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9 Supplementary Methods:

10

11 <u>Overview of the Study Design</u>

12

13 For the current study, we first included participants with confirmed coronavirus disease 2019 14 (COVID-19) enrolled as part of a randomized controlled trial (RCT) examining the effect of 15 several types of nasal irrigations on COVID-19-related outcomes. The detailed methods for this 16 RCT have been previously reported (1-3). Inclusion criteria included age ≥ 18 years, a qualitative 17 PCR test positive for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) performed 18 at Vanderbilt University Medical Center (VUMC) or one of its affiliated centers, planned self-19 quarantine after being diagnosed with COVID-19, and residence within a 30-mile radius of the 20 main VUMC campus in Nashville, Tennessee. Exclusion criteria included current use of nasal 21 saline irrigations or other nasal medications (such as nasal steroids), inability to perform nasal 22 irrigations or to collect upper respiratory tract (URT) samples in a separate house bathroom or 23 away from household contacts, and the need for hospitalization related to COVID-19. Thus, only 24 participants with symptomatic, mild-to-moderate COVID-19 (based on criteria from the World 25 Health Organization (4)) were included in the RCT. Eligible participants were contacted and 26 enrolled in the RCT within 24 hours of the initial diagnosis of COVID-19. Only participants who 27 were not randomized to one of the RCT's intervention arms -thus, were not assigned to one of 28 the nasal irrigations being tested– were included in the current study (n=24). 29

30 In parallel to conducting the aforementioned RCT, we also enrolled asymptomatic participants

within the VUMC community (including employees, students, and faculty, among others) to serve as controls (n=24). Inclusion criteria included age ≥ 18 years and absence of COVID-19-

related symptoms (e.g., runny nose, cough, or fever). Exclusion criteria included current use of COVID-19-

nasal saline irrigations or other nasal medications (such as nasal steroids) and current or prior

diagnosis of COVID-19. The asymptomatic participants were not related to those with COVID-

- 36 19 or lived in the same household.
- 37

Following adequate training, all of the above participants (n=48) were asked to collect a mid-

turbinate swab on the day of enrollment (day 1) using a self-collection kit (FLOQSwabs, Copan

- 40 Diagnostics Inc.). Those with COVID-19 were also asked to collect serial samples using the
- same method on follow-up days 3, 5, 7, 10, 14, and 21. This last day of follow-up (21 days) was
- 42 decided based on the presumed duration of SARS-CoV-2 transmission and the recommended

43 period of isolation for adults with COVID-19 at the time when the aforementioned RCT was

44 being conducted (5). The collection of all samples included in the current study occurred in

45 Spring-Summer of 2020. Each adult provided informed consent for his/her participation. The
 46 VUMC Institutional Review Board and Biosafety Committee approved this study.

46 47

48 SARS-CoV-2 Testing by Quantitative Reverse Transcription PCR

49 To measure viral load in participants with COVID-19 and rule out asymptomatic infection in

50 controls, quantitative reverse transcription PCR in all mid-turbinate swabs collected at

51 enrollment was performed. Total RNA was extracted from samples using a phenol-chloroform-

52 based method. Samples were placed in Red 1.5 mL RINO[®] screw-cap tubes (NextAdvance) pre-

53 filled with RNase-free zirconium oxide beads and QIAzol Lysis Reagent (Qiagen) was added.

54 Then, samples were homogenized in a Bullet Blender 24 Gold (NextAdvance) for 3 minutes at

55 maximum speed. Once samples were homogenized, genomic DNA was eliminated with gDNA

- Eliminator columns (Qiagen), and RNA was purified using the RNeasy Mini Plus Kit (Qiagen)
 following the manufacturers' protocols. RNA quality was measured using an Agilent 2100
- 57 Bioanalyzer (Agilent Technologies). The United States Centers for Disease Control and
- 59 Prevention primers and probes designed for the detection of SARS-CoV-2 (2019-nCoV) were
- 60 purchased from Integrated DNA Technologies (6). The SARS-CoV-2 nucleocapsid gene regions
- 61 1 and 2 were both targeted for the detection of SARS-CoV-2. RNase P was also examined as a
- 62 measure of RNA quality and quantity. RT-qPCR was performed using SuperScript III One-Step
- 63 RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen) on a Bio-Rad CFX96 Touch
- 64 Real-Time PCR Detection System (Bio-Rad) as per manufacturer's instructions. Plasmid
- 65 controls for SARS-CoV-2 nucleocapsid genes and RNase P were also ordered from Integrated
- DNA Technologies at a concentration of 66,666 copies/reaction. No-template-controls and an
 extraction negative control were used as negative controls. Reactions were prepared using 12.5
- μ SuperScript III Master Mix (ThermoFisher), 1 μ l each 400nm forward and reverse primer, 1 μ l
- 400nM FAM-labelled probe, 1 μl Platinum Taq polymerase, 3 μl of template RNA, and 7.25 μl
- 70 PCR Certified Water (Teknova). RNA was reverse transcribed at 50°C for 15 minutes, and PCR
- 71 conditions were run at 95°C denaturation step for 2 minutes, followed by 40 cycles of 95°C for
- 15 seconds and 55°C for 30 seconds. To measure SARS-CoV-2 viral load, the cycle threshold
- values were captured, analyzed, and calculated by the CFX Maestro software (Bio-Rad).
- 74

75 Characterization of the URT Microbiota

76

77 The methods used to characterize the URT microbiota have been previously described in detail 78 (3, 7-10). First, bacterial DNA from the mid-turbinate swabs using the DNeasy PowerSoil kit 79 (Qiagen) was extracted. Dual-indexed universal primers appended with Illumina-compatible 80 adapters were used to amplify the 16S ribosomal RNA (rRNA) gene. Primer sequences were 515F: 5'-AATGATACGGCGACCACCGAGATCTACAC NNNNNNN TATGGTAATT GT 81 82 GTGCCAGCMGCCGCGGTAA-3' and 806R: 5'-CAAGCAGAAGACGGCATACGAGAT 83 NNNNNNN AGTCAGTCAG CC GGACTACHVGGGTWTCTAAT-3', where the string of 84 8 N's represents the indices, and the bolded nucleotides represent the sites that bind to 16S rRNA 85 gene target sequence (7). For this, amplicons targeting the V4 region of the 16S rRNA gene were 86 generated by combining 7 µl of template, 12.5 µl MyTaq HS Mix (Bioline), 0.75 µl DMSO 87 (Sigma), 3 µl PCR Certified Water (Teknova), and 1 µl of each 10 µM primer. DNA was 88 denatured at 95°C for 2 min, followed by 30 cycles of 95°C for 20 seconds, 55°C for 15 seconds, 89 and 72°C for 5 minutes, and a final extension at 72°C for 10 minutes. Each amplified sample was 90 run on a 1% agarose gel to confirm reaction success. Amplicons were cleaned and normalized 91 with the SequalPrep Normalization Kit (ThermoFisher). Normalized amplicons were pooled and 92 cleaned with 1X AMPure XP beads (Beckman Coulter). The pool was then sequenced on an 93 Illumina MiSeq platform with 2x250 base pair reads. Two ZymoBIOMICS Microbial 94 Community DNA Standard (Zymo) mock community controls were processed concurrently with 95 participant samples. Negative controls (7 extraction and 15 PCR negative controls) were also 96 amplified and sequenced concurrently with participant samples. Following the sequencing 97 procedure, both mock community controls had a community composition very similar to what 98 was expected. In addition, only a small fraction of 16S rRNA gene sequences were found in the 99 negative controls compared to participant samples, and the bacterial sequences recovered had 100 little overlap with participant samples.

101

- 102 Next, the 16S rRNA gene sequences were processed using the R package dada2 by applying its
- 103 standard operating procedure (available at: https://benjjneb.github.io/dada2/tutorial.html) (11).
- 104 To this end, sequences were grouped into amplicon sequence variants (ASVs), and taxonomy
- 105 was assigned using the SILVA reference database version 132 (12). Low-quality sequences,
- 106 chimeras, and non-bacterial sequences were discarded as part of the dada2 pipeline. To remove
- 107 any suspected contaminants that were found in the negative controls, the remaining sequences
- 108 were processed using the R package decontam (13) using the "prevalence" method, with default
- 109 parameters. The full taxonomy of contaminant ASVs identified by decontam are listed in Table 110 S1. Those ASVs that were present in >1 sample were then retained and samples with <1,000
- 111 sequences (n=33) were discarded using the R package *phyloseq* (14, 15). Last, the relative
- 112 abundances of individual ASVs were calculated using simple proportions.
- 113
- 114 The raw 16S ribosomal RNA data discussed in this publication have been deposited in NCBI's 115 Sequence Read Archive and are accessible through accession number PRJNA726992 (16).
- 116
- 117 Statistical Analyses
- 118
- 119 General Approach: Statistical analyses were conducted at the ASV level in R version 3.1.10
- 120 (17). To compare the URT microbiota between groups in a comprehensive manner, different
- 121 types of statistical analyses that are frequently employed in microbial ecology were used,
- 122 including α -diversity (richness \pm evenness), β -diversity (overall composition), and differential
- 123 abundance (individual taxa) analyses. For α - and β -diversity analyses only, the processed dataset 124 was rarefied to the lowest library size of all samples (n=1,154). This rarefaction process was
- 125 repeated multiple times (n=400) and the results were averaged. Common α -diversity (observed
- 126 species, Shannon, and inverse Simpson indices) and β-diversity (Bray-Curtis [based on taxa
- 127 abundance] and Jaccard [based on taxa presence vs. absence] indices) metrics were calculated.
- 128 For differential abundance analyses, a non-rarified dataset was used and, to minimize the impact
- 129 of rare taxa, only ASVs with a relative abundance across all samples >0.01% were included. If a
- 130 particular ASV lacked species classification, then its identity at the next higher available
- 131 taxonomic level (e.g., genus or family) was used. Initial steps were conducted using the R
- 132 package *phyloseq* (14). Final comparisons were conducted using several R packages, which are
- 133 described below. All statistical models included age, sex, and the presence of at least one
- 134 comorbidity (i.e., obesity, diabetes, hypertension, lung disease, or heart disease, coded as yes vs. 135
- no or not reported) as covariates. Age was centered and scaled prior to statistical analyses. When 136
- appropriate, *p*-values were controlled for multiple testing using the Benjamini-Hochberg
- 137 procedure and the resulting q-values are reported (18). Statistical significance was defined as a p-138
- or q-value <0.05. Figures were created using the R packages ggplot2, ggalluvial, and 139 ComplexHeatmap (19). Minor aesthetic edits to figures (e.g., paneling, text insertion, or label
- 140 formatting) were made with Inkscape version 1.0.1 (available at: https://inkscape.org/).
- 141 Following statistical analyses, we attempted to identify the corresponding species of unclassified
- 142 ASVs that were found to be differentially abundant between groups using the Basic Local
- 143 Alignment Search Tool (BLAST) database and based on the highest expected value (E value)
- 144 and percent identity (20).
- 145

Comparisons between SARS-CoV-2-infected and -uninfected participants: First, the URT 146 147 microbiota of SARS-CoV-2-infected participants at each of the 7 study time points to that of controls at enrollment was compared. The comparisons of α -diversity metrics were conducted 148 149 using linear regression. To compare β -diversity metrics, non-metric-multidimensional scaling 150 (NMDS) and permutational multivariate analysis of variance (PERMANOVA) was used as 151 implemented in the metamds (with 999 iterations) and adonis2 (with 999 permutations and the 152 by = "margin" argument) functions of the R package *vegan*, respectively (21, 22). Differential 153 abundance analyses were performed using the R package DESeq2 (23). For the different types of 154 statistical analyses, the Benjamini-Hochberg procedure was used to control for multiple testing 155 (18). In the case of α - and β -diversity analyses, this was done separately for each index by 156 including all 7 p-values obtained from the individual comparisons in the false-discovery rate 157 calculations. Because DESeq2 already implements the Benjamini-Hochberg procedure to control 158 for multiple testing when performing analyses and to avoid overcorrection, no further correction 159 for multiple testing was performed in differential abundance analyses. However, based on pre-160 established criteria, ASVs were considered differentially abundant only if, in addition to 161 statistical significance, they 1) had an absolute fold change ≥ 2 (equivalent to an absolute fold

- 162 change ≥ 1 in the log₂ scale), and 2) were differentially abundant in ≥ 3 of the 7 individual 163 comparisons.
- 164

165 Longitudinal effect of SARS-CoV-2 viral load on the URT microbiota: Among participants with

166 COVID-19, the URT microbiota was then compared between those with and without high viral

167 load over time. High viral load was defined as a cycle threshold value for the detection of the

168 coronavirus nucleocapsid gene region 1 below the median of all samples collected on day 1 from

169 SARS-CoV-2-infected participants. The comparisons of α -diversity metrics were conducted

170 using generalized estimating equations (GEE) as implemented in the R package *geepack*

171 (clustering by participant, using an exchangeable correlation structure, and with robust standard 172

172 errors) (24). To compare β -diversity metrics, NMDS and PERMANOVA were used as

implemented in the *metamds* (with 999 iterations) and *adonis2* (with 999 permutations, the by =
"margin" argument, and restricted permutations by participant) functions of the R package

vegan, respectively (21, 22). Differential abundance analyses were performed using the

permuspliner function of the R package *SplinectomeR* (25). For this, we used the relative

abundances of individual ASVs, restricted the statistical analyses to the top 25 most predominant

ASVs in SARS-CoV-2-infected participants (which together represented 90.33% of all reads),

and controlled for multiple testing with the Benjamini-Hochberg procedure (18). Because

180 SplinectomeR does not allow for covariate adjustment, the association of SARS-CoV-2 viral load

181 with the relative abundance of ASVs found to be significant in *SplinectomeR* were then tested

182 using GEE as described above while adjusting for potential confounders. The statistical models

183 for α -diversity and differential abundance analyses using GEE also included the study time point

and a multiplicative interaction between SARS-CoV-2 viral load and the study time point as

additional covariates.

186 187 Supplementary Tables:

188 189 190
Table S1: Full taxonomy of all ASVs removed by the R *decontam* package (see attached .csv

file).

191 Supplementary Figure Legends:

- 192
- 193 Figure S1: Differential abundance of taxa of the upper respiratory tract microbiota between
- 194 SARS-CoV-2-uninfected and -infected participants. The figure shows box plots of *DESeq2*
- 195 normalized counts (y-axes) of selected ASVs by group (x-axes). Only ASVs that were
- 196 differentially abundant between groups using *DESeq2* models are shown (see text and Figure 4).
- 197 For SARS-CoV-2-infected participants, the y-axes represent the mean *DESeq2* normalized
- 198 counts across all follow-up days. *Definition of abbreviations:* ASV = Amplicon sequence
- 199 variant, SARS-CoV-2 = Severe acute respiratory syndrome coronavirus-2.
- 200

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203

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DESeq2 normalized counts

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SARS-CoV-2

							0
ASV_sequence	Kingdom	h Phylum	Class	Order	Family	Genus	Species
AACGTAGGGTGCAAGCGTTGTCCGGAATTACTGGGTGTAAAGGGAGCGCAGGCGGACCGGCAAGTTGGAAGTGAAAACTATGGGCTCAACCCATAAATTGCTTTCAAAACTGCTGGCCTTGAGTAGTGCAGAGGTAGGT	Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Subdoligranulum	NA
AACGTAGGTCACAAGCGTTGTCCGGAATTACTGGGTGTAAAGGGAGCGCAGGCGGGAGAACAAGTTGGAAGTGAAATCCATGGGCTCAACCCATGAACTGCTTTCAAAACTGTTTTCTTGAGTAGTGCAGAGGTAGGCGGAA	Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Faecalibacterium	prausnitzii
AACGTAGGTCACAAGCGTTGTCCGGAATTACTGGGTGTAAAGGGAGCGCGGGCGAGCGGCGATCAAGTTGGAAGTGAAATCCATGGGCTCAACCCATGAACTGCTTTCAAAACTGGTCGTCTTGAGTAGTGCAGAGGTAGGCGGA	A Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Faecalibacterium	NA
GACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGCGTCGCGTCGAATGTGAAATCCCCGAGGCTTGCATTCGGTCGG	Bacteria	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Actinonlanes	utahensis
	Bacteria	Proteobacteria	Gammaproteopacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	NA
TACGAAGGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGACTCTTAAGTCGGGGGTGAAAGCCCAGGGCTCAACCCTGGAATTGCCTTCGATACTGAGAGTCTTGAGTTCGGAAGAGGTTGGTGGA	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea	NA
TACGAAGGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGTGCGTAGGCGGGTCTTTAAGTCAGGGGTGAAATCCTGGAGCTCAACTCCAGAACTGCCTTTGATACTGAAGATCTTGAGTTCGGGAGAGGTGAGTGGAGTGGA	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	NA
TACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAA	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	NA
	I Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	salivae
		Dactoroldota					Salivae
TACGGAAGGTCCAGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCCGTGGATTAAGCGTGTTGTGAAATGTAGACGCTCAACGTCTGAATTGCAGCGCGAACTGGTTCACTTGAGTATGCGCAACGTAGGCCGGAAT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
TACGGAAGGTCCAGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCGGATTGTTAAGTCAGCGGTTAAAGGGTGTGGCTCAACCATGCATTGCCGTTGAAACTGGCGATCTTGAGTGCAGACAGGGATGCCGGAATT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella	NA
TACGGAAGGTCCAGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGTGTAGGCGGTCTGTTAAGCGTGTTGTGAAATTTAGGTGCTCAACATCTACCTTGCAGCGCGAACTGGCGGACTTGAGTGCACGCAACGTATGCGGAAT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	intermedia
	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	ΝΑ
	Duotoria	Daotoroidota	Bastaviala				
TACGGAAGGTCCAGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGTGTAGGCGGTTTGTTAAGCGTGTTGTGAAATTTAGGTGCTCAACATTTAACTTGCAGCGCGAACTGTCAGACTTGAGTACACGCAACGTATGCGGAATTG	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	pallens
TACGGAAGGTCCGGGCGTTATCCGGAATTATTGGGTTTAAAGGGAGCGCAGGCGGGAGTATAAGTCAGCTGTTAAATATCAGGGCCCAACTCTGTTATGCAGTTGAAACTATATTTCTTGAGTACGCACAGGGATGGCGGAATTCA	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella	NA
TACGGAAGGTCCGGGCGTTATCCGGAATTATTGGGTTTAAAGGGAGCGCAGGCGGGAGTGTAAGTCAGCTGTTAAATATCAGGGCCCAACTCTGTTATGCAGTTGAAACTATATTTCTTGAGTACGCACAGGGATGGCGGAATTC	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella	NA
TACGGAAGGTCCGGGCGTTATCCGGAATTATTGGGTTTAAAGGGAGCGCAGGCGGGAGTGTAAGTCAGCTGTTAAATATCAGGGCCCAACTCTGTTATGCAGTTGAAACTATATTTCTTGAGTACGCACAGGGATGGCGGAATTC	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella	NA
	Destaria	Destavaidate	Destavoidie	Destervideles	Drevetellesses		
	Dacteria	Dacteroluota	Dacteroiula	Bacteroidales	Frevolellaceae	Frevotella	INA
TACGGAAGGTCCGGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCCGGAGATTAAGTGTGTTGTGAAATGTAGACGCTCAACGTCTGACTTGCAGCGCATACTGGTTTCCTTGAGTACGCACAACGTTGGCGGAAT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
TACGGAAGGTCCGGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCGGATTGTTAAGTCAGCGGTTAAAGGGTGTGGCTCAACCATACATTGCCGTTGAAACTGGCGATCTTGAGTGCAGACAGGGATGCCGGAATT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella	NA
TACGGAAGGTCCGGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCTGGAGATTAAGTGTGTGT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	histicola
	Bactoria	Bacteroidota	Bacteroidia	Bacteroidales	Provotellaceae	Provotella	ΝΔ
		Dactoroldota					
TACGGAAGGTTCGGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCCGGAGATTAAGTGTGTTGTGAAATGTAGACGCTCAACGTCTGACTTGCAGCGCATACTGGTTTCCTTGAGTACGCACAACGTTGGCGGAATT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	veroralis
TACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGATGGATG	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	NA
TACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTGGACAGTTAAGTCAGTTGTGAAAGTTTGCGGCTCAACCGTAAAATTGCAGTTGATACTGGCTGTCTTGAGTACAGTAGAGGTGGGCGGAATT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	NA
TACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTGGGACTGGTAAGTCAGTTGTGAAAGTTTGCGGCTCAACCGTAAAATTGCAGTTGATACTGTCAGTCTGAGTACAGTAGAGGTGGGCGGGAATT	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	NA
	Duotonia	Daotoroidota	Bastasiala		Destauldessa		
	Bacteria	Bacteroldota	Bacteroidia	Bacteroidales	Bacteroldaceae	Bacteroides	NA
TACGGAGGATGCGAGCGTTATCCGGAATCATTGGGTTTAAAGGGTCTGTAGGCGGGCTGGTAAGTCAGAGGTGAAAGCGCTTAGCTCAACTAAGCAACTGCCTTTGAAACTGTTGGTCTTGAATGGTTGTGAAGTAGTTGGAAT	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	ochracea
TACGGAGGATGCGAGCGTTATCCGGAATTATTGGGTTTAAAGGGTGCGTAGGTTGCAAGGGAAGTCAGGGGTGAAAAGCCATAGCTCAACTATGGTCTTGCCTTTGAAACTCTCTAGCTAG	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	NA
	Racteria	Bacteroidota	Bacteroidia	Bacteroidales	Marinifilaceae	Odoribacter	splanchnicus
	Duotonia	Datata			Dhardaharata		
TACGGAGGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGACCGGAAAGTTGGGGGGTGAAATCCCGGGGGCTCAACCTCGGAACTGCCTTCAAAACTACTGGTCTTGAGTTCGAGAGAGGGGGAGGGGGGGG	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	NA
TACGGAGGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGATTGGAAAGTCAGAGGTGAAATCCCAGGGCTCAACCCTGGAACTGCCTTTGAAACTCCCAGTCTTGAGGTCGAGAGAGGTGAGTGA	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Haematobacter	NA
TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCA	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Salmonella	enterica
	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Salmonella	NA
TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCA	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae	Morganella	morganii
TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCA	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Escherichia-Shigella	NA
TACGGAGGGTGCAAGCGTTAATCGGAGTTACTGGGCGTAAAGCGCACGCA	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	NA	NA
	Bacteria	Campilobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	concisus
	Dacteria				·		
TACGTAAGGACCGAGCGTTGTCCGGAATCATTGGGCGTAAAGGGTACGTAGGCGGCTAGAAAAGCTAGAAGTCAAAGGCTATAGCTCAACTATAGTAAGCTTCTAAAACTATTTAGCTTGAGAAATGGAAGGGAAAGTGGAATTCO	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Iissierellales	Anaerococcus	NA	NA
TACGTAAGGACCGAGCGTTGTCCGGAATCATTGGGCGTAAAGGGTACGTAGGCGGGTCATTAAGTTAGAAGTCAAAGGCTATAGCTCAACTATAGTAAGCTCCTAAAACTGGAGACCTTGAGTAATGGAAGGGAAAGTGGAATTC	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerococcus	NA	NA
TACGTAGGATCCGAGCGTTATCCGGAGTGACTGGGCGTAAAGAGTTGCGTAGGCGGTTGTATAAGTGAATAGTGAAATCTGGTGGCTCAACCATACAGGCTATTATTCAAACTGTACAACTCGAGAGTGGTAGAGGTCACTGGAA	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	NA	NA
TACGTAGGGAGCAAGCGTTGTCCGGATTTACTGGGTGTAAAGGGTGCGTAGGCGGCTTTGCAAGTCAGATGTGAAATCTATGGGCTCAACCCATAAACTGCATTTGAAACTGTAGAGCTTGAGTGAAGTAGAGGCAGGC	Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Buminococcus	bromii
	Duotoria						
	Bacteria	Firmicutes	Ciostridia	Clostridia UCG-014	NA	NA	INA
TACGTAGGGAGCGAGCGTTGTCCGGATTTACTGGGTGTAAAGGGTGCGTAGGCGGCGAGGCAAGTCAGGCGTGAAATCTATGGGCTTAACCCATAAACTGCGCTTGAAACTGTCTTGCTTG	Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Incertae Sedis	NA
TACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTCTGTCGCGTCTGCTGTGAAATCCCGAGGCTCAACCTCGGGCTTGCAGTGGGGAACTAGAGTGCGGTAGGGGAGAATGGA	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	NA
TACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTTGTAGGCGGTTTGTCGCGTCTGCTGTGAAAGCCCGGGGCTTAACCCCGGGTTTGCAGTGGGGTACGGGCTAACTAGAGTGCAGTAGGGGAGACTGGA	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Rothia	NA
	Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	E0332	ΝΔ
	Dacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae		
TACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTTGTAGGCGGTTGGCCGCGTCTGCCGTGAAATCCTCTGGCTTAACTGGGGGGCGTGGGTACGGGTTGACTTGAGTGCGGTAGGGGAGACTGGA	Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	turicensis
TACGTAGGGGGGCAAGCGTTATCCGGAATTACTGGGTGTAAAGGGTGCGTAGGTGGTATGGCAAGTCAGAAGTGAAAACCCAGGGCTTAACTCTGGGACTGCTTTTGAAACTGTCAGACTGGAGTGCAGGAGAGGTAAGCGGAA	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerostipes	hadrus
TACGTAGGGGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGTGCGTAGGTGGTCTACTAAGCGCGAGGTGAAAGGCAATGGCTCAACCATTGTTAGCCTTGCGAACTGGCAGACTTGAGTGCAAGAGAGAG	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerovoracaceae	[Eubacterium] saphenum group	NA
	1 Bacteria	Actinobacteriota	Coriobacterija	Coriobacteriales	Atopobiaceae	Atopobium	ΝΔ
TACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGACGGAATGGCAAGTCTGAAGTGAAATACCCGGGCTCAACCTGGGAACTGCTTTGGAAACTGTTGTTCTAGAGTGTTGGAGAGGGAAGGTAAGTGGAA	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Oribacterium	sinus
TACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGACGGCAAGGCAAGTCTGAAGTGAAAGCCCGGTGCTTAACGCCGGGACTGCTTTGGAAACTGTTTGGCTGGAGTGCCGGAGAGGGAAGGCAAGCCGGA	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae NK4A136 group	NA
TACGTAGGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGACGGCAAGGCAAGTCTGATGTGAAAACCCAGGGCTTAACCCTGGGACTGCATTGGAAACTGTCTGGCTCGAGTGCCGGAGAGGTAAGCGGA	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Fusicatenibacter	saccharivorans
TACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGACGGCACGGCAAGCCAGATGTGAAAGCCCGGGGCTCAACCCCGGGACTGCATTTGGAACTGCTGAGCTAGAGTGTCGGAGAGGCAAGTGG	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Dorea	longicatena
	Destaria	Firmieutee	Cleatridia			Deres	NA
	Dacteria	Firmicules	Ciostridia	Lachnospirales	Lacinospiraceae	Dorea	INA
TACGTAGGGGGGGGGGGGGGGGAGCGTTATCCGGAATTATTGGGCGTAAAGAGTGCGTAGGTGGCACCTTAAGCGCAGGGGTTTAAGGCAATGGCTCAACCATTGTTCGCCTTGCGAACTGGGGTGCTTGAGTGCAGGAGGGGAAAGTGGAAT	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerovoracaceae	[Eubacterium] nodatum group	NA
TACGTAGGGGGGGGGGGGGGGGGTATCATTGGGCGTAAAGCGCGCGGGGGGGCCCGGCAGGCCGGGGGGGG	Bacteria	Actinobacteriota	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella	aerofaciens
TACGTAGGGGGGGGGGGGGGTGTCCGGAATTACTGGGCGTAAAGGGCACGCAGGCTGTGCTTCAAGTCAGCTGTAAAAGGATGCGGCTTAACCGTGTTATGCAGTTGAGACTGAGGTGCTGGAGTACCGGAGAGGGCAAGTGGAA	Bacteria	Synergistota	Synergistia	Synergistales	Synergistaceae	Fretibacterium	NA
	Bactoria	Firmicutes	Clostridia	Pontostrontococcales Tissierellales	Pentostrentococcaceae	Pentostrentococcus	stomatic
TACGTAGGGGGCTAGCGTTATCCGGATTTACTGGGCGTAAAGGGTGCGTAGGTGGTTTCTTAAGTCAGGAGTGAAAGGCTACGGCTCAACCGTAGTAAGCTCTTGAAACTGGGAAACTTGAGTGCAGGAGAGAGGGAAAGTGGAAT	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Ierrisporobacter	mayombei
TACGTAGGGGGGCTAGCGTTGTCCGGAATCACTGGGCGTAAAGGGTTCGCAGGCAG	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptoniphilus	NA	NA
TACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATATAAGACAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGTGACTGTATAGCTAGAGTACGGCAGAGGGGGGATGGAAT	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	NA
TACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGCAAGACAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTGTGACTGCATGGCTAGAGTGCGGCAGAGGGGGGGTGGAA	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA
	Bactoria	Actinobactoriata	Actinobactoria	Pifidabaatarialaa	Pifidabactariacaaa	Pifidobactorium	ΝΑ
		Actinobacteriota	Actinobacteria		Dilidobacteriaceae		
TACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCTGCGCCGGGTACGGGCGGG	Bacteria	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	NA
TACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTGTGTAAGACAGGTGTGAAATCCCCGGGCTTAACCTGGGAACTGCGCTTGTGACTGCACGGCTAGAGTATGGCAGAGGGGGGGG	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Methyloversatilis	NA
TACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTTGTAAGACAGGCGTGAAATCCCCGGGCTCAACCTGGGAATGGCGCTTGTGACTGCAAGGCTAGAGTGCGTCAGAGGGGGGGTAGA	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	gilardii
TACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTTGTAAGACAGGTGTGAAATCCCCGGGCTTAACCTGGGAACTGCACTGCAAGGCTAGAGTACGGCAGAGGGGGGGG	A Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Curvibacter	NA
	Bacteria	Actinobacteriota	Actinobacteria	Convnehacteriales	Convnehacteriaceae	Lawsonella	ΝΔ
	Duciella					Commente de la	
	A Bacteria	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	NA
TACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	NA
TACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	NA
TACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	NA
TACGTAGGTGGCAAGCGTTATCCCCCATTATTCCCCCCTAAACACTCCACCCCCTTTTCTAACTCCATCACATCCACACCCCTTTTCTAACTCCACCCCCTAAACACCTCCACCCCCTTTTCTAACTCCACCCCCTAAACACCTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCTTTTCTAACTCCACCCCCC	Dect-	Eirmioute -	Booilli				ΝΔ
							רא ר
	Bacteria	Firmicutes	Negativicutes	veilionellales-Selenomonadales	Veillonellaceae	NA	NA
TACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCGCGCGC	A Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Megasphaera	micronuciformis
TACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTCCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGGGGAGGTGGA	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	NA
TACGTAGGTGGCAAGCGTTGTCCGGATTTACTGGGTGTAAAGGGCGTGCAGCCGGGTCTGCAAGTCAGATGTGAAATCCATGGGCTCAACCCCATGAACTCCCAACCCCATGAAACTCTCAACTCTCAACTCTCCACCCCAATGCCAATGCCAACCCCATGAACTCCCATGAAACTCCAACCCCAACCCCAACCCCAACCCCAACCCCAACCCCAACCCC	Bacteric	Firmicutes	Clostridia	Oscillosnirales	Oscillospiraceae	UCG-002	NA
	Bacteria	Firmicutes	Ciostridia	Uscillospirales	Uscillospiraceae	UCG-002	NA
TACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	cecorum
TACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	NA
TACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGCGCGC	Bacteria	Firmicutes	Bacilli	Lactobacillales	Listeriaceae	Listeria	NA
	J Bacteria	⊢ırmıcutes	Bacilli	rysipelotrichales	rysipelatoclostridiaceae	Catenipacterium	mitsuokai
TACGTAGGTGGCGAGCGTTATCCGGATTTACTGGGTGTAAAGGGCGCGTAGGCGGGAATGCAAGTCAGATGTGAAATCCAAGGGCTCAACCCTTGAACTGCATTTGAAACTGTATTTCTTGAGTGTCGGAGAGGGTTGACGGAAT	Bacteria	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	NK4A214 group	NA
TACGTAGGTGGCGAGCGTTGTCCGGAATCATTGGGCGTAAAGGGAGCGCAGGCGGGCCGGTAAGTCTTACTTA	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	Selenomonas	NA
TACGTAGGTGGCGAGCGTTGTCCGGATTTACTGGGCGTAAAGGGAGCGTAGGCGGACTTTTAAGTGAGATGTGAAATACCCGGGCTCAACTTGGGTGCTGCATTTCAAACTGGAAGTCTAGAGTGCAGGAGAGGAGAGAGGAGAATGGAA	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium sensu stricto 1	NA
	Decl		Clockwist	Olootuidialaa			
	Bacteria	rirmicutes	Ciostridia	CIOSTIDIAIES	CIOSTIIDIACEAE	Ciostriaium sensu stricto 1	INA
TACGTATGGAGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGCAGACGGATATGCAAGTCTGAAGTGAAACCCCACGGCTCAACCGTGGGCTTGCTT	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Butyrivibrio	NA
TACGTATGGAGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGGCGGTCCTGCAAGTCTGATGTGAAAACCCGGGGGCTCAACCCCGGGACTGCATTGGAAACTGTAGGACTAGAGTGTCGGAGGGGTAAGTGGAA	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	NA	NA
TACGTATGGTGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGTGCGTAGGTGGTATGGCAAGTCAGAAGTGAAAGGCTGGGGCTCAACCCCGGGACTGCTTTTGAAACTGTCAAACTAGAGTACAGGAGAGGAAAGCGGAA	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	[Eubacterium] ruminantium aroup	NA
TACGTATGTCACGAGCGTTATCCGGATTTATTGGGCGTAAAGCGCGTCTAGGTGGTGATGTGAAAATGCAGGGCTCAACTCTGCAAACTGCAAAGTACTACAACTAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTACAACTAACAAC	Bacteria	Fusobacteriota	Fusobacterija	Fusobacteriales	Fusobacteriaceae	Fusobacterium	nucleatum
		Eucobast	Europe-1	Funchasterister			
	H Bacteria	rusobacteriota	rusobacterila	rusodacteriales	Leptotrichiaceae	Leptotrichia	INA
TACGTATGTCGCGAGCGTTATCCGGAATTATTGGGCATAAAGGGCATCTAGGCGGCCTTTCAAGTCAGGGGTGAAAACCTGCGGCTCAACCGCAGGCCTGCCT	Bacteria	Fusobacteriota	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	NA

Table S1

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