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Supplemental information

Antibody landscape against SARS-CoV-2 reveals significant differences between non-structural/ accessory and structural proteins

Yang Li, Zhaowei Xu, Qing Lei, Dan-yun Lai, Hongyan Hou, He-wei Jiang, Yun-xiao Zheng, Xue-ning Wang, Jiaoxiang Wu, Ming-liang Ma, Bo Zhang, Hong Chen, Caizheng Yu, Jun-biao Xue, Hai-nan Zhang, Huan Qi, Shu-juan Guo, Yandi Zhang, Xiaosong Lin, Zongjie Yao, Huiming Sheng, Ziyong Sun, Feng Wang, Xionglin Fan, and Sheng-ce Tao

Supplemental Information

Figure S1. SARS-CoV-2 proteome microarray and the assessment of reproducibility (related to Figure 1).

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Table S1. Serum Samples and patients (related to Figure 1).

Table S2. SARS-CoV-2 proteins included in the proteome microarray (related to Figure 1 and Figure S1).

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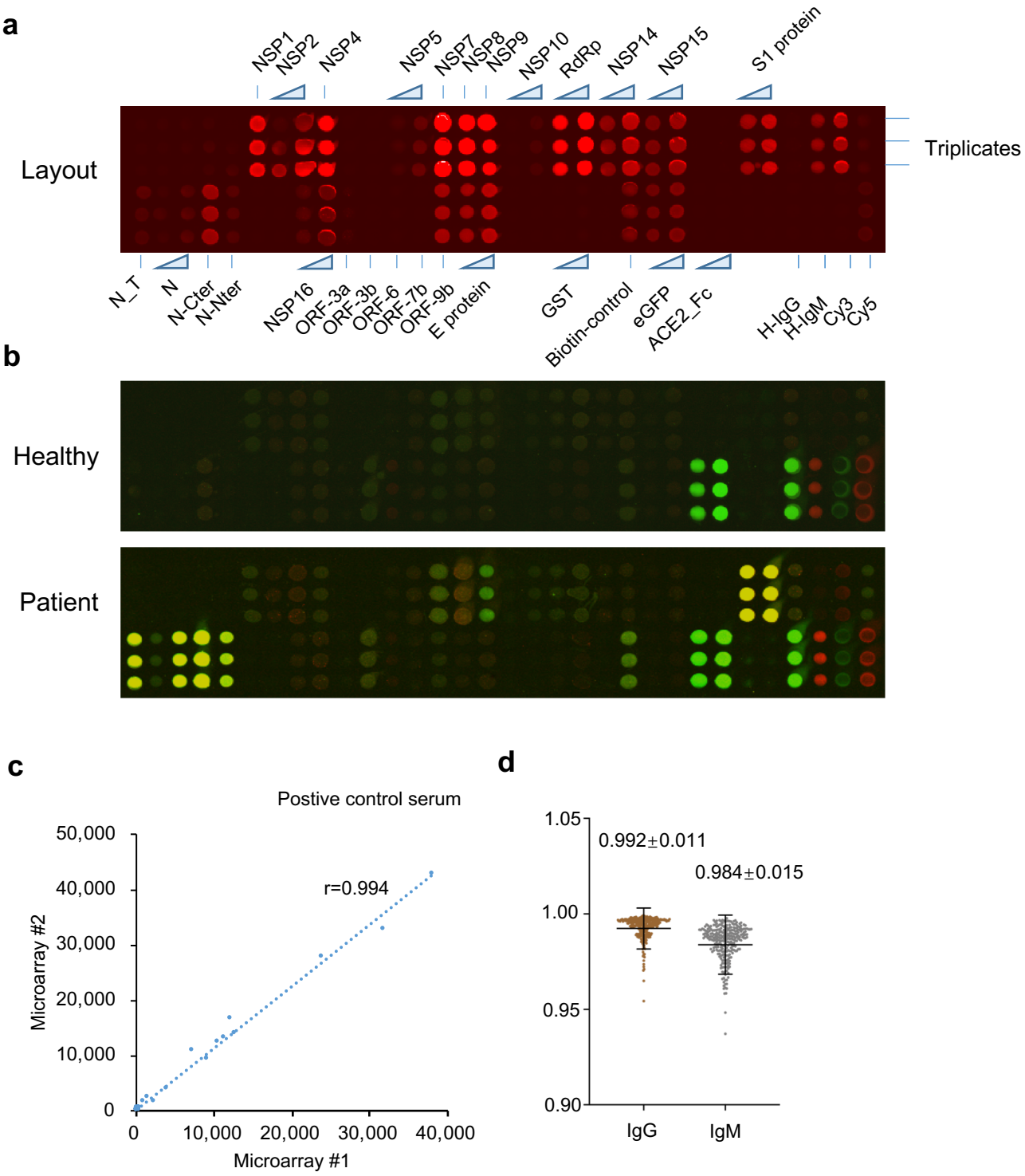


Figure S1. SARS-CoV-2 proteome microarray and the assessment of reproducibility (related to Figure 1). a. The layout of the SARS-CoV-2 proteome microarray. The locations of proteins and controls are indicated. **b**. Representative images of the microarray screened by sera from a healthy control and a COVID-19 patient. **c.** Correlation analysis between two microarrays probed independently with a positive control serum. **d**. Statistical analysis of the Pearson correlation coefficients between the microarrays incubated with the positive control serum with the averaged data set (see methods). The data are present as mean ± SD.

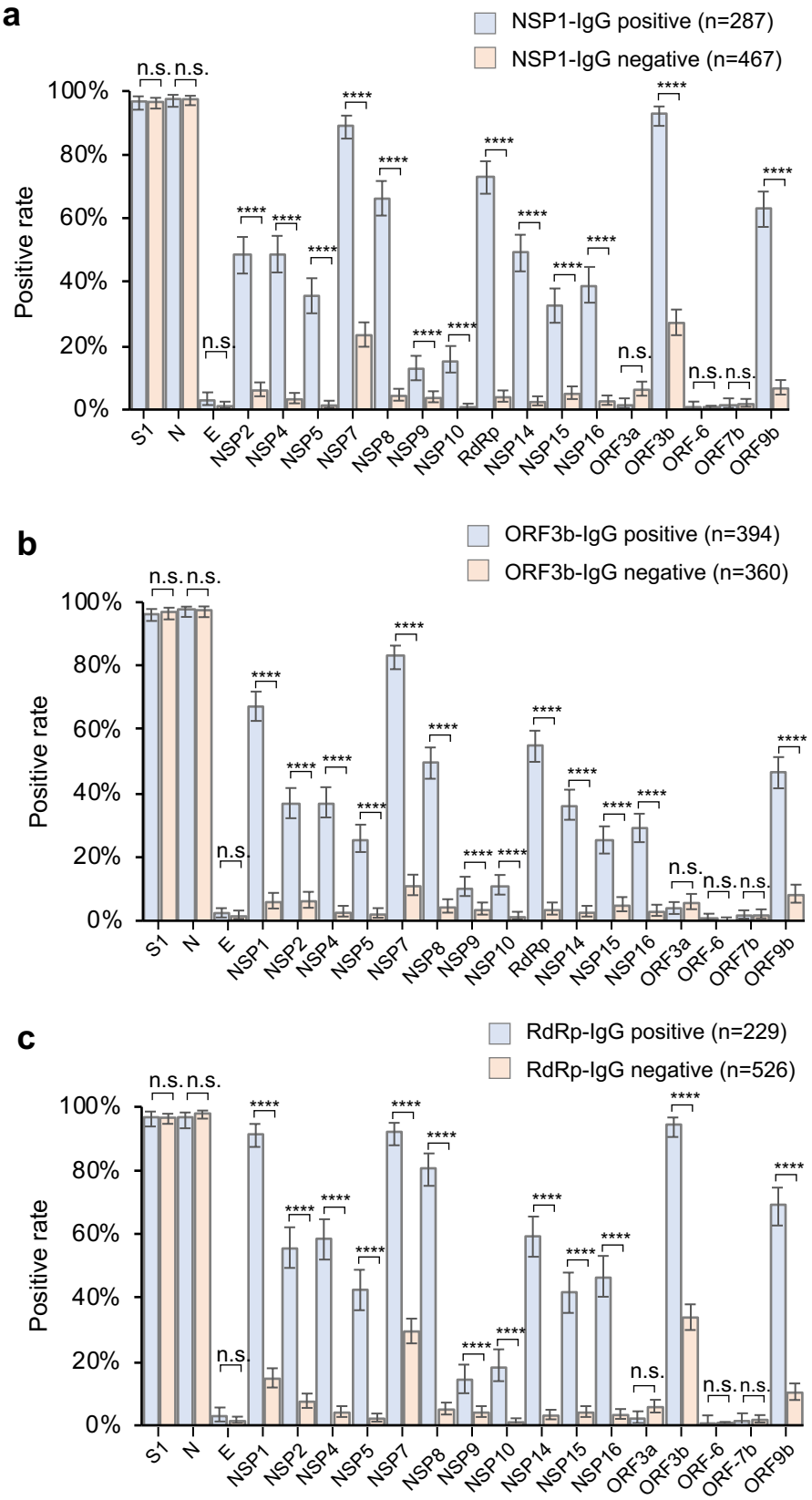
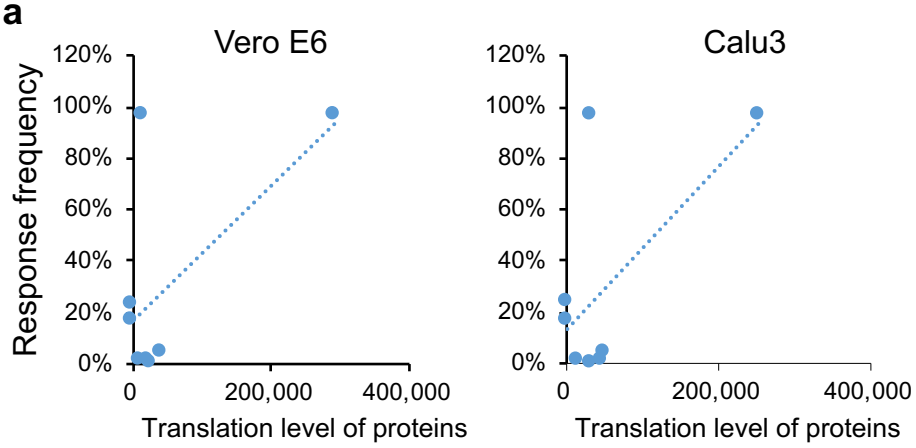


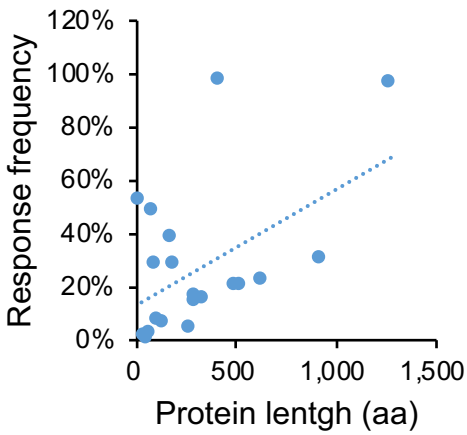
Figure S2. High associations among non-structural/ accessory proteins to elicit IgG response in patients (related to Figure 2). a-c, Antibody positive rates for all the SARS-CoV-2 proteins in two patient groups divided depending on positive or negative for NSP1 (a), ORF3b (b) and RdRP (c). Error bar was given as the 95% confidential interval. P-value was calculated by two-sided $\chi 2$ test. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$, n. s., not significant.

a



Protein	Translation level		response frequency
	Vero E6	Calu3	
ORF1a	659.2	1640.7	24%
ORF1b	394.3	852.9	17.30%
S protien	15648.7	34603.9	96.7%
ORF3a	40845.5	50519.3	4.5%
E protein	12351.5	14590.6	1.7%
ORF6	25280.8	33751.7	0.4%
ORF7b	20951.1	46634.2	1.6%
N protein	293655.1	252780.7	97.4%

b



Protein	**Length (aa)**	**Response frequency**
S	1273	96.7%
N protein	421	97.4%
E protein	75	1.7%
NSP1	180	38.0%
NSP2	638	22.1%
NSP4	500	20.5%
NSP5	306	14.3%
NSP7	83	48.4%
NSP8	198	27.9%
NSP9	113	7.0%
NSP10	139	6.2%
RdRp	932	30.3%
NSP14	527	20.1%
NSP15	345	15.3%
NSP16	298	16.4%
ORF3a	275	4.5%
ORF3b	22	52.1%
ORF6	61	0.4%
ORF7b	43	1.6%
ORF9b	99	28.0%

Figure S3. Antibody responses are not associated with protein abundance or length (related to Figure 2). a-b. Correlations between antibody positive rate and protein abundance (Finkel et al., 2020) (**a**), and protein length (**b**).

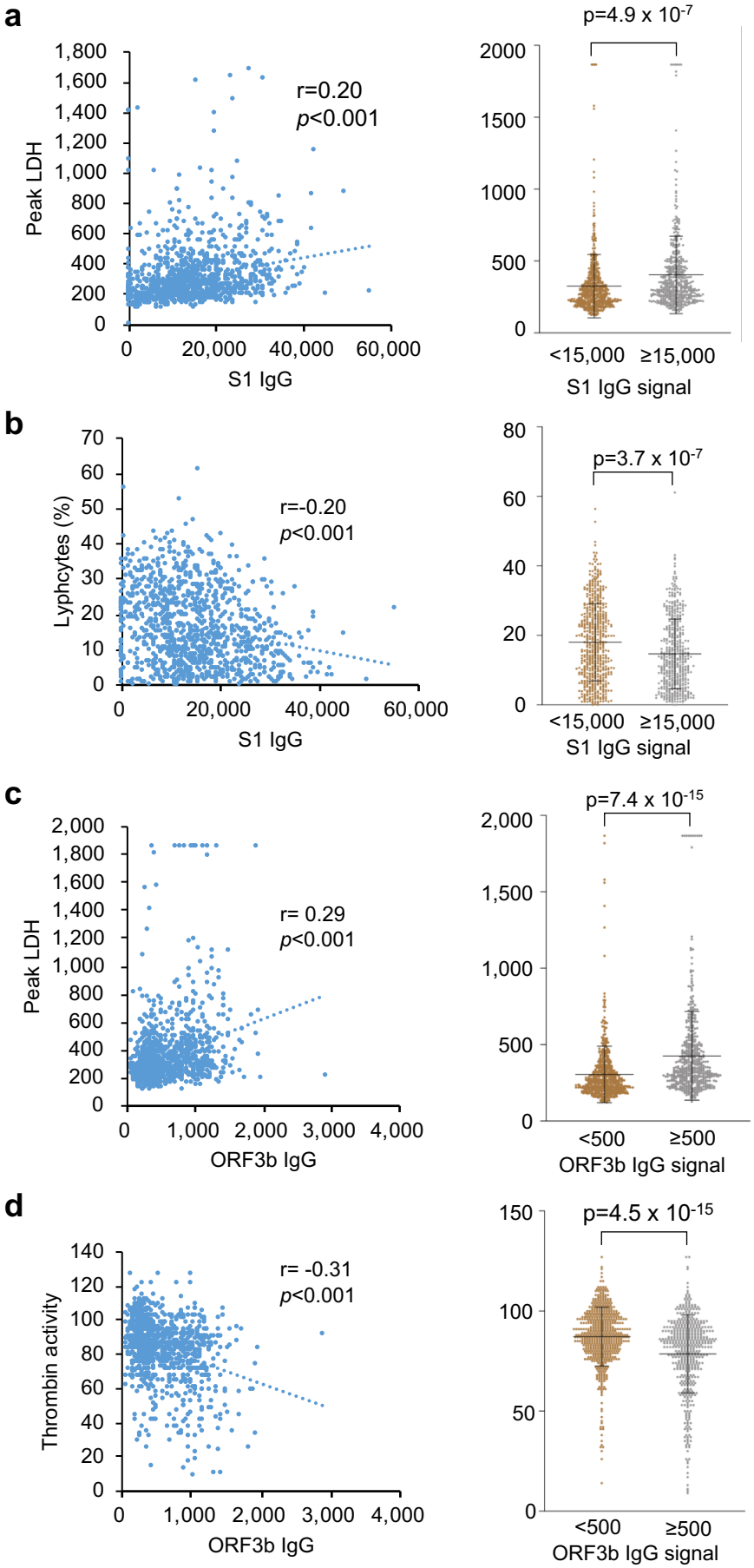


Figure S4. IgG responses are associated with clinical parameters (related to figure 3). a-d. Correlations and statistical analysis of IgG response against indicate proteins and clinical parameters. The right part for each panel depicts the distribution of the values for corresponding clinical parameter in lower and higher IgG response groups. *P*-values were calculated with two-sided *t* test.

Table S1. Serum samples and patients (related to Figure 1)

Group		COVID-19	Control-1	Control-2
Patients (n)		783	528	73
Serum samples (n)		2,360	528	73
Patients with samples >14 days after onset		756	-	-
Age		61.4±14.5	53.0 ± 20.5	N/A
Gender	**Male**	377	252	N/A
	Female	379	276	N/A
Severity/ outcome	**non-severe**	347	-	-
	Severe (survivors)	354		
	Severe (non-survivors)	55		
Source		Tongji Hospital, Wuhan	Tongren Hospital, Shanghai Ruijin Hospital, Shanghai	National Institutes for Food and Drug Control , Beijing, China
Subtype and number		-	Healthy: 142; Infection diseases: 141; Autoimmune diseases: 120; Lung cancer: 48; Other diseases: 77	Negative reference samples

Table S2. SARS-CoV-2 proteins included in the proteome microarray (related to Figure 1 and Figure S1)

Protein ID	Name	Resources	Concentration (mg/mL)	Tag(s)	Expression system
1	S1	Hangzhou Bioeast biotech (SC2S302)	0.17, 0.5	C-His	Mammalian Cells
2	N Protein	Our Lab	0.125	C-His	*E.coli*
	N Protein	VACURE Biotechnology (AG-PL-2101)	0.08, 0.25	C-His	Mammalian Cells
	N-Cter	Healthcode PROTN_nCoVN-CterHG01000	0.25	N-His/C-EGFP	Cell free(Yeast)
	N-Nter	Healthcode PROTN_nCoVN-NterHG01000	0.25	N-His/C-EGFP	Cell free(Yeast)
3	NSP1	Our Lab	0.125	C-His	*E.coli*
4	NSP2	Healthcode PROTN_nCoVNSP2HG01000	0.17, 0.5	N-His/C-EGFP	Cell free(Yeast)
5	NSP4	Our Lab	0.1	His-Trx/C-His	*E.coli*
6	NSP5	Healthcode PROTN_nCoV3ClpHG01000	0.17, 0.5	N-His/C-EGFP	Cell free(Yeast)
7	NSP7	Our Lab	0.125	C-His	*E.coli*
8	NSP8	Our Lab	0.25	C-His	*E.coli*
9	NSP9	Our Lab	0.25	C-His	*E.coli*
10	NSP10	Our Lab	0.17, 0.5	C-His	*E.coli*
11	RdRp	H. Eric Xu's Lab	0.17, 0.5	His	Insect Cells
12	NSP14	Healthcode PROTN_nCoVNSP14HG01000	0.17, 0.5	N-His/C-EGFP	Cell free(Yeast)
13	NSP15	Healthcode PROTN_nCoVNdUHG01000	0.17, 0.5	N-His/C-EGFP	Cell free(Yeast)
14	NSP16	Healthcode PROTN_nCoVOMTHG01000	0.17,0.5	N-His/C-EGFP	Cell free(Yeast)
15	ORF-3a	Our Lab	0.1	N-GST/C-His	*E.coli*
16	ORF-3b	Our Lab	0.1	N-GST/C-His	*E.coli*
17	ORF6	Our Lab	0.1	N-GST/C-His	*E.coli*
18	ORF-7b	Our Lab	0.125	N-GST/C-His	*E.coli*
19	ORF-9b	Our Lab	0.125	C-His	*E.coli*
20	E-protein	Healthcode PROTN_nCoVEHG01000	0.17, 0.5	N-His/C-EGFP	Cell free(Yeast)

Table S3 . IgG responses are associated with clinical parameters (related to Figure 3 and Figure S3)

	S1	N	NSP1	NSP7	NSP8	RdRp	ORF3b	ORF9b
Neutrophils(#)	0.13	0.04	0.18	0.15	0.08	0.18	0.26	0.11
Neutrophils(%)	0.23	0.12	0.23	0.18	0.11	0.22	0.29	0.14
LDH	0.2	0.11	0.21	0.15	0.1	0.23	0.29	0.13
Globulin	0.28	0.19	0.23	0.19	0.11	0.21	0.33	0.17
Urea	0.12	0.03	0.2	0.13	0.09	0.21	0.27	0.1
Bicarbonate	0.21	0.11	0.23	0.13	0.13	0.24	0.31	0.18
CRP	0.24	0.12	0.26	0.16	0.13	0.25	0.33	0.16
D-dimer	0.22	0.12	0.23	0.14	0.06	0.18	0.27	0.09
Fibrinogen	0.32	0.23	0.2	0.23	0.1	0.18	0.27	0.16
FDP	0.18	0.08	0.18	0.2	0.08	0.18	0.28	0.06
Myoglobin	0.06	-0.05	0.18	0.12	0.07	0.21	0.29	0.1
ESR	0.27	0.16	0.11	0.1	0.02	0.08	0.22	0.11
Lymphocyte(#)	-0.2	-0.12	-0.21	-0.15	-0.1	-0.2	-0.27	-0.13
Lymphocyte(%)	-0.23	-0.11	-0.22	-0.18	-0.1	-0.2	-0.28	-0.13
Platele count	-0.06	0.01	-0.18	-0.11	-0.07	-0.18	-0.26	-0.09
Eosinophils(#)	-0.17	-0.13	-0.18	-0.15	-0.12	-0.2	-0.25	-0.14
Eosinophils(%)	-0.18	-0.12	-0.21	-0.17	-0.14	-0.22	-0.27	-0.16
Plateletcrit	-0.07	0.01	-0.19	-0.11	-0.07	-0.18	-0.26	-0.1
Calcium	-0.25	-0.12	-0.25	-0.17	-0.14	-0.28	-0.36	-0.17
Total cholesterol	-0.13	-0.09	-0.21	-0.11	-0.06	-0.2	-0.26	-0.13
Albumin	-0.32	-0.16	-0.27	-0.18	-0.14	-0.27	-0.36	-0.19
Albumin/ globulin	-0.35	-0.23	-0.27	-0.2	-0.14	-0.26	-0.37	-0.21
Prothrombin activity	-0.14	-0.08	-0.23	-0.26	-0.11	-0.21	-0.31	-0.1
Phosphorus	-0.13	-0.07	-0.18	-0.16	-0.06	-0.17	-0.3	-0.08
Antithrombin	-0.09	-0.04	-0.23	-0.19	-0.1	-0.2	-0.28	-0.11
LDL	-0.04	-0.01	-0.17	-0.08	-0.05	-0.17	-0.29	-0.15
HDL	-0.09	-0.03	-0.2	-0.05	-0.06	-0.21	-0.32	-0.15
LDL+HDL	-0.06	-0.02	-0.2	-0.08	-0.06	-0.2	-0.33	-0.16
Cholinesterase	-0.12	-0.04	-0.21	-0.12	-0.08	-0.22	-0.32	-0.15
Prealbumin	-0.06	-0.02	-0.19	-0.1	-0.1	-0.2	-0.29	-0.16
Free T3	-0.19	0.01	-0.2	-0.12	-0.06	-0.13	-0.26	-0.07

Red color marks the correlation coefficients more than 0.2, and the green color marks the correlation coefficients less than -0.2.