Different pattern of pre-existing SARS-COV-2 specific T cell immunity in SARS-recovered and uninfected individuals

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Abstract

Memory T cells induced by previous infections can influence the course of new viral infections. Little is known about the pattern of SARS-CoV-2 specific pre-existing memory T cells in human. Here, we first studied T cell responses to structural (nucleocapsid protein, NP) and non-structural (NSP-7 and NSP13 of ORF1) regions of SARS-CoV-2 in convalescent from COVID-19 (n=24). In all of them we demonstrated the presence of CD4 and CD8 T cells recognizing multiple regions of the NP protein. We then show that SARS-recovered patients (n=23), 17 years after the 2003 outbreak, still possess long-lasting memory T cells reactive to SARS-NP, which displayed robust cross-reactivity to SARS-CoV-2 NP. Surprisingly, we observed a differential pattern of SARS-CoV-2 specific T cell immunodominance in individuals with no history of SARS, COVID-19 or contact with SARS/COVID-19 patients (n=18). Half of them (9/18) possess T cells targeting the ORF-1 coded proteins NSP7 and 13, which were rarely detected in COVID-19- and SARS-recovered patients. Epitope characterization of NSP7-specific T cells showed recognition of protein fragments with low homology to “common cold” human coronaviruses but conserved among animal betacoranaviruses.

Thus, infection with betacoronaviruses induces strong and long-lasting T cell immunity to the structural protein NP. Understanding how pre-existing ORF-1-specific T cells present in the general population impact susceptibility and pathogenesis of SARS-CoV-2 infection is of paramount importance for the management of the current COVID-19 pandemic.

Main Text:

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the cause of the coronavirus disease 2019 (COVID-19)[1]. This disease has spread pandemically placing lives and economies of the world under severe stress. SARS-CoV-2 infection is characterized by a broad spectrum of clinical syndromes, ranging from mild influenza-like symptoms to severe pneumonia and acute respiratory distress syndrome[2].

It is common to observe in human the ability of a single virus to cause different pathological manifestations. This is often due to multiple contributory factors including the quantity of viral inoculum, the genetic background of patients and the presence of concomitant pathological conditions. Moreover, an established adaptive immunity towards closely related or completely different viruses can increase protection[3] or enhance disease severity[4].

SARS-CoV-2 belongs to *Coronaviridae*, a family of large RNA viruses infecting many animal species. Six other coronaviruses are known to infect human. Four of them are endemically transmitted[5] and cause common cold (OC43, HKU1, 229E and NL63), while SARS-CoV (defined from now as SARS-CoV-1) and MERS-CoV have caused limited epidemics of severe pneumonia[6]. All of them trigger antibody and T cell responses in infected patients: however, antibody levels appear to wane relatively quicker than T cells. In SARS recovered patients, SARS-CoV-specific antibodies dropped below detection limit within 2 to 3 years[7], while SARS-CoV-specific memory T cells can be detected even at 11 years after infection[8]. Since the sequences of selected structural and non-structural proteins are highly conserved among different coronaviruses (i.e. NSP7 and NSP13 are 100% and 99% identical, respectively, between SARS-CoV-2, SARS-CoV-1 and the bat-SL-CoVZXC21[9]), we studied whether cross-reactive SARS-CoV-2-specific T cells are present in individuals who resolved from SARS-CoV-1 or SARS-CoV-2 infection. We also studied these T cells in individuals with no history of SARS or COVID-19 and who were also not in contact with SARS-CoV-2 infected cases. Collectively these individuals are hereon referred to as SARS-CoV-1/2 unexposed.

SARS-CoV-2-specific T cells have just started to be characterized in COVID-19 patients[10,11] and their potential protective role has been inferred from studies in SARS[12] and MERS[13] patients. To study SARS-CoV-2 specific T cells

associated with viral clearance, we collected peripheral blood of 24 individuals who recovered from mild to severe COVID-19 (demographic, clinical and virological information are summarized in **Extended Data Table 1**) and studied the T cell response against selected structural (nucleocapsid protein-NP) and non-structural proteins (NSP7 and NSP13 of ORF1) of the large SARS-CoV-2 proteome (**Figure 1A**). We selected nucleocapsid protein as it is one of the more abundant structural proteins produced and has large homology between different betacoranaviruses (**Extended Data Fig. 1**)[14].

NSP7 and NSP13 were selected for their complete homology between SARS-CoV-1, SARS-CoV-2 and other animal coronaviruses belonging to the betacoranavirus genus (**Extended Data Fig. 2**)[9], and because they are representative of the ORF1a/b polyprotein encoding the replicase-transcriptase complex[15]. This polyprotein is the first to be translated upon coronavirus infection. We synthesized 216 15-mer peptides overlapping by 10 amino acids (aa) covering the whole length of NSP7 (83aa), NSP13 (601aa) and NP (422aa) that were organized in 5 pools of approximately 40 peptides each (NP-1, NP-2, NSP13-1, NSP13-2, NSP13-3) and in a single pool of 15 peptides spanning NSP7 (**Figure 1B**). The unbiased method with overlapping peptides was utilized instead of peptide selection by bioinformatic approaches, since the performance of such algorithms in ethnically-diverse Asians is often sub-optimal[16].

Peripheral blood mononuclear cells (PBMC) of 24 recovered COVID-19 patients were stimulated for 18h with the different peptide pools and virus-specific T cell responses were analyzed by IFN-γ ELISpot assay. In all tested individuals (24/24) we detected IFN-γ spots following stimulation with the pools of synthetic peptides covering NP (**Figure 1C/D**). In nearly all individuals NP-specific responses could be identified for multiple regions of the protein: 23/24 for region 1-205aa (NP-1) and 24/24 for 206-422aa (NP-2). In sharp contrast, responses to NSP7 and NSP13 peptide pools were detected at low levels only in 3 out of 24 COVID-19 convalescents tested. Direct *ex vivo* intracellular cytokine staining (ICS) was performed to confirm and define the NP-specific IFN-γ ELISpot response. Due to the low frequency, NP-specific T cells were more difficult to visualize by ICS than by ELISpot, but a clear population of CD4 and/or CD8 T cells producing IFN-γ and/or TNF-α were detectable in 7 out of 9

tested subjects (**Figure 1E**). To confirm and further delineate the multispecificity of the NP-specific T cell response detected *ex vivo* in COVID-19 recovered patients, we defined in nine individuals, the distinctive sections of NP targeted by T cells. We organized the 82 overlapping peptides covering the entire NP into small peptide pools (7-8 peptides) that were used to stimulate PBMC either directly *ex vivo* or after an *in vitro* expansion protocol previously used in HBV[17] or SARS recovered subjects[18]. A schematic representation of the peptide pools is shown in **Figure 2A**. We found that 8 out of 9 COVID-19 recovered patients possess T cells that recognize multiple regions of NP of SARS-CoV-2 (**Figure 2A**). Importantly, we then defined single peptides that were able to activate T cells in 7 patients. Utilizing a peptide matrix strategy[18], we first deconvolute individual peptides responsible for the detected T cell response by IFN-γ ELISpot. Subsequently, we confirmed the identified single peptide by testing, with ICS, its ability to activate CD4 or CD8 T cells (**Figure 2B**). **Figure 2B** summarizes the different T cell epitopes defined by both ELISpot and ICS, in 7 COVID-19 recovered individuals. Remarkably, we observed that COVID-19 convalescents developed T cells specific to regions that were also targeted by T cells of SARS recovered subjects. For example, the NP region 101-120 which is a described CD4 T cell epitope in SARS-CoV-1 exposed individuals[8,18], also stimulated CD4 T cells of two COVID-19 recovered donors. Similarly, the NP region 321-340 contains epitopes triggering CD4 and CD8 T cells in both COVID-19 and SARS recovered patients[18]. The demonstration that COVID-19 and SARS recovered patients can mount T cell responses against shared viral determinants implicates that individuals with SARS-CoV-1 infection can induce T cells able to cross-react against SARS-CoV-2.

For the management of the current pandemic and for vaccine development against SARS-CoV-2, it is important to understand if acquired immunity will be long-lasting. Therefore, we tested if individuals who recovered from SARS 17 years ago still harbor memory T cells against SARS-CoV-1. Hence, their PBMC (n=15) were stimulated directly *ex vivo* with peptide pools covering SARS-CoV-1 NP (NP-1 and NP-2), NSP7 and NSP13 (**Figure 3A**). This revealed that 17 years after infection, those individuals still possess virus-specific memory T cells, and similar to COVID-19 recovered patients, we detected T cells reacting

almost exclusively to NP and not to the NSPs (**Figure 3B/C**). Subsequently, we tested if the NP-specific T cells detected in SARS recovered patients could cross-react with SARS-CoV-2 NP peptides (aa identity = 94%). Indeed, although at lower frequency, T cells in all 23 individuals tested reacted to SARS-CoV-2 NP (**Figure 3D, 4A**). In order to test whether these T cells could expand after encounter with SARS-CoV-2 NP, their PBMC were stimulated *in vitro* with the whole battery of NP, NSP7 and NSP-13 peptides and the quantity of T cells responding to SARS-CoV-2 NP, NSP7 and NSP13 was analyzed after 10 days of cell culture. A clear and robust expansion of NP-specific T cells was detected in 7 out of 8 individuals tested (**Figure 3E**). Importantly, and in sharp contrast to the T cell response to NP peptides, we could not detect any T cells reacting to the peptide pools covering NSP13 and only 1 out of 8 reacted to NSP7, despite *in vitro* expansion.

Thus, SARS-CoV-2 NP-specific cross-reactive T cells are part of the T cell repertoire of individuals with a history of SARS-CoV-1 infection and are able to robustly expand after encounter with SARS-CoV-2 NP peptides. These findings demonstrate that virus-specific memory T cells induced by betacoronanvirus infection are long-lasting, which supports the notion that COVID-19 patients would develop long-term T cell immunity. Furthermore, our findings also raise the intriguing possibility that infection with related viruses can also protect from or modify the pathology caused by SARS-CoV-2 infection.

To explore this possibility, we tested NP and NSP7/13-specific T cell responses in 18 SARS-CoV-1/2 unexposed donors. The blood samples were collected either before July 2019 or were serologically negative for both SARS-CoV-2 neutralizing antibodies and SARS-CoV-2 NP antibodies[19]. Different coronaviruses known to cause common cold in humans like OC43, HKU1, NL63 and 229E present different degrees of amino acid homology with SARS-CoV-2 (**Extended Data Fig. 1, 2**) and recent data demonstrated the presence of SARS-CoV-2 cross-reactive CD4 T cells (mainly specific for Spike) in SARS-CoV-2 unexposed donors[11]. Remarkably, we detected NP-specific T cells in some of our SARS-CoV-1/2 unexposed individuals. The pattern of T cell reactivity, however, was different compared to COVID-19 and SARS recovered. T cells from SARS-CoV-1/2 unexposed were directed against a single peptide

pool: i.e. none of the 18 donors responded to the NP-2 peptide pool (**Figure 4A**). Moreover, a different pattern was observed for NSP7- and NSP13-specific T cells. These cells were detected in only 3 out of 24 COVID-19 and in 2 out of 23 SARS recovered tested, but were present in 9 out of 18 unexposed donors (**Figure 4A/B**). The cumulative proportion of all studied subjects responding to NP and ORF-1-coded NSP7 and 13 proteins is shown in **Figure 4B**. These SARS-CoV-2 cross-reactive T cells from SARS-CoV-1/2 unexposed donors have the capacity to expand upon stimulation with SARS-CoV-2 peptides (**Figure 4C**). To better characterize the SARS-CoV-2 specific T cell reactivity detected in the SARS-CoV-1/2 unexposed individuals, fine-specificity and phenotype of the responding T cells were defined in selected donors. Characterization of the NP-specific T cells detected at high frequency in one donor (H-2) identified CD4 T cells reactive for an epitope comprised within the NP region 101-20. This same epitope was also detected in COVID-19 and SARS-recovered patients (**Figure 2B** and[8,18]). It has a high degree of homology to the MERS-CoV, OC43 and HKU1 NP sequences (**Figure 4D**). In two other SARS-CoV-1/2 unexposed donors (H-7 and H-3), we identified CD4 T cells specific for the NSP7 region 26-40 (SKLWAQCVQLHNDIL), and CD8 T cells specific for an epitope comprised within the NSP7 region 37-49 (NDILLAKDTTEAF), respectively (**Figure 4D, Extended Data Figure 3**).

These latter two T cell specificities were particularly intriguing since the homology between the two protein regions of SARS-CoV-1/2 and other “common cold” coronaviruses (OC43, HKU1 NL63 and 229E) was minimal (**Figure 4D**), especially for the CD8 peptide epitope. This may suggest that perhaps not only human “common cold” coronaviruses, but other presently unknown coronaviruses, possibly of animal origin, can induce cross-reactive SARS-CoV-2 memory T cells in the general population.

It was remarkable to find that NSP7/13-specific T cells were detected in 9 out of 18 (50%) SARS-CoV-1/2 unexposed donors, despite the fact that our analysis was performed with peptides that cover only 10% (684aa) of the ORF-1 proteome (7096aa). Notably, T cells specific for ORF-1-coded proteins were rarely detected in our SARS and COVID-19 convalescents. This is consistent with the findings of Grifoni et al[11]: using selected peptides, they detected ORF-

1 specific T preferentially in some SARS-CoV-2 unexposed donors while T cells of COVID-19 recovered donors preferentially recognized structural proteins.

The cause of this observed different pattern of immunodominance is presently unknown. We might speculate that a robust T cell response against structural proteins is induced by a productive infection (occurring in COVID-19 and SARS recovered patients). Individuals exposed to but not infected with possible unknown coronaviruses might just prime ORF-1-specific T cells. Indeed, induction of virus-specific T cells in "exposed but not infected" individuals has been demonstrated in other viral infections[20]. In coronavirus infected cells, the ORF-1 coded proteins are necessary for the formation of the viral replicase-transcriptase complex in which viral replication and transcription occur[14]. Therefore, an ORF-1-specific T cell can be envisioned to abort viral production in infected cells by lyses of SARS-CoV-2 infected cells even before the formation of mature virions.

Importantly, the ORF-1 region contains domains that are extremely conserved among many different coronaviruses[6]. The distribution of these viruses in different animal species might result in periodic human contact and subsequently induction of ORF-1-specific T cells with cross-reactive ability against SARS-CoV-2. Understanding the distribution, frequency and protective ability of the pre-existing structural or non-structural SARS-CoV-2 cross-reactive T cells could be of great importance to explain some of the differences in infection rate or pathology observed during this pandemic. T cells specific for viral structural proteins have protective ability in animal models of airway system infection[21,22]. Nevertheless, the impact that the presence of ORF-1 specific T cells could have in the differential modulation of SARS-CoV-2 infection will have to be carefully evaluated.

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Material and Methods:

Ethics statement: All donors provided written consent. The study was conducted in accordance with the Declaration of Helsinki and approved by the NUS institutional review board (H-20-006) and the SingHealth Centralised Institutional Review Board (reference CIRB/F/2018/2387).

Human samples: Donors were recruited based on their clinical history of SARS-CoV-1 or SARS-CoV-2 infection. Blood samples of recovered COVID-19 patients (n=24) were obtained 2 – 28 days post PCR negativity; of recovered SARS patients (n=23) 17 years post infection. Healthy donors' samples were either collected before June 2019 for studies of T cell function in viral diseases (n=10) or in March-April 2020 and tested negative for RBD neutralizing antibodies and negative in an ELISA for NP IgG[19].

PBMC isolation: Peripheral blood mononuclear cells (PBMC) were isolated by density-gradient centrifugation using Ficoll-Paque. Isolated PBMC were either studied directly or cryopreserved and stored in liquid nitrogen until used in the assays.

Peptide pools: 15-mer peptides overlapping by 10 amino acids spanning the entire protein sequence of SARS-CoV-2 NP, NSP7 and NSP 13, as well as SARS-CoV-1 NP were synthesized (GL Biochem Shanghai Ltd; see **Sup. Tables 1,2**). To stimulate PBMC, the peptides we divided into 5 pools of about 40 peptides covering NP (NP-1, NP-2) and NSP13 (NSP13-1, NSP13-2, NSP13-3) and one pool of 15 peptides covering NSP7. For single peptide identification, peptides were organized in a matrix of 12 numeric and 7 alphabetic pools for NP, and 4 numeric and 4 alphabetic pools for NSP7.

ELISpot assay: ELISpot plates (Millipore) were coated with human IFN-γ antibody (1-D1K, MabTech) overnight at 4ºC. 400,000 PBMC were seeded per well and stimulated with pools of SARS-CoV-1/2 peptides (2 µg/ml). For stimulation with peptide matrix pools or single peptides, a concentration of 5 µg/ml was used. Subsequently, the plates were developed with human biotinylated IFN-γ detection antibody (7-B6-1, MabTech), followed by

incubation with Streptavidin-AP (MabTech) and KPL BCIP/NBT Phosphatase Substrate (SeraCare).

Flow Cytometry: PBMC or Expanded T cell lines were stimulated for 5h at 37°C with or without SARS-CoV-1/2 peptide pools (2 µg/ml) in the presence of 10 µg/ml brefeldin A (Sigma-Aldrich). Cells were stained with the yellow LIVE/DEAD fixable dead cell stain kit (Invitrogen) and anti-CD3 (clone SK7), anti-CD4 (clone SK3), and anti-CD8 (clone SK1) antibodies. Cells were subsequently fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences-Pharmingen) and stained with anti-IFN-γ (clone 25723, R&D Systems) and anti-TNF-α (clone MAb11) antibodies and analyzed on a BD-LSR II FACS Scan. Data were analyzed by FlowJo (Tree Star Inc.). Antibodies were purchased from BD Biosciences-Pharmingen unless otherwise stated.

Cell culture for T cell expansion: T cell lines were generated as follows: 20% of PBMCs were pulsed with 10 µg/ml of the overlapping SARS-CoV-2 peptides for 1 hour at 37°C, subsequently washed, and cocultured with the remaining cells in AIM-V medium (Gibco; Thermo Fisher Scientific) supplemented with 2% AB human serum (Gibco; Thermo Fisher Scientific). T cell lines were cultured for 10 days in the presence of 20 U/ml of recombinant IL-2 (R&D Systems).

Sequence alignment:

Reference protein sequences for ORF1ab and Nucleocapsid Protein were downloaded from the NCBI database (see below). Sequences were aligned using the MUSCLE algorithm with default parameters and percentage identity was calculated in Geneious Prime 2020.1.2 (https://www.geneious.com). Alignment figures were made in Snapgene 5.1 (GSL Biotech).

Accession ID	ORF1ab	Nucleocapsid Protein
SARS-CoV-2	QHD43415.1	YP_009724397.2
SARS-CoV-1	NP_828849.2	AAP33707.1
MERS	YP_009047202.1	YP_009047211.1
OC43	YP_009555238.1	YP_009555245.1
HKU1	YP_173236.1	YP_173242.1
NL63	YP_003766.2	YP_003771.1
229E	NP_073549.1	NP_073556.1

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Author contributions:

NLB and ATT designed and performed experiments, analysed data, prepared the figures and edited the paper; KK, CYLT, MH, AC, ML, NT performed experiments and analysed data; MC, ML performed viral sequence homology and analysed data; WNC, LW provide antibody testing, MICC, EEO, SK, PAT, JGHL, YJT recruited patients and analysed data, YJT provided funding and AB designed and coordinated the study, provided funding, analysed the data, and wrote the paper.

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

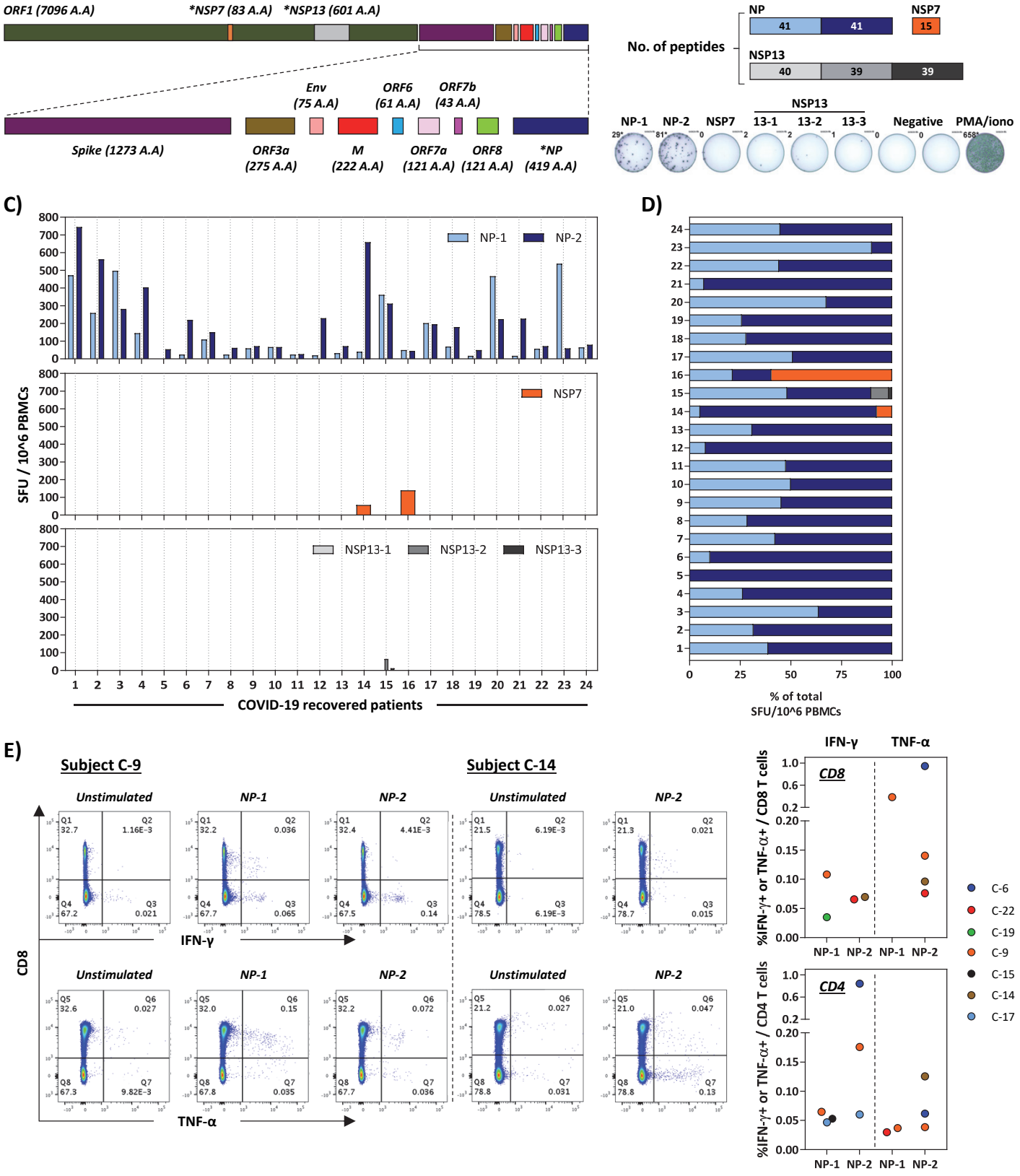
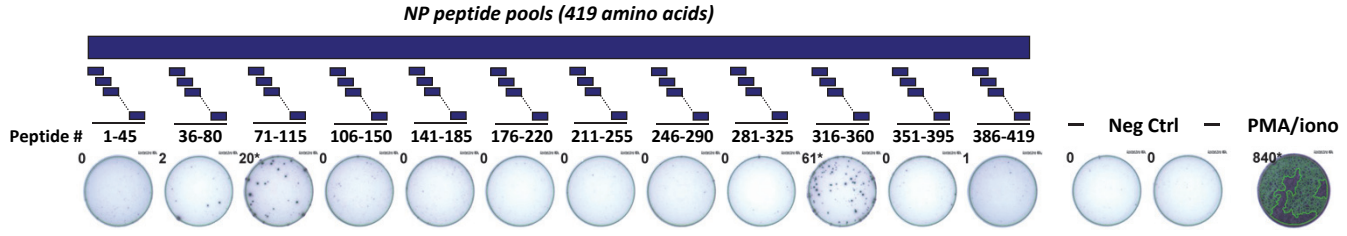


Fig 1: SARS-CoV-2-specific T cells in recovered COVID-19 patients

(A) SARS-CoV-2 proteome organization; analyzed proteins are marked by *. (B) For detection of SARS-CoV-2-specific T cells by IFN-γ ELISpot, 15-mer peptides overlapping by 10 amino acids covering nucleocapsid protein (NP) and the non-structural proteins (NSP) 7 and 13 were synthesized and split into 5 pools of about 40 peptides covering NP (NP-1, NP-2) and NSP13 (NSP13-1, NSP13-2, NSP13-3) and one pool of 15 peptides covering NSP7. (C) PBMC of 24 recovered COVID-19 patients were stimulated with the peptide pools. Bar graphs show frequency of spot forming units (SFU) of IFN-γ secreting cells. (C) Composition of the SARS-CoV-2-specific T cell repertoire is shown as percentage of SARS-CoV-2-specific T cells reacting to NP (NP-1 = light blue; NP2 = dark blue), NSP7 (orange) and NSP13 (grey) for the individual recovered COVID-19 patients tested. (D) PBMC were stimulated with the peptide pools covering NP (NP-1, NP-1) for 5h and analyzed by intracellular cytokine staining. Dot plots show examples of patients with CD4 and/or CD8 T cells producing IFN-γ and/or TNF-α in response to stimulation with NP-1 and/or NP2 peptides. The graphs summarize the percentage of SARS-CoV-2 NP-peptide-reactive CD4 and CD8 T cells in 7 individuals.

A)



Subjects	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6	Pool 7	Pool 8	Pool 9	Pool 10	Pool 11	Pool 12
C-1	-	-	+	-	-	-	-	-	-	+	-	-
C-4	-	-	-	+	-	-	-	+	+	-	-	-
C-18	-	-	+	-	-	-	+	+	+	+	-	-
*C-8	-	+	-	+	-	-	+	+	-	+	-	-
*C-10	-	-	-	-	+	+	+	-	-	+	-	-
*C-11	-	-	+	-	-	-	-	-	-	-	-	-
*C-12	+	-	-	+	-	-	+	-	-	+	-	-
*C-14	-	-	+	-	-	-	+	+	-	+	-	-
*C-15	-	-	+	+	-	-	+	+	-	-	-	-

+ *Positive*

- *Negative*

IFN-γ ELISPOT response against individual NP peptide pools

B)

Subjects	Type of response (CD8 / CD4)	Amino acid residue	SARS-CoV-2 Amino acid sequence	SARS-CoV-1 Amino acid sequence
C-1	CD4	NP 81-95	DDQIGYYRRATRR**I**R	DDQIGYYRRATRR**V**R
	CD8	NP 321-340	GMEVTPSGTWLTY**T**GAIKLD	GMEVTPSGTWLTY**H**GAIKLD
C-4	CD4	NP 266-280	K**A**YNVTQAFGRRGPE	K**Q**YNVTQAFGRRGPE
	CD4	NP 291-305	LIRQGTDYKHWPQIA	LIRQGTDYKHWPQIA
	CD4	NP 301-315	WPQIAQFAPSASAFF	WPQIAQFAPSASAFF
C-8	CD4	NP 51-65	SWFTALTQHGKE**D**L**K**	SWFTALTQHGKE**E**L**R**
	CD4	NP 101-120	MK**D**LSPRWYFYYLGTGPEA**G**	MK**E**LSPRWYFYYLGTGPEA**S**
C-10	CD8 / CD4	NP 321-340	GMEVTPSGTWLTY**T**GAIKLD	GMEVTPSGTWLTY**H**GAIKLD
C-12	CD8	NP 321-340	GMEVTPSGTWLTY**T**GAIKLD	GMEVTPSGTWLTY**H**GAIKLD
C-15	CD4	NP 101-120	MK**D**LSPRWYFYYLGTGPEA**G**	MK**E**LSPRWYFYYLGTGPEA**S**
C-16	CD4	NSP7 21-35	RVESSSKLWAQCVQL	RVESSSKLWAQCVQL

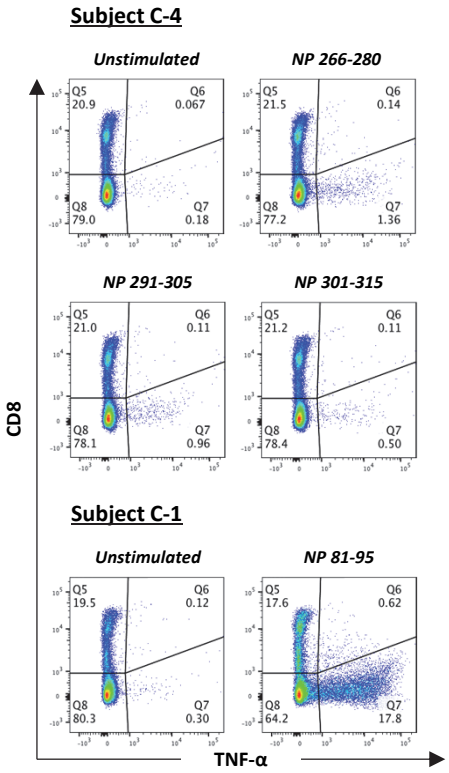


Fig 2: SARS-CoV-2-specific T cells in convalescent COVID-19 patients are targeting multiple regions of nucleocapsid protein
(A) PBMC of 9 COVID-19 recovered individuals were stimulated with 12 different pools of 7-8 NP-peptides to delineate the region to which their T cells react. The table shows IFN-γ ELISpot response against the individual NP peptide pools. (B) Following in vitro T cell expansion, a peptide pool matrix strategy was applied in 7 individuals. The distinct peptide epitopes to which the T cells react were identified by IFN-γ ELISpot and confirmed by ICS (representative dot-plots are shown). Previously described T cell epitopes for SARS-CoV-1 are highlighted in red; non-conserved amino acid residues between SARS-CoV-1 and -2 are bold and underlined.

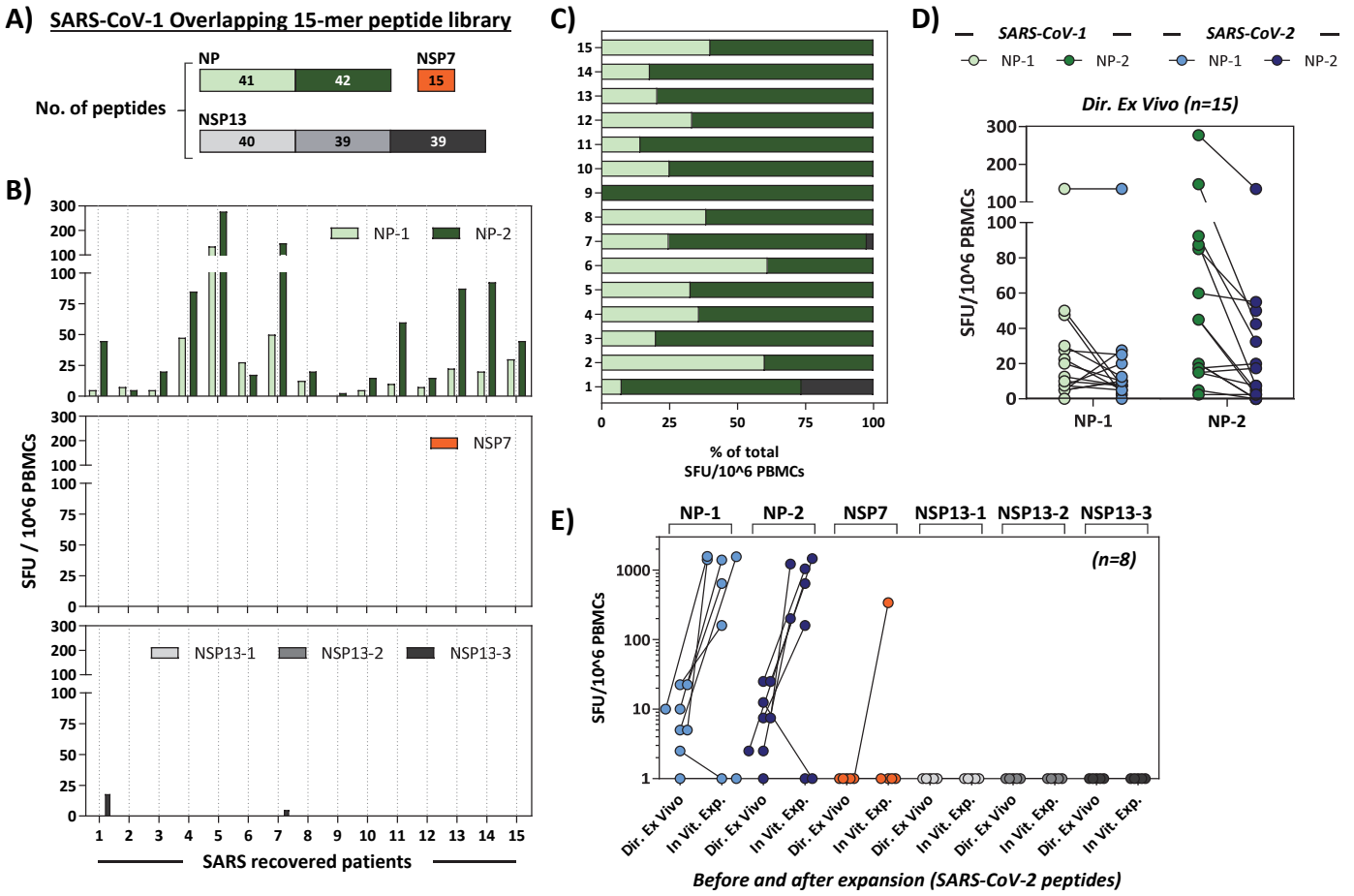
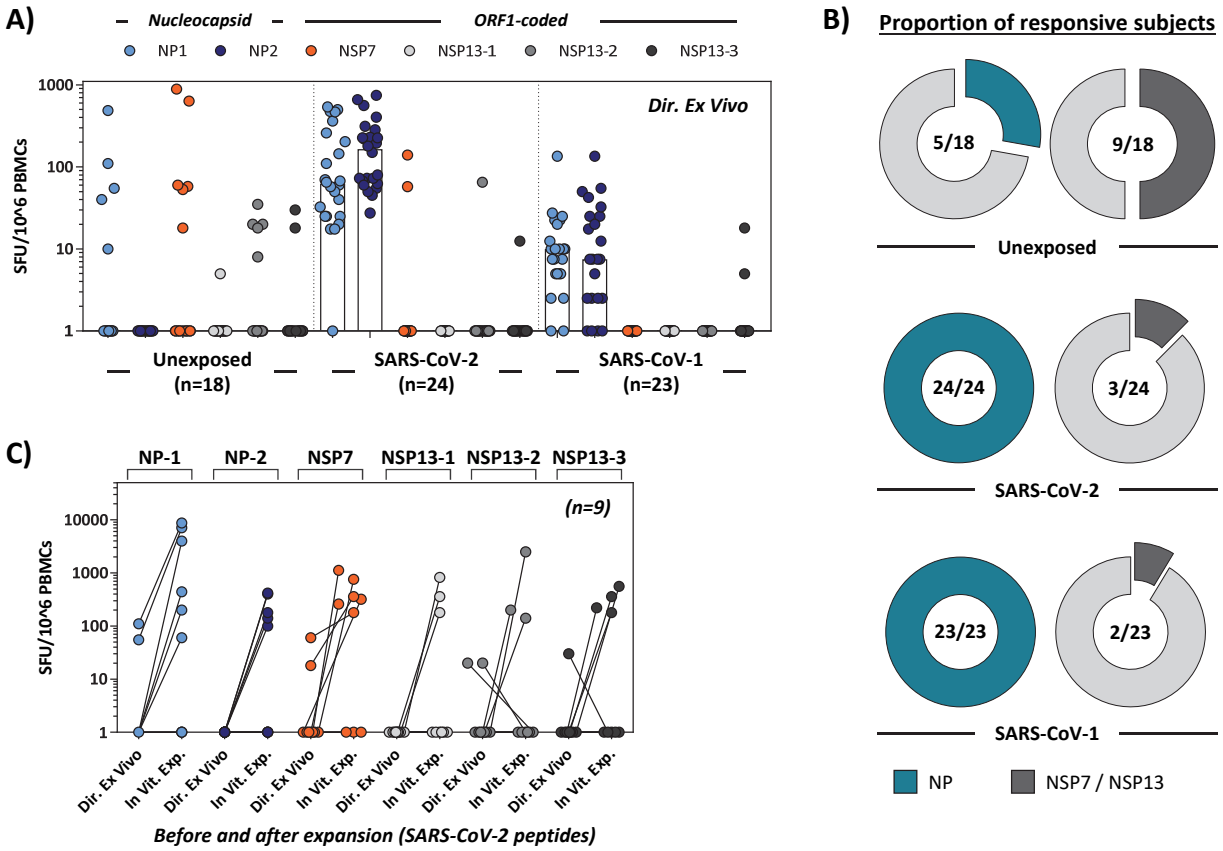


Fig 3: SARS-CoV-2 cross-reactive memory T cells are present in SARS-recovered patients

(A) PBMC isolated from 15 individuals who recovered from SARS 17 years ago were stimulated with SARS-CoV-1 NP, NSP7 and NSP13 peptide pools. (B) Bar graphs show spot forming units (SFU) of IFN-γ secreting cells per 1 million PBMC following overnight stimulation with the indicated peptide pools. (C) Composition of the SARS-CoV-1-specific T cells in individual recovered SARS patients. The percentage of SARS-CoV-1-specific T cells against NP (NP-1 = light green; NP2 = dark green), NSP7 (orange) and NSP13 (grey) in each patient is shown. (D) PBMC of 15 SARS-recovered individuals were stimulated in parallel with peptide pools covering NP of SARS-CoV-1 and of SARS-CoV-2 and their frequency is shown. (E) PBMC of 8 SARS-recovered individuals were stimulated with all peptides covering SARS-CoV-2 NP, NSP7 and NSP13 to expand peptide cross-reactive T cells. The graph shows the number of T cells reactive to the peptide pools indicated directly ex-vivo and after specific T cell expansion.



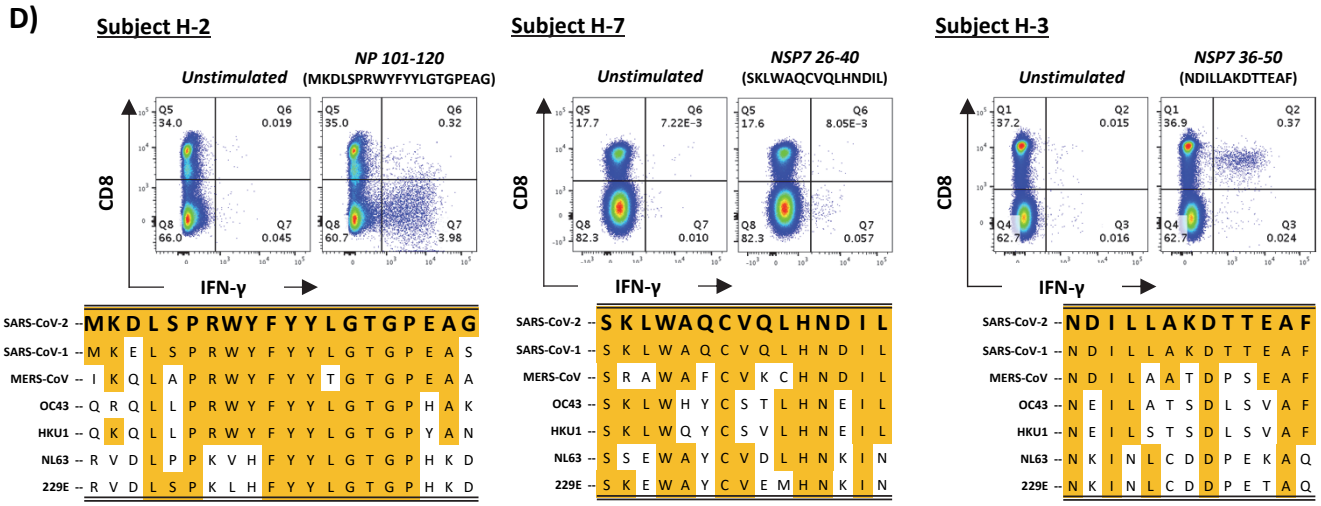


Fig 4: Differential protein immunodominance of SARS-CoV-2 specific T cells in COVID-19- and SARS-recovered patients and in unexposed individuals

(A) PBMC of individuals who are SARS-CoV-1/2 unexposed (n=18), recovered from SARS (n=23) or COVID-19 (n=24) were stimulated with peptide pools covering SARS-CoV-2 NP (NP-1, NP-2), NSP7 and NSP13 (NSP13-1, NSP13-2, NSP13-3) and analyzed by ELISpot. Frequency of peptide-reactive T cells is shown for each donor (dots) and the bars represent median frequency. (B) Pie charts represent percentage of individuals with NP-specific and NSP7/13-specific T cells for unexposed, SARS- and COVID-19-recovered individuals. (C) Frequency of SARS-CoV-2 reactive T cells in 9 unexposed donors to the indicated peptide pools directly ex vivo and after a 10-day expansion. (D) A peptide pool matrix strategy was applied in 3 SARS-CoV-1/2 unexposed individuals. The identified T cell epitopes were confirmed by ICS, and the sequences are aligned with the corresponding sequence of all coronaviruses known to infect humans.

Extended Data Table 1: Donor Characteristics

	COVID-19 recovered	SARS recovered	SARS-CoV-1/2 unexposed
Number	24	23	18
Median age in years (range)	49.5 (27-78)	49 (21-67)	40 (33-63)
Gender			
Male	58% (14/24)	26% (6/23)	50% (9/18)
Female	42% (10/24)	74% (17/23)	50% (9/18)
Residence			
Singapore	100%	100%	100%
Ethnicity			
Chinese	45.8% (11/24)	43.5% (10/23)	55.6% (10/18)
Caucasian	37.5% (9/24)	0% (0/23)	27.8% (5/18)
Indian	12.5% (3/24)	21.7% (5/23)	5.6% (1/18)
Japanese	4.2% (1/24)	0% (0/23)	0% (0/18)
Malay	0% (0/24)	30.4% (7/23)	11.1% (2/18)
Ceylonese	0% (0/24)	4.3% (1/23)	0% (0/18)
***Disease Severity**			
Mild	66.7% (16/24)	73.9% (17/23)	N/A
Moderate	16.7% (4/24)	13% (3/23)	N/A
Severe	16.7% (4/24)	13% (3/23)	N/A
Critical	0% (0/24)	0	N/A
Virological parameters			
SARS-CoV-1 PCR positive	N/A	100%	N/A
SARS-CoV-2 PCR positivity	100%	N/A	N/A
#SARS-CoV-2 NP Ig positivity	100%	100%	0%
#SARS-CoV-2 RBD Ig positivity	100%	0%	0%
Time since PCR negativity	2-28 days	17 years	N/A

**** WHO criteria***

 # reference: Yong et al., Lancet Infect Dis 2020

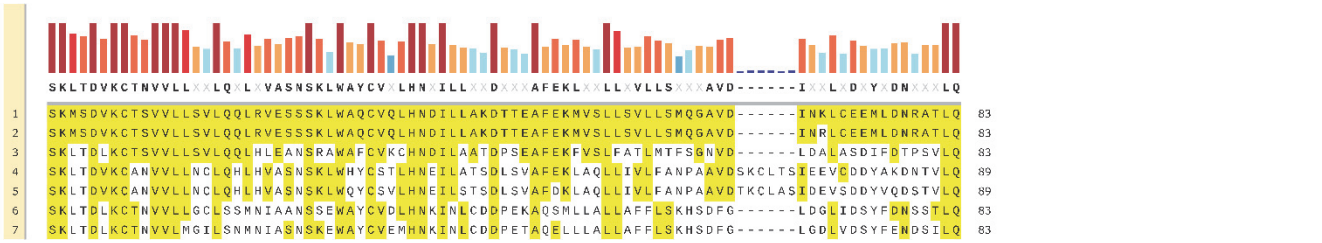
Nucleocapsid

1. SARS-CoV-2 2. SARS-CoV-1 3. MERS-CoV 4. OC43 5. HKU1 6. NL63 7. 229E

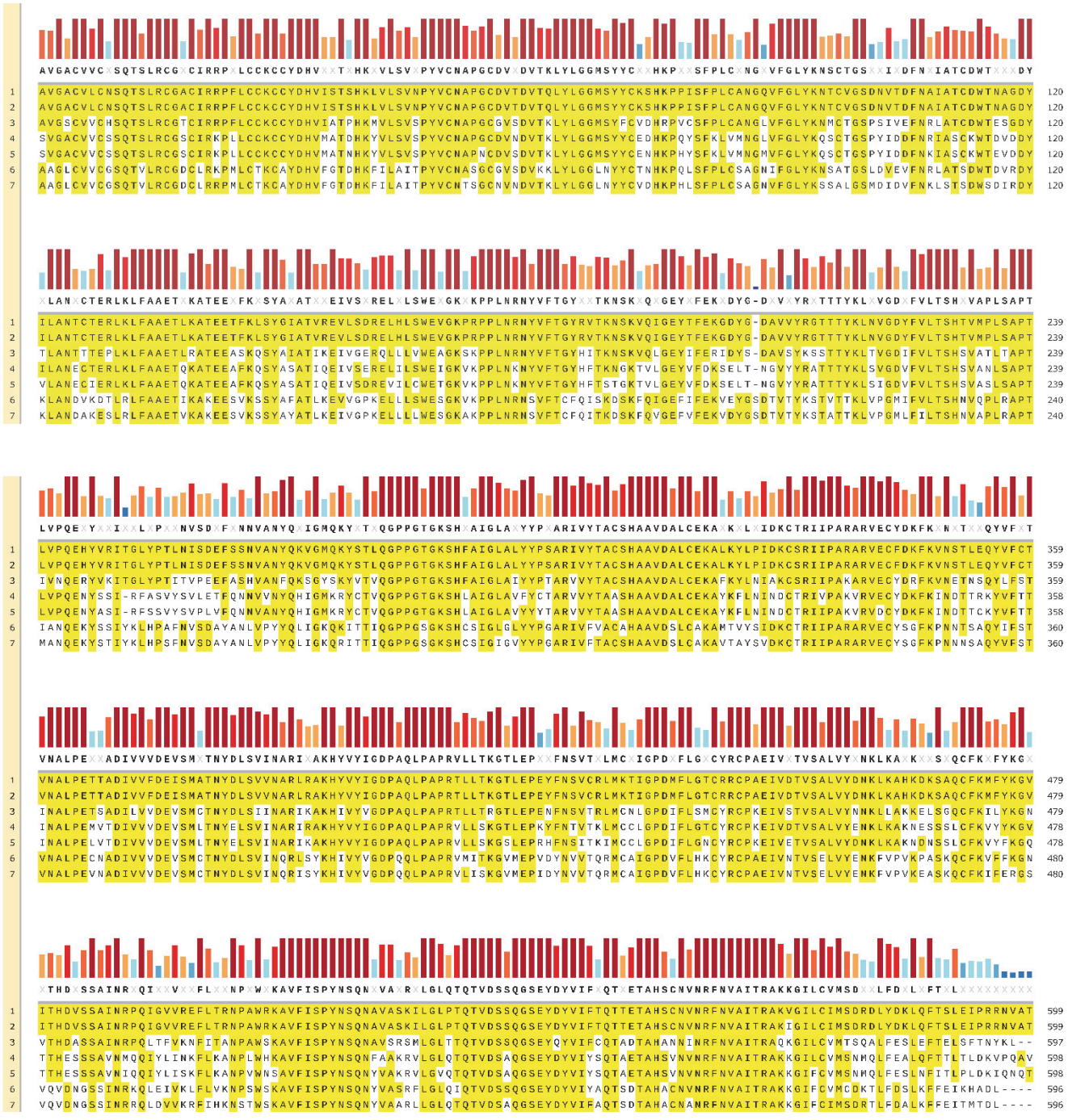
Extended Data Fig. 1: Sequence alignment of the nucleocapsid protein from all types of human coronaviruses
Amino acid sequences for Nucleocapsid Protein were downloaded from the NCBI database and aligned using the MUSCLE algorithm.

NSP7

1. SARS-CoV-2 2. SARS-CoV-1 3. MERS-CoV 4. OC43 5. HKU1 6. NL63 7. 229E

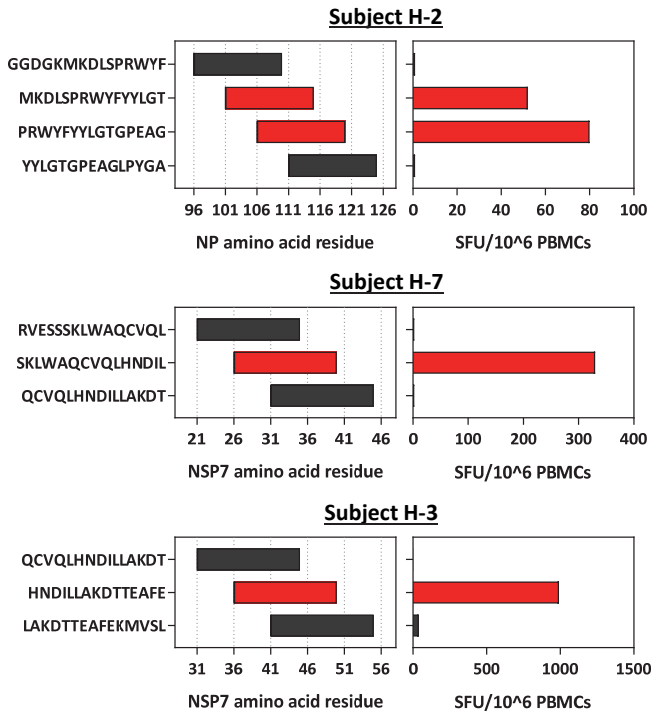


NSP13



Extended Data Fig. 2: Sequence alignment of the ORF-1-coded nonstructural proteins NSP7 and NSP13 from all types of human coronaviruses

Protein sequences for ORF1ab were downloaded from the NCBI database and aligned using the MUSCLE algorithm. The alignment for NSP7 and NSP13 is shown.



Extended Data Fig. 3: SARS-CoV-2 cross-reactive T cell epitope identification in three SARS-CoV-1/2 unexposed donors
PBMC were stimulated with the single peptides identified by the peptide matrix in parallel with the neighboring peptides and assayed by IFN-γ ELISpot. The amino acid residues are shown on the left; the frequency of IFN-γ-SFU/1 Million PBMC on the right. T cell-activating peptides in red, neighboring in black.

Supplementary Table 1: SARS-CoV-2 peptide libraries used in the study

SARS-CoV-2 OLP: nucleocapsid

NP-1 peptide pool			NP-2 peptide pool		
Peptide No	Peptide Sequence	aa	Peptide No	Peptide Sequence	aa
1	MSDNGPQNQRNAPRI	1-15	42	SPARMAGNGGDAALA	206-220
2	PQNQRNAPRITFGGP	6-20	43	AGNGGDAALALLLLD	211-225
3	NAPRITFGGPSDSTG	11-25	44	DAALALLLLDRLNQL	216-230
4	TFGGPSDSTGSNQNG	16-30	45	LLLLDRLNQLESKMS	221-235
5	SDSTGSNQNGERSGA	21-35	46	RLNQLESKMSGKGQQ	226-240
6	SNQNGERSGARSKQR	26-40	47	ESKMSGKGQQQQGQT	231-245
7	ERSGARSKQRRPQGL	31-45	48	GKGQQQQGQTVTKKS	236-250
8	RSKQRRPQGLPNNTA	36-50	49	QQGQTVTKKSAAEAS	241-255
9	RPQGLPNNTASWFTA	41-55	50	VTKKSAAEASKKPRQ	246-260
10	PNNTASWFTALTQHG	46-60	51	AAEASKKPRQKRTAT	251-265
11	SWFTALTQHGKEDLK	51-65	52	KKPRQKRTATKAYNV	256-270
12	LTQHGKEDLKFPRGQ	56-70	53	KRTATKAYNVTQAFG	261-275
13	KEDLKFPRGQGVPIN	61-75	54	KAYNVTQAFGRRGPE	266-280
14	FPRGQGVPINTNSSP	66-80	55	TQAFGRRGPEQTQGN	271-285
15	GVPINTNSSPDDQIG	71-85	56	RRGPEQTQGNFGDQE	276-290
16	TNSSPDDQIGYYRRA	76-90	57	QTQGNFGDQELIRQG	281-295
17	DDQIGYYRRATRRIR	81-95	58	FGDQELIRQGTDYKH	286-300
18	YYRRATRRIRGGDGK	86-100	59	LIRQGTDYKHWPQIA	291-305
19	TRRIRGGDGKMKDLS	91-105	60	TDYKHWPQIAQFAPS	296-310
20	GGDGKMKDLSPRWYF	96-110	61	WPQIAQFAPSASAFF	301-315
21	MKDLSPRWYFYYLGT	101-115	62	QFAPSASAFFGMSRI	306-320
22	PRWYFYYLGTGPEAG	106-120	63	ASAFFGMSRIGMEVT	311-325
23	YYLGTGPEAGLPYGA	111-125	64	GMSRIGMEVTPSGTW	316-330
24	GPEAGLPYGANKDGI	116-130	65	GMEVTPSGTWLTYTG	321-335
25	LPYGANKDGIIWVAT	121-135	66	PSGTWLTYTGAIKLD	326-340
26	NKDGIIWVATEGALN	126-140	67	LTYTGAIKLDDKDPN	331-345
27	IWVATEGALNTPKDH	131-145	68	AIKLDDKDPNFKDQV	336-350
28	EGALNTPKDHIGTRN	136-150	69	DKDPNFKDQVILLNK	341-355
29	TPKDHIGTRNPANNA	141-155	70	FKDQVILLNKHIDAY	346-360
30	IGTRNPANNAAIVLQ	146-160	71	ILLNKHIDAYKTFPP	351-365
31	PANNAAIVLQLPQGT	151-165	72	HIDAYKTFPPTEPKK	356-370
32	AIVLQLPQGTTLPKG	156-170	73	KTFPPTEPKKDKKKK	361-375
33	LPQGTTLPKGFYAEG	161-175	74	TEPKKDKKKKADETQ	366-380
34	TLPKGFYAEGSRGGS	166-180	75	DKKKKADETQALPQR	371-385
35	FYAEGSRGGSQASSR	171-185	76	ADETQALPQRQKKQQ	376-390
36	SRGGSQASSRSSSRS	176-190	77	ALPQRQKKQQTVTLL	381-395
37	QASSRSSSRSRNSSR	181-195	78	QKKQQTVTLLPAADL	386-400
38	SSSRSRNSSRNSTPG	186-200	79	TVTLLPAADLDDFSK	391-405
39	RNSSRNSTPGSSRGT	191-205	80	PAADLDDFSKQLQQS	396-410
40	NSTPGSSRGTSPARM	196-210	81	DDFSKQLQQSMSSAD	401-415
41	SSRGTSPARMAGNGG	201-215	82	QLQQSMSSADSTQA	406-419

SARS-CoV-2 OLP: NSP7

NSP7 peptide pool		
Peptide No	Peptide Sequence	aa
1	SKMSDVKCTSVVLLS	1-15
2	VKCTSVVLLSVLQQL	6-20
3	VVLLSVLQQLRVESS	11-25
4	VLQQLRVESSSKLWA	16-30
5	RVESSSKLWAQCVQL	21-35
6	SKLWAQCVQLHNDIL	26-40
7	QCVQLHNDILLAKDT	31-45
8	HNDILLAKDTTEAFE	36-50
9	LAKDTTEAFEKMVSL	41-55
10	TEAFEKMVSLLSVLL	46-60
11	KMVSLLSVLLSMQGA	51-65
12	LSVLLSMQGAVDINR	56-70
13	SMQGAVDINRLCEEM	61-75
14	VDINRLCEEMLDNRA	66-80
15	NRLCEEMLDNRATLQ	69-83

SARS-CoV-2 OLP: NSP13

NSP13-1 peptide pool			NSP13-2 peptide pool			NSP13-3 peptide pool		
Peptide No	Peptide Sequence	aa	Peptide No	Peptide Sequence	aa	Peptide No	Peptide Sequence	aa
1	AVGACVLCNSQTSLR	1-15	41	EKGDYGDAVVYRGTT	201-215	80	YVYIGDPAQLPAPRT	396-410
2	VLCNSQTSLRCGACI	6-20	42	GDAVVYRGTTTYKLN	206-220	81	DPAQLPAPRTLLTKG	401-415
3	QTSLRCGACIRRPFL	11-25	43	YRGTTTYKLNVGDYF	211-225	82	PAPRTLLTKGTLEPE	406-420
4	CGACIRRPFLCCKCC	16-30	44	TYKLNVGDYFVLTSH	216-230	83	LLTKGTLEPEYFNSV	411-425
5	RRPFLCCKCCYDHVI	21-35	45	VGDYFVLTSHTVMPL	221-235	84	TLEPEYFNSVCRLMK	416-430
6	CCKCCYDHVISTSHK	26-40	46	VLTSHTVMPLSAPTL	226-240	85	YFNSVCRLMKTIGPD	421-435
7	YDHVISTSHKLVLSV	31-45	47	TVMPLSAPTLVPQEH	231-245	86	CRLMKTIGPDMFLGT	426-440
8	STSHKLVLSVNPYVC	36-50	48	SAPTLVPQEHYVRIT	236-250	87	TIGPDMFLGTCRRCP	431-445
9	LVLSVNPYVCNAPGC	41-55	49	VPQEHYVRITGLYPT	241-255	88	MFLGTCRRCPAEIVD	436-450
10	NPYVCNAPGCDVTDV	46-60	50	YVRITGLYPTLNISD	246-260	89	CRRCPAEIVDTVSAL	441-455
11	NAPGCDVTDVTQLYL	51-65	51	GLYPTLNISDEFSSN	251-265	90	AEIVDTVSALVYDNK	446-460
12	DVTDVTQLYLGGMSY	56-70	52	LNISDEFSSNVANYQ	256-270	91	TVSALVYDNKLKAHK	451-465
13	TQLYLGGMSYYCKSH	61-75	53	EFSSNVANYQKVGMQ	261-275	92	VYDNKLKAHKDKSAQ	456-470
14	GGMSYYCKSHKPPIS	66-80	54	VANYQKVGMQKYSTL	266-280	93	LKAHKDKSAQCFKMF	461-475
15	YCKSHKPPISFPLCA	71-85	55	KVGMQKYSTLQGPPG	271-285	94	DKSAQCFKMFYKGVI	466-480
16	KPPISFPLCANGQVF	76-90	56	KYSTLQGPPGTGKSH	276-290	95	CFKMFYKGVITHDVS	471-485
17	FPLCANGQVFGLYKN	81-95	57	QGPPGTGKSHFAIGL	281-295	96	YKGVITHDVSSAINR	476-490
18	NGQVFGLYKNTCVGS	86-100	58	TGKSHFAIGLALYYP	286-300	97	THDVSSAINRPQIGV	481-495
19	GLYKNTCVGSDNVTD	91-105	59	FAIGLALYYPSARIV	291-305	98	SAINRPQIGVVREFL	486-500
20	TCVGSDNVTDFNAIA	96-110	60	ALYYPSARIVYTACS	296-310	99	PQIGVVREFLTRNPA	491-505
21	DNVTDFNAIATCDWT	101-115	61	SARIVYTACSHAAVD	301-315	100	VREFLTRNPAWRKAV	496-510
22	FNAIATCDWTNAGDY	106-120	62	YTACSHAAVDALCEK	306-320	101	TRNPAWRKAVFISPY	501-515
23	TCDWTNAGDYILANT	111-125	63	HAAVDALCEKALKYL	311-325	102	WRKAVFISPYNSQNA	506-520
24	NAGDYILANTCTERL	116-130	64	ALCEKALKYLPIDKC	316-330	103	FISPYNSQNAVASKI	511-525
25	ILANTCTERLKLFAA	121-135	65	ALKYLPIDKCSRIIP	321-335	104	NSQNAVASKILGLPT	516-530
26	CTERLKLFAAETLKA	126-140	66	PIDKCSRIIPARARV	326-340	105	VASKILGLPTQTVDS	521-535
27	KLFAAETLKATEETF	131-145	67	SRIIPARARVECFDK	331-345	106	LGLPTQTVDSSQGSE	526-540
28	ETLKATEETFKLSYG	136-150	68	ARARVECFDKFKVNS	336-350	107	QTVDSSQGSEYDYVI	531-545
29	TEETFKLSYGIATVR	141-155	69	ECFDKFKVNSTLEQY	341-355	108	SQGSEYDYVIFTQTT	536-550
30	KLSYGIATVREVLSD	146-160	70	FKVNSTLEQYVFCTV	346-360	109	YDYVIFTQTTETAHS	541-555
31	IATVREVLSDRELHL	151-165	71	TLEQYVFCTVNALPE	351-365	110	FTQTTETAHSCNVNR	546-560
32	EVLSDRELHLSWEVG	156-170	72	VFCTVNALPETTADI	356-370	111	ETAHSCNVNRFNVAI	551-565
33	RELHLSWEVGKPRPP	161-175	73	NALPETTADIVVFDE	361-375	112	CNVNRFNVAITRAKI	556-570
34	SWEVGKPRPPLNRNY	166-180	74	TTADIVVFDEISMAT	366-380	113	FNVAITRAKIGILCI	561-575
35	KPRPPLNRNYVFTGY	171-185	75	VVFDEISMATNYDLS	371-385	114	TRAKIGILCIMSDRD	566-580
36	LNRNYVFTGYRVTKN	176-190	76	ISMATNYDLSVVNAR	376-390	115	GILCIMSDRDLYDKL	571-585
37	VFTGYRVTKNSKVQI	181-195	77	NYDLSVVNARLRAKH	381-395	116	MSDRDLYDKLQFTSL	576-590
38	RVTKNSKVQIGEYTF	186-200	78	VVNARLRAKHYVYIG	386-400	117	LYDKLQFTSLEIPRR	581-595
39	SKVQIGEYTFEKGDY	191-105	79	LRAKHYVYIGDPAQL	391-405	118	FTSLEIPRRNVATLQ	587-601
40	GEYTFEKGDYGDAVV	196-210						

Supplementary Table 2: SARS-CoV-1 peptide libraries used in the study

SARS-CoV-1 OLP: nucleocapsid					
NP-1 peptide pool			NP-2 peptide pool		
Peptide No	Peptide Sequence	aa	Peptide No	Peptide Sequence	aa
1	MSDNGPQSNQRSAPR	1-15	43	MASGGGETALALLLL	211-225
2	PQSNQRSAPRITFGG	6-20	44	GETALALLLLDRLNQ	216-230
3	RSAPRITFGGPTDST	11-25	45	ALLLLDRLNQLESKV	221-235
4	ITFGGPTDSTDNNQN	16-30	46	DRLNQLESKVSGKGQ	226-240
5	PTDSTDNNQNGGRNG	21-35	47	LESKVSGKGQQQQGQ	231-245
6	DNNQNGGRNGARPKQ	26-40	48	SGKGQQQQGQTVTKK	236-250
7	GGRNGARPKQRRPQG	31-45	49	QQQGQTVTKKSAAEA	241-255
8	ARPKQRRPQGLPNNI	36-50	50	TVTKKSAAEASKKPR	246-260
9	RRPQGLPNNIASWFT	41-55	51	SAAEASKKPRQKRTA	251-265
10	LPNNIASWFTALTQH	46-60	52	SKKPRQKRTATKQYN	256-270
11	ASWFTALTQHGKEEL	51-65	53	QKRTATKQYNVTQAF	261-275
12	ALTQHGKEELRFPRG	56-70	54	TKQYNVTQAFGRRGP	266-280
13	GKEELRFPRGQGVPI	61-75	55	VTQAFGRRGPEQTQG	271-285
14	RFPRGQGVPINTNSG	66-80	56	GRRGPEQTQGNFGDQ	276-290
15	QGVPINTNSGPDDQI	71-85	57	EQTQGNFGDQDLIRQ	281-295
16	NTNSGPDDQIGYYRR	76-90	58	NFGDQDLIRQGTDYK	286-300
17	PDDQIGYYRRATRRV	81-95	59	DLIRQGTDYKHWPQI	291-305
18	GYYRRATRRVRGGDG	86-100	60	GTDYKHWPQIAQFAP	296-310
19	ATRRVRGGDGKMKEL	91-105	61	HWPQIAQFAPSASAF	301-315
20	RGGDGKMKELSPRWY	96-110	62	AQFAPSASAFFGMSR	306-320
21	KMKELSPRWYFYYLG	101-115	63	SASAFFGMSRIGMEV	311-325
22	SPRWYFYYLGTGPEA	106-120	64	FGMSRIGMEVTPSGT	316-330
23	FYYLGTGPEASLPYG	111-125	65	IGMEVTPSGTWLTYH	321-335
24	TGPEASLPYGANKEG	116-130	66	TPSGTWLTYHGAIKL	326-340
25	SLPYGANKEGIVWVA	121-135	67	WLTYHGAIKLDDKDP	331-345
26	ANKEGIVWVATEGAL	126-140	68	GAIKLDDKDPQFKDN	336-350
27	IVWVATEGALNTPKD	131-145	69	DDKDPQFKDNVILLN	341-355
28	TEGALNTPKDHIGTR	136-150	70	QFKDNVILLNKHIDA	346-360
29	NTPKDHIGTRNPNNN	141-155	71	VILLNKHIDAYKTFP	351-365
30	HIGTRNPNNNAATVL	146-160	72	KHIDAYKTFPPTEPK	356-370
31	NPNNNAATVLQLPQG	151-165	73	YKTFPPTEPKKDKKK	361-375
32	AATVLQLPQGTTLPK	156-170	74	PTEPKKDKKKKTDEA	366-380
33	QLPQGTTLPKGFYAE	161-175	75	KDKKKKTDEAQPLPQ	371-385
34	TTLPKGFYAEGSRGG	166-180	76	KTDEAQPLPQRQKKQ	376-390
35	GFYAEGSRGGSQASS	171-185	77	QPLPQRQKKQPTVTL	381-395
36	GSRGGSQASSRSSSR	176-190	78	RQKKQPTVTLLPAAD	386-400
37	SQASSRSSSRSRGNS	181-195	79	PTVTLLPAADMDDFS	391-405
38	RSSSRSRGNSRNSTP	186-200	80	LPAADMDDFSRQLQN	396-410
39	SRGNSRNSTPGSSRG	191-205	81	MDDFSRQLQNSMSGA	401-415
40	RNSTPGSSRGNSPAR	196-210	82	RQLQNSMSGASADST	406-420
41	GSSRGNSPARMASGG	201-215	83	SMSGASADSTQA	411-422
42	NSPARMASGGGETAL	206-220			