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Supplemental information

**Serological analysis reveals
an imbalanced IgG subclass composition
associated with COVID-19 disease severity**

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1 **Supplemental Table 1: Ig Isotype Average Index Values and Clinical Positivity Rate for the**
 2 **Convalescent Cohort (n=536), Related to Figure 2.**

	IgM				IgA				IgG			
	NP	RBD	S1	S2	NP	RBD	S1	S2	NP	RBD	S1	S2
Average Index Value [†]	1.35	6.45	9.75	0.50	1.51	3.86	6.06	3.14	8.49	12.43	19.68	23.21
Positive [‡]	22%	73%	79%	5%	22%	60%	70%	34%	97%	97%	98%	98%
Negative	78%	27%	21%	95%	78%	40%	30%	66%	3%	3%	2%	2%

3 [†] Index value measurements were calculated by dividing the raw MFI by 3 standard deviation cut-off value
 4 determined by a panel of 94 pre-pandemic normal human serum specimens.

5 [‡] Positivity is defined as 6 standard deviations above the mean MFI

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7 **Supplemental Table 2: Ig Isotype Index Values Stratified for COVID-19 Disease Severity for the Convalescent Cohort**
 8 **(n=481), Related to Figure 2.**

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		Mild				Moderate				Severe			
		N	RBD	S1	S2	N	RBD	S1	S2	N	RBD	S1	S2
IgM	Mean	0.995	5.544	7.853	0.468	1.574	7.273	11.490	0.522	1.574	7.168	11.120	0.621
	SD	1.492	6.318	10.610	0.677	2.270	8.687	17.140	0.736	1.367	6.703	10.480	0.966
	SEM	0.101	0.429	0.722	0.046	0.155	0.592	1.166	0.050	0.195	0.958	1.498	0.138
	Fold Change [†]					1.6**	1.3	1.5	1.1	1.6***	1.3	1.4**	1.3
IgA	Mean	1.268	3.348	4.981	2.903	1.374	3.661	6.077	3.399	3.072	5.982	10.030	3.072
	SD	2.255	3.510	5.449	6.824	2.015	5.769	10.690	10.590	4.564	7.367	13.030	4.190
	SEM	0.153	0.283	0.370	0.463	0.137	0.393	0.729	0.722	0.652	1.052	1.862	0.599
	Fold Change [†]					1.1	1.1	1.2	1.2	2.4****	1.8	2.0*	1.1
IgG	Mean	7.821	10.300	16.100	19.190	8.682	13.140	20.820	25.030	9.853	17.460	29.430	33.08
	SD	3.276	6.955	11.930	21.630	3.171	7.740	13.370	25.220	2.677	8.665	17.330	30.95
	SEM	0.222	0.472	0.810	1.468	0.216	0.528	0.912	1.720	0.382	1.238	2.475	4.422
	Fold Change [†]					1.1	1.3***	1.3*	1.3**	1.3***	1.7****	1.8****	1.7****

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11 †Fold change calculation based on the mean of the “mild” group

12 *denotes significance where *p<0.05 **p<0.01 ***p<0.0001 ****p<0.0001 as determined by Kruskal-Wallis, adjusted for multiple
 13 comparisons with Dunn’s test.

14 **Supplemental Table 3: IgG1-4 subclass Average Index Values and Clinical Positivity Rate for the**
 15 **Convalescent Cohort (n=536), Related to Figure 3.**

		Average Index Value[†]	Clinical Positive[‡]	Clinical Negative
IgG1	NP	19.03	97%	3%
	RBD	18.71	91%	9%
	S1	137.97	97%	3%
	S2	16.43	53%	47%
IgG2	NP	1.61	21%	10%
	RBD	1.09	10%	90%
	S1	1.69	14%	86%
	S2	3.20	8%	92%
IgG3	NP	4.93	64%	36%
	RBD	3.91	39%	61%
	S1	6.18	59%	41%
	S2	43.90	94%	6%
IgG4	NP	1.58	9%	91%
	RBD	0.61	1%	99%
	S1	0.36	1%	99%
	S2	0.24	0%	100%

16 [†]Index value measurements were calculated by dividing the raw MFI by 3 standard deviation cut-off value
 17 determined by a panel of 94 pre-pandemic normal human serum specimens.

18 [‡] Positivity is defined as 6 standard deviations above the mean MFI

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Supplemental Table 4: IgG1-4 Subclass Index Values Stratified by COVID-19 Disease Severity for the Convalescent

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Cohort (n=481), Related to Figure 3.

		Mild				Moderate				Severe			
		N	RBD	S1	S2	N	RBD	S1	S2	N	RBD	S1	S2
IgG1	Mean	16.68	14.30	103.30	10.87	20.04	20.22	147.80	19.15	23.12	29.66	239.20	31.37
	SD	11.51	13.52	114.60	30.61	12.33	17.01	138.90	49.20	10.43	19.53	190.20	71.19
	SEM	0.78	0.92	7.78	2.08	0.84	1.16	9.47	3.36	1.49	2.79	27.18	10.17
	Fold Change [†]					1.2*	1.4***	1.4**	1.8*	1.4***	2.1****	2.3****	2.9***
IgG2	Mean	1.36	1.17	1.71	1.16	1.83	0.84	1.45	6.04	2.08	0.45	1.79	2.58
	SD	2.41	4.18	6.50	5.09	3.90	2.55	3.91	53.51	2.33	0.74	4.35	8.70
	SEM	0.16	0.28	0.44	0.35	0.27	0.17	0.27	3.65	0.33	0.11	0.62	1.24
	Fold Change [†]					1.3	0.7	0.9	5.2	1.5**	0.4	1.0	2.2
IgG3	Mean	3.51	2.31	3.69	44.91	4.84	3.06	5.74	39.44	7.96	15.68	20.44	47.85
	SD	5.18	5.57	5.85	223.10	6.83	6.24	8.14	63.01	11.28	53.41	56.03	49.77
	SEM	0.35	0.38	0.40	15.14	0.47	0.43	0.56	4.30	1.61	7.63	8.01	7.11
	Fold Change [†]					1.4*	1.3*	1.6**	0.9	2.3****	6.8****	5.5****	1.1**
IgG4	Mean	1.15	0.50	0.35	0.27	1.86	0.79	0.43	0.24	1.85	0.61	0.40	0.27
	SD	8.00	0.27	0.20	0.61	12.91	3.52	0.71	0.15	7.41	0.32	0.26	0.28
	SEM	0.54	0.02	0.01	0.04	0.88	0.24	0.05	0.01	1.06	0.05	0.04	0.04
	Fold Change [†]					1.6	1.6	1.2	0.9	1.6**	1.2	1.1	1.0

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†Fold change calculation based on the mean of the "mild" group

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*denotes significance where *p<0.05 **p<0.01 ***p<0.0001 ****p<0.0001 as determined by Kruskal-Wallis, adjusted for multiple comparisons with Dunn's test.

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Supplemental Table 5: Ordered Probit Regression, Related to Figure 4a.

UNIVARIATE PROPORTIONAL ODDS PROBIT REGRESSION				MODEL PERFORMANCE ON TESTING DATA	
Variable	Coefficient (SE)	p-value	AIC	Accuracy (95% CI)	p-value ACC>NIR
DPO	0.00842029 (0.06290226)	8.94E-01	635.23	0.4214 (0.3385, 0.5077)	7.77E-01
Age	0.1792742 (0.06333862)	4.65E-03	627.22	0.5286 (0.4425, 0.6134)	3.75E-02
Gender (Male)	-0.1172834 (0.13943004)	4.00E-01	634.54	0.4214 (0.3385, 0.5077)	7.77E-01
PRNT90	0.1958114 (0.06226123)	1.66E-03	625.25	0.45 (0.3659, 0.5363)	5.33E-01
PRNT50	0.1824153 (0.06318260)	3.89E-03	626.90	0.4643 (0.3797, 0.5505)	3.98E-01
IgM N	0.1192098 (0.06165475)	5.32E-02	631.52	0.5571 (0.4708, 0.641)	7.02E-03
IgM RBD	0.07171105 (0.06237824)	2.50E-01	633.93	0.4786 (0.3935, 0.5646)	2.76E-01
IgM S1	0.08198261 (0.06196963)	1.86E-01	633.50	0.5 (0.4144, 0.5856)	1.35E-01
IgM S2	0.05418636 (0.06214503)	3.83E-01	634.49	0.4214 (0.3385, 0.5077)	7.77E-01
IgA N	0.2121957 (0.06457068)	1.02E-03	624.14	0.5 (0.4144, 0.5856)	1.35E-01
IgA RBD	0.1038650 (0.06144069)	9.09E-02	632.39	0.4429 (0.359, 0.5292)	6.00E-01
IgA S1	0.1057137 (0.06116121)	8.39E-02	632.27	0.4286 (0.3453, 0.5149)	7.23E-01
IgA S2	-0.006981466 (0.06471904)	9.14E-01	635.23	0.45 (0.3659, 0.5363)	5.33E-01
IgG N	0.2282152 (0.06524374)	4.69E-04	622.79	0.5286 (0.4425, 0.6134)	3.75E-02
IgG RBD	0.3024209 (0.06405524)	2.34E-06	612.70	0.5214 (0.4354, 0.6065)	5.36E-02
IgG S1	0.3205334 (0.06427497)	6.14E-07	610.04	0.5214 (0.4354, 0.6065)	5.36E-02
IgG S2	0.1731082 (0.06260303)	5.69E-03	627.59	0.5643 (0.478, 0.6478)	4.32E-03
IgG1 N	0.1873592 (0.06359648)	3.22E-03	626.54	0.55 (0.4637, 0.6341)	1.11E-02
IgG1 RBD	0.3164681 (0.06377248)	6.96E-07	610.31	0.5357 (0.4495, 0.6203)	2.56E-02
IgG1 S1	0.3069176 (0.06392332)	1.58E-06	611.87	0.5071 (0.4214, 0.5926)	1.02E-01
IgG1 S2	0.1230611 (0.06162206)	4.58E-02	631.28	0.5071 (0.4214, 0.5926)	1.02E-01
IgG2 N	0.09762629 (0.06173738)	1.14E-01	632.76	0.4714 (0.3866, 0.5575)	3.35E-01
IgG2 RBD	-0.07505903 (0.07014432)	2.85E-01	634.03	0.4786 (0.3935, 0.5646)	2.76E-01
IgG2 S1	-0.0594108 (0.07129139)	4.05E-01	634.50	0.45 (0.3659, 0.5363)	5.33E-01
IgG2 S2	0.0397431 (0.05994935)	5.07E-01	634.81	0.4643 (0.3797, 0.5505)	3.99E-01
IgG3 N	0.2194403 (0.06429924)	6.43E-04	623.24	0.5286 (0.4425, 0.6134)	3.75E-02
IgG3 RBD	0.2619276 (0.08327653)	1.66E-03	622.19	0.5071 (0.4214, 0.5926)	1.02E-01
IgG3 S1	0.3560372 (0.07712245)	3.90E-06	609.82	0.5071 (0.4214, 0.5926)	1.02E-01
IgG3 S2	0.1104608 (0.06175604)	7.37E-02	632.07	0.4571 (0.3728, 0.5434)	4.65E-01
IgG4 N	0.007072853 (0.06156942)	9.09E-01	635.23	0.45 (0.3659, 0.5363)	5.33E-01
IgG4 RBD	0.03380058 (0.05986258)	5.72E-01	634.93	0.45 (0.3659, 0.5363)	5.33E-01
IgG4 S1	0.1080485 (0.06200676)	8.14E-02	632.20	0.5 (0.4144, 0.5856)	1.35E-01
IgG4 S2	-0.03506842 (0.07450493)	6.38E-01	635.01	0.4429 (0.359, 0.5292)	6.00E-01
All variables			648.02	0.5071 (0.4214, 0.5926)	1.02E-01
Selected variables			600.80	0.6 (0.5139, 0.6818)	2.56E-04

Supplemental Table 6: Ordered Probit Regression, Related to Figure 4a.

PROPORTIONAL ODDS PROBIT REGRESSION WITH AGE AS A COVARIATE				MODEL PERFORMANCE ON TESTING DATA	
Variable	Coefficient (SE)	p-value	AIC	Accuracy (95% CI)	p-value ACC>NIR
DPO	-0.004119273 (0.06338651)	9.48E-01	629.22	0.5286 (0.4425, 0.6134)	3.75E-02
Gender (Male)	-0.1293131 (0.13987964)	3.55E-01	628.36	0.5429 (0.4566, 0.6272)	1.71E-02
PRNT90	0.1559160 (0.06547446)	1.73E-02	623.51	0.5429 (0.4566, 0.6272)	1.71E-02
PRNT50	0.1449436 (0.06572414)	2.74E-02	624.36	0.5214 (0.4354, 0.6065)	5.36E-02
IgM N	0.07836274 (0.06409214)	2.21E-01	627.73	0.5214 (0.4354, 0.6065)	5.36E-02
IgM RBD	0.01943351 (0.06557456)	7.67E-01	629.13	0.55 (0.4637, 0.6341)	1.11E-02
IgM S1	0.02322937 (0.06622030)	7.26E-01	629.10	0.5571 (0.4708, 0.641)	7.02E-03
IgM S2	0.05514419 (0.06216877)	3.75E-01	628.44	0.5643 (0.478, 0.6478)	4.32E-03
IgA N	0.1710039 (0.06742154)	1.12E-02	622.63	0.5429 (0.4566, 0.6272)	1.71E-02
IgA RBD	0.09119512 (0.06167864)	1.39E-01	627.04	0.5357 (0.4495, 0.6203)	2.56E-02
IgA S1	0.09161639 (0.06145615)	1.36E-01	627.01	0.5143 (0.4284, 0.5996)	7.47E-02
IgA S2	-0.0227294 (0.06522780)	7.27E-01	629.10	0.5214 (0.4354, 0.6065)	5.36E-02
IgG N	0.1942685 (0.06750208)	4.00E-03	620.84	0.5571 (0.4708, 0.641)	7.02E-03
IgG RBD	0.2820277 (0.06481207)	1.35E-05	610.10	0.5929 (0.5067, 0.675)	4.76E-04
IgG S1	0.2993826 (0.06512311)	4.28E-06	607.84	0.5357 (0.4495, 0.6203)	2.56E-02
IgG S2	0.1487403 (0.06345589)	1.91E-02	623.72	0.5857 (0.4995, 0.6683)	8.61E-04
IgG1 N	0.1443364 (0.06732723)	3.20E-02	624.62	0.5929 (0.5067, 0.675)	4.76E-04
IgG1 RBD	0.2937825 (0.06482250)	5.84E-06	608.46	0.5786 (0.4923, 0.6615)	1.52E-03
IgG1 S1	0.2837305 (0.06491573)	1.24E-05	609.88	0.5214 (0.4354, 0.6065)	5.36E-02
IgG1 S2	0.09804867 (0.06240420)	1.16E-01	626.76	0.5571 (0.4708, 0.641)	7.02E-03
IgG2 N	0.08248615 (0.06185257)	1.82E-01	627.45	0.5286 (0.4425, 0.6134)	3.75E-02
IgG2 RBD	-0.06202069 (0.07071954)	3.80E-01	628.41	0.5357 (0.4495, 0.6203)	2.57E-02
IgG2 S1	-0.05381328 (0.07181056)	4.54E-01	628.62	0.5357 (0.4495, 0.6203)	2.57E-02
IgG2 S2	0.03299862 (0.06017975)	5.83E-01	628.92	0.5357 (0.4495, 0.6203)	2.57E-02
IgG3 N	0.1925910 (0.06495653)	3.03E-03	620.21	0.5714 (0.4851, 0.6547)	2.59E-03
IgG3 RBD	0.2625168 (0.08549222)	2.14E-03	616.70	0.5 (0.4144, 0.5856)	1.35E-01
IgG3 S1	0.3463172 (0.07767068)	8.24E-06	605.61	0.5429 (0.4566, 0.6272)	1.71E-02
IgG3 S2	0.1169920 (0.06213372)	5.97E-02	625.71	0.5 (0.4144, 0.5856)	1.35E-01
IgG4 N	-0.008487359 (0.06277202)	8.92E-01	629.20	0.5286 (0.4425, 0.6134)	3.75E-02
IgG4 RBD	0.04314625 (0.06013673)	4.73E-01	628.70	0.5286 (0.4425, 0.6134)	3.75E-02
IgG4 S1	0.1071146 (0.06217504)	8.49E-02	626.25	0.5286 (0.4425, 0.6134)	3.75E-02
IgG4 S2	-0.02925658 (0.07612034)	7.01E-01	629.06	0.5286 (0.4425, 0.6134)	3.75E-02

Supplemental Table 7: IgG Subclass Index Ratios, Related to Figure 4b.

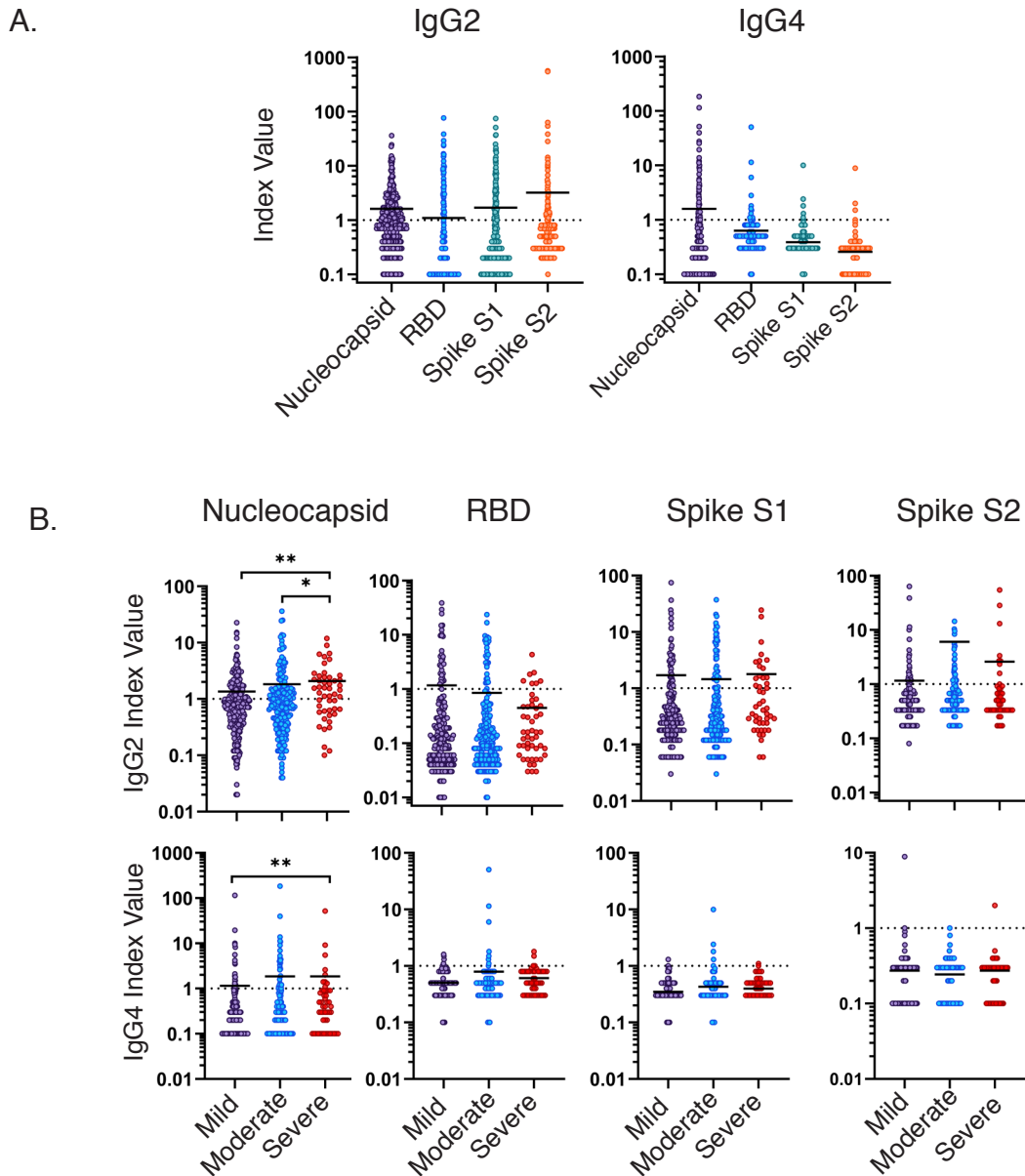
		IgG1/IgG				IgG3/IgG			
		Mean	SD	SEM	Fold Change [†]	Mean	SD	SEM	Fold Change [†]
Mild	N	2.925	4.475	0.304		0.256	0.399	0.027	
	RBD	2.043	3.077	0.209		0.198	0.288	0.020	
	S1	4.918	2.475	0.168		0.230	0.398	0.027	
	S2	0.260	0.350	0.024		2.170	2.493	0.169	
Moderate	N	2.770	2.845	0.194	0.9	0.243	0.289	0.020	0.9
	RBD	2.351	3.304	0.225	1.2	0.203	0.346	0.024	1.0
	S1	5.652	2.656	0.181	1.1	0.230	0.261	0.018	1.0
	S2	0.364	0.470	0.032	1.4	1.853	1.976	0.135	0.9
Severe	N	2.232	0.730	0.104	0.8	0.314	0.398	0.057	1.3
	RBD	1.504	0.523	0.075	0.7	0.666	1.842	0.263	3.4**
	S1	6.747	2.857	0.408	1.4****	0.516	1.100	0.157	2.2****
	S2	0.494	0.545	0.078	1.9***	1.979	1.931	0.276	0.9
Validation Cohort	N	2.471	1.199	0.212	0.8	0.490	0.815	0.144	2.0*
	RBD	3.898	1.579	0.279	1.9****	0.544	0.757	0.134	2.7**
	S1	18.730	12.930	2.285	3.8****	1.103	1.459	0.258	4.8****
	S2	0.993	1.960	0.346	3.8****	2.257	3.304	0.584	1.0

[†] Fold change calculation based on the mean of the "mild" group

*denotes significance where *p<0.05 **p<0.01 ***p<0.0001 ****p<0.0001 as determined by Kruskal-Wallis, adjusted for multiple comparisons with Dunn's test

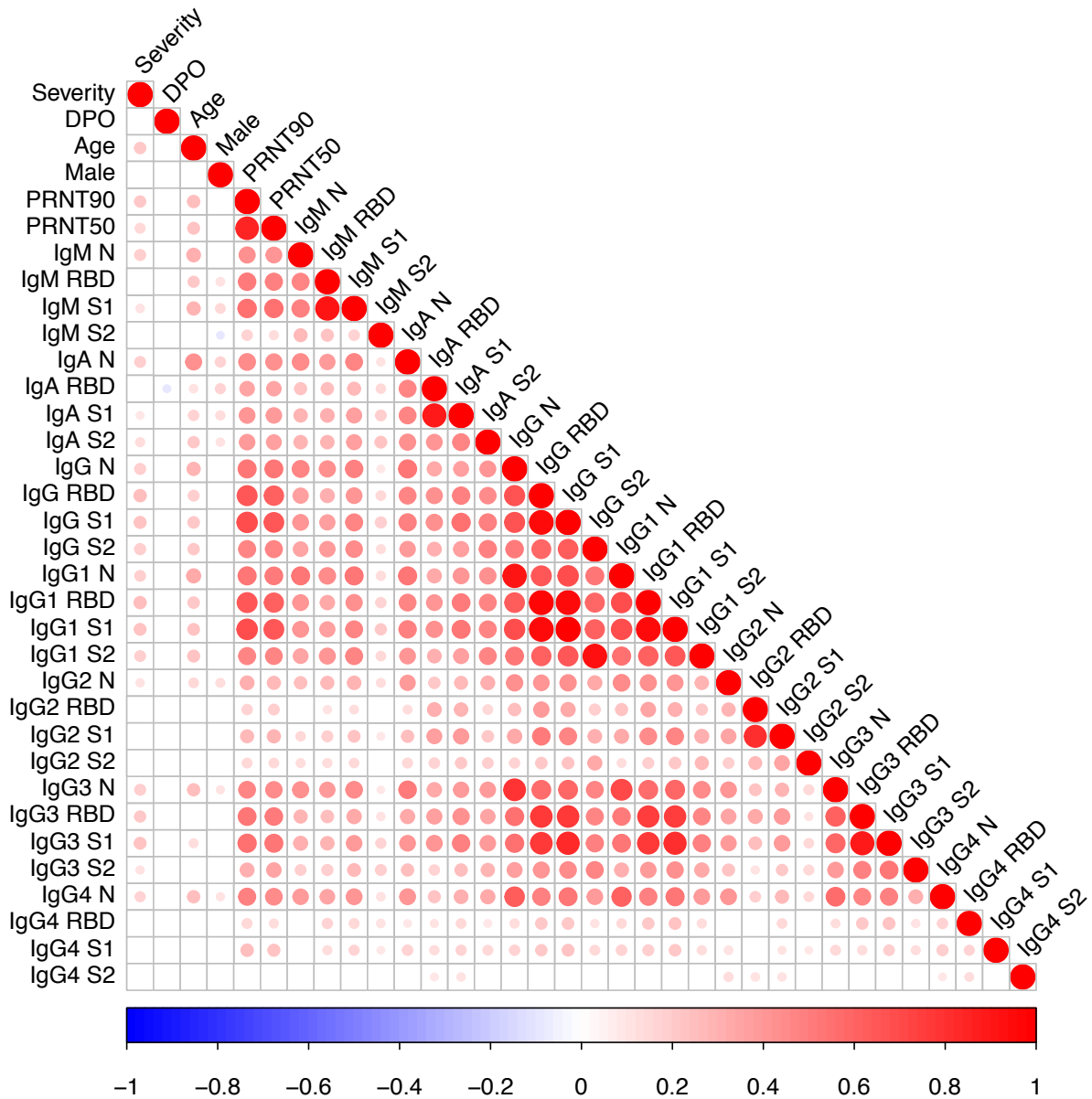
Supplemental Table 8: Clinical Characteristics of the Validation Cohort, Related to STAR Methods.

	All	Male	Female
Count	32	12(37.5%)	20(62.5%)
Mean Age (years)	60	59	61

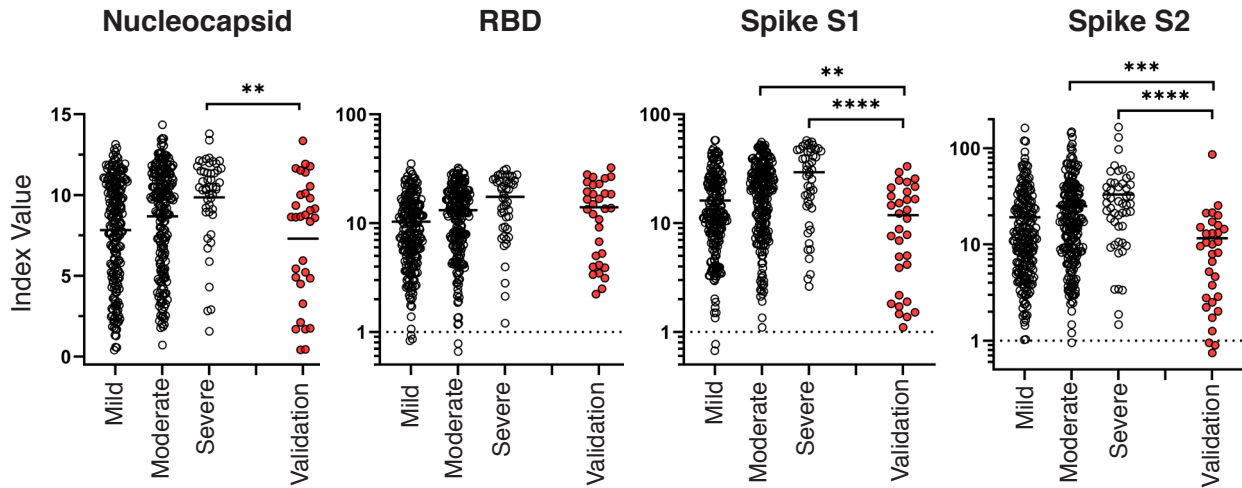


Supplemental Figure 1: IgG Subclass and Antigen Distribution of the SARS-CoV-2 specific Antibody Profile Across COVID-19 Disease Severities, Related to Figure 3.

Serum specimens from convalescent COVID-19 donors were analyzed for reactivity of IgG2, and IgG4 specific to the SARS-CoV-2 nucleocapsid, RBD, S1 subunit, or S2 subunit. Index values represent the raw MFI divided by the cutoff value (3 standard deviations above the mean) determined by a panel of 94 pre-pandemic normal human serum specimens (cutoff = dashed line). (a) Reactivity of IgG1, IgG2, and IgG3 to SARS-CoV-2 nucleocapsid, RBD, S1 subunit, or S2 subunit of the full patient cohort (b) or grouped by disease severity. Index value represents the raw MFI divided by the background cutoff value determined by a panel of 93 normal human serum specimens. Statistical significance was determined by the non-parametric Kruskal-Wallis test where * $p < 0.05$ ** $p < 0.001$ *** $p < 0.0001$, and **** $p < 0.00001$ adjusted for multiple comparisons by Dunn's test.



Supplemental Figure 2: Correlation Matrix of Antibody Measurements and Clinical Features, Related to Figure 5. Correlation matrix of antibody reactivities, neutralization titers, patient features, and COVID-19 disease severity. Circles representing Spearman's correlations are displayed, with color and size of the circle reflecting the strength of the correlation. Only correlations with $p < 0.05$ are represented by a circle. All p-values are adjusted via the Benjamini and Hochberg method.



Supplemental Figure 3: SARS-CoV-2 specific IgG of the Validation Cohort compared to the Convalescent Cohort Across COVID-19 Disease Severities, Related to Figures 3, 4, and 6.

IgG reactivity to SARS-CoV-2 nucleocapsid, RBD, S1, or S2 of the convalescent cohort (n=481) grouped by disease severity, and compared to the validation cohort (n=32). Index values represent the raw MFI divided by the background cutoff value determined by a panel of 94 normal human serum specimens. Statistical significance was determined by the non-parametric Kruskal-Wallis test where *p < 0.05 **p < 0.001 ***p < 0.0001, and ****p < 0.00001 adjusted for multiple comparisons by Dunn's test.