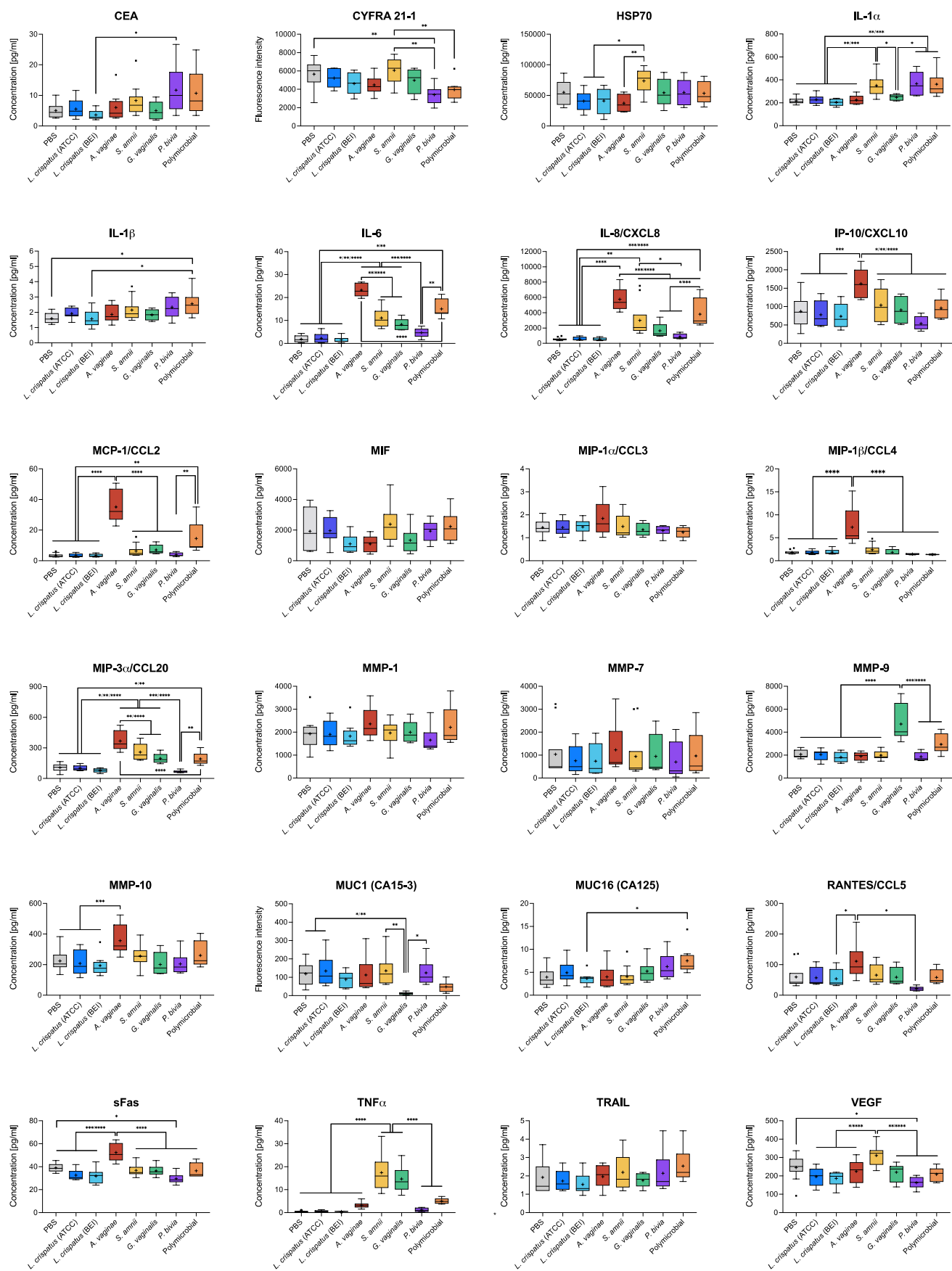
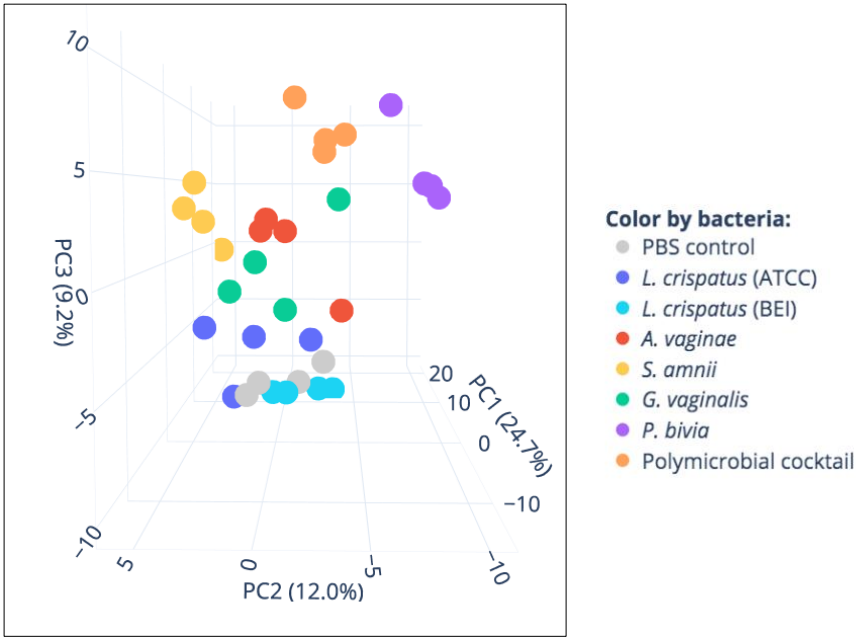




**Supplementary Figure 1. Levels of protein targets in human 3D cell models following mono- and polymicrobial infections with *Lactobacillus* and BVAB.** Human 3D cervical models were infected with bacteria for 24 h under anaerobic conditions. Levels of cytokines/chemokines, epithelial barrier targets and growth factors and cellular stress-related proteins evaluated in the cell culture supernatants using cytometric bead arrays. The concentrations of protein

targets are shown as floating bar graphs. The boxes represent the median and interquartile range and Tukey whiskers. A plus sign (+) on plots indicates mean values. Statistical differences between the mean levels of protein targets among the groups were determined using an analysis of variance (ANOVA) with Tukey's adjustment for multiple comparisons. \* ( $p<0.05$ ), \*\* ( $p<0.01$ ), \*\*\* ( $p<0.001$ ), \*\*\*\* ( $p<0.0001$ ).



**Supplementary Figure 2. Principal component analysis of metabolic profiles of human 3D human cervical models infected with *Lactobacillus* and BVAB.** Human 3D cervical models were infected with bacteria for 24 h under anaerobic conditions. Metabolic profiles were evaluated using cell culture supernatants and UPLC/MS-MS analysis. Principal component analysis (PCA) was performed on metabolomes using Plotly. First three principal components, which explain 45.9% of the variance in the data, are displayed. Each point represents a single sample colored according to the infection group.

**Supplementary Table 1. Total numbers of metabolites significantly altered in 3D cervical cell culture models following colonization with cervicovaginal bacteria.** Human 3D cervical models were infected with bacteria for 24 h under anaerobic conditions. Metabolic profiles were evaluated using cell culture supernatants and UPLC/MS-MS analysis. Statistical differences between the mean intensities of metabolites among the groups were determined using Welch's two-sample *t*-test with FDR correction. Gold- and purple colors indicate numbers of metabolites that were enriched and depleted, respectively.

| Compared groups<br>(Welch's <i>t</i> -test) | Number of altered<br>metabolites<br>( $p \leq 0.05$ ) | Number of enriched<br>and depleted<br>metabolites (↑↓) |
|---------------------------------------------|-------------------------------------------------------|--------------------------------------------------------|
| <i>L. crispatus</i> (ATCC) vs. PBS          | 40                                                    | 24   16                                                |
| <i>L. crispatus</i> (BEI) vs. PBS           | 42                                                    | 33   9                                                 |
| <i>A. vaginae</i> vs. PBS                   | 55                                                    | 35   20                                                |
| <i>S. amnii</i> vs. PBS                     | 72                                                    | 50   22                                                |
| <i>G. vaginalis</i> vs. PBS                 | 43                                                    | 25   18                                                |
| <i>P. bivia</i> vs. PBS                     | 67                                                    | 47   20                                                |
| Polymicrobial vs. PBS                       | 105                                                   | 72   33                                                |

**Supplementary Table 2. Unique and shared metabolites altered following infections with single BVAB species and the polymicrobial cocktail in 3D cervical cell culture models.** Human 3D cervical models were infected with bacteria for 24 h. Metabolic profiles were evaluated using cell culture supernatants and UPLC/MS-MS analysis. Statistical differences between the mean intensities of metabolites among the groups were determined using Welch's two-sample *t*-test with FDR correction.

|                                                                                               | Metabolite name                | Superpathway | Subpathway                                           |
|-----------------------------------------------------------------------------------------------|--------------------------------|--------------|------------------------------------------------------|
| <i>Unique metabolites altered by the polymicrobial community</i>                              | Dimethylarginine               | Amino Acid   | Urea cycle; arginine and proline metabolism          |
|                                                                                               | Ethylmalonate                  | Amino Acid   | Leucine, isoleucine and valine metabolism            |
|                                                                                               | Caproate (6:0)                 | Lipid        | Medium chain fatty acid                              |
|                                                                                               | Glutarate                      | Lipid        | Fatty acid, dicarboxylate                            |
|                                                                                               | Glycerol                       | Lipid        | Glycerolipid metabolism                              |
|                                                                                               | Octanoylcarnitine              | Lipid        | Fatty acid metabolism (acyl carnitine, medium chain) |
|                                                                                               | N6-Carbamoyl-threonyladenosine | Nucleotide   | Purine metabolism, adenine containing                |
| <i>Metabolites shared across the four single BVAB species and the polymicrobial infection</i> | $\alpha$ -Hydroxyisocaproate   | Amino acid   | Leucine, isoleucine and valine metabolism            |
|                                                                                               | Phenyllactate (PLA)            | Amino acid   | Phenylalanine metabolism                             |
|                                                                                               | 5-Methylthioadenosine (MTA)    | Amino acid   | Polyamine metabolism                                 |
|                                                                                               | Phenylalanylglycine            | Peptide      | Dipeptide                                            |
|                                                                                               | Cytosine                       | Nucleotide   | Pyrimidine metabolism, cytidine containing           |