# SUPPLEMENTARY MATERIAL

## Drinking pattern and sex modulate the impact of ethanol consumption on the mouse gut microbiome

Carla B P Silva [1*], Edson Alexandre Nascimento-Silva [2], Lívia Soares Zaramela [2], Bruno Ruiz Brandão da Costa [3], Vanessa Fernandes Rodrigues [2], Bruno Spinosa De Martinis [4], Daniela Carlos [2], Rita C Tostes [1]

## Material and Methods

*Analysis of the gut microbiota by real-time quantitative PCR (qPCR)*

To evaluate the maltodextrin effects on the gut microbiota, we used feces samples from control mice, collected before and 9 hours after intragastric gavage with maltodextrin [9 $g.kg^{-1}$ p.c., 45% solution (m/v)] (Infinity Pharma®, Nova Iguaçu, RJ, Brazil). DNA extraction was performed according to the recommendations of the DNeasy® PowerSoil® Pro Kit (QIAGEN, Germany). DNA quantity and quality were assessed using NanoDrop 2000 device (Thermo Fisher Scientific, USA). Then the samples were immediately frozen at −20°C until molecular analysis. For PCR analysis, 10 ng of DNA and 1 μM of forward and reverse primers [*Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Tenericutes*, and *Eubacteria* (normalizer gene)] were used. Specific primers sequences for all bacteria phyla are reported in Table S2. Differences (ΔCT) between *Eubacteria* cycle threshold (CT) values and the evaluated phyla were used to obtain normalized levels of each bacteria phylum ($2^{-\Delta\Delta CT}$) (1). The fecal samples collected before the maltodextrin exposure was used as a normalizer to define the relative abundance of each phylum.

## REFERENCE

1. **da Silva JL**, **Barbosa LV**, **Pinzan CF**, **Nardini V**, **Brigo IS**, **Sebastião CA**, **Elias-Oliveira J**, **Brazão V**, **Júnior JC do P**, **Carlos D**, **Cardoso CR de B**. The Microbiota-Dependent Worsening Effects of Melatonin on Gut Inflammation. *Microorganisms* 11, 2023. doi: 10.3390/microorganisms11020460.