

Supplementary Materials

A 14-day Double-blind, Randomized, Controlled Cross-over Intervention Study with Anti-bacterial Benzyl Isothiocyanate from Nasturtium (*Tropaeolum majus*) on Human Gut Microbiome and Host Defense

Simon P. Pfäffle ^{1,2}, Corinna Herz ¹, Eva Brombacher ^{3,6,7,8}, Michele Proietti ⁴, Michael Gigl ^{9,10}, Christoph Hofstetter ⁵, Verena Mittermeier-Kleßinger ⁵, Sophie Claßen ¹, Hoai Tran ¹, Corinna Dawid ^{5,9,10}, Clemens Kreutz ^{3,8}, Stefan Günther ^{2,*} and Evelyn Lamy ^{1,*}

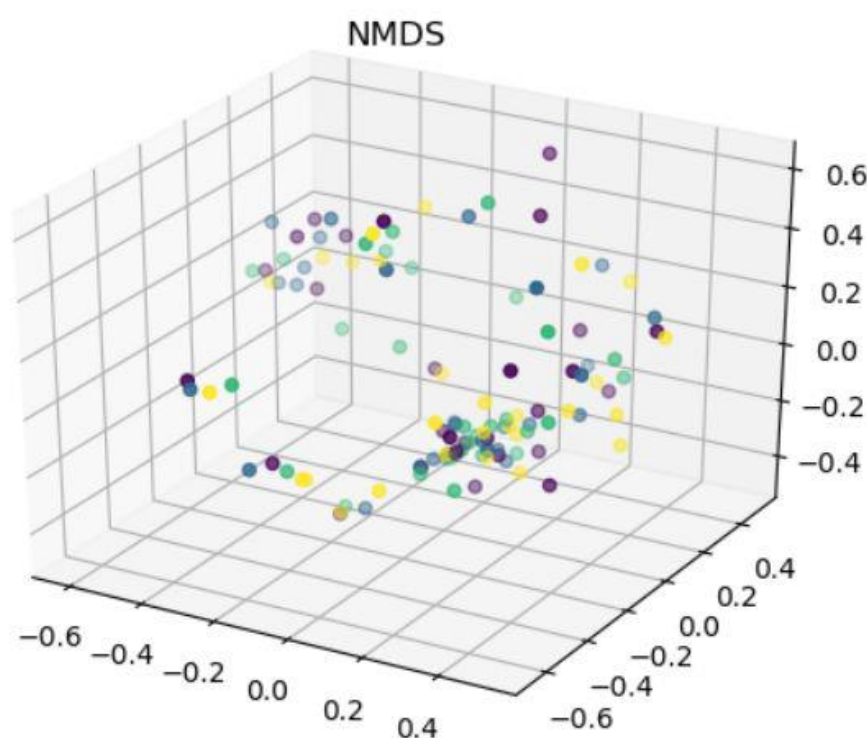


Figure S1. NMDS plot of thetaYC distances for three dimensions. Stress value: 0.15; R-squared: 0.87; colored by sampling day (T1–T4); T1: start of the intervention with *verum* (pre); T2: end of the intervention with *verum* (post); T3: start of intervention with control (pre), T4: end of the intervention with control (post).

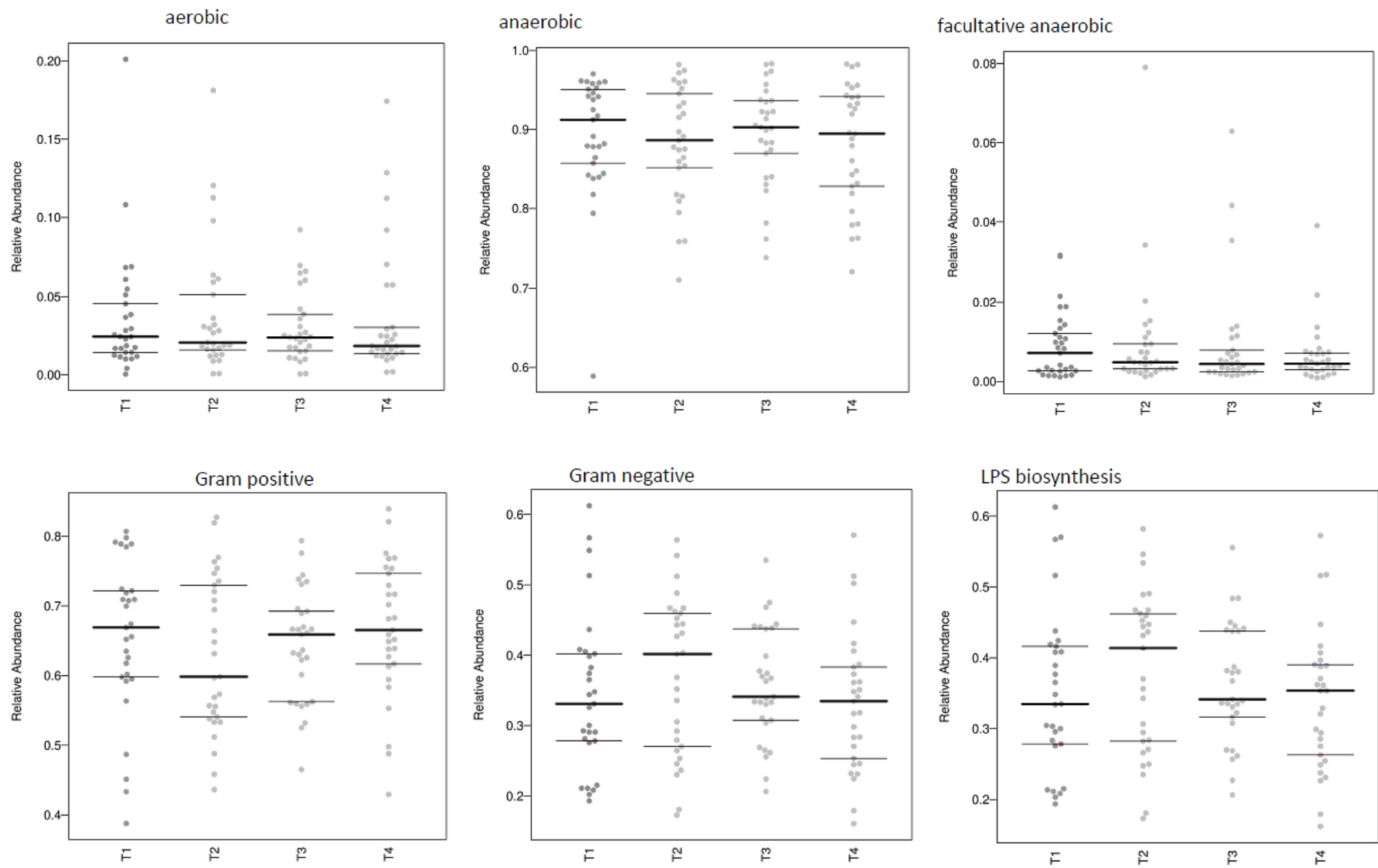
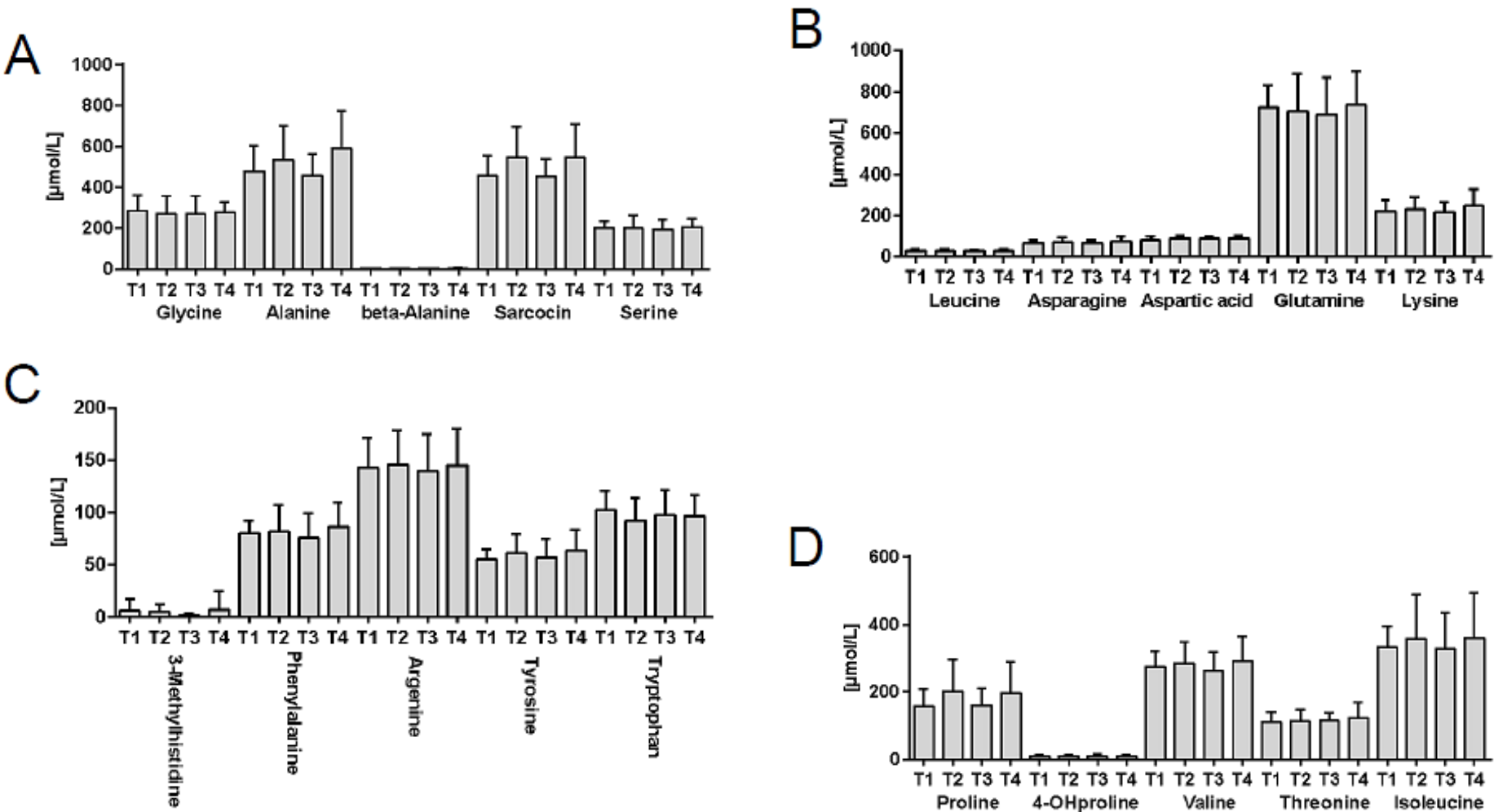
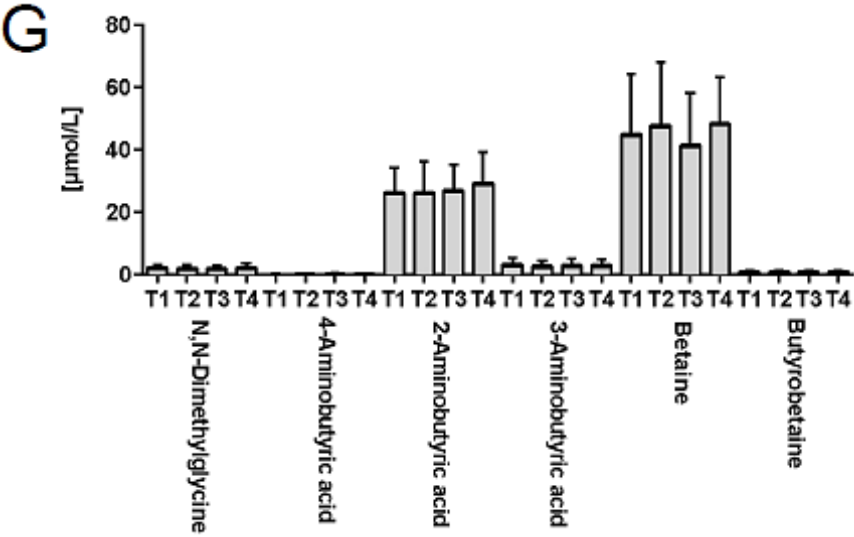
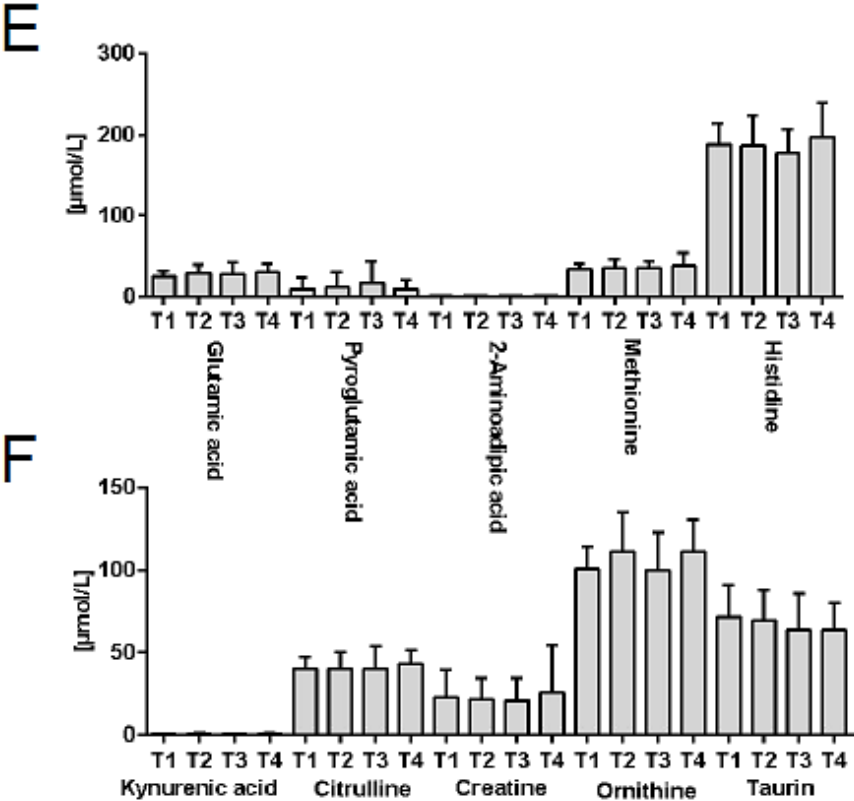


Figure S2. High-level phenotypes. Relative Abundance of high-level phenotypes (n = 29 subjects) using BugBase web application, showing median and 25th/75th percentile. T1: start of the intervention with *verum* (pre); T2: end of the intervention with *verum* (post); T3: start of intervention with control (pre), T4: end of the intervention with control (post).





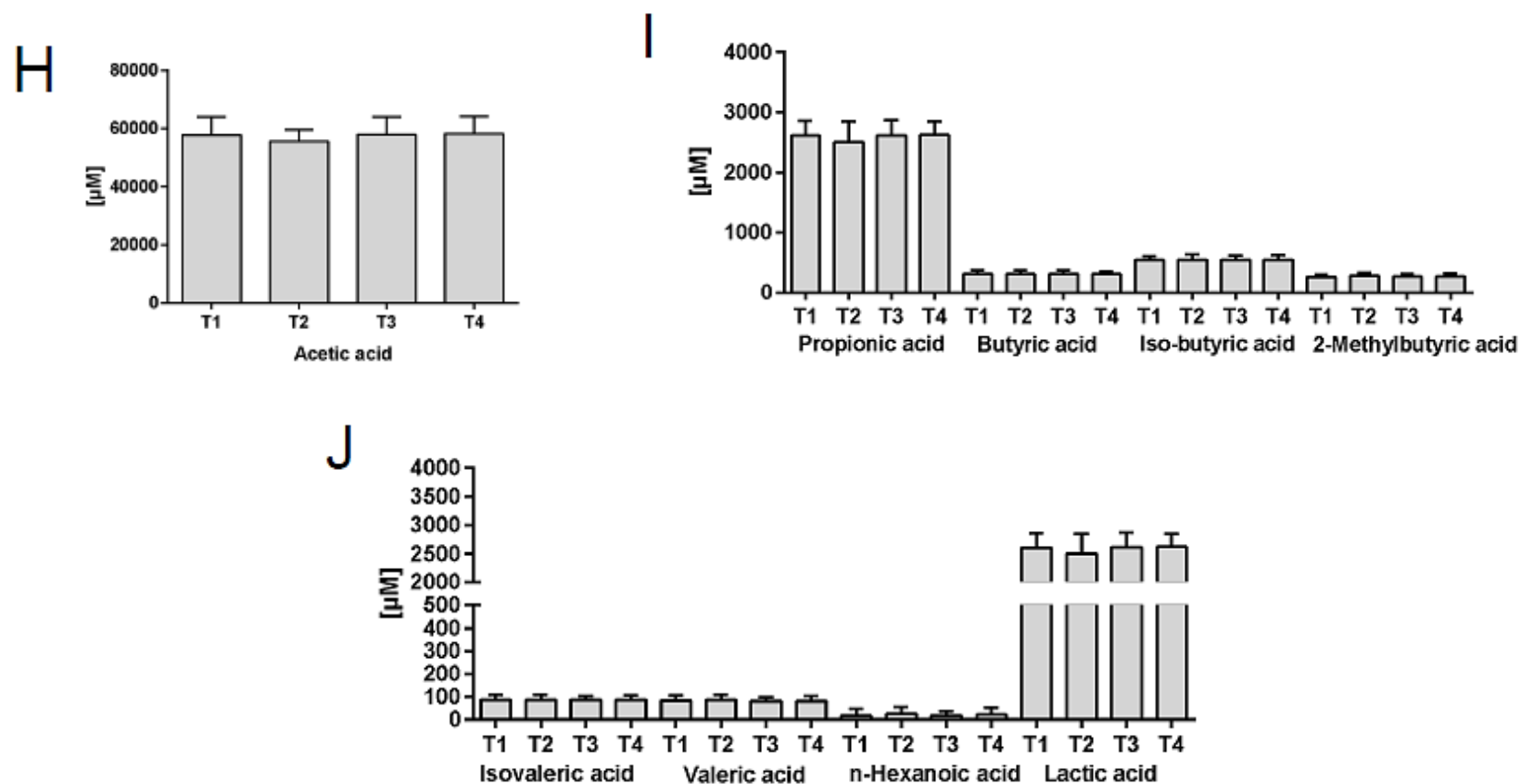
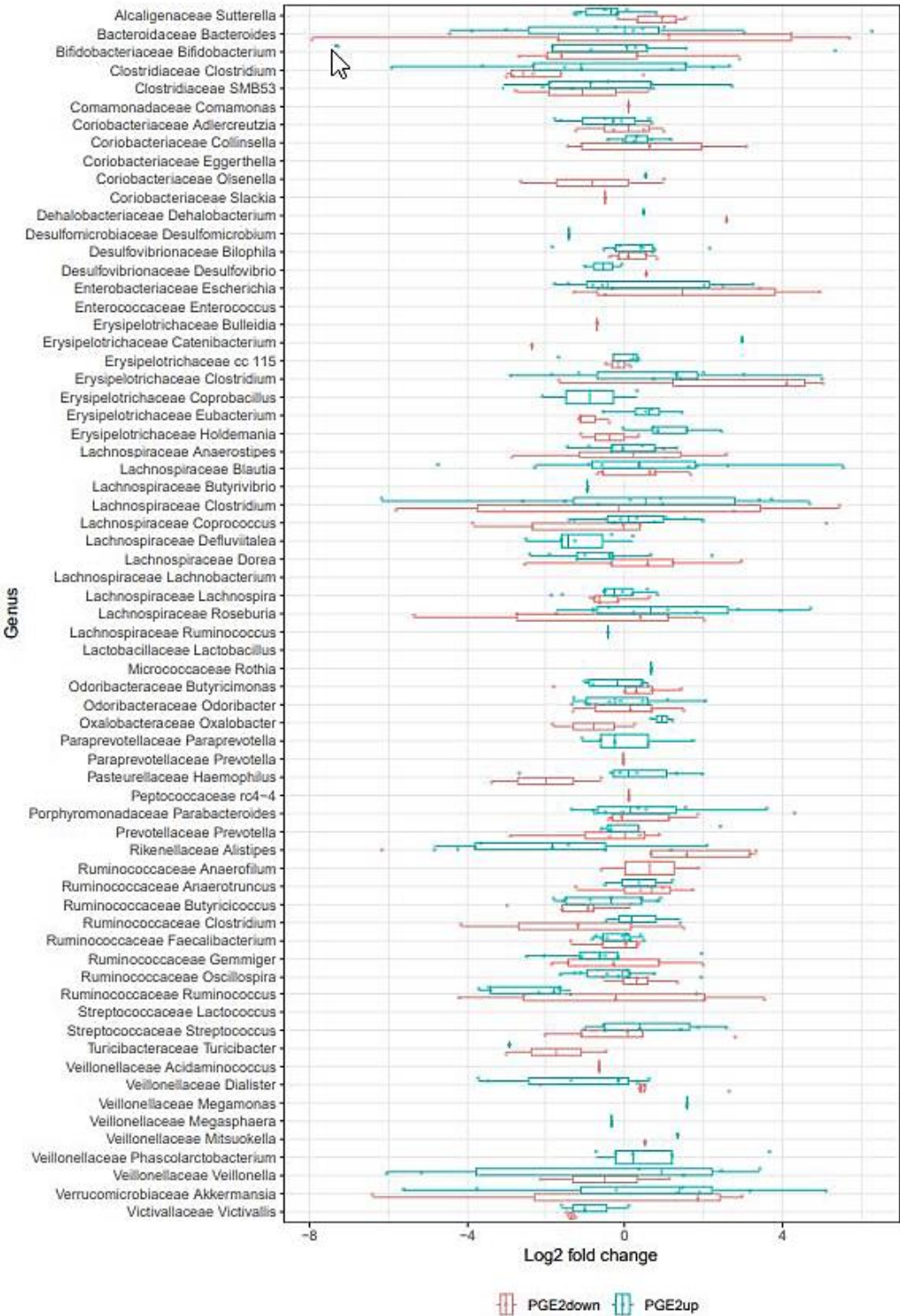


Figure S3. Metabolome analysis using QTRAP. Measurement of different amino acids (A-G, n=19) and of SCFA (H-J, n=19) in serum of participants. T1: start of the intervention with *verum* (pre); T2: end of the intervention with *verum* (post); T3: start of intervention with control (pre), T4: end of the intervention with control (post).

A



B

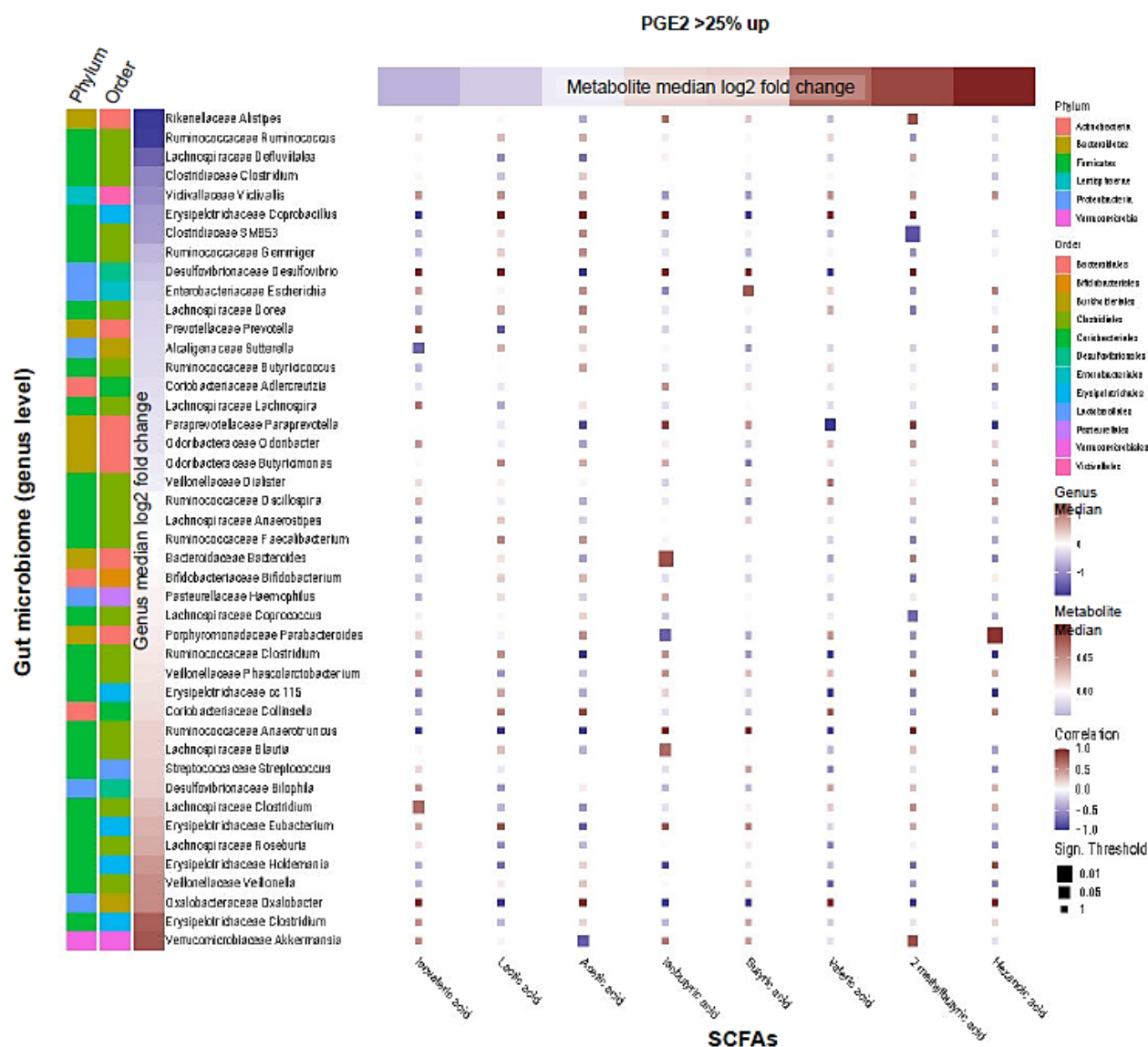


Figure S4. Corellation of bacteria abundance between subgroups (A) and Spearman correlation of gut microbiome and SCFAs (B). (A) Analysis is conducted on the difference in the log₂-fold changes of bacteria genera between pre vs. post treatment with *verum* in the subgroup with a >25% downregulated serum PGE₂ (blue boxes) and subjects with a >25% upregulated serum PGE₂ (red boxes). Results are presented as box-plots. The line inside the box shows the median of the values. (B) Spearman correlation analysis is conducted on the difference in the log₂-fold changes of bacteria genera between pre vs. post treatment with *verum* and the log₂-fold changes of SCFAs between *verum* and control. Spearman correlation of the difference in bacteria genera and the difference in SCFAs for subjects with a >25% upregulated serum PGE₂. Correlation with an unadjusted p-value < 0.05 or 0.01 are emphasized by bigger squares in the correlation matrix. Positive correlation coefficients are displayed in red, negative ones in blue. To the left of the matrix, color-coded bars of the phylum and order of the respective genera are displayed. Also, to the left and above the correlation matrix the median of the absolute difference in the log₂-transformed abundance of bacteria genera (left) and metabolites (top) between *verum* and control is displayed as a color-coded bar, where blue corresponds to negative and red to positive values. No significant correlations were observed between changes in microbiota and changes in SCFAs for the subgroup of subjects with a >25% downregulated serum PGE₂.