

1 **Early induction of SARS-CoV-2 specific T cells associates with rapid viral clearance and mild**
2 **disease in COVID-19 patients**

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1 **Summary**

2 Virus-specific humoral and cellular immunity act synergistically to protect the host from viral
3 infection. We interrogated the dynamic changes of virological and immunological
4 parameters in 12 patients with symptomatic acute SARS-CoV-2 infection from disease onset
5 to convalescence or death. We quantified SARS-CoV-2 viral RNA in the respiratory tract in
6 parallel with antibodies and circulating T cells specific for various structural (NP, M, ORF3a
7 and spike) and non-structural proteins (ORF7/8, NSP7 and NSP13). We observed that while
8 rapid induction and quantity of humoral responses were associated with increased disease
9 severity, an early induction of SARS-CoV-2 specific T cells was present in patients with mild
10 disease and accelerated viral clearance. These findings provide further support for a
11 protective role of SARS-CoV-2 specific T cells over antibodies during SARS-CoV-2 infection
12 with important implications in vaccine design and immune-monitoring.

13

1 **Introduction**

2 In December 2019, a new coronavirus was detected in Wuhan, China in several patients
3 with pneumonia and was later named SARS-CoV-2(Zhou et al., 2020). The illness (COVID-19)
4 resulting from SARS-CoV-2 infection is reported to be multi-faceted with inflammation of
5 the respiratory tract causing the leading symptoms of fever and dry cough {Chen:2020kl}.

6 Both humoral (Long et al., 2020a) (Gudbjartsson et al., 2020; Sun et al., 2020) and cellular
7 (Grifoni et al., 2020) (Braun et al., 2020; Le Bert et al., 2020) (Weiskopf et al., 2020)
8 components of adaptive immunity are induced in SARS-CoV-2 infected individuals but their
9 roles in viral control or disease pathogenesis still needs to be clarified. Viral clearance and
10 reduced disease severity have been associated with a coordinated activation of humoral and
11 cellular anti-viral immunity (Rydyznski Moderbacher et al., 2020) and robust T cell responses
12 (Takahashi et al., 2020). On the other hand, a positive relation between the magnitude of
13 SARS-CoV-2 antibodies (Hung et al., 2020; Long et al., 2020b) or T cells(Peng et al., 2020)
14 with disease severity have also been reported. However, most of these studies have
15 analysed patients during the convalescent phase of infection and only a single study
16 reported the dynamic changes of viral and immunological parameters in severe COVID-19
17 patients during the initial phases of infection (Weiskopf et al., 2020). To fill this gap, we
18 longitudinally followed-up twelve patients with SARS-CoV-2 infection from symptom onset
19 to convalescence or death. We quantified SARS-CoV-2 viral load in the upper respiratory
20 tract and virus-specific antibodies and T cells at multiple time points. Our data reveal a
21 direct association between early induction of SARS-CoV-2 specific T cells and rapid control of
22 viral infection.

23

1 **Results**

2 Dynamics of SARS-CoV-2 replication

3 Relative quantities of SARS-CoV-2 in the respiratory tract and its persistence in each
4 individual patient was calculated by utilizing the number of RT-PCR cycles as a proxy of viral
5 quantity (Figure 1). Duration of infection was defined from symptom onset till RT-PCR
6 negativity (two negative SARS-CoV-2 RT-PCR 24 hours apart). The patients displayed
7 different profiles of virus quantity and persistence: short duration of infection was detected
8 in four patients (P18, 04, 02 and 15) who became RT-PCR negative within 15 days of
9 symptom onset. Five (P11, 16, 06, 09 and 03) were RT-PCR negative at around day 17-24,
10 while two (P10 and 12) became RT-PCR negative almost 1 month after onset of symptoms.
11 One patient (P05) who succumbed to infection was persistently SARS-CoV-2 RT-PCR positive
12 till day 31 post symptom onset when he demised. Peak viral quantities was also positively
13 correlated with the duration of infection (Figure 1 insert). In addition, while patients who
14 eliminated the virus within 15 days experienced mild respiratory symptoms (presence of
15 fever or respiratory symptoms but not requiring supplemental oxygen), patients with
16 moderate (requiring oxygen supplementation $FiO_2 < 0.5$) and severe (requiring oxygen
17 supplementation $FiO_2 > 0.5$, high flow oxygen and/or mechanical ventilation) symptoms
18 eliminated the virus from upper respiratory tract later.

19

20 Dynamics of SARS-CoV-2 specific antibody response

21 We then characterized the kinetics of anti-SARS-CoV-2 antibody appearance. Virus
22 neutralization ability was tested longitudinally utilizing the surrogate virus neutralization

1 test (sVNT) that quantified the ability of serum antibodies to inhibit the binding of Spike RBD
2 to ACE2 receptor *in vitro* (Tan et al., 2020). We also quantified anti-RBD, anti-S1 (S1 domain
3 of spike) and anti-NP (nucleoprotein) IgG and IgM antibodies with a Luminex–based
4 quantification test that utilize beads coated with RBD, S1 domain of Spike and NP proteins
5 respectively (Figure 2A).

6 Figure 2B shows that all COVID-19 patients developed neutralising antibodies with the
7 exception of patient P02 who nonetheless cleared SARS-CoV-2 at day 13 post symptom
8 onset (Figure 1). The peak neutralizing activity was achieved within 9-15 days post symptom
9 onset. Of note, the two patients who first reached 90% virus neutralisation developed
10 severe disease (P05-deceased and P03-severe). The kinetics of anti-RBD, anti-S1 and anti-NP
11 IgG also peaked around the 10-20 days period, similar to previous observations (Isho et al.,
12 2020; Ripperger et al., 2020)with higher peak levels of antibodies against NP and Spike
13 detected in patient P05 who succumbed to the infection. Interestingly, when we analysed in
14 parallel the kinetics of appearance of antibody responses against different proteins, we
15 observed that patients with severe disease have an early NP-biased antibody response,
16 while those with mild/moderate symptoms had either a spike-dominant or balanced
17 response (Figure 2C). These kinetics of appearance is consistent with recent data (Atyeo et
18 al., 2020; Sun et al., 2020) showing that an anti-nucleocapsid humoral response is
19 preferentially induced over a Spike IgG response in severe COVID-19 patients.

20

21 Dynamics of SARS-CoV-2 specific cellular responses

22 We next analysed the kinetics of SARS-CoV-2 specific T cell appearance during the RT-PCR
23 positive phase of disease. Overlapping 15-mer peptide libraries covering the whole NP,

1 Membrane (M), ORF7ab, ORF8, ORF3a, the NSP7 and NSP13 of ORF1ab and a pool of ~40
2 peptides containing all the confirmed T cell epitopes of Spike were used to stimulate PBMC
3 in an IFN- γ ELISPOT assay (Figure 3A). Since samples of PCR+ patients could not be handled
4 outside a BSL-3 laboratory, a more detailed immunological analysis performed by flow
5 cytometry could only be performed after viral clearance (Supplementary Figure 1). During
6 the initial phase of SARS-CoV-2 infection, the quantity of IFN- γ secreting cells after
7 stimulation by the different peptides pools increased progressively with the peak of
8 frequency detected within ~15 days after symptom onset in the majority of the tested
9 patients (8 out of 12, Figure 3B and 3C) in line with a previous report (Weiskopf et al., 2020).
10 In two patients, peak responses were detected beyond 20 days after symptom onset, while
11 patient P05, who succumbed to disease before viral clearance, had no detectable IFN- γ
12 secreting cells when stimulated with the different peptide pools until day 26 when
13 stimulation with Spike peptides activated a weak response (Figure 3B). Importantly, large
14 production of IFN- γ was detected at all time points in all patients after stimulation with
15 PMA+Ionomycin, showing that global cellular functionality was not compromised (Figure 4A
16 insert). The frequency of IFN- γ secreting cells reactive to all the different peptide pools was
17 also quantified at least 1 month after resolution of infection. The magnitude of the IFN- γ
18 response declined massively in 7 out of the 8 patients tested (Figure 3B), consistent with the
19 waning of the cellular immune response that follows resolution of acute infection. The
20 quantity and time of appearance of SARS-CoV-2 peptide-reactive cells was then analysed in
21 relation to the virological and clinical parameters.

22 First, we observed that in contrast to the antibody quantity, the overall magnitude of SARS-
23 CoV-2 peptide-reactive cells was not proportional to the severity of disease. Figure 2B shows
24 that while higher quantities of IgG were observed in patients with severe compared to mild

1 COVID-19, an opposite pattern was detected when the total IFN- γ response detected after
2 stimulation by all the peptide pools was calculated. Higher frequencies of IFN- γ secreting
3 cells both in the early (day 1-15) as well as late stages (day 15-30) were present in mild but
4 not in the severe COVID-19 patients (Figure 3B and 3C). In addition, by analysing the
5 association between the time of SARS-CoV-2 T cell appearance and the length of infection,
6 we observed a statistically significant direct correlation between the early appearance of
7 SARS-CoV-2 peptide-reactive cells (specific for NP, ORF7/8, ORF3a, M and Spike) and shorter
8 duration of infection (Figure 3D). In contrast, no correlation was observed when we
9 analysed the time of antibody and the length of infection. The temporal association of SARS-
10 CoV-2 specific T cell appearance with reduced length of infection suggest that T cells play an
11 essential role in the control of SARS-CoV-2 infection.

12

13 Hierarchy of T cell immunogenicity towards different SARS-CoV-2 proteins

14 Finally, we performed a granular analysis of the ability of different SARS-CoV-2 proteins to
15 stimulate IFN- γ production in severe and mild cases of SARS-CoV-2 infection. Peptide pools
16 covering all the different structural proteins (M, NP, ORF3a and Spike) and the ORF7/8
17 accessory proteins stimulate IFN- γ production from the PBMC of all the acute patients with
18 the exception of patient P05 (Figure 4A). In line with our previous report, NSP7 and NSP13
19 pools were rarely able to trigger IFN- γ response in COVID-19 patients(Le Bert et al., 2020). In
20 most of the acute patients, we observed the simultaneous presence of IFN- γ producing cells
21 specific for all the different peptide pools both during the acute phase of infection and at
22 convalescence (Figure 4A). Of note, we observed that the ORF 7/8 peptide pool triggered a
23 robust IFN- γ response preferentially in the early phases of infection and only in patients

1 with mild disease (Figure 3B and 4A). When we calculated the proportion of IFN- γ producing
2 cells triggered by different SARS-CoV-2 proteins at the time of first detection and at
3 convalescence (>30 days after viral clearance), we observed that ORF7/8 responses waned
4 almost completely at convalescence. In contrast, the proportion of cells stimulated by the
5 peptide pools covering other SARS-CoV-2 proteins remained unchanged (M, ORF3a and
6 Spike) or increased (NP) over time (Figure 4B). To demonstrate unequivocally that IFN- γ
7 secreting cells detected in our assays after peptide stimulation were indeed T cells, we
8 performed a flow cytometry phenotypic analysis of IFN- γ producing cells expanded after
9 SARS-CoV-2 peptide stimulation of PBMCs. Unfortunately, such characterisations was
10 performed only with PBMCs of SARS-CoV-2 patients at convalescence due to biosafety
11 regulations that prevented us from analysing PBMCs collected during active infection (RT-
12 PCR positive) outside a BSL-3 laboratory. As expected and already demonstrated, CD3+ T
13 cells produced IFN- γ after peptide pool stimulation (Supplementary Figure 1A). Most of the
14 peptide responsive T cells were CD4 cells but CD8 T cells specific to NP peptide pools were
15 also detected (Supplementary Figure 1B). We were also able to expand ORF7 or ORF8
16 specific T cells in 6 out of 7 patients in whom we detected, in the early phases of infection, a
17 robust population of IFN- γ producing cells after activation with the combined ORF7/8
18 peptide pools. Phenotypic analysis showed that these cells were all CD4 T cells. Thus even
19 though we were unable to directly demonstrate that CD4 T cells were responsible for the
20 IFN- γ response triggered by ORF7/8 pools during the early phases of infection, such
21 interpretation was strongly supported by the exclusive expansion of ORF7 and ORF8 specific
22 CD4 T cells in the convalescents.

23

1 Discussion

2 Despite the small number of patients analysed, our longitudinal analysis of the dynamics of
3 virological and virus-specific immunological parameters during the acute phase of SARS-
4 CoV-2 infection revealed a positive relation between induction of SARS-CoV-2 specific T cell
5 responses and early control of infection. In addition, the quantity of virus-specific T cells
6 present during the acute phase of infection was not proportional to the COVID-19 severity
7 but was actually more robust in patients with mild disease. These data contrasted with the
8 recent analysis of SARS-CoV-2 specific T cells in COVID-19 convalescent patients that
9 reported a positive association of the frequency of SARS-CoV-2 specific T cells with disease
10 severity(Peng et al., 2020). However in this work, the T cell response was measured in
11 patients who were already in the convalescent phase. Since our longitudinal analysis
12 showed that the frequency of SARS-CoV-2 specific T cells rapidly waned after SARS-CoV-2
13 clearance, the time of T cell analysis can significantly influence the magnitude of T cell
14 response detected. The most robust T cell response was detected in patients with
15 mild/moderate symptoms who cleared the virus early, while for example the patient with
16 severe disease who succumbed to infection developed only a weak and monospecific T cell
17 response detectable 26 days after symptom onset.

18 An opposite scenario was observed for antibodies. The two most severe COVID-19 patients
19 studied here showed the most rapid and robust ability to achieve peak virus neutralisation
20 and the overall quantities of SARS-CoV-2 specific antibodies were higher in severe than
21 milder COVID-19 cases. These data confirm the numerous observations that have linked
22 virus-specific antibody production (Hung et al., 2020; Long et al., 2020b)
23 Ripperger:2020fq}(Ripperger et al., 2020) and B cell hyperactivation (Woodruff et al., 2020)

1 with increased disease severity. Due to the significant link between early induction of T cells
2 and shorter duration of the infection, the demonstration of the early induction of ORF7/8
3 specific cellular immunity can be of particular significance in viral control. For example, a
4 382 nucleotide deletion that truncates ORF7b and ORF8 leading to the elimination of ORF8
5 transcription had been reported at low frequencies in multiple countries (Su et al., 2020).
6 While the mechanism leading to the acquisition of the genomic change is unresolved, the
7 early induction of ORF7/8 specific T cells and a recent report of the robust early antibody
8 response to ORF8 in SARS-CoV-2 infection would suggest that an immune-driven selection
9 process could be involved (Hachim et al., 2020). Given that this viral variant was associated
10 with a milder disease (Young et al., 2020), the early induction of ORF7/8 immunity is worthy
11 of further investigation. It remains difficult to explain why ORF7/8 specific T cells were
12 preferentially detected during the acute phase of infection. Recent findings show a
13 corresponding increase in ORF8-specific antibodies during the early phases of SARS-CoV-2
14 infection (Hachim et al., 2020). However, there is no experimental evidences of a
15 preferential early expression of ORF7/8 proteins in SARS-CoV-2 infected cells that might
16 contribute to an increased immunogenicity of these accessory proteins in the early phases
17 of SARS-CoV-2 infection. An alternative hypothesis is that pre-existing immunological
18 memory to ORF7 or ORF8 might have caused a selective accelerated expansion of ORF7/8 T
19 cells since ORF7/8 specific T cells can be occasionally detected in archived PBMC samples
20 collected from healthy individuals before 2019 (Mateus et al., 2020). However, ORF7/8 is
21 only expressed by SARS-CoV-1 and SARS-CoV-2 with little homology to other seasonal
22 coronaviruses, hence the role of such peptide cross-reactive cells is puzzling and calls for a
23 more detailed analysis of the effect of pre-existing immunity in the control or pathogenesis

- 1 of SARS-CoV-2 infection and of the role of T cells specific for different antigens in SARS-CoV-
- 2 2 protection.
- 3

1 **Acknowledgments**

2 We want to express our gratitude to the study participants and personnel involved in
3 ensuring the safety of the BSL3 laboratory operations.

4

5 **Author contributions**

6 A.T.T., N.L.B. and A.B. designed the experiments. M.L., C.W.T, Y.Z. and G.J.S. performed the
7 experiments in the BSL3 laboratory. W.N.C., C.W.T and L.W. performed the antibody
8 analysis. K.K., C.T. and A.C. performed all other experiments. A.T.T., M.L., N.L.B. and A.B.
9 analysed and interpreted the data. A.T.T. and A.B. prepared the figures and wrote the
10 paper. B.Y., S.K., J.L. and D.L. recruited patients and provided all clinical data. A.B. designed,
11 coordinated the study and provided funding.

12

13 **Declaration of interest**

14 A.B. is a cofounder and A.T.T. consults for Lion TCR, a biotech company developing T cell
15 receptors for treatment of virus-related diseases and cancers. None of the other authors has
16 any competing interest related to the study.

17

1 **Figure Legends**

2 **Figure 1. Relative quantities of SARS-CoV-2 in the upper respiratory tract of symptomatic**

3 **COVID-19 patients during acute infection.** Longitudinal RT-PCR quantification of SARS-CoV-

4 2 RNA in the upper respiratory tract of COVID-19 patients (n=12) with variable symptom

5 severity from symptom onset till RT-PCR negativity. Dotted lines denote the positive cut-off.

6 Insert shows the correlation between the peak relative quantities of SARS-CoV-2 and the

7 duration of infection.

8

9 **Figure 2. Longitudinal analysis of SARS-CoV-2 specific antibody-related responses in acute**

10 **COVID-19 patients.** A) Schematic representation of the surrogate virus neutralisation assay

11 and the Luminex-based assay to quantify SARS-CoV-2 RBD-, S1- and NP-specific IgG and IgM

12 antibodies. A cut-off to define significant virus neutralisation or antibody quantities was set

13 at 20% inhibition for the sVNT assay (as defined in ref) and an MFI >100 for the Luminex

14 based assay respectively. B) SARS-CoV-2 neutralisation and the relative quantities of specific

15 IgG and IgM antibodies. C) Rose plots represent the quantity of RBD-, S1- and NP-specific

16 IgG and IgM antibodies at the time of first detectable antibody response. Patient P02 has no

17 detectable antibody response throughout and the corresponding rose plot is not shown.

18

19 **Figure 3. Longitudinal analysis of SARS-CoV-2 T responses in COVID-19 patients during**

20 **acute infection and at convalescence.** A) SARS-CoV-2 proteome organization; analysed

21 proteins are marked by an asterisk. 15-mer peptides, which overlapped by 10 amino acids,

22 comprising the nucleoprotein, membrane, ORF7ab, ORF8, ORF3a, NSP7 and NSP13 were

23 grouped into 10 pools with the indicated number of peptides in each pool. 15-mer predicted

1 peptides previously shown to activate Spike-specific CD8 and CD4 T cells were grouped into
2 a single pool. PMA/ionomycin was used as a positive control for all samples analysed. B)
3 Longitudinal analysis of the total SARS-CoV-2 T cell response in COVID-19 patients from
4 onset of disease until convalescence. Individual lines represent single patients. C) Total
5 SARS-CoV-2 T cell response detected in all COVID-19 patients (n=12) at days 1-15, 16-30 and
6 >50 days after symptom onset. Patients with mild/moderate or severe symptoms are
7 indicated. D) Correlation between the duration of infection and the number of days to the
8 first detectable T cell response (Total, NP-specific, ORF7/8-specific, ORF3a-specific, M-
9 specific or Spike-specific T cell response) or antibody-related response (sVNT, RBD-, S1- or
10 NP-specific IgG and IgM) are shown in the respective dot plots. P-values and the
11 corresponding r^2 values are shown.

12

13 **Figure 4. Hierarchy of cellular responses towards different SARS-CoV-2 proteins.** A)
14 Stacked bars denotes the frequency of peptide reactive cells in all COVID-19 patients against
15 the indicated SARS-CoV-2 protein at all time points tested. Green shaded areas denote the
16 convalescence phase of the disease. Positive controls are inserted for each patient. B) Plots
17 show the proportion of peptide reactive cells attributed to the respective SARS-CoV-2
18 protein at the peak response during the acute phase and the convalescence phase of the
19 disease (n=8). Wilcoxon matched-pairs test was used to evaluate the differences and the p-
20 values are shown.

21

22

1 **Methods**

2 Study design

3 Patients (n=12) were enrolled in this study after being admitted to the hospital and
4 confirmed to be infected with SARS-CoV-2 based on a positive SARS-CoV-2 RT-PCR test as
5 part of the PROTECT (National Healthcare Group Domain Specific Review Board reference
6 number 2012/00917) and Novel Pathogens (CIRB ref. 2018/3045) studies. All participants
7 provided written informed consent. Peripheral blood of acutely infected patients was
8 collected in Mononuclear Cell Preparation tubes (CPT, BD Vacutainer) and transferred at 4°C
9 to the biosafety level-3 (BSL3) facility for same-day processing. Blood from study
10 participants at convalescent time points was obtained and processed in BSL2 laboratories.
11 Six patients were male, six were female, their median age at time of admission was 52.5
12 years, ranging from 27 to 78 years. None of the patients received immunomodulatory
13 treatments during the study period.

14

15 Surrogate virus neutralization assay

16 sVNT assay to quantify the neutralising antibody response were performed as previously
17 described (Tan et al., 2020). Briefly, sera from acutely infected patients were prepared in
18 BSL3 containment and heat-inactivated prior to sVNT assay. HRP-conjugated RBD
19 (Genscript) were pre-incubated with 1:20 diluted serum at 37°C for 1h, followed by addition
20 to the Fc-chimeric human ACE2-coated MaxiSORP ELISA plate (Nunc) for an hour at room
21 temperature. Colorimetric signal was developed using TMB substrate (KPL) after extensive

1 PBST washes and the reaction was stopped with 1M HCl. Absorbance reading at 450 nm and
2 570 nm were obtained using Hidex Sense microplate reader (Hidex).

3

4 Luminex analysis

5 SARS-CoV-2 RBD, S1 and N proteins (Genscript) were conjugated onto MagPlex microsphere
6 (Luminex) using xMAP antibody coupling kit (Luminex). SARS-CoV-2 spike and N proteins
7 specific antibodies were detected by pre-incubation of 100-fold diluted serum (in 1% BSA
8 PBS) with conjugated microspheres (1250 beads/antigen) for 1h at room temperature,
9 followed by 1:1000 diluted PE-conjugated anti-human IgG polyclonal antibody (eBioscience)
10 or PE-conjugated anti-human IgM antibody for 1h at room temperature. The signal was
11 detected using Luminex MAGPIX instrument.

12

13 IFN- γ ELISPOT assay

14 15-mer peptides that spanned the entire ORF of genes eight SARS-CoV-2 proteins (NP, M,
15 ORF7, ORF8, ORF3, S, NSP7, NSP13) and antibody responses to two SARS-CoV-2 proteins
16 (NP, S) were synthesised with 10 amino acids overlap and were grouped into pools of
17 approximately 40 peptides (Table S1). CPT tubes were centrifuged at 1500 rcf for 15 mins
18 and approximately 2 ml of mononuclear cells located on top of the polyester gel were
19 aliquoted and stored at -80°C. ELISPOT plates were prepared with IFN- γ coating antibody
20 (MabTech) and peptides pools in 50 μ l AIM-V medium (Gibco; Thermo Fisher Scientific)
21 supplemented with 2% AB human serum (Gibco; Thermo Fisher Scientific) on the day of
22 incubation with PBMCs. Vials containing PBMCs were thawed at RT, 2U Benzonase

1 (SigmaAldrich) to remove remaining nucleic acids was added when fully thawed and
2 incubated for 15 mins before centrifugation at 1000 rcf for 10 mins. The cell pellet was
3 dissolved in 1 ml of AIM-V medium (Gibco; Thermo Fisher Scientific) supplemented with 2%
4 AB human serum (Gibco; Thermo Fisher Scientific) before quantification at undiluted and
5 1:10 dilution using a Scepter 2.0 cell counter (Millipore). Approximately 2×10^5 PBMCs in
6 100 μ l per well were incubated in the presence of peptides overnight at 37°C and 5% CO₂.
7 After 24 hours, inoculum was removed and plates were washed six times with PBS.
8 Biotinylated anti-IFN- γ antibody (MabTech) at a 1:2000 dilution in PBS/0.5% FCS was
9 incubated at RT for two hours, followed by six wash steps with PBS and incubation of
10 Streptavidin-AP (MabTech) at a 1:2000 dilution in PBS/0.5% FCS at RT for one hour. After
11 another six PBS washes, 50 μ l of KLP BCIP/NBT phosphatase substrate (SeraCare) was added
12 and incubated at RT in the dark for 5-15 mins. The reaction was stopped by washing the
13 plate with water extensively when the chromogenic reaction produced clearly visible spots.
14 Subsequently, the plates were allowed to air-dry and spot forming units (SFU) were
15 analysed using an Immunospot reader and software (Cellular Technology).

16

17 SARS-CoV-2 specific T cell lines

18 T cell lines were generated as follows: 20% of PBMC were pulsed with 10 μ g/ml of the
19 overlapping SARS-CoV-2 peptides (all pools combined) or single peptides for 1 hour at 37°C,
20 subsequently washed, and co-cultured with the remaining cells in AIM-V medium (Gibco;
21 Thermo Fisher Scientific) supplemented with 2% AB human serum (Gibco; Thermo Fisher
22 Scientific). T cell lines were cultured for 10 days in the presence of 20 U/ml of recombinant
23 IL-2 (R&D Systems).

1

2 Flow cytometry analysis

3 Expanded T cell lines were stimulated for 5h at 37°C with or without SARS-CoV-2 peptide
4 pools (2 µg/ml) in the presence of 10 µg/ml brefeldin A (Sigma-Aldrich). Cells were stained
5 with the yellow LIVE/DEAD fixable dead cell stain kit (Invitrogen) and anti-CD3 (clone SK7;
6 3:50), anti-CD4 (clone SK3; 3:50), and anti-CD8 (clone SK1; 3:50) antibodies.. Cells were
7 subsequently fixed and permeabilised using the Cytofix/Cytoperm kit (BD Biosciences-
8 Pharmingen) and stained with anti-IFN-γ (clone 25723, R&D Systems; 1:25) and anti-TNF-α
9 (clone MAb11; 1:25) antibodies and analysed on a BD-LSR II FACS Scan. Data were analysed
10 by Kaluza (Beckman Coulter). Antibodies were purchased from BD Biosciences-Pharmingen
11 unless otherwise stated.

12

13 Statistical analysis

14 Regression analyses were performed using GraphPad Prism v7 software. Figures were
15 prepared in Adobe Illustrator Creative Cloud.

16

1 **Supplementary Figure Legends**

2 **Supplementary Figure 1. In vitro expanded SARS-CoV-2 specific T cell lines.** A) Short-term T
3 cell lines were generated using the respective SARS-CoV-2 peptide pools. Each line was then
4 stimulated with the corresponding peptide pool used for expansion and the frequency of
5 IFN- γ producing T cells was quantified. The flow cytometry gating strategy is shown above.
6 Representative dot plots of ORF7/8, M and NP-2 specific T cell lines generated from patient
7 P06 are displayed. C) Frequencies of IFN- γ producing CD4 or CD8 T cells of all in vitro
8 expanded T cell lines generated from the convalescent PBMCs of respective COVID-19
9 patients (n=7).

10

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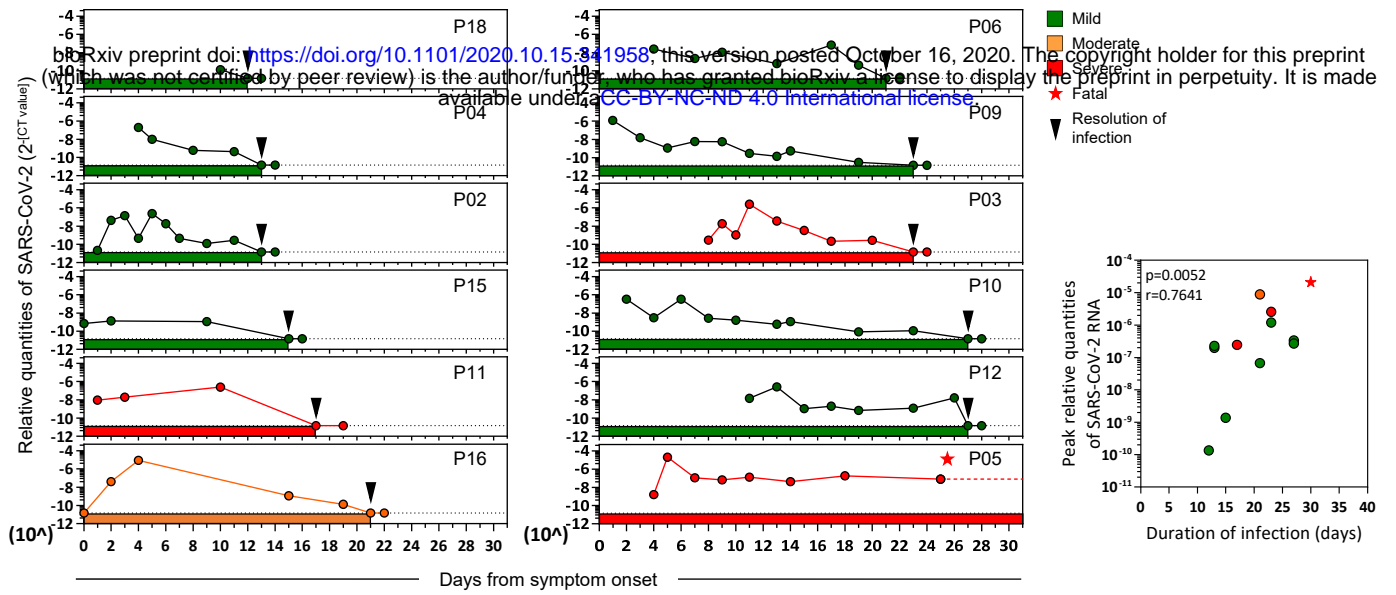
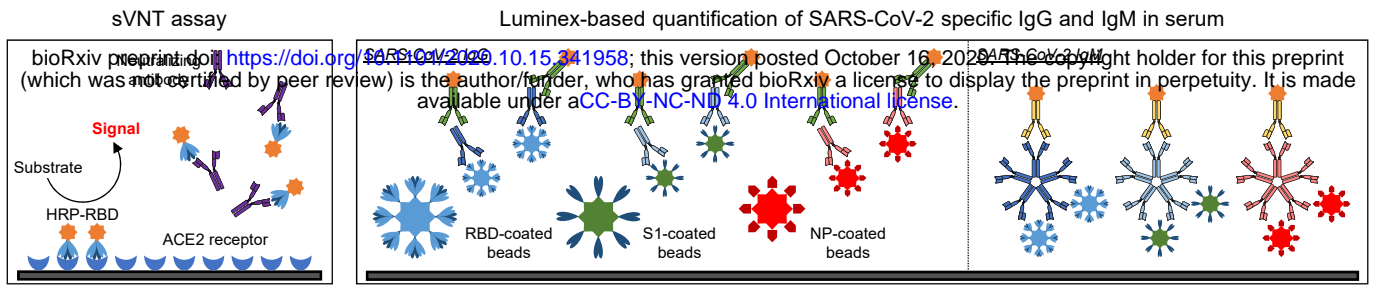
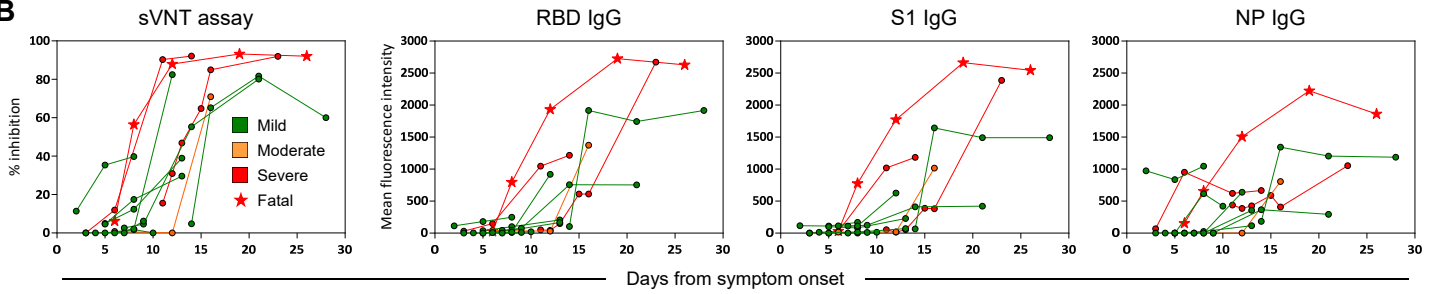
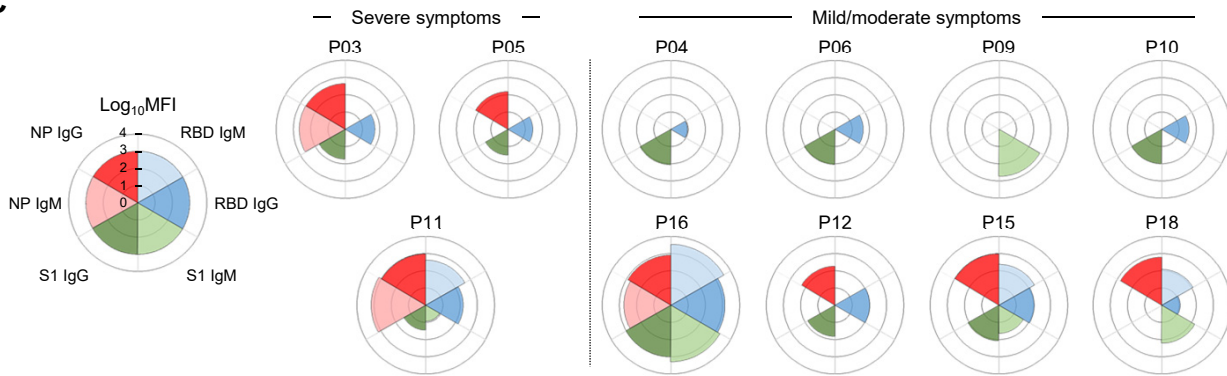


Figure 1

A**B****C****Figure 2**

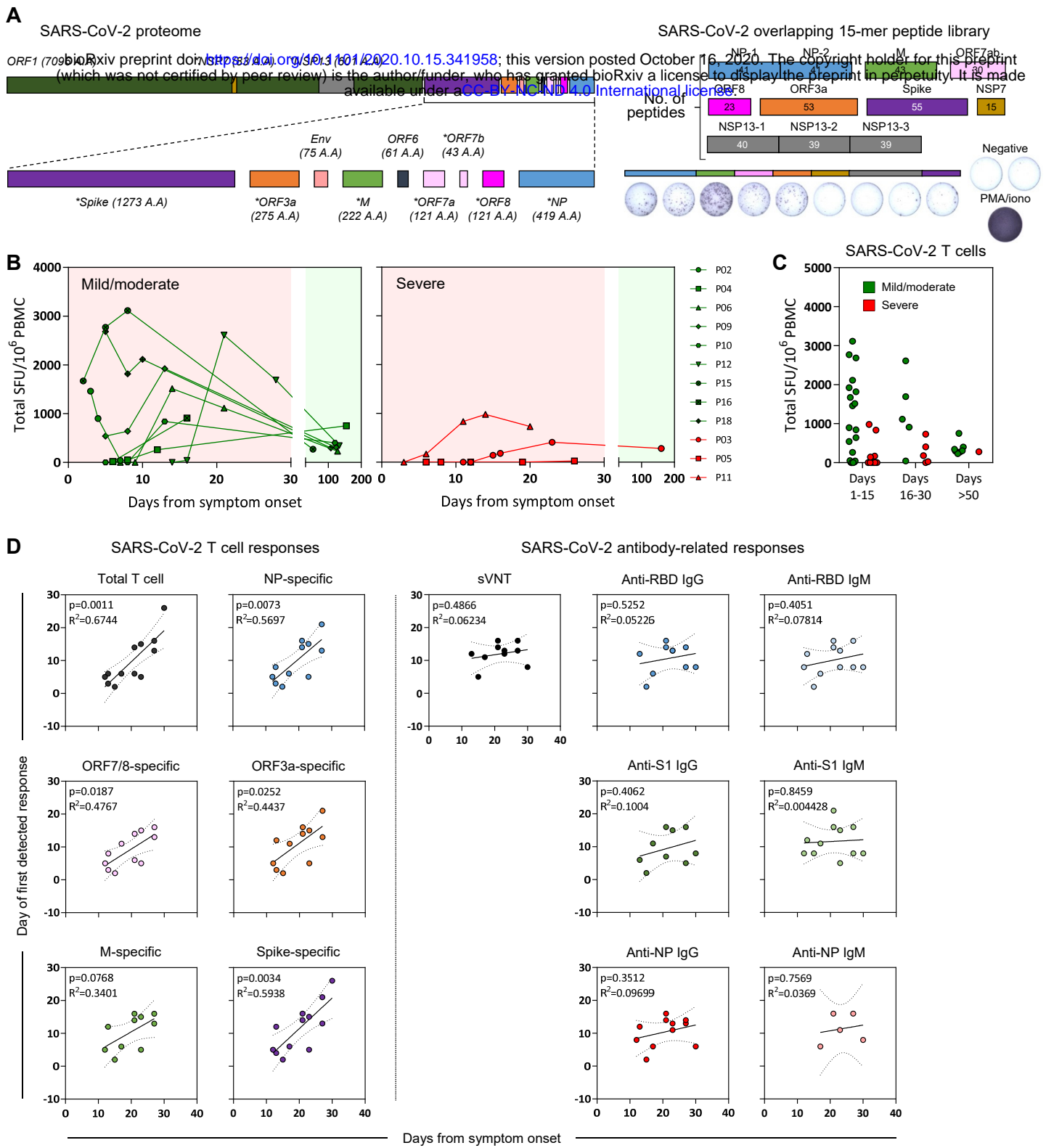


Figure 3

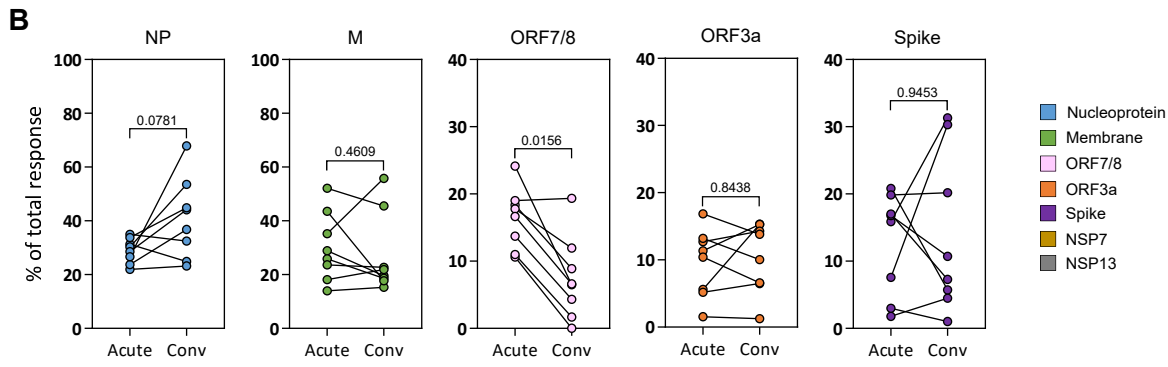
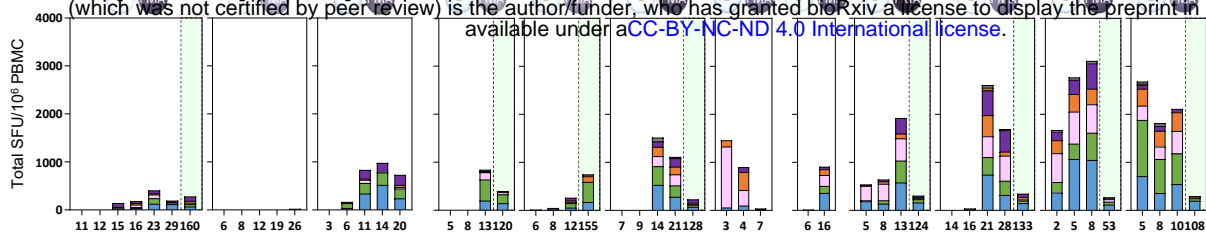


Figure 4

