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Supplemental information

Periodontal disease is associated with increased gut colonization of pathogenic *Haemophilus parainfluenzae* in patients with Crohn's disease

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Supplementary Figure 1

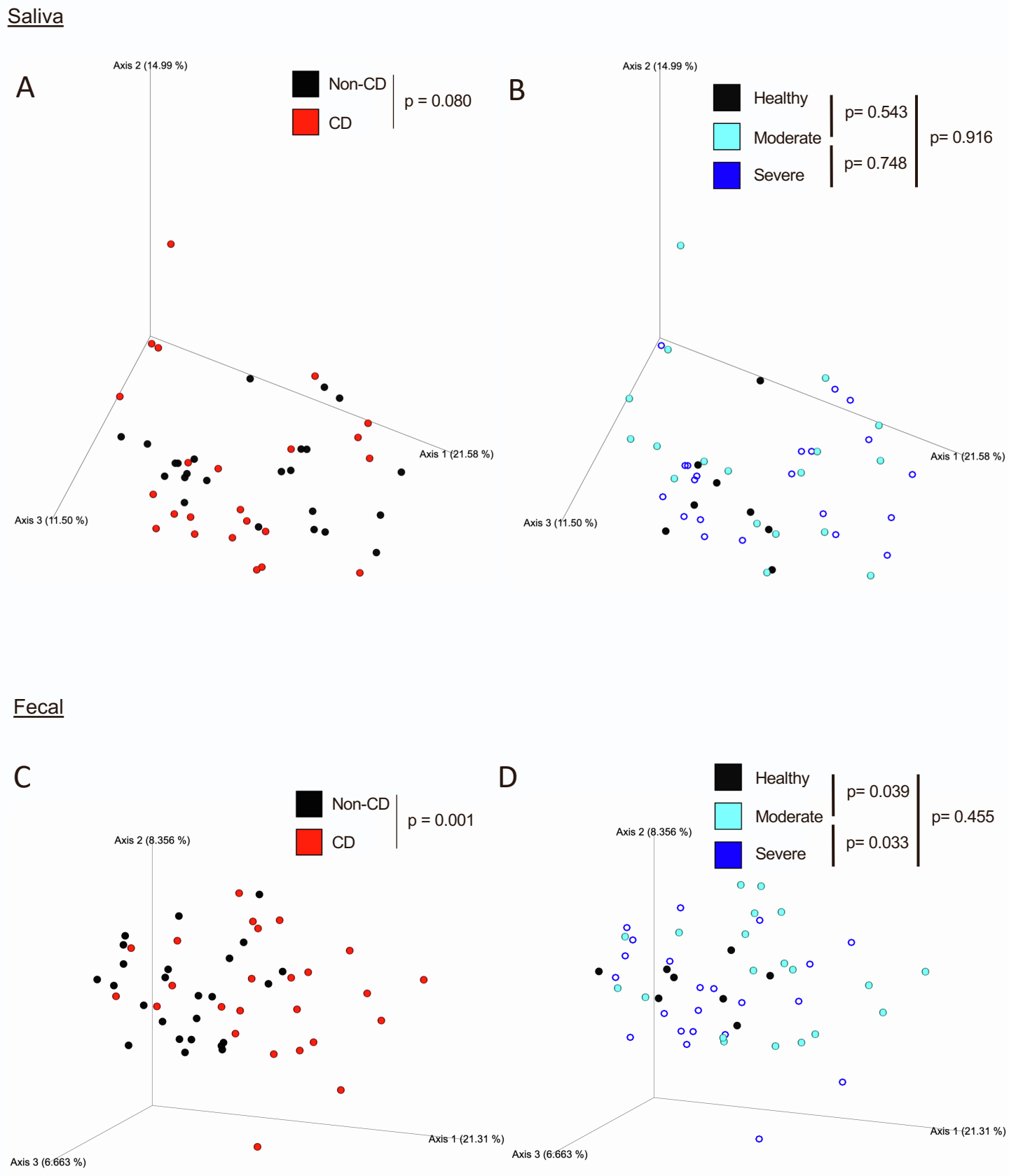


Figure S1. Salivary and fecal beta diversity measurements associated with CD or PD. Related to Figure 1. Salivary and fecal beta diversity was measured by unweighted-UNiFrac distance based on (A,C) CD ((n=25 in non-CD and n =25 in CD) or (B,D) PD (n=8 in healthy, n=21 in moderate, and n=21 in severe) status. Statistical testing was performed using PERMANOVA.

Supplementary Figure 2

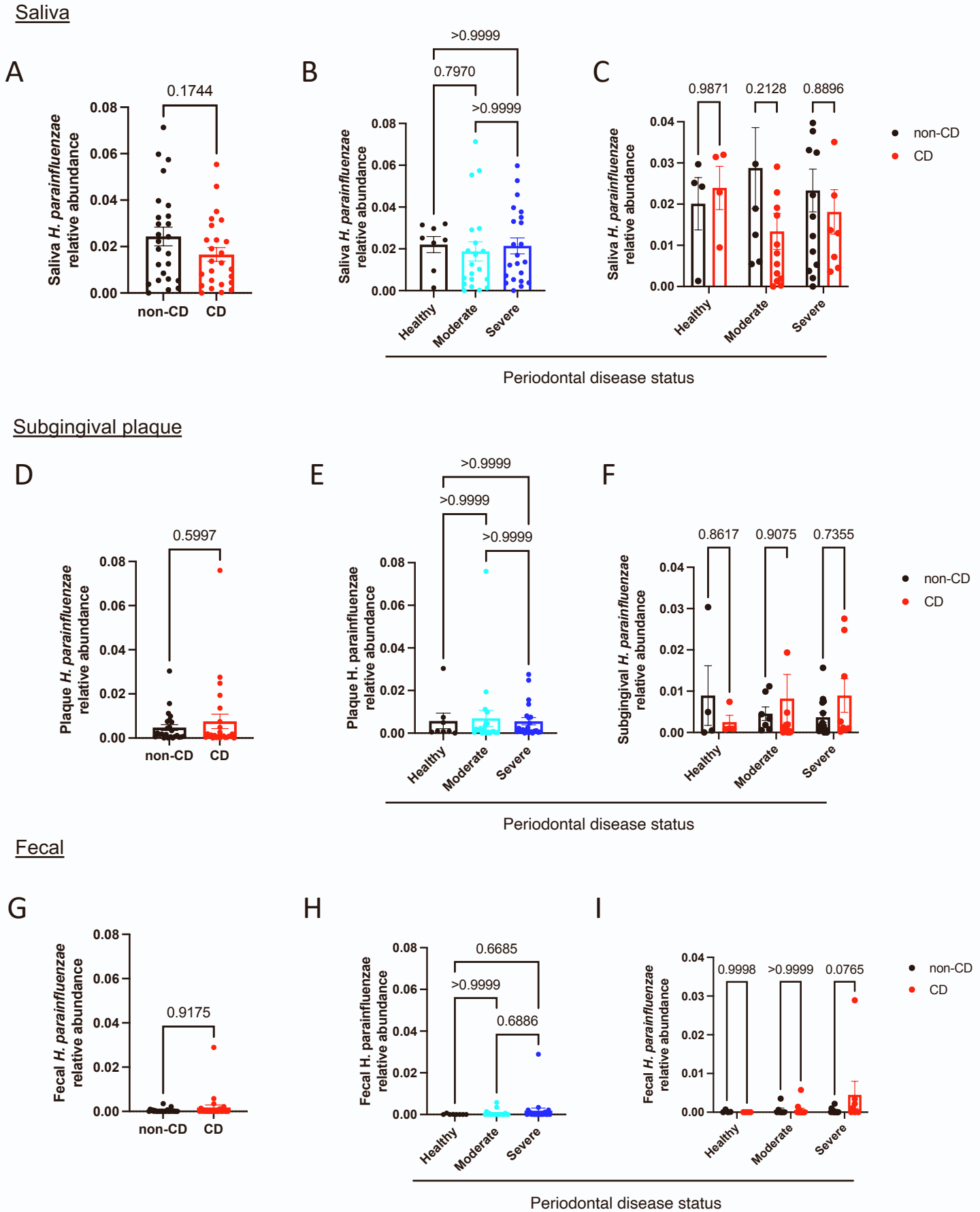
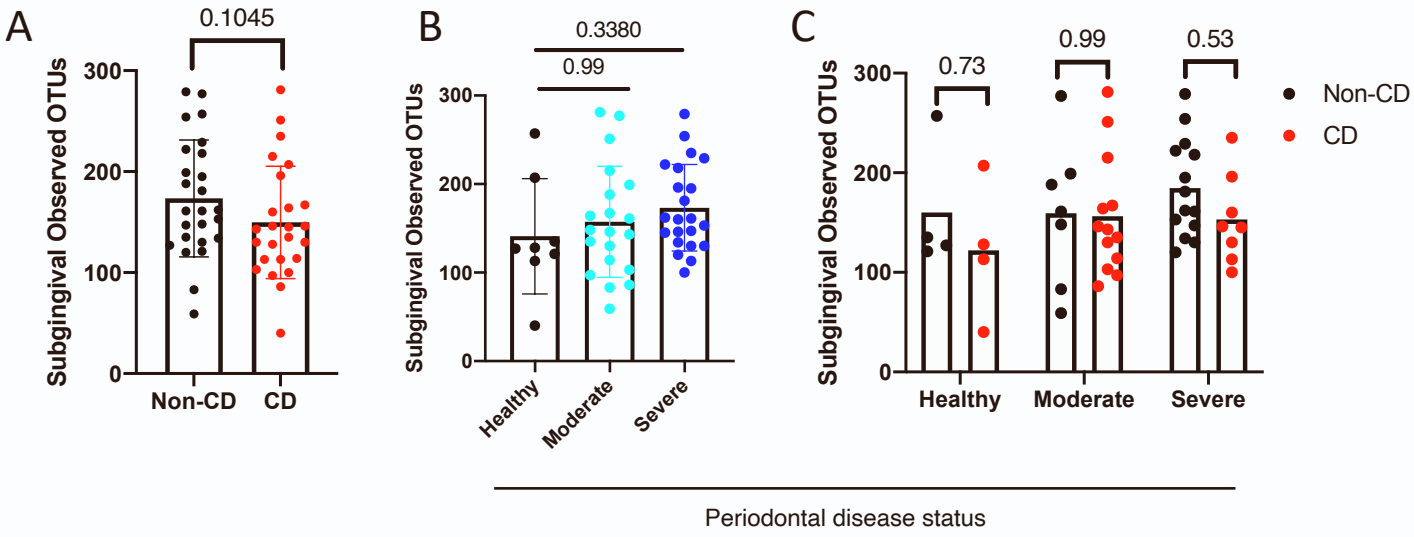
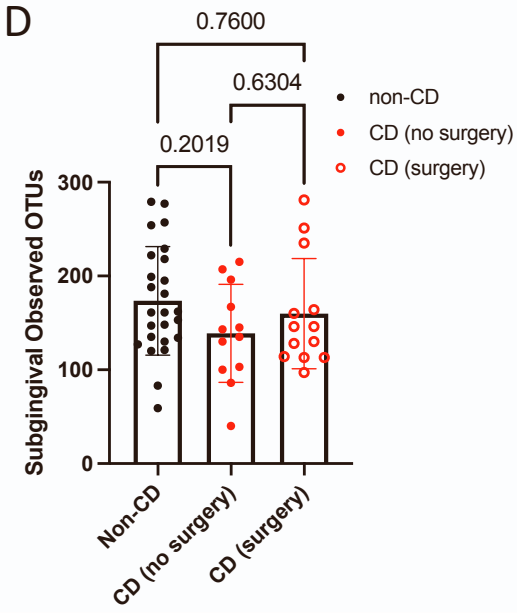
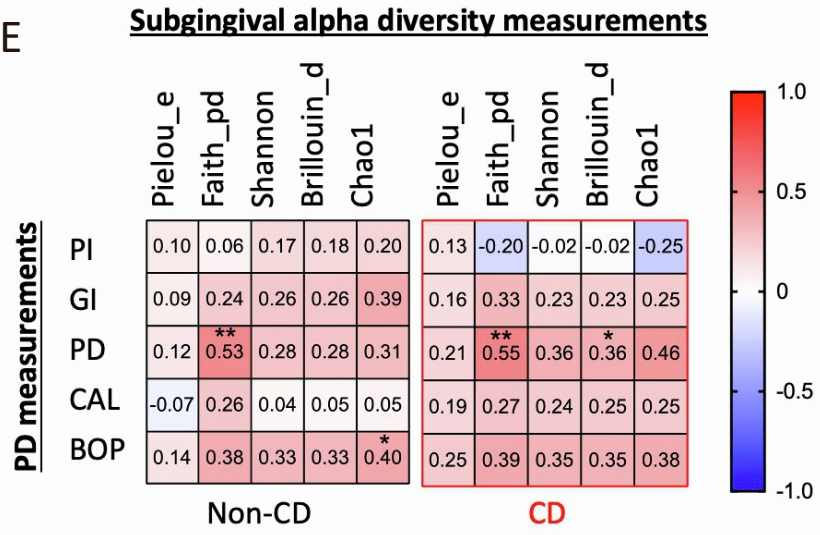


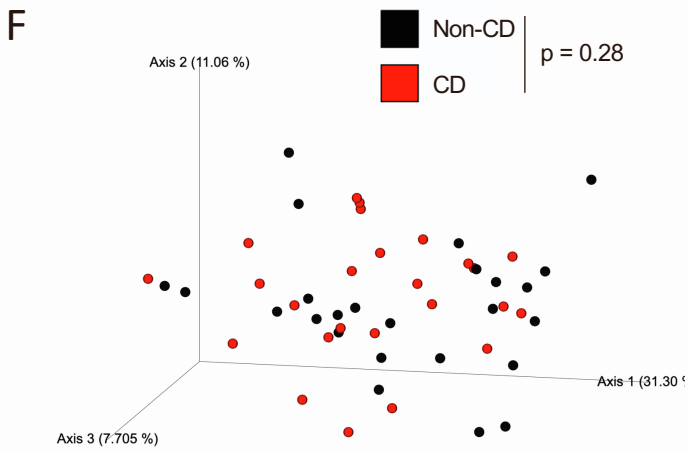
Figure S2. The relative abundance of *H. parainfluenzae* is unchanged in saliva, subgingival or fecal samples. Related to Figure 2. The relative abundance of *H. parainfluenzae* in fecal samples based on (A,D,G) CD (n=25 in non-CD and n =25 in CD) , (B,E,I) PD (n=8 in healthy, n=21 in moderate, and n=21 in severe) status, or (C,G,J) stratified by CD and PD status (n=4 in healthy PD and non-CD, n= 4 in healthy PD and CD, n=7 in moderate PD and non-CD, n=13 in moderate PD and CD, n=14 in severe PD and non-CD, and n=8 in severe PD and CD) . Statistical testing was performed using (A,D,H) Mann-Whitney U, (B,E,H) Kruskal-Wallis followed by Dunn's multiple comparisons test, and (C,F,I) two-way ANOVA followed by Sidak's multiple comparisons tests. Data are represented as mean ± SEM.

Supplementary Figure 3









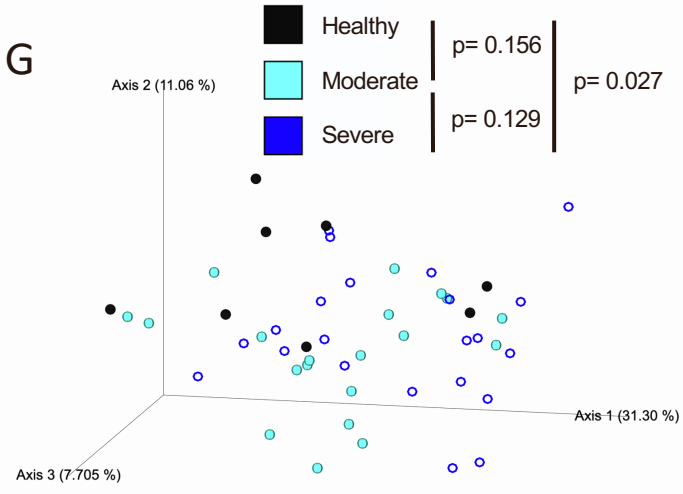


Figure S3. No significant changes in subgingival microbiota alpha-diversity with CD or PD. Related to Figure 1. Subgingival alpha diversity based on (A) CD (n=25 in non-CD and n =25 in CD), (B) PD (n=8 in healthy, n=21 in moderate, and n=21 in severe), (C) stratified by CD and PD status (n=4 in healthy PD and non-CD, n= 4 in healthy PD and CD, n=7 in moderate PD and non-CD, n=13 in moderate PD and CD, n=14 in severe PD and non-CD, and n=8 in severe PD and CD), or (D) CD surgery status ((n=25 in non-CD, n=12 in CD no surgery, n=13 in CD surgery) represented by observed OTUs. (E) Correlation matrix of subgingival alpha diversity measures and whole mouth periodontal clinical measurements (n=25 each group). pielou_e: Pielou's Evenness index, faith_pd: Faith's phylogenetic diversity, shannon: Shannon diversity index, brillouin_d: Brillouin index, chao1: Chao1 richness estimate, PI: plaque index, GI: gingival index, PD: mean pocket depth, GR: gingival recession, CAL: mean clinical attachment loss, BOP: bleeding on probing. Beta diversity was measured by unweighted-UNiFrac distance based on (A) CD or (B) PD status. Statistical testing was performed using (A) Mann-Whitney U, (B,D) Kruskal-Wallis followed by Dunn's multiple comparisons test, (C)Two-way ANOVA followed by Sidak's multiple comparisons test. (E) Pearson correlation tests with coefficients shown in color scale. A two-tailed test was used to calculate p-values. (F,G) Beta diversity comparison was performed using PERMANOVA. Data are represented as mean ± SEM.

Supplementary Figure 4

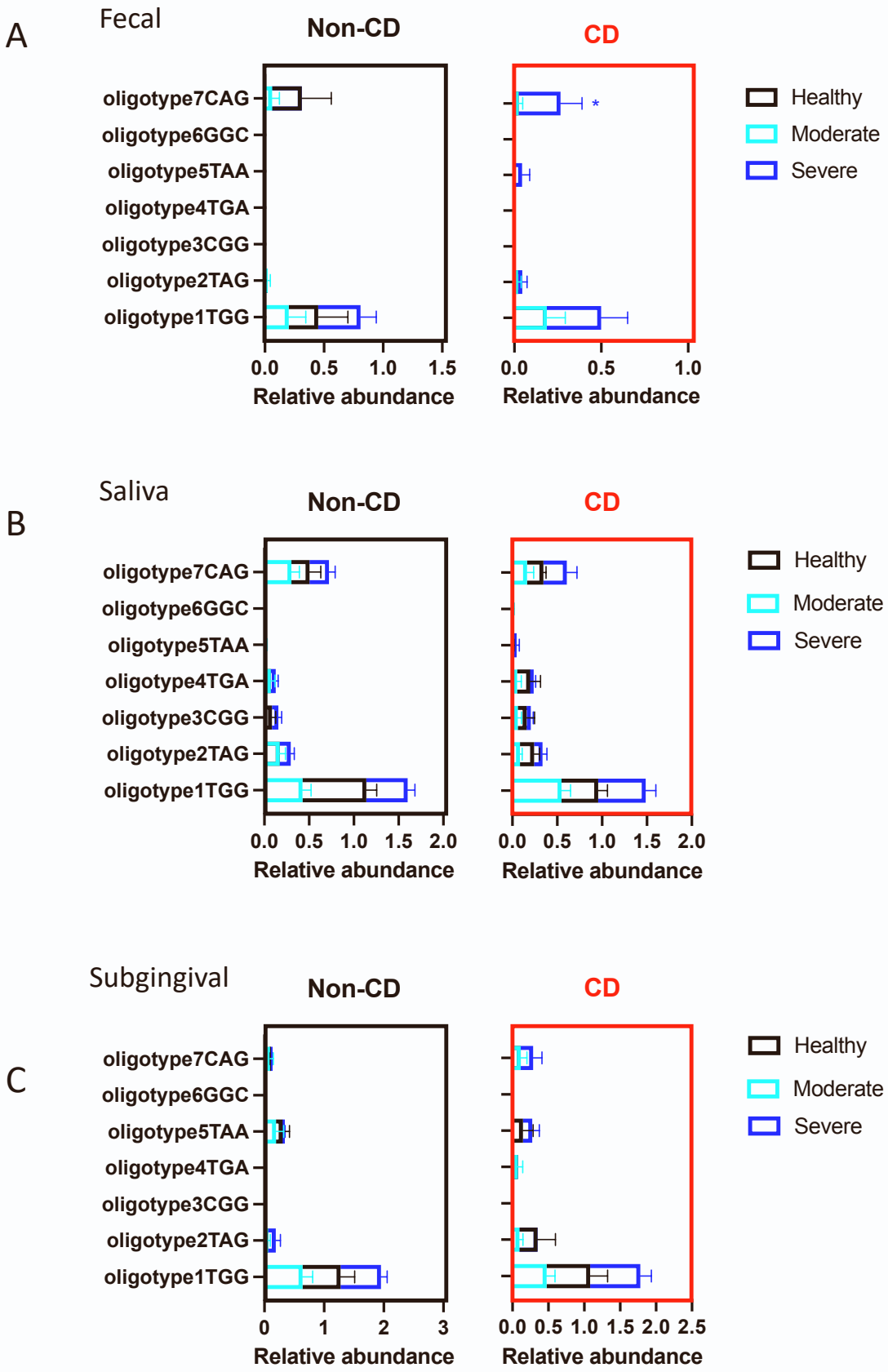
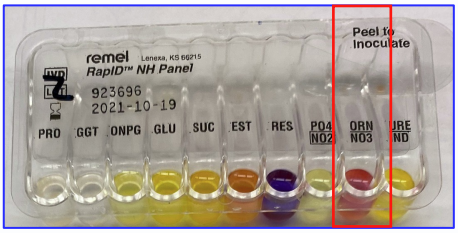


Figure S4. Oligotyping analysis of *H. parainfluenzae* 16S rRNA amplicon. Related to Figure 2. Oligotypes of *H. parainfluenzae* in (A) fecal, (B) saliva, and (C) subgingival based on CD or PD status (n=4 in healthy PD and non-CD, n= 4 in healthy PD and CD, n=7 in moderate PD and non-CD, n=13 in moderate PD and CD, n=14 in severe PD and non-CD, and n=8 in severe PD and CD) represented on stacked bar plot. Statistical testing was performed using two-way ANOVA followed by Sidak's multiple comparisons test. *Adjusted p-value <0.05

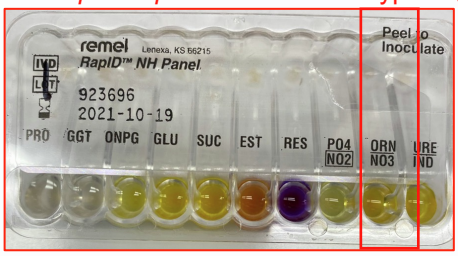
Supplementary Figure 5

A

Haemophilus parainfluenzae biotype III (S1p)



Haemophilus parainfluenzae biotype V (S2)



B

ID	Location	Biotype
S1p	Saliva	III
S2	Saliva	V
F2	Fecal	III
F3p	Fecal	V

C

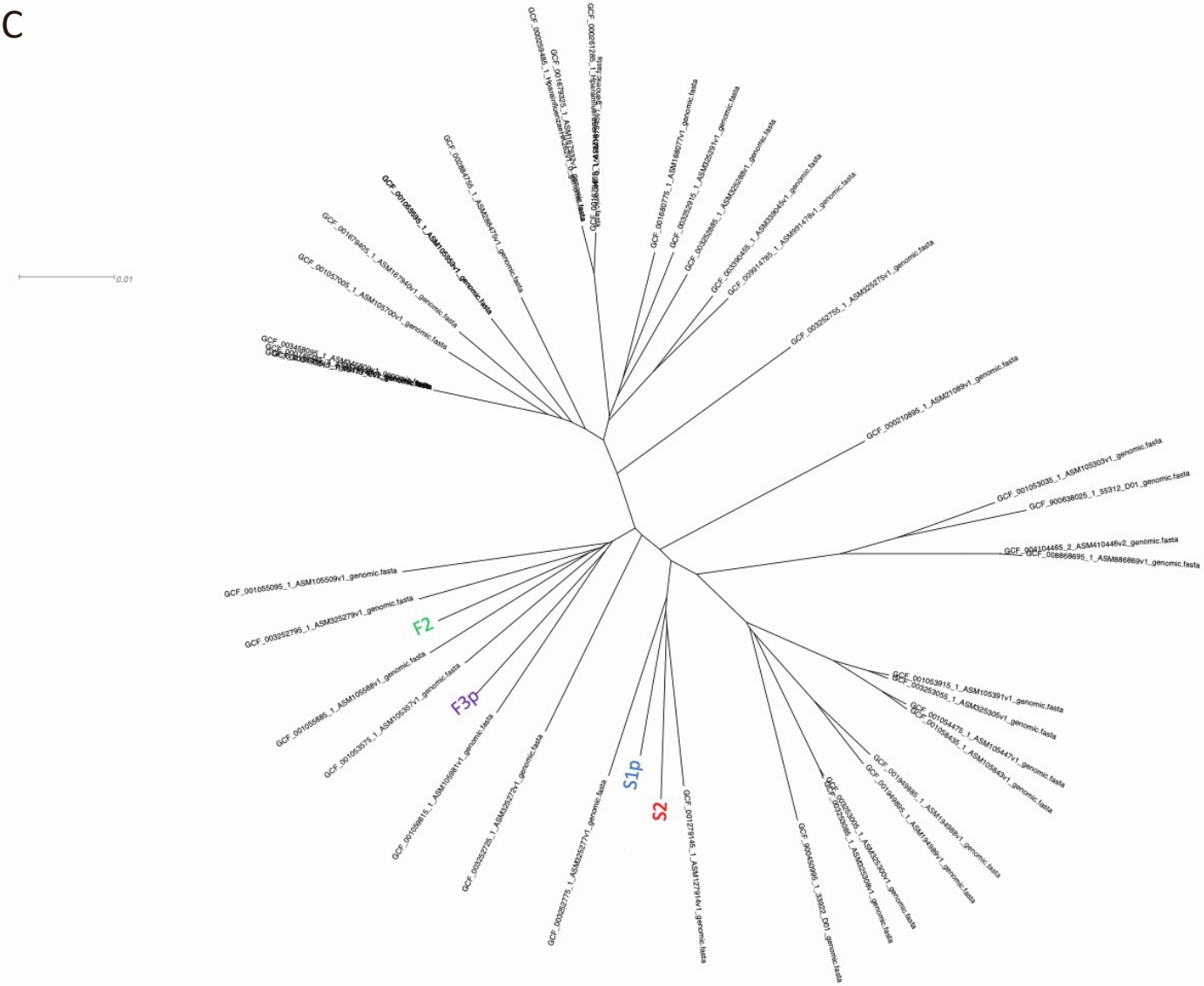
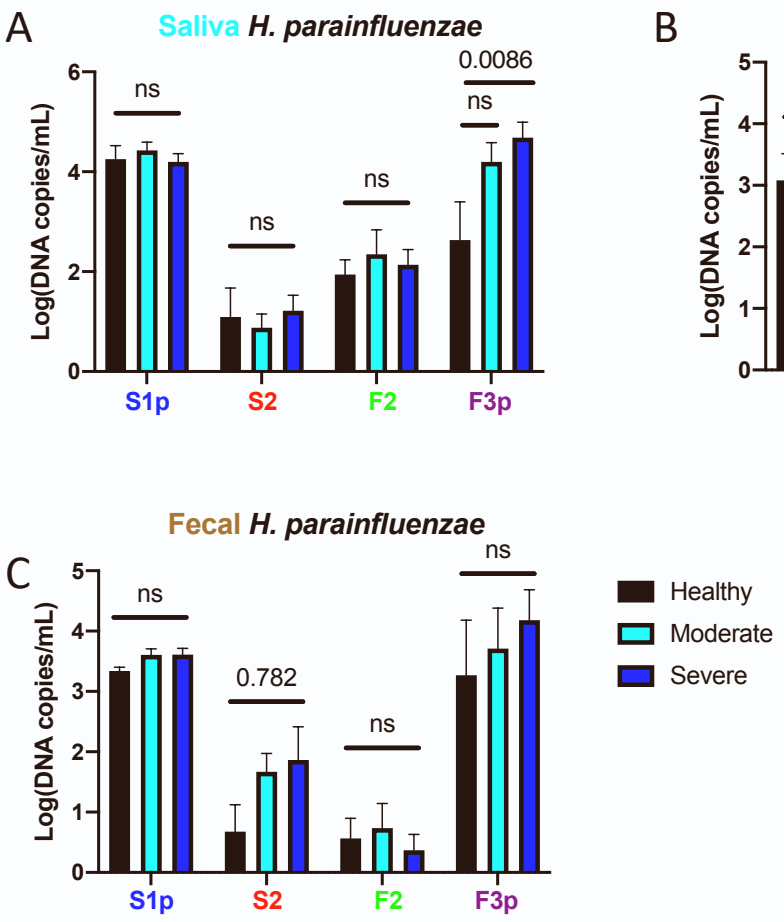


Figure S5. Biochemical and genomic analysis of *H. parainfluenzae* strains. Related to Figure 2. Biochemical assay to determine *H. parainfluenzae* biotype. (A) *H. parainfluenzae* biotype II (S1p and F2 strains) metabolizes ornithine, and *H. parainfluenzae* biotype V (S2 and F3p strains) does not metabolize ornithine. **(**B) Table summarizing *H. parainfluenzae* strain and biotype. (C) Whole genome-based phylogeny of H. parainfluenzae genomes. Isolated strains (S1p, S2, F2, and F3p) are highlighted in colors.

Supplementary Figure 6



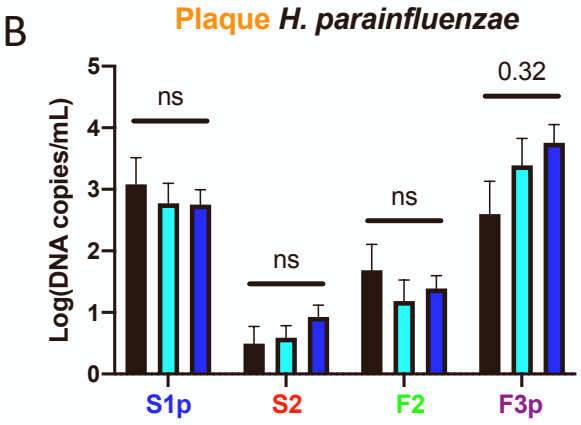
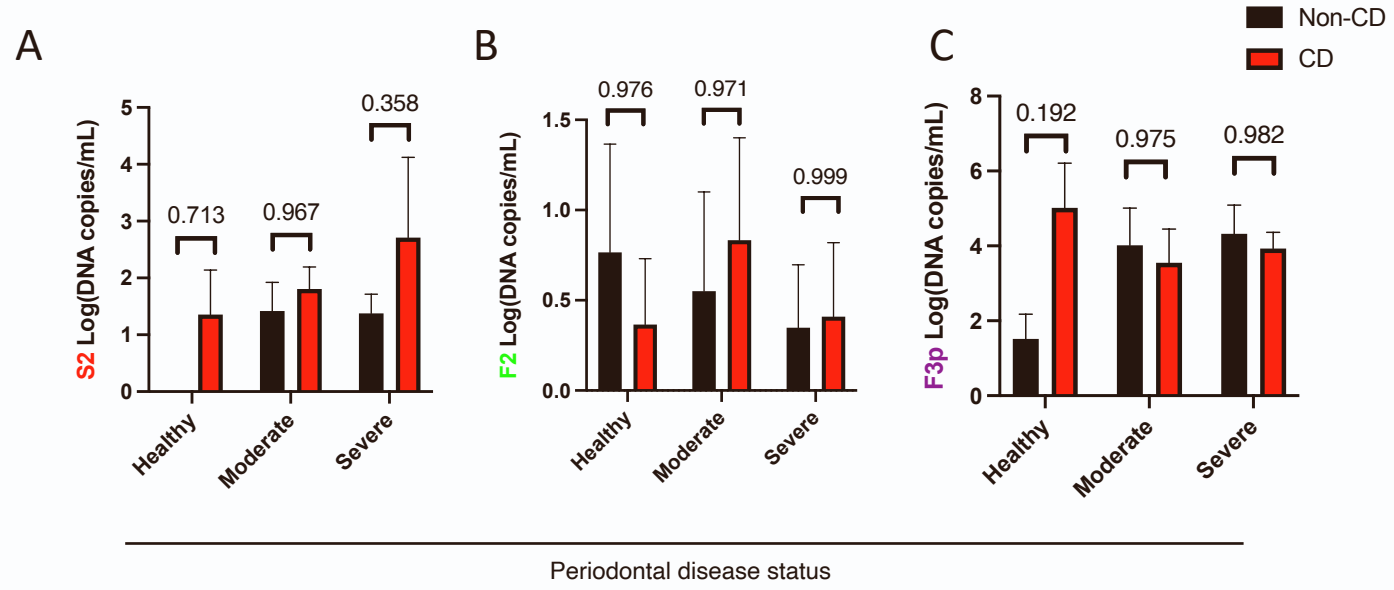


Figure S6. Quantification of H. parainfluenzae strains in (A) saliva, (B) subgingival, and (C) fecal samples based on PD status. Related to Figure 2. (n=8 in healthy, n=21 in moderate, and n=21 in severe)Statistical testing was performed using 2-way ANOVA and Sidak's multiple comparison test.

Supplementary Figure 7



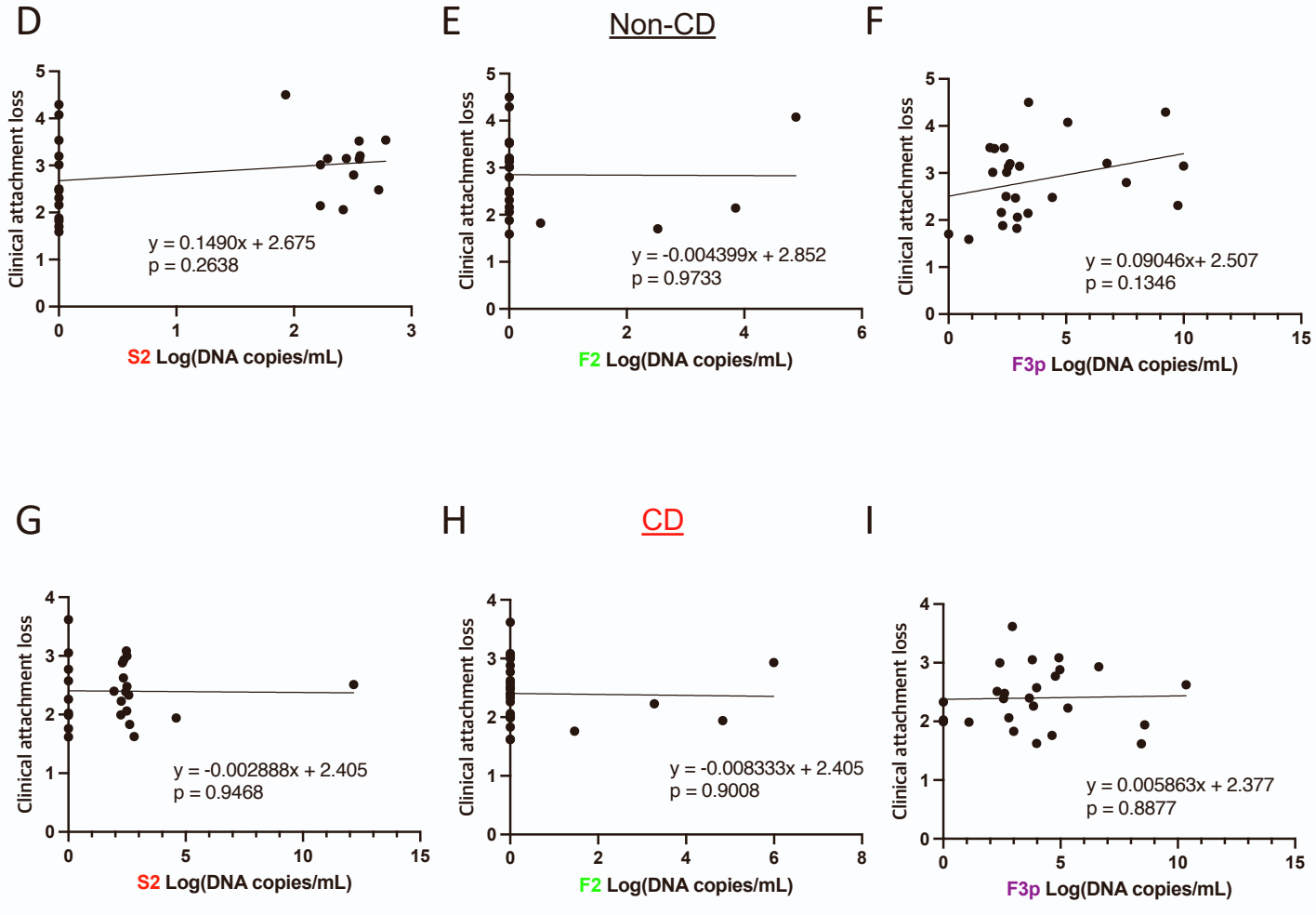


Figure S7. Association between *H. parainfluenzae* strains and periodontal disease severity. Related to Figure 2. (A) qPCR quantification of *H. parainfluenzae* strains (A) S2, (B) F2, and (C) F3p in fecal samples based on periodontal disease status in CD patients (n=4 in healthy PD and non-CD, n= 4 in healthy PD and CD, n=7 in moderate PD and non-CD, n=13 in moderate PD and CD, n=14 in severe PD and non-CD, and n=8 in severe PD and CD). (D-I) Pearson correlation analysis of fecal *H. parainfluenzae* strains S2, F2, and F3p log (DNA copies/mL) with clinical attachment loss in non-CD (n=25) and CD (n=25). Statistical testing was performed using (A-C) 2-way ANOVA and Sidak's multiple comparison test, and (D-I) two-tailed statistical test.

Strain	Fprward_seq	Reverse_seq	Internal Oligo
S1p	TTTTCACGCAGTTTCACCAA	CCCACAATAACGCAGAACCT	CAACCCGTGGCAAGGGCTTG
S2	GGACTGTAATAGCGGAAACGA	TTACCGGCAGAAACTGAACC	TGCCCCACAAAGCGATGGTT
F2	ATTTTGGATCGAAGGGGAAG	GGCATCGTCTTGTTTGTTCC	TGGCGGGAGTTTGCGAGAGA
F3p	TCTTTACCCTGCGCATTTTT	ACACACCGTTTGGGAAAGAG	TGCCATCATCATCCACCGCC

Table S1. H. parainfluenzae strain-specific qPCR primer and probe sequences. Related to the design of strain-specific primers for *H. parainfluenzae* strains in STAR Methods section.

Dataset 1 (MTX|EC)	Dataset 2 (MGX|Species)	Spearman	P value	FDR p
4.1.1.17: Ornithine decarboxylase	Haemophilus parainfluenzae	0.626	4.41E-84	9.08E-80
4.1.1.17: Ornithine decarboxylase	Veillonella unclassified	0.422	3.72E-34	5.37E-31
4.1.1.17: Ornithine decarboxylase	Veillonella dispar	0.307	4.96E-18	1.59E-15
4.1.1.17: Ornithine decarboxylase	Veillonella atypica	0.299	3.29E-17	1.00E-14
4.1.1.17: Ornithine decarboxylase	Streptococcus parasanguinis	0.298	5.18E-17	1.55E-14
4.1.1.17: Ornithine decarboxylase	Streptococcus salivarius	0.247	5.19E-12	8.51E-10
4.1.1.17: Ornithine decarboxylase	Veillonella parvula	0.232	9.64E-11	1.35E-08
4.1.1.17: Ornithine decarboxylase	Oscillibacter unclassified	-0.145	6.27E-05	1.77E-03
4.1.1.17: Ornithine decarboxylase	Lachnospiraceae bacterium	-0.142	8.37E-05	2.23E-03
4.1.1.17: Ornithine decarboxylase	Flavonifractor plautii	-0.141	9.91E-05	2.55E-03
4.1.1.17: Ornithine decarboxylase	Klebsiella pneumoniae	0.135	1.93E-04	4.27E-03
4.1.1.17: Ornithine decarboxylase	Clostridium bolteae	-0.134	2.20E-04	4.71E-03
4.1.1.17: Ornithine decarboxylase	Parabacteroides goldsteinii	-0.123	6.50E-04	1.07E-02
4.1.1.17: Ornithine decarboxylase	Clostridium symbiosum	-0.113	1.73E-03	2.18E-02
4.1.1.17: Ornithine decarboxylase	Bacteroides intestinalis	-0.113	1.84E-03	2.27E-02
4.1.1.17: Ornithine decarboxylase	Parabacteroides distasonis	-0.104	3.97E-03	3.93E-02

Table S4. Significantly associated species with microbial ornithine decarboxylase activity. Related to Figure 6. This table was adapted from cross-data type association analysis from the original paper describing iHMP2 dataset[1]. Subject-specific random effect and covariate effects were minimized by first residualizing using a linear mixed effect model. Spearman associations using HAllA 0.8.17 (hierarchical all-against-all association testing) and FDR correction were performed for statistical testing. MTX: metatranscriptomics, MGX: metagenomics.

Reference

1. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature. 2019;569(7758):655-62; doi: 10.1038/s41586-019-1237-9.