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Supplemental information

Pre-existing humoral immunity to human common cold coronaviruses negatively impacts the protective SARS-CoV-2 antibody response

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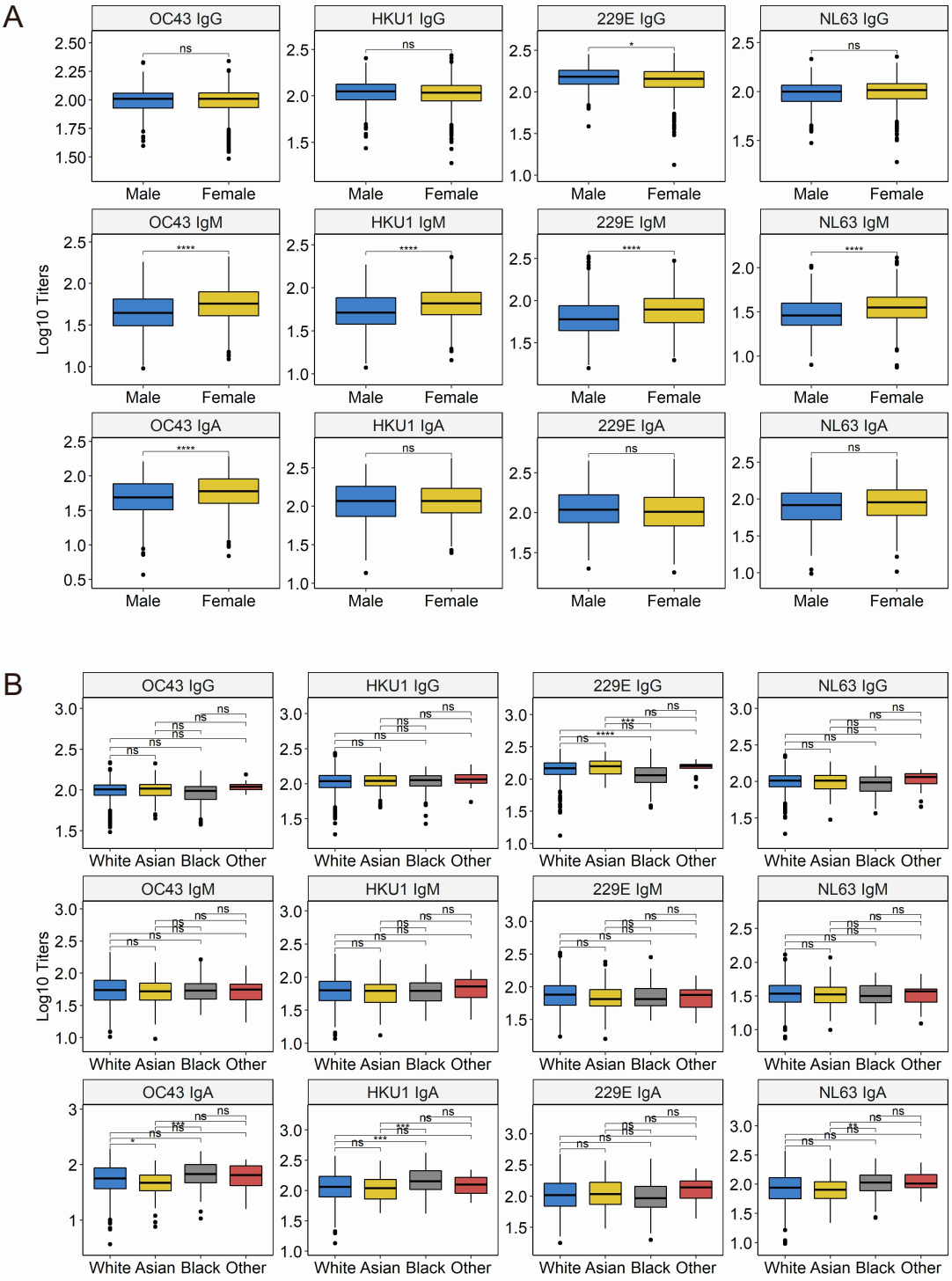
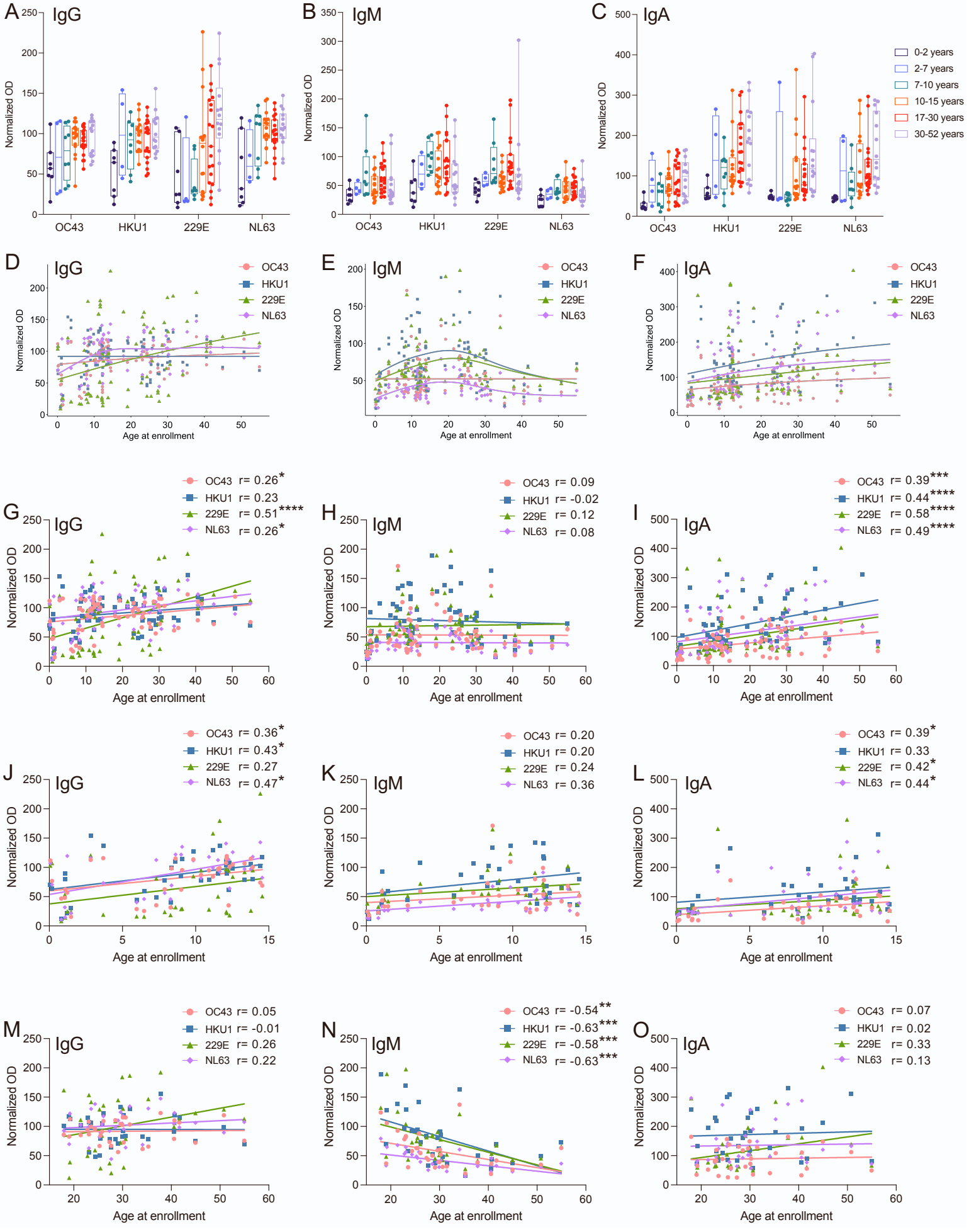
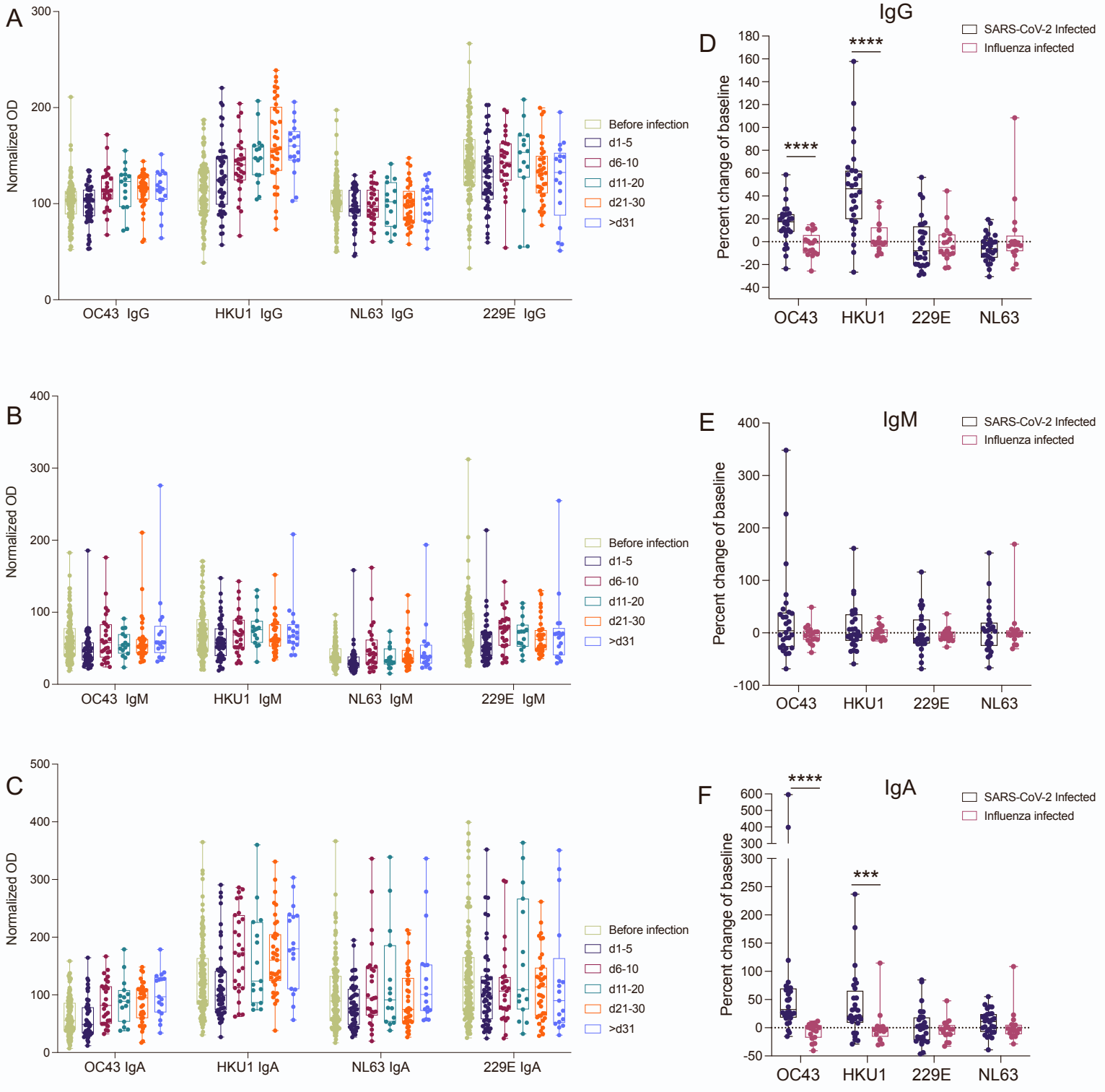


Supplemental Figure 1. hCCCoV antibody level associations with gender and ethnicity, Related to Figure 2. Samples from 1202 individuals taken prior to SARS-CoV-2 infection were analyzed by ELISA for hCCCoV as described in Figure 1. Levels of antibodies (normalized ODs) were compared in (**A**) male versus female participants and across (**B**) ethnicities. Statistical significance was determined by the Wilcoxon–Mann–Whitney test with Bonferroni adjustment (ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

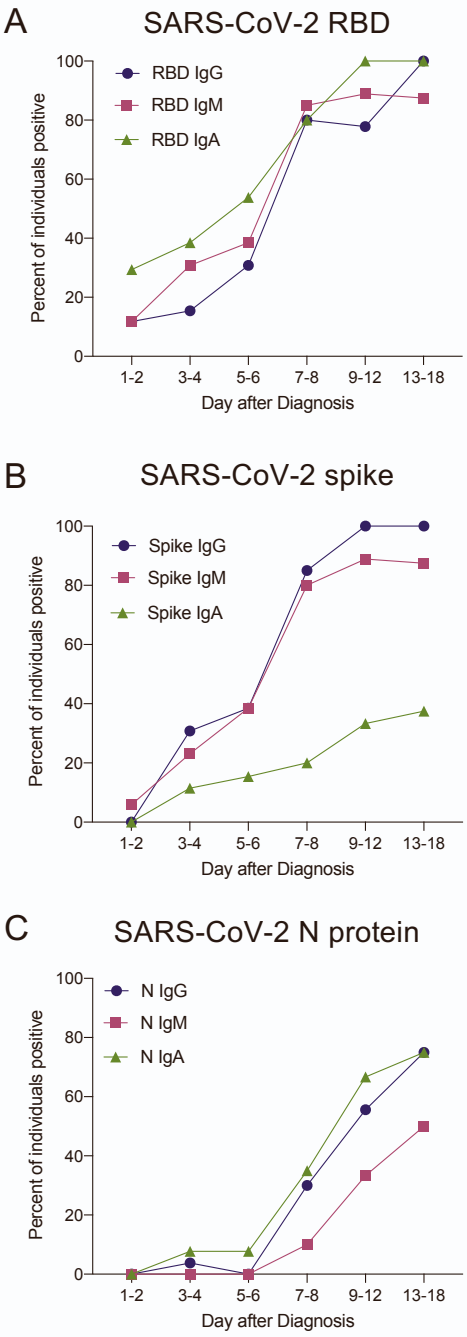
Supplemental Figure 2. Older individuals have higher levels of hCCCoV IgG and IgA compared to younger individuals.



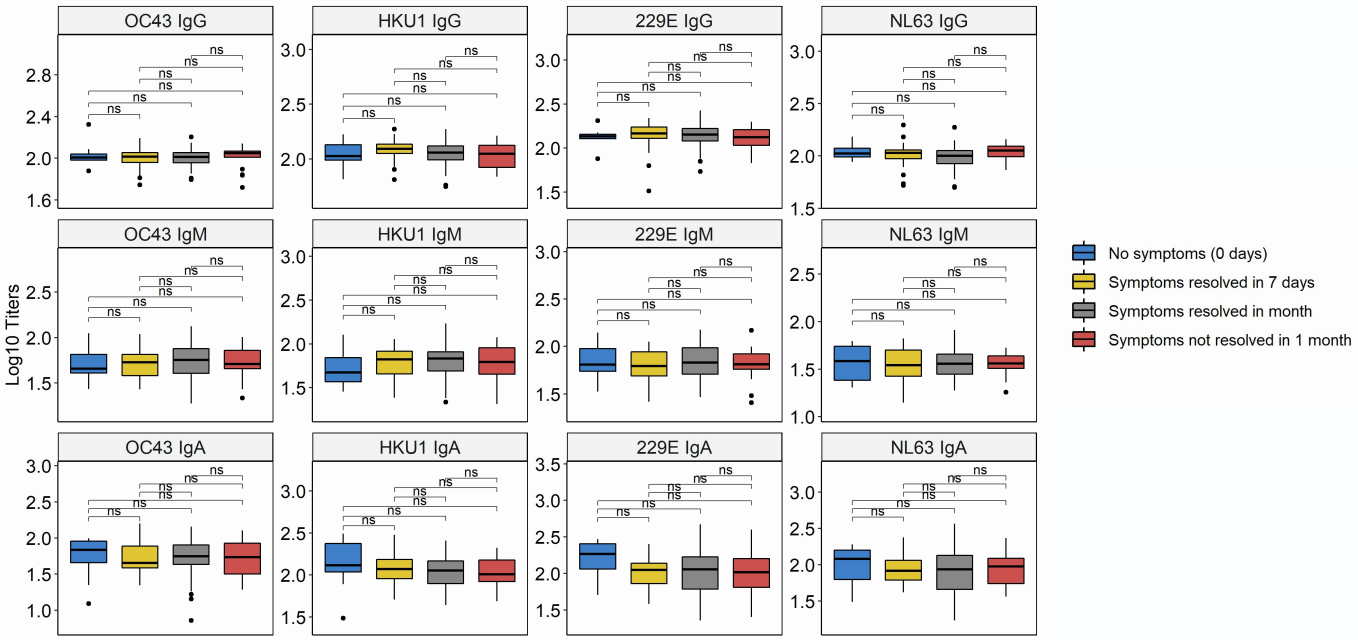
Supplemental Figure 2. Older individuals have higher levels of hCCCoV IgG and IgA compared to younger individuals, Related to Figure 2. Banked plasma samples collected in a previous study with the FLU09 cohort were analyzed by ELISA for IgG, IgM, and IgA antibodies specific for the spike proteins of OC43, HKU1, 229E, and NL63. (**A-C**) The normalized OD of each sample is depicted for the different age groups. The samples are grouped by age as follows: 0-2 years (n=7), 2-7 years (n=5), 7-10 years (n=11), 10-15 years (n=28), 17-30 years (n=23), and 30-54 years (n=13). (**D-E**) The normalized OD for all samples is plotted along with age. The smooth lines are estimated by fitting into generalized additive models. (**G-I**) The normalized OD for all age groups, (**J-L**) 0-14 years old, or (**M-O**) 17- 54 years old is plotted along with age with a simple linear regression line. Associations with age were tested by the Spearman method with the Benjamini, Krieger, and Yekutieli method correction for multiple comparisons. The correlation coefficients and adjusted p-values are indicated in the legends. (p *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).



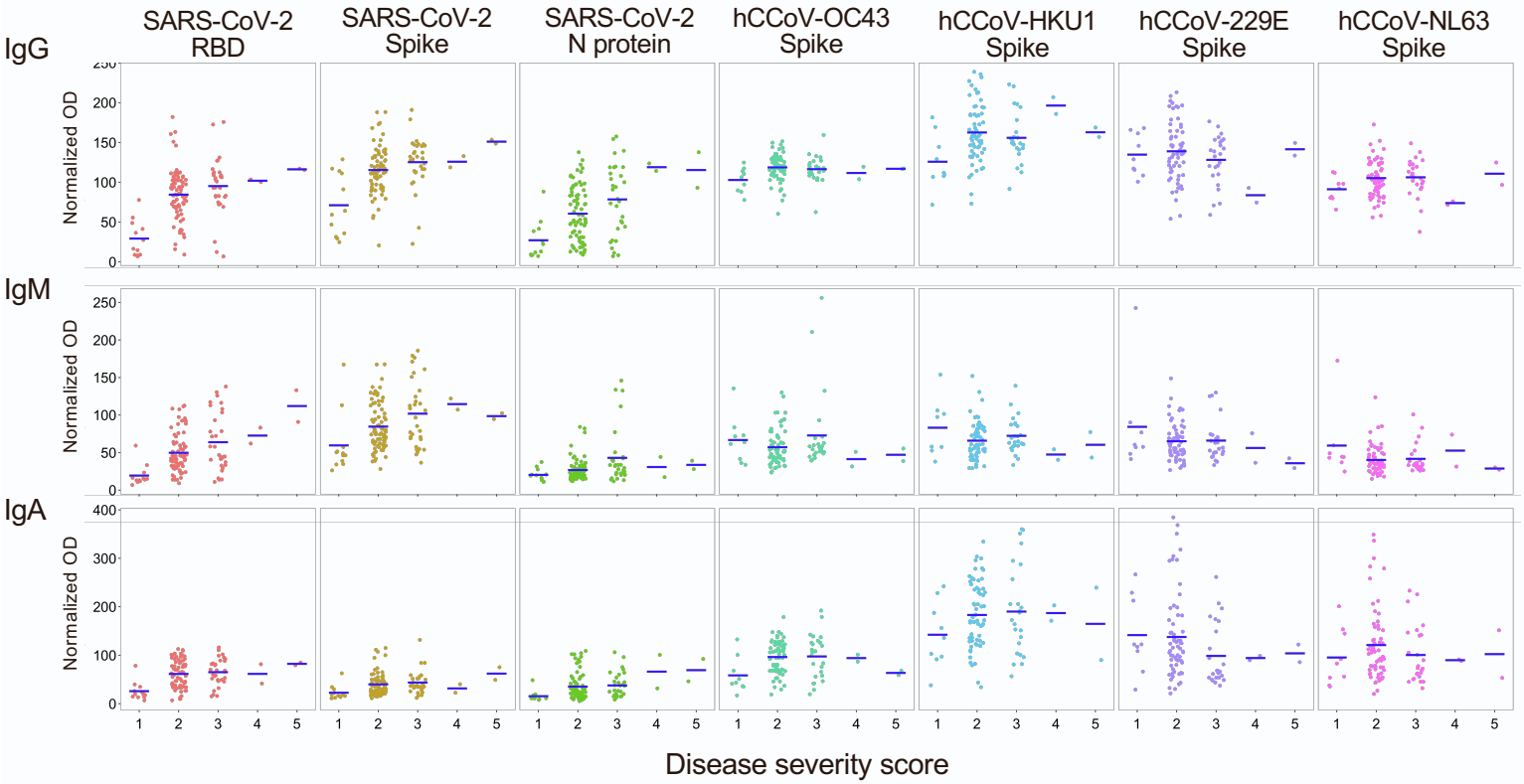
Supplemental Figure 3. The increase in hCCCoV antibody levels after infection is specific to SARS-CoV-2, Related Figure 3. Normalized OD of hCCCoV (**A**) IgG, (**B**) IgM, and (**C**) IgA antibodies in the baseline sample and at different times after confirmed SARS-CoV-2 diagnosis. (**D-F**) Samples taken from individuals during the convalescent phase (greater than 30 days after infection) after infection with SARS-CoV-2 or influenza virus were analyzed by ELISA for (**D**) IgG, (**E**) IgM, and (**F**) IgA antibodies specific for the spike proteins of hCCCoV. The percent change of each antibody in the convalescent sample relative to the baseline for each individual was calculated. Samples from individuals infected with influenza virus were collected in a prior study with the FLU09 cohort. P-values were calculated using the Mann-Whitney test with the Holm-Sidak multiple comparisons method. Adjusted p values are shown. (***, $p < 0.001$; ****, $p < 0.0001$).



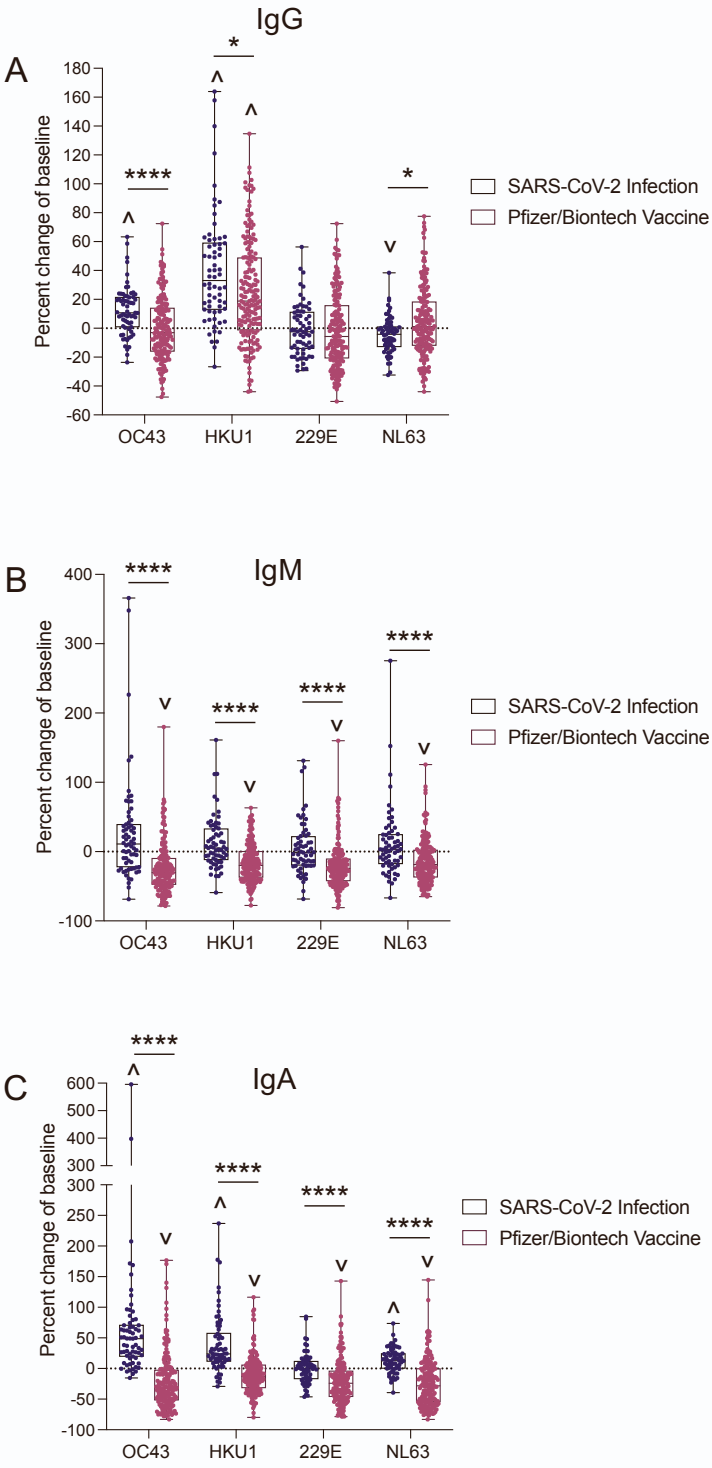
Supplemental Figure 4. IgM antibodies do not precede IgG or IgA antibodies following SARS-CoV-2 infection, Related to Figure 3. Samples taken from individuals within the first five days of symptom onset and PCR confirmation of SARS-CoV-2 infection were analyzed by ELISA for antibodies specific for SARS-CoV-2 (**A**) RBD, (**B**) spike, and (**C**) N protein. Samples with a normalized OD greater than two times the mean of the baseline samples for the entire cohort were considered positive.



Supplemental Figure 5. Baseline hCCCoV antibody levels do not correlate with symptom duration, Related to Figure 4. Participants with a confirmed SARS-CoV-2 infection were grouped according to the self-reported duration of symptoms. Baseline hCCCoV antibodies (normalized ODs) were compared between individuals with no symptoms (0 days) (n=8), symptoms that resolved within in 7 days (n=26), 30 days (n= 56), or greater than 30 days (n=17). Statistical significance was determined by the Wilcoxon–Mann–Whitney test with Bonferroni adjustment. "ns", not significant.



Supplemental Figure 6. Correlation of antibody levels after SARS-CoV-2 infection with severity scores, Related to Figure 6. Infected individuals were given a score based on the severity of self-reported symptoms, ranging from 1 (asymptomatic; n=8), 2 (any symptoms other than shortness of breath and not requiring hospitalization or supplemental oxygen; n=69), 3 (shortness of breath, but not requiring hospitalization or supplemental oxygen; n=26), 4 (hospitalization or requiring supplemental oxygen for more than 1 hour; n=2), to 5 (ICU admission; n=2). Antibody levels were analyzed in samples taken between 16-40 days following confirmed SARS-CoV-2 infection and compared across severity groups.



Supplemental Figure 7. Betacoronavirus IgG levels increase after Pfizer/BioNTech BNT162b2 vaccination, Related to Figure 6. Samples taken from individuals 20-60 days after SARS-CoV-2 diagnosis or the second dose of BNT162b2 were analyzed by ELISA for (**A**) IgG, (**B**) IgM, and (**C**) IgA antibodies specific for the spike proteins of OC43, HKU1, 229E, and NL63. The percent change of each antibody normalized OD relative to the baseline sample is depicted for individuals after SARS-CoV-2 infection (n=66) or vaccination (n=181). Carrot symbols indicate groups that are significantly increased or decreased compared to no fold change as determined by the Wilcoxon signed-rank test and adjusted using the Benjamini, Krieger, and Yekutieli method. Asterisks indicate significant differences between infected and vaccinated samples that were calculated using the Mann-Whitney test with the Holm-Sidak multiple comparisons method. Adjusted p values are shown. (*, $p < 0.05$; ****, $p < 0.0001$).

	SARS-CoV-2 negative	SARS-CoV-2 positive	Total
Cases	1081	121	1202
Age at enrollment, median (Range)	44 (20-83)	41 (20-68)	43 (20-83)
Age at enrollment, median (IQR)	44 (34-54)	41 (32-52)	43 (34-54)
Age at enrollment, mean (SD)	44 (12.1)	41.8 (11.7)	43.9 (12.1)
Gender, n (%)			
Female	783 (72.4)	100 (82.6)	883 (73.5)
Male	298 (27.6)	21 (17.4)	319 (26.5)
Race (self-reported), n (%)			
White, Caucasian	870 (80.5)	99 (81.8)	969 (80.6)
Asian	107 (9.9)	6 (5.0)	113 (9.4)
Black or African American	79 (7.3)	10 (8.3)	89 (7.4)
All others	15 (1.4)	4 (3.3)	19 (1.6)
Not reported	10 (0.9)	2 (1.7)	12 (1.0)
Ethnicity, n (%)			
Hispanic	38 (3.5)	4 (3.3)	42 (3.5)
Non-Hispanic	1029 (95.2)	114 (94.2)	1143 (95.1)
Not reported	14 (1.3)	3 (2.5)	17 (1.4)
Immunocompromised, n (%)	35 (3.2)	2 (1.7)	42 (3.5)
Patient care role, n (%)			
Direct	384 (35.5)	54 (44.6)	438 (36.4)
Indirect	112 (10.4)	16 (13.2)	128 (10.6)
None	585 (54.1)	51 (42.1)	636 (52.9)

Supplemental Table 1. SJTRC cohort data, Related to Figures 1-6.

From Baseline to Acute				
hCCCoV Antibodies	N	Fold change, Median (Range)	Adjusted P-value	
OC43 IgG	96	1.01 (0.60, 1.90)	>0.99	
HKU1 IgG	96	1.14 (0.66, 2.56)	<0.0001	***
NL63 IgG	96	0.95 (0.50, 2.34)	0.0092	**
229E IgG	96	0.95 (0.48, 1.70)	0.65	
OC43 IgM	96	0.86 (0.31, 4.61)	0.27	
HKU1 IgM	95	0.94 (0.23, 4.36)	>0.99	
NL63 IgM	95	0.89 (0.28, 4.92)	0.19	
229E IgM	95	0.85 (0.28, 3.14)	0.08	
OC43 IgA	96	1.08 (0.21, 12.37)	0.18	
HKU1 IgA	96	1.06 (0.33, 2.64)	0.86	
NL63 IgA	96	0.98 (0.61, 3.15)	>0.99	
229E IgA	95	0.92 (0.48, 2.47)	0.045	*
From Baseline to Convalescent				
hCCCoV Antibodies	N	Fold change, Median (Range)	Adjusted P-value	
OC43 IgG	60	1.11 (0.76, 1.63)	<0.0001	***
HKU1 IgG	60	1.37 (0.73, 2.64)	<0.0001	***
NL63 IgG	60	0.95 (0.68, 1.38)	0.022	*
229E IgG	60	0.98 (0.71, 1.56)	>0.99	
OC43 IgM	60	1.10 (0.31, 4.66)	>0.99	
HKU1 IgM	57	1.05 (0.41, 2.61)	>0.99	
NL63 IgM	57	1.03 (0.33, 3.75)	>0.99	
229E IgM	57	0.99 (0.32, 2.31)	>0.99	
OC43 IgA	60	1.44 (0.85, 6.96)	<0.0001	***
HKU1 IgA	60	1.24 (0.71, 3.37)	<0.0001	***
NL63 IgA	60	1.12 (0.61, 1.55)	0.0087	**
229E IgA	60	1.00 (0.54, 1.85)	>0.99	

Supplemental Table 2. Change in hCCCoV antibody levels after SARS-CoV-2 infection, Related to Figure 3. The fold change of antibody normalized ODs was calculated as the ratio of hCCCoV antibody normalized OD in the acute or convalescent sample to the normalized OD of the baseline sample. Red text indicates a negative fold change relative to the baseline sample. P-values for Wilcoxon signed-rank test adjusted using the Bonferroni method.

	Univariate analysis			Multivariate analysis		
Variable	HR	95% CI	P value	HR	95% CI	P value
log10 229E IgA	1.15	[0.58, 2.28]	0.68	1.23	[0.61, 2.49]	0.5586
log10 229E IgG	0.72	[0.22, 2.31]	0.579	0.77	[0.22, 2.62]	0.6702
log10 229E IgM	0.56	[0.24, 1.28]	0.166	0.41	[0.16, 1.02]	0.0562
log10 HKU1 IgA	0.97	[0.44, 2.12]	0.933	1.05	[0.46, 2.42]	0.8999
log10 HKU1 IgG	3.12	[0.85, 11.47]	0.0865	3.02	[0.82, 11.18]	0.098
log10 HKU1 IgM	1.46	[0.62, 3.40]	0.386	1.13	[0.44, 2.91]	0.803
log10 NL63 IgA	0.94	[0.45, 1.94]	0.858	0.88	[0.41, 1.87]	0.7302
log10 NL63 IgG	2.02	[0.50, 8.23]	0.327	1.38	[0.32, 5.91]	0.6609
log10 NL63 IgM	3.17	[1.18, 8.50]	0.0219	2.68	[0.92, 7.82]	0.0722
log10 OC43 IgA	0.93	[0.47, 1.82]	0.825	0.76	[0.38, 1.53]	0.4368
log10 OC43 IgG	2.08	[0.41, 10.47]	0.375	1.62	[0.31, 8.42]	0.5662
log10 OC43 IgM	1.42	[0.62, 3.26]	0.411	1.04	[0.42, 2.60]	0.9313

Supplemental Table 3. Cox regression analysis of baseline hCCCoV antibody levels associated with time to SARS-CoV-2 infection, Related to Figure 4. Cox regression analysis of baseline hCCCoV antibody levels associated with time to SARS-CoV-2 infection was performed among 1202 subjects. Multivariable Cox proportional hazards models were performed to control possible confounding factors, including age at enrollment, gender, race, and patient contact