SARS-CoV-2-reactive T cell receptors isolated from convalescent COVID-19 patients confer potent T cell effector function

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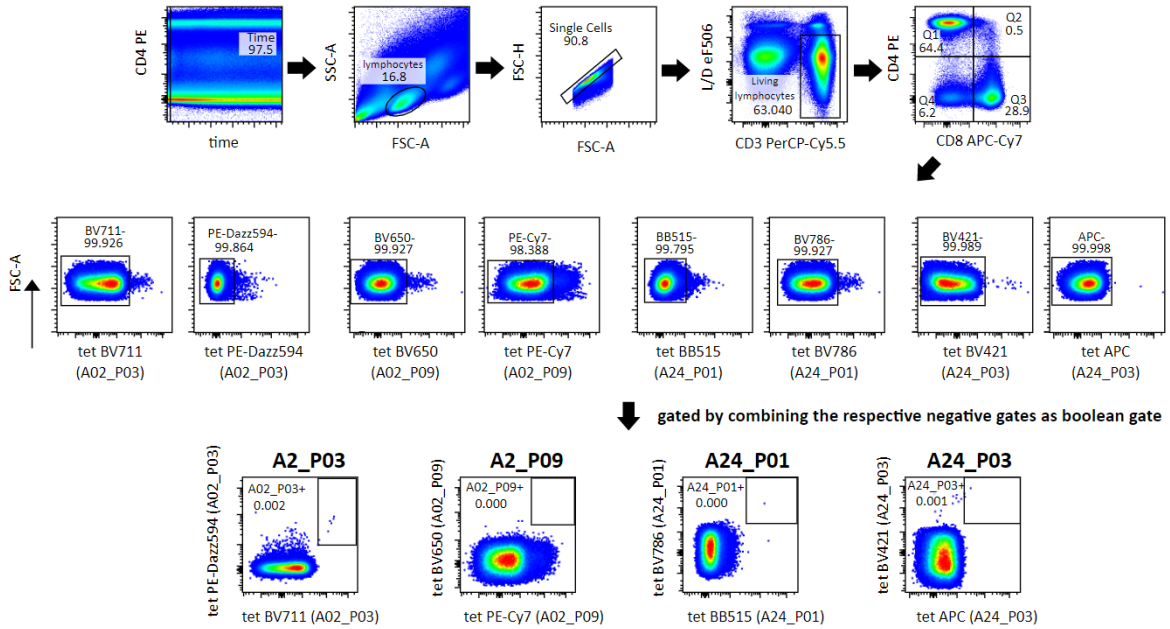
Supplementary Material

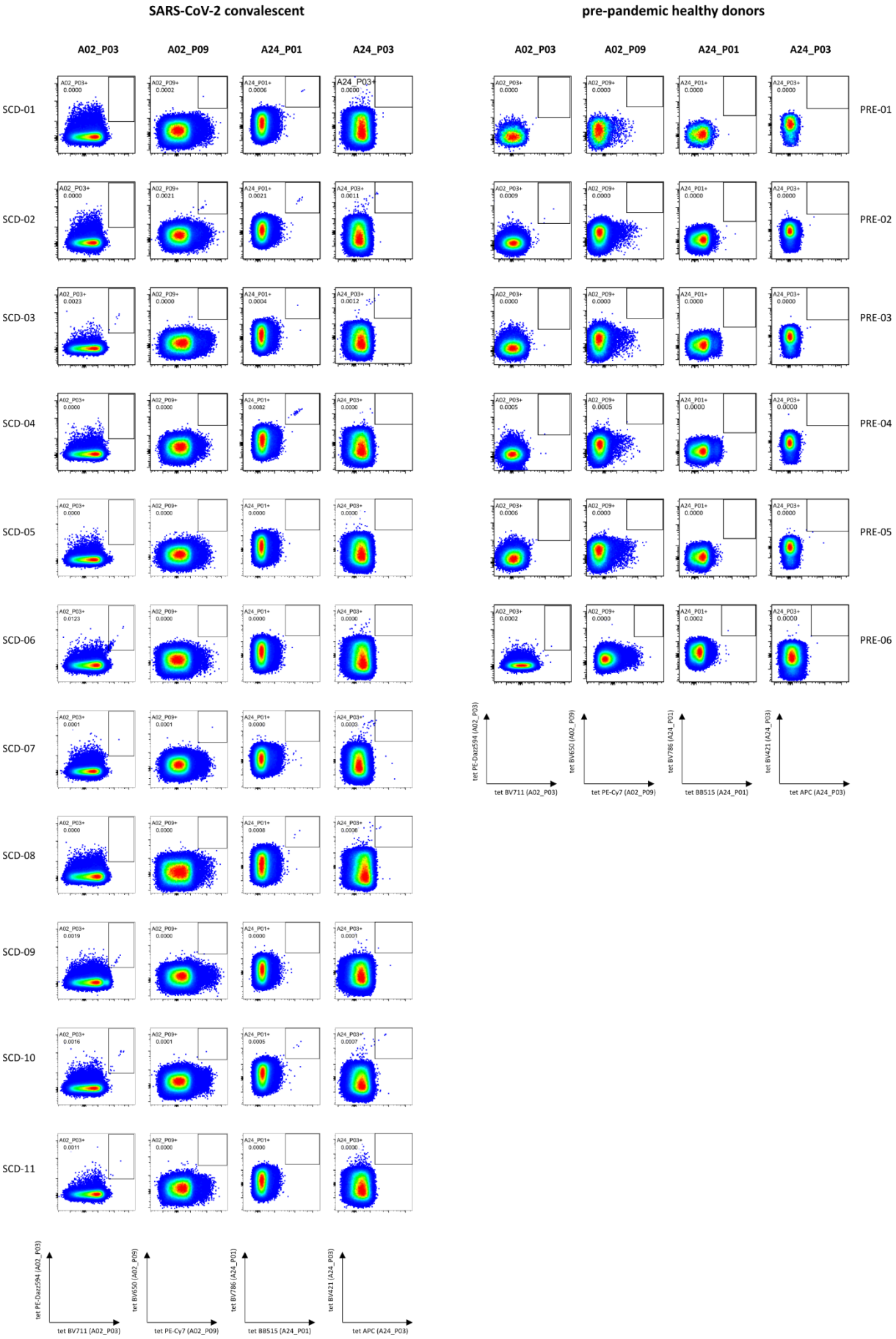
Suppl. Fig. 1



Supplementary Fig. 1 Gating strategy for tetramer staining

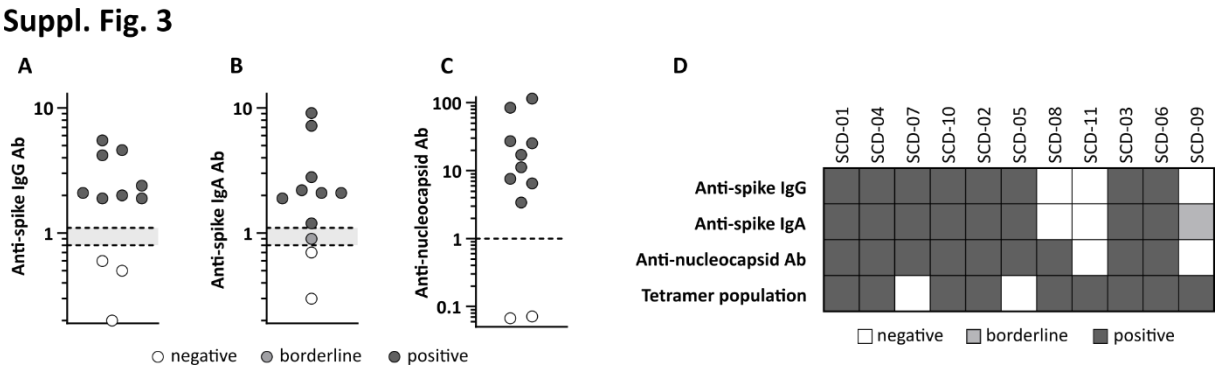
Exemplary representation of the gating strategy for tetramer staining. Cells were gated on time, excluding the initial burst (to avoid “polluting” background events). A standard lymphocyte gate was applied, and FSC-A vs. FSC-H was used for doublet exclusion. Viable CD3+ cells were gated and CD8+ (CD4-) cells sub-gated. Boolean gating was applied to reduce background of tetramer staining. To this end, CD8+ T cells were gated for each of the individual tetramer fluorochromes and a combinatorial gate of all non-target fluorochromes was applied (middle row) before applying the final 2D tetramer gate (bottom row). For example, BV650- AND PE-Cy7- AND BB515- AND BV786- AND BV421- AND APC- cells were pre-gated before applying the PE-Dazz594 vs. BV711 gate for A02_P03. The example shows SCD-03.

Suppl. Fig. 2


SARS-CoV-2 convalescent
pre-pandemic healthy donors
A02_P03
A02_P09
A24_P01
A24_P03
SCD-01
SCD-02
SCD-03
SCD-04
SCD-05
SCD-06
SCD-07
SCD-08
SCD-09
SCD-10
SCD-11
PRE-01
PRE-02
PRE-03
PRE-04
PRE-05
PRE-06
tet PE-Daz594 (A02_P03)
tet BV711 (A02_P03)
tet BV650 (A02_P09)
tet PE-Cy7 (A02_P09)
tet BV786 (A24_P01)
tet BB515 (A24_P01)
tet BV421 (A24_P03)
tet APC (A24_P03)

Supplementary Fig. 2 Overview of 2D tetramer stainings from SARS-CoV-2 convalescent and pre-pandemic donor samples

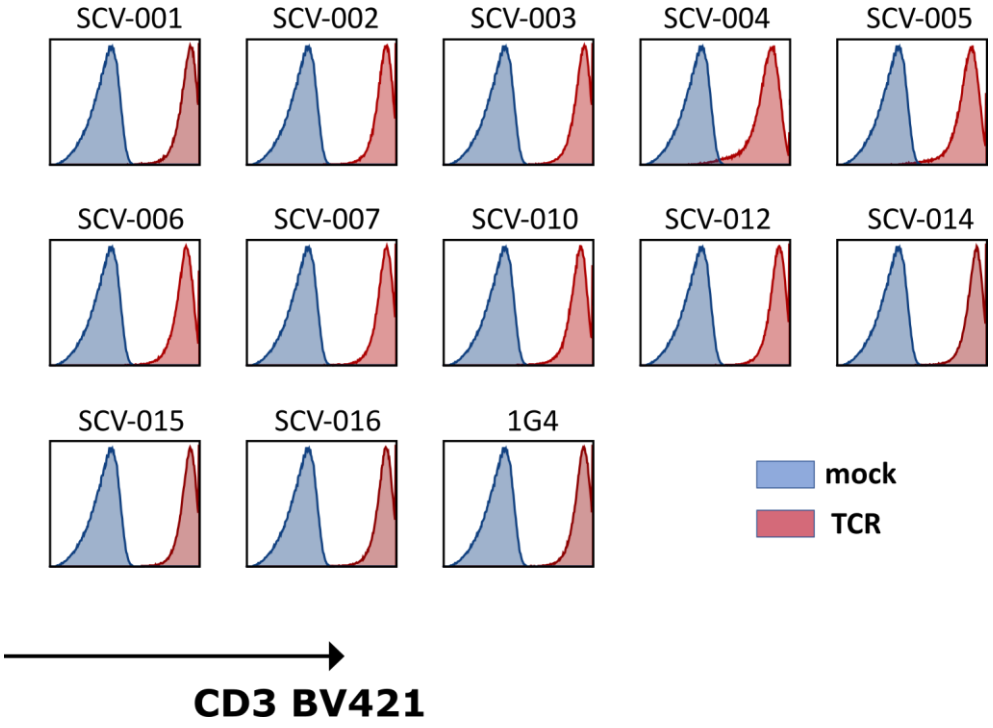
PBMC samples of SARS-CoV-2 convalescent and pre-pandemic healthy donor controls were stained with tetramers specific to SARS-CoV-2 target epitopes A02_P03, A02_P09, A24_P01 and A24_P03. On the left the SARS-CoV-2 convalescent samples are shown, on the right the pre-pandemic healthy donor controls (HLA-A02+ and HLA-A24+ double positive).



Supplementary Fig. 3 SARS-CoV-2-directed antibody responses in analysed donors

SARS-CoV-2-directed antibody responses. (A-C) Antibody responses in convalescents (n = 11) for anti-S1 (A) IgG and (B) IgA (EUROIMMUN) or (C) anti-nucleocapsid (Elecsys immunoassay). Donors with negative or borderline responses are marked in white or light grey, respectively. (D) Heatmap indicating positive (dark grey), borderline (light grey), or negative (white) antibody responses as well as successful TCR identification. Ab, antibody.

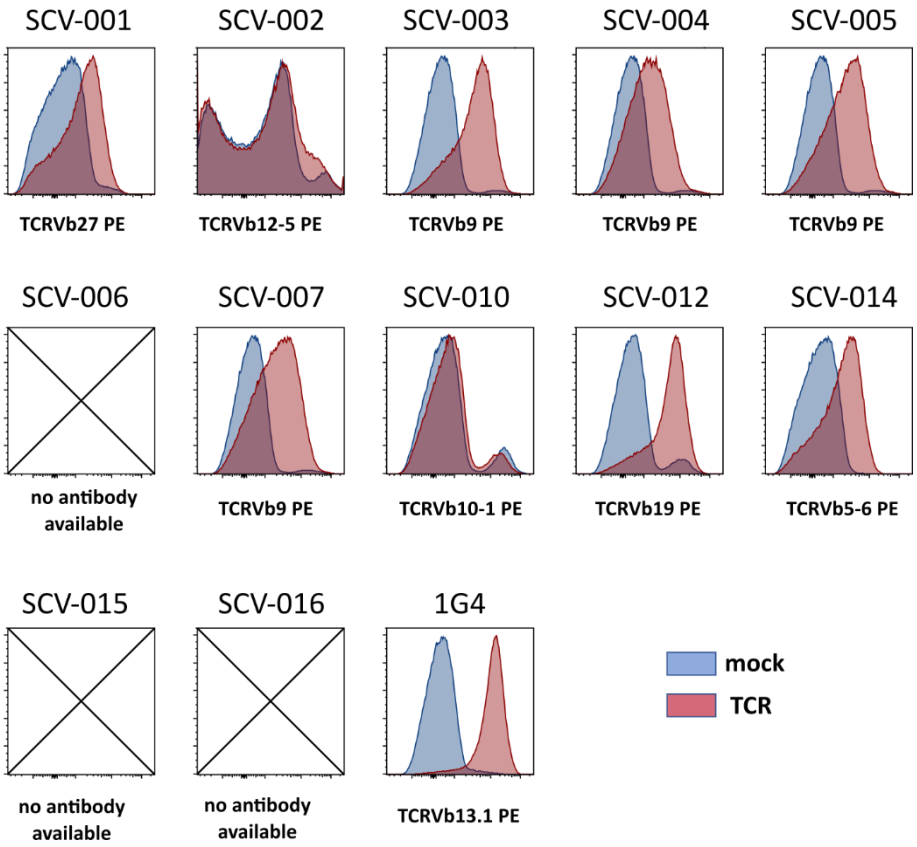
Suppl. Fig. 4



Supplementary Fig. 4 TCR expression control of electroporated TCR-KO Jurkat reporter cells

An equal number ($0.2x10^6$) of electroporated TCR-KO Jurkat cells were stained with fixable viability dye eFluor780 and CD3 BV421 and subsequently analysed by flow cytometry. The histogram overlay shows mock electroporated Jurkats in blue and TCR electroporated Jurkats in red.

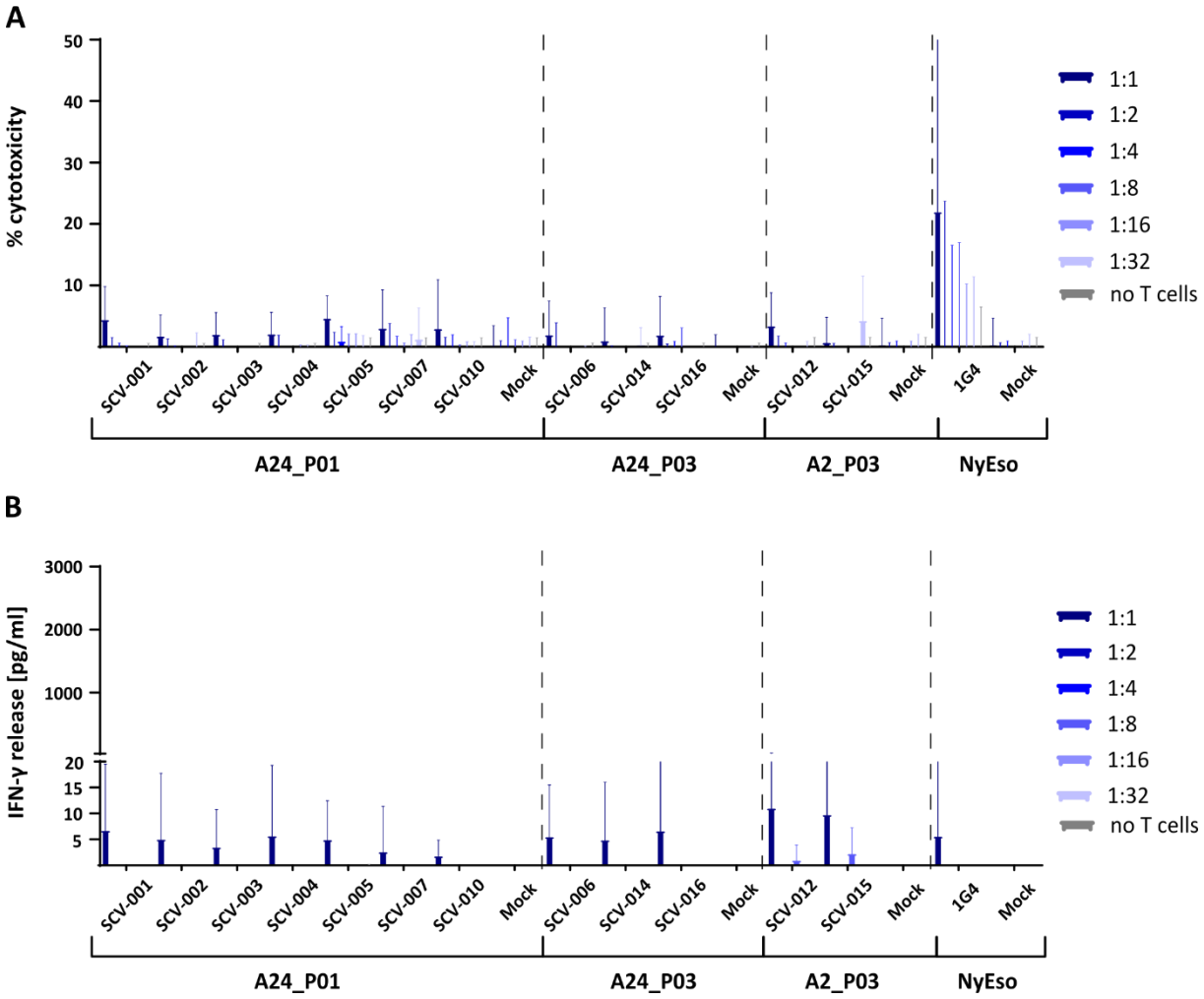
Suppl. Fig. 5



Supplementary Fig. 5 TCR expression control of electroporated primary CD8+ T cells

An equal number ($0.2x10^6$) of electroporated CD8+ T cells were stained with fixable viability dye eFluor780 and a PE-conjugated antibody directed against the respective TCRVβ chain. Mock electroporated cells were labelled with the respective matching TCRVβ antibody. Labelled cells were subsequently analysed by flow cytometry. The histogram overlay shows mock electroporated CD8+ T cells in blue and TCR electroporated CD8+ T cells in red. For SCV-006, SCV-015 and SCV-016 matching TCRVβ (or -Vα) antibodies were not available.

Suppl. Fig. 6



Supplementary Fig. 6 Irrelevant peptide controls for SARS-CoV-2-reactive TCRs in primary CD8+ T cells

SARS-CoV-2-reactive TCRs passing the 3-fold cut-off criterium were electroporated into primary CD8+ T cells. T2-A2 and T2-A24 cells were pulsed with 100nM of an HLA-matched irrelevant peptide and plated at a fixed number of $30x10^3$ cells/well (96-well U-bottom plate) as target cells. Electroporated CD8+ T cells were added in titrated effector/target (E/T) ratios of 1:1, 1:2, 1:4, 1:8, 1:16 and 1:32 ($30x10^3$ - ~$1x10^3$). The experiment was conducted three times (n = 3) using primary CD8+ samples from 5 different donors in total. Error bars represent standard deviation.

A Bar graph summarizing the cytotoxicity shown as % cytotoxicity. Triton-lysed cells were used as maximum lysis (100% killing) reference. Dark blue to light blue indicates the titrated E/T ratio.

B Bar graph summarizing the IFN-□ response as measured by ELISA (in pg/ml). Dark blue to light blue indicates the titrated E/T ratio.

Supplementary Table 1 SARS donor characteristics for TCR sequencing

Summary of patient and clinical characteristics. Awareness of symptoms indicates patient-subjective disease severity. n, number.

Number of donors		11
Age [years]		
	Range	22 - 59
	Median	33
Sex [n (%)]		
	Female	5 (45)
	Male	6 (55)
Sample collection date		04/2020 - 05/2020
SARS-CoV-2 PCR positivity [n (%)]		11 (100)
Antibody response, anti-spike IgG [n (%)]		
	Positive	8 (73)
	Borderline	0 (0)
	Negative	3 (27)
Antibody response, anti-spike IgA [n (%)]		
	Positive	8 (73)
	Borderline	1 (9)
	Negative	3 (18)
Antibody response, anti-nucleocapsid [n (%)]		
	Positive	9 (82)
	Negative	2 (18)
Interval positive test to sample collection [days]		
	Range	31 - 56
	Mean	44
Awareness of symptoms [n (%)]		
	No	2 (18)
	Mild	2 (18)
	Moderate	1 (9)
	Severe	6 (55)
Febrile illness (≥ 38.0°C)		
	Yes	6 (55)
	No	5 (45)

Awareness of symptoms indicates patient-subjective disease severity. n, number.