Supplemental information

Specific host metabolite and gut

microbiome alterations are associated

with bone loss during spaceflight

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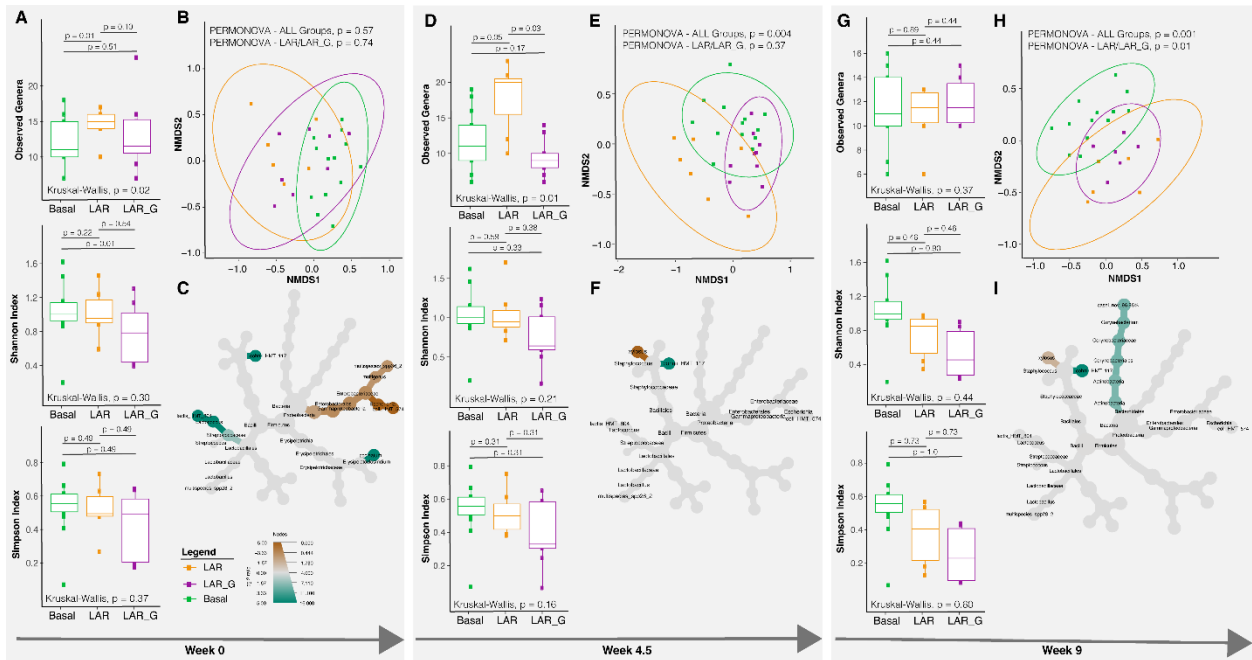
Figure S1 Longitudinal Analysis of the Oral Microbiome in the LAR Flight Cohort versus LAR_G Ground Control.

(A, D, G) Longitudinal differences in alpha diversity of Observed genera, Shannon, and Simpson diversity indices when comparing LAR versus LAR_G or Basal cohorts at Pre-Flight (week 0), Live Animal Return to Earth (week 4.5), and Termination (week 9) timepoints. The non-parametric Kruskal-Wallis statistical test was used to compare groups of two or more. The Wilcoxon Rank-Sum statistical test was employed for pairwise comparisons. Statistical significance is indicated accordingly.

(B, E, H) Beta diversity analysis comparing LAR versus LAR_G or Basal cohorts at Pre-Flight (week 0), Live Animal Return to Earth (week 4.5), and the Termination (week 9) timepoints. Nonparametric multivariate analysis of variance (PERMANOVA) was used to calculate a statistical comparison of variance using distance matrices. Statistical significance is indicated accordingly.

(C, F, I) Comparative analysis of genera enriched or lost in the LAR versus LAR_G cohorts at Pre-Flight (week 0), Live Animal Return to Earth (week 4.5), and the Termination (week 9) timepoints. Taxa enriched or lost in the LAR cohort at a threshold of p<0.05 compared to taxa present in the LAR_G cohort are represented in the *metacoder* heat tree by a color intensity Log_2 median ratio scale. P-values are indicated in the text and TableS4-6.
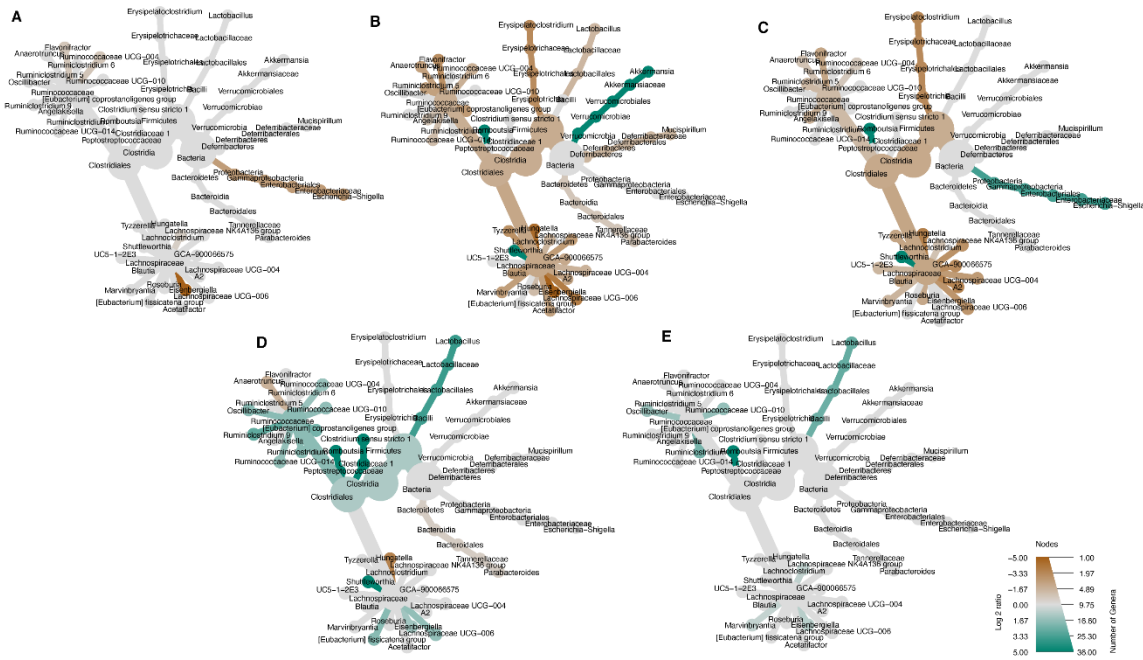
Figure S2 Fecal Necropsy Termination Analysis of Enriched or Lost Genera Evaluating Caging Effects.

(A-C) (A) Analysis of genera enriched or lost in the LAR versus LAR_G cohort at necropsy, (B) Analysis of genera enriched or lost in the LAR versus Basal cohort, and (C) Analysis of genera enriched or lost in the LAR_G versus Basal cohort (week 9). Taxa enriched or lost in the comparison cohorts in Figure S1A-C was at a threshold of $p<0.05$ and are represented in the *metacoder* heat tree by a color intensity Log_2 median ratio scale.

(D) Analysis of genera enriched or lost in the ISS versus Basal cohort (week 9). Taxa enriched or lost in the ISS cohort at a threshold of $p<0.05$ compared to taxa present in the Basal cohort are represented in the *metacoder* heat tree by a color intensity Log_2 median ratio scale.

(E) Analysis of genera enriched or lost in the ISS_G versus Basal cohort (week 9). Taxa enriched or lost in the ISS_G cohort at a threshold of $p<0.05$ compared to taxa present in the Basal cohort are represented in the *metacoder* heat tree by a color intensity Log_2 median ratio scale.