Supplementary materials and methods

Patients

Between January 25, 2020 and May 21, 2020, ninety-two qRT-PCR (Mabsky Biotech Co., Ltd.) confirmed SARS-CoV-2 infected individuals were admitted to Beijing Ditan Hospital, Capital Medical University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Beijing Ditan Hospital, Capital Medical University (No. 2020-020-02) and the Ethics Committees of Institute of Microbiology, Chinese Academy of Sciences (APIMCAS2020057). Patients were classified into asymptomatic, mild or moderate (mild/moderate, MM), severe or critical cases (severe/critical, SC) according to the guideline of COVID-19 infection from the National Health Commission of the People's Republic of China issued on 3 March, 2020 (version 7) (China, 2020). The viral shedding period was defined as the interval from the symptom onset to last positive nasopharyngeal swab (days after symptom onset (dao) of last positive). According to the duration of viral shedding, ninety-two mild/moderate and severe/critical patients were divided into forty-five short viral shedding periods (SVS) group (less or equal to 30 dao for virus shedding) and forty-seven long viral shedding periods group (LVS, more than 30 dao).

Detection of the SARS-CoV-2 specific IgG and IgM antibody

A total of 338 sequential serum/plasma samples were collected for antibody test including SARS-CoV-2 spike receptor-binding domain (S-RBD)-immunoglobulin (Ig) M and IgG (S-RBD-IgM and S-RBD-IgG)

and nucleocapsid protein IgM and IgG (NP-IgM and NP-IgG). The S-RBD-IgM, S-RBD-IgG, NP-IgM and NP-IgG against SARS-CoV-2 were tested using indirect enzyme-linked immunosorbent assay (ELISA) kits (Beijing Hotgen and Guangzhou Qianxun Biotechnology Co., Ltd., China). For the kits, 100 ng S-RBD or NP protein was coated in 96 well plates and stored at 4℃. Serum/plasma samples were inactivated at 56℃ for 30 minutes and a serial of two-fold dilutions were performed for the antibodies test. Anti-human IgG-horseradish peroxidase (HRP) and anti-human IgM-HRP conjugated monoclonal antibody were used for detection at 450nm.

Neutralization assay

Neutralization activity of serum/plasma from COVID-19 patients was measured using a single-round HIV-1 based pseudovirus infection of 293T/ACE2+TMPRSS2 cells as previously described (Zhang. et al., 2021). Pseudovirus of SARS-CoV-2 was generated by co-transfection of 293T cells with pNL4-3.Luc.R-E-backbone and viral spike protein expression plasmids pSectaq2-SARS-CoV-2-S. 293T cells transfected with angiotensin-converting enzyme 2 (ACE2) and TMPRSS2 were seeded in a 96-well plate at a concentration of 10^4 cells per well and cultured for 12 h. Heat-inactivated serum/plasma samples were diluted in serial five-fold dilutions commencing with a dilution of 1:100, mixed with equal volume of SARS-CoV-2 pseudotyped virus (10ng of p24) and incubated at 37℃ for 1 h. Then the mixture was added to pre-seeded 293T/ACE2+TMPRSS2 for infection. The culture medium was refreshed after 12 h and incubated for an additional 48 h. Assays were developed with a luciferase assay system (Promega), and the relative light units (RLU) were read on a Promega GloMax Luminometer. The titers of neutralizing

antibodies (NAbs) were calculated as 50% inhibitory concentration (IC_{50}), expressed as the highest dilution of plasma which resulted in 50% reduction of luciferase luminescence compared with virus control. IC_{50} was interpolated from the neutralization curves determined using the log (inhibitor) *vs.* response--Variable slope (four parameters) fit using automatic outlier detection in Graphpad Prism Software.

Statistical analysis

Statistical analysis was carried out using SPSS 22.0 or Graphpad 8.0 (GraphPad Software, Inc., CA, USA). Data are indicated as mean ± Standard Deviation (SD) or medians (25^{th}, 75^{th} percentile) as indicated. The cumulative seropositive rates of S-RBD-IgM, S-RBD-IgG, NP-IgM and NP-IgG antibodies were analyzed by Kaplan-Meier survival curves and Log-rank (Mantel-cox) test was applied. The dynamic change of antibodies titer was demonstrated by line plot along the days after the disease onset, and LOWESS (locally weighted scatterplot smoothing) curve were fitted to display and compare the trends of different antibodies response among different group. Wilcox test was applied to compare the difference among different groups. $P < 0.05$ was considered statistically significant.

List of abbreviations

COVID-19: coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; ELISA: enzyme-linked immunosorbent assay; SVS: short viral shedding period; LVS: long viral shedding period; S-RBD: spike receptor-binding domain; NP: nucleocapsid protein; angiotensin-converting enzyme 2 (ACE2); RLU: relative light units; IC_{50}: 50% inhibitory concentration; LOWESS: locally weighted

scatterplot smoothing; Standard Deviation (SD); MM: mild/moderate; SC: severe/critical; dao: days after symptom onset; SE: standard error.

Compliance and ethics

All authors declare that they do not have any Conflict of Interest. All authors state that they conformed with the Helsinki Declaration in 1975. The study was approved by the Ethics Committee of the Beijing Ditan Hospital, Capital Medical University (No. 2020-020-02) and the Ethics Committee of Institute of Microbiology, Chinese Academy of Sciences (APIMCAS2020057). All patients gave written informed consent.

Table S1 Demographics and baseline characteristics of the COIVD-19 patients with differing severity

Characteristics	Total (N= 92)	Mild/moderate (MM) patients (N =49)	Severe/critical (SC) patients (N =43)	P values
Age (Year)	47 (2, 88)	36 (2, 76)	60 (20, 88)	<0.0001
Gender				0.223
Male (%)	55 (59.8)	27 (55.1)	28 (65.1)	
Female (%)	37 (40.2)	22 (44.9)	15 (34.9)	
Viral shedding periods (dao)				0.07
SVS (%)	45 (100)	28 (57.1)	17 (39.5)	
LVS (%)	47 (100)	21 (42.9)	26 (60.5)	
Duration of viral shedding (days)	31±19	26±16	37±20	0.07
Hubei exposure (%)	22 (23.9)	11 (22.4)	11 (25.6)	0.925
Imported case (%)	23 (25)	9 (18)	14 (33)	0.369
Complications (n, %)				<0.0001
Hypertension	20 (21.7)	4 (8.2)	16 (37.2)	0.001
Cardiovascular disease	4 (4.3)	0	4 (9.3)	0.044

Chronic Pulmonary disease	8 (8.7)	0	8 (18.6)	0.002
Diabetes	10 (1.09)	1 (2)	9 (20.9)	0.004
Hyperlipemia	3 (3.3)	2 (4.1)	1 (2.3)	0.549
Chronic kidney disease	5 (5.4)	1 (2)	4 (9.3)	0.143
Immune disorders	2 (2.2)	0	2 (4.7)	0.098
Others	1 (1.1)	0	1	0.467
Sign and symptoms				
Fever	62 (67.4)	34 (9.4)	28 (65.1)	0.415
Dry cough	43 (46.7)	19 (38.8)	24 (55.8)	0.077
Dyspnea	4 (4.3)	1 (2)	3 (7)	0.261
Shortness of breath	9 (9.8)	4 (8.2)	5 (11.6)	0.417
Muscle soreness	21 (22.8)	10 (20.4)	11 (25.6)	0.366
Fatigue	28 (30.4)	15 (30.6)	13 (30.2)	0.575
Diarrhea	3 (3.3)	2 (4.1)	1 (2.3)	0.549
Headache	6 (6.5)	4 (8.2)	2 (4.7)	0.403
Sore throat	15 (16.3)	11 (22.4)	4 (9.3)	0.076
Nasal congestion	4 (4.3)	3 (6.1)	1 (2.3)	0.620
Runny nose	2 (2.2)	2 (4.1)	0	0.281
Laboratory data				
WBC ($\times 10^9$/L)	6.2 ±3.4	5.3 ±1.7	7.5 ±4.6	0.002
Lymphocytes ($\times 10^9$/L)	1.6 ±1.0	1.9 ±1.2	1.2 ±0.4	0.071
Neutrophils ($\times 10^9$/L)	4.1 ±3.4	2.9 ±1.2	5.7 ±4.6	0.004
Hemoglobin (g/L)	131.9 ±17.7	136.3 ±17.9	125.7 ±15.9	0.624
Platelets ($\times 10^9$/L)	252.0 ±102.3	279.4 ±100.9	213.9 ±94.0	0.109
CRP (mg/L)	43.7 ±59.5	9.9 ±17.5	77.4 ±67.2	<0.0001
LDH (U/L)	289.0 ±189.2	222.5 ±63.0	374.5 ±142.9	0.002
PT (s)	13.4 ±2.1	11.9 ±0.6	14.1 ±2.3	0.024
APTT (s)	32.7 ±5.0	31.3 ±1.7	33.4 ±6.0	0.035
D-dimer (mg/L)	3.2 ±7.0	0.4 ±0.2	4.6 ±8.4	0.030
Blood Potassium (mmol/L)	3.9 ±0.5	3.9 ±0.4	3.9 ±0.6	0.129
Blood sodium (mmol/L)	137.4 ±3.7	139.8 ±1.8	135.0 ±3.5	0.001
Albumin (g/L)	38.9 ±6.2	42.4 ±5.0	34.9 ±4.9	<0.0001
ALT (U/L)	33.2 ±28.6	28.8 ±21.8	38.1 ±34.5	0.197
AST (U/L)	35.2 ±24.4	24.9 ±11.2	46.7 ±29.9	<0.0001

Notes: mild/moderate: MM; severe/critical: SC; SVS: short viral shedding period; LVS: long viral shedding period; dao: days after symptom onset; WBC: white blood cells; CRP: C-reactive protein; LDH: lactate dehydrogenase; PT: prothrombin time; APTT: activated partial thromboplastin time; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Table S2 Demographics and baseline characteristics of the COIVD-19 patients with short (SVS) and long viral shedding periods (LVS)

Characteristics	Total (N= 92)	SVS (N =45)	LVS (N =47)	P values
Age (Year)	47 (2, 88)	44 (3, 88)	50 (2, 86)	0.073
Gender				0.025
Male (%)	55 (59.8)	32 (71.1)	23 (48.9)	
Female (%)	37 (40.2)	13 (28.9)	24 (51.5)	
Classification				0.07
Non-severe (%)	49 (53.2)	28 (62.2)	21 (44.7)	
Severe (%)	43 (46.8)	17 (37.8)	26 (55.3)	
Viral shedding periods (dao)	31±19	16±7	46±14	<0.001
Hubei exposure (%)	22 (23.9)	12(26.7)	10 (21.3)	0.545
Imported case (%)	23 (25)	9 (18)	14 (33)	0.245
Complications (n, %)				0.327
Hypertension	20 (21.7)	10 (22.2)	10 (21.3)	0.556
Cardiovascular disease	4 (4.3)	0	4 (8.5)	0.064
Chronic Pulmonary disease	8 (8.7)	2 (4.4)	6 (12.8)	0.148
Diabetes	10 (1.09)	5 (11.1)	5 (13.6)	0.602
Hyperlipemia	3 (3.3)	2 (4.4)	1 (2.1)	0.484
Chronic kidney disease	5 (5.4)	2 (4.4)	3 (6.4)	0.521
Immune disorders	3 (3.3)	0	3 (6.4)	0.129
Others	1 (1.1)	1 (2.2)	0	0.489
Sign and symptoms				
Fever	62 (67.4)	30 (66.7)	32 (68.1)	0.531
Dry cough	43 (46.7)	21 (46.7)	22 (46.8)	0.577
Dyspnea	4 (4.3)	2 (4.3)	2 (4.3)	0.675
Shortness of breath	9 (9.8)	2 (4.4)	7 (14.9)	0.09
Muscle soreness	21 (22.8)	11 (24.4)	10 (21.3)	0.455
Fatigue	28 (30.4)	12 (26.7)	16 (34.0)	0.294
Diarrhea	3 (3.3)	3 (6.7)	0 (0)	0.113
Headache	6 (6.5)	4 (8.9)	2 (4.3)	0.318
Sore throat	15 (16.3)	7 (15.6)	8 (17.0)	0.537

Nasal congestion	4 (4.3)	3 (6.7)	1 (2.1)	0.292
Runny nose	2 (2.2)	1 (2.2)	1 (2.2)	0.742
Laboratory data				
WBC ($\times 10^9$/L)	6.2±3.4	5.6±2.5	6.9±4.1	0.073
Lymphocytes ($\times 10^9$/L)	1.6±1.0	1.7±1.0	1.5±1.0	0.027
Neutrophil ($\times 10^9$/L)	4.1±3.4	3.1±1.1	5.0±4.5	0.008
Hemoglobin (g/L)	131.9±17.7	133.9±19.1	129.9±16.5	0.095
Platelets ($\times 10^9$/L)	252.0±102.3	270.0±108.9	234.8±94.9	0.585
CRP (mg/L)	43.7±59.5	35.5±55.1	53.9±64.2	0.545
LDH (U/L)	289.0±189.2	284.4±131.2	294.5±129.4	0.691
PT (s)	13.4±2.1	12.1±0.9	13.9±2.3	0.047
APTT (s)	32.7±5.0	31.0±2.3	33.5±5.9	0.069
D-dimer (mg/L)	3.2±7.0	3.6±7.3	3.0±7.2	0.691
Blood Potassium (mmol/L)	3.9±0.5	3.9±0.5	3.9±0.6	0.287
Blood sodium (mmol/L)	137.4±3.7	138.5±3.3	136.2±3.8	0.360
Albumin (g/L)	38.9±6.2	39.7±6.4	37.3±5.9	0.170
ALT (U/L)	33.2±28.6	38.3±33.9	25.7±16.2	0.033
AST (U/L)	35.2±24.4	33.9±22.3	36.9±27.6	0.940

Notes: SVS: short viral shedding period; LVS: long viral shedding period; dao: days after symptom onset; WBC: white blood cells; CRP: C-reactive protein; LDH: lactate dehydrogenase; PT: prothrombin time; APTT: activated partial thromboplastin time; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Table S3 The number of proportion of short viral shedding periods (≤ 30 days, SVS) and long viral shedding periods (> 30 days, LVS) among asymptomatic, mild/moderate and severe/critical patients

Viral shedding / Group	SVS	LVS	P value
Asymptomatic or symptomatic			1.000
Asymptomatic	3 (50%)	3 (50%)	
Symptomatic	45 (48.9%)	47 (51.1%)	
Disease severity			0.141
mild/moderate, MM	28 (57.1%)	21 (42.9%)	
severe/critical, SC	17 (39.5%)	26 (60.5%)	

Notes: SVS: short viral shedding period; LVS: long viral shedding period; mild/moderate: MM; severe/critical: SC.

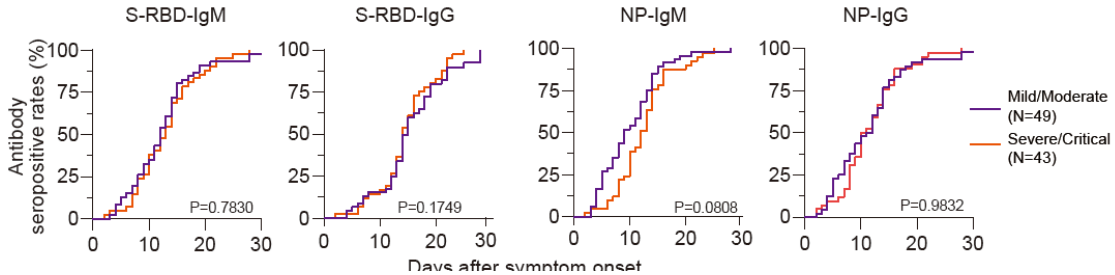

Figure S1 The antibody seropositive rates of mild/moderate and severe/critical COVID-19 patients.

Kaplan-Meier survival curves of the antibody seropositive rates of spike receptor-binding domain (S-RBD)-immunoglobulin (Ig) M, S-RBD-IgG, nucleocapsid protein (NP)-IgM and NP-IgG among 49 mild/moderate (MM) and 43 severe/critical (SC) coronavirus disease 2019 (COVID-19) patients since illness onset to 30 days after symptom onset (dao). Log-rank (Mantel-cox) test was applied.

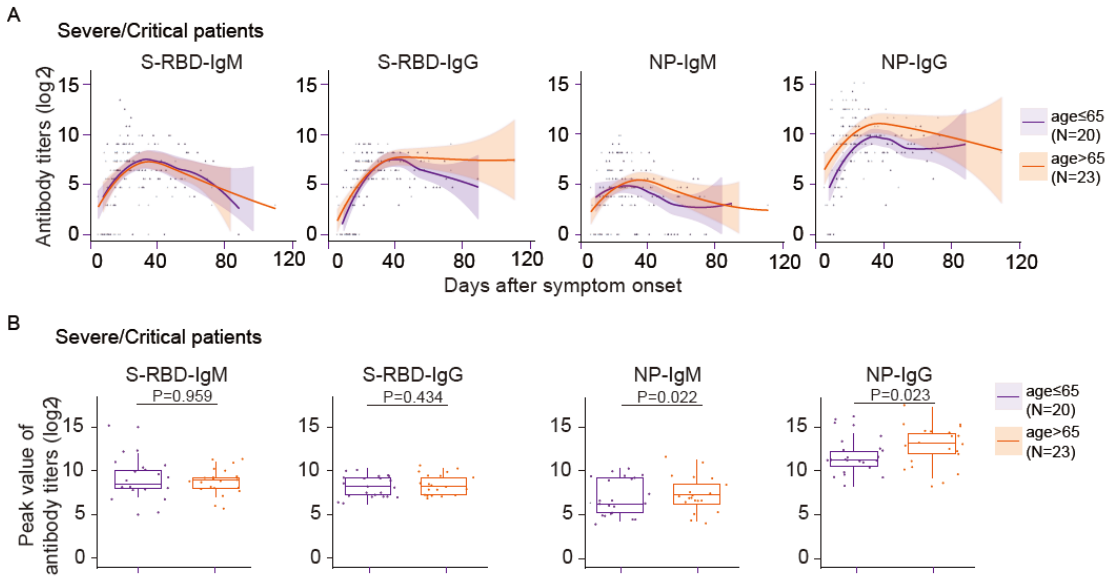

Figure S2 The dynamic trend profiling of antibody titers and the peak antibody titers between age ≤ 65 (18-65) and age > 65 of severe/critical patients.

(A) Line plot demonstrating the dynamic trend profiling of S-RBD-IgM, S-RBD-IgG, NP-IgM and

NP-IgG antibody titers (loess smoothed normalized counts ± standard error (SE)) over time after symptom onset in age ≤ 65 (18–65) and age > 65 of severe/critical COVID-19 patients.

(B) The peak antibody titers (the highest antibody titer) of S-RBD-IgM, S-RBD-IgG, NP-IgM, and NP-IgG in age ≤ 65 and age > 65 of severe/critical COVID-19 patients. Wilcox test was applied to compare the antibody titers between groups.

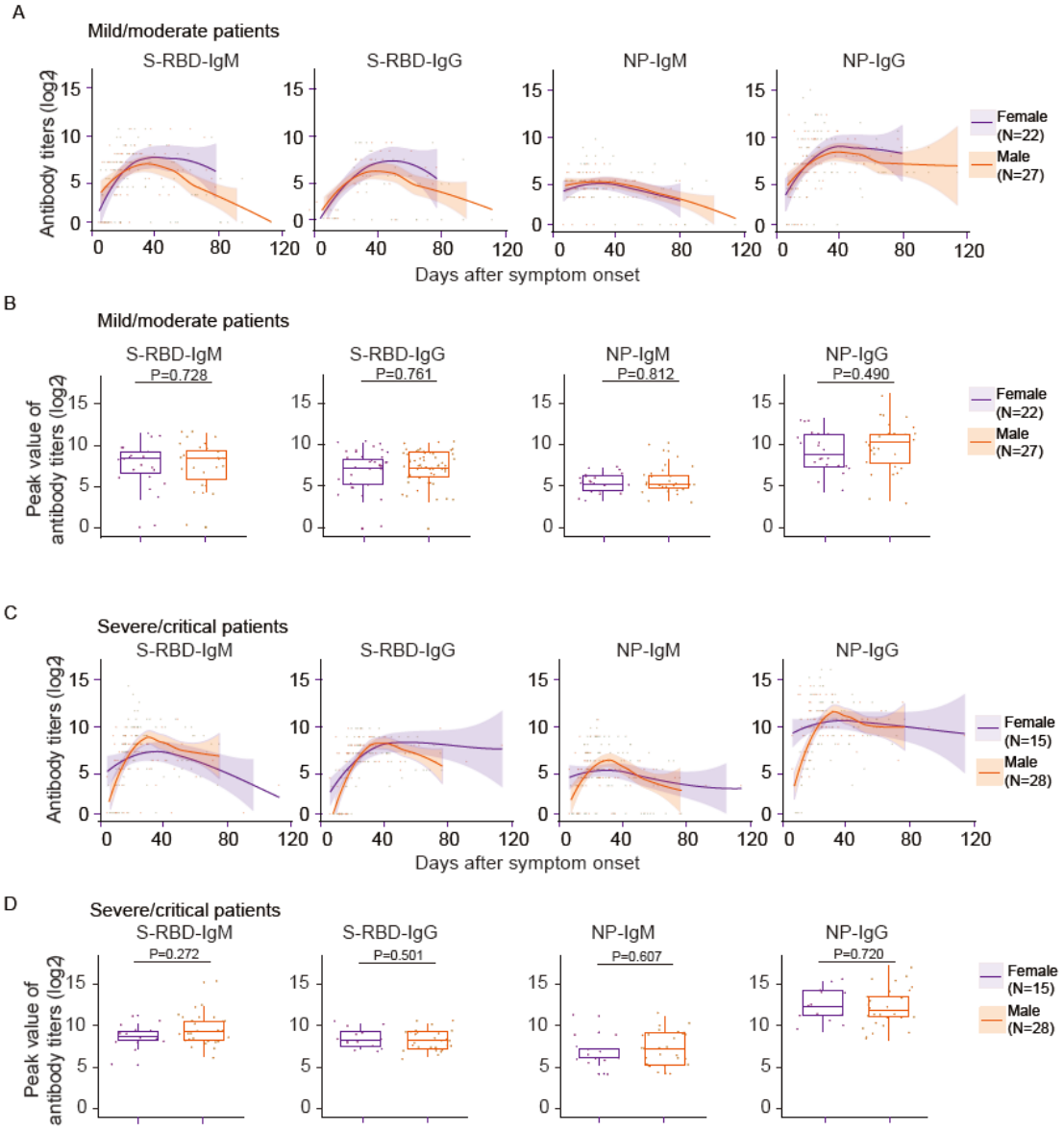

Figure S3 The dynamic trend profiling of antibody titers and the peak antibody titers among female and male of mild/moderate or severe/critical patients.

(A) Line plot demonstrating the dynamic trend profiling of S-RBD-IgM, S-RBD-IgG, NP-IgM and NP-IgG antibody titers (loess smoothed normalized counts ± SE) over time after symptom onset in female and male of mild/moderate COVID-19 patients.

(B) The peak antibody titers (the highest antibody titer) for S-RBD-IgM, S-RBD-IgG, NP-IgM, and NP-IgG in female and male of mild/moderate COVID-19 patients. Wilcox test was applied to compare the antibody titers between groups.

(C) Line plot demonstrating the dynamic trend profiling of S-RBD-IgM, S-RBD-IgG, NP-IgM, and NP-IgG antibody titers (loess smoothed normalized counts ± SE) over time after symptom onset in female and male of severe/critical COVID-19 patients.

(D) The peak antibody titers (the highest antibody titer) for S-RBD-IgM, S-RBD-IgG, NP-IgM, and NP-IgG in female and male of severe/critical COVID-19 patients. Wilcox test was applied to compare the antibody titers between groups.

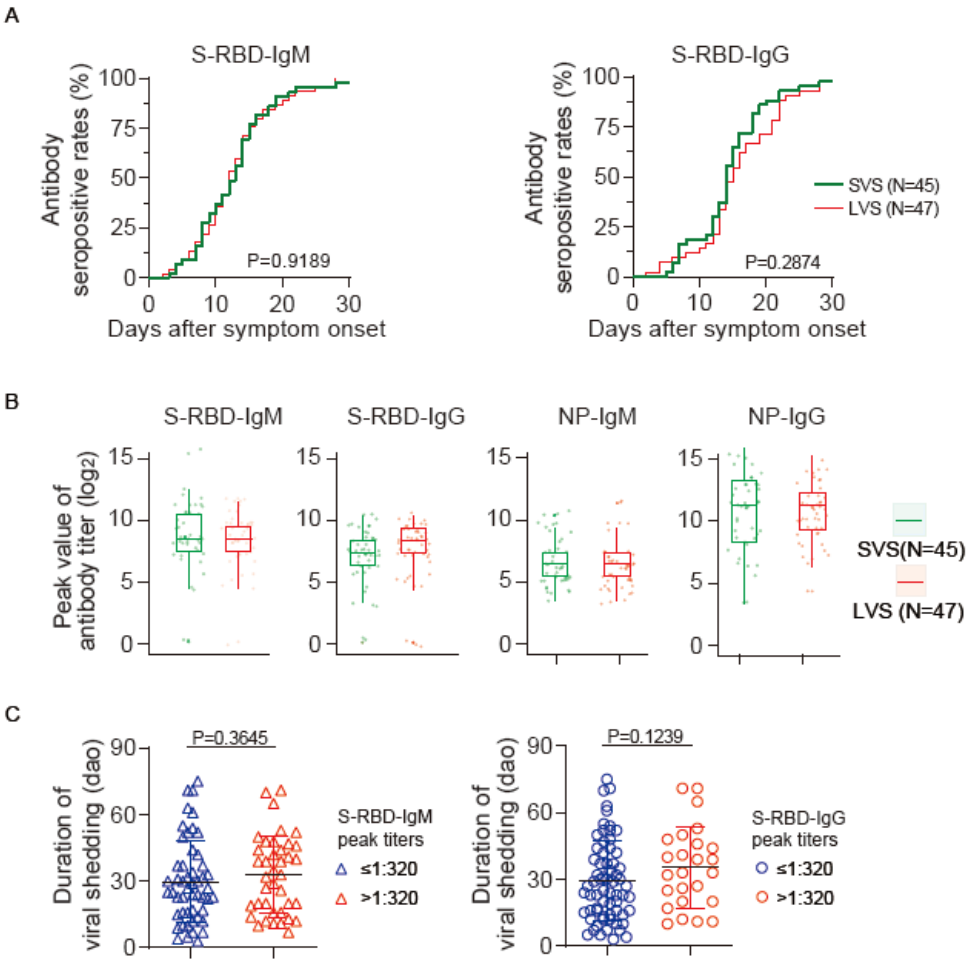

Figure S4 Antibody responses in patients with short and long viral shedding periods.

A) Kaplan-Meier survival curves of the antibody seropositive rates of S-RBD-IgM and S-RBD-IgG among 45 short viral shedding period (SVS) and 47 long viral shedding period (LVS) COVID-19 patients since illness onset to 30 dao. Log-rank (Mantel-cox) test was applied.

B) The peak antibody titers (the highest antibody titer) of S-RBD-IgM, S-RBD-IgG, NP-IgM and NP-IgG among SVS and LVS patients were shown. Wilcox test was applied to compare the difference among four groups.

C) The virus shedding periods of patients with different peak value of S-RBD-IgM titers (≤ 1:320 and > 1:320) and S-RBD-IgG titers (≤ 1:320 and > 1:320) were shown and compared with Wilcox test.

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