*Sequence data processing*

Before processing, primers were removed using Cutadapt (v. 3.7) (1). Raw sequence reads were then imported to R version 4.1.1 to be processed with the DADA2 package version 1.21 (2). Briefly, trimmed reads were quality filtered using the trimandFilter() command with the following parameters: truncQ = 2, trimRight = c(10,30), maxN = 0, maxEE = c(2,2). The error learning step of the dada2 pipeline was conducted with nbases = 1 x 109. Paired-end sequences were then merged with a minimum of a 12 base pair overlap region and zero mismatches. Chimeras were removed using the removeBimeraDenovo() function, with method = “consensus”. The final sequence table was then trimmed to only include sequences with read lengths ranging from 367-375 base pairs. The Silva V138 (3) database was used to assign taxonomy. The final ASV table, sample metadata, and taxonomic assignments were imported to Phyloseq (4) for downstream processing.

All non-bacterial reads and those assigned to chloroplasts or mitochondria were removed before downstream analyses. ASV tables were then rarefied to 1400 reads per sample for comparison of alpha diversity between the two treatment groups. PERMANOVA testing was applied to within-sample transformed abundances (reads assigned to taxon / total number of reads per sample) using the Adonis function in the vegan package (5) to test for multivariate differences between the two treatment groups.

To assess differences in the functional potential of the host-associated microbiomes of the two treatment groups, we used the PICRUST 2 software (6) with its default settings on our rarefied ASV table. This software uses taxonomic annotations of 16S rRNA sequences to predict the metabolic pathways present in a microbiome sample. To determine pathways, KEGG orthologs, and Enzyme Commission numbers that were differentially abundant between the two treatment groups, we used a Kruskal-Wallis test with α = 0.005.

*Data and code accessibility*

# Raw sequence reads were deposited into the NCBI SRA under BioProject number: PRJNA927293. Reviewer link [here](https://dataview.ncbi.nlm.nih.gov/object/PRJNA927293?reviewer=qnj9310bu2bdo7q4t7djjid1l6). All code used for processing raw amplicon reads, statistical analyses, and PICRUST2 annotations can be found at <https://github.com/gcuster1991/ayayee_mouse_2023.git> (this is private now. We will make it public at the time of pub/review).

1. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10.

2. Callahan BJ, Sankaran K, Fukuyama JA, McMurdie PJ, Holmes SP. 2016. Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses. F1000Research 5:1492.

3. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596.

4. McMurdie PJ, Holmes S. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8:1–11.

5. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O’Hara RB, L. G, Simpson P, Solymos M, Stevens HH, Szoecs E, Wagner H. 2018. vegan: Community Ecology Package. R package version 2.5-2.

6. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI. 2020. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38:685–688.