

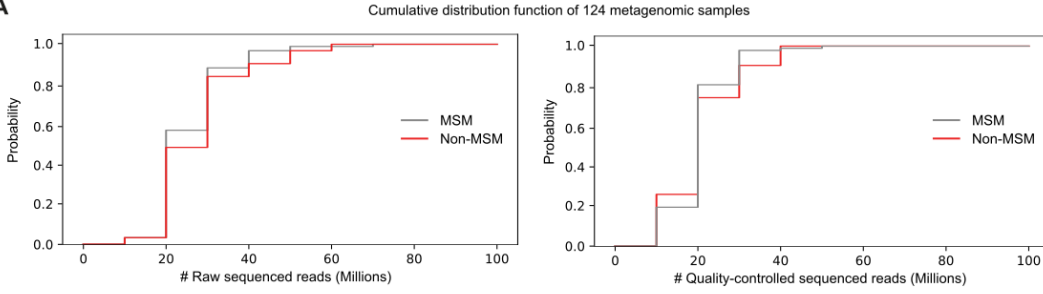
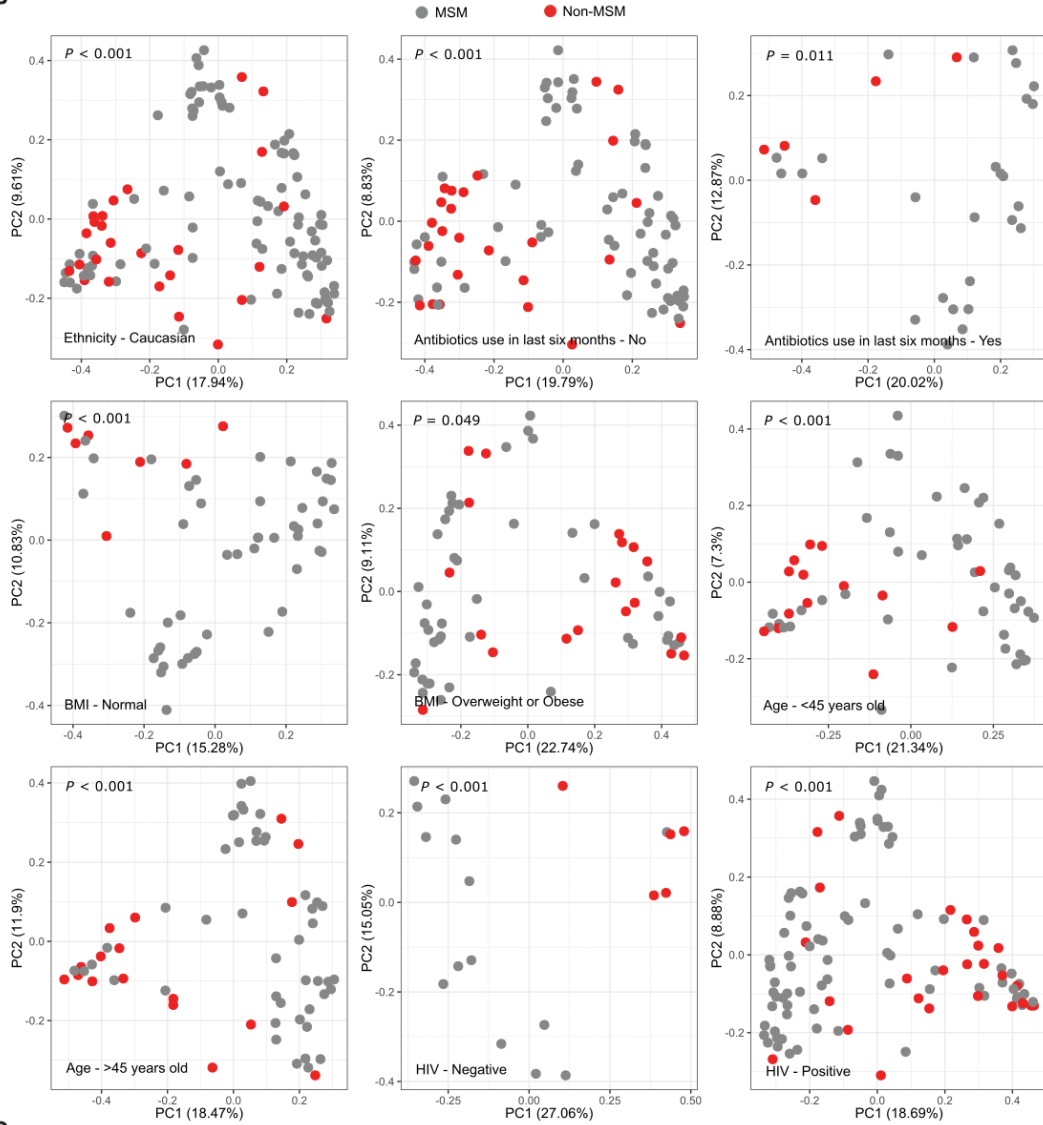
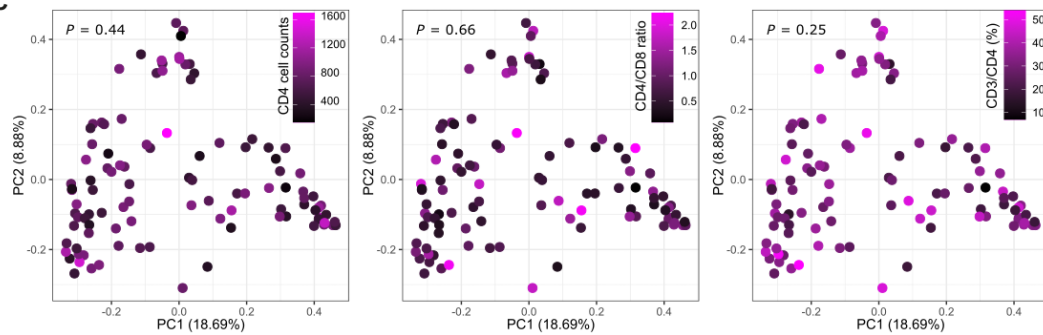
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**Supplemental information**

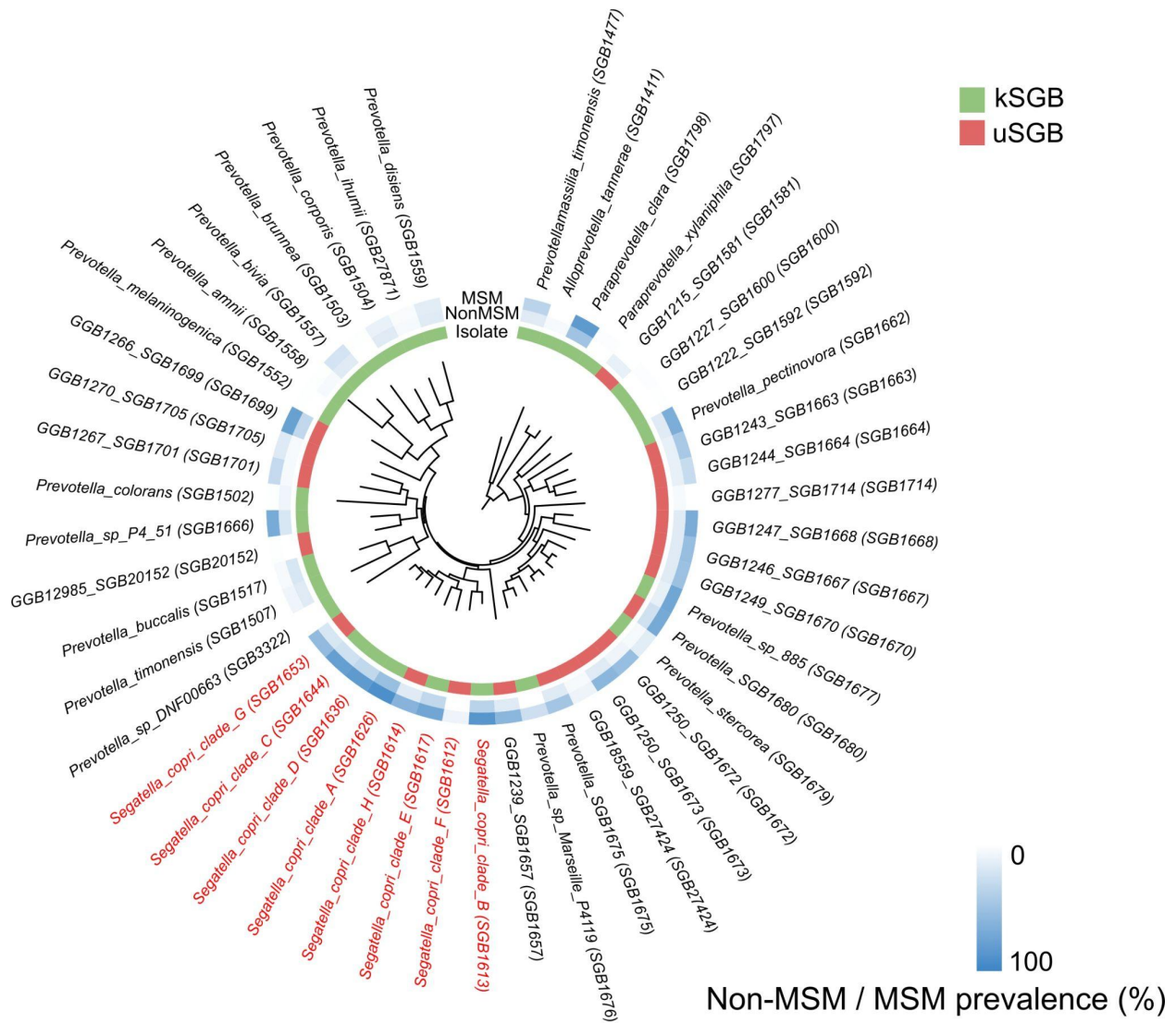
**Establishment of a non-Westernized  
gut microbiota in men who have sex with men  
is associated with sexual practices**

**Kun D. Huang, Lena Amend, Eric J.C. Gálvez, Till-Robin Lesker, Romulo de Oliveira, Agata Bielecka, Aitor Blanco-Míguez, Mireia Valles-Colomer, Isabel Ruf, Edoardo Pasolli, Jan Buer, Nicola Segata, Stefan Esser, Till Strowig, and Jan Kehrman**

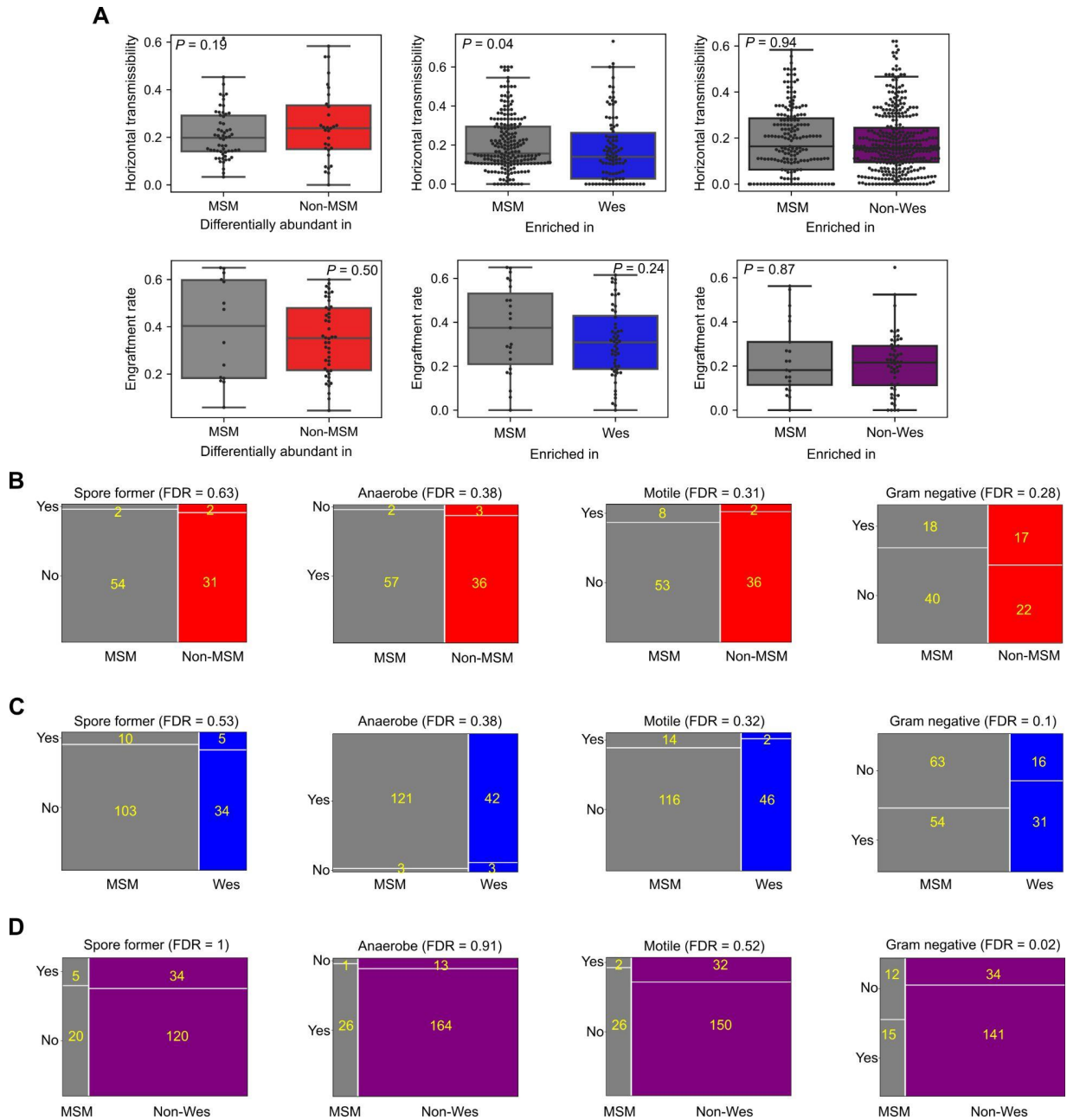
## Supplemental Information

**A****B****C**

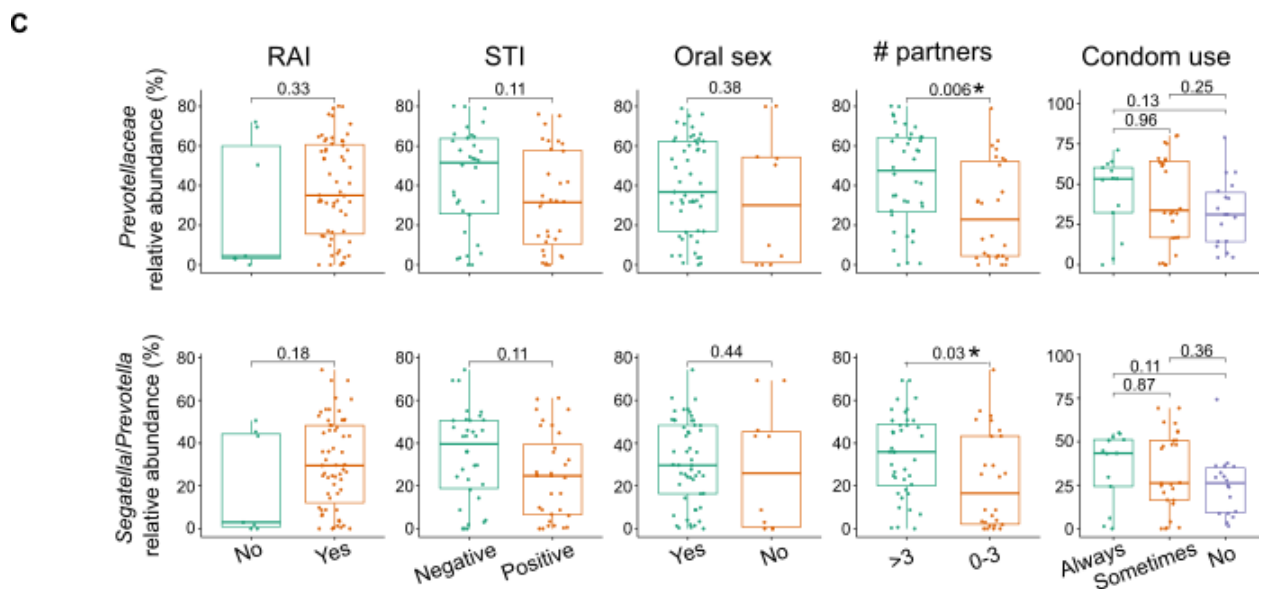
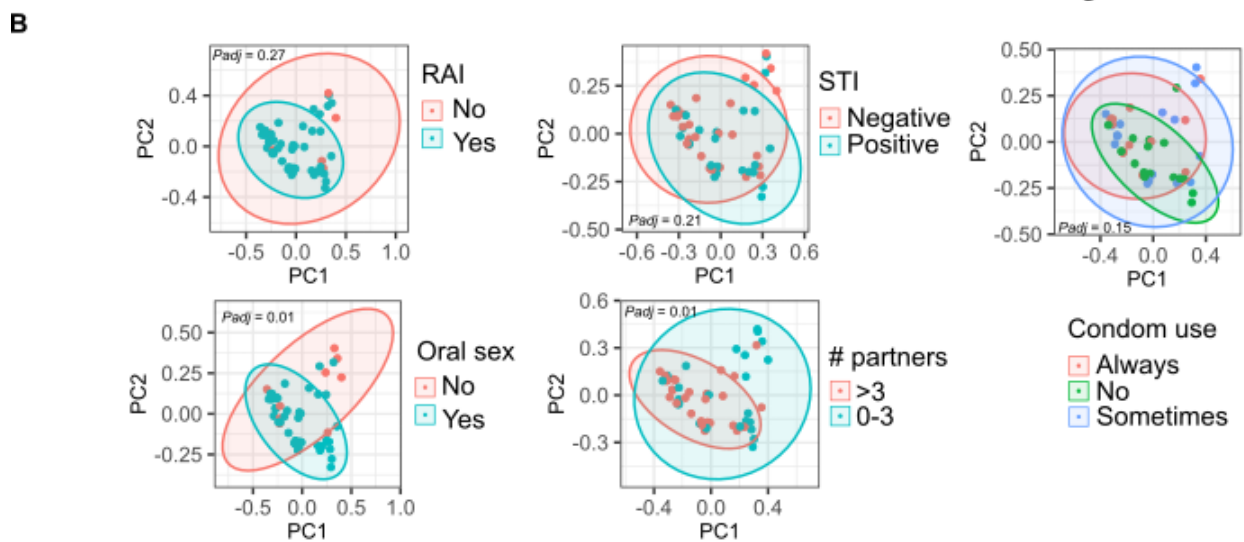
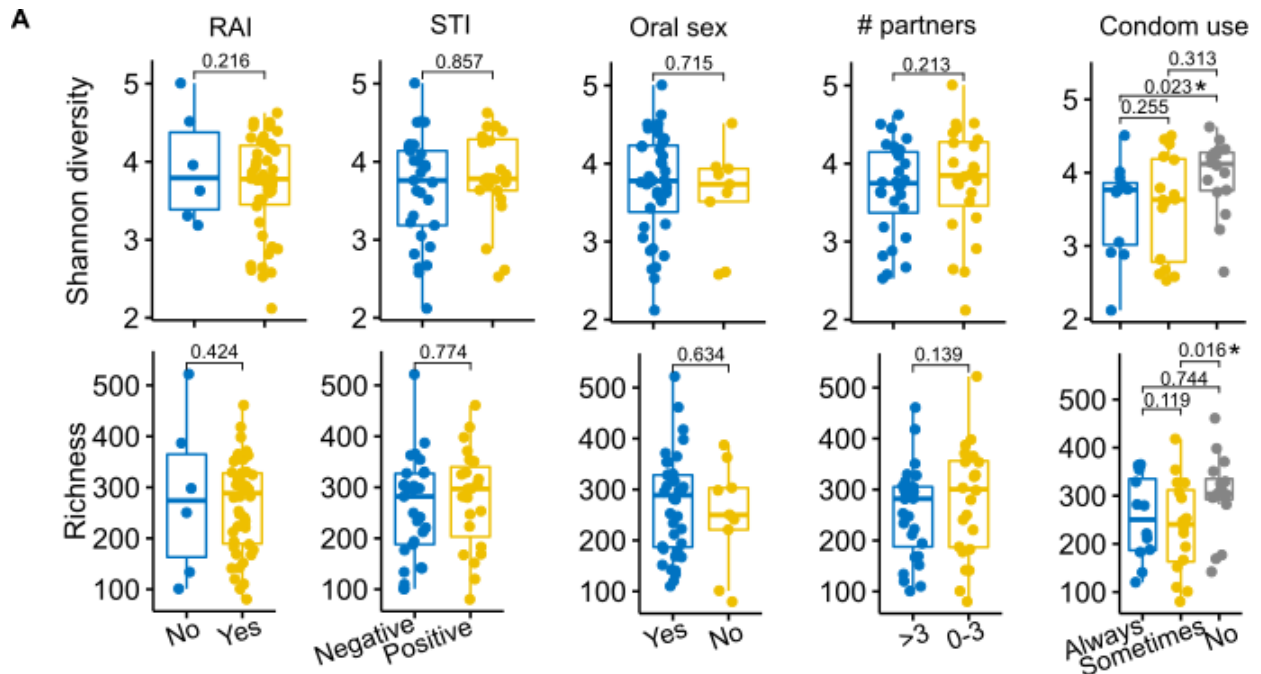
**Figure S1. Basic statistics of sequencing reads and comparisons of the taxonomic composition of MSM samples with that of Non-MSM samples for anthropometric factors and HIV infections, Related to Figure 1, Table 1 and Table 2.** **A** Cumulative distribution function of sequenced reads in MSM and non-MSM samples (Wilcoxon rank-sum test, raw reads (left)  $P = 0.4308$ ; quality-controlled reads (right)  $P = 0.9608$ ). **B** Principal coordinate analysis (PCoA) based on MetaPhlAn 4 <sup>[S1]</sup> abundance profiles between MSM and non-MSM samples for each subset of samples stratified by covariates (PERMANOVA, all  $P < 0.05$ ). **C** PCoA analysis based on MetaPhlAn 4 abundance profiles of a HIV-infected individuals ( $N = 103$ , Table 2) regarding HIV infection factors including CD4 cell counts, CD4/CD8 ratio and CD3/CD4 (%) (PERMANOVA, all  $P > 0.05$ ).



**Figure S2.** The phylogenetic structure of *Prevotellaceae* species members reconstructed using PhyloPhIAn 3.0 <sup>[S2]</sup> and visualized using iTOL v6 <sup>[S3]</sup>, Related to **Figure 2**. Labels of *S. copri* complex clades were highlighted in red. Prevalence indicates the percentage of individuals carrying that species member in the MSM or non-MSM population, respectively.



**Figure S3. Assessment of engraftment rate and horizontal transmissibility in MSM-associated species, Related to STAR Methods.** **A** Boxplots reporting the horizontal transmissibility and engraftment rates of species enriched in MSM (gray), non-MSM (red), Westernized (blue) and non-Westernized subjects (purple).  $P$  values were estimated using the Wilcoxon rank-sum test. **B**, **C** and **D** Mosaic plots showing the number of species enriched in MSM (gray) compared to non-MSM subjects (red), Westernized subjects (blue) and non-Westernized subjects (purple) for possessing transmission-related phenotypes, respectively. FDRs were estimated using Fisher's exact test followed by Benjamini-Hochberg correction for multiple hypotheses comparison.



**Figure S4. Association analysis of MSM gut microbiome and sexual practices, Related to Figure 4.** **A** Boxplots reporting Shannon diversity and richness for each sexual practice. The significance was estimated by fixed effects linear models which control confounding effects. Asterisk denotes the statistical significance ( $P_{adj} < 0.05$ ). **B** Principal coordinate analysis (PCoA) based on microbial abundance profiled using MetaPhlAn 4 between groups in each sexual behavior. Covariate-controlled PERMANOVA was used to test the significance of sample separation as input using log-transformed relative abundances. **C** Boxplots reporting MetaPhlAn 4 relative abundances of members at the level of *Prevotellaceae* and *Segatella/Prevotella* for each sexual activity, and the statistical significance was measured using Wilcoxon rank-sum test. Asterisk denotes the statistical significance ( $P < 0.05$ ).



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