

Supplemental information

**IgG targeting distinct seasonal coronavirus-
conserved SARS-CoV-2 spike subdomains correlates
with differential COVID-19 disease outcomes**

Jose L. Garrido, Matías A. Medina, Felipe Bravo, Sarah McGee, Francisco Fuentes-Villalobos, Mario Calvo, Yazmin Pinos, James W. Bowman, Christopher D. Bahl, Maria Ines Barria, Rebecca A. Brachman, and Raymond A. Alvarez

Supplemental Information

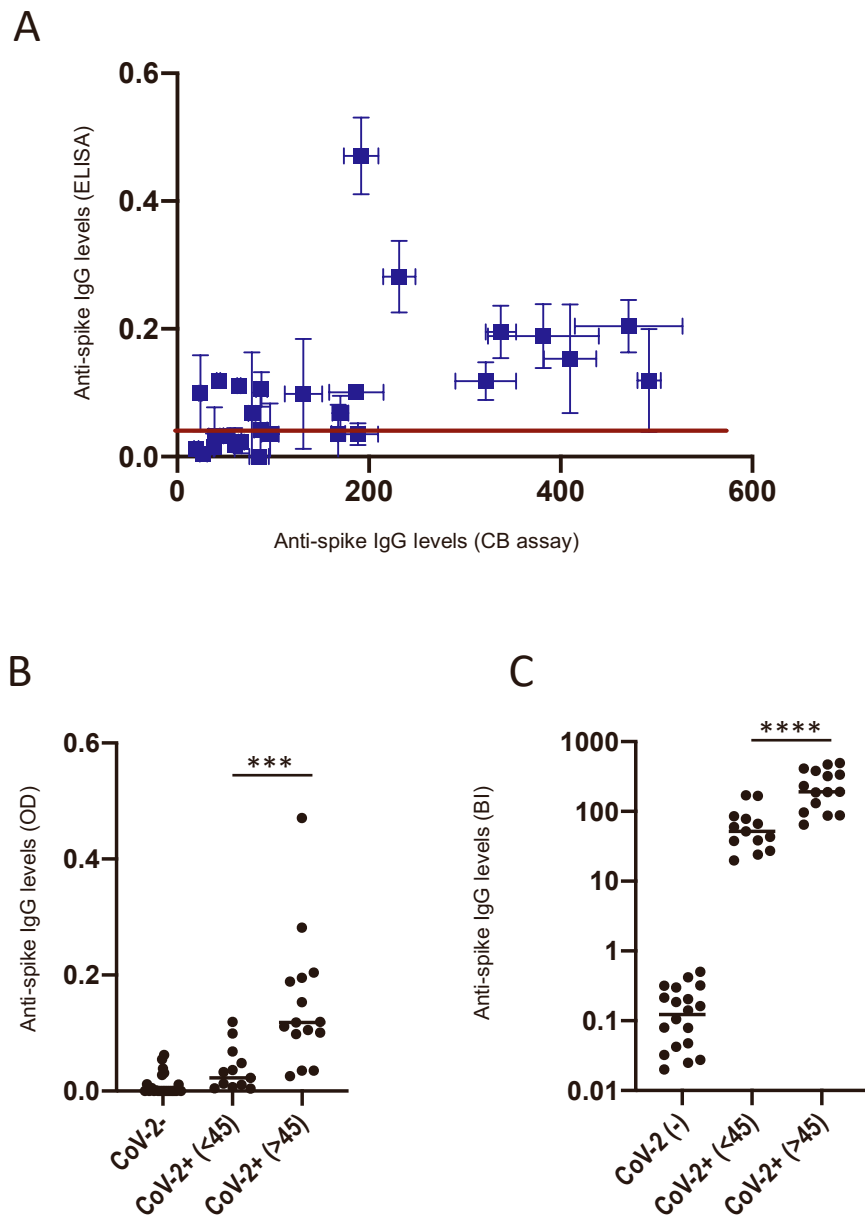
Clinical Characteristics	SARS-CoV-2 Convalescent vs Naive	
	Convalescent	Naive
Sex, female/male	12/16	12/8
(Total)	(28)	(20)
Median Age \pm SD	38 \pm 10.1073292	38 \pm 13.5418741
Days from onset of disease to blood draw (median \pm SD)	43 \pm 17.1284933	55.5 \pm 22.8414827
Blood type		
A+	13	4
B+	5	7
B-	0	1
AB+	1	1
O+	8	7
O-	1	1
Underlying conditions	vit d3 deficient, depression, allergies, high cholesterol, high blood pressure, asthma, hypertension, high uric acid (gout), diabetes, pregnant, HIV	high cholesterol, high blood pressure, acid indigestion, inflammatory conditions, hypertension, overweight, seizures, ADHD, herpes, hypothyroid

Table S1. Characteristics of SARS-CoV-2 convalescent and naïve donors

Symptoms	All convalescent donors			Mild convalescent donors			Severe convalescent donors		
	Frequency of symptoms (N=28)	Average symptom severity score (1-10)	Average Duration of Symptoms (Days)	Frequency of symptoms (N=13)	Average symptom severity score (1-10)	Average Duration of Symptoms (Days)	Frequency of symptoms (N=15)	Average symptom severity score (1-10)	Average Duration of Symptoms (Days)
Runny Nose	14/28 (50.0%)	5.14	9.20	7/13 (53.9%)	3.43	8.00	7/15 (46.7%)	6.86	8.67
Sore throat	16/28 (57.1%)	5.06	5.25	6/13 (53.8%)	3.75	3.50	10/15 (66.7%)	5.7	4.6
Cough	21/28 (75.0%)	5.33	8.50	9/13 (69.2%)	4.00	6.43	12/15 (80%)	6.33	9.00
Fatigue	25/28 (89.3%)	6.56	7.24	12/13 (92.3%)	4.36	4.73	13/15 (86.7%)	8.39	10.0
Myalgia	24/28 (87.5%)	6.83	6.33	11/13 (84.6%)	4.46	3.40	13/15 (86.7%)	8.62	8.69
Diarrhea	7/28 (25.0%)	6.14	8.14	2/13 (15.4%)	5.50	2.00	5/15 (33.3%)	6.40	9.80
Fever	19/28 (67.9%)	6.11	4.47	9/13 (69.2%)	4.09	2.78	10/15 (66.7%)	7.70	6.22
Vomiting	5/28 (17.9%)	4.30	4.00	3/13 (23.1%)	4.00	4.00	2/15 (13.3%)	4.75	3.00
Nausea	4/28 (14.3%)	5.50	4.00	2/13 (15.4%)	4.50	6.00	2/15 (13.3%)	6.50	7.00
Headache	24/28 (85.7%)	5.50	7.69	11/13 (86.6%)	3.85	3.70	13/15 (86.7%)	6.77	10.0
Shortness of breath	11/28 (39.3%)	4.00	8.00	2/13 (15.4%)	3.00	2.00	9/15 (60%)	4.22	8.11

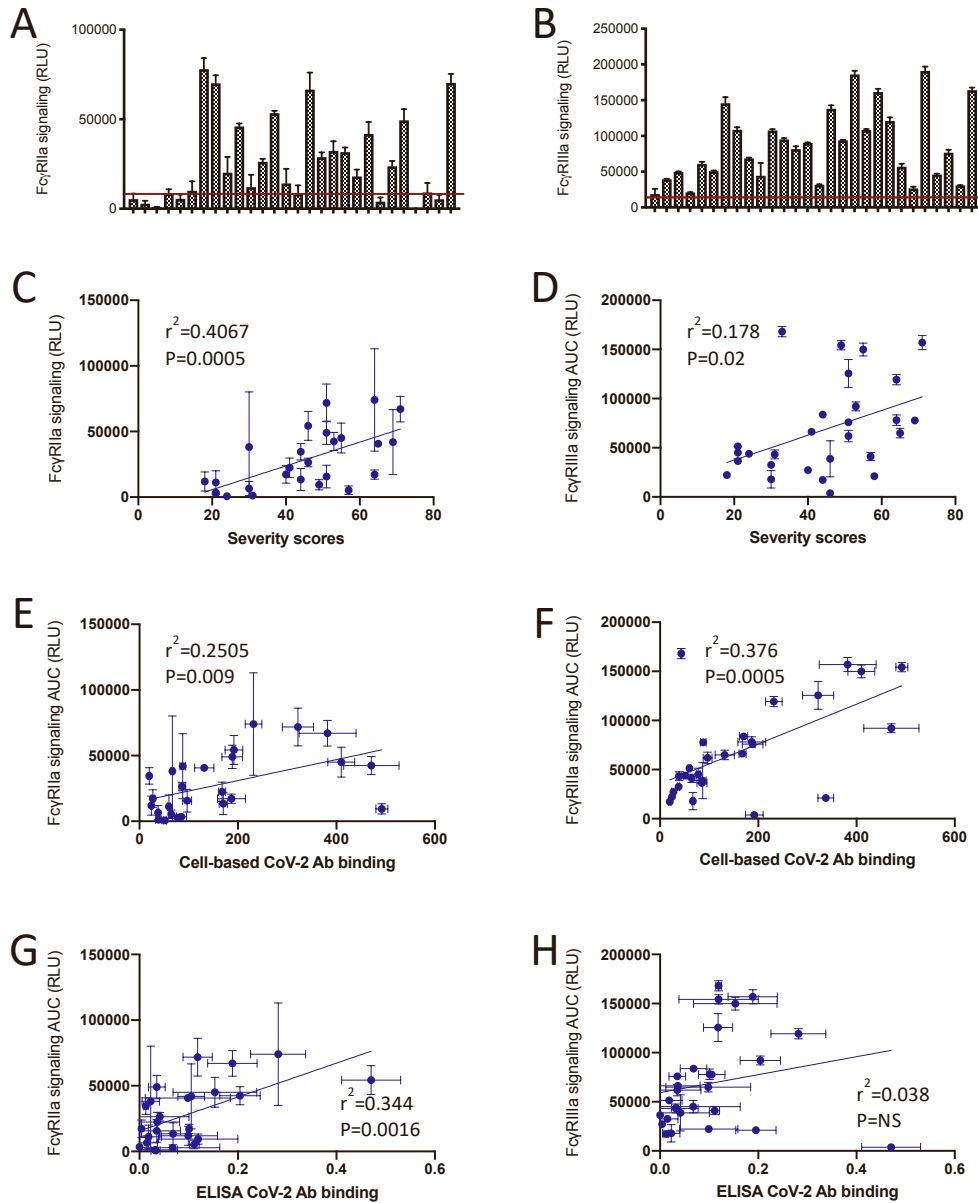
Table S2. Symptomology of SARS-CoV-2 convalescent donors. Related to Table 1.

Table depicts frequency of COVID-19 symptoms experienced by convalescent donors. Symptom intensity was scored out of 10, with 10 being the most severe, and 0 not being experienced. Mild convalescent donors were defined as donors with composite symptom intensity scores below 45. Severe convalescent donors were defined as donors with composite symptom intensity scores above 45.



Supplementary Figure 1. Comparison of anti-spike IgG levels in convalescent donors as quantified by ELISA vs Cell-based assays. Related to Figure 1.

(A) Anti-spike IgG titers in SARS-CoV-2 naïve (CoV-2-) and SARS-CoV-2 positive convalescent (CoV-2+) donors (n=20 and 28, respectively), as quantified by recombinant spike protein ELISA vs Cell-based (CB) binding assay. Red line represents 3-fold above the mean anti-spike levels of naïve (CoV-2-) donors, as quantified by ELISA. **(B,C)** Levels of anti-spike IgG titers as quantified by (B) ELISA, or (C) Cell based IgG binding assay. SARS CoV-2 naïve (CoV-2-) and convalescent (CoV-2+) donors are shown with convalescent donors split by COVID19 severity scores. (n=20 naïve, 13 mild, 15 more severe and 17 ICU). The SEM of N=3 experiments are shown.



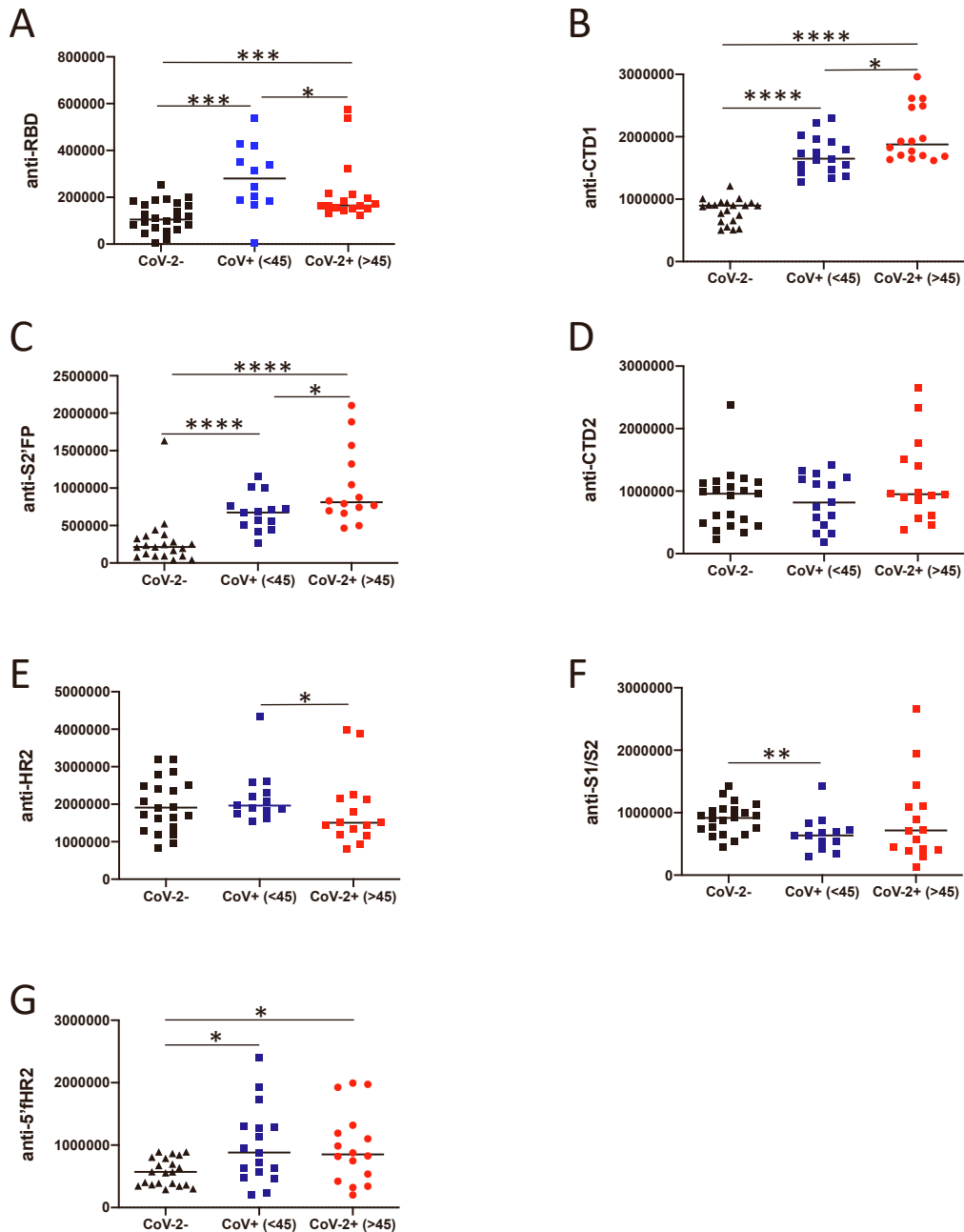
Supplementary Figure 2. Levels of anti-spike IgG-induced Fc γ R-activation correlates with COVID-19 severity and anti-spike titers. Related to Figure 2.

Figure depicts the levels of Fc γ R-signaling induced by purified IgG derived from SARS-CoV-2 convalescent donors in response to SARS-CoV-2 spike protein expressed on the surface of 293T cells (n=28). Graphs show the levels of (A) Fc γ RIIa and (B) Fc γ RIIIa signaling induced by 25 μ g/ml of purified IgG from all SARS CoV-2 convalescent donors. Red line represents 2-fold above the mean anti-spike levels of all naïve (CoV-2⁻) donors in each Fc γ R signaling assay. Scatter plots show the area under the (C) Fc γ RIIa or (D) Fc γ RIIIa signaling curve versus COVID-19 severity scores. Scatter plots show the area under the (E) Fc γ RIIa or (F) Fc γ RIIIa signaling curve versus the levels of anti-spike IgG titers as quantified by cell-based IgG binding assay. Scatter plots show the area under the (G) Fc γ RIIa or (H) Fc γ RIIIa signaling curve versus the levels of anti-spike IgG titers as quantified by ELISA. All Fc γ R signaling assays were conducted using a three-point titration curve of purified donor IgG (25ug/ml, 5ug/ml, & 1ug/ml). Area under all points was used to calculate AUC. The SEM of N=3 experiments are shown, along with significance of slopes and r^2 values.

Sequence Numbering	SARS CoV-2 Spike protein sequence	Functional region	Domain Homology with other hCoV spike protein				
			betacoronaviruses			alphacoronaviruses	
			SARS1	OC43	HKU1	NL63	229E
S-1-1273	SARS CoV-2	Full spike protien	78%	32%	30%	24%	26%
S1-319-541	RVQPTESIVRFP NITNLCPFGEVF NATRFASVYAW NRKRISNCVADY SVLYNSASFSTF KCYGVSPTKLND LCFTNVYADSFVI RGDEVRQIAPGQ TGKIADYNYKLP DDFTGCVIAWNS NNLDSKVGNGY NYLYRFLFRKSNL KPFERDISTEIYQ AGSTPCNGVEG FNCYFPLQSYGF QPTNGVGYQPY RVVWLSFELLHA PATVCGPKKSTN LVKNKCVNF	Receptor Binding Domain (RBD)	73.0%	20.7%	20.3%	17.1%	12.6%
S1-556-576 (Pep4)	NKKFLPFQQFGR DIADTTDAV	C-terminus domain (CTD1)	66.7%	14.9%	9.5%	4.8%	14.3%
S1-618-638 (Pep1)	TEVPVAIHADQL TPTWRVYST	C-terminus domain (CTD2)	76.2%	19.0%	14.3%	4.8%	14.3%
S1/S2-672-687 (Pep2)	ASYQTQTNSPR RARSV	Furin cleavage site (S1/S2)	37.5%	25.0%	31.3%	6.3%	0.0%
S2-805-823 (Pep5)	ILPDPSKPSKRSF IEDLLF	S2' Fusion protein (S2'FP)	89.5%	63.2%	47.4%	52.6%	47.4%
S2-1143-1167 (Pep6)	PELDSFKEELDK YFKNHTSPDVL G	5' HR2 flanking region (5'fHR2)	100.0%	60.0%	44.0%	12.0%	16.0%
S2-1179-1213 (Pep3)	IQKEIDRLNEVAK NLNESLIDLQELG KYE QYIKWP	Heptad Repeat-2 (HR2) region	100.0%	60.0%	42.9%	34.3%	42.9%

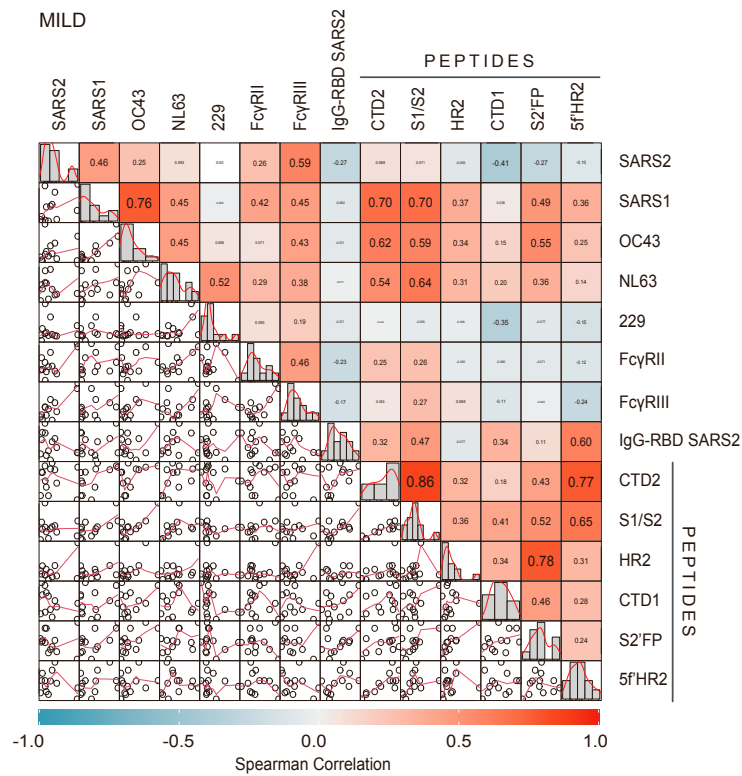
Table S3. Sequence identity of SARS CoV-2 Immunodominant epitopes and functional regions in comparison to seasonal hCoVs. Related to Figure 2.

The level of sequence identity between spike proteins were assessed using PRALINE software (IBIVU), by comparing SARS-CoV-2 spike protein sequence (Genbank YP_009724390.1) to spike protein sequences of SARS1 (Genbank AAP13567.1), OC43 (Genbank AVR40344.1), HKU1 (YP_173238.1), NL63 (APF29071.1), or 229E (APT69883.1). Percent sequence identity was measured by the level of exact AA conservation in reference to the SARS-CoV-2 spike sequence. Gaps in hCoV sequences were treated as no conservation.

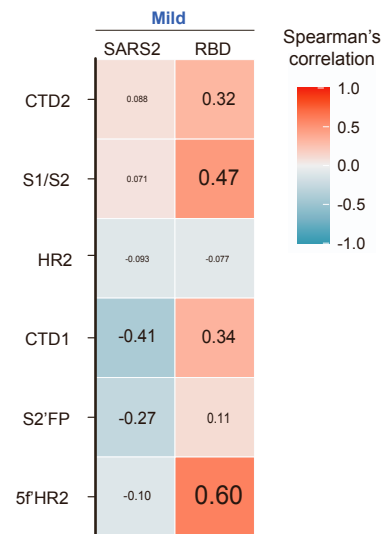


Supplementary Figure 3. SARS-CoV-2 convalescent IgG differentially target seasonal CoV-conserved and non-conserved SARS-CoV-2 immunodominant epitopes. Related to Figure 5. Graphs compare the levels of IgG-binding to (A) RBD, (B) CTD1, (C) S2'FP, (D) CTD2 (E) HR2, (F) S1/S2 (G) 5'F HR2 regions in in SARS-CoV-2 naïve (CoV-2-, n=20) and SARS-CoV-2 positive convalescent (CoV-2+, n=28) donors. Convalescent donors were split into two groups based on COVID19 severity scores, MILD (<45, n=13), and more severe (>45, n=15). The SEM of N=3 experiments are shown.

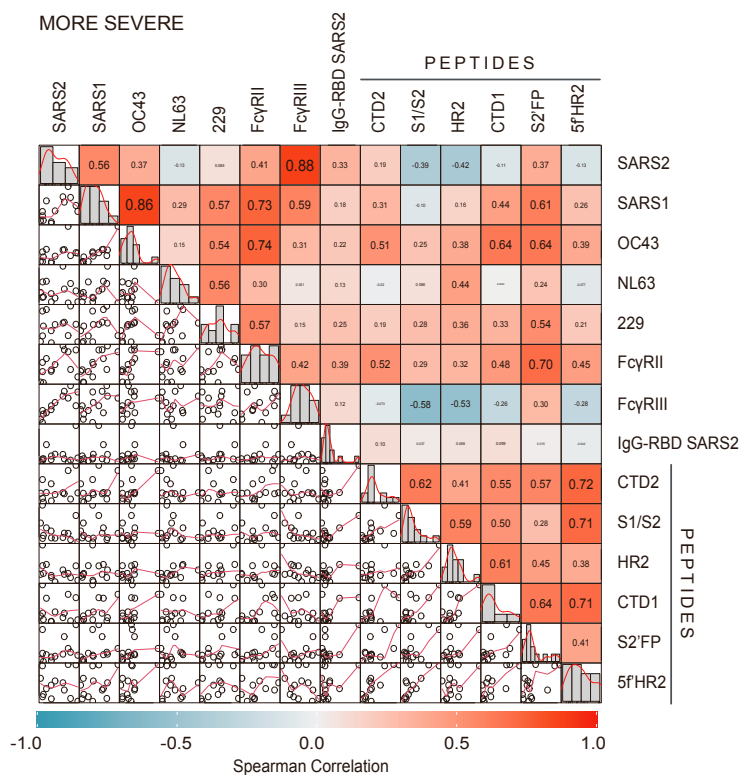
A



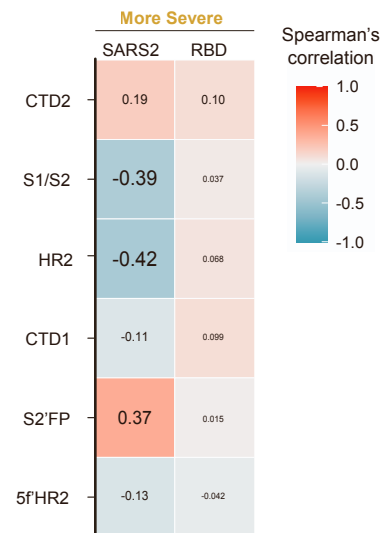
B



C



D



Supplementary Figure 4. Preferential antibody-binding to different regions of the SARS CoV-2 spike correlates with COVID-19 severity. Related to Figure 6.

(A,C) Scatter matrix chart summarizes the Spearman's correlation (r values, upper) and the scatter plots (lower) between all analyzed variables for samples separated by mild (n=13) or more severe (n=15) symptoms, respectively. The small bar graphs (diagonal) represent the distribution of data for each variable. (B,D) Heatmaps show the Spearman's correlations (r values) between IgG-Spike or IgG-RBD and the levels of IgG targeting the six functional spike domains for samples separated by B) mild (n=13) or D) more severe (n=15) symptoms.