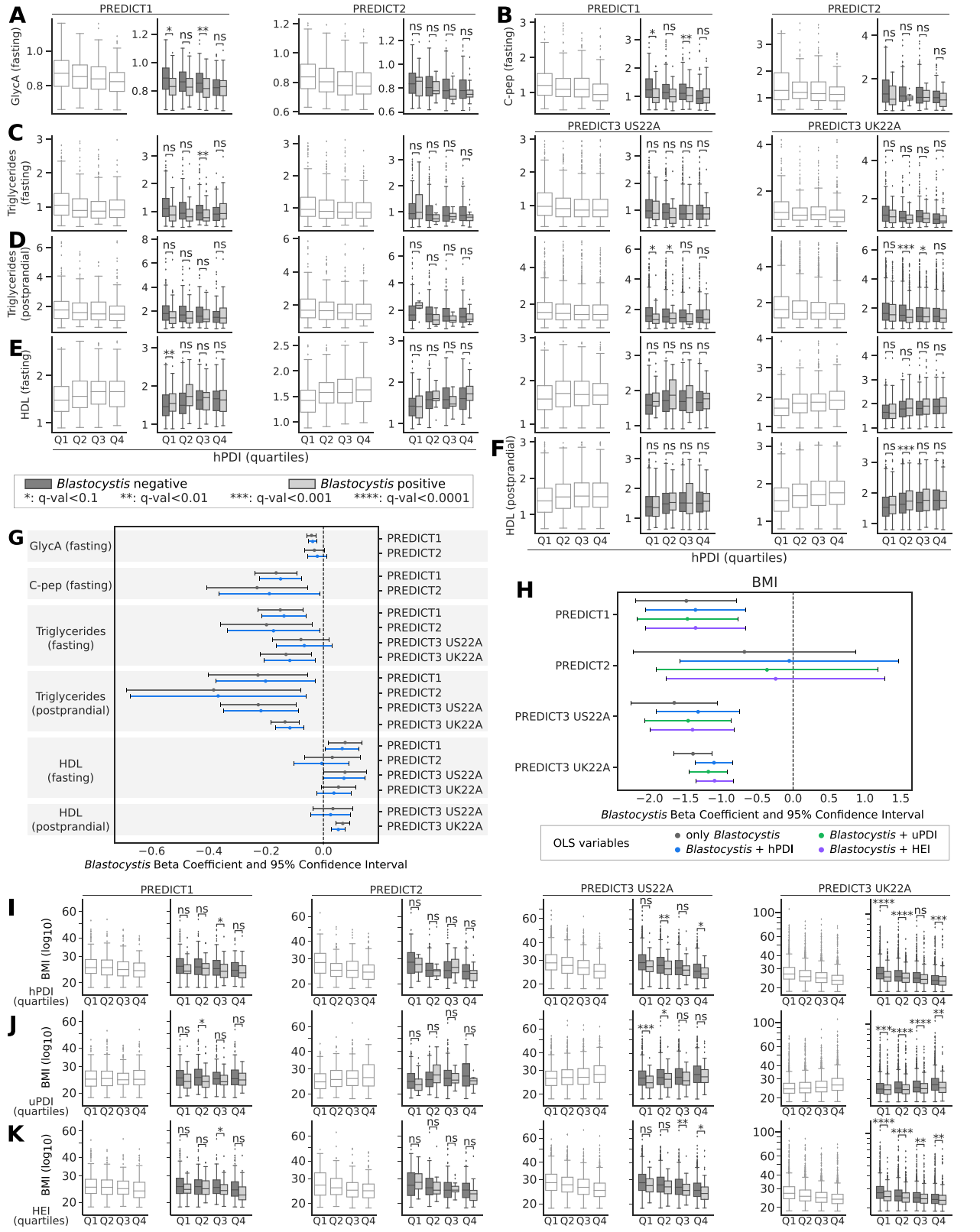

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Figure S4. Among individuals with similar diet quality, *Blastocystis*-positive ones generally have more favorable cardiometabolic profiles and a lower BMI, and *Blastocystis* is a significant independent predictor of cardiometabolic marker values and BMI, related to Figures 2 and 3

(A–F) Participants were divided into quartiles according to their hPDI values, from less (Q1) to more (Q4) healthful diet. Within each quartile of dietary quality, *Blastocystis*-positive individuals show lower triglycerides, C-peptide, and GlycA values and higher HDL values compared with *Blastocystis*-negative ones.

(G) Linear regression models to predict different marker values using only *Blastocystis* presence/absence as an independent variable (in gray) or including both *Blastocystis* presence/absence and hPDI (in blue). *Blastocystis* appears to be a significant independent predictor in both univariable and multivariable modeling across PREDICT cohorts.

(H) The forest plot shows the beta coefficients and 95% confidence intervals for linear regression models built to predict BMI using only *Blastocystis* presence/absence as an independent variable (in gray) or using *Blastocystis* presence/absence in combination with different dietary scores (hPDI, uPDI, and HEI, in blue, green, and purple, respectively).

(I–K) Participants were divided into quartiles based on their hPDI (I), uPDI (J), and HEI (K) values, respectively. Within hPDI, uPDI, and HEI quartiles, *Blastocystis*-positive individuals generally had a lower BMI compared with *Blastocystis*-negative individuals.

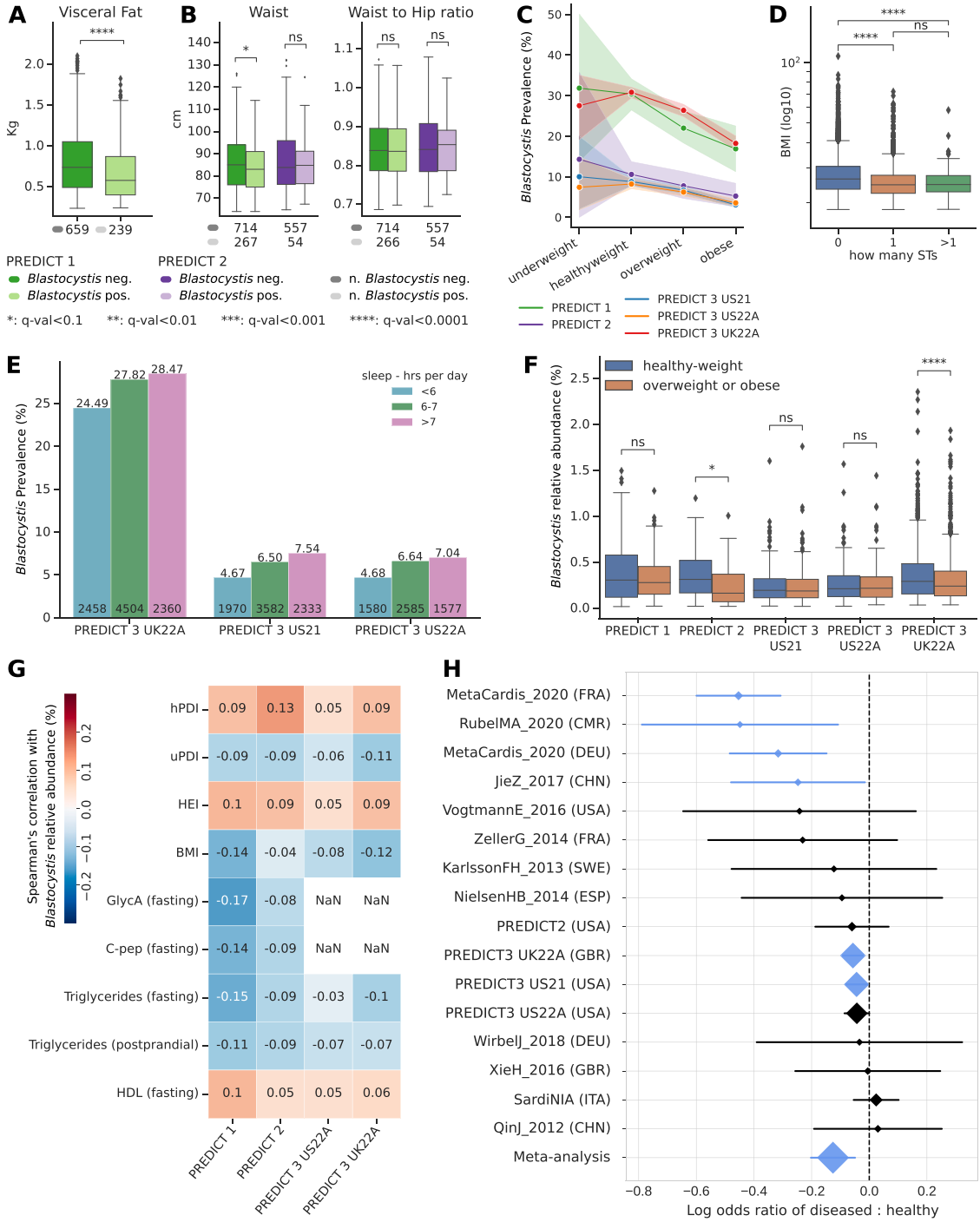

Figure S5. Associations between *Blastocystis* presence and relative abundance with markers of a healthier lifestyle and diet, favorable cardiometabolic markers, and decreased odds of chronic disease, related to Figure 5 and STAR Methods

(A) Visceral fat measurements according to presence-absence of *Blastocystis* in the PREDICT 1 cohort. Individuals with *Blastocystis* showed significantly lower visceral fat, as previously reported.[7]

(B) Waist circumference and waist-to-hip (WHR) ratio are occasionally used as surrogate measures of body adiposity and show no association with *Blastocystis* carriage. Both markers were available only for PREDICT 1 and PREDICT 2.

(C) *Blastocystis* prevalence differs by established BMI categories for each cohort in the PREDICT studies. Individuals with "healthy-weight" (and underweight) BMI tend to have higher *Blastocystis* prevalence compared with individuals with overweight and obesity. Underweight individuals were excluded from Figure 5 due to comparatively low numbers (i.e., n = 22 in P1, n = 14 in P2, n = 50 in P3_US21, n = 54 in P3_US22A, and n = 120 in P3_UK22A) and the impossibility to exclude other diseases associated with low BMI values.

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(D) Individuals colonized by two or more *Blastocystis* subtypes showed no significant difference in BMI values from those mono-colonized.
(E) *Blastocystis* prevalence was linked to increased sleeping time. Individuals self-reported their average sleeping time, and those with more than 7 h per night showed higher *Blastocystis* prevalence across all PREDICT 3 cohorts. Number of individuals in each sleeping category is reported.
(F) Among *Blastocystis*-positive participants, those with "healthy-weight" BMI tended to have a higher relative abundance of *Blastocystis* compared with overweight or obese ones.
(G) *Blastocystis* relative abundance was inversely correlated with adherence to uPDI, the inflammatory marker GlycA, C-peptide, circulating triglycerides, and BMI. Conversely, it was positively correlated with adherence to the healthy plant-based dietary index (hPDI), healthy eating index (HEI), and HDL.
(H) Meta-analysis of 11,042 individuals with various chronic diseases and their 25,161 controls revealed a consistent inverse association between *Blastocystis* relative abundance and disorders linked to gut microbial disturbances. Note: for the MLVS/MBS and DMP datasets, *Blastocystis* relative abundance could not be estimated due to missing data on coverage depth. ASCVD, atherosclerotic cardiovascular disease; T2D, type 2 diabetes; IGT, impaired glucose tolerance; IBDs, inflammatory bowel diseases; STH, soil-transmitted helminths; CRC, colorectal cancer (STAR Methods).

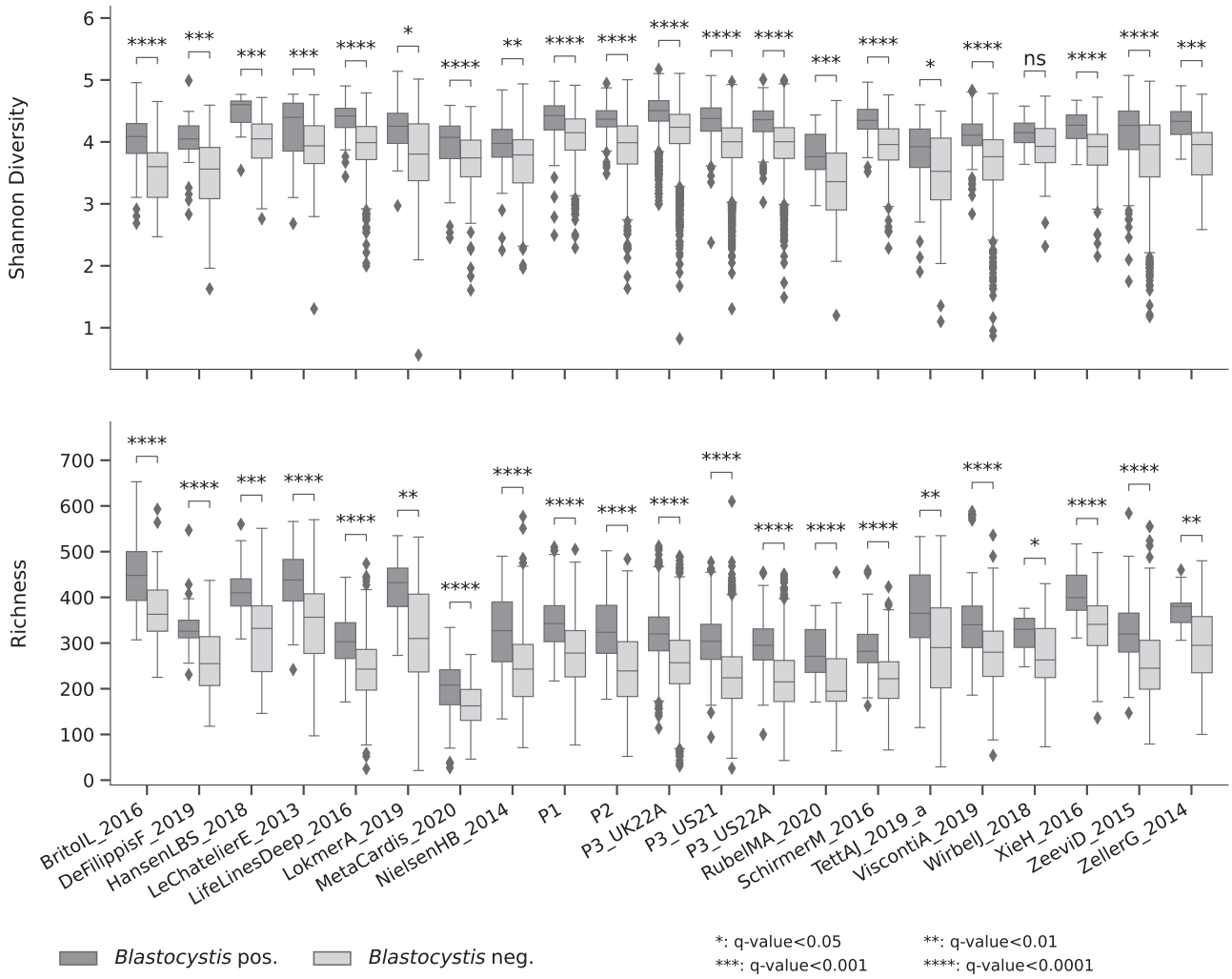

Figure S6. *Blastocystis* carriage was consistently linked to increased gut microbial alpha diversity, related to Figure 6

The presence of *Blastocystis* in the gut microbiome was associated with increased alpha diversity computed both as the number of species detected (richness) and the Shannon's diversity index across multiple international cohorts. The boxplots show results only from healthy adult samples with the first and third quartiles delimited by boxes and the median shown as the middle line; whiskers extend up to 1.5× the IQR. The same datasets used in the machine learning analysis were considered in these analyses (STAR Methods).

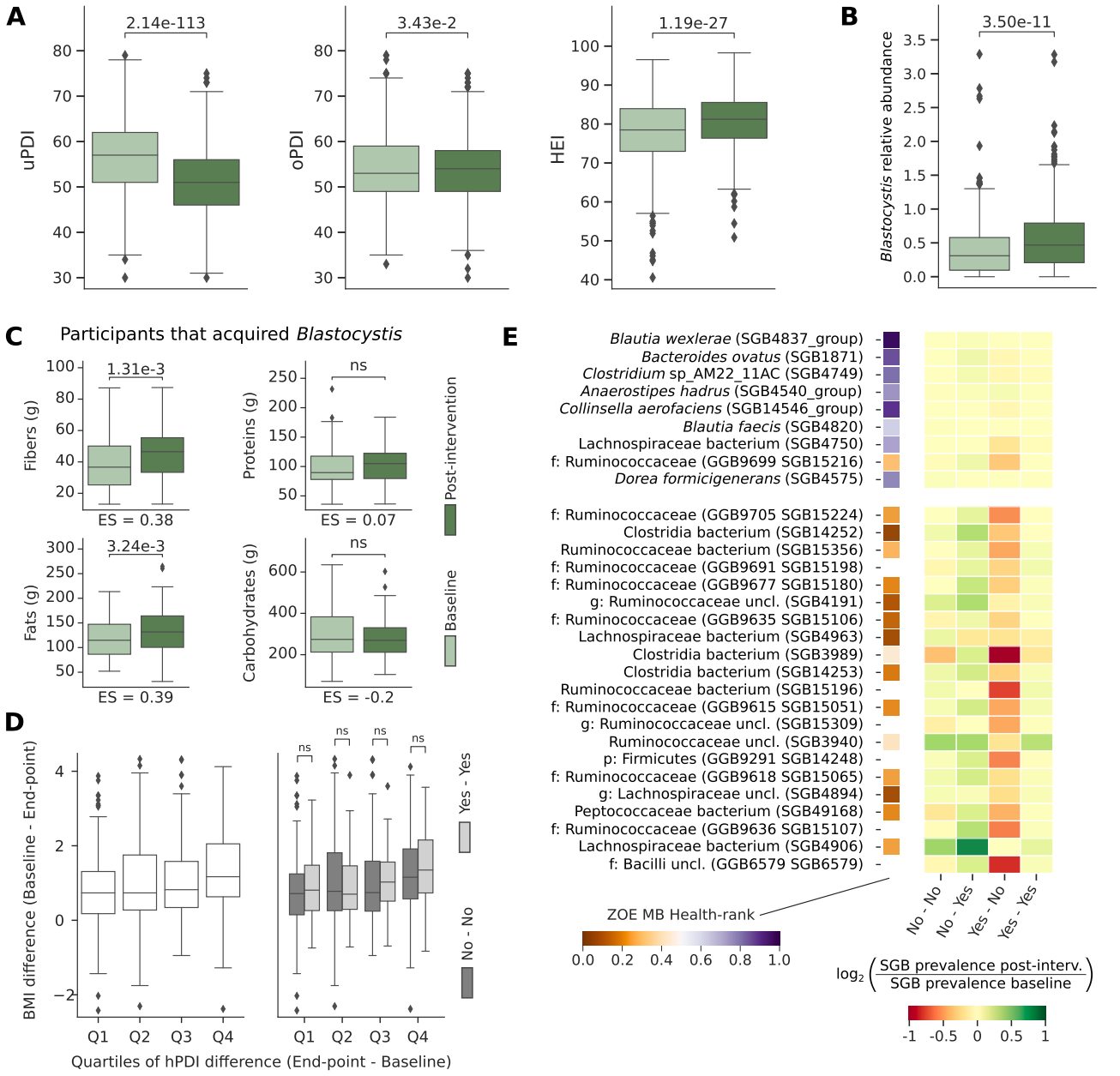

Figure S7. Personalized dietary intervention impacts *Blastocystis* presence and abundance, macronutrients intake, and BMI, related to Figures 5 and 6

(A) Overall, after following a personalized dietary program (PDP), the uPDI values (indicating increased intake of unhealthy plant-based foods) were significantly decreased, while oPDI and HEI values were modestly but significantly higher (indicating improved dietary quality) post-intervention.

(B) Post-intervention, *Blastocystis* relative abundance was significantly increased compared with baseline. Boxplots include participants who stayed *Blastocystis*-positive throughout the PDP intervention and those who either lost or acquired *Blastocystis*.

(C) Individuals who acquired *Blastocystis* during the PDP showed a significant increase in fiber and fat consumption (Wilcoxon p value < 0.05) and no significant changes in the intake of proteins and carbohydrates. ES, effect size.

(D) Among the "*Blastocystis*-stable" participants (i.e., either *Blastocystis*-negative or positive at both time points, n = 1,038), we considered those who improved their diet following the PDP according to the hPDI index (difference in hPDI between post-intervention and baseline greater than 0, n = 857). Participants who improved their diet the most (Q4) experienced a higher weight loss, and in particular, *Blastocystis*-positive individuals ("Yes-Yes") usually lost more weight, though not statistically significant.

(E) Heatmap of the species most associated with *Blastocystis* presence/absence from the cross-sectional ML predictions (Figure 5). For each species, we calculated the $\log_2$-fold change of its prevalence before and after the PDP, in each of the four groups of *Blastocystis* acquisition. No striking patterns were evident

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for individuals with *Blastocystis* stably present or absent (“No-No” and “Yes-Yes”). Conversely, participants who acquired *Blastocystis* (“No-Yes”) showed an increase in prevalence of SGBs enriched in *Blastocystis*-positive subjects (i.e., favorably ranked SGBs and reported in the bottom side of this heatmap and of Figure 5B), while participants who lost *Blastocystis* (“Yes-No”) showed an opposite decreasing trend in prevalence for the same SGBs.