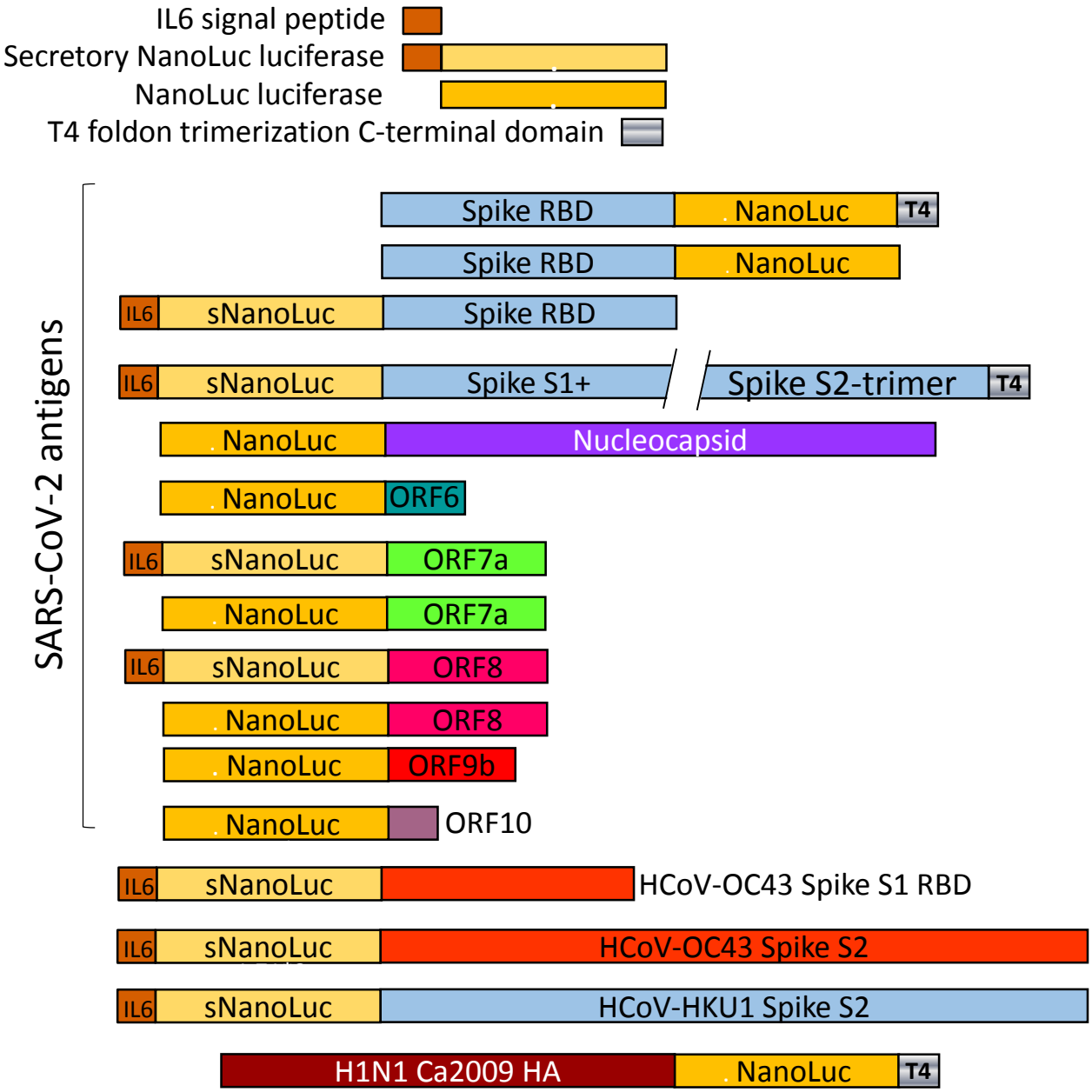
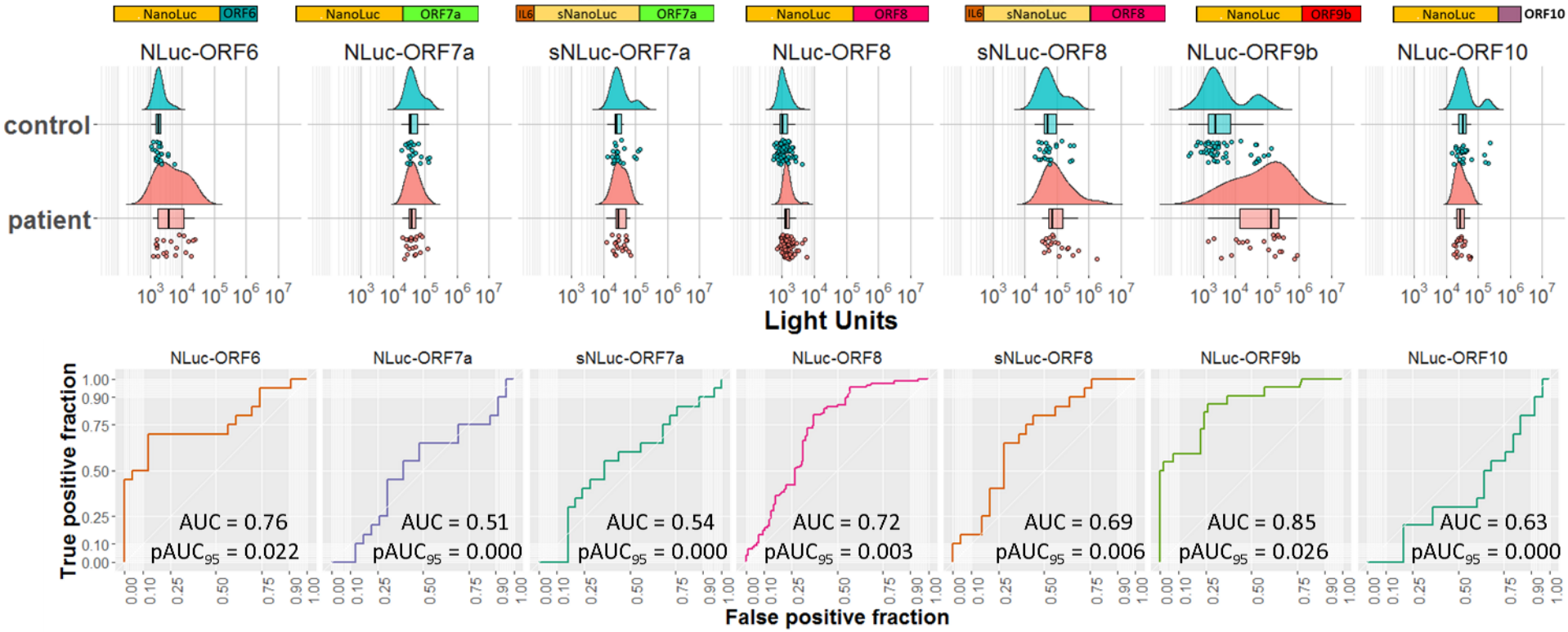
Supplemental Figure 1



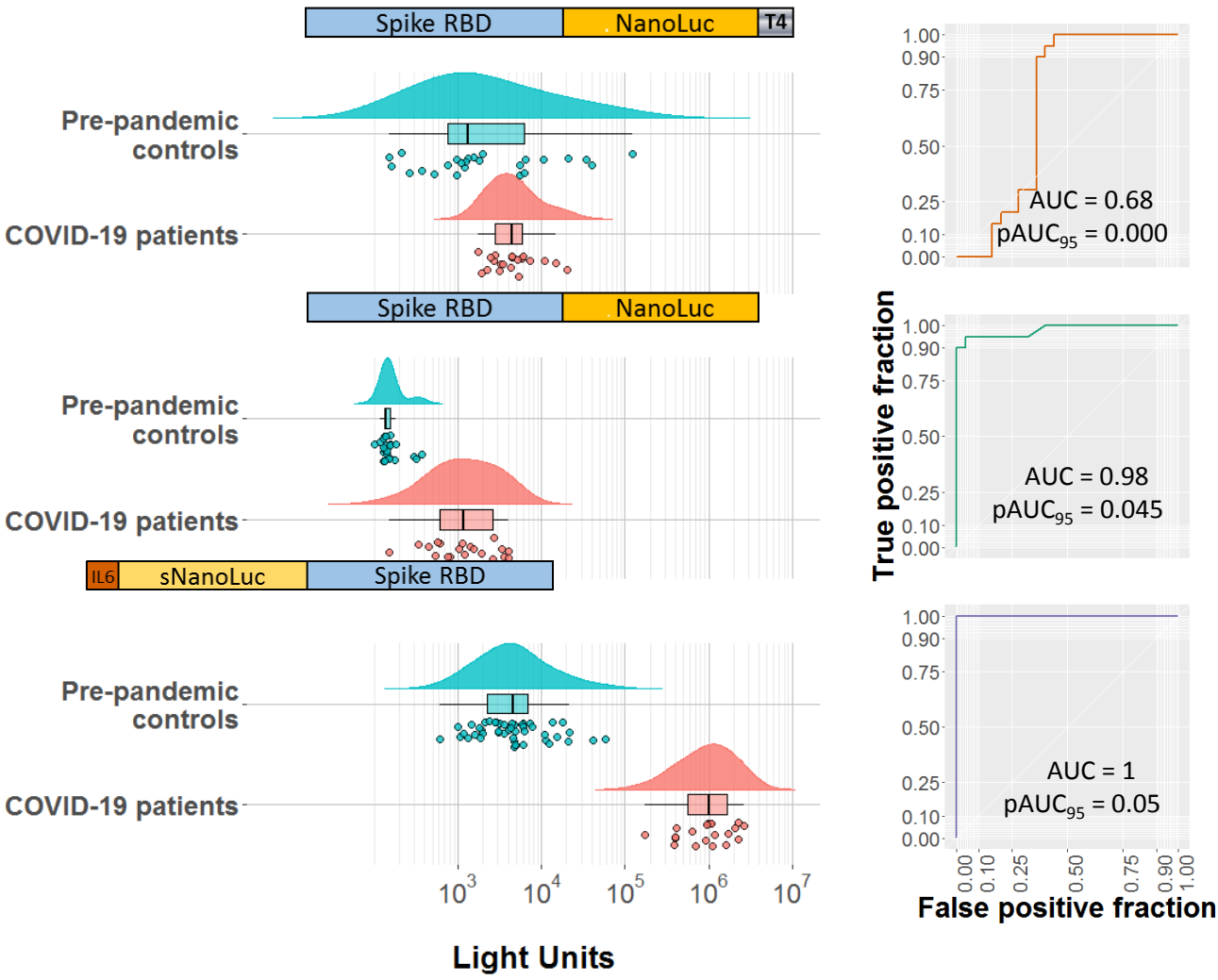
Schematic representation of the recombinant nanoluciferase tagged antigens used in the study



IgG antibody responses in COVID-19 and control sera against SARS-CoV-2 ORF proteins. *The top panel show raw data (Light Units) measured after LIPS using the shown recombinant SARS-COV-2 antigens. For each ORF are shown the probability density estimates, boxplot and individual measurement in COVID-19 patient (magenta circle) or control (light blue circle) sera. The diagnostic performance of each LIPS assay is shown in the bottom panels as ROC curves.*

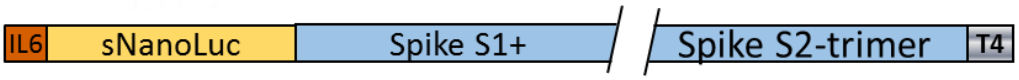
Supplemental Figure 2

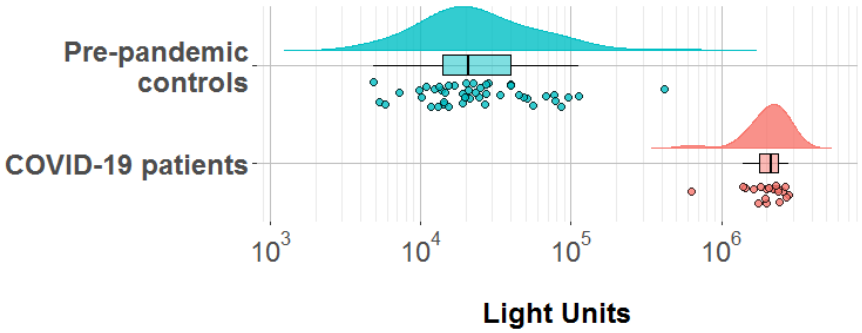
Supplemental Figure 3

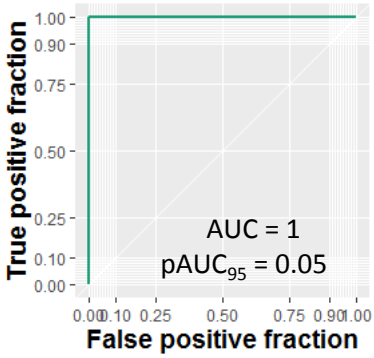


Alternative SARS-CoV-2 spike protein RBD antigens - IgG antibody responses in COVID-19 and control sera and LIPS performance. Raw data (Light Units) measured after LIPS using the shown recombinant RBD antigens. In the left panels are shown the probability density estimates, boxplot and individual measurement in COVID-19 patient (magenta circle) or control (light blue circle) sera. The right panels show the corresponding ROC curves

Supplemental Figure 4

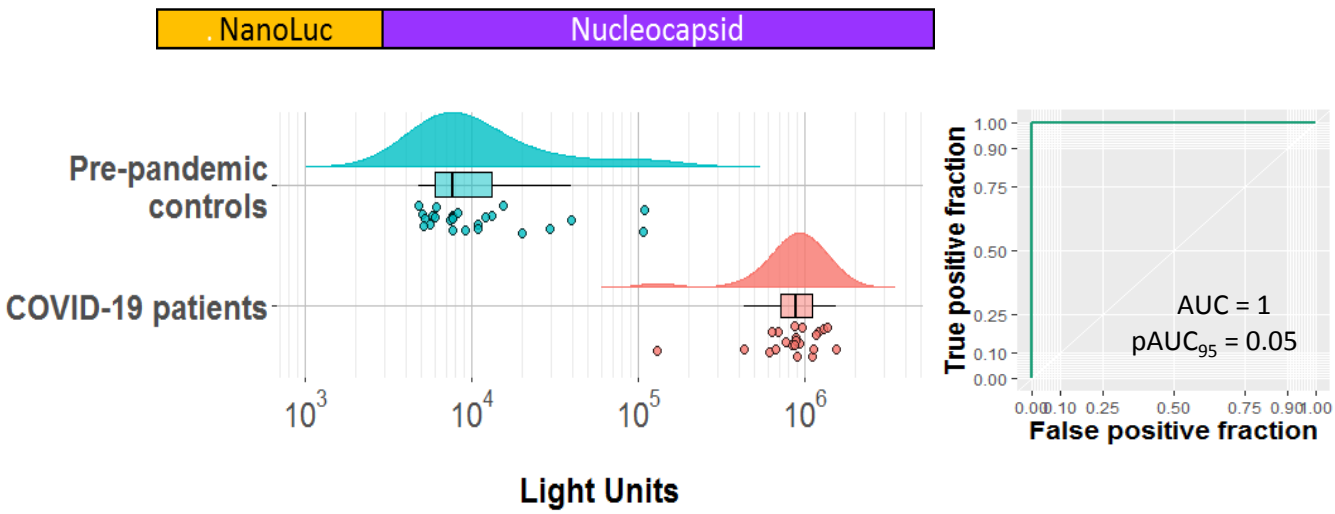




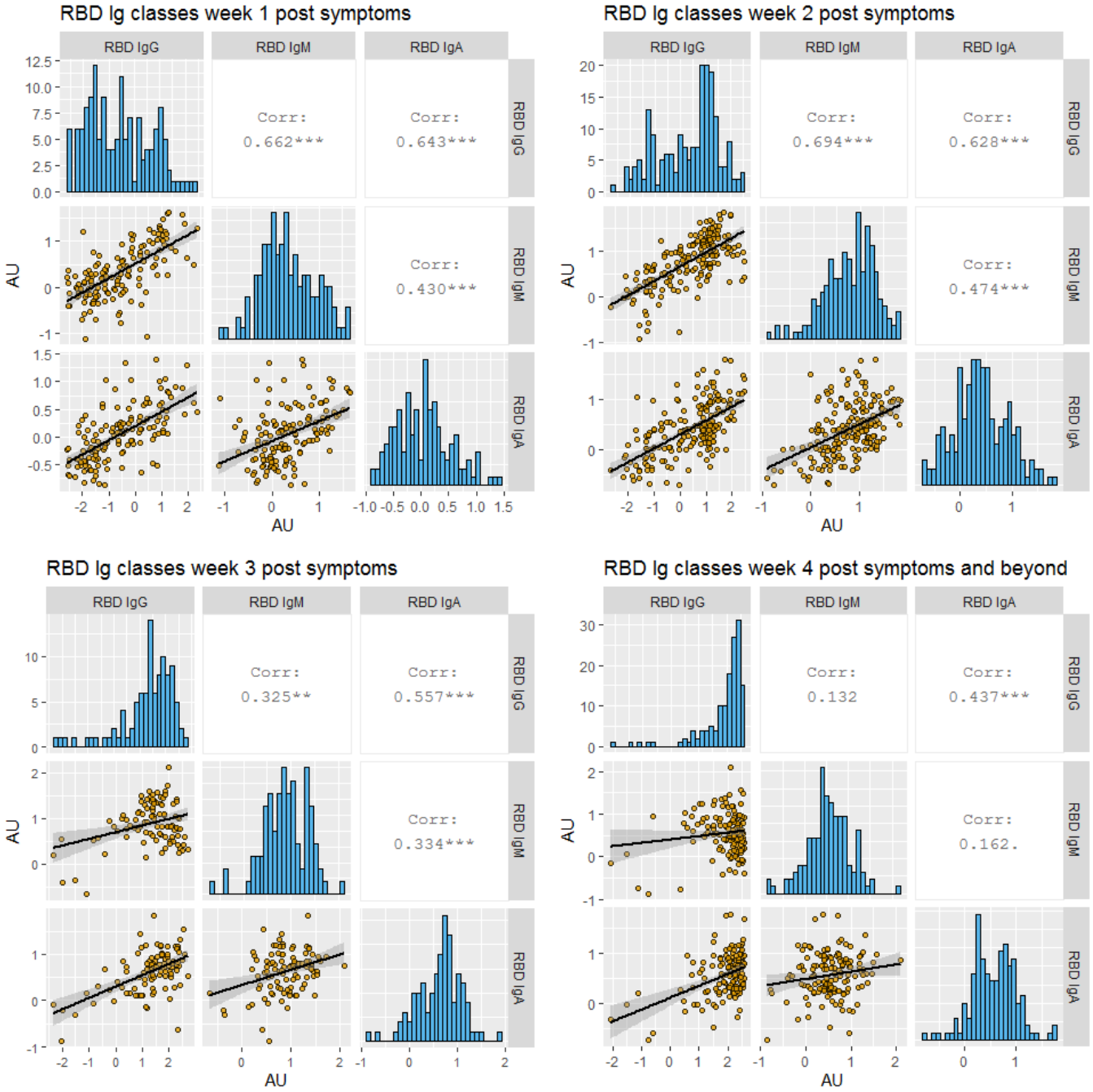


SARS-CoV-2 spike S1+S2 antigen - IgG antibody responses in COVID-19 and control sera and LIPS performance. Raw data (Light Units) measured after LIPS using the shown recombinant S1+S2 antigen. In the left panel are shown the probability density estimates, boxplot and individual measurement in COVID-19 patient (magenta circles) or control (light blue circles) sera. The right panels show the corresponding ROC curve.

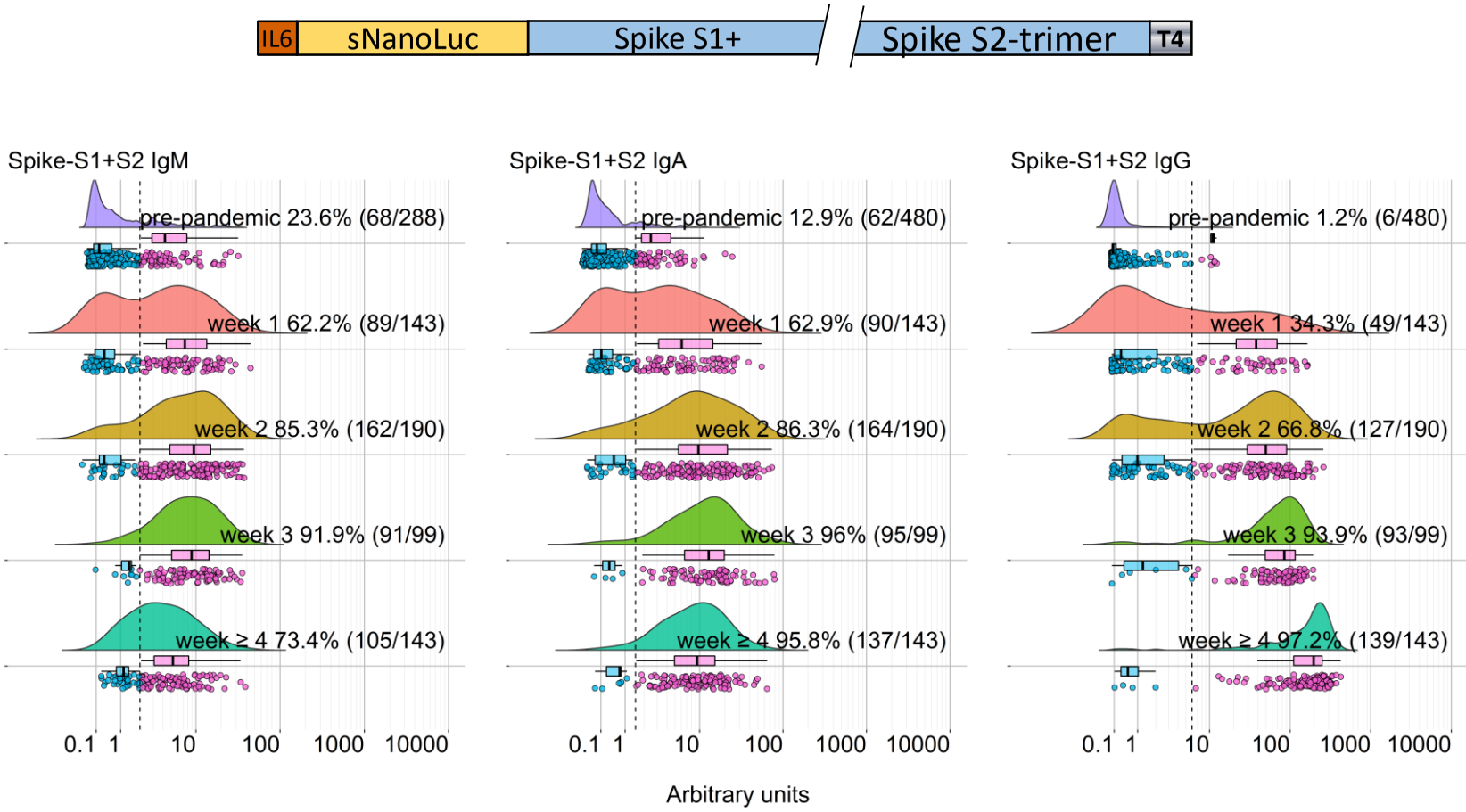
Supplemental Figure 5



SARS-CoV-2 Nucleocapsid protein - IgG antibody responses in COVID-19 and control sera and LIPS performance. Raw data (Light Units) measured after LIPS using the shown recombinant Nucleocapsid antigen. In the left panel are shown the probability density estimates, boxplot and individual measurement in COVID-19 patient (magenta circles) or control (light blue circles) sera. The right panels show the corresponding ROC curve.



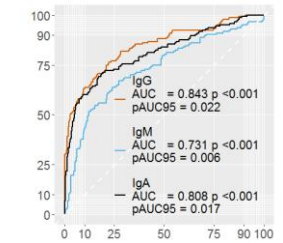
Correlation plots of anti-SARS-CoV2 spike RBD antibodies of IgG, IgM, IgA class. *COVID-19 sera collected at the indicated time points from symptoms onset were measured by the LIPS indicated in grey labels above each row/column. Boxes under the diagonal show each correlation plot of arbitrary units after log10 conversion. Orange circles correspond to individual measurements, the black line represents the regression line and the grey area its 95% CI. Boxes on the diagonal show as histograms the distribution of arbitrary units in each assay. Boxes above the diagonal show the corresponding Pearson correlation analysis coefficients.*

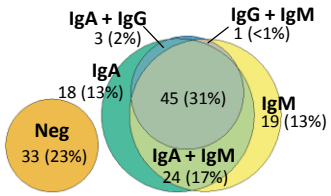


Kinetics of anti-SARS-CoV-2 spike S1+S2 antibody development in COVID-19: *results are stratified by the symptom duration (weeks 1, 2, 3, ≥ 4) at serum sampling and by immunoglobulin class in COVID-19 patients and control sera. For each assay and time point are shown the percentage and count of Ab positive among the indicated number of tested patients, the arbitrary units measured in each sample (circles), their probability density estimate and a boxplot showing median, IQR, whiskers extending to 1.96 times the median. Circle and boxplot fill colour correspond to an antibody positive (magenta) or negative (light blue) score.*

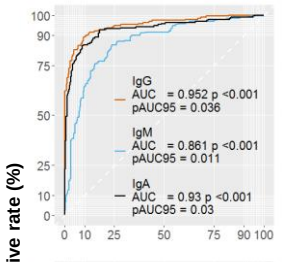
Supplemental Figure 7

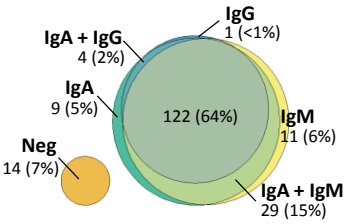
Supplemental Figure 8



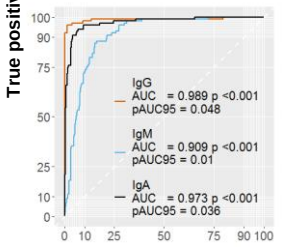


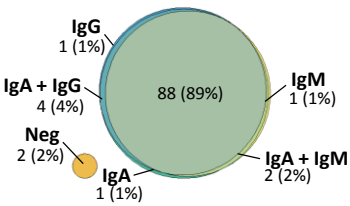
anti SARS-CoV-2 S1+S2 week 1 post symptoms onset				
Ab class	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG	34 (27-43)	99 (97-100)	89 (78-96)	83 (80-86)
IgM	62 (54-70)	76 (71-81)	57 (49-65)	80 (75-85)
IgA	63 (54-71)	87 (84-90)	59 (51-67)	89 (86-91)



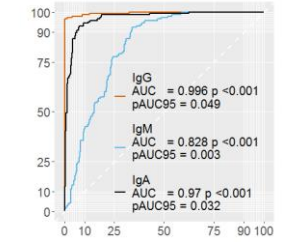


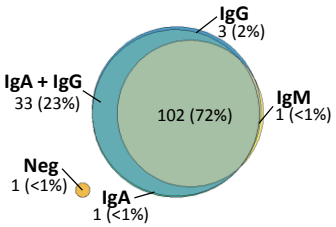
anti SARS-CoV-2 S1+S2 week 2 post symptoms onset				
Ab class	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG	67 (60-73)	99 (97-100)	95 (90-98)	88 (85-91)
IgM	85 (79-90)	76 (71-81)	70 (64-76)	89 (84-92)
IgA	86 (81-91)	87 (84-90)	73 (66-78)	94 (92-96)





anti SARS-CoV-2 S1+S2 week 3 post symptoms onset				
Ab class	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG	94 (87-98)	99 (97-100)	94 (87-98)	99 (97-100)
IgM	92 (85-96)	76 (71-81)	57 (49-65)	96 (93-98)
IgA	96 (90-99)	87 (84-90)	61 (52-68)	99 (98-100)

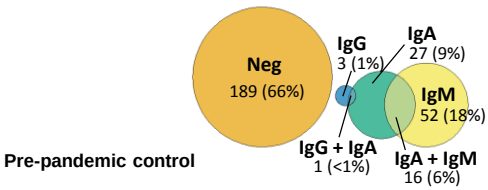




anti SARS-CoV-2 S1+S2 week 4 and beyond post symptoms onset				
Ab class	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG	97 (93-99)	99 (97-100)	96 (91-98)	99 (98-100)
IgM	73 (65-80)	76 (71-81)	61 (53-68)	85 (80-89)
IgA	96 (91-98)	87 (84-90)	69 (62-75)	99 (97-99)

True positive rate (%)

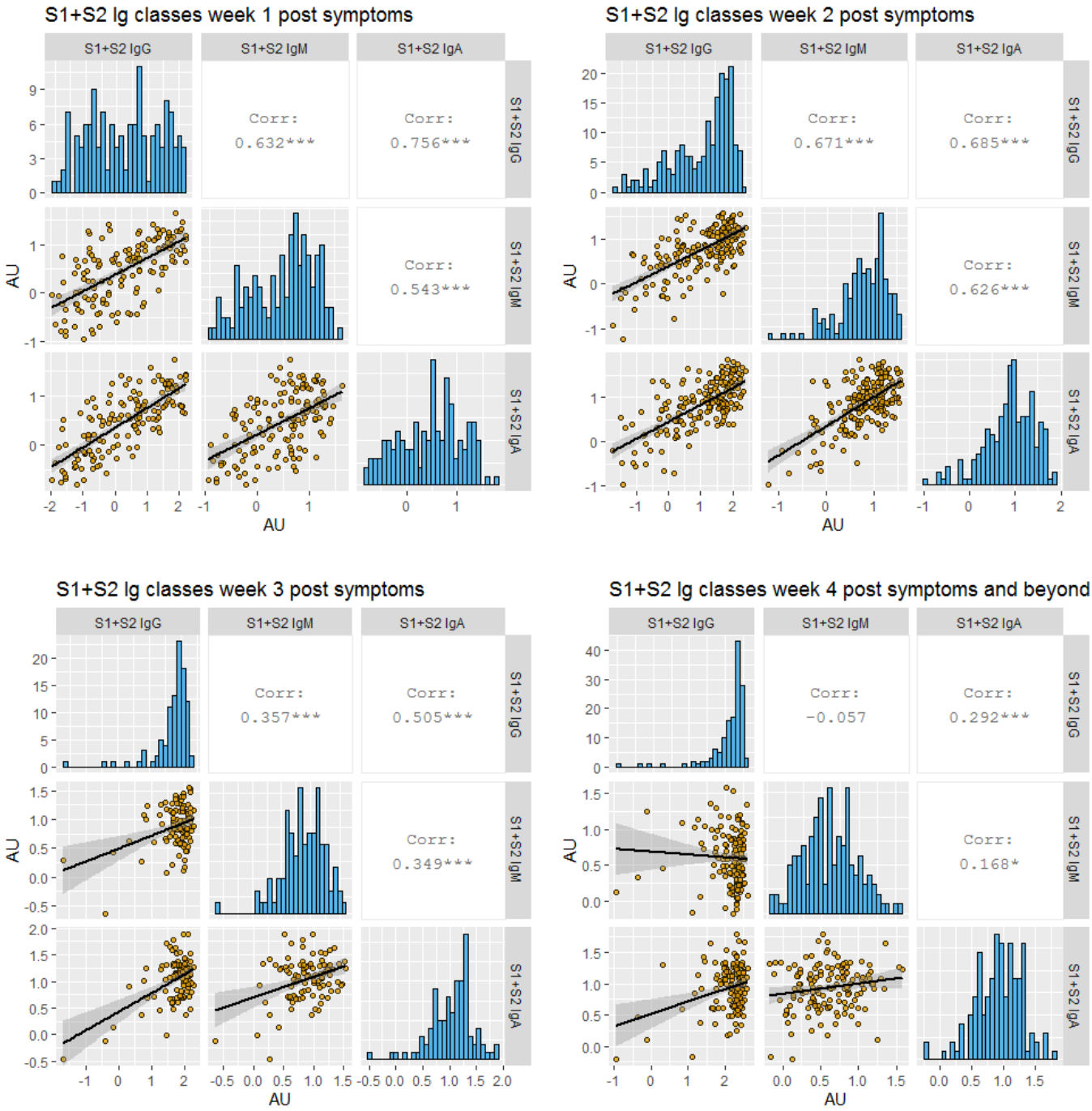
False positive rate (%)



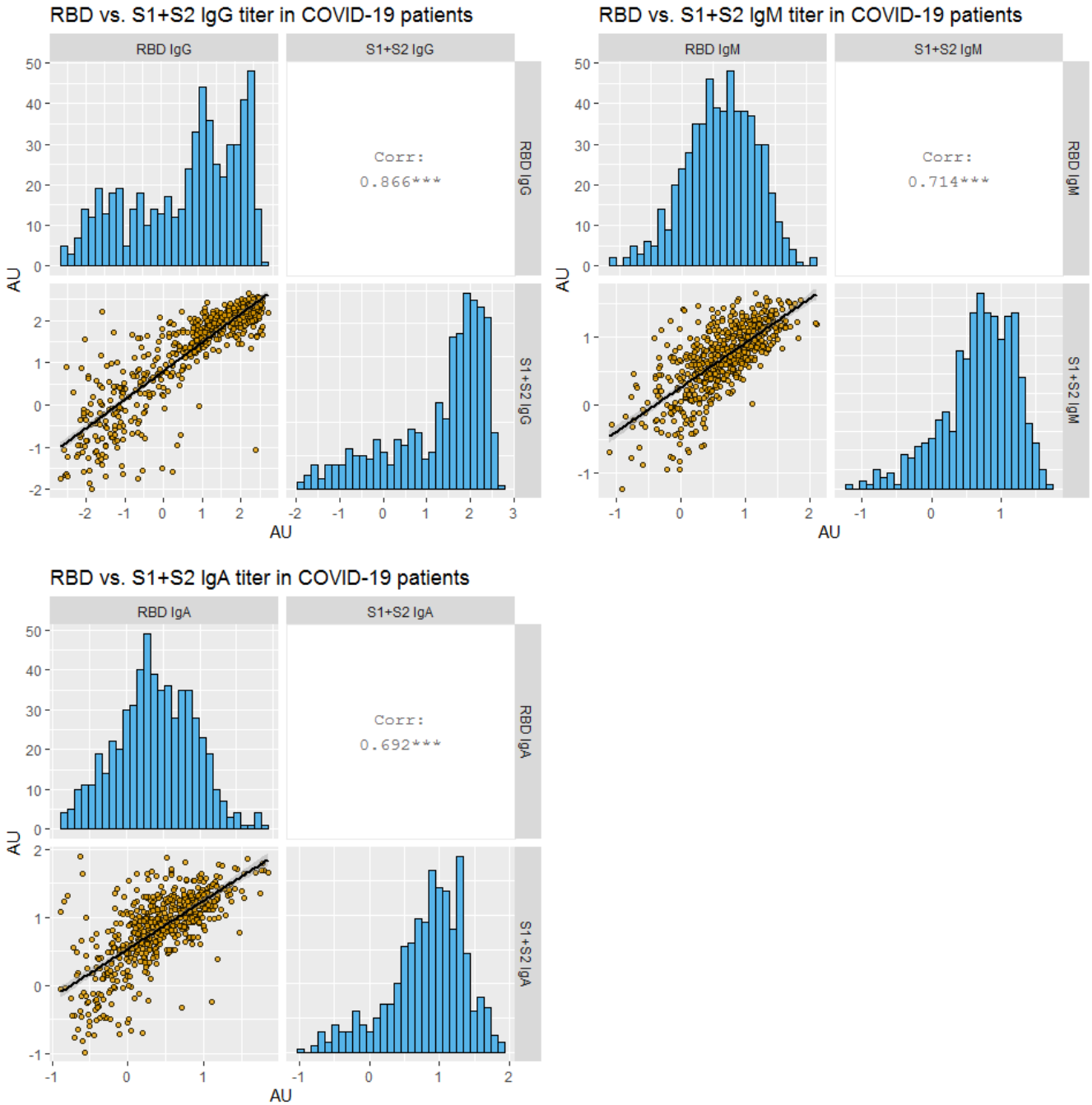
Pre-pandemic control

Performance of the SARS-CoV-2 spike S1+S2 LIPS in COVID-19 after patient stratification by symptoms duration at serum sampling. *Left panels: ROC curve analysis of SARS-CoV-2 spike S1+S2 assays measuring either IgG or IgM or IgA at 1 to ≥ 4 weeks post symptoms onset. Shown are the total ROC-AUC and its p value and the pAUC95. Middle panels: Venn diagrams of anti-spike S1+S2 antibody positive or negative score combinations for different immunoglobulin classes at the same time points in samples tested for all 3 subclasses. Right panels: sensitivity, specificity, positive and negative predictive values of the corresponding LIPS assays for each immunoglobulin class*

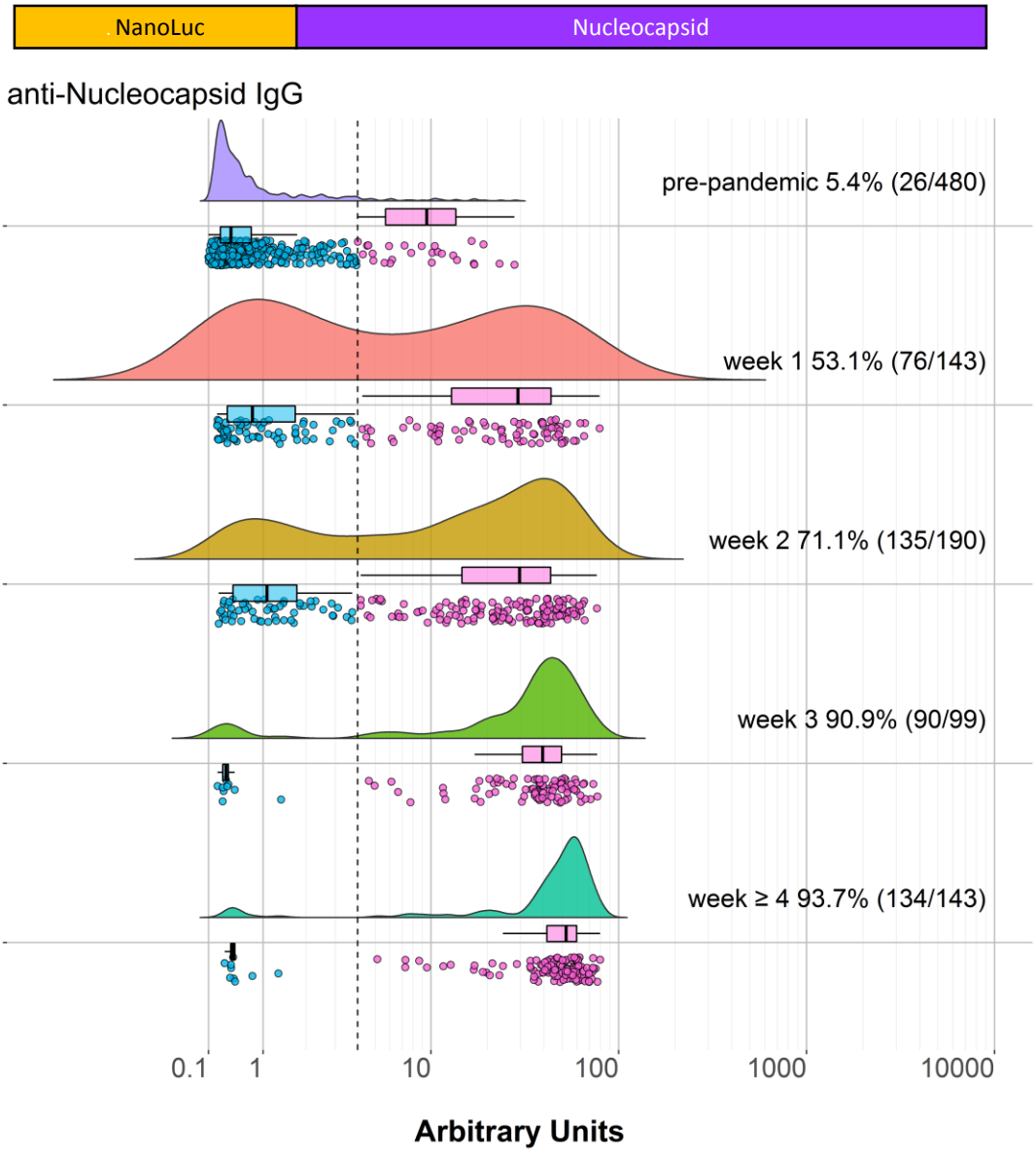
Supplemental Figure 9



Correlation plots of anti-SARS-CoV2 spike S1+S2 antibodies of IgG, IgM, IgA class. *COVID-19 sera collected at the indicated time points from symptoms onset were measured by the LIPS indicated in grey labels above each row/column. Boxes under the diagonal show each correlation plot of arbitrary units after log10 transformation. Orange circles correspond to individual measurements, the black line represents the regression line and the grey area its 95% CI. Boxes on the diagonal show as histograms the distribution of arbitrary units in each assay. Boxes above the diagonal show the corresponding Pearson correlation analysis coefficients.*

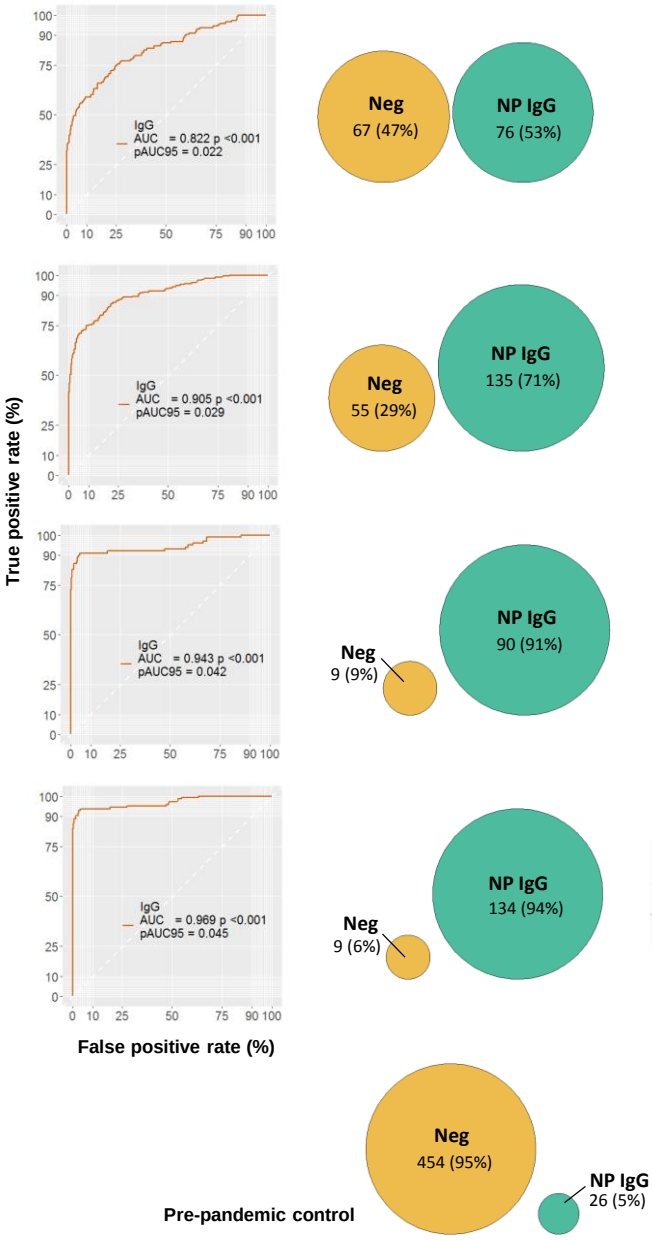


Correlation plots of anti-SARS-CoV2 spike S1+S2 and anti RBD IgG antibodies of IgG, IgM, IgA class. *Antibodies were measured in COVID-19 sera by the LIPS indicated in grey labels above each row/column. Boxes under the diagonal show each correlation plot of arbitrary units after log10 transformation. Orange circles correspond to individual measurements, the black line represents the regression line and the grey area its 95% CI. Boxes on the diagonal show as histograms the distribution of arbitrary units in each assay. Boxes above the diagonal show the corresponding Pearson correlation analysis coefficients.*



Kinetics of anti-SARS-CoV-2 Nucleocapsid IgG antibody development in COVID-19: *patients were stratified by the indicated symptoms duration at serum sampling. The results are shown as the arbitrary units measured in each sample (circles), their probability density estimate and a boxplot showing median, IQR with whiskers extending to 1.96 times the median and outlier omission. Circle and boxplot fill color corresponds to an antibody positive (magenta fill) or negative (light blue circle) score.*

Supplemental Figure 12



IgG anti SARS-CoV-2 Nucleocapsid in COVID-19 patients week 1 post symptoms onset

Ab class	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG	53 (45-62)	95 (92-96)	75 (65-83)	87 (84-90)

IgG anti SARS-CoV-2 Nucleocapsid in COVID-19 patients week 2 post symptoms onset

Ab class	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG	71 (64-77)	95 (92-96)	84 (77-89)	89 (86-92)

IgG anti SARS-CoV-2 Nucleocapsid in COVID-19 patients week 3 post symptoms onset

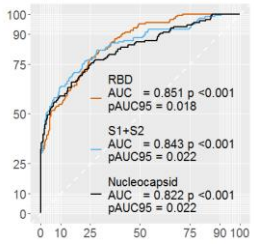
Ab class	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG	91 (83-96)	95 (92-96)	78 (69-85)	98 (96-99)

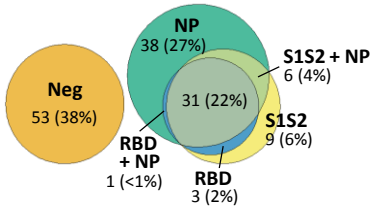
IgG anti SARS-CoV-2 Nucleocapsid in COVID-19 patients week 4 and beyond post symptoms onset

Ab class	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG	94 (88-97)	95 (92-96)	84 (77-89)	98 (96-99)

Performance of the SARS-CoV-2 Nucleocapsid (NP) IgG LIPS in COVID-19 after patient stratification by symptoms duration at serum sampling. *Left panels: ROC curve analysis of the anti-NP IgG LIPS at different weeks post symptoms onset. Shown are the values of total ROC-AUC and pAUC95. Middle panels: Venn diagrams of anti-NP IgG antibody positive and negative scores in the same patients. Right panels, sensitivity, specificity, positive and negative predictive value of the anti-NP IgG LIPS at the same time points*

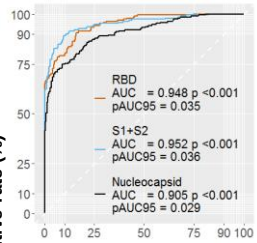
Supplemental Figure 13

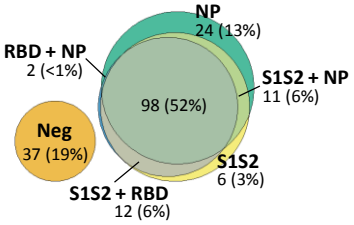




IgG anti-SARS-CoV-2 Ags in COVID-19 patients week 1 post symptoms onset

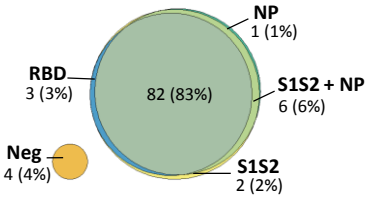
Antigen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RBD	26 (19-34)	100 (99-100)	100 (91-100)	82 (79-85)
S1+S2	34 (27-43)	99 (97-100)	89 (78-96)	83 (80-86)
Nucleocapsid	53 (45-62)	95 (92-96)	75 (65-83)	87 (84-90)





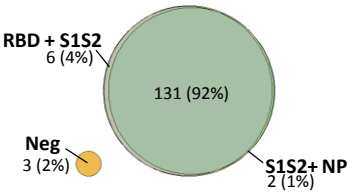
IgG anti-SARS-CoV-2 Ags in COVID-19 patients week 2 post symptoms onset

Antigen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RBD	59 (52-66)	100 (99-100)	100 (97-100)	86 (83-89)
S1+S2	67 (60-73)	99 (97-100)	95 (90-98)	88 (85-91)
Nucleocapsid	71 (64-77)	95 (92-96)	84 (77-89)	89 (86-92)



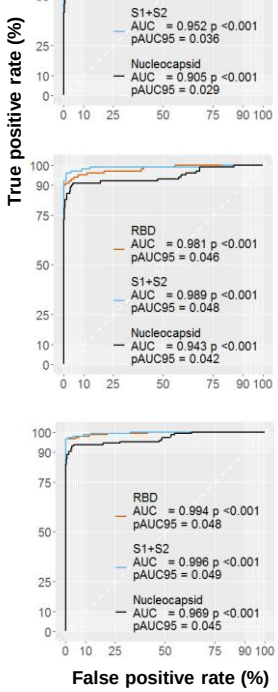
IgG anti-SARS-CoV-2 Ags in COVID-19 patients week 3 post symptoms onset

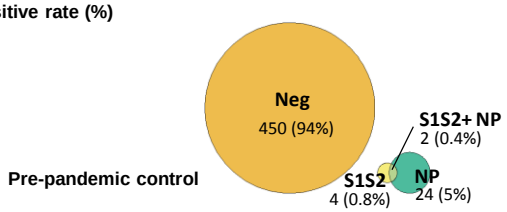
Antigen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RBD	87 (79-93)	100 (99-100)	100 (96-100)	97 (96-99)
S1+S2	94 (87-98)	99 (97-100)	94 (87-98)	99 (97-100)
Nucleocapsid	91 (83-96)	95 (92-96)	78 (69-85)	98 (96-99)



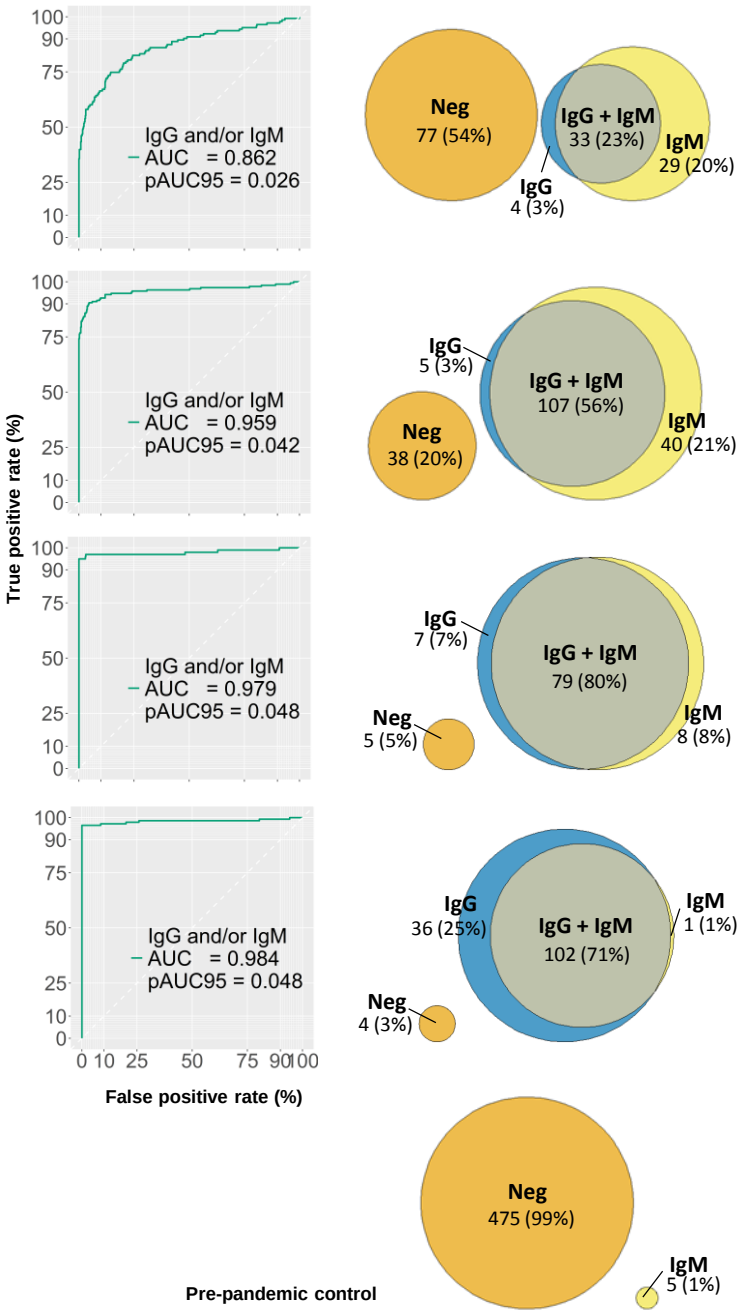
IgG anti-SARS-CoV-2 Ags in COVID-19 patients week 4 and beyond post symptoms onset

Antigen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RBD	97 (92-99)	100 (99-100)	100 (97-100)	99 (98-100)
S1+S2	97 (93-99)	99 (97-100)	96 (91-98)	99 (98-100)
Nucleocapsid	94 (88-97)	95 (92-96)	84 (77-89)	98 (96-99)





Performance of the SARS-CoV-2 spike S1+S2, spike RBD, Nucleocapsid IgG LIPS combination in COVID-19 after patient stratification by symptoms duration at serum sampling. *Left panels: ROC curve analysis of SARS-CoV-2 LIPS assays measuring IgG to each Ag at 1 to ≥ 4 weeks post symptoms onset. Shown are the total ROC-AUC and its p value and the pAUC95. Middle panels: Venn diagrams of SARS-CoV2 antibody positive or negative score combinations for different antibodies at the same time points. Right panels: sensitivity, specificity, positive and negative predictive values of the corresponding LIPS assays*



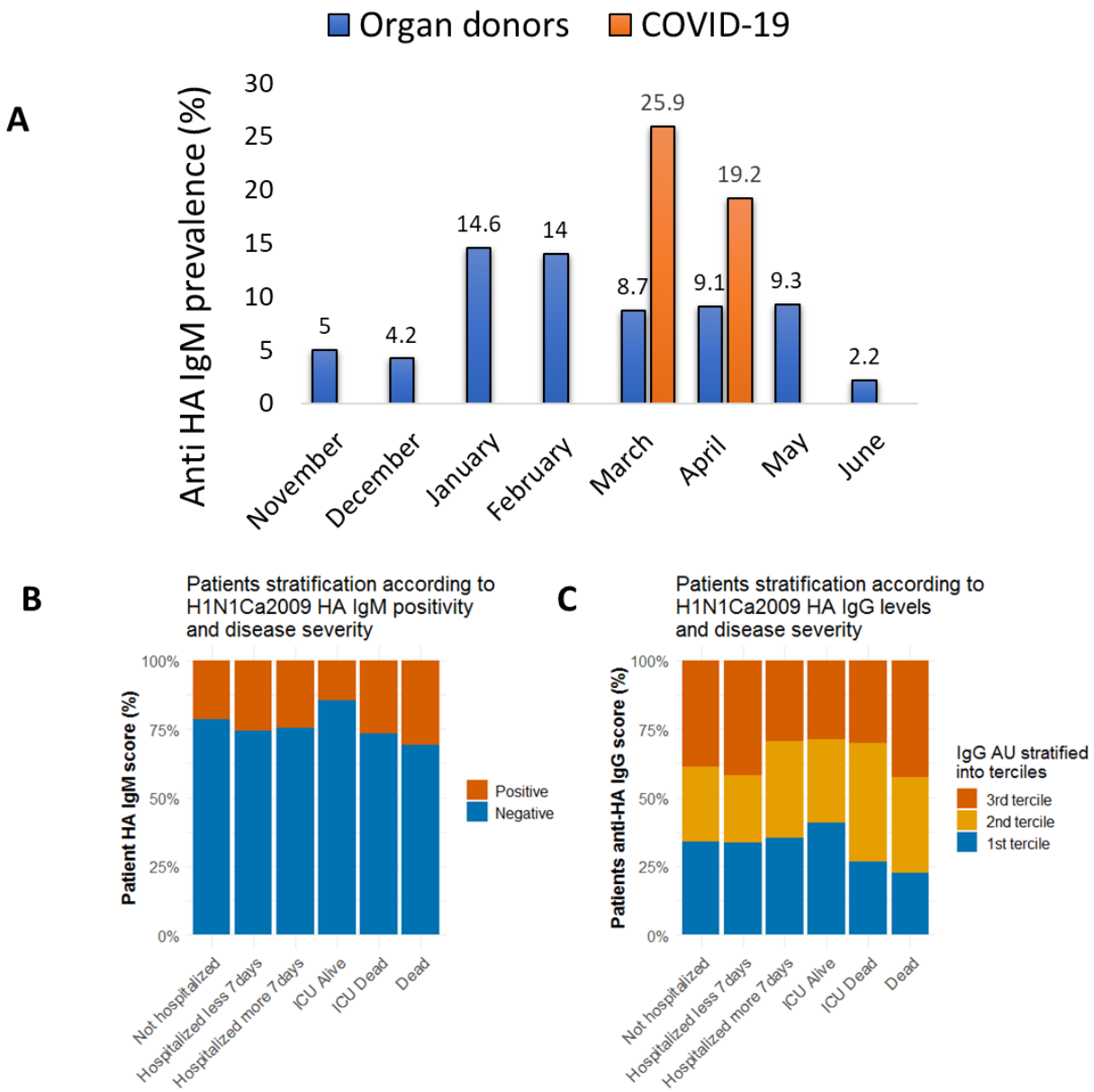
week 1	
parameter	**% (95 c.i.)**
Sensitivity	48 (40-57)
Specificity	99 (97-99)
ppv	91 (82-96)
npv	86 (83-89)

week 2	
parameter	**% (95 c.i.)**
Sensitivity	83 (76-88)
Specificity	99 (97-99)
ppv	96 (91-98)
npv	93 (91-95)

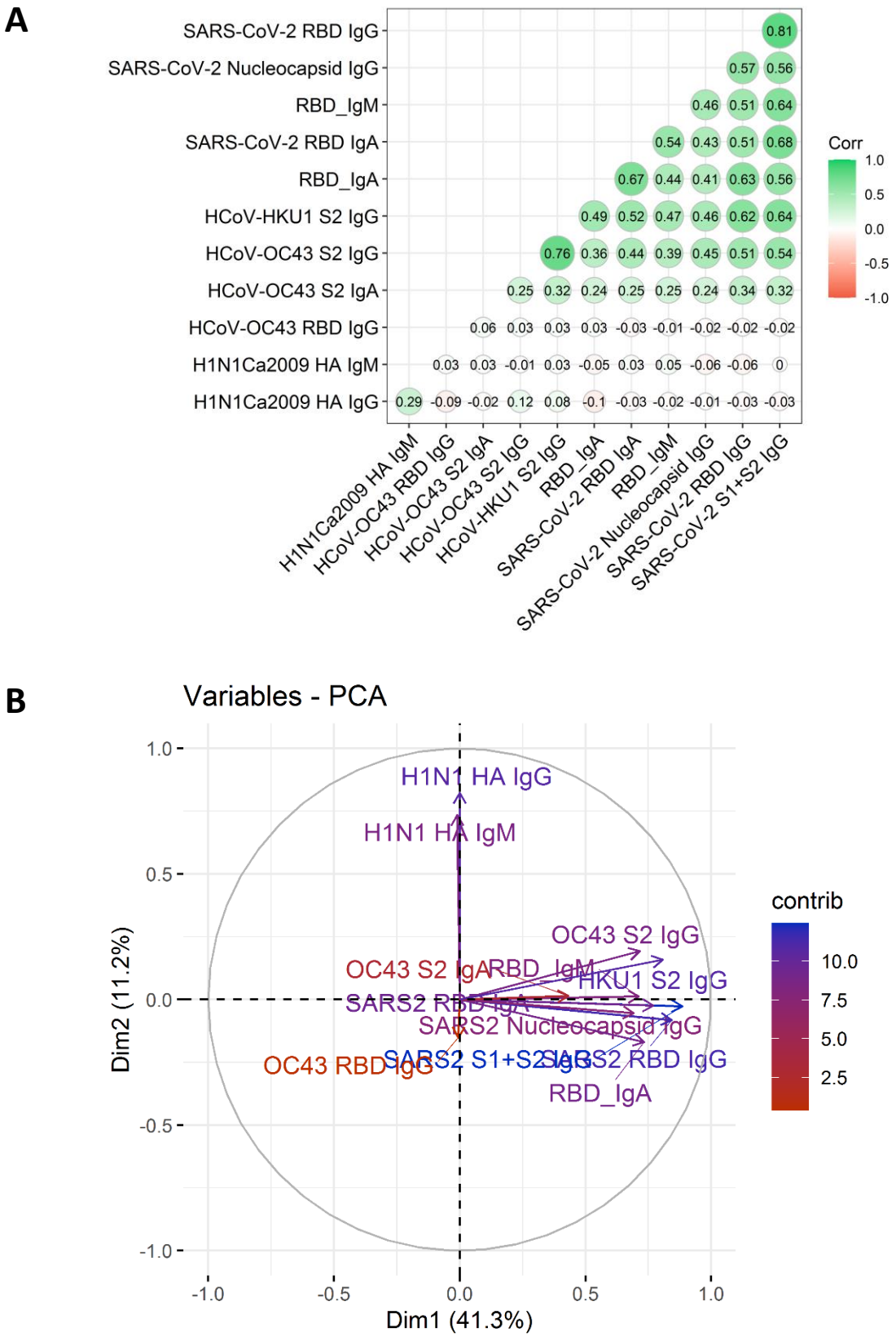
week 3	
parameter	**% (95 c.i.)**
Sensitivity	95 (89-98)
Specificity	99 (97-99)
ppv	93 (86-97)
npv	99 (98-100)

week 4	
parameter	**% (95 c.i.)**
Sensitivity	97 (93-99)
Specificity	99 (97-99)
ppv	95 (90-98)
npv	99 (98-100)

Performance of the SARS-CoV-2 spike RBD IgG and IgM LIPS combined in COVID-19 after patient stratification by symptoms duration at serum sampling. *Left panels: ROC curve analysis of SARS-CoV-2 spike RDB LIPS assays measuring IgG or IgM at 1 to ≥ 4 weeks post symptoms onset. Shown are the total ROC-AUC and its p value and the pAUC95. Middle panels: Venn diagrams of SARS-CoV2 spike RDB antibody positive or negative score combinations for different antibodies at the same time points. Right panels: sensitivity, specificity, positive and negative predictive values of the two assays combination*

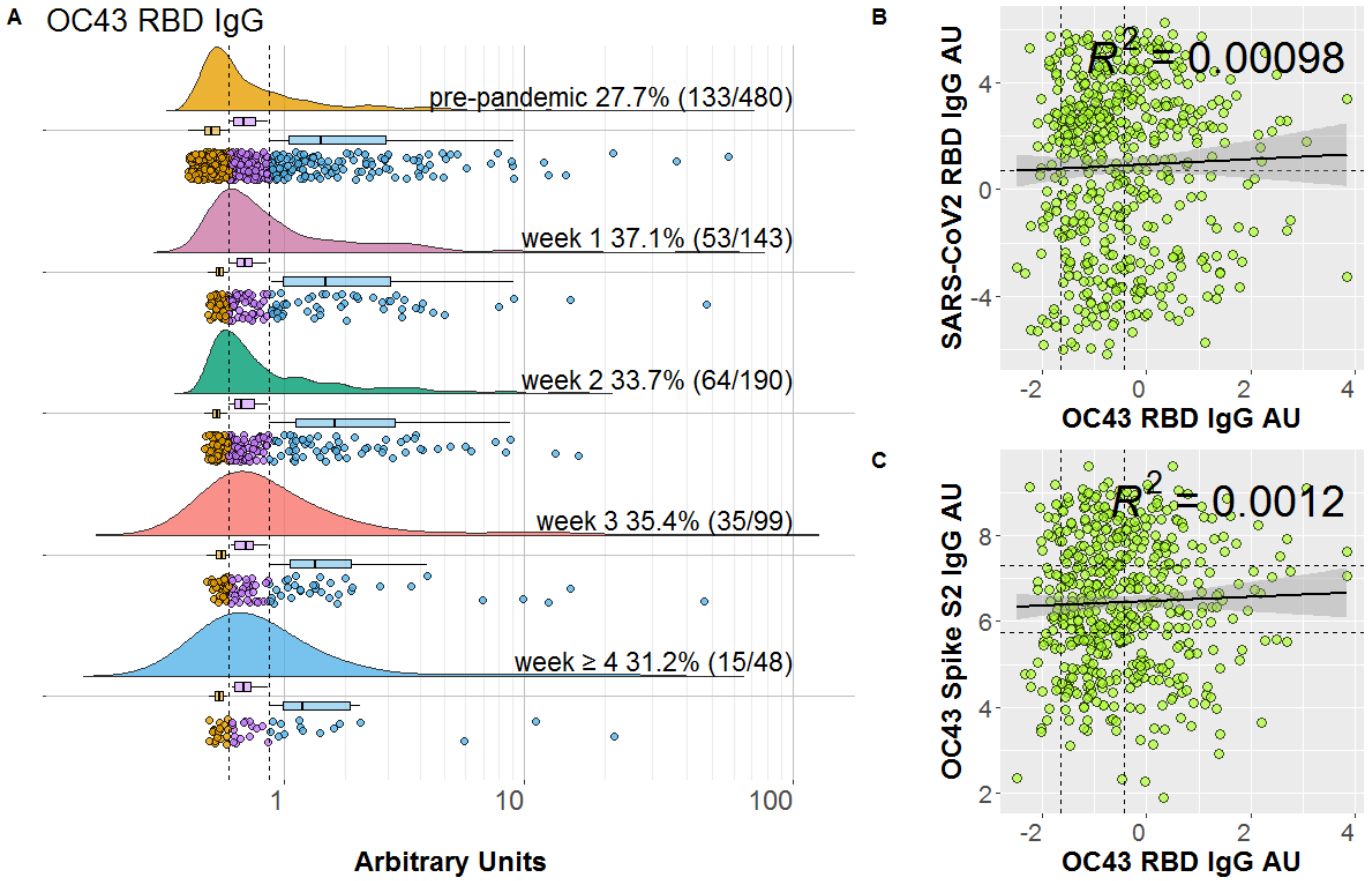


Antibody response to influenza (A/H1N1) hemagglutinin in COVID-19. *Panel A: Prevalence of IgM positivity to H1N1Ca2009 hemagglutinin in confirmed COVID-19 (n=509) and pre-pandemic control(n=469) according to the month of sampling. Panel B. Percentage of positive anti-HA IgM responses and the percentage of patients divided into terciles of anti-HA IgG responses are represented stratified into six groups based on increasing disease severity: discharged without hospitalization (57 out of 509, 11.2%), hospitalized [≤7days, 78 out of 509 (15.3%); >7days 232 out of 509 (45.6%)], hospitalized and admitted to ICU [alive, 49 out of 509 (9.6%); dead, 30 out of 509 (5.9%)], hospitalized and dead (63 out of 509, 12.4%).*



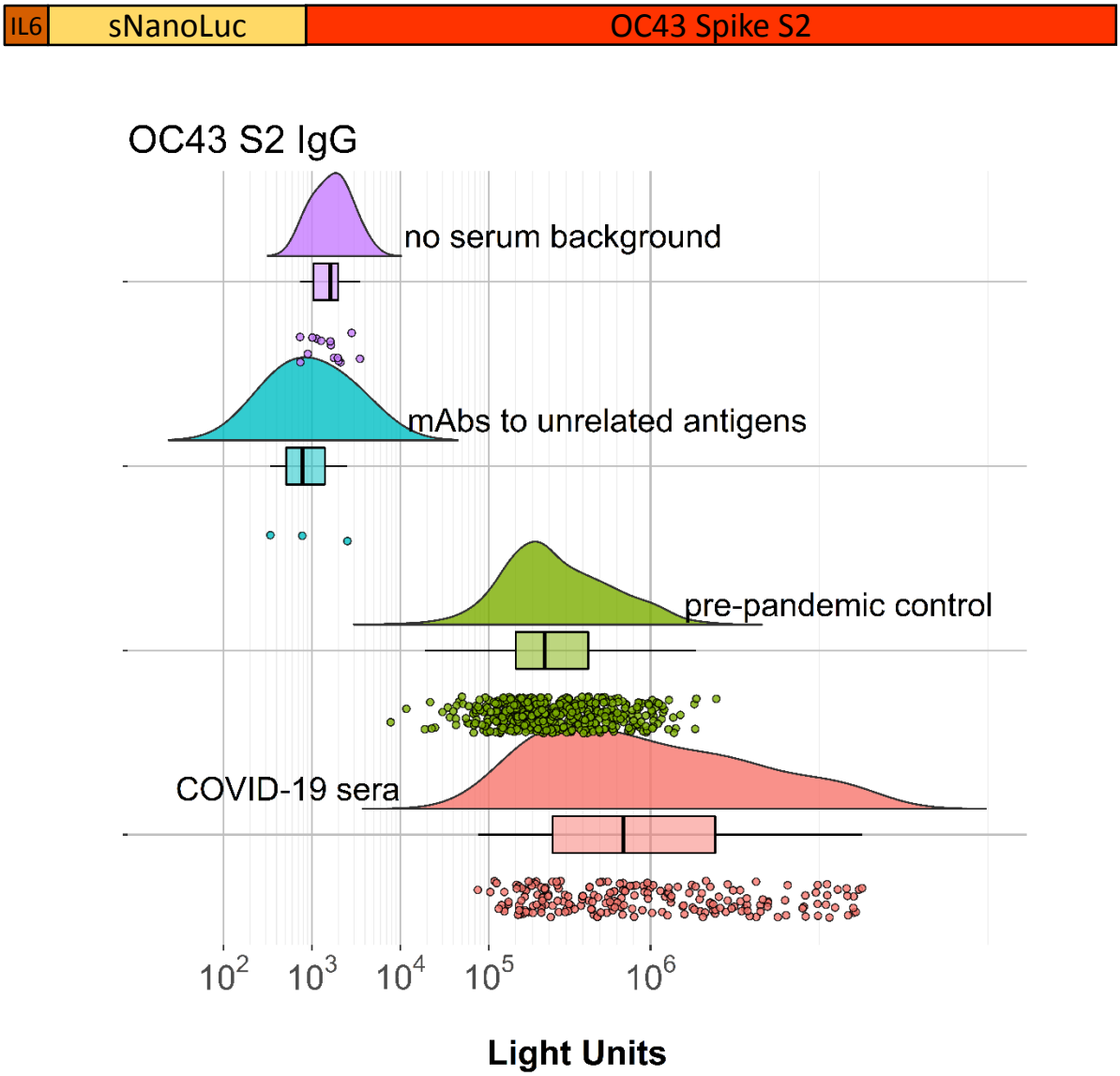
Correlation and PCA analysis of antibody responses in COVID-19. *Panel A: Correloplot of antibody responses. The circle fill colour is proportional to the shown regression coefficient of the respective combination. Panel B: Principal Component Analysis (PCA) of antibody responses. The colour scale of the components represents their relative contribution to the PCA.*

Supplemental Figure 17



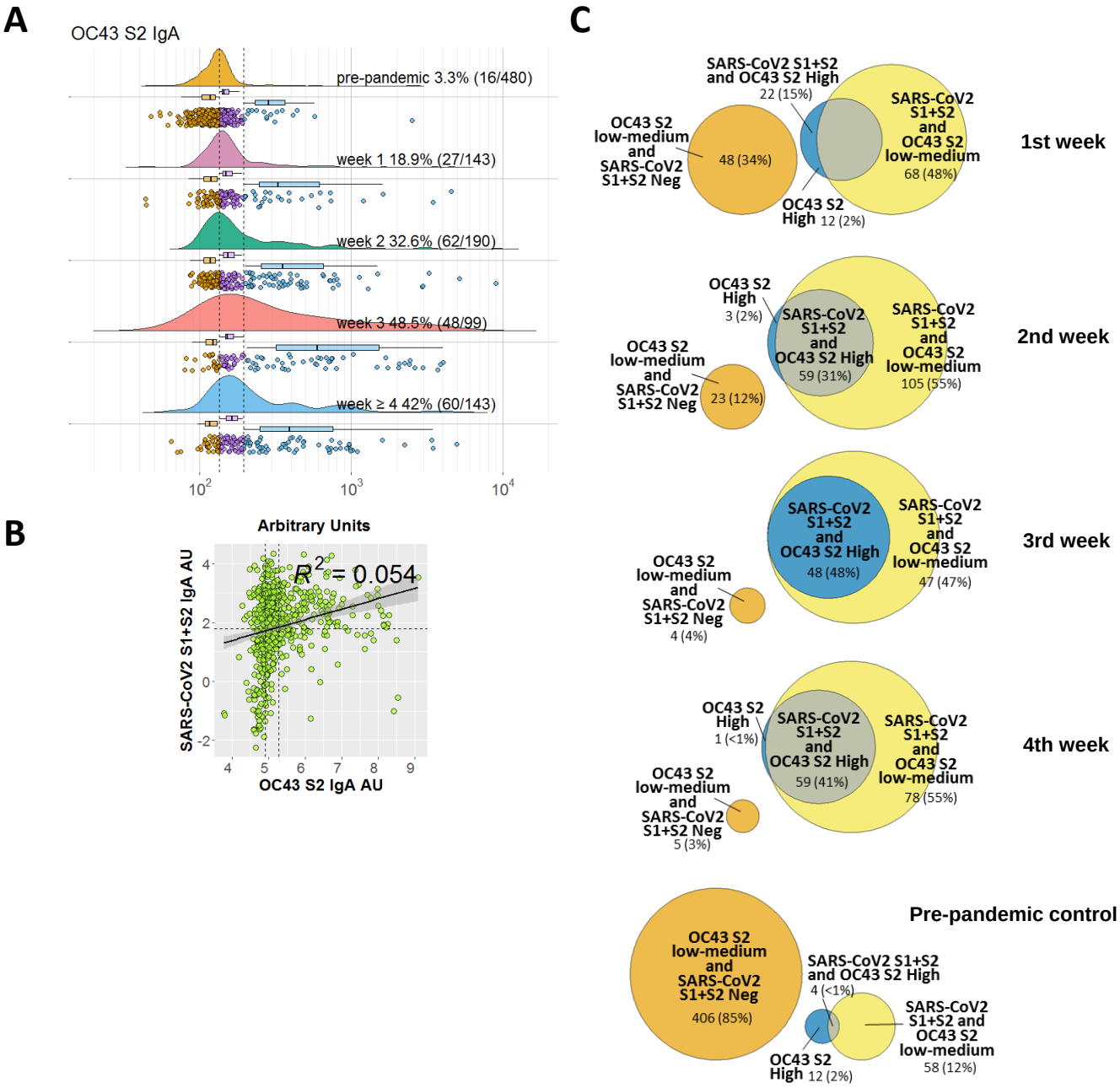
Anti-OC43 Spike RBD IgG antibodies in COVID-19. *Panels A: Kinetics of anti-OC43 spike RBD IgG antibodies in COVID-19 stratified by the indicated symptoms duration at serum sampling. IgG titre was grouped according to terciles in COVID19 patients. The results show the arbitrary units measured in each sample (circles), their probability density estimate and boxplot (median, IQR, and whiskers extending to 1.5 times the IQR), and the prevalence of high titre subjects. Circle and boxplot fill color corresponds to high (light blue fill), medium (purple circle), low (orange fill) titre. Panel B: Correlation of OC43 spike RBD and SARS-CoV-2 RBD IgG in COVID-19 sera. Panel C: Correlation of OC43 spike RBD and spike S2 IgG in COVID-19 sera. The black line and grey area stand for the regression line and its 95% c.i.; shown are the respective regression coefficients.*

Supplemental Figure 18



HCoV-OC43 spike S2 antigen - IgG antibody responses in COVID-19 and control sera. Raw data (Light Units) measured after LIPS using the shown recombinant S2 antigen. Shown are the probability density estimates, boxplot and individual measurement in COVID-19 patient (magenta circles), pre-pandemic control (light blue circles) sera, mAbs to three irrelevant antigens (2 against insulin, 1 against the IA-2 type 1 diabetes autoantigen), and measurements in wells without serum.

Supplemental Figure 19



Anti-HCoV-OC43 spike S2 IgA antibodies in COVID-19. *Panel A: Kinetics of anti-HCoV-OC43 spike S2 IgA antibodies expansion in COVID-19stratified by the indicated symptoms duration at serum sampling. The results show the arbitrary units measured in each sample (circles), their probability density estimate and boxplot (median, IQR, and whiskers extending to 1.5 times the IQR) and the prevalence of high titre subjects. IgG titre was grouped according to terciles in COVID19 patients, circle and boxplot fill color corresponds to high (light blue fill), medium (purple circle), low (orange fill) titre. Panel B: Correlation of OC43 spike S2 and SARS-CoV-2 S1+S2 IgA in COVID-19 sera. The black line and grey area stand for the regression line and its 95% CI; shown is the regression coefficient. Panel C: Venn diagrams of anti-HCoV-OC43 S2 IgA antibodies (grouped into high and medium-low titre) and anti-SARS-CoV-2 S1+S2 antibodies (score as positive or negative).*

ESM TABLES

ESM Table 1A - Baseline characteristics of the study subjects

	Symptomatic COVID-19 (n=509)			Paucisymptomatic COVID-19 (n=8)			Organ donors (n=480)		
	median (IQR)	%	NA[§]	median (IQR)	%	NA[§]	median (IQR)	%	NA[§]
Age (years)	63 (54-75)		0	53.5 (39.5-62)		0	60 (47-71)		0
Sex Male		66.2	0		50.0	0		54.8	0
Body Mass Index	26.8 (24.2-30.4)		127	24.9 (19.4-28.7)		0	25.2 (23.4-27.7)		3
Ethnicity			0			0			
Caucasian		84.1			100				
Hispanic		10.0			0				
Asian		2.8			0				
African		3.1			0				
Median time from symptoms			29			0			
to last FU	99 (93-103)								
to admission	7 (4.5-10)								
to blood sampling:	10 (7-16)								
Days ≤7		29.6			0				
Days 8-14		39.8			0				
Days 15-21		20.6			0				
Days>21		10.0			100				
Median time from admission									
to last FU (CI 95%)	59 (58-60)		0		no admission				
N of comorbidities	1 (0-2)		5	1 (0-2)		0			
None		41.3			100				
1		29.2			0				
2		17.9			0				
3		8.5			0				
4		2.8			0				
≥5		0.4			0				
Comorbidities°			5			0			
Hypertension		45.7			0				
CAD		11.5			0				
T1D/T2D		0.8/15.4			0				
COPD		5.5			0				
CKD		10.7			0				
Cancer		9.9			0				
ND		4.6			0				
Symptoms at onset			0			0			
General									
Fever		87.4			50.0				
Fatigue/Malaise		8.3			87.5				
Myalgia/Arthralgia		5.6			75.0				
Respiratory		82.2							
Cough		49.7			62.5				
Dyspnea		63.1			0				
Sore Throat		1.7			62.5				
Chest Pain		6.3			12.5				
Gastrointestinal (any)		15.3							
Diarrhea		8.9			75.0				
Vomiting/Nausea		7.7			25.0				
Abdominal Pain		2.5			12.5				
Others									
Headache		5.9			25.0				
Conjunctivitis		0.4			12.5				
Hypo/Anosmia		3.9			50.0				
Hypo/dysgeusia		3.7			50.0				
Skin Rash		0.8			0				

[§] **NA:** Missing values

°COPD: Chronic obstructive pulmonary disease; **ND:** Neurodegenerative disease; **T1D/T2D:** type 1/type 2 diabetes; **CAD:** coronary artery diseases; **CKD:** Chronic Kidney disease.

ESM Table 1B - Baseline characteristics of the study subjects

	Symptomatic COVID-19 (n=509)			Paucisymptomatic COVID-19 (n=8)			Organ donors (n=480)		
	median (IQR)	%	NA[§]	median (IQR)	%	NA[§]	median (IQR)	%	NA[§]
Laboratory at blood sampling°									
WBC, $x10^9$/L	7.0 (5.2-9.7)		22						
Lymph count, $x10^9$/L	1.0 (0.7-1.4)		46						
Neu count, $x10^9$/L	5.1 (3.5-7.9)		46						
Mo count, $x10^9$/L	0.5 (0.3-0.7)		46						
N/Ly ratio	5.3 (2.6-8.5)		46						
HGB, g/dl	13.1 (11.6-14.3)		22						
PLT count, $x10^9$/L	235 (178-318)		22						
Bil, mg/dl	0.56 (0.36-0.88)		103						
ALT, U/L	37.0 (24-62)		67						
AST, U/L	44.5 (31-65)		63						
Crea, mg/dl	0.98 (0.78-1.26)		31						
LDH, U/L	361 (273-467)		74						
CRP, mg/L	68.9 (21-137)		25						
D-Dimer, µg/mL	1.1 (0.51-2.1)		274						
IL-6, pg/ml	39.2 (18-89.3)		352						
Ferritin, ng/mL	1149 (566-1870)		314						
PaO2/Fi02 ratio (mmHg)	200 (111-323)		380						
Antibodies°°									
SARS-CoV-2									
IgG RBD	5.9	58.3	0	58.5	100	0	0.06	0.0	0
IgM RBD	4.6	68.8	0				0.14	1.0	0
IgA RBD	1.9	61.1	0				0.06	2.9	0
IgG S1/S2	32.3	65.4	0	138	100	0	0.04	1.3	192
IgM S1/S2	6.0	79.8	0				0.44	23.6	192
IgA S1/S2	7.5	82.5	0				0.09	15.3	192
IgG Nucleocapsid	21.3	70.9	0				0.46	5.4	0
HCoV-OC43									
IgG RBD	0.4	4.1	0				0.26	2.9	0
IgG S2	641		0	19524		0	88.2		0
IgA S2	153		0				128		0
A/H1N1 (Flu)									
IgM fluHA	2.99	23.6	0				1.11	9.2	11
IgG fluHA	7136		0						
HCoV-HKU1									
IgG S2	848		0				46.2		0

[§] **NA:** Missing values

° ALT: Alanine Amino Trasferase; **AST:** Aspartate Amino Transaminase; **Bil:** total bilirubin; **Crea:** creatinine; **CRP:** C-reactive protein; **LDH:** Lactate dehydrogenase; **HGB:** Hemoglobin; **Lymph:** Lymphocyte; **Neu:** Neutrophil; **Mo:** Monocytes; **PLT:** Platelet; **PaO2:** partial pressure of oxygen; **WBC:** White Blood cell.

°° Median Antibody titers (Arbitrary Units) and the percentage of positives are reported

ESM Table 2 - Percent of positive samples and titre in Ab positives*

	IgG antibodies			IgM antibodies			IgA antibodies		
SARS-CoV-2 Spike RBD	**Positivity %(count/total)**	**Arbitrary Units (IQR)**	**ANOVA p value****	**Positivity %(count/total)**	**Arbitrary Units (IQR)**	**ANOVA p value****	**Positivity %(count/total)**	**Arbitrary Units (IQR)**	**ANOVA p value****
Pre-pandemic control	0 (0/480)	- -		1 (5/480)	2.5 (2.2-2.7)		2.9 (14/480)	1.8 (1.5-3.3)	
COVID-19 week 1	25.9 (37/143)	10.7 (5.6-17.0)		43.4 (62/143)	6.2 (3.3-13.8)	0.01	34.3 (49/143)	2.5 (1.7-4.6)	0.33
COVID-19 week 2	58.9 (112/190)	13.9 (7.4-27.4)	0.49	77.4 (147/190)	9.2 (4.7-16.9)	0.17	65.8 (125/190)	3.4 (2.3-8.9)	0.06
COVID-19 week 3	86.9 (86/99)	44.5 (18.3-116.8)	$<10^{-7}$	87.9 (87/99)	8.6 (4.7-20.2)	0.99	80.8 (80/99)	5.5 (3.3-8.9)	0.42
COVID-19 week 4	96.5 (138/143)	153.4 (63.6-240.1)	$<10^{-6}$	72 (103/143)	5.1 (3.0-8.8)	$<10^{-3}$	87.4 (125/143)	4.6 (2.2-7.7)	0.53
SARS-CoV-2 Spike S1+S2									
Pre-pandemic control	1.2 (6/480)	10.9 (10.3-11.5)		23.6 (68/288)	4.2 (3.1-7.3)		12.9 (62/480)	2.6 (2.0-3.6)	
COVID-19 week 1	34.3 (49/143)	37.8 (21.4-69)	$<10^{-2}$	62.2 (89/143)	7.4 (4.5-13.4)	$<10^{-2}$	62.9 (90/143)	6 (3.4-13.0)	$<10^{-7}$
COVID-19 week 2	66.8 (127/190)	49.8 (29.5-90.5)	0.24	85.3 (162/190)	9.5 (4.9-14.8)	0.44	86.3 (164/190)	9.7 (5.4-20.8)	$<10^{-4}$
COVID-19 week 3	93.9 (93/99)	84.4 (48.9-115.9)	$<10^{-2}$	91.9 (91/99)	8.9 (5.3-14.3)	0.99	96 (95/99)	12.9 (6.9-19.6)	0.35
COVID-19 week 4	97.2 (139/143)	195.8 (110.8-249.3)	$<10^{-7}$	73.4 (105/143)	5.3 (3.1-8.0)	$<10^{-4}$	95.8 (137/143)	9.3 (5.1-15.1)	0.04
SARS-CoV-2 Nucleocapsid									
Pre-pandemic control	5.4 (26/480)	9.5 (5.7-13.6)							
COVID-19 week 1	53.1 (76/143)	29.1 (12.9-43.7)	$<10^{-7}$						
COVID-19 week 2	71.1 (135/190)	29.7 (14.7-43.5)	0.99						
COVID-19 week 3	90.9 (90/99)	39.4 (30.8-49.7)	$<10^{-3}$						
COVID-19 week 4	93.7 (134/143)	52.5 (41.6-59-8)	0.02						
OC43 Spike RBD									
Pre-pandemic control	27.7 (133/480)	1.5 (1.1-2.9)							
COVID-19 week 1	37.1 (53/143)	1.6 (1.0-3.1)	0.99						
COVID-19 week 2	33.7 (64/190)	1.8 (1.2-3.2)	0.99						
COVID-19 week 3	35.4 (35/99)	1.5 (1.1-2.1)	0.99						
COVID-19 week 4	31.2 (15/48)	1.3 (1.0-2.1)	0.99						
OC43 Spike S2									
Pre-pandemic control	1.7 (8/480)	88 (39-206)					3.3 (16/480)	128.4 (112.6-143.4)	
COVID-19 week 1	14 (20/143)	259 (96-712)	$<10^{-7}$				18.9 (27/143)	141.5 (126.9-169.1)	$<10^{-3}$
COVID-19 week 2	31.6 (60/190)	655 (208-2174)	$<10^{-6}$				32.6 (62/190)	149.6 (123.2-242.7)	0.07
COVID-19 week 3	49.5 (49/99)	1472 (457-2883)	$<10^{-2}$				48.5 (48/99)	190.2 (140.1-585.3)	$<10^{-5}$
COVID-19 week 4	59.4 (85/143)	2024 (751-4052)	0.33				41.9 (60/143)	180.5 (136.7-341.5)	0.03
HKU1 Spike S2									
Pre-pandemic control	0 (0/480)	46.2 (23.1-89.2)							
COVID-19 week 1	14 (20/143)	263 (129-820)	$<10^{-7}$						
COVID-19 week 2	38.9 (74/190)	854 (279-3173)	$<10^{-7}$						
COVID-19 week 3	58.6 (58/99)	2432 (731-4874)	$<10^{-5}$						
COVID-19 week 4	63.6 (91/48)	2282 (1058-5183)	0.99						

*= for the OC43 and HKU1 abs the percent of subjetcs in the first tercile replaces the percent of positive samples
and arbitrary units from all samples are used to calculate ANOVA
** = with Tukey HSD post hoc correction

ESM Table 3 - SARS-CoV-2 Spike RBD antibody titers over time

IgG antibodies

	Baseline	month 1 post hospital discharge follow-up		month 3 post hospital discharge follow-up	
	Arbitrary Units (IQR)	Arbitrary Units (IQR)	Fold change (IQR)	Arbitrary Units (IQR)	Fold change (IQR)
Baseline in week 1 (n= 7)	0.7 (0.5-1.1)	121 (80, 259)	126 (112-260)	303 (248-311)	366 (274-718)
Baseline in week 2 (n=17)	8.9 (2.1-24)	216 (143-293)	16 (8-40)	284 (247-315)	24 (10.8-68)
Baseline in week 3 (n=11)	61 (39-111)	181 (146-278)	3 (1.8-4.4)	306 (266-314)	4.8 (2.9-7.7)

IgM antibodies

	Baseline	month 1 post hospital discharge follow-up		month 3 post hospital discharge follow-up	
	Arbitrary Units (IQR)	Arbitrary Units (IQR)	Fold change (IQR)	Arbitrary Units (IQR)	Fold change (IQR)
Baseline in week 1 (n= 7)	5.9 (4.9-9.6)	1.6 (1.3-3.2)	0.43 (0.3-0.7)	0.8 (0.5-2.7)	0.1 (0.1-0.3)
Baseline in week 2 (n=17)	7.8 (5.6-23.2)	3.0 (2.5-8.4)	0.40 (0.3-1.2)	1.5 (0.7-6.0)	0.24 (0.1-0.5)
Baseline in week 3 (n=11)	26.5 (8.4-31)	2.8 (1.1-4.1)	0.14 (0.1-0.3)	0.8 (0.5-3.3)	0.14 (0.02-0.2)

IgA antibodies

	Baseline	month 1 post hospital discharge follow-up		month 3 post hospital discharge follow-up	
	Arbitrary Units (IQR)	Arbitrary Units (IQR)	Fold change (IQR)	Arbitrary Units (IQR)	Fold change (IQR)
Baseline in week 1 (n= 7)	1.4 (0.7-2.0)	3.6 (3.0-8.0)	3.1 (2.3-6.6)	5.2 (3.9-13.0)	4.7 (4.3-8.2)
Baseline in week 2 (n=17)	5.0 (1.9-7.0)	5.4 (2.6-7.1)	1.0 (0.9-1.4)	5.4 (2.9-8.4)	1.4 (0.9-2.6)
Baseline in week 3 (n=11)	5.7 (4.3-7.4)	3.9 (3.2-12.4)	0.8 (0.5-2.0)	6.4 (4.5-20.4)	1.0 (0.8-2.8)

ESM Table 4A - Baseline characteristics of study population according to COVID-19 severity

	Discharged (n=57)		Hospitalized (n= 310)				ICU (n=79)				Dead (n=63)		p-value
			≤7days (n=78)		>7days (n=232)		Alive (n=49)		Dead (n=30)				
	median (IQR)	%	median (IQR)	%	median (IQR)	%	median (IQR)	%	median (IQR)	%	median (IQR)	%	
Age (years)	49 (44.5-61)		61 (49.7-68)		64.5 (56-76)		61 (52-67)		69.5 (62-72)		80 (73.5-85)		<0.001
Sex (Male)		47.4		62.8		68.1		89.8		66.7		61.9	<0.001
Body Mass Index	27.6 (24.3-33.7)		25.7 (23.4-30.1)		27.4 (24.2-30.4)		27.7 (25-31.2)		26.5 (24.4-28.7)		24.8 (23.1-28.4)		0.028
Ethnicity													
Caucasian		70.2		79.5		83.2		91.8		90.0		96.8	0.052
Hispanic		15.8		14.1		10.3		6.1		10.0		1.6	
Asian		5.3		3.8		3.0		0.0		0.0		1.6	
African		8.8		2.6		3.4		2.0		0.0		0.0	
Time from symptoms													
to last FU*	53 (50-56)		59 (56-62)		58 (56-60)		70 (67-72)		28 (21-35)		17 (14-20)		<0.001
to admission	7.0 (3-10)		9.0 (5-12)		7.0 (5-10)		7.0 (5.5-10)		7.0 (4-10)		5.5 (3-7.5)		0.057
to blood sampling:	9.0 (4-16)		11 (7-14)		10 (7-15)		15.5 (10-24)		13.5 (11-21)		8.0 (5-14)		<0.001
Days ≤7		41.5		26.7		30.1		16.7		9.1		39.6	<0.001
Days 8-14		32.1		49.3		41.9		25.0		45.5		35.8	
Days 15-21		13.2		16.0		22.7		29.2		27.3		15.1	
Days>21		13.2		8.0		5.2		29.2		18.2		9.4	
to swab negativization*	32 (30-34)		36 (29-42)		41 (38-44)		46 (36-56)		48.0 (41-54.5)		30 (20-40)		<0.001
Time from admission													
to last FU (95%CI)	43 (39-47)		49 (47-51)		49 (47-51)		58 (52-64)		21 (15-27)		9 (7-11)		<0.001
Hospital stay (95%CI)			5 (4-6)		17 (15-19)		42 (27-57)		21 (17-25)		8 (6-10)		<0.001
N of Comorbidities	0 (0-1)		0 (0-1)		1 (0-2)		1 (0-1)		1 (0-2)		2 (1-3)		<0.001
None		71.9		53.8		39.4		41.7		34.5		6.6	<0.001
1		15.8		25.6		32.5		37.5		34.5		24.6	
2		8.8		11.5		20.3		12.5		13.8		31.1	
3		3.5		7.7		6.1		6.3		13.8		23.0	
4		0.0		1.3		1.3		0.0		3.4		14.8	
≥5		0.0		0.0		0.4		2.1		0.0		0.0	
Comorbidities°													
Hypertension		22.8		35.9		46.6		41.7		51.7		75.8	<0.001
CAD		5.3		6.4		9.9		8.3		20.7		27.4	<0.001
T1D/T2D		0/8.8		0/9.0		0/17.2		2.1/14.6		3.4/17.2		3.2/22.6	0.038
COPD		0.0		6.4		4.3		4.2		3.4		16.1	0.003
CKD		3.6		6.4		7.4		14.6		10.3		29	<0.001
Cancer		3.5		9.0		8.6		6.3		6.9		25.8	0.001
ND		0.0		3.8		4.8		4.2		3.4		9.8	0.23

*95% confidence interval

°COPD: Chronic obstructive pulmonary disease; **ND:** Neurodegenerative disease; **T1D/T2D:** type 1/type 2 diabetes; **CAD:** coronary artery diseases; **CKD:** Chronic Kidney disease.

ESM Table 4B - Baseline characteristics of study population according to COVID-19 severity

	Discharged (n=57)		Hospitalized (n= 310)				ICU (n=79)				Dead (n=63)		p-value
			≤7days (n=78)		>7days (n=232)		Alive (n=49)		Dead (n=30)				
	median (IQR)	%	median (IQR)	%	median (IQR)	%	median (IQR)	%	median (IQR)	%	median (IQR)	%	
Symptoms at onset													
General													
Fever		83.9		88.0		89.3		97.8		95.8		70.7	<0.001
Fatigue/Malaise		16.1		4.0		6.3		4.3		12.5		15.5	0.023
Myalgia/Arthralgia		12.5		4.0		6.3		0.0		0.0		5.2	0.082
Respiratory													
Cough		57.1		52.0		55.4		34.8		45.8		31.0	0.005
Dyspnea		37.5		56.0		68.3		73.9		75.0		63.8	<0.001
Sore Throat		3.6		0.0		0.0		8.7		8.3		0.0	<0.001
Chest pain		12.5		12.0		5.4		0.0		4.2		1.7	0.018
Any		75.0		78.7		85.3		87.0		95.8		72.4	0.04
Gastrointestinal													
Diarrhea		10.7		6.7		9.4		13.0		0.0		8.6	0.539
Vomiting/nausea		8.9		10.7		6.7		13.0		0.0		5.2	0.33
Abdominal Pain		1.8		1.3		3.1		4.3		0.0		1.7	0.805
Any		16.1		16.0		15.6		21.7		0.0		13.8	0.309
Others													
Headache		3.6		1.3		2.7		0.0		0.0		0.0	0.514
Conjunctivitis		0.0		0.0		0.9		0.0		0.0		0.0	0.803
Hypo/Anosmia		7.1		9.3		3.6		0.0		0.0		0.0	0.027
Hypo/Dysgeusia		8.9		6.7		3.6		0.0		0.0		0.0	0.052
Skin Rash		0.0		0.0		1.6		0.0		0.0		0.0	0.634
Laboratory at blood sampling[§]													
WBC, $x10^9$/L	5.9 (4.8-7.6)		6.2 (5.1-8.4)		6.6 (5.1-9.1)		9.4 (7.7-11.8)		11.7 (8.3-20.1)		9.0 (6-11.1)		<0.001
Lymph count, $x10^9$/L	1.2 (0.9-1.65)		1.0 (0.9-1.5)		1.0 (0.7-1.4)		0.9 (0.6-1.2)		0.8 (0.6-1.35)		0.8 (0.5-1.12)		<0.001
Neu count, $x10^9$/L	3.7 (3.1-5.2)		4.3 (3.1-6.2)		4.8 (3.3-7.2)		7.5 (5.9-10.7)		10.9 (6.35-15.9)		7.4 (4.4-9.6)		<0.001
Mo count, $x10^9$/L	0.4 (0.4-0.7)		0.6 (0.4-0.7)		0.5 (0.3-0.7)		0.5 (0.3-0.7)		0.8 (0.4-1.05)		0.4 (0.2-0.5)		<0.001
N/Ly ratio	3.14 (2.2-4.7)		3.6 (2.2-5.7)		4.8 (2.6-7.9)		8.3 (5.4-12.7)		13.5 (8.8-17.9)		9.9 (5.3-13.9)		<0.001
HGB, g/dl	14.25 (13-15)		13.3 (12.1-14.4)		13.2 (11.9-14.3)		12.35 (11-13.8)		11.9 (10.8-12.8)		11.9 (10.3-13.4)		<0.001
PLT count, $x10^9$/L	220 (185-283)		236 (190-323)		241 (185-322)		294 (203-382)		289 (175-346)		174 (119-268)		<0.001
Bil, mg/dl	0.39 (0.28-0.69)		0.56 (0.35-0.83)		0.55 (0.37-0.82)		0.72 (0.48-1.22)		1.02 (0.61-1.88)		0.53 (0.29-0.91)		<0.001
ALT, U/L	30 (23-49)		41 (21.5-52.5)		45 (26-67)		60 (44.5-84)		46 (25-62)		27 (17-33.5)		<0.001
AST, U/L	27 (22-43)		34 (27-61)		47 (34-69)		62 (42-89.5)		54 (39-72.5)		43 (30.5-62.5)		0.001
Crea, mg/dl	0.8 (0.7-1)		0.89 (0.72-1.1)		0.95 (0.79-1.22)		1.1 (0.91-1.6)		1.44 (0.99-1.9)		1.3 (0.96-1.94)		<0.001
LDH, U/L	262 (212-298)		299 (241-384)		363 (280-455)		510 (379-632)		557 (396-789)		443 (366-673)		<0.001
CRP, mg/L	10.2 (2.5-24.7)		47.7 (16-87.5)		70.9 (27.3-127.6)		142.7 (45.8-225)		143.9 (55.6-189)		118 (62.3-175)		<0.001
D-Dimer, µg/mL	0.31 (0.27-0.46)		0.75 (0.41-1.44)		1.03 (0.53-1.76)		3.25 (1.64-6.41)		1.96 (1.48-3.47)		1.8 (0.7-3.66)		<0.001
IL-6, pg/ml			18 (9.3-39.2)		35.5 (18.6-74.7)		85 (24.8-107)		130 (45-175)		90.5 (50-114)		<0.001
Ferritin, ng/mL			555 (217-1222)		1043 (589-1725)		2745 (1294-3571)		1001 (735-1957)		1295 (665-1747)		<0.001
PaO2/Fi02 ratio (mmHg)	375 (338-409)		300 (257-339)		214 (124-327)		91.6 (0.71-1.33)		136 (101-208)		133 (57-198)		<0.001

§ **ALT:** Alanine Amino Trasferase; **AST:** Aspartate Amino Transaminase; **Bil:** total bilirubin; **Crea:** creatinine; **CRP:** C-reactive protein; **LDH:** Lactate dehydrogenase; **HGB:** Hemoglobin; **Lymph:** Lymphocyte; **Neu:** Neutrophil; **Mo:** Monocytes; **PLT:** Platelet; **PO2:** partial pressure of oxygen; **WBC:** White Blood cell.

ESM Table 4C - Baseline characteristics of study population according to COVID-19 severity

	Discharged (n=57)		Hospitalized (n= 310)				ICU (n=79)				Dead (n=63)		p-value
			≤7days (n=78)		>7days (n=232)		Alive (n=49)		Dead (n=30)				
	median (IQR)	% pos	median (IQR)	% pos	median (IQR)	% pos	median (IQR)	% pos	median (IQR)	% pos	median (IQR)	% pos	
Antibodies													
SARS-CoV-2													
IgG RBD	0.8 (0.02-15.9)		3.2 (0.15-19.3)		6.9 (0.31-24.1)		31.3 (10.7-93.4)		31.8 (2.4-189)		0.13 (0.02-3.4)		<0.001
		45.6		55.1		61.6		87.8		80.0		28.6	<0.001
IgM RBD	2.6 (0.95-4.8)		5.7 (1.5-10.9)		6.1 (1.9-13.7)		9.1 (3.3-17.2)		5.5 (3.2-11.9)		1.75 (0.65-4.2)		<0.001
		54.4		69.2		72.8		83.7		83.3		47.6	<0.001
IgA RBD	1.46 (0.2-5)		1.61 (0.72-3.5)		2.1 (0.76-5.1)		6.1 (3.4-8.9)		4.4 (1.6-15)		0.81 (0.25-2.3)		<0.001
		52.6		57.7		60.3		91.8		86.7		39.7	<0.001
IgG S1/S2	5.7 (0.37-54.7)		30.3 (2.9-58.3)		35 (2.9-58.3)		89.4 (43.6-126)		66.7 (20.4-96.4)		2.4 (0.42-36.5)		<0.001
		49.1		65.4		68.5		89.8		83.3		41.3	<0.001
IgM S1/S2	3.95 (1.1-6.4)		6.7 (3.3-13.3)		6.6 (2.6-13.5)		6.8 (3.9-13.3)		7.9 (4-13.8)		4.23 (1-10.8)		<0.001
		71.9		84.6		80.2		87.8		90.0		68.3	0.03
IgA S1/S2	5.5 (0.4-11.4)		6.03 (2.5-16.2)		7.5 (3-16.9)		11.96 (6.6-20.7)		10.65 (4.9-19.2)		3.95 (1.1-12.9)		<0.001
		64.9		83.3		84.5		98.0		93.3		73.0	<0.001
IgM NP	21.8 (1.7-44)		18.1 (2.1-39.7)		23.0 (2.9-14.5)		35.4 (13.5-46.6)		33.9 (10.9-44.7)		5.3 (0.5-31.5)		0.002
		63.2		69.2		72.8		87.8		86.7		52.4	<0.001
A/H1N1 (flu)													
IgM HA	2.8 (1.2-9)		2.7 (1-7.2)		2.9 (1.2-9.8)		2.1 (1.4-5.6)		3.5 (2-12.9)		3.8 (1.3-16.5)		0.452
		22.8		20.5		24.1		14.3		26.7		31.7	0.38
IgG HA	7599 (2709-19933)		8411 (2402-27263)		5950 (2883-14505)		5824 (1454-15271)		7809 (3577-20524)		9382 (3959-29607)		0.234
(terciles)	35.1/26.3/38.6		34.6/25.6/39.7		34.1/36.6/29.3		40.8/30.6/28.6		26.7/43.3/30		23.8/34.9/41.3		0.336
HCoV-OC43													
IgG RBD	0.31 (0.15-0.88)		0.45 (0.16-1.1)		0.39 (0.14-1.2)		0.31 (0.14-.0.75)		0.53 (0.18-1.1)		0.31 (0.11-0.79)		0.571
		5.3		2.6		4.8		6.1		0.0		3.2	0.73
IgG S2	454 (193-1628)		544 (243-1272)		643 (190-2210)		1571 (402-3485)		1656 (588-3736)		259 (89-1041)		<0.001
(terciles)	38.6/35.1/26.3		33.3/43.6/23.1		31.9/32.8/35.3		22.4/26.5/51		13.3/33.3/53.3		50.8/27/22.2		0.001
IgA S2	138 (120-214)		158 (131-290)		153 (128-247)		182 (145-394)		209 (143-385)		139 (121-165)		<0.001
(terciles)	43.9/26.3/29.8		33.3/32.1/34.6		31.5/36.2/32.3		18.4/34.7/46.9		23.3/20/56.7		46/36.5/17.5		0.005
HCoV-HKU1													
IgG S2	463 (204-1153)		651 (276-1903)		953 (255-3148)		2928 (736-5698)		4640 (1007-8800)		339 (148-1039)		<0.001
(terciles)	31.6/43.9/24.6		24.4/44.9/30.8		25.9/32.8/41.4		10.2/30.6/59.2		6.7/23.3/70		46/31.7/22.2		<0.001

ESM Table 5 - Multivariate Cox Regression analyses*

Time to Death

Model 1

	HR (95% CI)	p-value
Age	1.05 (1.02-1.08)	0
Sex (Male)	1.16 (0.66-2.04)	0.603
Time to swab	0.95 (0.91-0.98)	0.004
Hypertension	1.40 (0.74-2.64)	0.303
CAD	1.88 (0.96-3.67)	0.065
CKD	1.65 (0.85-3.21)	0.137
Cancer	1.23 (0.64-2.37)	0.534
Neu count, $x10^9$/L	2.13 (1.50-3.04)	0.000
Lymph count, $x10^9$/L	0.59 (0.43-0.81)	0.001
Mo, $x10^9$/L	0.72 (0.54-0.96)	0.025
HGB, g/dl	0.62 (0.2-1.94)	0.413
SARS-CoV-2 RBD IgG+	0.47 (0.25-0.87)	0.016

Model 2

	HR (95% CI)	p-value
Age	1.04 (1.01-1.07)	0.012
Sex (Male)	2.44 (1.17-5.07)	0.017
Time to swab	0.97 (0.93-1.01)	0.158
Hypertension	2.08 (0.99-4.37)	0.054
CAD	2.46 (1.16-5.21)	0.018
Cancer	3.63 (1.61-8.18)	0.002
PLT, $x10^9$/L	0.52 (0.33-0.82)	0.004
Crea, mg/dl	1.55 (1.08-2.23)	0.017
ALT, U/L	0.60 (0.39-0.92)	0.019
LDH, 100 U/L	5.19 (2.6-10.37)	0.000
CRP, 10 mg/L	1.18 (0.96-1.45)	0.114
SARS-CoV-2 RBD IgG+	0.43 (0.19-0.97)	0.041

Time to swab negativization

	HR (95% CI)	p-value
Age	0.98 (0.97-1.00)	0.013
Sex (Male)	1.08 (0.77-1.51)	0.668
BMI	1.02 (0.98-1.05)	0.347
Time to swab	0.98 (0.96-1.01)	0.202
ND	0.62 (0.27-1.41)	0.252
HGB, g/dl	0.98 (0.47-2.05)	0.960
LDH, 100 U/L	0.68 (0.50-0.92)	0.012
SARS-CoV-2 S1+S2 IgA+	1.74 (1.10-2.73)	0.017

*Adjusted for sex, age and variables significant at $p<0.05$ in the univariate analysis and with less than 100 missing values; analysis stratified for time from symptoms to sampling.

ND: Neurodegenerative disease; **CAD:** coronary artery diseases; **CKD:** Chronic Kidney disease; **BMI:** Body Mass Index; **ALT:** Alanine Amino Trasferase; **Crea:** creatinine; **CRP:** C-reactive protein; **LDH:** Lactate dehydrogenase; **HGB:** Hemoglobin; **Lymph:** Lymphocyte; **Neu:** Neutrophil; **PLT:** Platelet; **Mo:** Monocytes.

ESM table 6 - Symptoms at onset according to anti-HA IgM

	Negative (%) n=389	Positive (%) n=120	p-value
General			
Fever	88.8	82.9	0.11
Fatigue/Malaise	9.3	5.1	0.18
Myalgia/Arthralgia	5.5	6.0	0.82
Respiratory			
Cough	50.8	46.2	0.38
Dyspnea	61.7	67.5	0.27
Sore Throat	1.6	1.7	1
Chest Pain	6.0	6.8	0.75
Any	81.7	83.8	0.68
Gastrointestinal			
Diarrhea	9.6	6.8	0.37
Vomiting/nausea	8.5	5.1	0.24
Abdominal pain	3.0	0.9	0.31
Any	16.7	11.1	0.18
Others			
Headache	1.6	2.6	0.46
Conjunctivitis	0.5	0	1
Hypo/Anosmia	3.0	6.8	0.09
Hypo/Dysgeusia	3.3	5.1	0.4
Skin Rash	1	0	0.58