Supplemental Material and Methods

Control samples

The specificity control samples comprised serum samples from 130 hospitalized patients with pneumonia, of whom 46 were treated at an ICU (59 female, 71 male, median age: 61 years, range: 1-93). These samples had been collected between July 2019 and February 2020 and were sent to the Centre for Virology for serological routine screening testing by complement binding fixation (CBF). PCR from corresponding respiratory specimens and/or serology confirmed infection with Influenza A (INA) and B (INB) viruses (n=7), Rhinoviruses (n=3), Respiratory Syncytial virus (n=3; RSV), Adenovirus (n=1; ADV), Herpes simplex virus type 1 (n=2), Human Cytomegalovirus (n=2), Mycoplasma pneumoniae (n=2, MCP) or Legionella pneumophila (n=1). In all other cases, serum samples tested negative for INA, INB, RSV, ADV, MCP, parainfluenza, and enteroviruses using CBF. Serum samples collected between January and February 2020 (n=13) were only included when there was a corresponding respiratory sample, and this sample tested negative for SARS-CoV-2 by PCR. After routine testing, these samples were anonymized and integrated into a sample bank for future comparative immunoassay studies. Since these anonymized samples had been acquired in the past, the local ethics committee concluded that no written consent from control individuals was required for this study (EK 2156/2019).

PCR

For PCR analyses, nucleic acid was extracted from the nasopharyngeal swab and tracheal aspirate samples using the NucliSens EasyMag extractor, according to the manufacturer's instructions (Biomerieux, Marcy l'Etoile, France). SARS-CoV-2 real-time TaqMan PCR was performed with primers and probe recommended by the WHO and located in the E-gene, as

described previously [1]. Sensitive detection was confirmed using a proficiency panel from Instand (Instand, Düsseldorf, Germany).

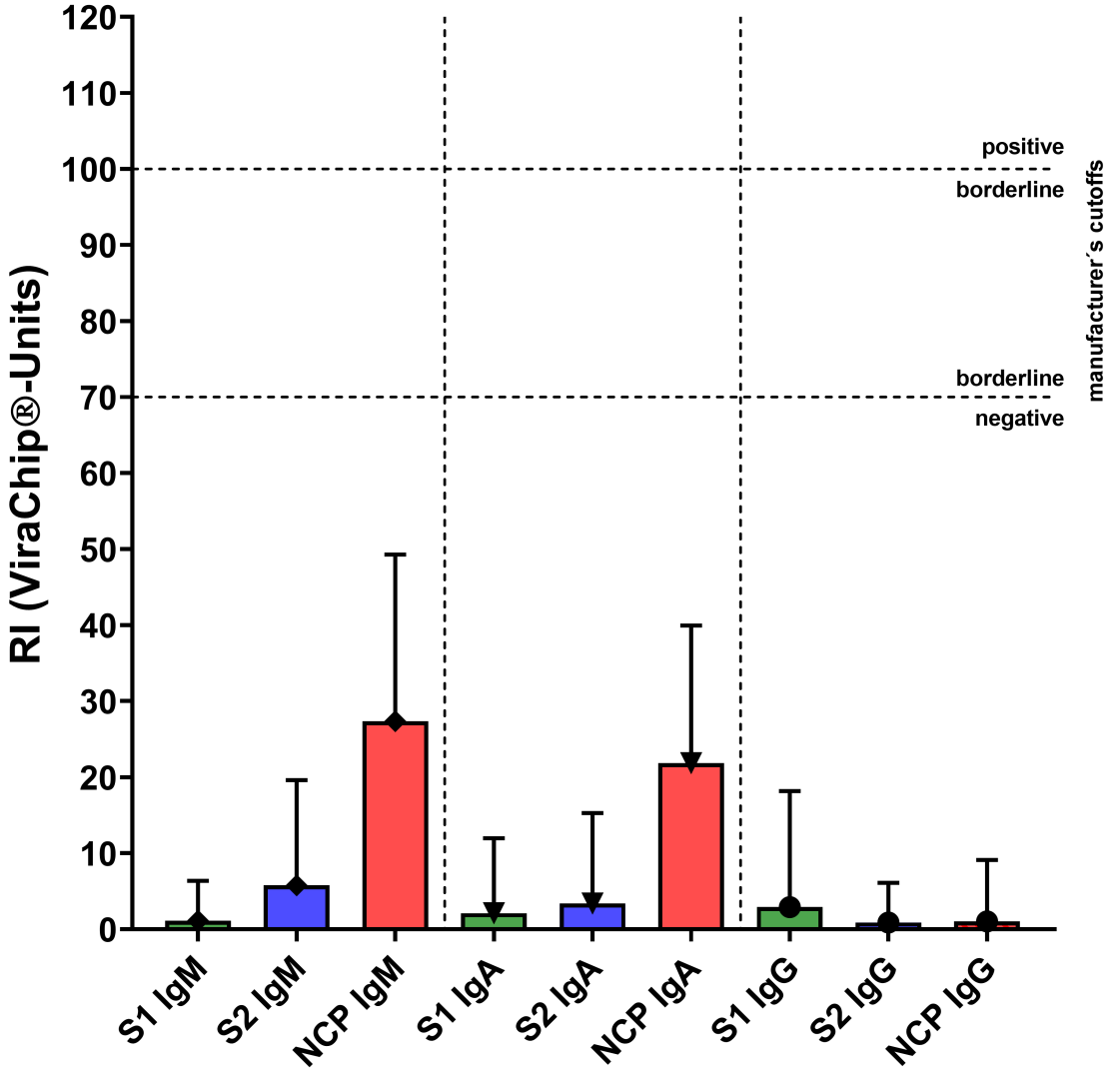
Serum dilution

The automated ELISA system for the assessement of S1-specific IgA antibodies (Euroimmun SARS-CoV-2 IgA ELISA on an Euroimmun Analyzer I; both Lübeck, Germany) indicates when the measured optical density (OD) exceeds the linear limit of the standard curve at a dilution of 1:101 (the dilution factor the manufacturer recommends), and in this case does not report results as antibody ratios for respective samples. In order to correlate IgA antibody levels with disease severity nevertheless, we then further diluted thesamples from 1:5120 to 1: 20.480 and remeausred the samples until the measured OD decreased linearly with dilution and then recalculated the antibody Ratio accordingly.

Reference:

1. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill **2020**; 25.

Supplemental Figure S1: S1, S2 and NCP-specific IgM, IgA, and IgG antibody levels in controls



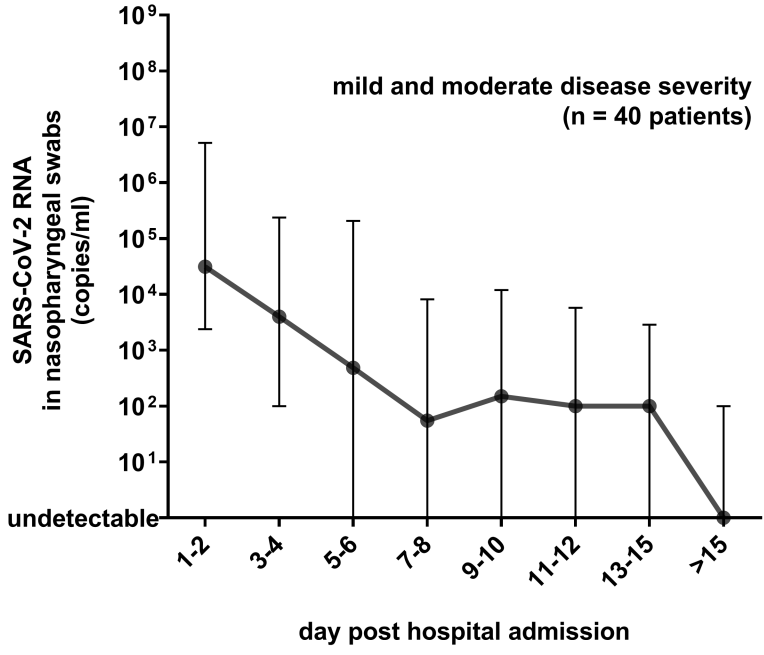
bars indicate the mean
error bars indicate Std. Deviation

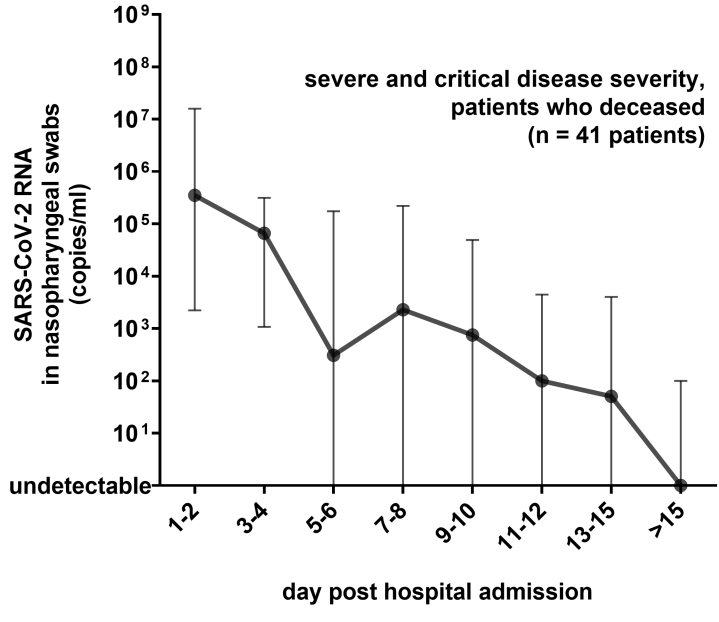
	IgM			IgA			IgG		
	S1	S2	NCP	S1	S2	NCP	S1	S2	NCP
mean (RI, ViraChip®-Units)	1.13	5.77	27.36	2.12	3.39	21.83	2.90	0.89	1.02
Std. Deviation	5.24	13.83	21.31	8.92	11.89	18.12	15.28	5.21	8.10
cut-off level (RI, ViraChip®-Units)	20	40	70	20	30	60	40	20	20

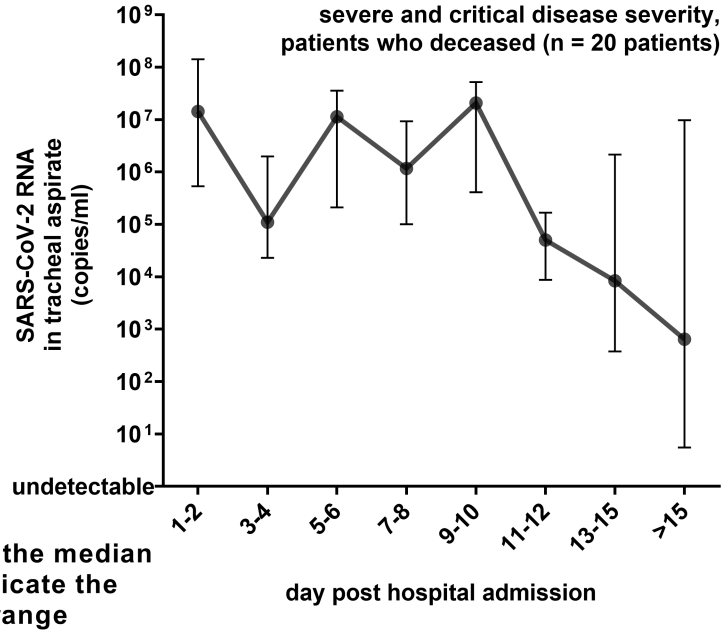
Supplemental Table S1: Overview of the SARS-CoV-2 immunoassays (ELISAs/CLIAs) used in the study

Test	Manufacturer	Principle	Instrument	Target Antigen	Immunglobulin class	Unit	cutoff		
							negative	borderline	positive
SARS-CoV-2 IgM, IgA, and IgG ViraChip® assay	Viramed, Planegg, Germany	Microarray	ViraChip® Scanner	S1, S2, NCP, respectively	IgM, IgA, IgG, respectively	ViraChip®-Units	shown in Supplemental Figure 1		
SARS-CoV-2 IgA ELISA	Euroimmun, Lübeck, Germany	ELISA	Euroimmun Analyzer I	S1	IgA	Ratio	<0.8	0.8-1.1	>1.1
SARS-CoV-2 IgG ELISA					IgG				
NCP-SARS-CoV-2 IgM ELISA				NCP	IgM				
NCP-SARS-CoV-2 IgG ELISA					IgG				
Wantai SARS-CoV-2 IgM ELISA	Wantai Biological Pharmacy Ent, Beijing, China		Bio-Tek Instruments ELx808	RBD of S1	IgM		<0.9	0.9-1.1	>1.1
Wantai SARS-CoV-2 Ab ELISA					total Ig				
LIAISON® SARS-CoV-2 IgG CLIA	Diasorin, Saluggia, Italy	CLIA	Diasorin XL Liasion	S1, S2	IgG	AU/ml	<12	12-15	>15
Platelia SARS-CoV-2 Total Ab Assay	Bio-Rad Laboratories, Inc., Hercules, USA	ELISA	Bio-Tek Instruments ELx808	NCP	total Ig	Index R	<0.8	0.8-1	>1
Elecsys Anti-SARS-CoV-2 ECLIA	Roche, Basel, Switzerland	ECLIA	Cobas e411	NCP	total Ig	COI	<1		≥1
COVID-19 ELISA IgM+IgA	Vircell, Valencia, Spain	ELISA	Bio-Tek Instruments ELx808	S, NCP	IgM, IgA	Index	<6	6-8	>8
COVID-19 ELISA IgG					IgG		<4	4-6	>6

Supplemental Figure S2: SARS-CoV-2 RNA concentration





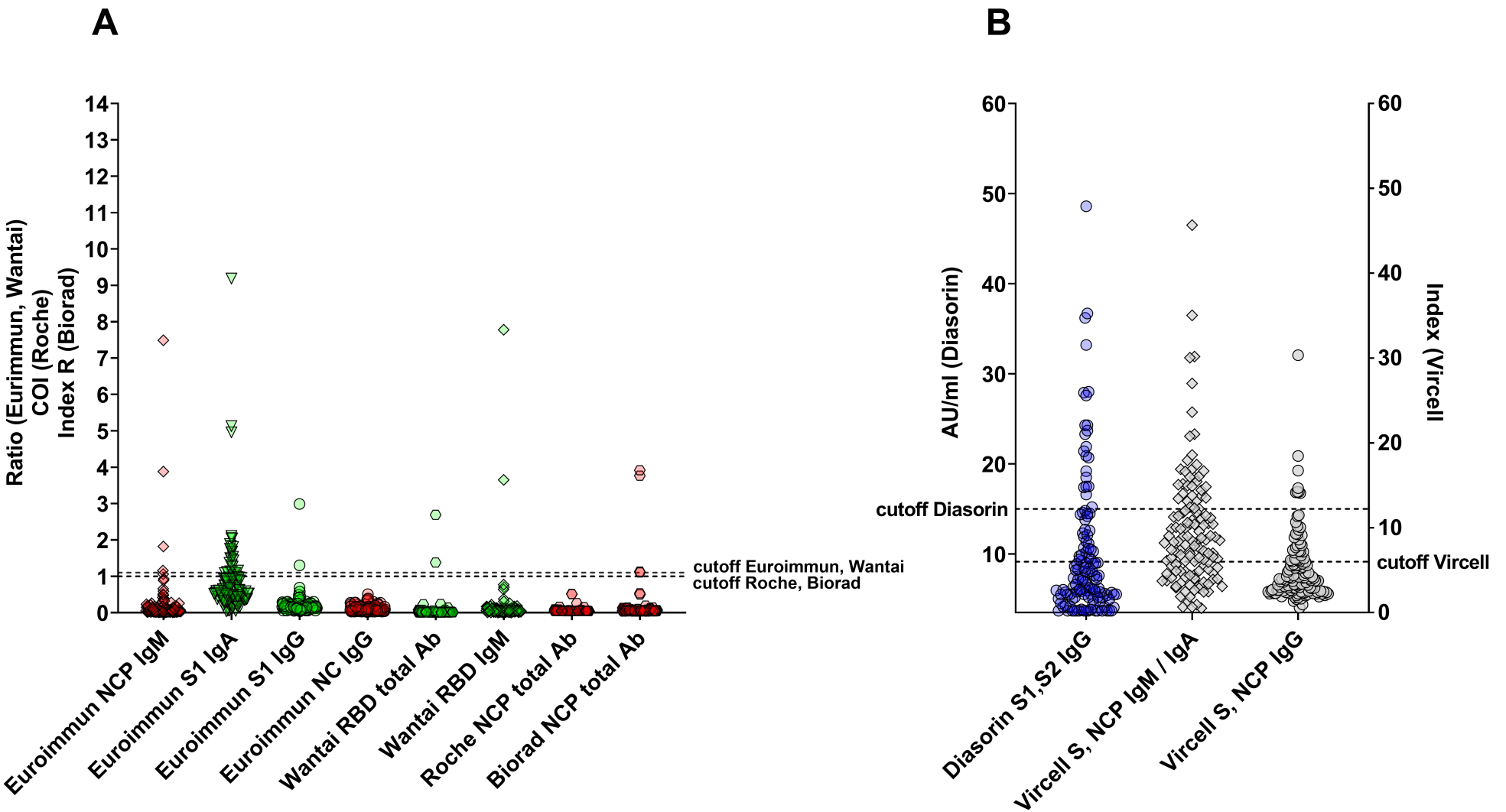


lines indicate the median
error bars indicate the interquartile range

Supplemental Table S2: sample number per interval (using one sample per patient per interval-step)

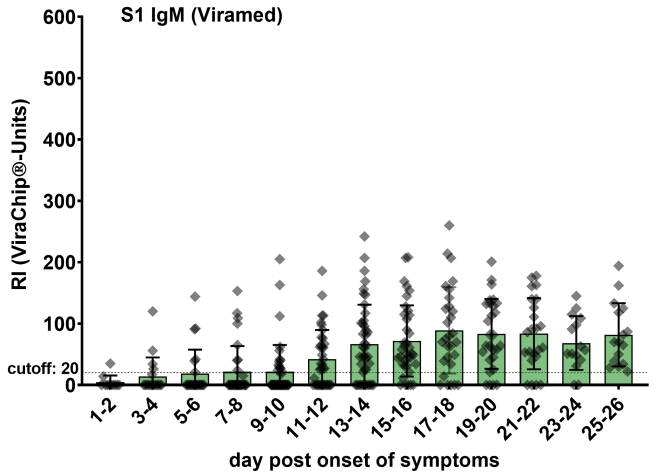
days post onset of symptoms	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26
number of samples (n)	11	18	23	31	42	38	43	37	29	28	23	16	15

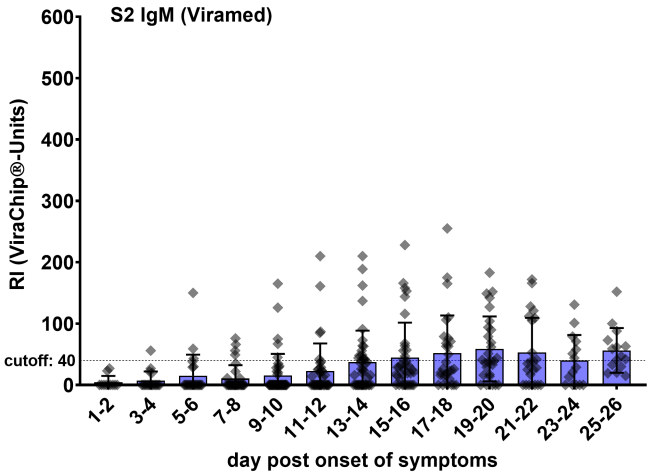
Supplemental Figure S3: antibody levels in controls

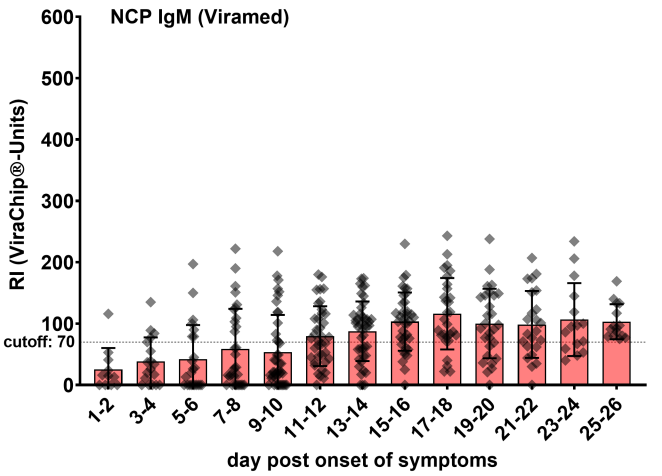


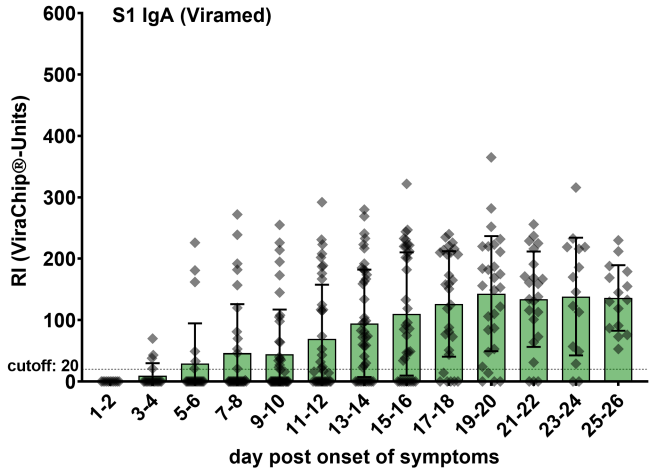
Supplemental Figure S4: Antibody levels (Microarray)

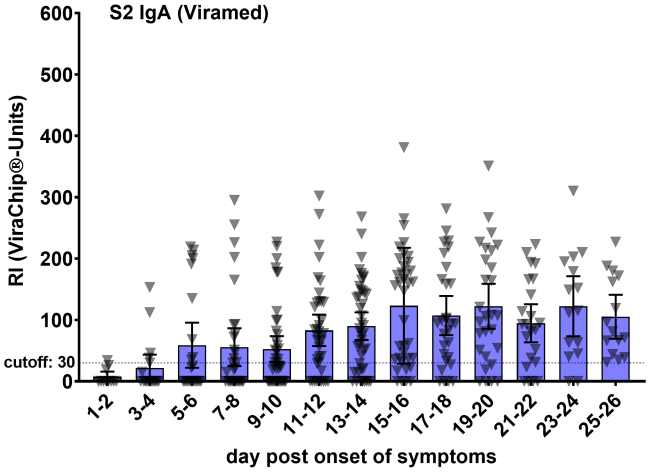
bars indicate the mean,
error bars indicate the standard deviation

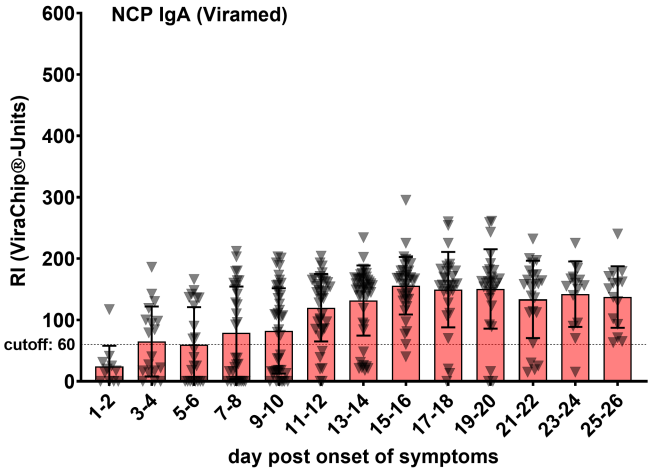


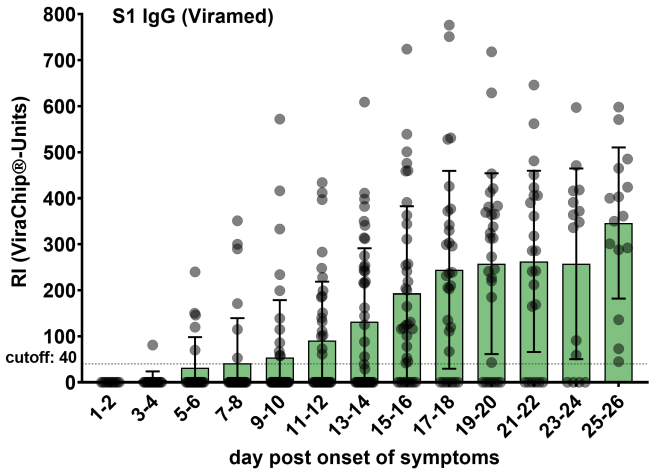


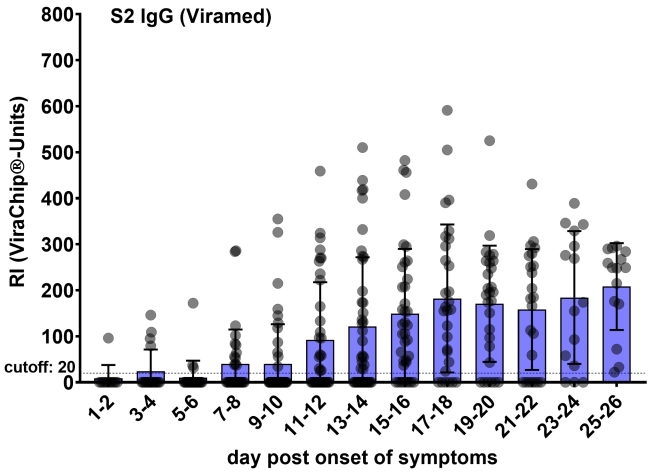


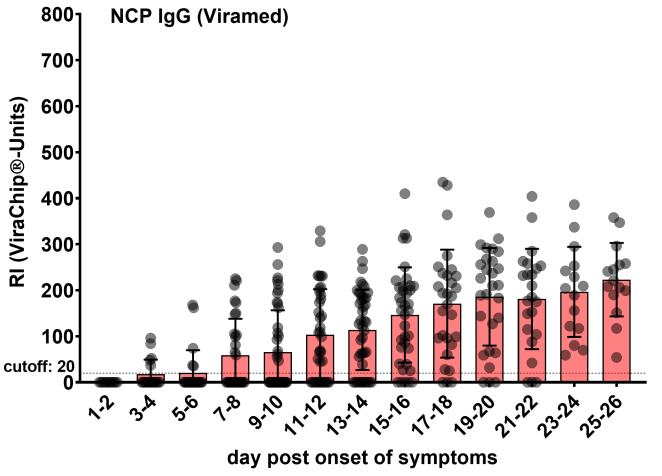






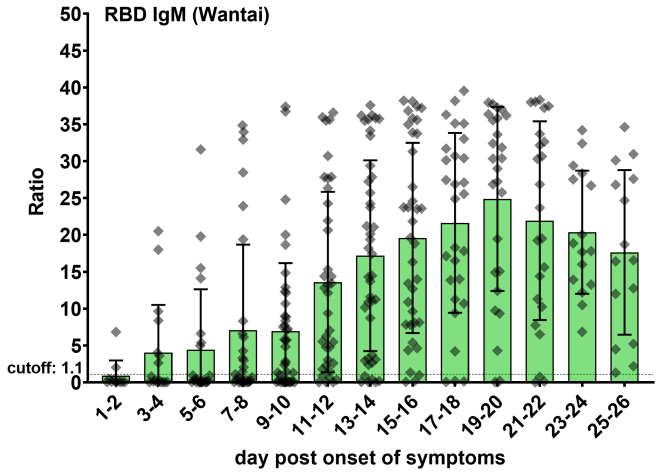


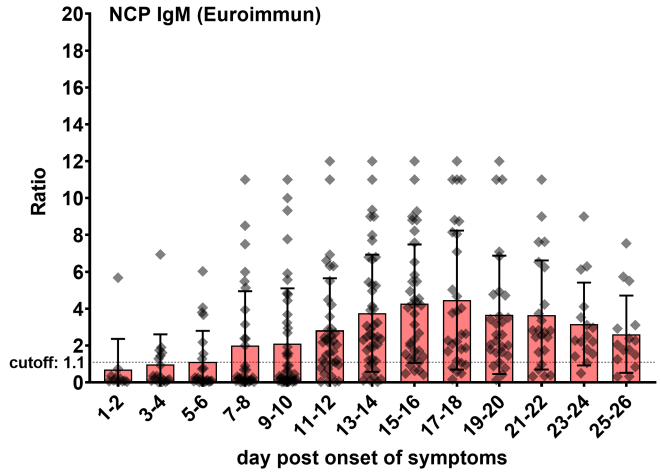


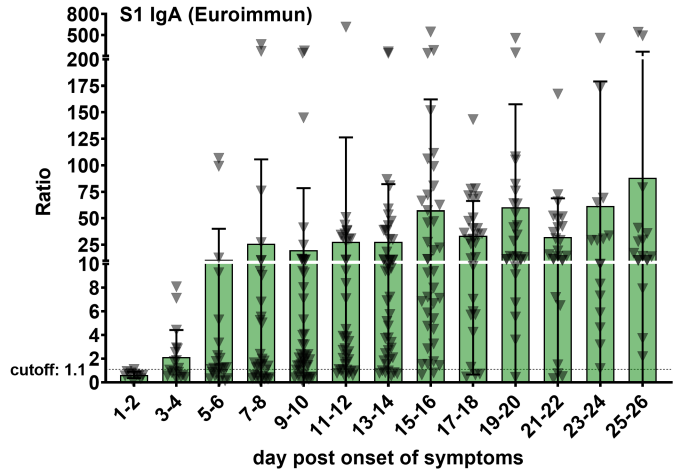


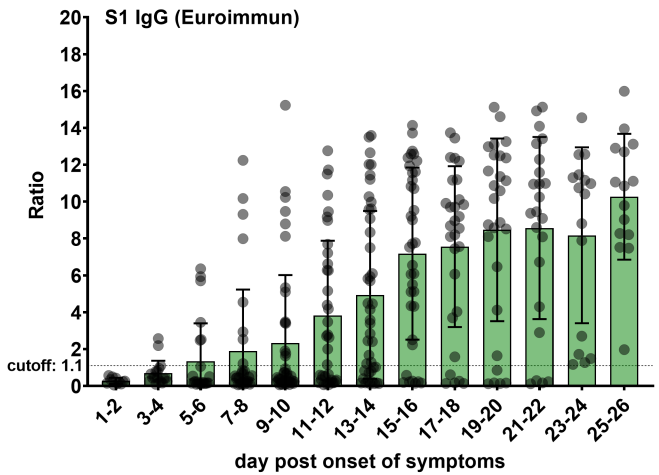
Supplemental Figure S5: Antibody levels (ELISAs and CLIAs)

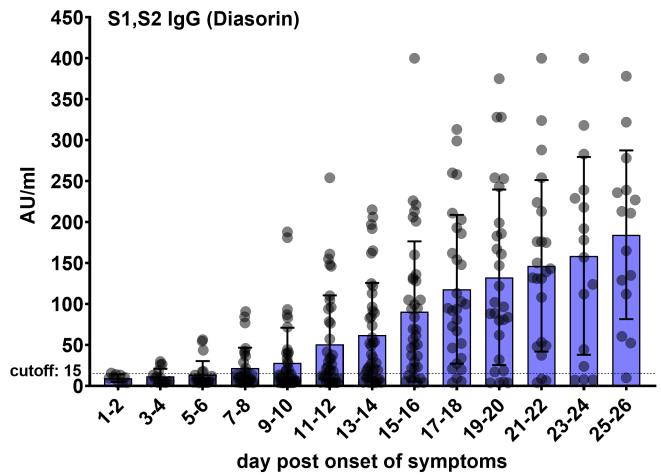
bars indicate the mean,
error bars indicate the standard deviation

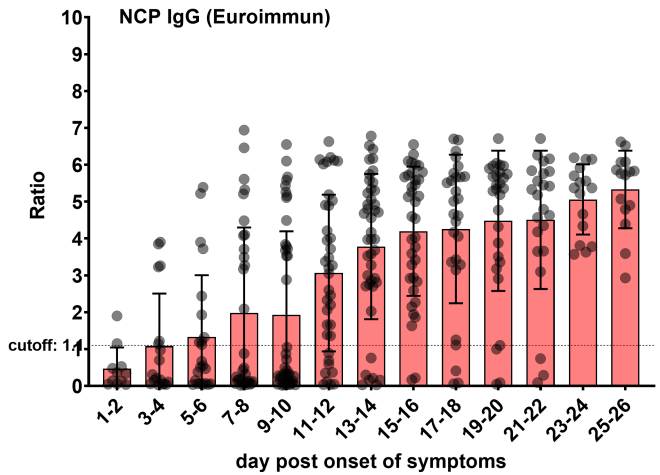


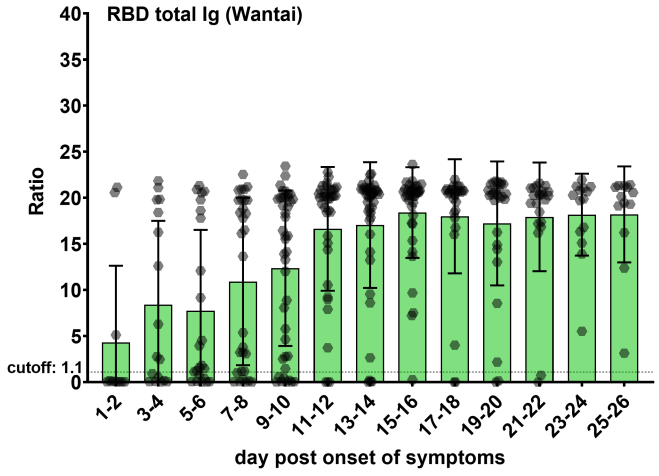


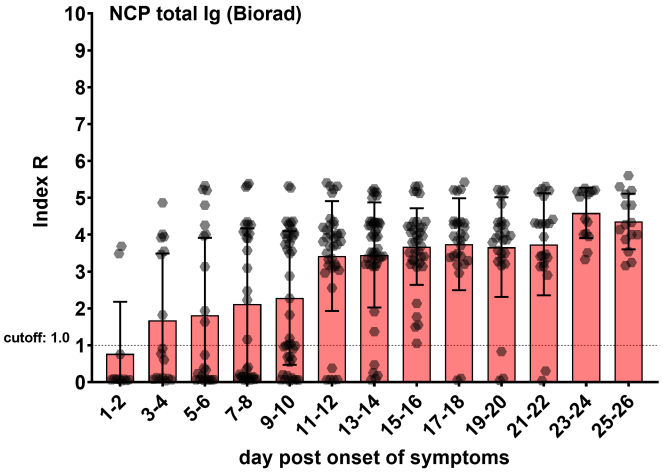


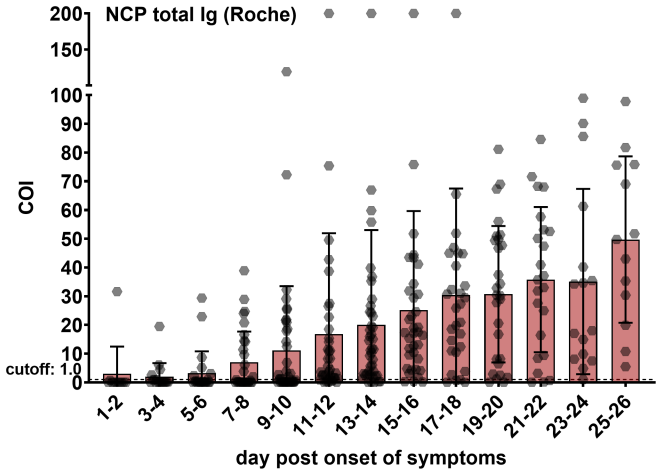




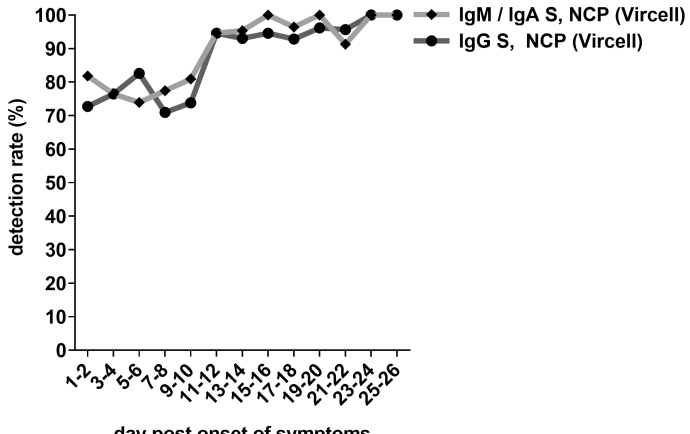


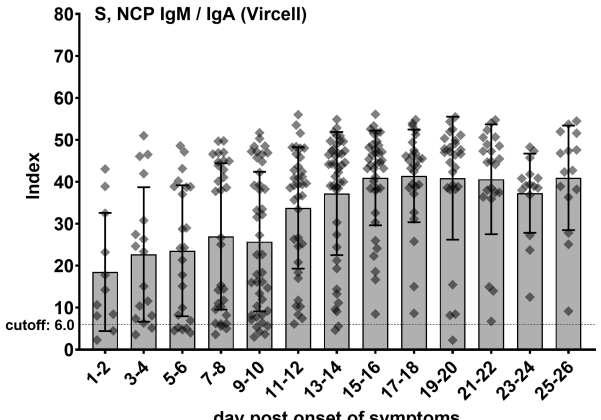


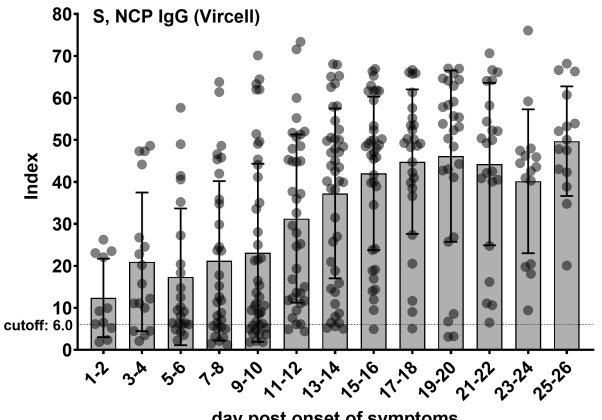




Supplemental Figure S6: detection rates and antibody levels of immunoassays with low specifity

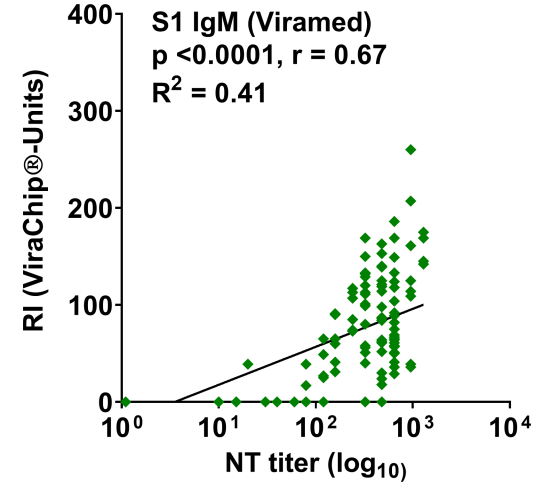


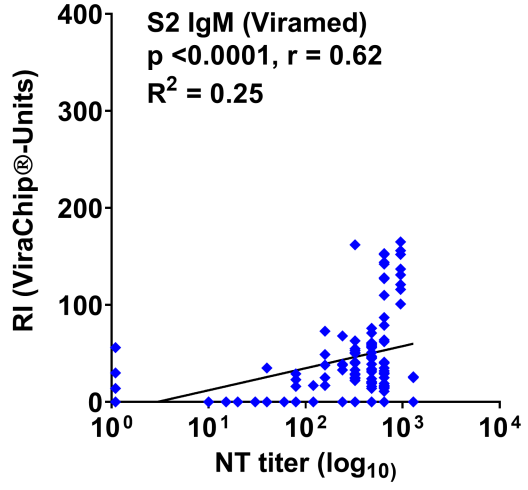


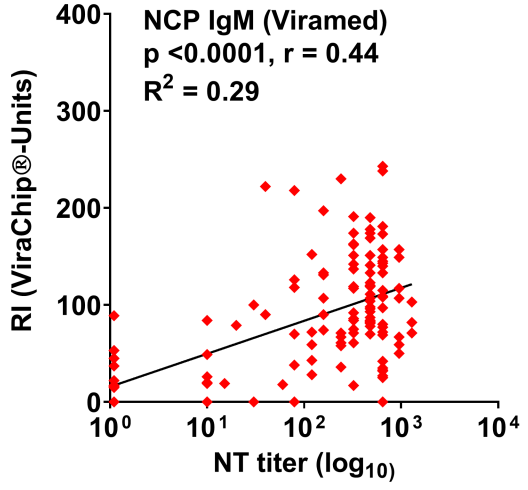


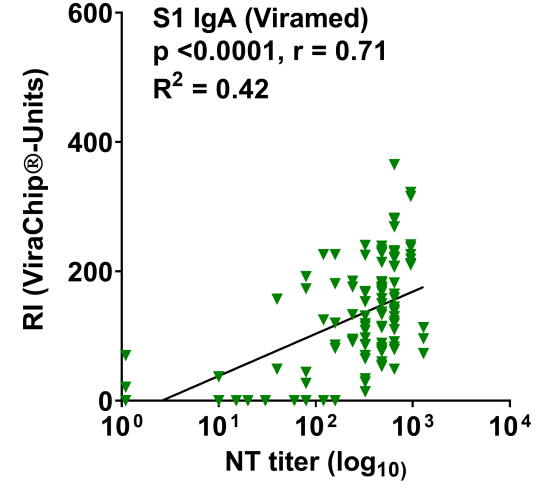
bars indicate the mean, error bars indicate the standard deviation

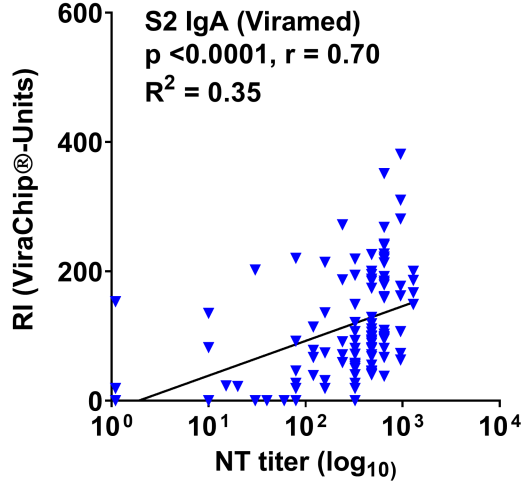
Supplemental Figure S7: correlation of the results by the microarray and the NT

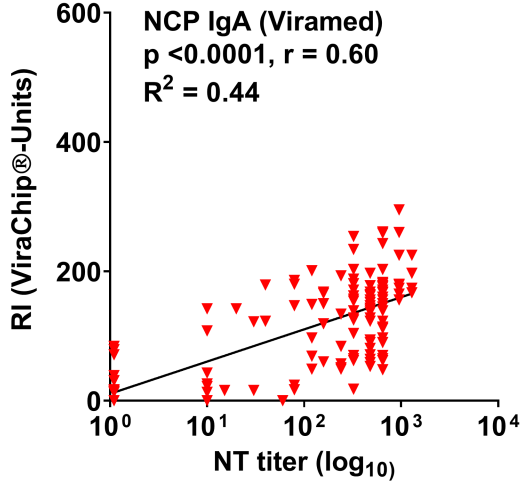


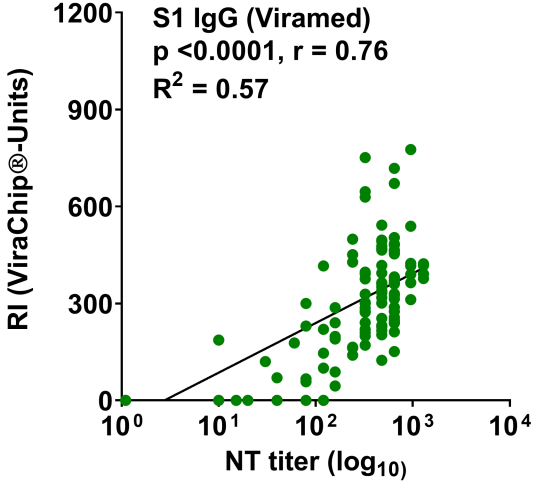


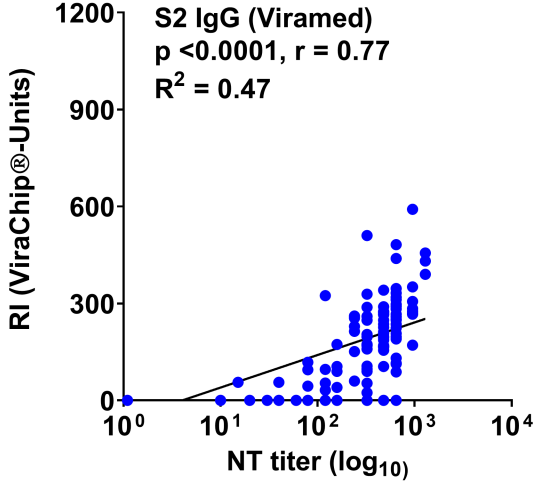


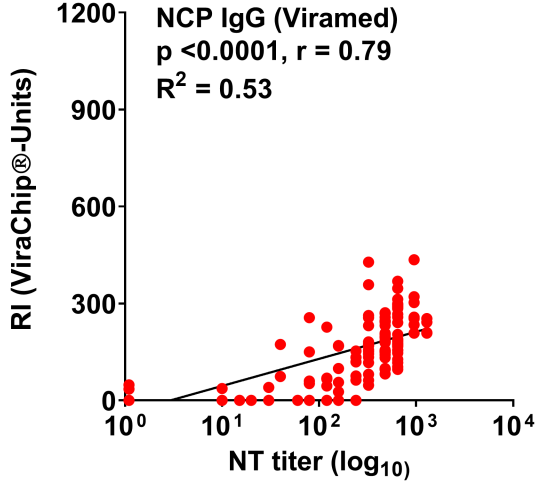




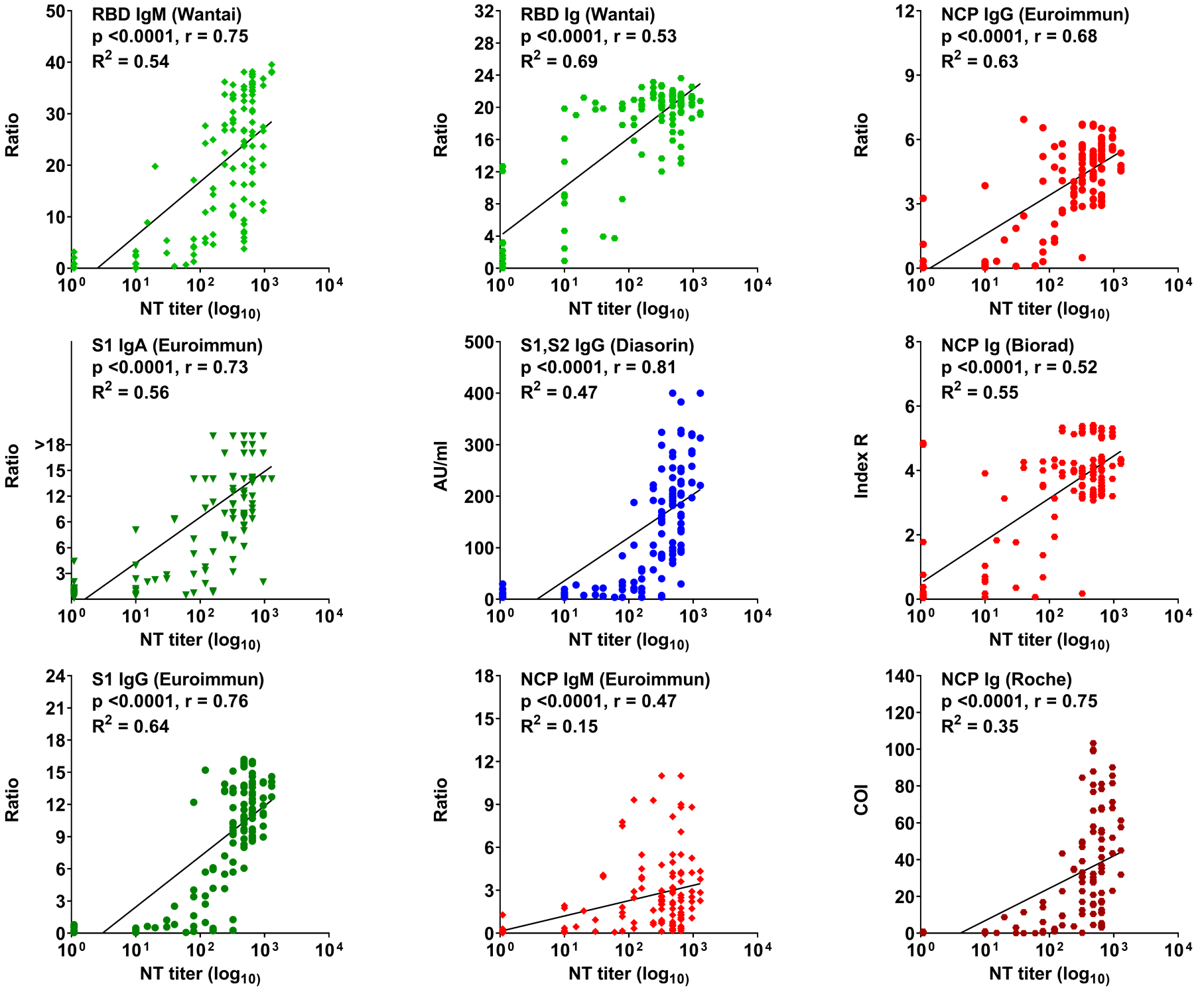








Supplemental Figure S8: correlation of the results by the ELISAs/CLIAs and the NT



Supplemental Figure S9:
Antibody levels in relation to disease severity as assessed with the other immunoassays (not shown in Figure 7)