**Supplementary Information**

# Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity

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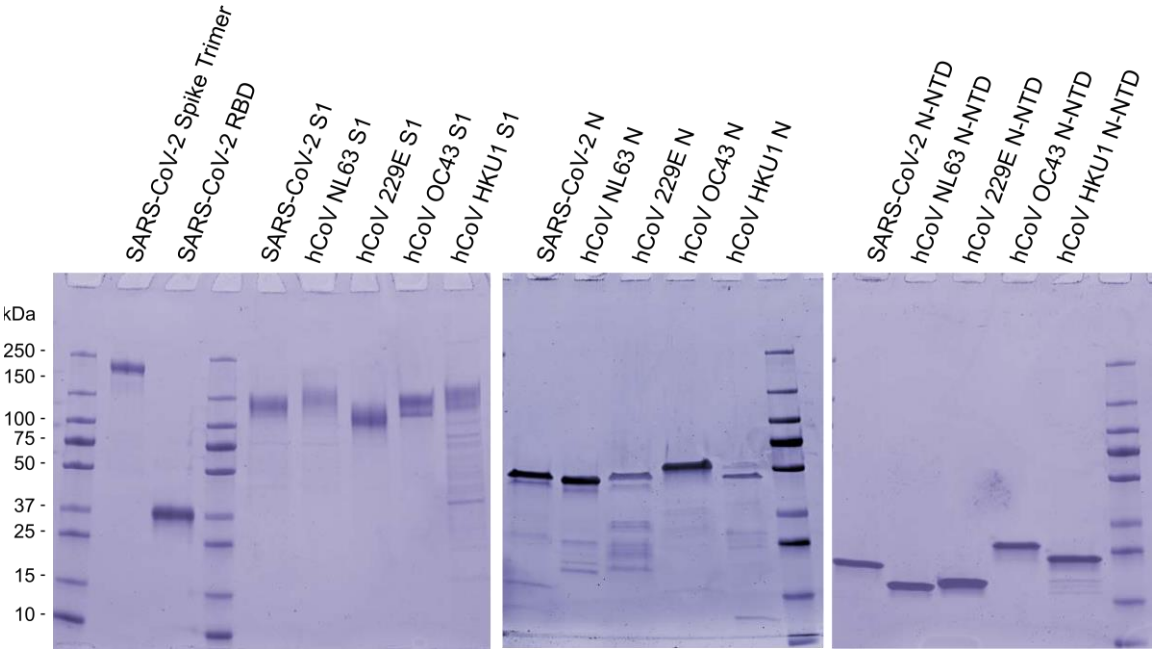
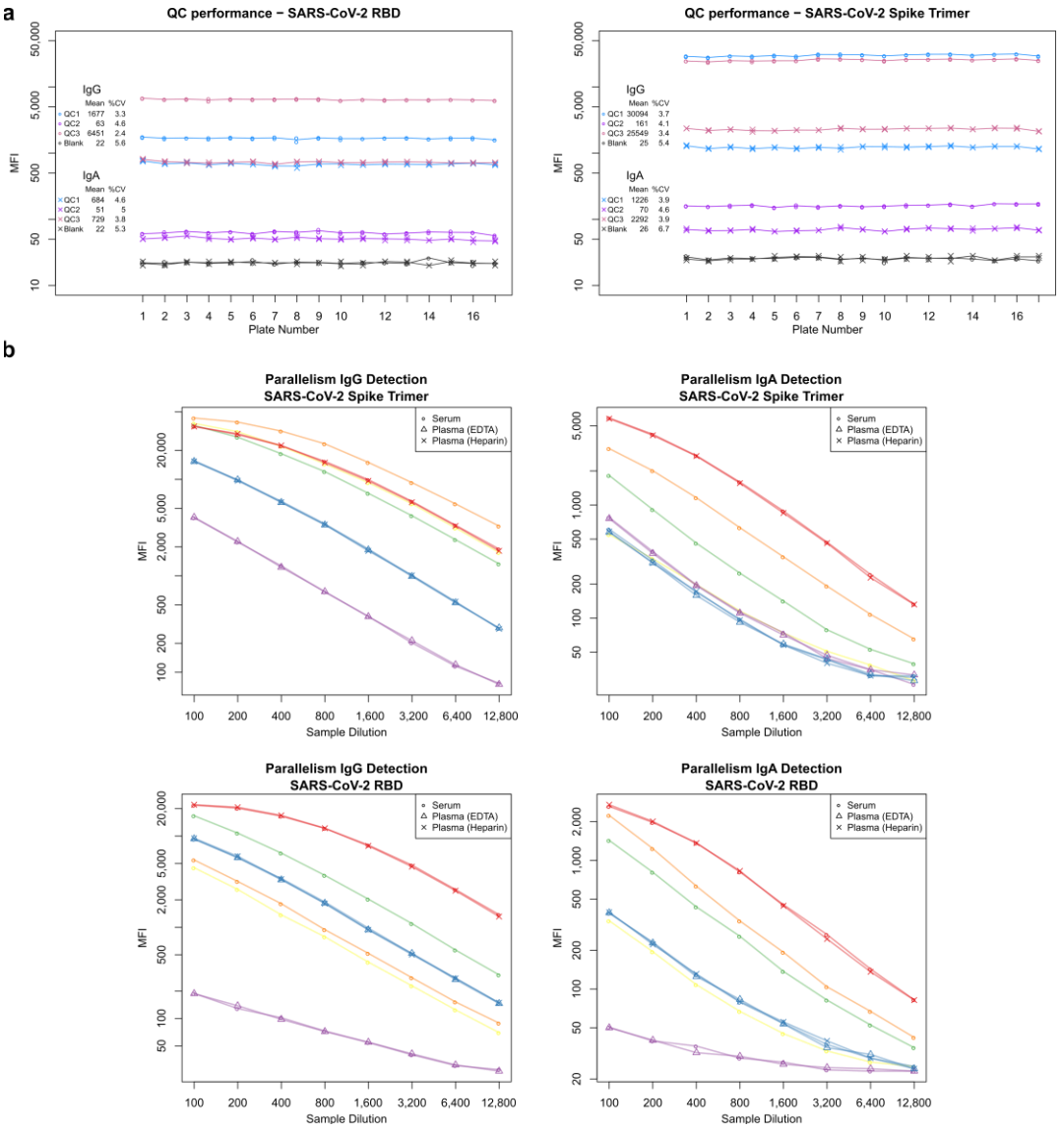


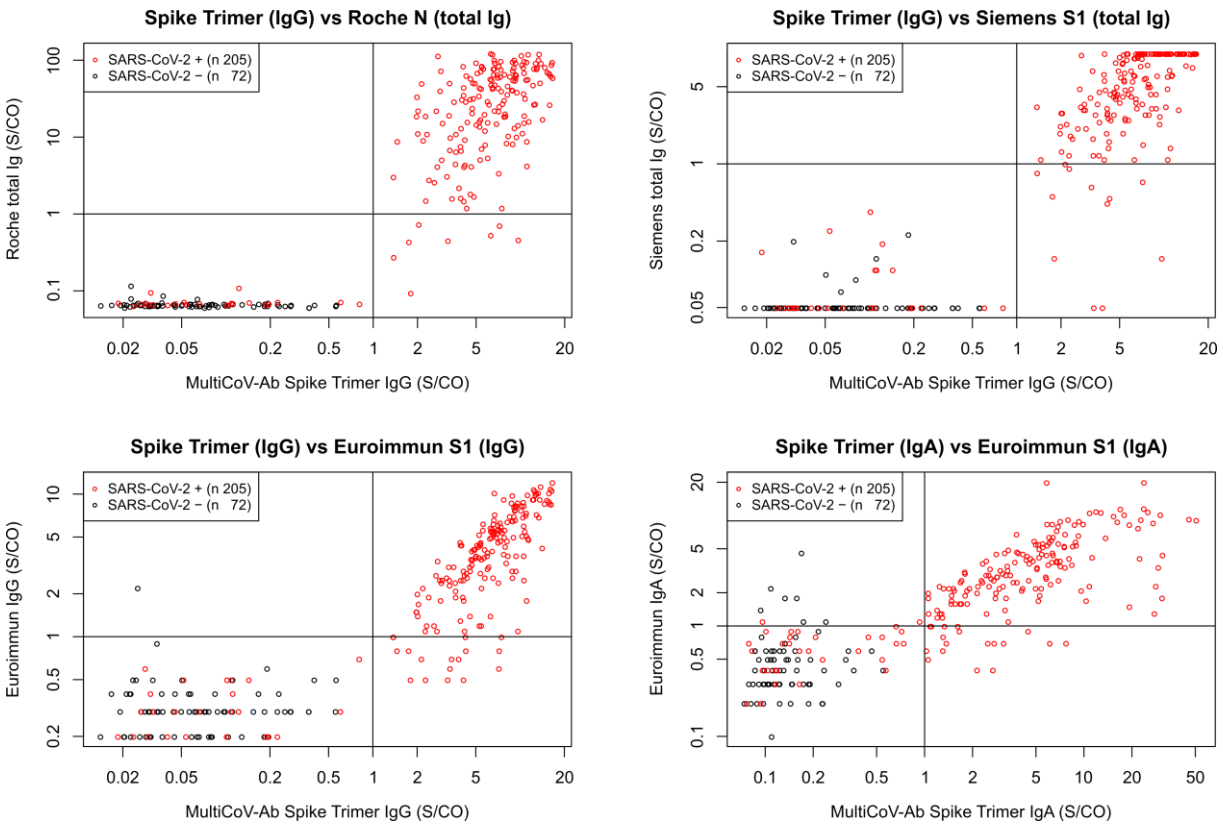

**Supplementary Figure 1**

SDS-PAGE analysis of the recombinant viral antigens used in this study. To test for purity and integrity 1 - 2 µg of indicated recombinant proteins were boiled in reducing SDS-sample buffer and subjected to a gradient (4 – 20 %) SDS-PAGE followed by Coomassie staining. SARS-CoV-2 Spike Trimer, SARS-CoV-2 RBD and the S1-domains of SARS-CoV-2, hCoV-NL63, hCoV-229E, hCoV-OC43 and hCoV-HKU1 were produced in ExpiHEK™ cells. Nucleocapsid (N) and N-terminal domain of nucleocapsid (N-NTD) of SARS-CoV-2, hCoV-NL63, hCoV-229E, hCoV-OC43 and hCoV-HKU1 were produced in *E.coli*. The image is an assembly of all expressed proteins and is representative for single protein purity after purification. Source data are provided as a Source Data file.



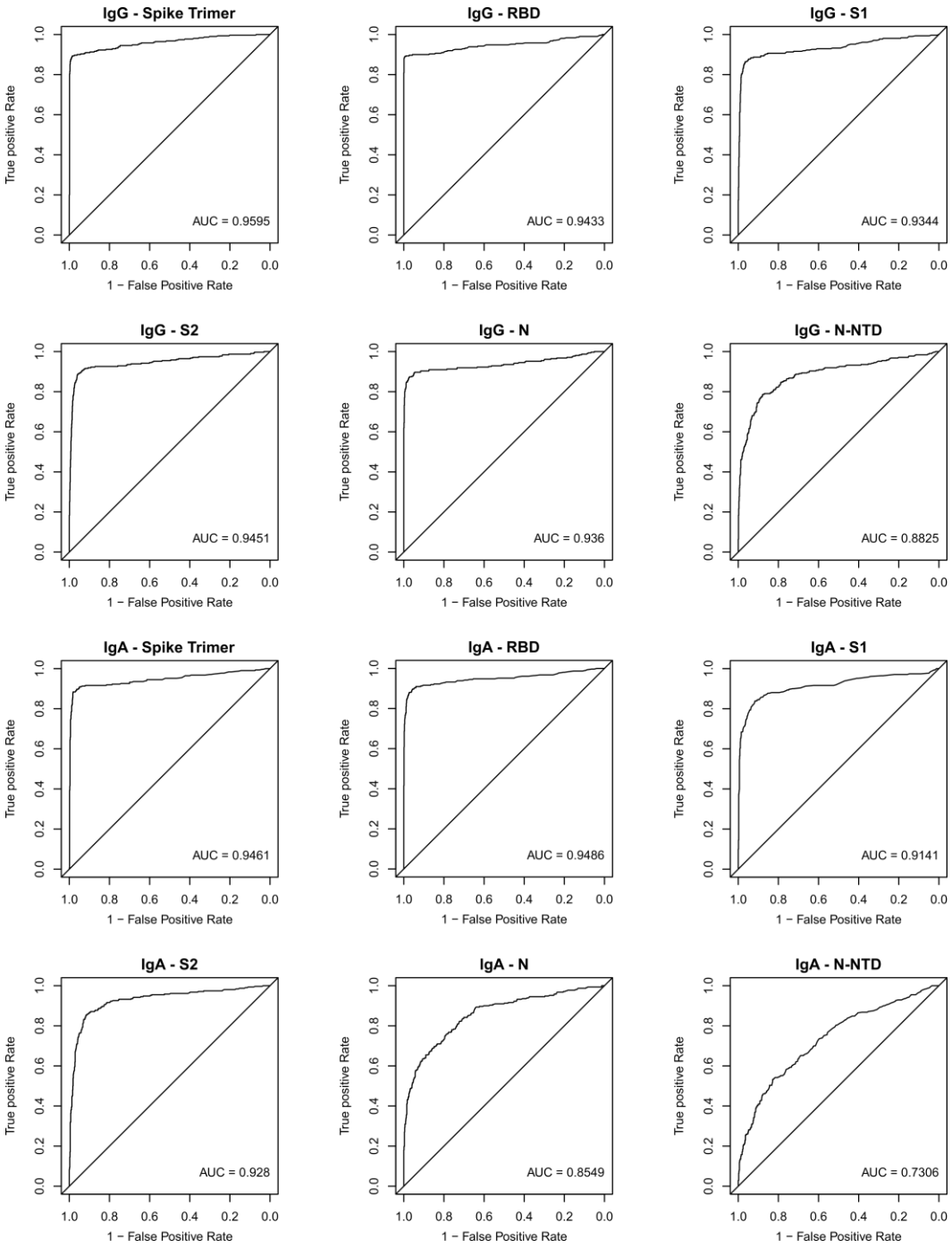

**Supplementary Figure 2**

**a,** Three quality control (QC) samples, as well as a sample of assay buffer (blank sample) were processed in duplicates on every plate. Performance across 17 assay runs is depicted and mean and %CVs are shown on the left side (n=34). For plate 14, a processing error lead to exclusion of one blank sample from this evaluation. **b,** To assess parallelism of signals from different samples, 6 unique serum samples were processed over a dilution series of 8 steps from 1:100 to 1:12,800. For 3 samples, paired plasma (EDTA and/or Heparin) were available and processed together. For IgG and IgA detection of Spike Trimer and RBD, MFI are plotted against sample dilution. Color indicates unique sample and shapes indicate sample type. The data represent a single measurement per sample and dilution (n=1). Source data are provided as a Source Data file.



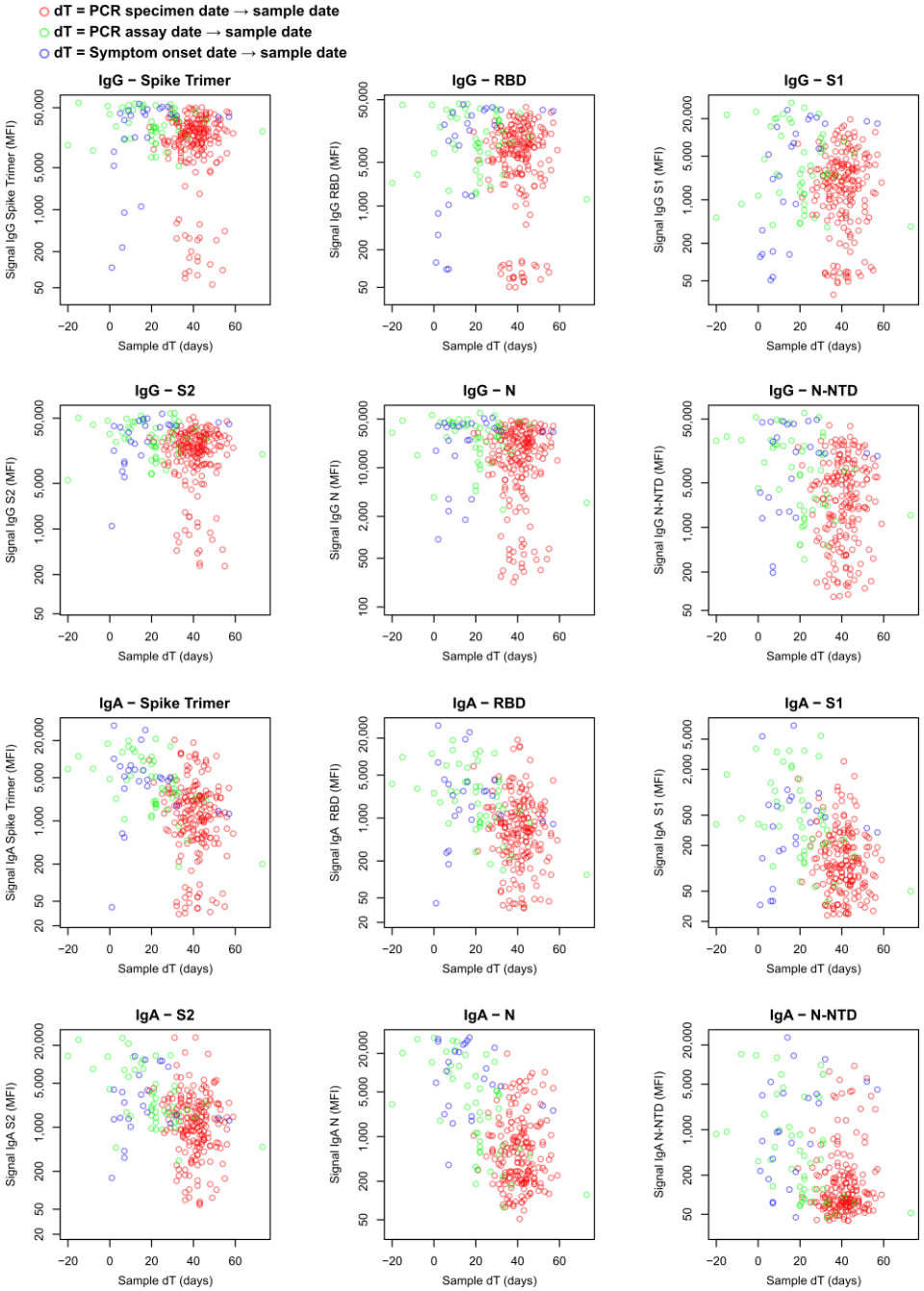

**Supplementary Figure 3**

Scatterplots of sample set with defined SARS-CoV-2 infection status (infected: red, n=205; uninfected: black, n=72) to compare performance of the MultiCoV-Ab Spike Trimer vs indicated antigens of commercial SARS-CoV-2 test kits. Signals are depicted as Signal to cut-off ratios (S/CO) on a logarithmic scale. Lines indicate the respective cut-off values as defined by the manufacturer to determine positive and negative test results. Source data are provided as a Source Data file.



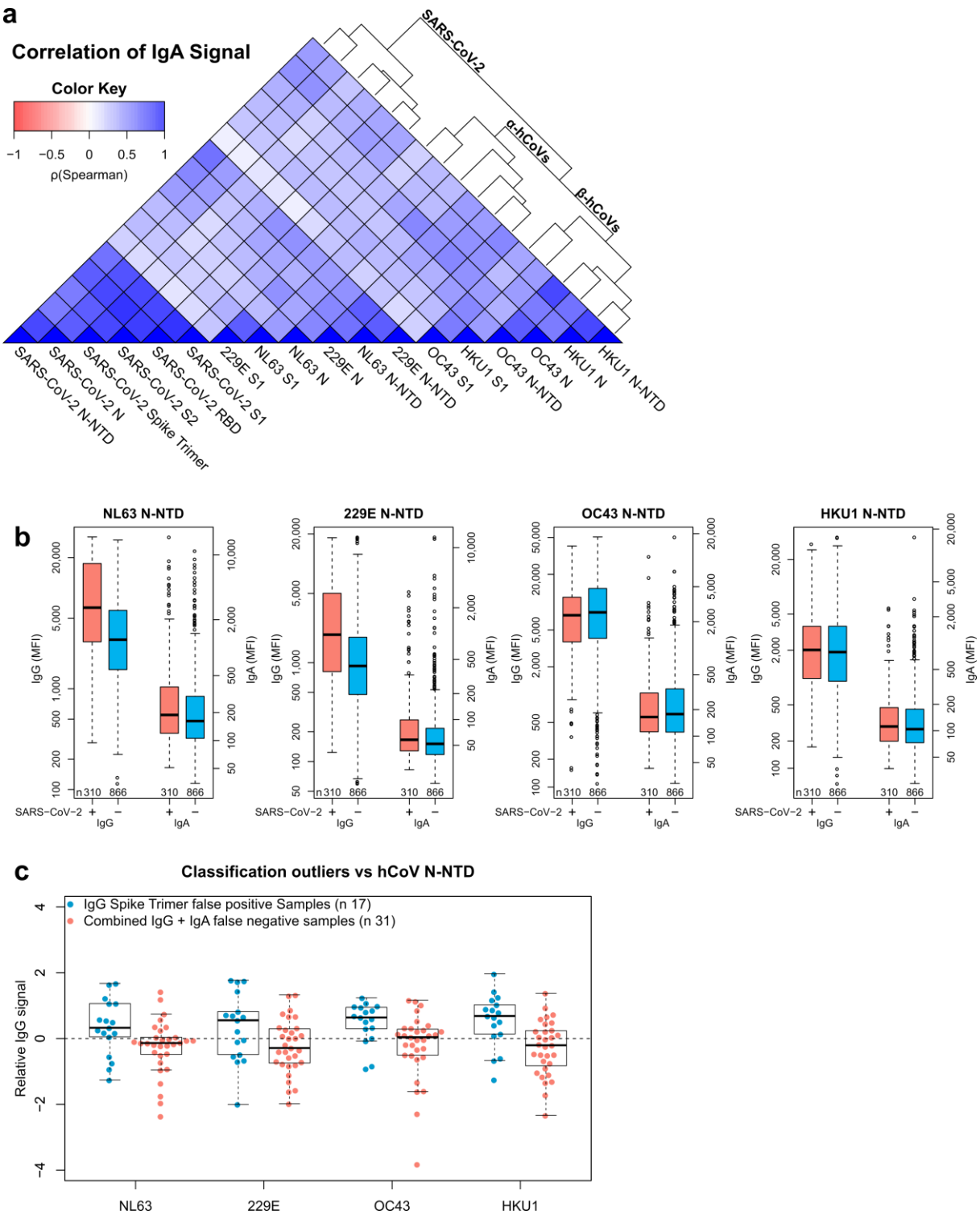

**Supplementary Figure 4**

ROC analysis[1,2] for IgG and IgA detection of SARS-CoV-2 antigens based on the extended sample set of 866 uninfected and 310 infected samples used for clinical validation for MultiCoV-Ab. True positive rate is displayed against 1 - false positive rate, corresponding to sensitivity and specificity at a given cut-off. AUC-values indicating individual antigen performance are shown. Source data are provided as a Source Data file.



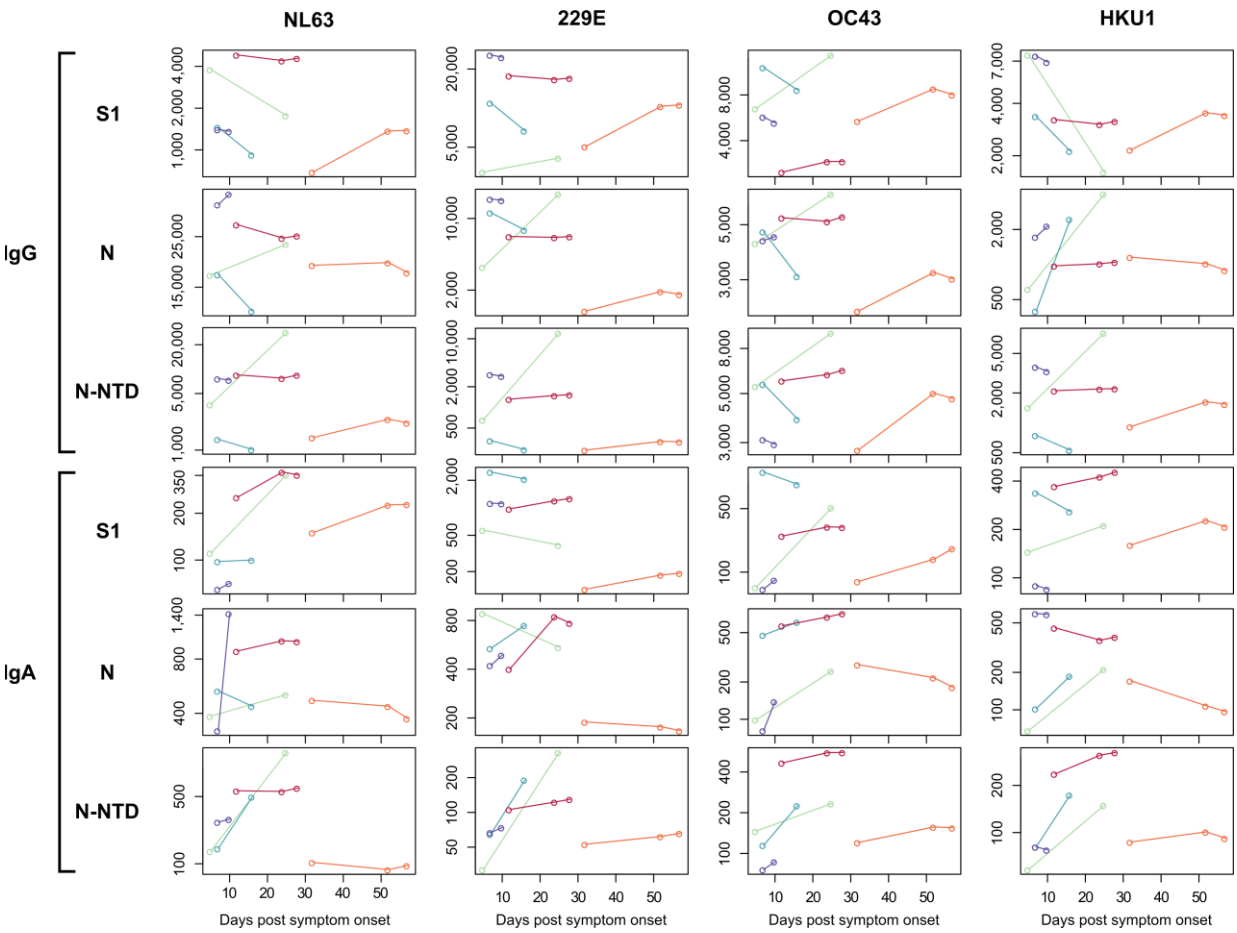

**Supplementary Figure 5**

Impact of sample time on antibody response is visualized by scatter plots. Sample dT in days is displayed against the observed MFI signal per antigen. Definition of dT is not consistent as samples measured in this study were taken from various sources. dT was calculated from the day of a positive PCR result (red circles), from the day of the PCR test itself (green circles) or from the day of symptom onset (blue circles). Source data are provided as a Source Data file.


a
Correlation of IgA Signal
Color Key
−1 −0.5 0 0.5 1
ρ(Spearman)
SARS-CoV-2
α-hCoVs
β-hCoVs
SARS-CoV-2 N-NTD
SARS-CoV-2 N
SARS-CoV-2 Spike Trimer
SARS-CoV-2 S2
SARS-CoV-2 RBD
SARS-CoV-2 S1
229E S1
NL63 S1
NL63 N
229E N
NL63 N-NTD
229E N-NTD
OC43 S1
HKU1 S1
OC43 N-NTD
OC43 N
HKU1 N
HKU1 N-NTD
b
NL63 N-NTD
229E N-NTD
OC43 N-NTD
HKU1 N-NTD
IgG (MFI)
IgA (MFI)
n 310 866
SARS−CoV−2 + − + −
IgG
IgA
c
Classification outliers vs hCoV N-NTD
IgG Spike Trimer false positive Samples (n 17)
Combined IgG + IgA false negative samples (n 31)
Relative IgG signal
NL63
229E
OC43
HKU1

**Supplementary Figure 6**

**a,** Correlation of IgA response for the entire sample set (n=1176) is visualized as heatmap based on Spearman's ρ coefficient; dendrogram on the right side displays antigens after hierarchical clustering was performed. **b,** Immune response (IgG and IgA) towards hCoV N-NTD proteins are presented as Box-Whisker plots of sample MFI on a logarithmic scale for SARS-CoV-2-infected (red, n=310) and uninfected (blue, n=866) individuals. Box represents the median and the 25th and 75th percentiles, whiskers show the largest and smallest values. Outliers determined by 1.5 times IQR of log-transformed data are depicted as circles. **c,** Relative levels of IgG-specific immune response towards hCoV N-NTD proteins are presented as Box-Whisker plots / stripchart overlays of log-transformed and per-antigen scaled and centred MFI for the sample subsets of Spike Trimer false positives (blue, n=17) and combined IgG + IgA false negatives (red, n=31). Box represents the median and the 25th and 75th percentiles, whiskers show the largest and smallest values, excluding outliers as determined by 1.5 times IQR. Source data are provided as a Source Data file.




**Supplementary Figure 7**

Kinetic of hCoV antigen-specific IgA and IgG responses is shown for indicated days after symptom onset for the three used hCoV antigens across five different patients. Colored lines indicated kinetic of respective SARS-CoV-2 antigen per patient. Source data are provided as a Source Data file.

**Supplementary Table 1 | Assay Variance and LOD**

Intra- and inter-assay variance were determined by repeated measurement of QC samples and blank sample as replicates on one plate and in duplicates over 17 plates, respectively. Standard deviation relative to mean (%CV) is given for each antigen. A limit of detection (LOD) was calculated from 24 blank sample replicates on the same plate as the mean MFI + 3 times standard deviation.

| | | | SARS-CoV-2 | | | | | | hCoV NL63 | | | hCoV 229E | | | hCoV OC43 | | | hCoV HKU1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Spike Trimer | RBD | S1 | S2 | N | N-NTD | S1 | N | N-NTD | S1 | N | N-NTD | S1 | N | N-NTD | S1 | N | N-NTD |
| Inter-assay variance (%CV) n = 34, duplicates, 17 plates | IgG | QC1 | 3.7 | 3.3 | 3.4 | 3.7 | 2.8 | 7.4 | 3.5 | 3.2 | 4.4 | 3.3 | 2.8 | 5.2 | 3.1 | 6.0 | 4.4 | 3.4 | 4.7 | 5.4 |
| | | QC2 | 4.1 | 4.6 | 6.9 | 3.4 | 5.3 | 4.8 | 3.0 | 2.2 | 6.3 | 2.4 | 2.1 | 6.7 | 2.7 | 4.5 | 2.3 | 2.7 | 5.1 | 2.8 |
| | | QC3 | 3.4 | 2.4 | 2.3 | 3.6 | 2.1 | 4.6 | 3.1 | 2.5 | 3.5 | 2.9 | 2.0 | 4.7 | 2.9 | 6.4 | 4.6 | 3.2 | 3.2 | 3.5 |
| | | Blank | 5.4 | 5.6 | 6.7 | 6.4 | 5.6 | 6.1 | 6.3 | 7.1 | 5.7 | 9.1 | 6.1 | 6.1 | 5.6 | 7.3 | 4.1 | 4.9 | 6.1 | 8.3 |
| | IgA | QC1 | 3.9 | 4.6 | 4.9 | 4.0 | 5.1 | 5.0 | 4.2 | 3.6 | 3.9 | 4.0 | 5.3 | 7.4 | 4.3 | 7.4 | 5.0 | 4.2 | 6.0 | 5.0 |
| | | QC2 | 4.6 | 5.0 | 5.1 | 3.9 | 3.9 | 4.2 | 3.7 | 2.4 | 7.6 | 2.9 | 2.2 | 6.0 | 4.1 | 16.4 | 4.2 | 4.3 | 5.5 | 3.9 |
| | | QC3 | 3.9 | 3.8 | 4.5 | 3.4 | 3.4 | 4.8 | 3.9 | 2.8 | 4.0 | 3.0 | 5.1 | 4.5 | 3.6 | 6.1 | 4.1 | 3.7 | 4.5 | 5.1 |
| | | Blank | 6.7 | 5.3 | 8.2 | 6.3 | 5.3 | 5.3 | 3.3 | 5.0 | 5.0 | 6.7 | 7.0 | 6.1 | 5.3 | 7.1 | 4.7 | 6.0 | 6.8 | 6.3 |
| Intra-assay variance (%CV) n = 24 | IgG | QC1 | 2.5 | 1.9 | 2.0 | 2.1 | 1.8 | 2.1 | 2.4 | 1.7 | 2.8 | 2.0 | 2.7 | 3.2 | 1.9 | 2.0 | 2.2 | 2.7 | 2.4 | 2.2 |
| | | QC2 | 5.9 | 4.3 | 4.1 | 2.8 | 2.7 | 3.2 | 1.9 | 1.9 | 2.6 | 2.0 | 2.2 | 2.7 | 2.2 | 1.6 | 2.1 | 2.5 | 3.1 | 2.5 |
| | | QC3 | 1.6 | 4.3 | 5.1 | 1.9 | 1.9 | 4.5 | 4.2 | 1.7 | 3.2 | 3.3 | 4.1 | 5.7 | 3.2 | 3.1 | 5.5 | 5.6 | 6.0 | 8.4 |
| | | Blank | 6.0 | 5.6 | 5.2 | 5.8 | 5.2 | 4.2 | 5.0 | 4.8 | 4.8 | 7.3 | 6.2 | 6.3 | 6.5 | 6.2 | 4.4 | 6.1 | 6.0 | 6.2 |
| | IgA | QC1 | 2.5 | 3.3 | 5.2 | 3.8 | 3.7 | 4.2 | 3.2 | 2.3 | 2.2 | 2.0 | 4.8 | 4.7 | 2.9 | 4.7 | 3.4 | 3.3 | 4.5 | 4.3 |
| | | QC2 | 4.8 | 5.7 | 5.7 | 3.2 | 4.1 | 4.3 | 3.4 | 2.0 | 5.7 | 2.1 | 2.1 | 6.1 | 3.0 | 3.1 | 1.9 | 3.9 | 6.4 | 3.8 |
| | | QC3 | 3.1 | 4.7 | 5.5 | 3.0 | 4.1 | 4.4 | 3.7 | 2.7 | 3.7 | 2.7 | 5.4 | 5.8 | 2.6 | 4.1 | 3.1 | 2.4 | 4.5 | 4.3 |
| | | Blank | 5.8 | 5.3 | 6.3 | 5.0 | 5.4 | 5.5 | 4.5 | 5.6 | 5.3 | 7.2 | 6.3 | 7.0 | 6.7 | 9.5 | 7.3 | 5.2 | 8.8 | 7.0 |
| LOD (MFI) n = 24 | IgG | | 32 | 26 | 23 | 29 | 38 | 33 | 29 | 28 | 26 | 65 | 35 | 24 | 33 | 30 | 33 | 37 | 33 | 25 |
| | IgA | | 31 | 26 | 26 | 27 | 37 | 32 | 57 | 28 | 40 | 28 | 36 | 22 | 35 | 28 | 32 | 33 | 39 | 28 |

**Supplementary Table 2 | Complete overview of study sample set divided into columns by age groups and sex.**
Samples from SARS-CoV-2-infected donors are further split up by hospitalization status. Age and gender of patients from which multiple samples were available for time course analyses are indicated. SARS-CoV-2-uninfected samples are further divided into samples drawn during the pandemic, which was defined as all samples taken on 01.01.2020 or later, and pre-pandemic samples. 147 samples with previous hCoV infection were included in the SARS-CoV-2-uninfected group. Detailed diagnosis of hCoV subspecies is indicated where available. Other sample conditions for special groups of uninfected samples are listed. NA: Information was not available.

| Age | ≤39 | | | | 40-59 | | | | ≥60 | | | | NA | | | | ∑ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n | 299 (25.4 %) | | | | 241 (20.5 %) | | | | 475 (40.4 %) | | | | 161 (13.7 %) | | | | 1176 |
| Sex | male | | female | | male | | female | | male | | female | | male | | female | | NA | | |
| n | 139 | (11.8 %) | 160 | (13.6 %) | 144 | (12.2 %) | 97 | (8.2 %) | 271 | (23.0 %) | 204 | (17.3 %) | 5 | (0.4 %) | 3 | (0.3 %) | 153 | (13.0 %) | 1176 |
| SARS-CoV-2-infected (total) | 60 | (19.4 %) | 51 | (16.5 %) | 71 | (22.9 %) | 63 | (20.3 %) | 42 | (13.5 %) | 17 | (5.5 %) | 3 | (1.0 %) | 3 | (1.0 %) | 0 | (0.0 %) | 310 |
| Hospitalized (for COVID19) | 6 | (10.9 %) | 2 | (3.6 %) | 14 | (25.5 %) | 6 | (10.9 %) | 23 | (41.8 %) | 4 | (7.3 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 55 |
| Non-Hospitalized | 52 | (25.0 %) | 43 | (20.7 %) | 49 | (23.6 %) | 43 | (20.7 %) | 13 | (6.3 %) | 8 | (3.8 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 208 |
| Hospitalisation NA | 2 | (4.3 %) | 6 | (12.8 %) | 8 | (17.0 %) | 14 | (29.8 %) | 6 | (12.8 %) | 5 | (10.6 %) | 3 | (6.4 %) | 3 | (6.4 %) | 0 | (0.0 %) | 47 |
| Patients with time series | 2 | (40.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 2 | (40.0 %) | 1 | (20.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 5 |
| SARS-CoV-2-uninfected (total) | 79 | (9.1 %) | 109 | (12.6 %) | 73 | (8.4 %) | 34 | (3.9 %) | 229 | (26.4 %) | 187 | (21.6 %) | 2 | (0.2 %) | 0 | (0.0 %) | 153 | (17.7 %) | 866 |
| Sample during pandemic | 10 | (15.4 %) | 10 | (15.4 %) | 12 | (18.5 %) | 14 | (21.5 %) | 7 | (10.8 %) | 5 | (7.7 %) | 1 | (1.5 %) | 0 | (0.0 %) | 6 | (9.2 %) | 65 |
| Sample pre-pandemic | 69 | (8.6 %) | 99 | (12.4 %) | 61 | (7.6 %) | 20 | (2.5 %) | 222 | (27.7 %) | 182 | (22.7 %) | 1 | (0.1 %) | 0 | (0.0 %) | 147 | (18.4 %) | 801 |
| Previous hCoV Infection | 19 | (12.9 %) | 18 | (12.2 %) | 45 | (30.6 %) | 20 | (13.6 %) | 29 | (19.7 %) | 16 | (10.9 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 147 |
| confirmed NL63 | 2 | (20.0 %) | 0 | (0.0 %) | 3 | (30.0 %) | 1 | (10.0 %) | 2 | (20.0 %) | 2 | (20.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 10 |
| confirmed 229 | 5 | (25.0 %) | 1 | (5.0 %) | 4 | (20.0 %) | 1 | (5.0 %) | 5 | (25.0 %) | 4 | (20.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 20 |
| confirmed OC43 | 0 | (0.0 %) | 1 | (3.7 %) | 14 | (51.9 %) | 1 | (3.7 %) | 6 | (22.2 %) | 5 | (18.5 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 27 |
| confirmed HKU1 | 3 | (20 %) | 1 | (6.7 %) | 4 | (26.7 %) | 2 | (13.3 %) | 5 | (33.3 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 15 |
| unknown hCoV | 9 | (12 %) | 15 | (20.0 %) | 20 | (26.7 %) | 15 | (20.0 %) | 11 | (14.7 %) | 5 | (6.7 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 75 |
| Pregnant | 0 | (0.0 %) | 9 | (90.0 %) | 0 | (0.0 %) | 1 | (10.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 10 |
| RF/HAMA samples | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 6 | (100 %) | 6 |
| PCT > 3 ng/mL | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 21 | (100 %) | 21 |
| Neuroinflammatory disease | 6 | (40.0 %) | 6 | (40.0 %) | 1 | (6.7 %) | 0 | (0.0 %) | 1 | (6.7 %) | 1 | (6.7 %) | 0 | (0.0 %) | 0 | (0.0 %) | 0 | (0.0 %) | 15 |

**Supplementary Table 3 - Percentage Identity of sequence alignments of used hCoV and corresponding SARS-CoV-2.**
Alignments were calculated using version 1.2.4. of Clustal Omega[3]. Sequences of constructs used in alignment are provided as a Source Data file.

| | | % of sequence identity of corresponding antigen | | | | |
|---|---|---|---|---|---|---|
| Protein | Identifier | hCoV-NL63 | hCoV-229E | SARS-CoV-2 | hCoV-OC43 | hCoV-HKU1 |
| hCoV-NL63 S1 | APF29071.1 | 100.0 | 50.2 | 17.8 | 19.7 | 18.1 |
| hCoV-229E S1 | APT69883.1 | 50.2 | 100.0 | 18.5 | 20.2 | 18.7 |
| SARS-CoV-2 S1 | QHD43416.1 | 17.8 | 18.5 | 100.0 | 24.2 | 24.2 |
| hCoV-OC43 S1 | AVR40344.1 | 19.7 | 20.2 | 24.2 | 100.0 | 58.0 |
| hCoV-HKU1 S1 | AGW27881.1 | 18.1 | 18.7 | 24.2 | 58.0 | 100.0 |
| hCoV-NL63 N | YP_003771.1 | 100.0 | 47.5 | 30.9 | 29.0 | 29.7 |
| hCoV-229E N | NP_073556.1 | 47.5 | 100.0 | 30.4 | 30.1 | 32.2 |
| SARS-CoV-2 N | QHD43423.2 | 30.9 | 30.4 | 100.0 | 37.1 | 36.7 |
| hCoV-OC43 N | YP_009555245.1 | 29.0 | 30.1 | 37.1 | 100.0 | 65.5 |
| hCoV-HKU1 N | YP_173242.1 | 29.7 | 32.2 | 36.7 | 65.5 | 100.0 |
| hCoV-NL63 N-NTD | YP_003771.1 | 100.0 | 63.4 | 35.3 | 34.4 | 35.8 |
| hCoV-229E N-NTD | NP_073556.1 | 63.4 | 100.0 | 38.2 | 37.9 | 39.2 |
| SARS-CoV-2 N-NTD | QHD43423.2 | 35.3 | 38.2 | 100.0 | 42.0 | 42.8 |
| hCoV-OC43 N-NTD | YP_009555245.1 | 34.4 | 37.9 | 42.0 | 100.0 | 68.3 |
| hCoV-HKU1 N-NTD | YP_173242.1 | 35.8 | 39.2 | 42.8 | 68.3 | 100.0 |

**Supplementary Table 4 | Overview of primers used in this study with respective sequence.**

| Primer | Sequence |
|---|---|
| pRSET2b down-for | GGTAAGCTTGATCCGGCTGCTAA |
| SARS-CoV2_NTD-rev | GGGAAGCTTACTCAGCATAGAAGCCCTTTGG |
| OC43_NTD-rev | GGGAAGCTTATTCGATATAATAGCCCTGCGG |
| NL63_NTD-rev | GGGAAGCTTATTCAACAACGCTCAGTTCCG |
| 229E_NTD-rev | GGGAAGCTTATTCAACAACGGTAACACCATTC |
| HKU1_NTD-rev | GGGAAGCTTATTCCACATAGTAGCCCTGAGGC |
| S1 CoV2-for | CTTCTGGCGTGTGACCGG |
| S1 CoV2-rev | GTTGCGGCCGCTTAGTGGTGGTGGTGGTGGTGGGGGCTGTTTGTCTGTGTCTG |

**Supplementary Table 5 | Overview of antigens used in this study**.

| Construct | Manufacturer | Sequence Identifier | Fragment | Mutations | Expression system | Tag | Tag position |
|---|---|---|---|---|---|---|---|
| SARS-CoV-2 Spike Trimer | In-house expressed | QHD43416.1 | 1-1213 | $^{682}$RRAR to A, K986P and V987P | Expi293 | Thrombin cleavage-site/ T4 foldon/ $His_6$ | C-terminus |
| SARS-CoV-2 RBD | In-house expressed | QHD43416.1 | 1-14 + 319-541 | | Expi293 | $His_6$ | C-terminus |
| SARS-CoV-2 S2 | Sino Biological #40590-V08B | YP_009724390.1 | 686-1213 | | Baculovirus-Insect cells | $His_6$ | C-terminus |
| SARS-CoV-2 S1 | In-house expressed | QHD43416.1 | 1-681 | | Expi293 | $His_6$ | C-terminus |
| hCoV-OC43 S1 | In-house expressed | AVR40344.1 | 1-760 | | Expi293 | $His_6$ | C-terminus |
| hCoV-NL63 S1 | In-house expressed | APF29071.1 | 1-744 | | Expi293 | $His_6$ | C-terminus |
| hCoV-229E S1 | In-house expressed | APT69883.1 | 1-561 | | Expi293 | $His_6$ | C-terminus |
| hCoV-HKU1 S1 | In-house expressed | AGW27881.1 | 1-755 | | Expi293 | $His_6$ | C-terminus |
| SARS-CoV-2 N | Aalto Bioreagents #CK 6406-b | NA | full length | | *E. coli* | $His_6$ | C-terminus |
| SARS-CoV-2 N-NTD | In-house expressed | QHD43423.2 | 1-174 | | *E.coli* BL21 | $His_6$ | N-terminus |
| hCoV-OC43 N | In-house expressed | YP_009555245.1 | 1-448 | | *E.coli* BL21 | $His_6$ | N-terminus |
| hCoV-OC43 N-NTD | In-house expressed | YP_009555245.1 | 1-189 | | *E.coli* BL21 | $His_6$ | N-terminus |
| hCoV-NL63 N | In-house expressed | YP_003771.1 | 1-377 | | *E.coli* BL21 | $His_6$ | N-terminus |
| hCoV-NL63 N-NTD | In-house expressed | YP_003771.1 | 1-139 | | *E.coli* BL21 | $His_6$ | N-terminus |
| hCoV-229E N | In-house expressed | NP_073556.1 | 1-389 | | *E.coli* BL21 | $His_6$ | N-terminus |
| hCoV-229E N-NTD | In-house expressed | NP_073556.1 | 1-141 | | *E.coli* BL21 | $His_6$ | N-terminus |
| hCoV-HKU1 N | In-house expressed | YP_173242.1 | 1-441 | | *E.coli* BL21 | $His_6$ | N-terminus |
| hCoV-HKU1 N-NTD | In-house expressed | YP_173242.1 | 1-188 | | *E.coli* BL21 | $His_6$ | N-terminus |

For commercial antigens, catalogue number is given and information is provided as available from the data sheets. NA: Information was not available.

**Supplementary References**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3 Madeira, F. *et al.* The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic acids research* **47**, W636-W641 (2019).