**Supplementary Methods**

**In-Silico Analysis of Bacterial Genomes**

16S rRNA gene sequences of *Prevotella* strains (**Table 1**) were downloaded from the Silva database [1] (<https://www.arb-silva.de>). The sequences were aligned using MAFFT version 7 pipeline, the multiple alignment program for nucleotide sequences [2]. The tree was constructed using unweighted pair group method with arithmetic mean (UPGMA) clustering method. The tree was visualized using interactive tree of life [3] (<https://itol.embl.de>) display tool. Metabolic pathway and enzyme analyses were performed using MetaCyc database [4] (<https://metacyc.org>) by extracting the predicted functions, reactions, and metabolites based on the genomes included in the database. Once the analysis results were downloaded, comparative analyses at enzyme, reaction, metabolite and pathway level were performed. In order to better integrate experimental and predicted function data, reactions unique to the evaluated *Prevotella* strains or species were extracted and summarized in **Supplementary Table 2** and **Supplementary Table 3**. Additionally, in order to provide genomic insights into mucin metabolism, we extracted known enzymatic reactions that are involved in mucin degradation. However, it is important to note that there might be other unspecific or unknown reactions that might be contributing to the mucin metabolism.

**Bacterial Strains and Growth Conditions**

*Prevotella* strains and *Gardnerella vaginalis* strain JCP8151B (**Table 1**) were obtained from Biodefense and Emerging Infections Research Resources Repository (BEI) (<https://www.beiresources.org>) (Manassas, VA) or the American Type Culture Collection (ATTC). All strains were grown on tryptic soy agar (TSA) (Becton, Dickinson and Company, Franklin Lakes, NJ) supplemented with 5% defibrinated sheep blood (Quad Five, Ryegate, MT) at 37 οC under anaerobic conditions generated using GasPak EZ Anaerobe Container System. Prior to each assay or experiment, each strain was cultured directly from the frozen stocks on TSA supplemented with 5% defibrinated sheep blood and sub-cultured only once on the fresh TSA plates. Following the bacterial inoculation, the plates were immediately placed in a GasPak EZ Anaerobe Container Systems and incubated overnight at 37 οC.

**Human Endometrial Epithelial Cell Culture and Generation of 3-D Model**

Human endometrial adenocarcinoma cell line (HEC-1A) (ATCC HTB-112) was cultured as monolayers in supplemented modified McCoy’s 5A medium at 37 °C and in 5% carbon dioxide atmosphere. Human 3-D endometrial epithelial cell (EEC) model was generated as previously described [5]. Briefly, cells were grown on collagen-coated dextran microcarrier beads (Cytodex-3; Sigma-Aldrich, St. Louis, MO) in a rotating wall vessel (RWV) bioreactor (Synthecon, Houston, TX) for 21 days and then differentiated 3-D aggregates were transferred into 24-well plates (~2.5 × 106 cells/mL) for infection assays.

**Infection Assays**

*Prevotella* and *Gardnerella* strains were grown for 20 hours as described above, resuspended in sterile Dulbecco’s phosphate-buffer saline (DPBS) to the optical density at 600 nm (OD600) of 0.5. Prior to the infection assays, the bacterial suspensions were serially diluted, plated on TSA supplemented with 5% defibrinated sheep blood and incubated for 48 hours under anaerobic conditions. This standard plating assay also demonstrated viability of the isolates as part of our experimental protocol. The number of colony forming units (CFUs) were counted and used to calculate the concentration of bacteria (CFU/mL) in each bacterial suspension. Monolayer and 3-D EEC were infected at a multiplicity of infection (MOI) of 10 for 24 hours under anaerobic conditions using the GasPak EZ Anaerobe Container System. The MOI of 10 was selected based on *Prevotella* growth rates and optimization of infection assays in our in vitro models with regard to dose, colonization and time of infection with a wide range of vaginal microbiota species to avoid massive bacterial overgrowth. Cytotoxicity was evaluated via trypan blue exclusion and crystal violet staining, as described previously [6]. The quantification of the cell density was performed using Image J software [7]. Briefly, light microscopy images were converted into 8-bit format and background staining was removed. These consistent parameters were used in the processing of all the images.

**Scanning Electron Microscopy (SEM)**

Samples were fixed and processed for SEM as described previously [8] and imaged with a scanning electron microscope (model JSM-6300; JEOL). Images were acquired using an IXRF model 500 digital processor (IXRF Systems Inc., Houston, TX and pseudo-colored using Adobe Photoshop CS5.1 (Adobe, San Jose, CA).

**Gene Expression Assays**

The 3-D EEC model was infected with the *Prevotella* strains or treated with microbial products, poly(I:C) (100 μg/ml) and flagellin (FLA) (0.5 μg/mL), as positive controls for 24 hours. Then, the 3-D aggregates were harvested, and pellets were stored at -80 οC until RNA extraction. Total RNA was extracted from 3-D EEC aggregates using Quick-RNA Miniprep Kit (Zymo Research, Irvine, CA) according to the manufacturer’s instructions. cDNA was synthesized from 1 µg of total RNA using iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA). The gene expression was analyzed by quantitative real-time PCR assays using primers in **Supplementary Table 1** and iTaq Universal SYBR Green Supermix (Bio-Rad) as described previously [5].

**Sialidase Assay**

Sialidase activity was determined in overnight *Prevotella* cultures using AmplexTM Red Neuroaminidase (Sialidase) Assay Kit (A-22178) according to the manufacturer’s instructions. The assay was performed using three independent cultures of each *Prevotella* strain tested.

**Statistical Methods**

All experiments were performed at least in triplicate. Statistical analyses were performed using Prism v8 software (GraphPad, San Diego, CA). Analysis of variance (ANOVA) with Tukey’s adjustment for multiple comparison was used to determine the differences in the means and . An adjusted *p* value less than 0.05 was considered significant. Hierarchical clustering analysis of the cytokines was performed using ClustVis tool [9].

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