Supplemental Figures and Figure Legends

Figure S1

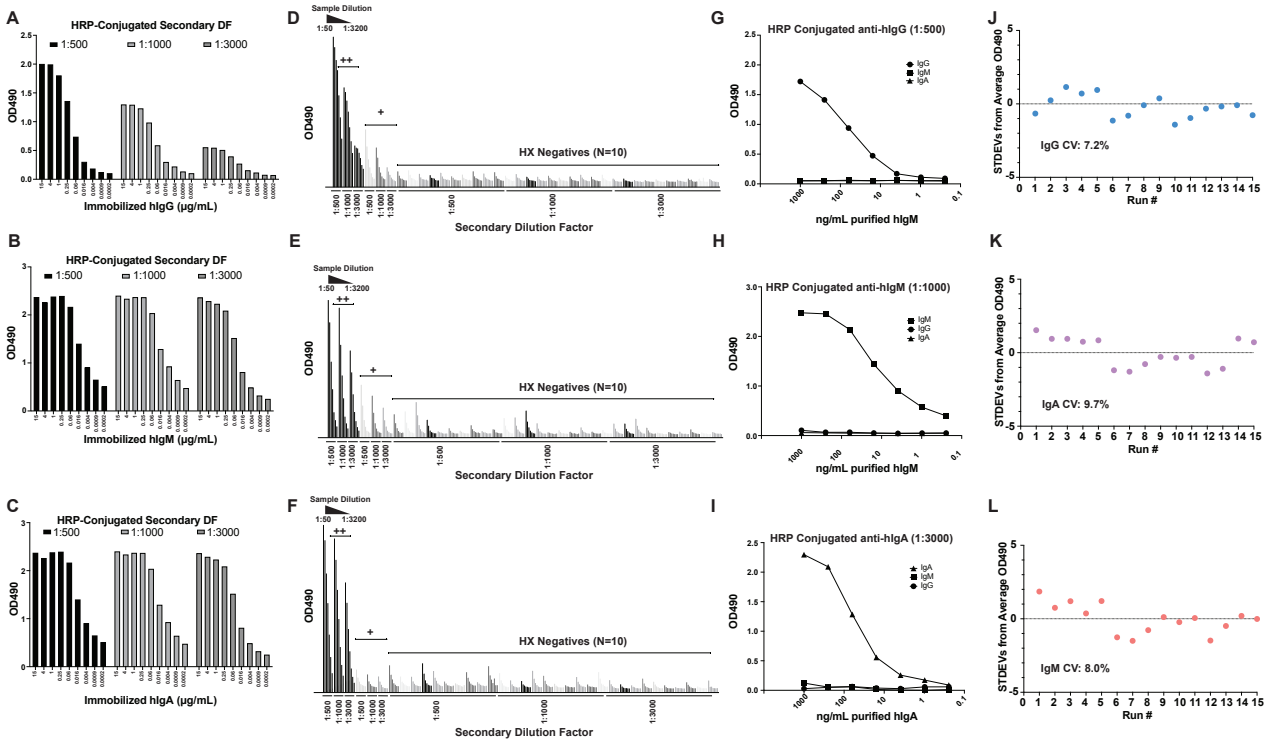

Figure S1 Optimization of detection reagents and precision analysis. (A-C) Different dilutions (1:500, 1:1000, and 1:3000) of HRP-conjugated anti human IgG antibody were tested using purified human IgG, IgA and IgM from human serum (Sigma) immobilized on high bind ELISA plates. (D) Serum dilutions of 1:50 to 1:3200 with different conjugate dilutions were tested in a selection of historical negative and PCR-confirmed COVID-19 samples with high and intermediate levels of reactivity in each assay. Based on the above data and the known normal levels of total serum IgA/M (60-400mg/dL) to be in a range approximately half that of IgG (700-1,500 mg/dL), a serum/plasma dilution of 1:100 was selected for IgA and IgM assays while a dilution of 1:200 was selected for the IgG assay. Conjugate dilutions of 1:500 for IgG, 1:1000 for IgM, and 1:3000 for IgA were selected based on retention of signal over the dilutions tested in D-F and the level of background

observed in historical negative samples. Using the selected parameters, all three assays exhibited background OD values in the range of 0.04-0.07. (G-H) Analysis of conjugate specificity at chosen dilutions using purified human IgG, IgA, and IgM. (J-L) Levy-Jennings analysis for single dilution SARS CoV-2 IgG, IgA, and IgM serology tests over 15 independent runs.

Figure S2

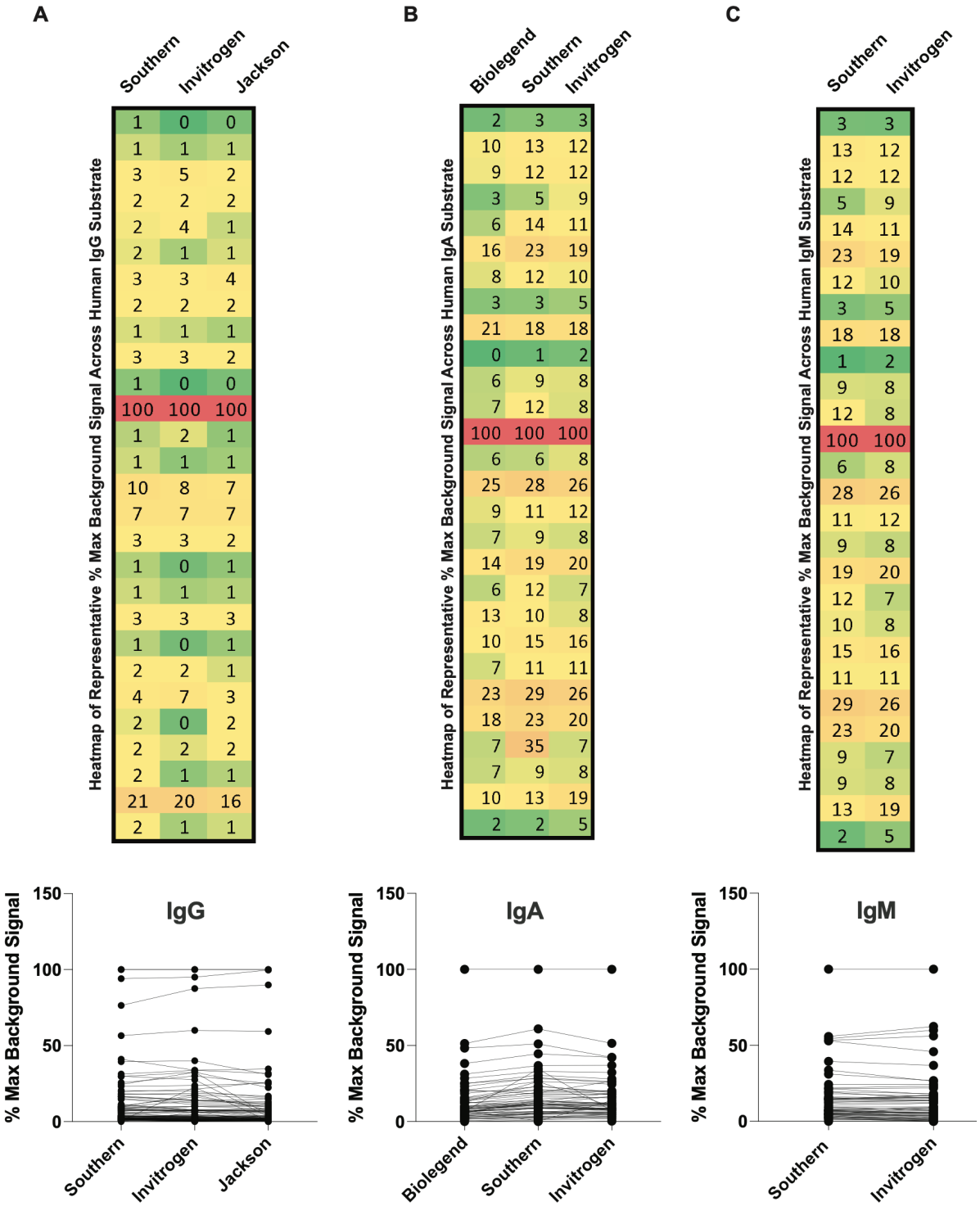

Figure S2. Comparison of commercial detection reagents in pre-pandemic serum samples. (A-C) Pre-pandemic samples from healthy blood donors and patients being assessed for infection by other pathogens were analyzed by RBD ELISA using HRP detection reagents from multiple

companies (N=103 samples for IgG; 63 for IgA and IgM). To assess overall agreement between detection reagents, signal intensity relative to maximum background signal observed for each conjugate in the sample set is plotted and connected for each sample across the tested secondary reagents. Heatmaps showing the intensity of background signal relative to the maximum for each conjugate are shown for a representative sample set below each plot.

Figure S3

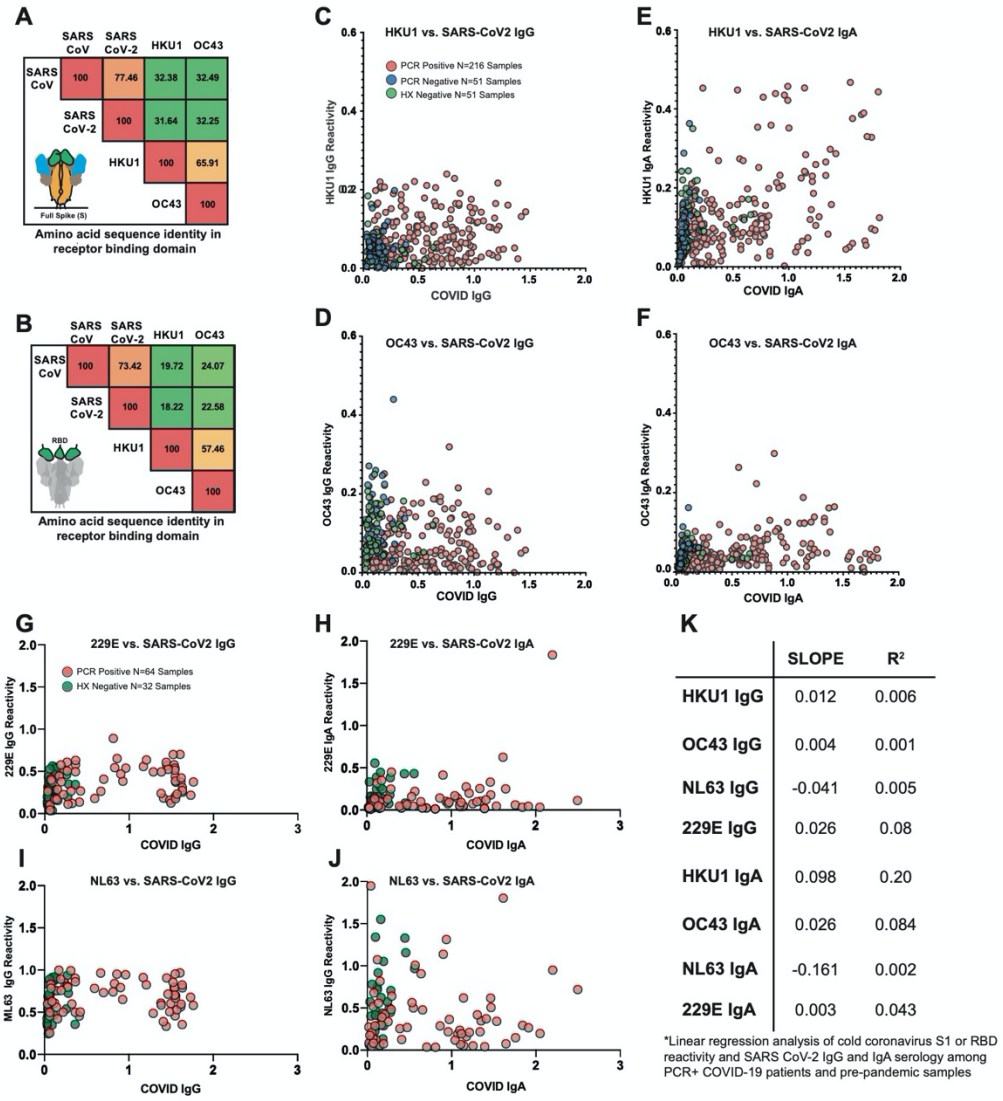

Figure S3 Correlative analysis of SARS-CoV-2 RBD serology results with beta and alphacoronavirus S1 and RBD ELISA reactivity. (A-B) Amino acid sequence comparison of full-length spike protein (A) and the receptor binding domain (RBD) (B) of human zoonotic coronaviruses SARS-CoV and SARS-CoV-2 with endemic human cold-causing betacoronaviruses OC43 and HKU1. (C-E) OD values from the COVID-19 RBD-ELISA are plotted against reactivity in an ELISA using immobilized S1 domain from OC43 or HKU1 for a subset of samples from PCR positive, PCR negative, and historical pre-pandemic samples. (G-H) A similar analysis was performed using recombinant receptor binding domains from human

alphacoronaviruses 229E and NL63 using a subset of PCR positive and historical negative samples. (K) Slopes and R^2 values from linear regression analyses of SARS CoV-2 RBD ELISA OD values against reactivity in each cold-coronavirus assay.

Figure S4

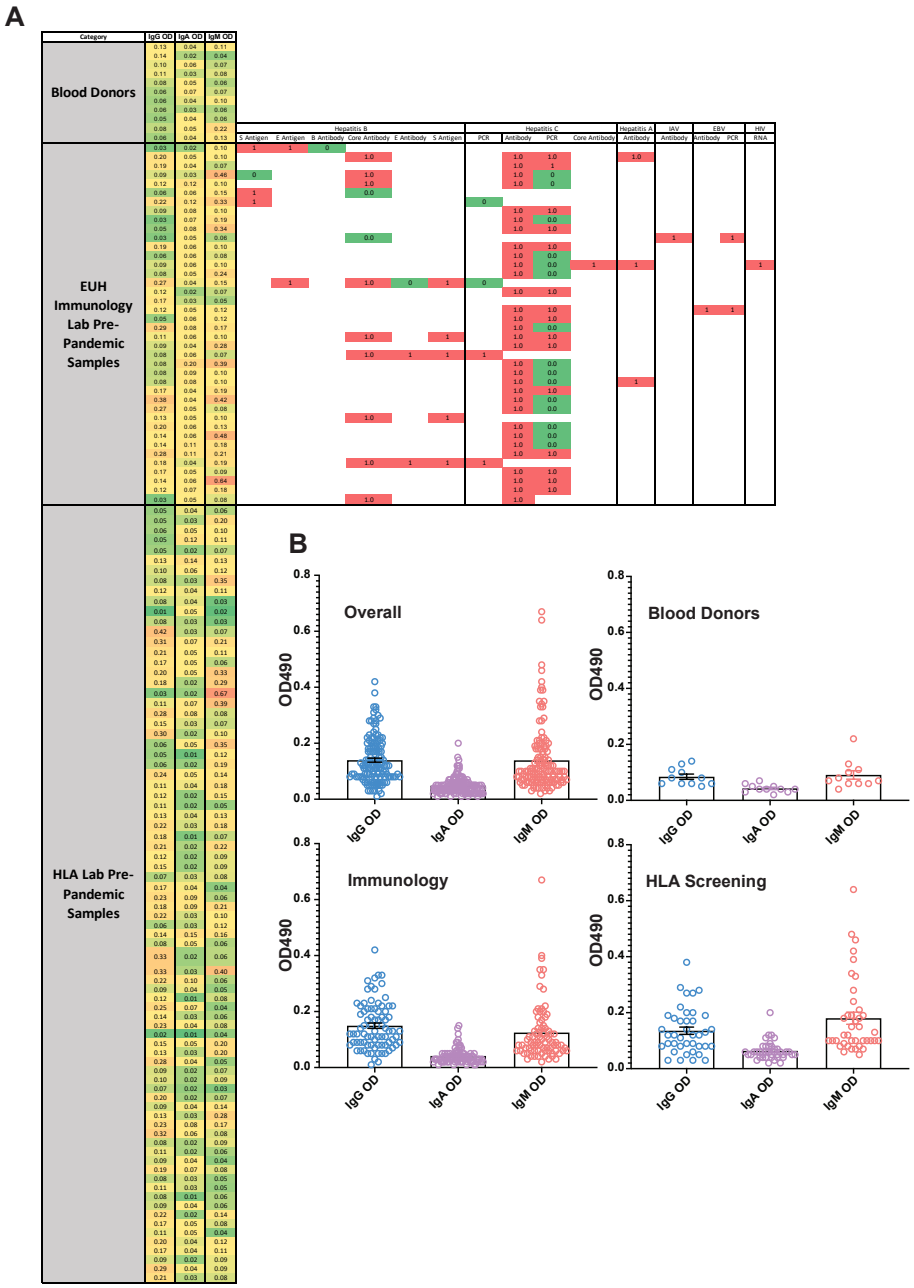

Figure S4 (A) heat-mapped OD values from each group of pre-pandemic samples used in the specificity analysis. (B) OD values of the historical negative cohort are plotted for each assay and subgroup.

Figure S5

A

PCR +

#	Samples	Age	Sex	Severity at Presentation	Intubation	IgG	IgA	IgM
1	4	65	M	(1)	YES	1	1	1
2	16	42	M	(3)	YES	1	1	1
3	30	87	M	(2)	YES	1	1	1
4	33	76	M	(2)	YES	1	1	1
5	25	62	M	(2)	YES	1	1	1
6	1	71	F	(2)	YES	1	1	1
7	2	77	M	(1)	YES	1	1	1
8	1	67	F	(1)	NO	1	1	1
9	4	81	M	(3)	NO	1	1	1
10	2	50	M	(3)	NO	1	1	1
11	5	28	F	(2)	YES	1	1	1
12	7	56	F	(3)	YES	1	1	1
13	1	38	M	(2)	NO	1	1	1
14	1	31	M	(1)	NO	1	1	1
15	2	63	M	(4)	YES	1	1	1
16	20	72	M	(3)	YES	1	1	1
17	6	70	M	(2)	YES	1	1	1
18	3	79	M	(1)	NO	1	1	1
19	2	93	F	(2)	NO	1	1	1
20	2	45	M	(1)	NO	1	1	1
21	7	55	F	(3)	NO	1	1	1
22	6	65	F	(2)	NO	1	1	1
23	3	68	F	(4)	YES	0	0	0
24	2	53	F	(1)	NO	1	0	1
25	2	76	M	(2)	NO	0	0	0
26	1	74	M	(4)	YES	1	0	0
27	3	68	M	(4)	YES	1	1	1
28	1	61	F	(2)	NO	1	0	0
29	1	37	M	(2)	NO	0	1	1
30	3	77	F	(2)	NO	0	0	1
31	3	85	M	(2)	NO	1	1	1
32	6	66	M	(2)	YES	1	1	1
33	19	22	F	(3)	YES	1	1	1
34	2	51	M	(1)	NO	0	0	0
35	2	77	F	(2)	YES	1	1	1
36	1	53	M	(2)	NO	1	0	0
37	2	27	F	(1)	NO	0	0	0
38	6	39	F	(2)	YES	1	1	1
39	2	89	M	(1)	YES	0	0	0
40	1	69	M	(2)	NO	1	1	1
41	1	80	F	(2)	NO	1	1	1
42	3	49	F	(2)	NO	0	0	0
43	1	53	F	(2)	NO	1	1	1
44	20	57	M	(3)	YES	1	1	1
45	3	76	M	(1)	NO	0	0	1
46	20	49	M	(2)	YES	1	1	1
47	5	61	F	(2)	YES	1	1	1
48	1	62	M	(2)	NO	1	0	0
49	16	96	M	(4)	YES	1	1	1
50	3	73	M	(2)	NO	1	1	1
51	3	82	F	(2)	YES	1	1	1
52	1	66	F	(2)	NO	1	1	1
53	20	63	M	(1)	NO	1	1	1
54	4	27	F	(2)	YES	1	1	1
55	20	90	M	(4)	YES	1	1	1
56	2	57	M	(2)	NO	1	0	0
57	4	57	M	(2)	YES	1	1	1
58	16	46	M	(4)	YES	1	1	1
59	5	51	F	(2)	NO	1	1	1
60	1	50	F	(2)	NO	0	0	0
61	20	89	M	(4)	YES	1	1	1
62	2	100	M	(2)	NO	1	0	0
63	1	38	F	(2)	NO	0	0	0
64	1	79	F	(1)	NO	0	0	0
65	10	58	M	(2)	YES	1	1	1
66	2	54	M	(3)	NO	1	1	1
67	2	92	F	(2)	NO	0	1	0
68	16	65	M	(2)	YES	1	1	1
69	13	69	F	(2)	YES	1	1	0
70	16	67	M	(2)	YES	1	1	1
71	2	65	M	(2)	NO	1	1	1
72	1	33	M	(2)	NO	0	0	0
73	10	91	F	(2)	NO	1	1	1
74	2	77	F	(2)	NO	1	1	1
75	2	70	M	(1)	YES	0	0	1
76	2	95	F	(1)	NO	0	1	1
77	15	88	F	(4)	YES	1	1	1
78	1	77	F	(2)	NO	0	0	0

B

PCR -

#	Samples	Age	Sex	Severity at Presentation	Intubation	IgG	IgA	IgM
1	2	63	M	(1)	NO	0	0	1
2	3	58	F	(1)	NO	0	0	0
3	3	50	M	(2)	NO	1	1	1
4	5	70	F	(2)	NO	0	0	0
5	12	55	M	(2)	YES	0	0	0
6	2	24	M	(4)	NO	0	0	0
7	3	47	M	(4)	YES	0	0	0
8	2	66	F	(2)	NO	0	0	1
9	1	60	M	(1)	NO	0	0	0
10	1	53	F	(2)	NO	0	0	0
11	3	61	F	(2)	NO	0	0	0
12	3	54	M	(2)	NO	0	0	0
13	12	67	M	(1)	NO	1	1	1
14	5	69	F	(2)	NO	0	0	1
15	3	47	F	(2)	NO	0	0	0
16	15	72	M	(4)	YES	1	1	0
17	9	44	M	(4)	YES	0	0	1
18	11	20	M	(4)	YES	0	0	0
19	14	67	M	(4)	NO	0	0	1
20	13	54	F	(1)	NO	0	0	0
21	3	66	M	(2)	NO	0	0	1
22	16	74	F	(1)	YES	0	0	0
23	14	75	M	(2)	NO	0	0	0
24	8	85	F	(2)	NO	1	1	0
25	6	66	M	(2)	NO	1	1	0
26	2	47	F	(1)	NO	0	0	0
27	3	96	F	(2)	NO	0	0	0
28	13	55	M	(4)	YES	1	0	1
29	4	69	M	(4)	YES	1	0	0
30	15	73	M	(1)	NO	0	0	0
31	4	34	M	(2)	NO	0	0	0
32	1	87	F	(2)	NO	0	0	0
33	4	76	F	(2)	NO	0	0	0
34	2	59	M	(2)	NO	0	0	0
35	11	57	M	(1)	NO	0	0	0
36	1	53	M	(1)	NO	1	0	0
37	11	70	F	(1)	NO	0	0	1
38	2	52	F	(1)	NO	0	0	0
39	1	54	M	(2)	NO	0	0	0
40	1	57	F	(2)	NO	0	0	0
41	1	52	M	(1)	NO	0	0	0
42	10	32	M	(4)	NO	1	0	0
43	3	39	F	(1)	NO	0	0	0
44	4	77	M	(1)	NO	0	0	0
45	4	40	F	(1)	NO	0	0	0
46	11	77	M	(1)	NO	0	0	0
47	4	48	F	(4)	YES	0	0	0
48	3	78	F	(4)	NO	0	0	0
49	2	88	F	(4)	NO	0	0	0
50	4	93	F	(2)	NO	0	0	1
51	2	84	F	(2)	NO	0	0	0
52	7	56	M	(4)	NO	0	0	0
53	1	57	M	(2)	NO	0	0	0
54	3	39	F	(4)	NO	0	0	1
55	3	50	F	(4)	NO	0	1	0
56	1	61	F	(4)	YES	0	0	0
57	1	76	M	(1)	NO	0	0	0
58	1	21	F	(1)	NO	0	0	0
59	2	80	M	(4)	YES	0	0	0
60	1	52	M	(2)	NO	0	0	0
61	1	41	F	(2)	NO	0	0	0

Figure S5. Cohort and sampling characteristics of SARS-CoV-2 PCR+ and PCR- study populations. (A) Individual patients are tabulated by patient ID from the PCR-confirmed cohort with sampling, age, sex, and basic clinical information along with the overall alpha response (ie. whether any sample tested positive by a given assay in the column). Note that the majority of

individuals in this set who tested negative, had very little sampling and the available samples were collected early during infection (B) The same information as in A, but for the cohort of PCR negative individuals.