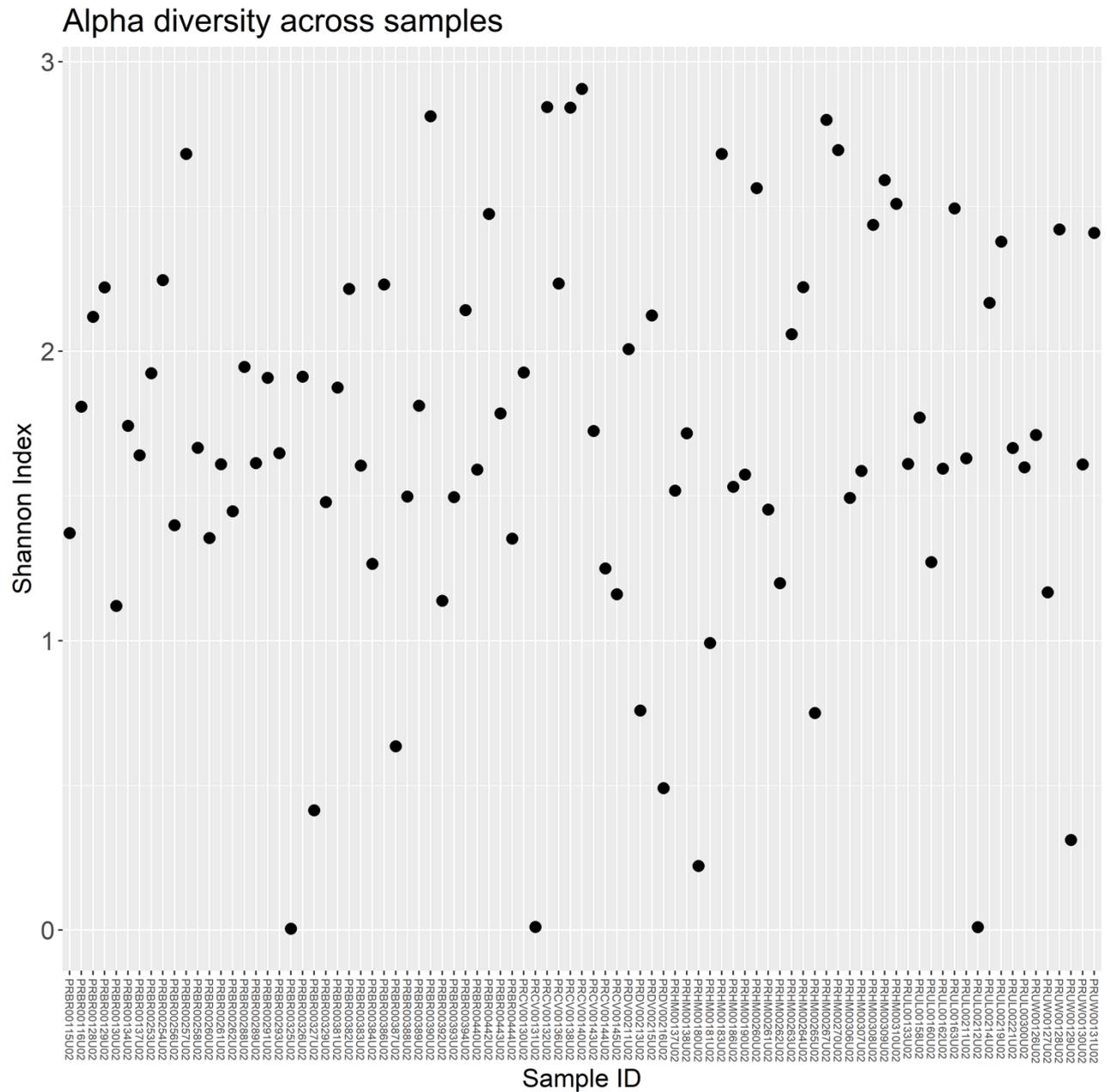


Figure S1. Alpha diversity (Shannon index) per sample from the PROVENT cohort (n = 89). Higher Shannon index values indicate greater microbial diversity.

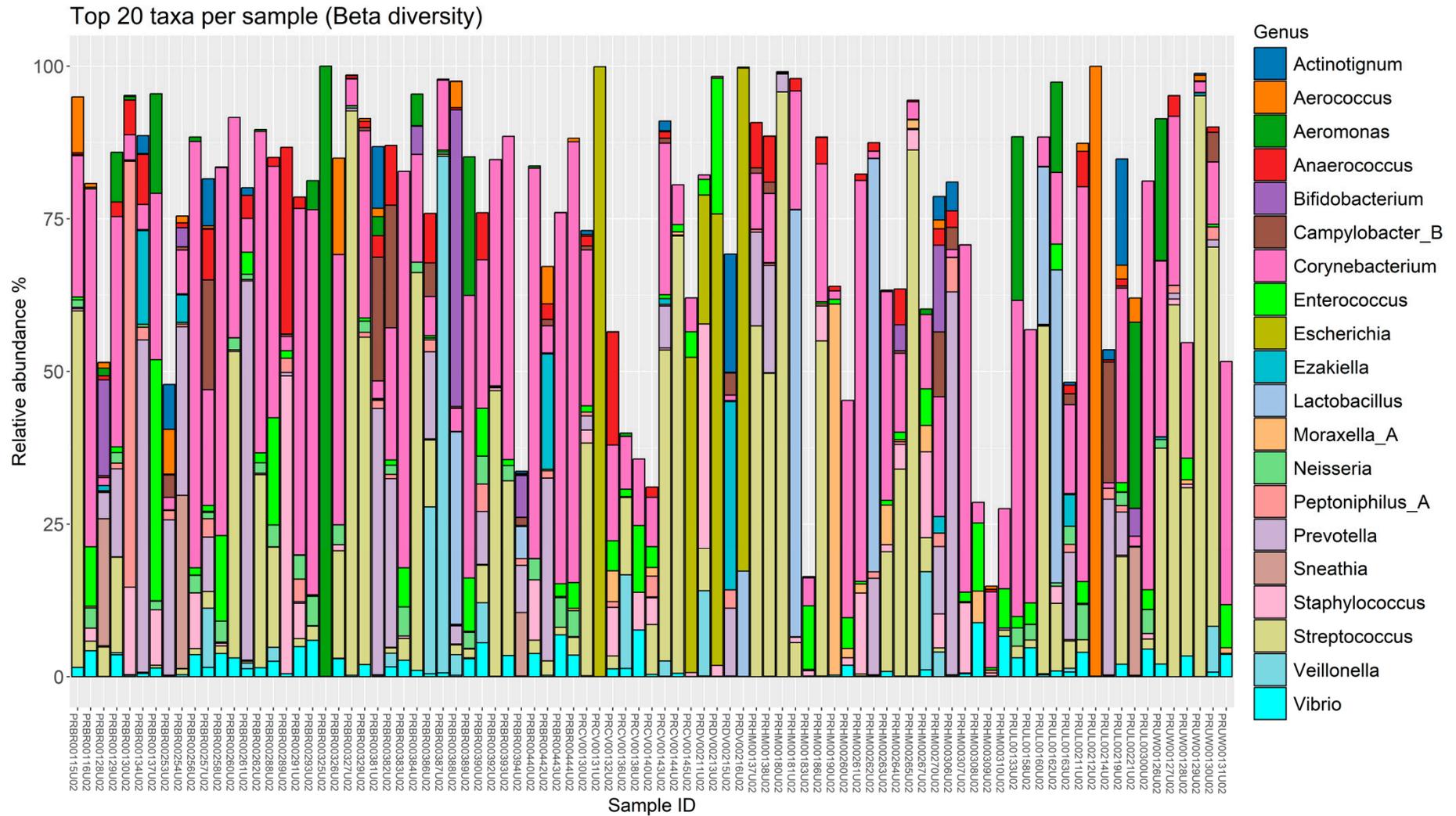


Figure S2. Beta diversity representation of the samples from the PROVENT cohort (n = 89) using the 20 most abundant genera. Samples whose relative abundance does not amount to 100% are due to the exclusion of unidentified ASVs to the Genus level as well as due to the exclusion of ASVs not belonging to the 20 most abundant genera.

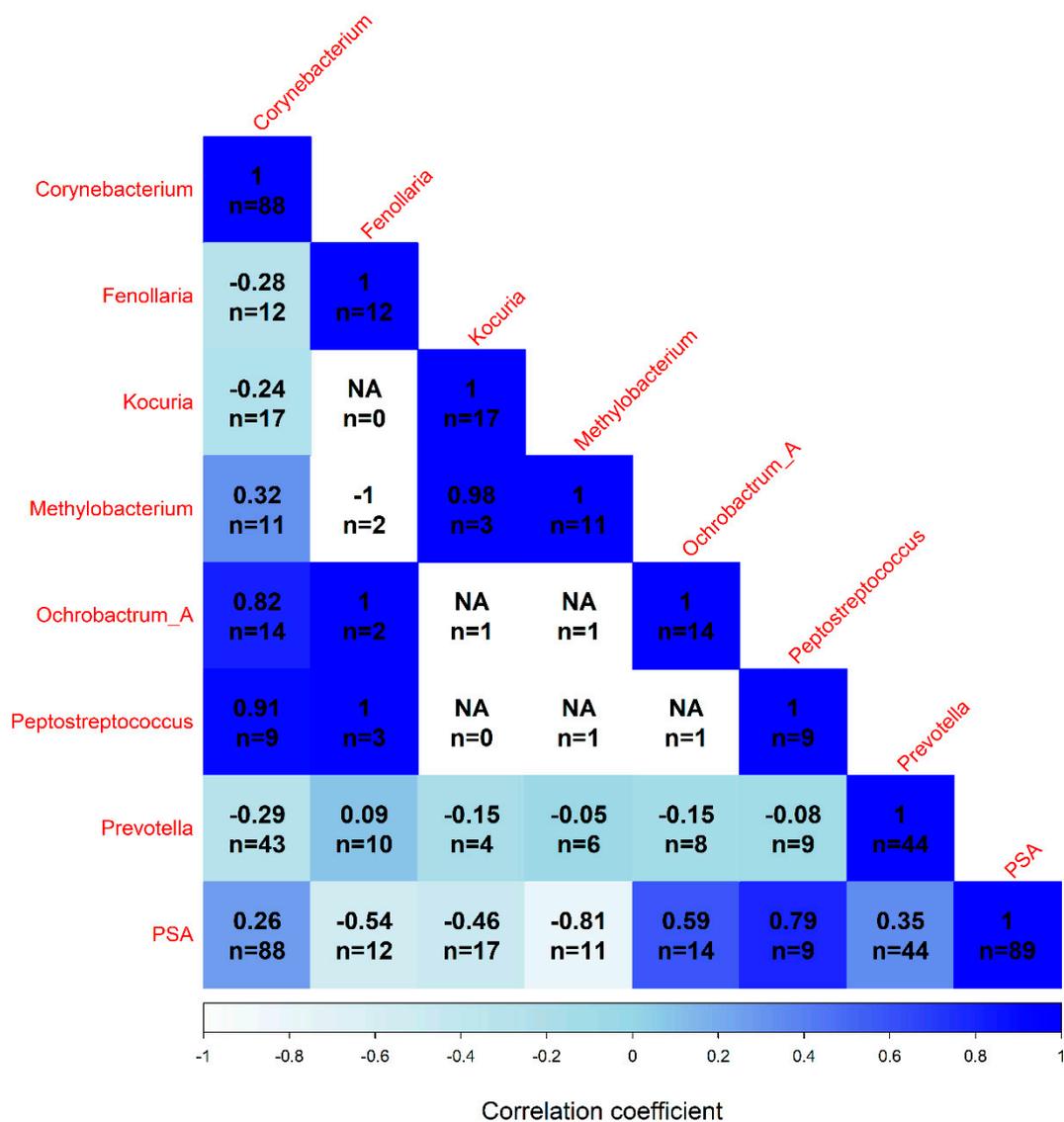


Figure S4 Correlation matrix illustrating the relationships between the relative abundance of genera and PSA levels. All displayed genera had a Pearson correlation with PSA with a p-value < 0.1. The Pearson correlation coefficient and the number of samples taken for each comparison is displayed in each cell. NA: correlation coefficient could not be estimated due to lack of an appropriate number of samples.