Mobiluncus mulieris Alters the Transcriptomic Profile of Cervicovaginal Epithelial Cells, Shedding Light on Molecular Drivers of Adverse Reproductive Outcomes

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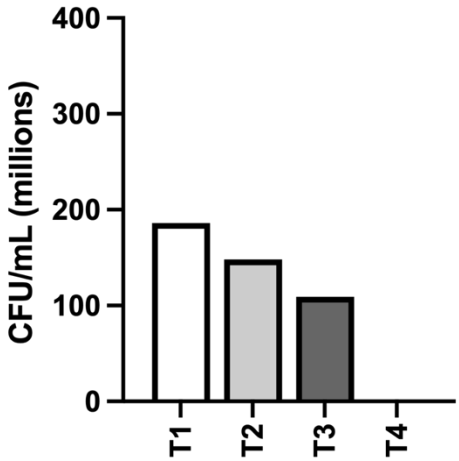
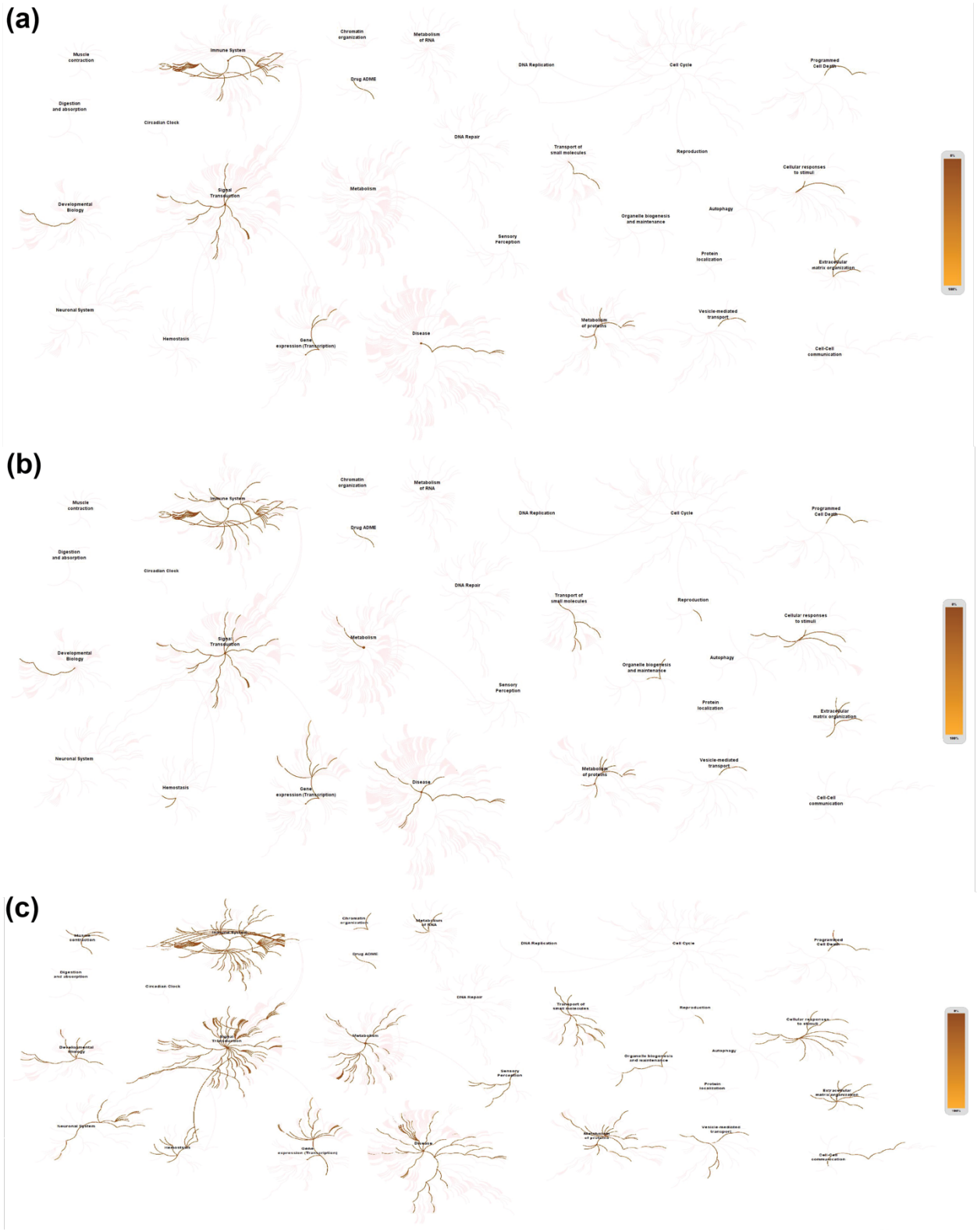
Supplementary Information

Supplementary Figures



Supplementary Figure 1. The viability of the live *M. mulieris* culture was tracked from the sample preparation steps to 24 hours after incubation in a CO2 incubator (the same conditions used during bacterial exposure). Colony-forming unit (CFU) counts of the same bacterial culture were monitored at four different time points: before (T1) and after (T2) centrifuging the *M. mulieris* culture at 3,500 g for 30 minutes to prepare the live bacterial pellet; after resuspending the bacterial pellet in KSF media (the same conditions used to prepare the live bacterial sample for exposure to cervicovaginal epithelial cells, T3); and 24 hours after incubation in a CO2 incubator (T4).

Supplementary Information



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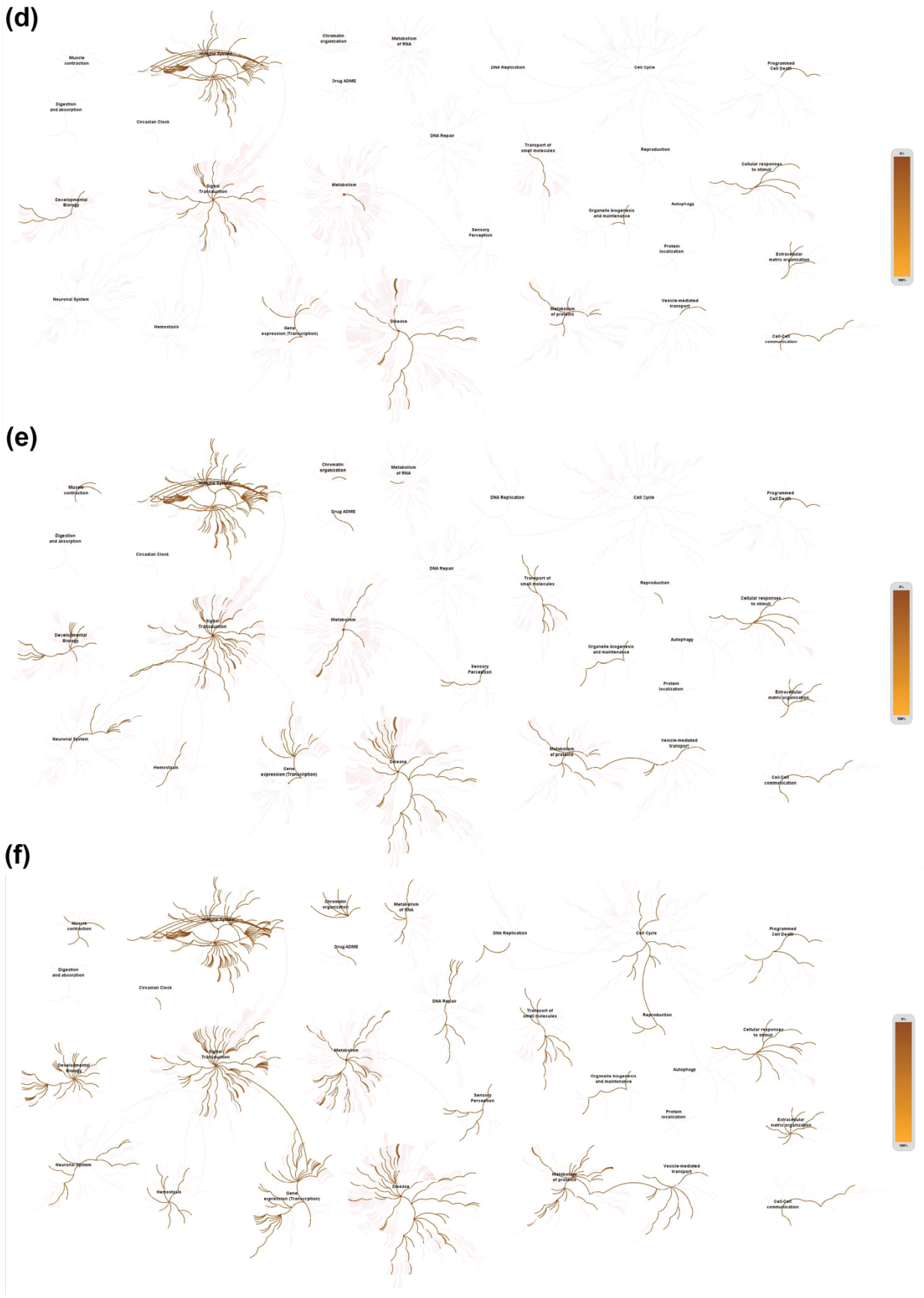

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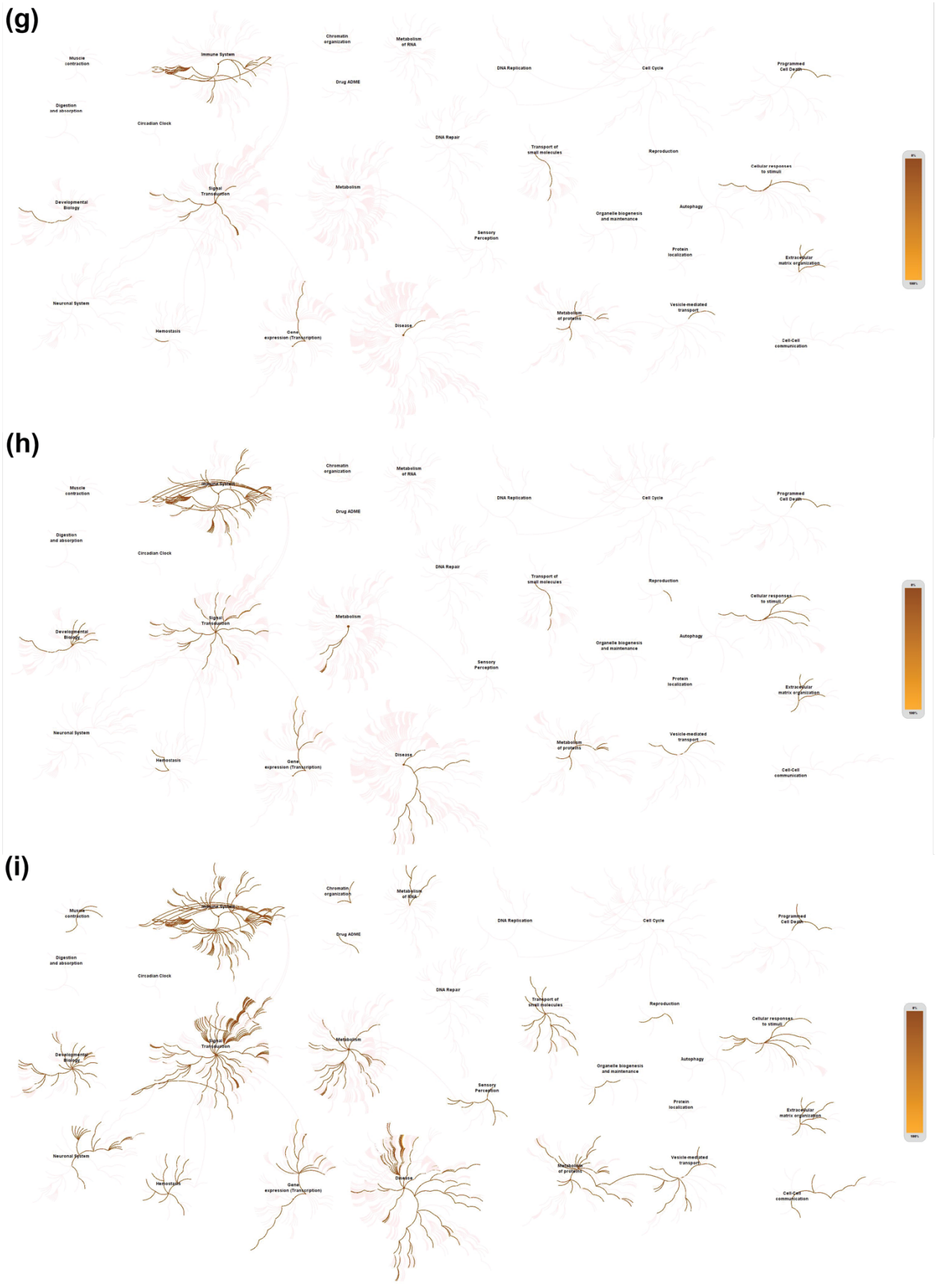
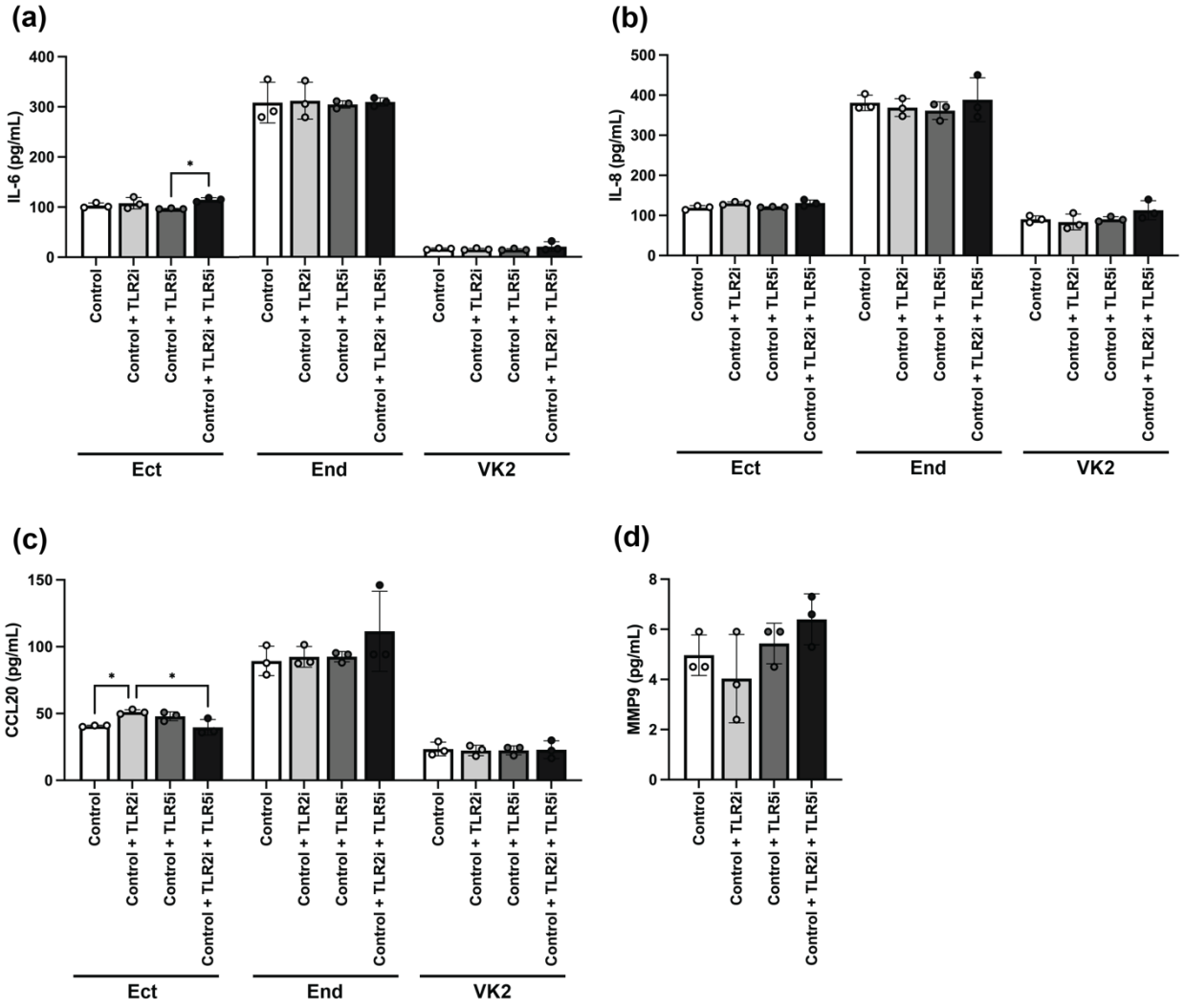
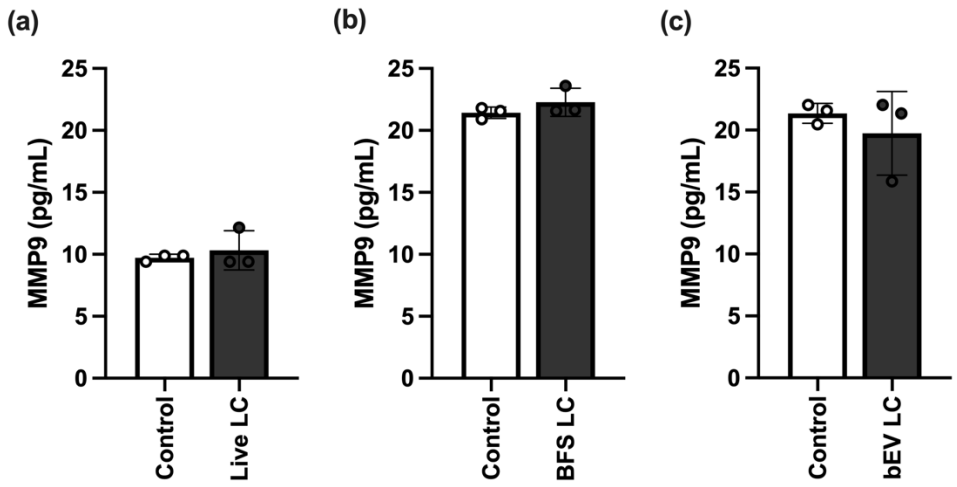


Supplementary Figure 2. Genome-wide overview of the over-representation analysis arranged in a hierarchical structure. The center of each cluster represents the root of a main pathway, with each subsequent node corresponding to lower-level pathways within the hierarchy. Results are shown for Ect cells treated with (a) Live, (b) BFS, and (c) bEV; End cells treated with (d) Live, (e) BFS, and (f) bEV; and VK2 cells treated with (g) Live, (h) BFS, and (i) bEV *M. mulieris* exposure. Color intensity reflects the number of entities enriched within each pathway.



Supplementary Figure 3. Negative control test confirming that hTLR2 inhibitor (TLR2i) and hTLR5 inhibitor (TLR5i) did not affect markers of interest. Bar plots show the levels of (a) IL-6, (b) CCL20, and (c) IL-8 in Ect, End, and VK2 cells. (d) MMP9 treated with TLR2i and/or TLR5i in VK2 cells. Bar plots represent the mean and the error bars for the standard deviation. A one-way ANOVA was used to compare the differences between the groups, and one-way ANOVA was used, followed by Tukey's multiple comparison test. The statistical significance denoted by "*" ($p<0.05$).



Supplementary Fig 4. MMP9 protein levels produced by VK2 cells in response to *L. crispatus* (LC) exposure. MMP9 levels were measured in response to (a) Live, (b) BFS, and (c) bEV LC. Bar plots represent the mean and the error bars for the standard deviation. An unpaired t-test was used to compare MMP9 concentrations between groups.

Supplementary Information

Supplementary Data Legends

Supplementary Data 1. Raw counts of gene expression from RNA-seq analysis when Ect cells were exposed to either live *M. mulieris* or cell culture media (control, NT).

Supplementary Data 2. Raw counts of gene expression from RNA-seq analysis when Ect cells were exposed to either BFS *M. mulieris* or a 1:100 dilution of NYCIII media in cell culture media (control, Sup-ctrl).

Supplementary Data 3. Raw counts of gene expression from RNA-seq analysis when Ect cells were exposed to either bEV *M. mulieris* or cell culture media containing the same volume of bEV suspension media as the treated samples in the bEV groups (control, bEV-ctrl).

Supplementary Data 4. Raw counts of gene expression from RNA-seq analysis when End cells were exposed to either live *M. mulieris* or cell culture media (control, NT).

Supplementary Data 5. Raw counts of gene expression from RNA-seq analysis when End cells were exposed to either BFS *M. mulieris* or a 1:100 dilution of NYCIII media in cell culture media (control, Sup-ctrl).

Supplementary Data 6. Raw counts of gene expression from RNA-seq analysis when End cells were exposed to either bEV *M. mulieris* or cell culture media containing the same volume of bEV suspension media as the treated samples in the bEV groups (control, bEV-ctrl).

Supplementary Data 7. Raw counts of gene expression from RNA-seq analysis when VK2 cells were exposed to either live *M. mulieris* or cell culture media (control, NT).

Supplementary Data 8. Raw counts of gene expression from RNA-seq analysis when VK2 cells were exposed to either BFS *M. mulieris* or a 1:100 dilution of NYCIII media in cell culture media (control, Sup-ctrl).

Supplementary Information

Supplementary Data 9. Raw counts of gene expression from RNA-seq analysis when VK2 cells were exposed to either bEV *M. mulieris* or cell culture media containing the same volume of bEV suspension media as the treated samples in the bEV groups (control, bEV-ctrl).

Supplementary Data 10. DEGs identified by Live, BFS, and/or bEV *M. mulieris* exposure in Ect, End, and VK2 cells. DEGs identified in two forms of *M. mulieris* exposure are summarized as combinations (e.g., Live/BFS).

Supplementary Data 11. Summary of pathway analysis results on DEGs uniquely altered by bEV treatment.

Supplementary Data 12. The fold change (FC) and FDR-corrected p-values (adj p-value) for the significantly altered main pathways by *M. mulieris* bEV treatment in VK2 cells.

Supplementary Data 13. The fold change (FC) and FDR-corrected p-values (adj p-value) for the significantly altered sub-pathways by *M. mulieris* bEV treatment in VK2 cells.

Supplementary Data 14. The fold change (FC) and FDR-corrected p-values (adj p-value) for the DEGs by *M. mulieris* bEV treatment in VK2 cells.