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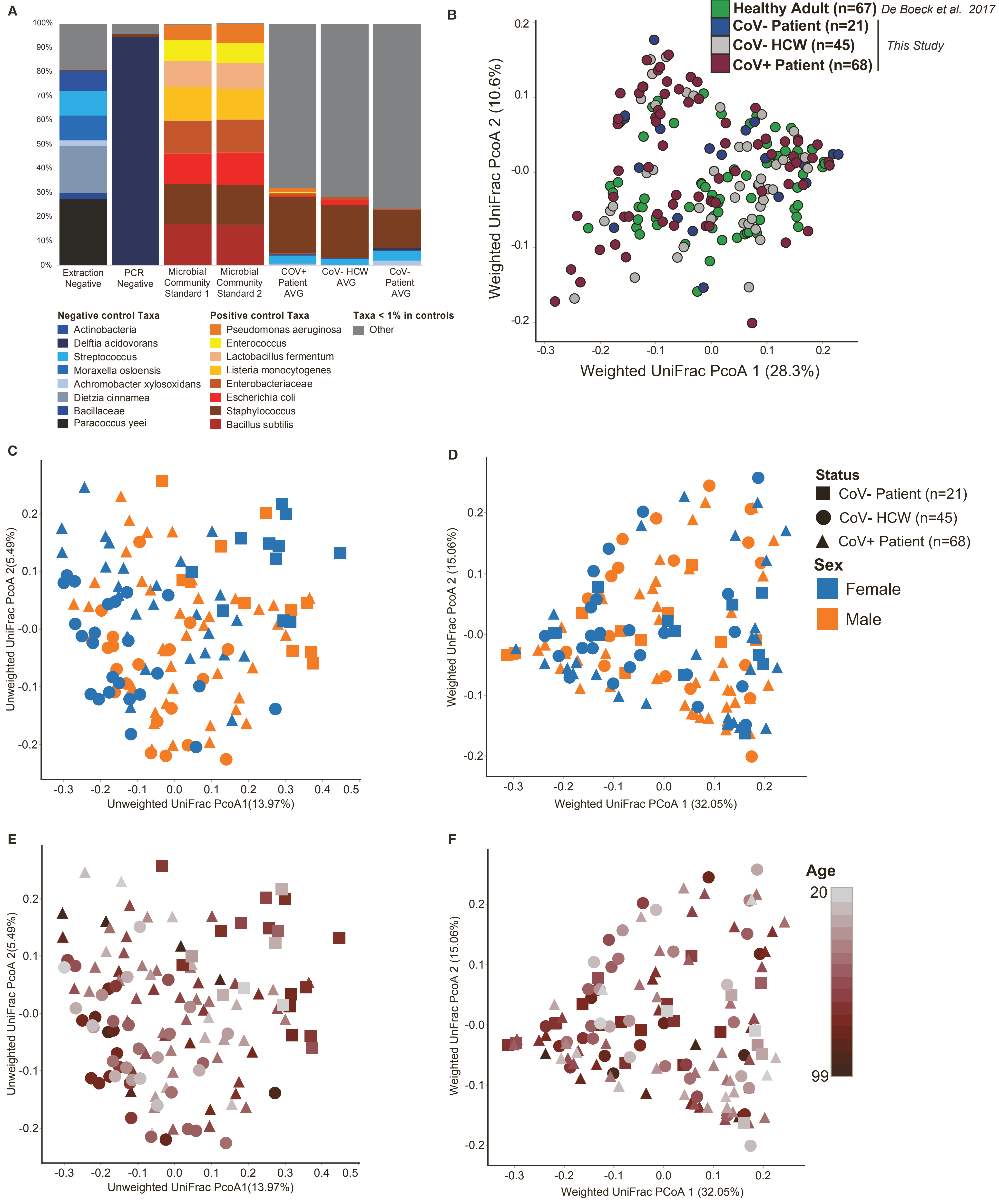
Supplemental information

Acute SARS-CoV-2 infection is associated with an increased abundance of bacterial pathogens, including *Pseudomonas aeruginosa* in the nose

Nicholas S. Rhoades, Amanda N. Pinski, Alisha N. Monsibais, Allen Jankeel, Brianna M. Doratt, Isaac R. Cinco, Izabela Ibraim, and Ilhem Messaoudi



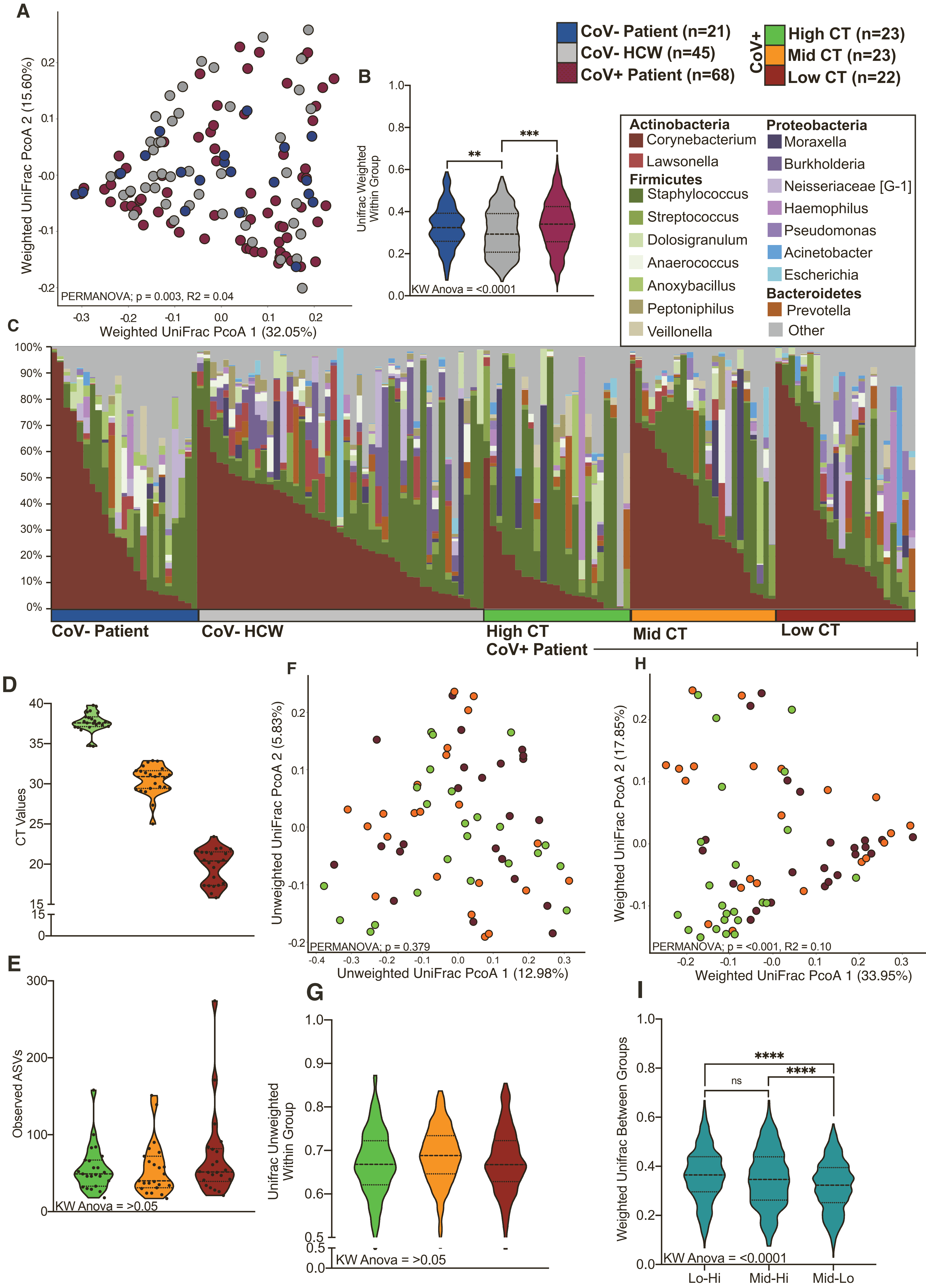
G

Unweighted UniFrac PERMANOVA	R^2	p
Status	**0.093**	**0.001**
Sex	**0.012**	**0.015**
Age	0.007	0.404
Sex within Status	0.014	0.279
Age within Status	0.012	0.78

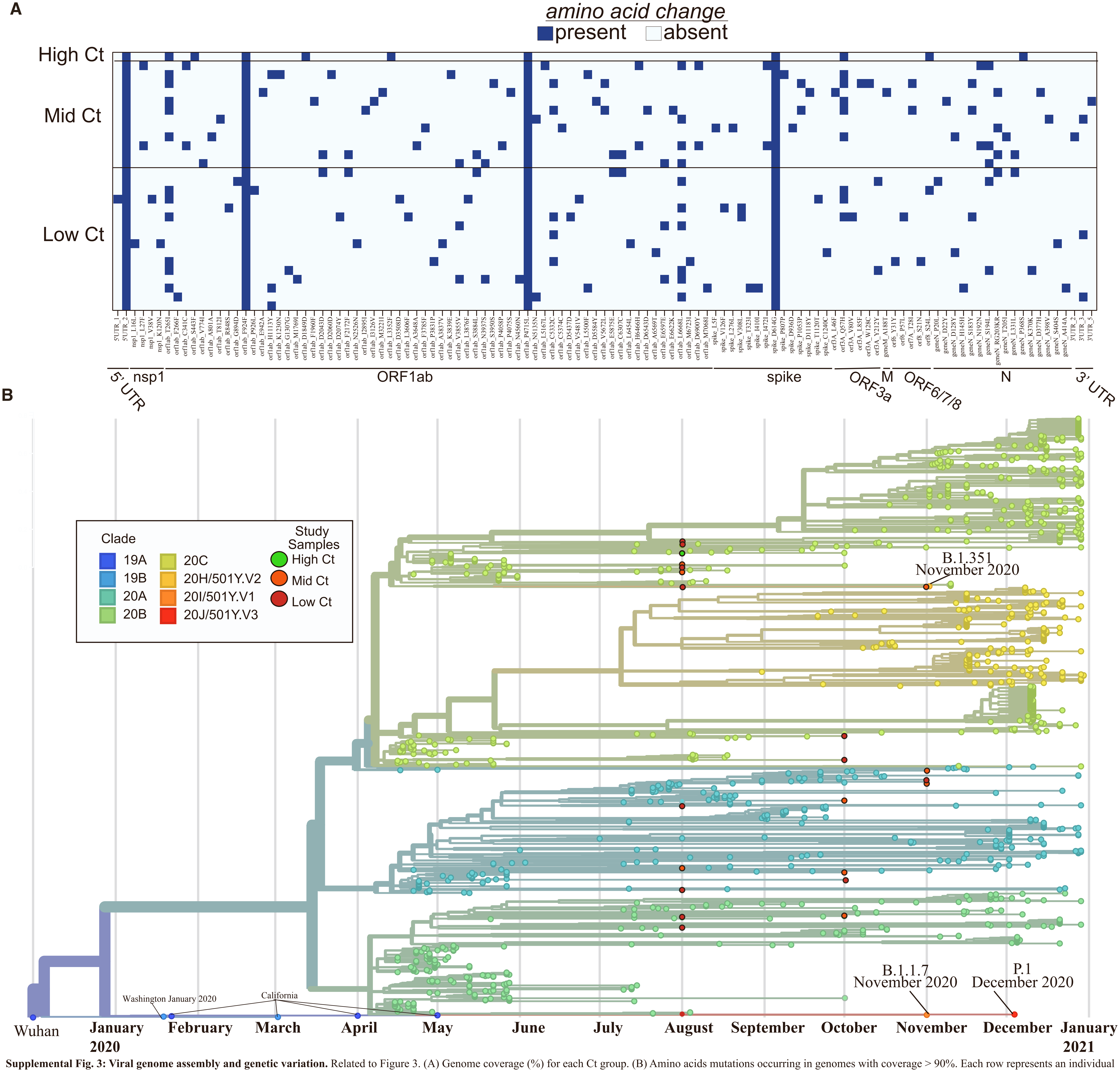
H

Weighted UniFrac PERMANOVA	R^2	p
Status	**0.04**	**0.003**
Sex	0.006	0.506
Age	**0.016**	**0.033**
Sex within Status	0.014	0.428
Age within Status	0.016	0.318

Supplemental Fig. 1: DNA co-eluted following RNA exraction from viral transport medium is reflective of a nasal microbiome identified using conventional DNA extraction methods. Related to Figure 1. (A) Stacked bar plot highlighting taxa found in positive and negative control samples after removal of potential contaminating sequences, overlap of streptococcus between the extraction control and patient samples was not shared at the ASV level. (B) Principal-coordinate analysis of nasal microbiome weighted UniFrac distance including samples from this study and previously published "healthy" nasal microbiome data. (C-F) Principal-coordinate analysis of nasal microbiome unweighted (C,E) and weighted (D,F) distance including data only from this study colored by sex (C,D) and age (E,F). (G,H) The contribution of age, sex, age within status and sex within status to the total variance in the unweighted (G) and unweighted (H) UniFrac distance measured using PERMANOVA (Adonis with 999 permutations).



Supplemental Fig. 2: Additional features of the nasal microbiome associated with SARS-CoV-2 infection. Related to Figure 2. (A) Principal-coordinate analysis of nasal microbiome weighted UniFrac distance colored by host status. The contribution of host status to the total variance in the weighted UniFrac dissimilarity matrices was measured using PERMANOVA (Adonis with 10,000 permutations). (B) Violin plot illustrating average weighted UniFrac distances within each group. (C) Stacked bar of bacterial genera found at greater than 1% average abundance across the entire study population. Each vertical bar represents an individual sample and are ordered horizontally by host status. (D) Violin plot of Ct values generated via reverse transcription quantitative PCR of the SARS-CoV-2 Spike gene showing the distribution of samples within each CT group. (E) Violin plot of observed amplicon sequencing variants split by host Ct group within CoV+ patients. (F) Principal-coordinate analysis of nasal microbiome unweighted UniFrac distance colored by Ct group. The contribution of Ct value to the total variance in the weighted UniFrac dissimilarity matrices was measured using PERMANOVA (Adonis with 10,000 permutations). (G) Violin plot illustrating average unweighted UniFrac distances within each group. (H) Principal-coordinate analysis of nasal microbiome weighted UniFrac distance colored by Ct group. The contribution of the Ct to the total variance in the weighted UniFrac dissimilarity matrices was measured using PERMANOVA (Adonis with 10,000 permutations). (I) Violin plot illustrating average weighted UniFrac distances between each CT group. Significance for panels B,E,G,I was determined using Kruskal Wallis non-parametric ANOVA, with Dunn's multiple comparison * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.



Supplemental Fig. 3: Viral genome assembly and genetic variation. Related to Figure 3. (A) Genome coverage (%) for each Ct group. (B) Amino acids mutations occurring in genomes with coverage > 90%. Each row represents an individual subject. Horizontal axis is order 3' to 5'. (B) Nextstrain phylogenetic tree of genomes with >90% coverage, variants of concern (P.1, B.1.1.7, B.1.351, B.1.427, B.1.429) and all genomes collected from Orange County, California January 2020 to December 2020.

Supplmental Table 1: Sample Metadata, Related to Figure 1. NA = Not Applicable.

SampleID	Status	CT_Value	CT_CAT	Age	Sex	Viral Genome	RNAseq	Microbiome
HCW_1	CoV- HCW	NA	NA	78	F	NA	No	Yes
HCW_2	CoV- HCW	NA	NA	55	F	NA	No	Yes
HCW_3	CoV- HCW	NA	NA	77	F	NA	No	Yes
HCW_4	CoV- HCW	NA	NA	76	F	NA	No	Yes
HCW_5	CoV- HCW	NA	NA	82	M	NA	No	Yes
HCW_6	CoV- HCW	NA	NA	55	M	NA	No	Yes
HCW_7	CoV- HCW	NA	NA	37	M	NA	No	Yes
HCW_8	CoV- HCW	NA	NA	55	M	NA	No	Yes
HCW_9	CoV- HCW	NA	NA	29	M	NA	No	Yes
HCW_10	CoV- HCW	NA	NA	80	M	NA	Yes	Yes
HCW_11	CoV- HCW	NA	NA	76	F	NA	No	Yes
HCW_12	CoV- HCW	NA	NA	83	M	NA	No	Yes
HCW_13	CoV- HCW	NA	NA	71	F	NA	No	Yes
HCW_14	CoV- HCW	NA	NA	91	M	NA	No	Yes
HCW_15	CoV- HCW	NA	NA	55	M	NA	No	Yes
HCW_16	CoV- HCW	NA	NA	48	M	NA	No	Yes
HCW_17	CoV- HCW	NA	NA	76	F	NA	No	Yes
HCW_18	CoV- HCW	NA	NA	28	M	NA	No	Yes
HCW_19	CoV- HCW	NA	NA	68	F	NA	No	Yes
HCW_20	CoV- HCW	NA	NA	53	F	NA	No	Yes
HCW_21	CoV- HCW	NA	NA	55	M	NA	No	Yes
HCW_22	CoV- HCW	NA	NA	50	M	NA	No	Yes
HCW_23	CoV- HCW	NA	NA	49	F	NA	No	Yes
HCW_24	CoV- HCW	NA	NA	91	F	NA	No	Yes
HCW_25	CoV- HCW	NA	NA	39	M	NA	No	Yes
HCW_26	CoV- HCW	NA	NA	66	F	NA	No	Yes
HCW_27	CoV- HCW	NA	NA	24	F	NA	No	Yes
HCW_28	CoV- HCW	NA	NA	36	M	NA	No	Yes
HCW_29	CoV- HCW	NA	NA	32	M	NA	No	Yes
HCW_30	CoV- HCW	NA	NA	30	F	NA	No	Yes
HCW_31	CoV- HCW	NA	NA	48	M	NA	No	Yes
HCW_32	CoV- HCW	NA	NA	30	F	NA	No	Yes
HCW_33	CoV- HCW	NA	NA	37	F	NA	No	Yes
HCW_34	CoV- HCW	NA	NA	29	M	NA	No	Yes
HCW_35	CoV- HCW	NA	NA	61	F	NA	No	Yes
HCW_36	CoV- HCW	NA	NA	33	F	NA	No	Yes
HCW_37	CoV- HCW	NA	NA	26	F	NA	No	Yes
HCW_38	CoV- HCW	NA	NA	35	F	NA	Yes	Yes
HCW_39	CoV- HCW	NA	NA	43	M	NA	No	Yes
HCW_40	CoV- HCW	NA	NA	50	M	NA	Yes	Yes
HCW_41	CoV- HCW	NA	NA	68	F	NA	No	Yes
HCW_42	CoV- HCW	NA	NA	82	F	NA	No	Yes
HCW_43	CoV- HCW	NA	NA	61	F	NA	No	Yes
HCW_44	CoV- HCW	NA	NA	42	M	NA	Yes	Yes
HCW_45	CoV- HCW	NA	NA	67	F	NA	No	Yes
COV_2	CoV+	38.076218	Hi	99	F	< 90% genome coverage	No	Yes
COV_3	CoV+	36.700247	Hi	55	M	< 90% genome coverage	No	Yes
COV_8	CoV+	37.806442	Hi	51	M	< 90% genome coverage	No	Yes
COV_10	CoV+	37.121967	Hi	26	F	No Library generated	No	Yes
COV_16	CoV+	39.08479	Hi	32	F	< 90% genome coverage	No	Yes
COV_17	CoV+	37.37857	Hi	67	F	< 90% genome coverage	No	Yes
COV_19	CoV+	37.56185	Hi	59	F	< 90% genome coverage	No	Yes
COV_24	CoV+	37.584984	Hi	79	M	< 90% genome coverage	No	Yes
COV_42	CoV+	37.14862	Hi	69	M	Yes	No	Yes
COV_48	CoV+	37.538563	Hi	34	M	< 90% genome coverage	No	Yes
COV_49	CoV+	38.944378	Hi	70	M	< 90% genome coverage	No	Yes

COV_50	CoV+	38.812202	Hi	30	M	< 90% genome coverage	No	Yes
COV_51	CoV+	37.67764	Hi	62	M	< 90% genome coverage	No	Yes
COV_53	CoV+	39.622738	Hi	91	F	< 90% genome coverage	No	Yes
COV_54	CoV+	38.347515	Hi	40	M	< 90% genome coverage	No	Yes
COV_55	CoV+	37.016968	Hi	25	M	No Library generated	No	Yes
COV_56	CoV+	38.229053	Hi	77	M	No Library generated	No	Yes
COV_57	CoV+	37.764614	Hi	62	M	No Library generated	No	Yes
COV_59	CoV+	39.776764	Hi	24	M	< 90% genome coverage	No	Yes
COV_61	CoV+	37.07317	Hi	45	M	No Library generated	No	Yes
COV_62	CoV+	37.32942	Hi	59	F	< 90% genome coverage	No	Yes
COV_63	CoV+	34.675777	Hi	49	F	< 90% genome coverage	No	Yes
COV_67	CoV+	34.74406	Hi	45	F	< 90% genome coverage	No	Yes
COV_1	CoV+	20.173272	Low	61	F	Yes	No	Yes
COV_5	CoV+	20.293746	Low	24	M	Yes	No	Yes
COV_18	CoV+	22.005283	Low	59	F	< 90% genome coverage	No	Yes
COV_21	CoV+	17.307197	Low	98	F	Yes	No	Yes
COV_23	CoV+	20.507776	Low	58	M	Yes	No	Yes
COV_25	CoV+	21.307083	Low	71	M	Yes	No	Yes
COV_26	CoV+	21.88542	Low	44	M	Yes	No	Yes
COV_27	CoV+	21.656952	Low	49	F	Yes	No	Yes
COV_28	CoV+	15.81649	Low	59	M	< 90% genome coverage	Yes	Yes
COV_30	CoV+	17.344656	Low	56	M	Yes	Yes	Yes
COV_33	CoV+	21.483639	Low	47	M	Yes	No	Yes
COV_34	CoV+	21.459263	Low	87	F	Yes	No	Yes
COV_35	CoV+	17.365662	Low	34	F	Yes	No	Yes
COV_39	CoV+	20.509508	Low	46	M	Yes	No	Yes
COV_40	CoV+	18.368208	Low	32	F	< 90% genome coverage	No	Yes
COV_41	CoV+	23.435507	Low	60	F	Yes	No	Yes
COV_43	CoV+	17.242144	Low	62	M	Yes	Yes	Yes
COV_44	CoV+	16.328108	Low	49	F	Yes	No	Yes
COV_46	CoV+	19.594623	Low	95	F	Yes	No	Yes
COV_64	CoV+	21.307083	Low	71	M	No Library generated	No	Yes
COV_66	CoV+	17.60019	Low	47	M	< 90% genome coverage	No	Yes
COV_68	CoV+	21.933756	Low	76	F	< 90% genome coverage	No	Yes
COV_4	CoV+	31.215872	Mid	30	M	< 90% genome coverage	No	Yes
COV_6	CoV+	29.00934	Mid	83	F	Yes	No	Yes
COV_7	CoV+	29.424534	Mid	51	M	Yes	No	Yes
COV_9	CoV+	29.59582	Mid	26	F	Yes	No	Yes
COV_11	CoV+	31.63975	Mid	33	F	< 90% genome coverage	No	Yes
COV_12	CoV+	31.136995	Mid	28	F	Yes	No	Yes
COV_13	CoV+	31.890806	Mid	65	M	No Library generated	No	Yes
COV_14	CoV+	30.049786	Mid	75	F	< 90% genome coverage	No	Yes
COV_15	CoV+	32.22774	Mid	52	M	< 90% genome coverage	No	Yes
COV_20	CoV+	30.898294	Mid	46	M	Yes	Yes	Yes
COV_22	CoV+	32.890465	Mid	51	M	< 90% genome coverage	No	Yes
COV_29	CoV+	25.006283	Mid	38	M	Yes	No	Yes
COV_31	CoV+	29.639048	Mid	70	M	Yes	No	Yes
COV_32	CoV+	32.694588	Mid	66	M	< 90% genome coverage	No	Yes
COV_36	CoV+	29.281885	Mid	56	M	Yes	No	Yes
COV_37	CoV+	31.368456	Mid	73	M	Yes	No	Yes
COV_38	CoV+	32.829716	Mid	77	F	< 90% genome coverage	No	Yes
COV_45	CoV+	31.100018	Mid	50	M	< 90% genome coverage	No	Yes
COV_47	CoV+	30.700317	Mid	32	M	< 90% genome coverage	No	Yes
COV_52	CoV+	29.120705	Mid	65	M	Yes	No	Yes
COV_58	CoV+	31.564772	Mid	36	F	Yes	No	Yes
COV_60	CoV+	29.432646	Mid	75	M	Yes	No	Yes
COV_65	CoV+	27.339397	Mid	40	M	< 90% genome coverage	No	Yes
NonCoV_1	CoV- patient	NA	NA	32	F	NA	No	Yes

NonCoV_2	CoV- patient	NA	NA	30	F	NA	No	Yes
NonCoV_3	CoV- patient	NA	NA	75	F	NA	No	Yes
NonCoV_4	CoV- patient	NA	NA	77	M	NA	No	Yes
NonCoV_8	CoV- patient	NA	NA	73	M	NA	No	Yes
NonCoV_9	CoV- patient	NA	NA	71	M	NA	No	Yes
NonCoV_10	CoV- patient	NA	NA	77	M	NA	No	Yes
NonCoV_12	CoV- patient	NA	NA	72	F	NA	No	Yes
NonCoV_13	CoV- patient	NA	NA	20	M	NA	No	Yes
NonCoV_14	CoV- patient	NA	NA	24	F	NA	No	Yes
NonCoV_16	CoV- patient	NA	NA	33	F	NA	No	Yes
NonCoV_17	CoV- patient	NA	NA	62	F	NA	No	Yes
NonCoV_18	CoV- patient	NA	NA	72	M	NA	No	Yes
NonCoV_19	CoV- patient	NA	NA	59	M	NA	No	Yes
NonCoV_20	CoV- patient	NA	NA	36	F	NA	No	Yes
NonCoV_22	CoV- patient	NA	NA	77	F	NA	No	Yes
NonCoV_23	CoV- patient	NA	NA	70	F	NA	No	Yes
NonCoV_24	CoV- patient	NA	NA	68	M	NA	No	Yes
NonCoV_26	CoV- patient	NA	NA	65	M	NA	No	Yes
NonCoV_27	CoV- patient	NA	NA	56	F	NA	No	Yes
NonCoV_28	CoV- patient	NA	NA	73	M	NA	No	Yes

Supplmental Table 2: Full LEFsE results, Related to Figure 2

Taxa	Group	Log10 LDA score	FDR p-value
g__Ochrobactrum	CoV+	2.811758395	0.0367078
g__Jeotgalicoccus	CoV+	3.076247372	0.0078099
g__Schaalia	CoV+	3.483370495	0.0175861
f__Bacillaceae___	CoV+	3.030009565	0.0343436
g__Dermabacter	CoV+	3.064796147	0.0036059
g__Micrococcus	CoV+	3.391801863	0.006672
g__Fastidiosipila	CoV+	3.070355684	0.0298342
g__Paracoccus	CoV+	2.916667243	0.0249822
g__Peptoniphilaceae__G_3_	CoV+	3.30527293	0.0098114
g__Fusobacterium	CoV+	3.289965814	0.002674
g__Pseudomonas	CoV+	4.001548907	0.0087463
g__Prevotella	CoV+	3.991703718	0.0225291
g__Moraxella	CoV+	4.211959633	0.0142522
g__Acinetobacter	CoV+	3.834024999	0.0110778
f__Enterobacteriaceae___	CoV+	3.035617222	0.0010228
g__Rothia	CoV+	3.044886859	0.0019198
g__Porphyromonas	CoV+	3.290269729	0.0162161
g__Brevundimonas	CoV+	2.825426652	0.0311487
g__Peptoniphilus	HCW	3.991241674	0.0002256
g__Capnocytophaga	HCW	2.997119911	0.0380345
g__Mycobacterium	HCW	3.362911231	0.0008491
g__Cardiobacterium	HCW	3.661581553	0.0243386
g__Klebsiella	HCW	3.744210715	0.0427925
c__Gammaproteobacteria_________	HCW	3.197456319	1.84E-09
g__Acidovorax	HCW	3.028770995	0.0241977
g__Finegoldia	HCW	3.515115664	0.0012149
g__Lawsonella	HCW	4.228442269	0.0001613
g__Lactococcus	HCW	3.675104466	0.0002873
g__Sphingomonas	HCW	3.437903649	0.0089651
c__Alphaproteobacteria_________	HCW	3.115002019	6.26E-06
g__Turicella	HCW	3.584602911	0.0003532
g__Burkholderia	HCW	4.546294251	7.17E-16
g__Escherichia	HCW	3.993386794	0.0196662
g__Cutibacterium	HCW	3.504843758	5.48E-15
g__Kocuria	HCW	3.143013983	0.0051622
g__Delftia	CoV-	3.692288734	0.0278583
g__Paenibacillus	CoV-	4.335964038	2.12E-19
g__Brevibacterium	CoV-	3.087982656	0.0394132
g__Anaerococcus	CoV-	3.995463034	0.0123125
g__Anoxybacillus	CoV-	4.223854276	9.19E-12
c__Betaproteobacteria_________	CoV-	4.065762597	8.34E-18