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Memory T cells induced by previous pathogens can shape the susceptibility to, and clinical severity of, subsequent infections[1]. Little is known about the presence of pre-existing memory T cells in humans with the potential to recognize SARS-CoV-2. 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 COVID-19 convalescents (n=36). In all of them we demonstrated the presence of CD4 and CD8 T cells recognizing multiple regions of the NP protein. We then showed that SARS-recovered patients (n=23) still possess long-lasting memory T cells reactive to SARS-NP 17 years after the 2003 outbreak, which displayed robust cross-reactivity to SARS-CoV-2 NP. Surprisingly, we also frequently detected SARS-CoV-2 specific T cells in individuals with no history of SARS, COVID-19 or contact with SARS/COVID-19 patients (n=37). SARS-CoV-2 T cells in uninfected donors exhibited a different pattern of immunodominance, frequently targeting the ORF-1-coded proteins NSP7 and 13 as well as the NP structural protein. Epitope characterization of NSP7-specific T cells showed recognition of protein fragments with low homology to "common cold" human coronaviruses but conserved amongst animal betacoranaviruses. Thus, infection with betacoronaviruses induces multispecific and long-lasting T cell immunity to the structural protein NP. Understanding how pre-existing NP- and 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.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the cause of the coronavirus disease 2019 (COVID-19)[2]. This disease has spread pandemically, placing lives and economies around the world under severe stress. SARS-CoV-2 infection is characterized by a broad spectrum of clinical syndromes, ranging from asymptomatic or mild influenza-like symptoms to severe pneumonia and acute respiratory distress syndrome[3].

It is common to observe the ability of a single virus to cause widely differing pathological manifestations in humans. This is often due to multiple contributory factors including the size of viral inoculum, the genetic background of patients and the presence of concomitant pathological conditions. Moreover, an established adaptive immunity towards closely related viruses[4] or other microbes[5] can reduce susceptibility[6] or enhance disease severity[7].

SARS-CoV-2 belongs to *Coronaviridae*, a family of large RNA viruses infecting many animal species. Six other coronaviruses are known to infect humans. Four of them are endemically transmitted[8] and cause the 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[9]. All of them trigger antibody and T cell responses in infected patients: however, antibody levels appear to wane faster than T cells. SARS-CoV-specific antibodies dropped below the detection limit within 2 to 3 years[10], whereas SARS-CoV-specific memory T cells have been detected even 11 years after SARS infection[11]. Since the sequences of selected structural and non-structural proteins are highly conserved amongst different coronaviruses (e.g. NSP7 and NSP13 are 100% and 99% identical, respectively, between SARS-CoV-2, SARS-CoV-1 and the bat-SL-CoVZXC21[12]), we investigated whether cross-reactive

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SARS-CoV-2-specific T cells are present in individuals who resolved SARS-CoV-1, comparing responses with those present in resolvers of SARS-CoV-2 infection. We also studied these T cells in individuals with no history of SARS or COVID-19 or of 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 in recovered patients with COVID-19

SARS-CoV-2-specific T cells have just started to be characterized in COVID-19 patients[13,14] and their potential protective role has been inferred from studies in SARS[15] and MERS[16] patients. To study SARS-CoV-2 specific T cells associated with viral clearance, we collected peripheral blood from 36 individuals after recovery 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 (Fig. 1a). We selected nucleocapsid protein as it is one of the more abundant structural proteins produced[17] and has a high degree of homology between different betacoranaviruses (Extended Data Fig. 1)[18].

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)[12], and because they are representative of the ORF1a/b polyprotein encoding the replicase-transcriptase complex[19]. This polyprotein is the first to be translated upon coronavirus infection and is essential for the subsequent transcription of the genomic and sub-genomic RNA species coding for structural proteins[19]. 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 split into 5 pools of approximately 40 peptides each (NP-1, NP-2, NSP13-1, NSP13-2, NSP13-3) and a single pool of 15 peptides spanning NSP7 (Fig. 1b). This unbiased method with overlapping peptides was utilized instead of bioinformatic selection of peptides, since the performance of such algorithms is often sub-optimal in Asian populations[20].

Peripheral blood mononuclear cells (PBMC) of 36 recovered COVID-19 patients were stimulated for 18h with the different peptide pools and virus-specific responses were analyzed by IFN-γ ELISpot assay. In all individuals tested (36/36) we detected IFN-γ spots following stimulation with the pools of synthetic peptides covering NP (Fig. 1c, d). In nearly all individuals NP-specific responses could be identified against multiple regions of the protein: 34/36 for region 1-215aa (NP-1) and 36/36 for 206-419aa (NP-2). In sharp contrast, responses to NSP7 and NSP13 peptide pools were detected at very low levels in 12 out of 36 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 their relative 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 (Fig. 1e; Extended Data Figs. 3 and 4). Moreover, despite the small sample size, we compared the frequency SARS-CoV-2-specific IFN-γ spots with the presence of virus neutralizing antibodies, duration of infection and disease severity, but found no correlations (Extended Data Fig. 5). To confirm and further delineate the multispecificity of the NP-specific responses detected *ex vivo* in COVID-19 recovered patients, we mapped the precise regions of NP able to activate IFN-γ responses in nine individuals. We organized the 82 overlapping peptides covering the entire NP into small peptide pools (of 7-8 peptides) that were used to stimulate PBMC either directly *ex vivo* or after an *in vitro* expansion protocol previously used in HBV[21] or SARS recovered subjects[22]. A schematic representation of the peptide pools is shown in Fig. 2a. We found that 8 out of 9 recovered COVID-19 patients possess PBMC that recognize multiple regions of NP of SARS-CoV-2 (Fig. 2a). Importantly, we then defined single peptides that were able to activate T cells in 7 patients. Utilizing a peptide matrix strategy[22], we first deconvoluted individual peptides responsible for the detected 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 (Table 1 and Fig. 2b). Table 1 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 from SARS recovered subjects. For example, the NP region 101-120, which is a described CD4 T cell epitope in SARS-CoV-1 exposed individuals[11,22], also stimulated CD4 T cells of two COVID-19 convalescents. Similarly, the NP region 321-340 contained epitopes triggering CD4 and CD8 T cells in both COVID-19 and SARS recovered patients[22]. The demonstration that COVID-19 and SARS recovered patients can mount T cell responses against shared viral determinants implies that SARS-CoV-1 infection can induce T cells able to cross-react against SARS-CoV-2.

SARS-CoV-2-specific T cells in recovered patients with SARS

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. We have previously demonstrated that patients who recovered from SARS harbor T cells specific for epitopes within different SARS-CoV-1 proteins that persist for 11 years after infection[11]. Here, we collected PBMC 17 years post SARS-CoV-1 infection and tested if they still harbor cells reactive against SARS-CoV-1 and whether these have cross- reactive potential against SARS-CoV-2 peptides. PBMC from SARS resolvers (n=15) were stimulated directly *ex vivo* with peptide pools covering SARS-CoV-1 NP (NP-1 and NP-2), NSP7 and NSP13 (Fig. 3a). This revealed that 17 years after infection, IFN-γ responses to SARS-CoV-1 peptides were still present and were almost exclusively focused on NP rather than the NSP peptide pools (Fig. 3b). Subsequently, we tested if SARS-CoV-2 NP peptides (aa identity = 94%) induce IFN-γ responses from PBMC of SARS resolvers. Indeed, PBMC from all 23 individuals tested reacted to SARS-CoV-2 NP peptides (Fig. 3c, d). In order to test whether these low frequency responses in SARS-resolvers could expand after encounter with SARS-CoV-2 NP, the quantity of IFN-γ producing cells responding to SARS-CoV-2 NP, NSP7 and NSP13 was analyzed after 10 days of cell culture in the presence of the relevant peptides. A clear and robust expansion of NP-reactive cells was detected in 7 out of 8 individuals tested (Fig. 3e) and ICS staining confirmed that SARS recovered have memory SARS NP specific CD4 and CD8 T cells[11] (Extended Data Fig. 6). Importantly, and in sharp contrast to the response to NP peptides, we could not detect any cells reacting to the peptide pools covering NSP13 and only 1 out of 8 reacted to NSP7 (Fig. 3e).

Thus, SARS-CoV-2 NP-specific 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 T cells induced by betacoronanvirus infection are long-lasting, supporting the notion that COVID-19 patients will develop long-term T cell immunity. Our findings also raise the intriguing possibility that long-lasting T cells generated following infection with related viruses may be able to protect against, or modify the pathology caused by, SARS-CoV-2 infection.

SARS-CoV-2-specific T cells in SARS-CoV-1/2 unexposed donors

To explore this possibility, we tested NP- and NSP7/13-peptide-reactive IFN-γ responses in 37 SARS-CoV-1/2 unexposed donors. Donors were

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either sampled before July 2019 (n=26) or were serologically negative for both SARS-CoV-2 neutralizing antibodies and SARS-CoV-2 NP antibodies[23] (n=11). Different coronaviruses known to cause common colds in humans like OC43, HKU1, NL63 and 229E present different degrees of amino acid homology with SARS-CoV-2 (Extended Data Figs. 1 and 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[14]. Remarkably, we detected SARS-CoV-2-specific IFN-γ responses in 19 out of 37 SARS-CoV-1/2 unexposed individuals (Fig. 4a, b). The cumulative proportion of all studied individuals responding to peptides covering NP and ORF-1-coded NSP7 and 13 proteins is shown in Fig. 4b. SARS-CoV-1/2 unexposed individuals showed a distinct pattern of reactivity; whilst COVID-19 and SARS recovered donors reacted preferentially to NP peptide pools (66% COVID-19 and 91% SARS recovered individuals responded only to NP pools), the unexposed group showed a mixed response to NP and NSP7/13 (Fig. 4a–c). In addition, whereas NSP peptides stimulated a dominant response in only 1 out of 59 COVID-19/SARS resolvers, they triggered dominant reactivity in 9 out of 19 unexposed donors with SARS-CoV-2-reactive cells (Fig. 4c, Extended Data Fig. 7). These SARS-CoV-2 reactive cells from SARS-CoV-1/2 unexposed donors had the capacity to expand upon stimulation with SARS-CoV-2 peptides (Fig. 4d). We then delineated the SARS-CoV-2 specific response detected in the SARS-CoV-1/2 unexposed individuals in more detail. Characterization of the NP-specific response in one donor (H-2) identified CD4 T cells reactive for an epitope within the NP region 101-20. This same epitope was also detected in COVID-19 and SARS recovered patients (Fig. 2b and[8,22]). It has a high degree of homology to the MERS-CoV, OC43 and HKU1 NP sequences (Fig. 4e). In the same donor, we analyzed the PBMC collected at multiple timepoints, demonstrating the persistence of the NP-101-20 response over the period of 1 year (Extended Data Fig. 8a). In three other SARS-CoV-1/2 unexposed donors, we identified CD4 T cells specific for the NSP7 region 26-40 (SKLWAQCVQLHNDIL; donor H-7), and CD8 T cells specific for an epitope comprised within the NSP7 region 36-50 (HNDILLAKDTTEAFE; donors H-3 and H-21; Fig. 4e, Extended Data Fig. 8b).

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 (Fig. 4e), especially for the CD8 peptide epitope. Indeed, the low homology peptides covering the sequences of "common cold" coronaviruses failed to stimulate PBMC of the NSP7 36-50 responsive individuals (Extended Data Fig. 8c). Even though we cannot exclude that some SARS-CoV-2 reactive T cells might be naïve or induced by completely unrelated pathogens[5], this finding suggests that other presently unknown coronaviruses, possibly of animal origin, might induce cross-reactive SARS-CoV-2 T cells in the general population.

We further characterized the NSP7-specific CD4 and CD8 T cells present in the three uninfected individuals. The reactive T cells expanded efficiently *in vitro* and were mainly double IFN-γ and TNF-α (CD8 T cells) or single IFN-γ (CD4 T cells) producers (Extended Data Fig. 9a). We also determined that the NSP7-36-50 specific CD8 T cells are HLA-B35 restricted and of effector memory/terminal differentiated phenotype (CCR7/CD45RA$^{-/+}$) (Extended Data Fig. 9b, c).

Conclusions

Why NSP7/13-specific T cells are detected and often dominant in SARS-CoV-1/2 unexposed donors, while representing a minor population in SARS-Cov-1/2 recovered individuals is unclear. It is however consistent with the findings of Grifoni et al[11], who detected ORF-1-specific T cells preferentially in some SARS-CoV-2 unexposed donors whilst T cells of COVID-19 recovered preferentially recognized structural proteins. Induction of virus-specific T cells in "exposed but uninfected" individuals has been demonstrated in other viral infections[24–26]. Theoretically, individuals exposed to coronaviruses might just prime ORF-1-specific T cells, since the ORF-1-coded proteins are produced first in coronavirus-infected cells and are necessary for the formation of the viral replicase-transcriptase complex essential for the subsequent transcription of the viral genome leading to various RNA species[18]. Therefore, ORF-1-specific T cells could hypothetically abort viral production by lysing SARS-CoV-2-infected cells before the formation of mature virions. In contrast, in COVID-19 and SARS patients, the NP protein, which is abundantly produced in cells secreting mature virions[17], would be expected to result in preferential boosting of NP-specific T cells.

Importantly, the ORF-1 region contains domains that are extremely conserved among many different coronaviruses[9]. The distribution of these viruses in different animal species might result in periodic human contact inducing ORF-1-specific T cells with cross-reactive ability against SARS-CoV-2. Understanding the distribution, frequency and protective capacity of 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 rates or pathology observed during this pandemic. T cells specific for viral proteins have protective ability in animal models of airway infections[27,28], but the impact that pre-existing NP- and/or ORF-1-specific T cells could have in the differential modulation of SARS-CoV-2 infection will have to be carefully evaluated.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2550-z.

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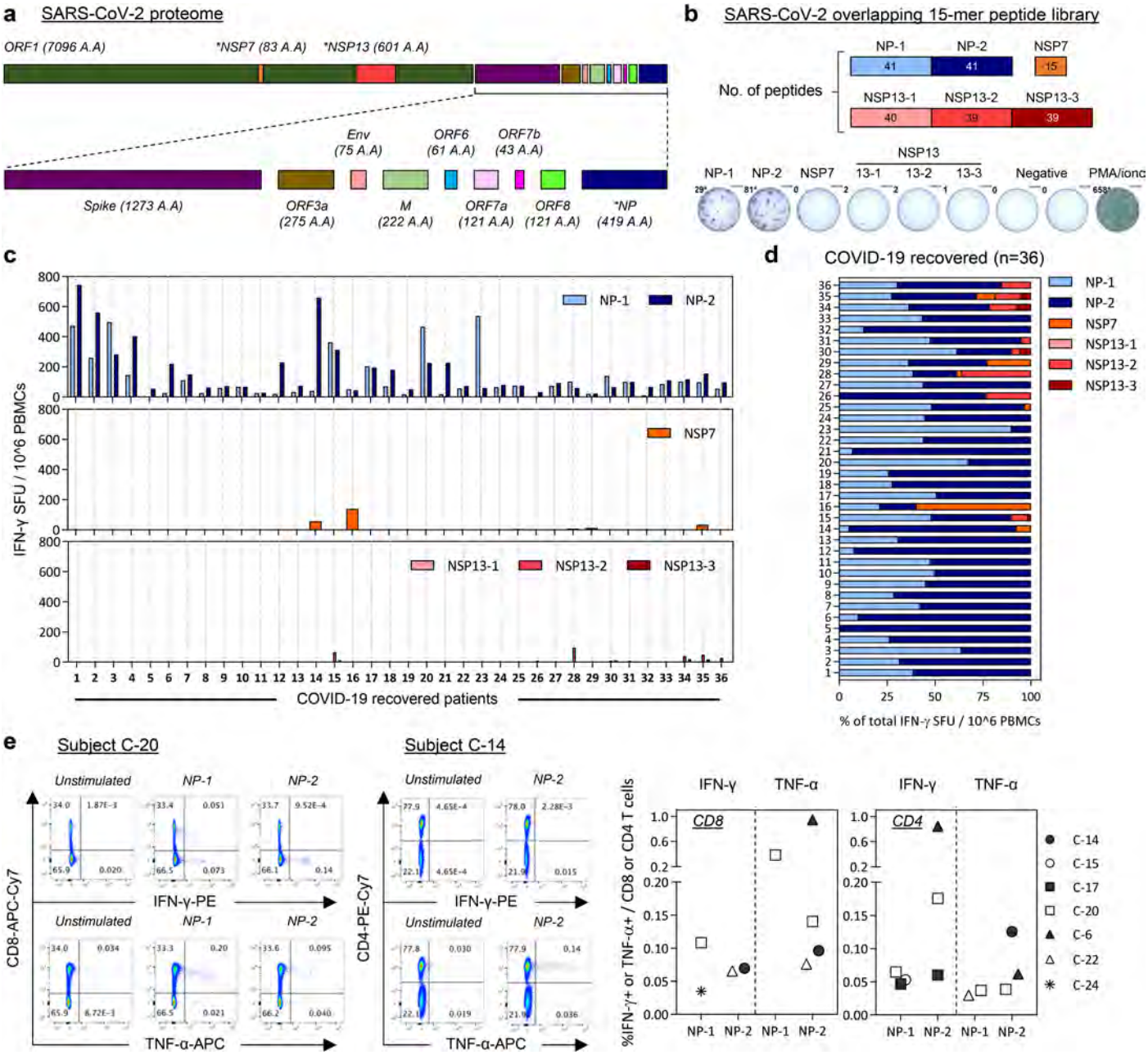
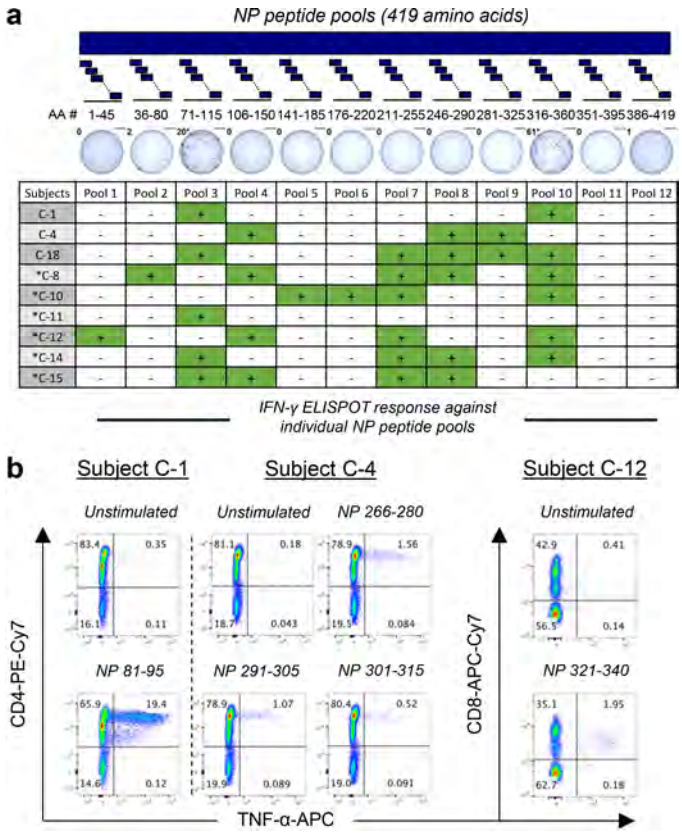


Fig. 1 | SARS-CoV-2-specific responses in recovered COVID-19 patients. **a**, SARS-CoV-2 proteome organization; analyzed proteins are marked by *. **b**, 15-mer peptides overlapping by 10 amino acids covering nucleocapsid protein (NP) and the non-structural proteins (NSP) 7 and 13 were split into 6 pools covering NP (NP-1, NP-2), NSP7 and NSP13 (NSP13-1, NSP13-2, NSP13-3). **c**, PBMC of recovered COVID-19 patients (n=36) were stimulated with the peptide pools. Bar graphs show frequency of spot forming units (SFU) of IFN-γ secreting cells. **d**, The composition of the SARS-CoV-2 response in each individual is shown as a percentage of the total detected response (NP-1 = light blue; NP-2 = dark blue; NSP7 = orange; NSP13-1 = light red; NSP13-2 = red; NSP13-3 = dark red). **e**, PBMC were stimulated with the peptide pools covering NP (NP-1, NP-2) for 5h and analyzed by intracellular cytokine staining. Dot plots show examples of patients (2/7) with CD4 and/or CD8 T cells producing IFN-γ and/or TNF-α in response to stimulation with NP-1 and/or NP-2 peptides. The graphs summarize the percentage of SARS-CoV-2 NP-peptide-reactive CD4 and CD8 T cells in 7 individuals (unstimulated controls were subtracted for each response).



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	-	-	+	+	-	-	+	+	-	-	-	-

Fig. 2 | SARS-CoV-2-specific T cells in COVID-19 convalescents 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. The table shows IFN-γ ELISpot response against the individual NP peptide pools. *denotes responses detected after *in vitro* expansion. **b**, Following *in vitro* cell expansion, a peptide pool matrix strategy was applied. T cells reacting to distinct peptides were identified by IFN-γ ELISpot and confirmed by ICS. Representative dot plots of 3/7 patients are shown.

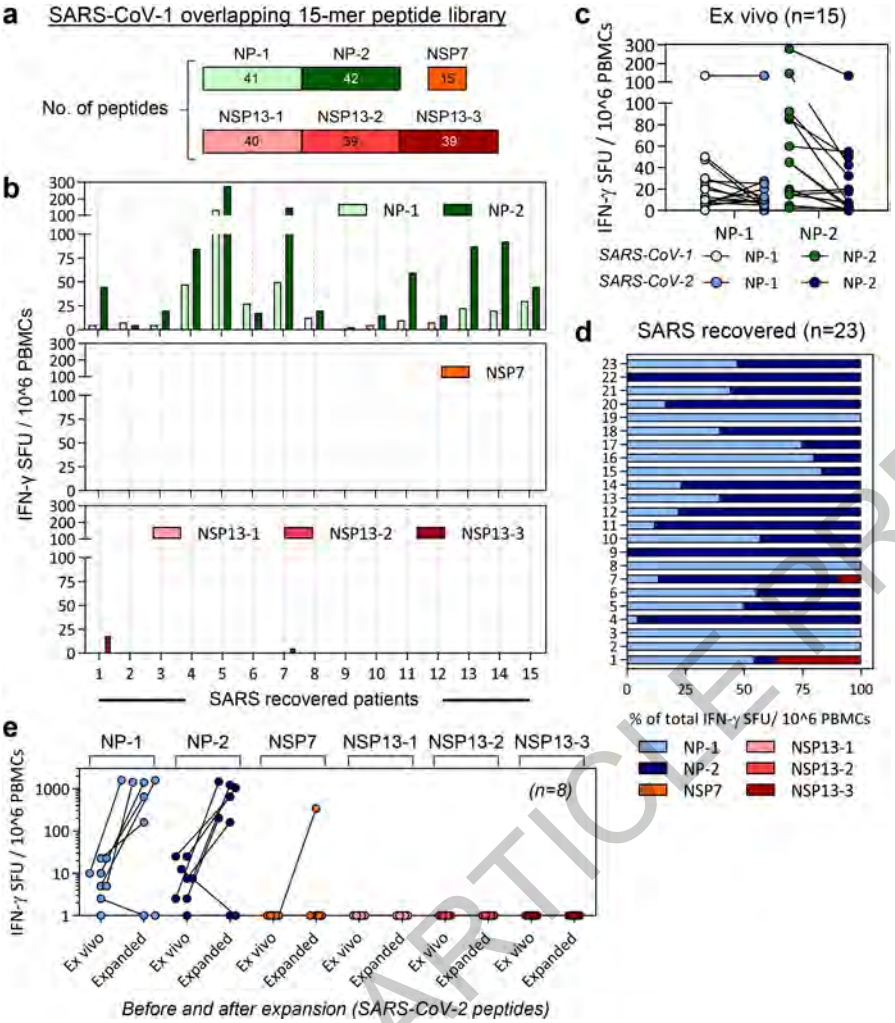


Fig. 3 | SARS-CoV-2 cross-reactive responses are present in SARS-recovered patients. a, PBMC isolated from 15 individuals who recovered from SARS 17 years ago were stimulated with SARS-CoV-1 NP, NSP7 and NSP13 peptide pools. **b**, Bar graphs show spot forming units (SFU) of IFN-γ secreting cells following overnight stimulation with the indicated peptide pools. **c**, 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 the frequency IFN-γ producing cells is shown. **d**, The composition of the SARS-CoV-2 response in each SARS recovered subject (n=23) is shown as a percentage of the total detected response (NP-1 = light blue; NP-2 = dark blue; NSP7 = orange; NSP13-1 = light red; NSP13-2 = red; NSP13-3 = dark red). **e**, PBMC of 8 SARS-recovered individuals were stimulated with all peptides covering SARS-CoV-2 NP, NSP7 and NSP13 to detect cross-reactive responses. The graph shows the number of cells reactive to the different peptide pools directly *ex vivo* and after *in vitro* expansion.

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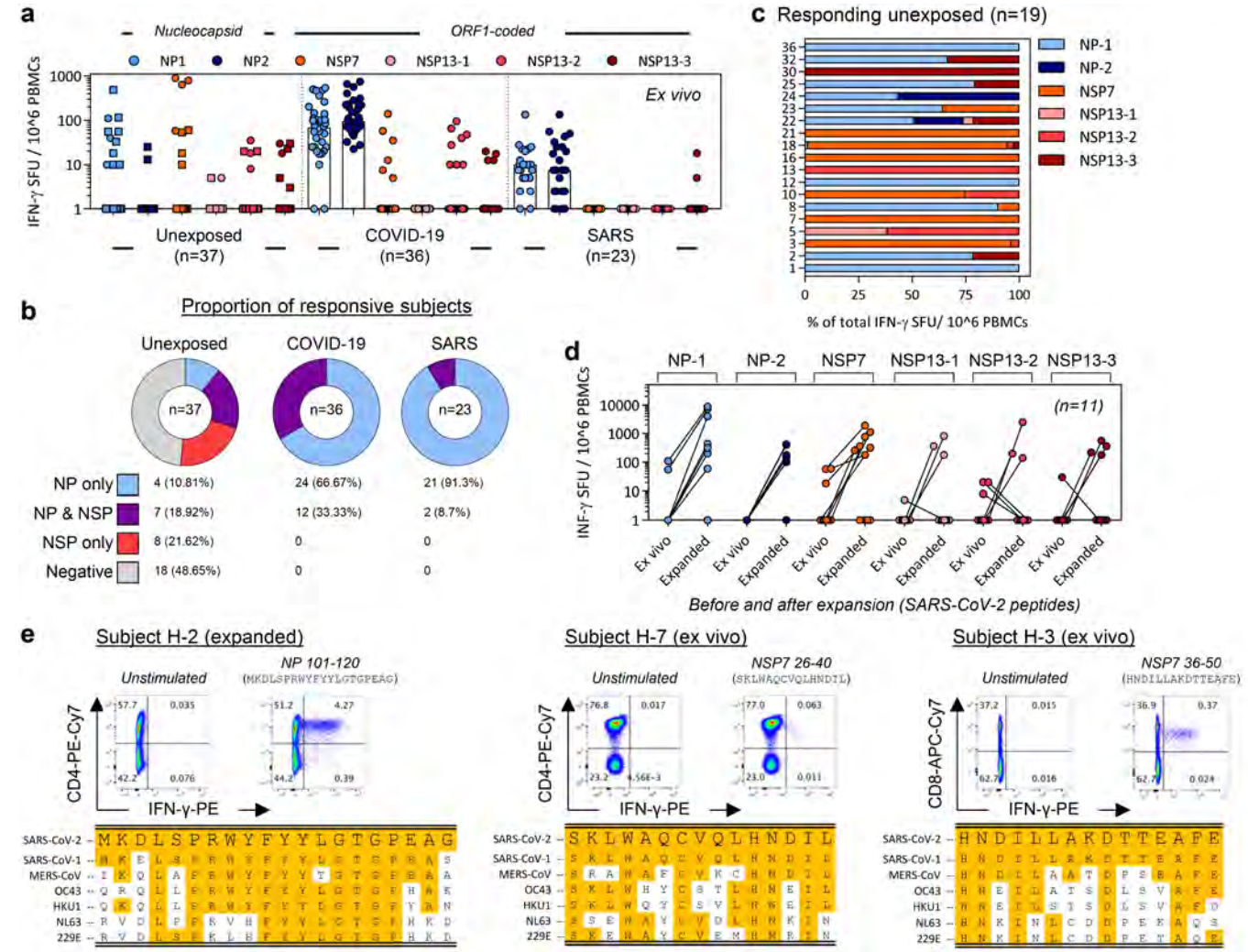

Fig. 4 | Immunodominance of SARS-CoV-2 responses in COVID-19- and SARS-recovered patients and in unexposed individuals. a, PBMC of individuals who are SARS-CoV-1/2 unexposed (n=37), recovered from SARS (n=23) or COVID-19 (n=36) were stimulated with peptide pools covering SARS-CoV-2 NP (NP-1, NP-2), NSP7 and NSP13 (NSP13-1, NSP13-2, NSP13-3) and analyzed by ELISpot. Frequency of peptide-reactive cells is shown for each donor (dots/square) and the bars represent median frequency. Square points denote PBMC samples collected before July 2019. **b**, Pie charts represent percentage of individuals with NP-specific, NSP7/13-specific or both responses in cohort. **c**, The composition of the SARS-CoV-2 response in each responding unexposed donor (n=19) is shown as a percentage of the total detected response (NP-1 = light blue; NP-2 = dark blue; NSP7 = orange; NSP13-1 = light red; NSP13-2 = red; NSP13-3 = dark red). **d**, Frequency of SARS-CoV-2 reactive cells in 11 unexposed donors to the indicated peptide pools directly *ex vivo* and after a 10-day expansion. **e**, 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.

Table 1 | SARS-CoV-2 T cell epitopes

Participants	T cell phenotype	Amino acid residue	SARS-CoV-2 Amino acid sequence	SARS-CoV-1 Amino acid sequence
C-1	CD4	NP 81-95	DDQIGYYRRATRRIR	DDQIGYYRRATRRVR
	CD8	NP 321-340	GMEVTPSGTWLTYTGAIKLD	GMEVTPSGTWLTYHGAIKLD
C-4	CD4	NP 266-280	KAYNVTQAFGRRGPE	KQYNVTQAFGRRGPE
	CD4	NP 291-305	LIRQGTDYKHWPQIA	LIRQGTDYKHWPQIA
	CD4	NP 301-315	WPQIAQFAPSASAFF	WPQIAQFAPSASAFF
C-8	CD4	NP 51-65	SWFTALTQHGKEDLK	SWFTALTQHGKEELR
	CD4	NP 101-120	MKDLSPRWYFYYLGTGPEAG	MKELSPRWYFYYLGTGPEAS
C-10	CD4/CD8	NP 321-340	GMEVTPSGTWLTYTGAIKLD	GMEVTPSGTWLTYHGAIKLD
C-12	CD8	NP 321-340	GMEVTPSGTWLTYTGAIKLD	GMEVTPSGTWLTYHGAIKLD
C-15	CD4	NP 101-120	MKDLSPRWYFYYLGTGPEAG	MKELSPRWYFYYLGTGPEAS
C-16	CD4	NSP7 21-35	RVESSSKLWAQCVQL	RVESSSKLWAQCVQL

T cells reacting to distinct peptides were identified by IFN-γ ELISpot and confirmed by ICS. 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 underlined.

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=36) 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=26) or in March-April 2020 and tested negative for RBD neutralizing antibodies and negative in an ELISA for NP IgG (n=11)[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 NSP13, as well as SARS-CoV-1 NP were synthesized (GL Biochem Shanghai Ltd; see Supplementary Tables 1, 2). To stimulate PBMC, the peptides were 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; 5 µg/ml) overnight at 4 °C. 400,000 PBMC were seeded per well and stimulated for 18h 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; 1:2000), followed by incubation with Streptavidin-AP (Mabtech) and KPL BCIP/NBT Phosphatase Substrate (SeraCare). Spot forming units (SFU) were quantified with ImmunoSpot. To quantify positive peptide-specific responses, 2x mean spots of the unstimulated wells were subtracted from the peptide-stimulated wells, and the results expressed as SFU/10^6 PBMC. We excluded the results if negative control wells had >30 SFU/10^6 PBMC or positive control wells (PMA/Ionomycin) were negative.

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; 3:50), anti-CD4 (clone SK3; 3:50), and anti-CD8 (clone SK1; 3:50) antibodies. For analysis of T cell differentiation status, cells were additionally stained with anti-CCR7 (clone 150503; 1:10) and anti-CD45RA (clone HI100; 1:10). 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; 1:25) and anti-TNF-α (clone MAb11; 1:25) 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.

Expanded T cell lines
T cell lines were generated as follows: 20% of PBMC were pulsed with 10 µg/ml of the overlapping SARS-CoV-2 peptides (all pools combined) or single 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).

HLA-restriction assay
The HLA-type of healthy donor H-3 was determined and different EBV transformed B cells lines with one common allele each were selected for presentation of peptide NSP7 36-50 (see below). B cells were pulsed with 10 µg/ml of the peptide for 1 hour at 37 °C, washed three times, and cocultured with the expanded T cell line at a ratio of 1:1 in the presence of 10 µg/ml brefeldin A (Sigma-Aldrich). Non-pulsed B cell lines served as a negative control detecting potential allogeneic responses and autologous peptide-pulsed cells served as a positive control. HLA-class I haplotype of the different B cell lines: CM780 = A*24:02, A*33:03, B*58:01, B*55:02, Cw*07:02, Cw*03:02; WGP48 = A*02:07, A*11:01, B*15:25, B*46:01, Cw*01:02, Cw*04:03; NP378 = A*11:01, A*33:03, B*51:51, B*35:03, Cw*07:02, Cw*14:02; NgaBH = A*02:01, A*33:03, B*58:01, B*13:01, Cw*03:02.

Sequence alignment
Reference protein sequences for ORF1ab (Accession IDs: QHD43415.1, NP_828849.2, YP_009047202.1, YP_009555238.1, YP_173236.1, YP_003766.2, NP_073549.1) and Nucleocapsid Protein (Accession IDs: YP_009724397.2, AAP33707.1, YP_009047211.1, YP_009555245.1, YP_173242.1, YP_003771.1, NP_073556.1) were downloaded from the NCBI database. 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).

Surrogate virus neutralization assay
A novel surrogate virus neutralization test (sVTN) was used. Specifically, it measures the quantity of anti-Spike antibodies that block protein-protein interaction between the binding domain of spike (RBD) to the ACE2 receptor using an ELISA-based assay[29].

Statistical analyses
All statistical analyses were performed in Prism (GraphPad Software), and details are provided in the figure legends.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
Reference protein sequences for ORF1ab and Nucleocapsid Protein were downloaded from the NCBI database, see above. All data are available in the main text or the supplementary materials.

29. Tan, C. W. *et al.* A SARS-CoV-2 surrogate virus neutralization test (sVNT) based on antibody-mediated blockage of ACE2-spike (RBD) protein-protein interaction. Preprint at Research Square: https://doi.org/10.21203/rs.3.rs-24574/v1 (2020).

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ACCELERATED ARTICLE PREVIEW

Author contributions NLB and ATT designed all experiments and analysed all the data, prepared the figures and edited the paper; KK, CYLT, MH, AC, ML, NT performed ELISpots, intracellular cytokine staining and short-term T cell lines; MC, ML performed viral sequence homology and analysed data; WNC, LW provide antibody testing, MICC, EEO, SK, PAT, JGHL, YJT selected and recruited patients and analysed clinical data, YJT provided funding and designed the study, AB designed and coordinated the study, provided funding, analysed the data, and wrote the paper.

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

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41586-020-2550-z.

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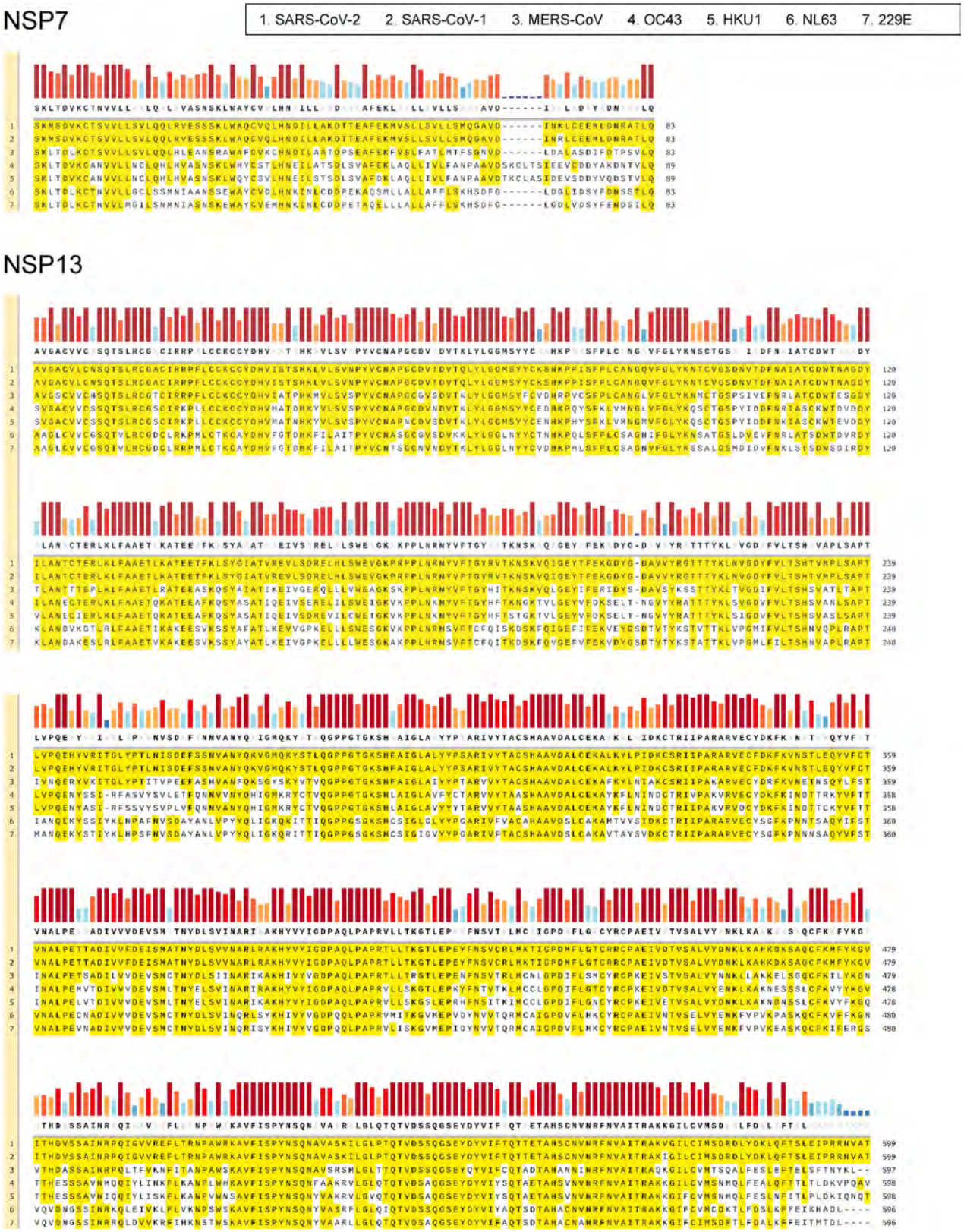
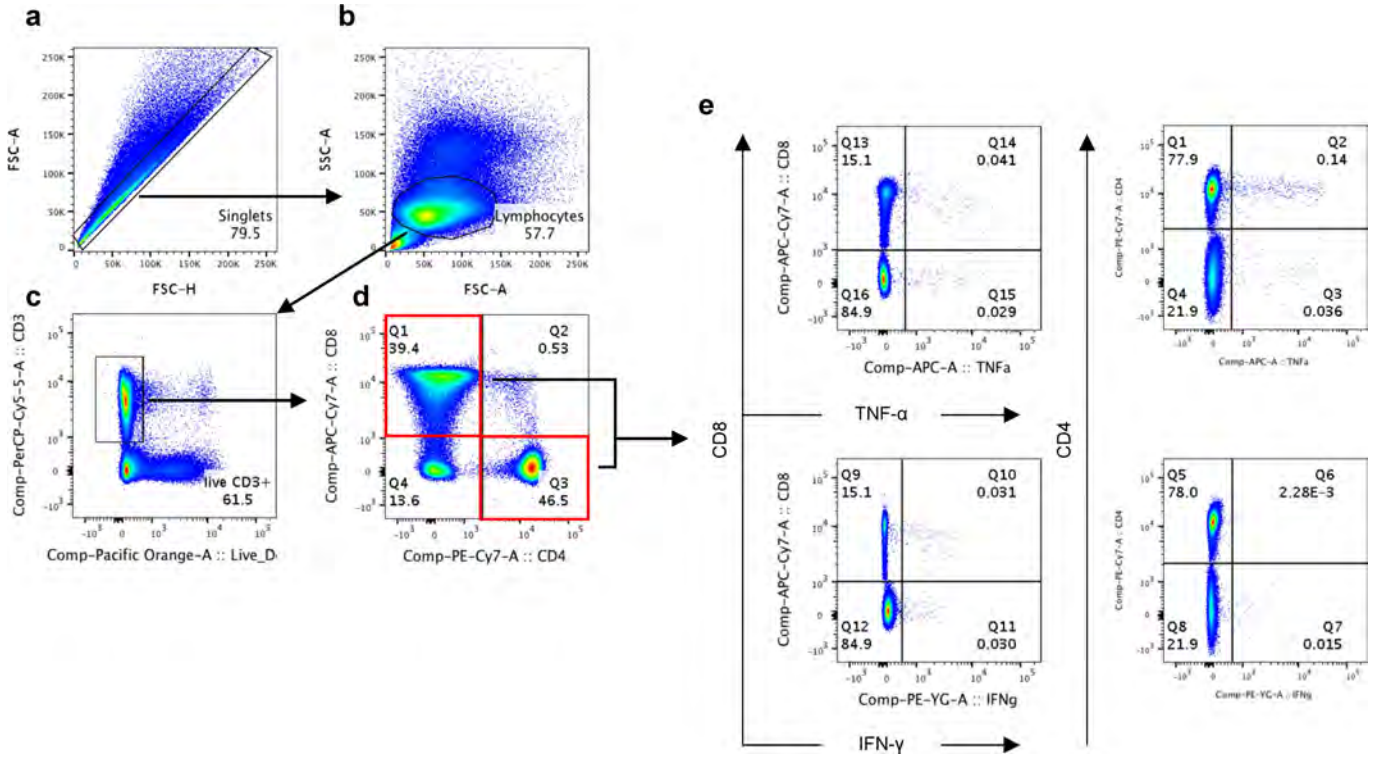
Nucleocapsid

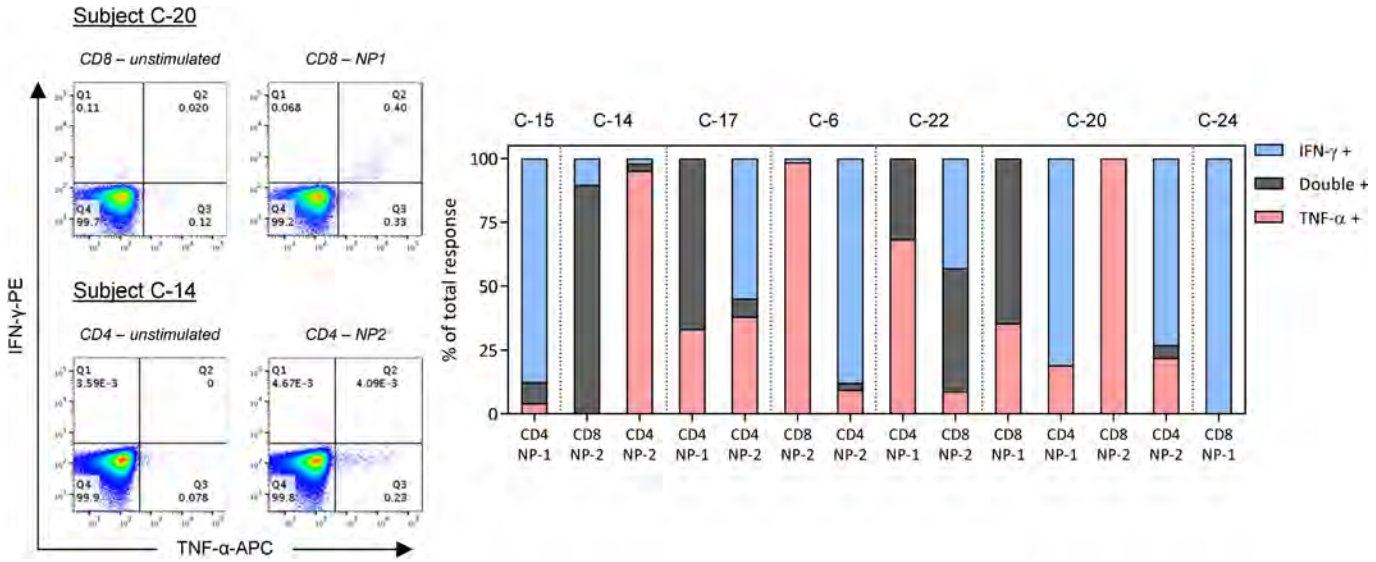
1. SARS-CoV-2 2. SARS-CoV-1 3. MERS-CoV 4. OC43 5. HKU1 6. NL63 7. 229E



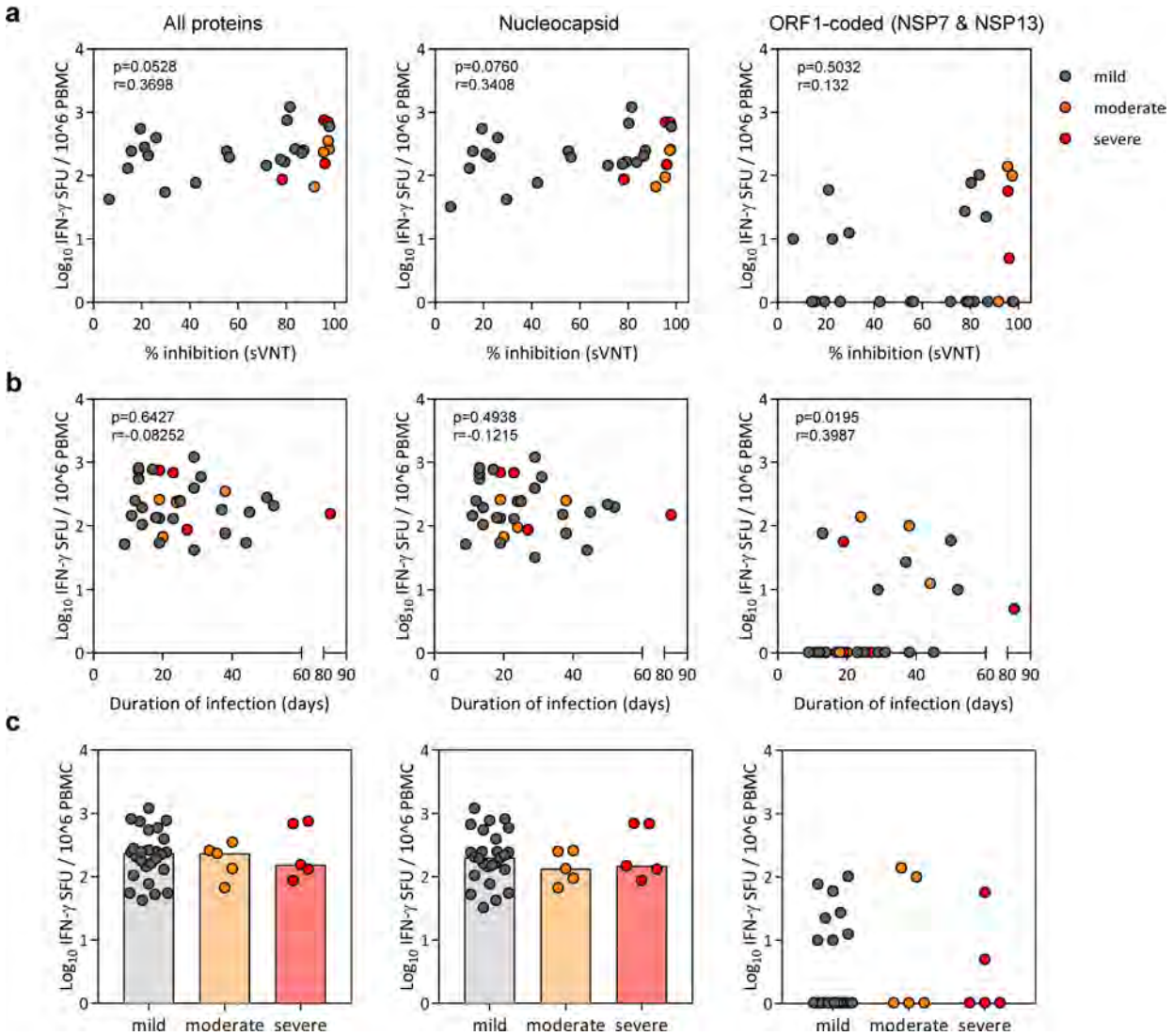
Extended Data Fig. 2 | Sequence alignment of the ORF-1-coded nonstructural proteins NSP7 and NSP13 from all types of human coronaviruses. Protein sequences for ORF1ab were downloaded from the NCBI database and aligned using the MUSCLE algorithm. The alignment for NSP7 and NSP13 is shown.



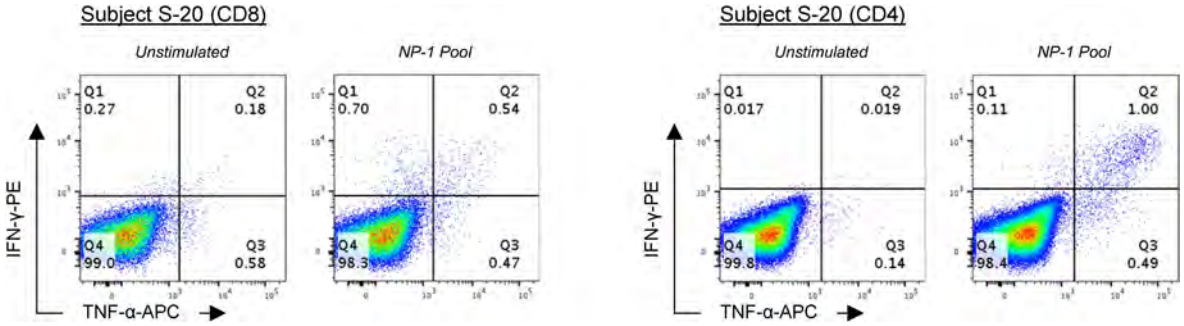
Extended Data Fig. 3 | Flow cytometry gating strategy. a, Forward scatter area versus forward scatter height density plot for doublet exclusion. **b**, Forward and side scatter density plots to identify the lymphocyte population. **c**, Live T cells were gated based on CD3 expression and a live/dead discrimination dye. **d**, Only single expressing CD8 and CD4 T cells were Boolean gated and **e**, used for IFN-γ and/or TNF-α analysis.



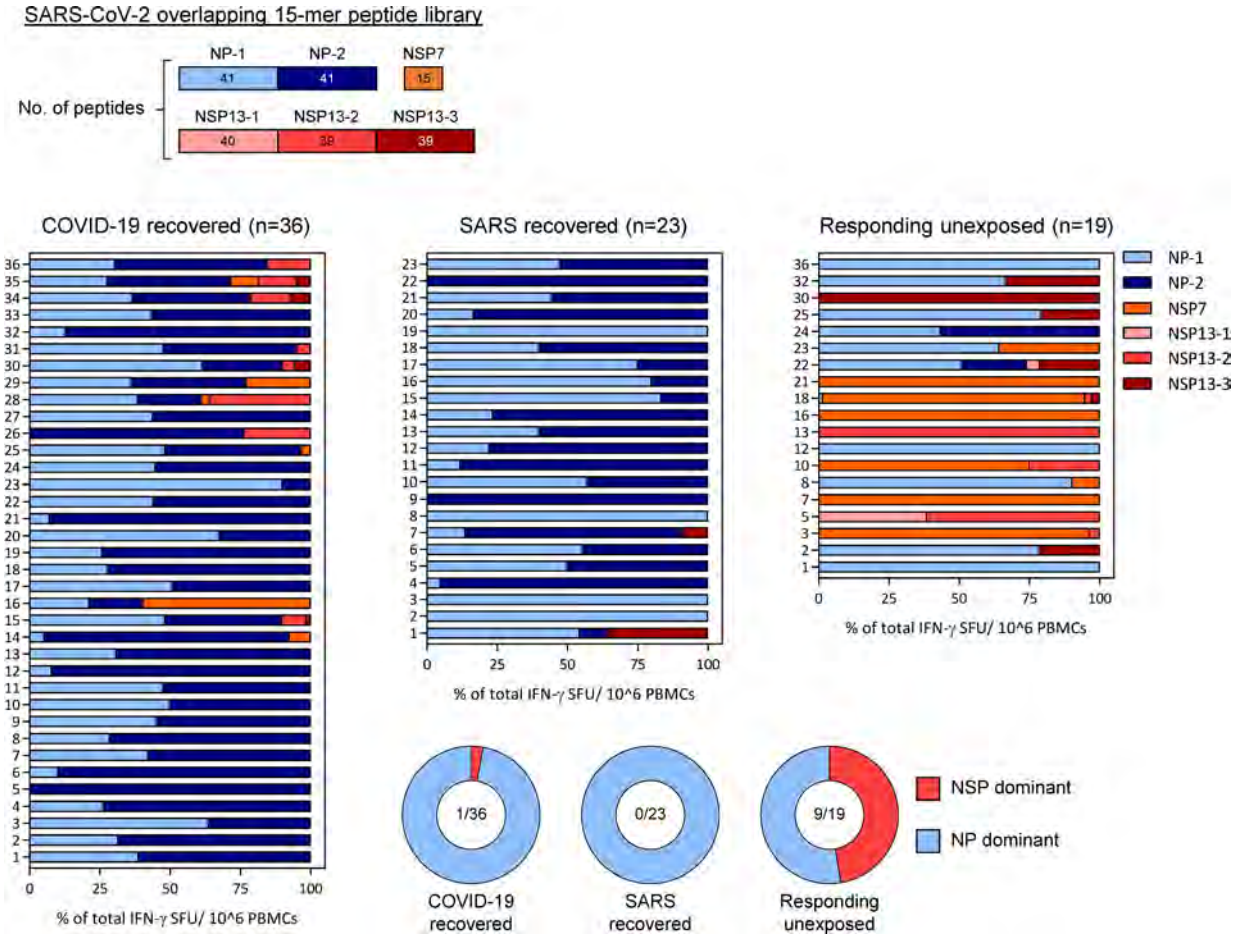
Extended Data Fig. 4 | IFN-γ and TNF-α production profile of SARS-CoV-2 specific T cells of COVID-19 recovered patients. PBMC from COVID-19 recovered patients (n=7) were stimulated with the peptide pools covering NP (NP-1, NP-2) for 5h and analyzed by intracellular cytokine staining for IFN-γ and TNF-α. Dot plots show examples of patients with CD8 (top) or CD4 (bottom) T cells producing IFN-γ and/or TNF-α in response to stimulation with NP-1 or NP-2 peptide pools. The bars show the respective single and double cytokine producers as a proportion of the total detected response after stimulation with the corresponding NP peptide pools in each COVID-19 recovered patient.



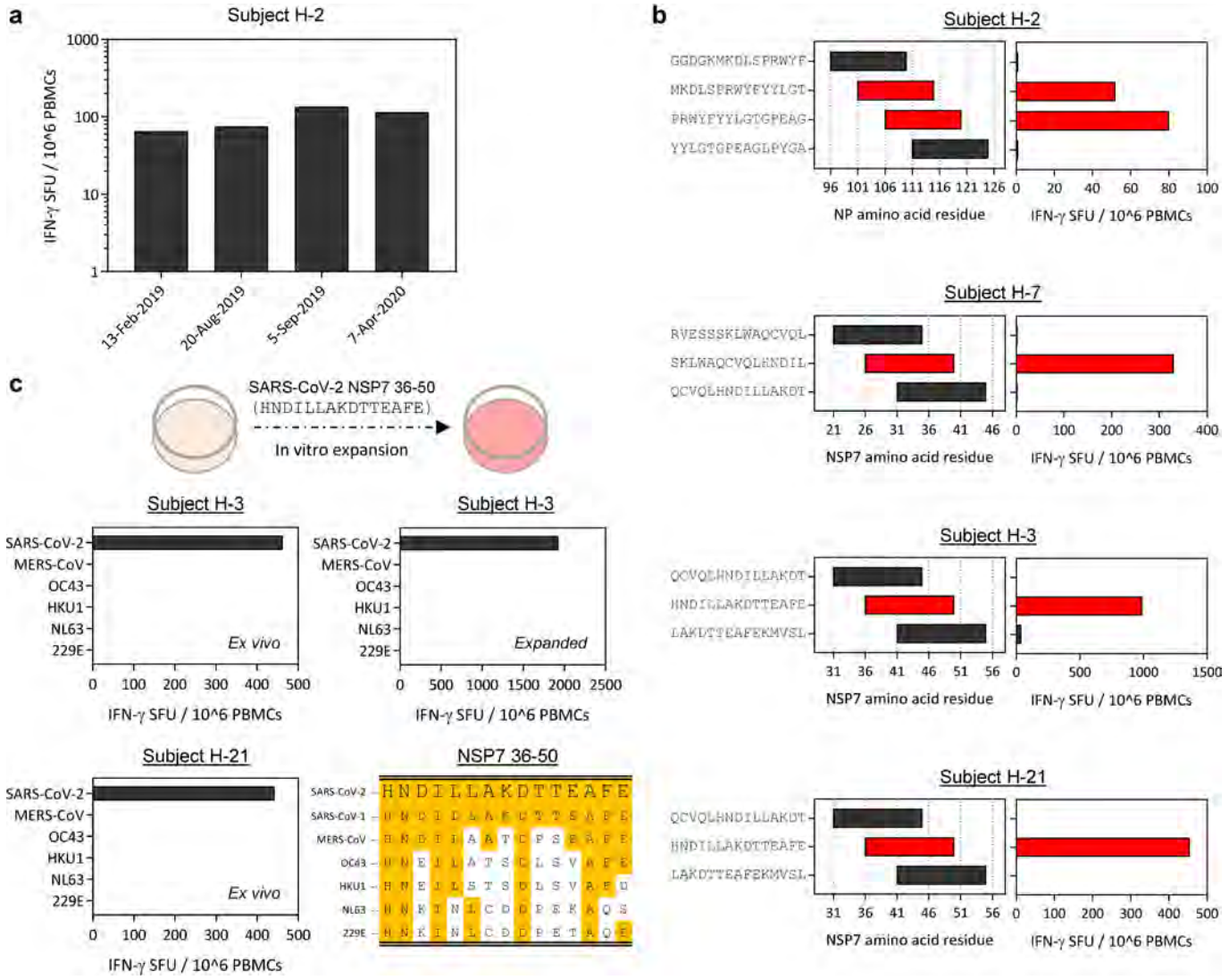
Extended Data Fig. 5 | Correlation analysis of SARS-CoV-2 specific IFN-γ responses with the presence of virus neutralizing antibodies, duration of infection and disease severity. The magnitude of SARS-CoV-2 specific responses, as quantified by IFN-γ ELISpot, against all (NP, NSP7 and NSP13) SARS-CoV-2 proteins tested (left), NP (middle) or NSP7/13 (right) were correlated with **a**, level of virus neutralizing antibodies assayed using a surrogate virus neutralization assay (n=28) and **b**, the duration of SARS-CoV-2 PCR positivity (n=34). The respective p-values (two-tailed) and correlation coefficients (Spearman correlation) are indicated. Patients who present with a mild (grey), moderate (orange) or severe (red) disease are indicated. **c**, Magnitude of SARS-CoV-2 specific responses stratified by mild (n=26), moderate (n=5) and severe (n=5) disease. The bars represent the median magnitude of the response. Mild disease: with or without CXR changes; not requiring oxygen supplement. Moderate disease: oxygen supplement less than 50%. Severe disease: oxygen supplement 50% or more or high flow oxygen or intubation.



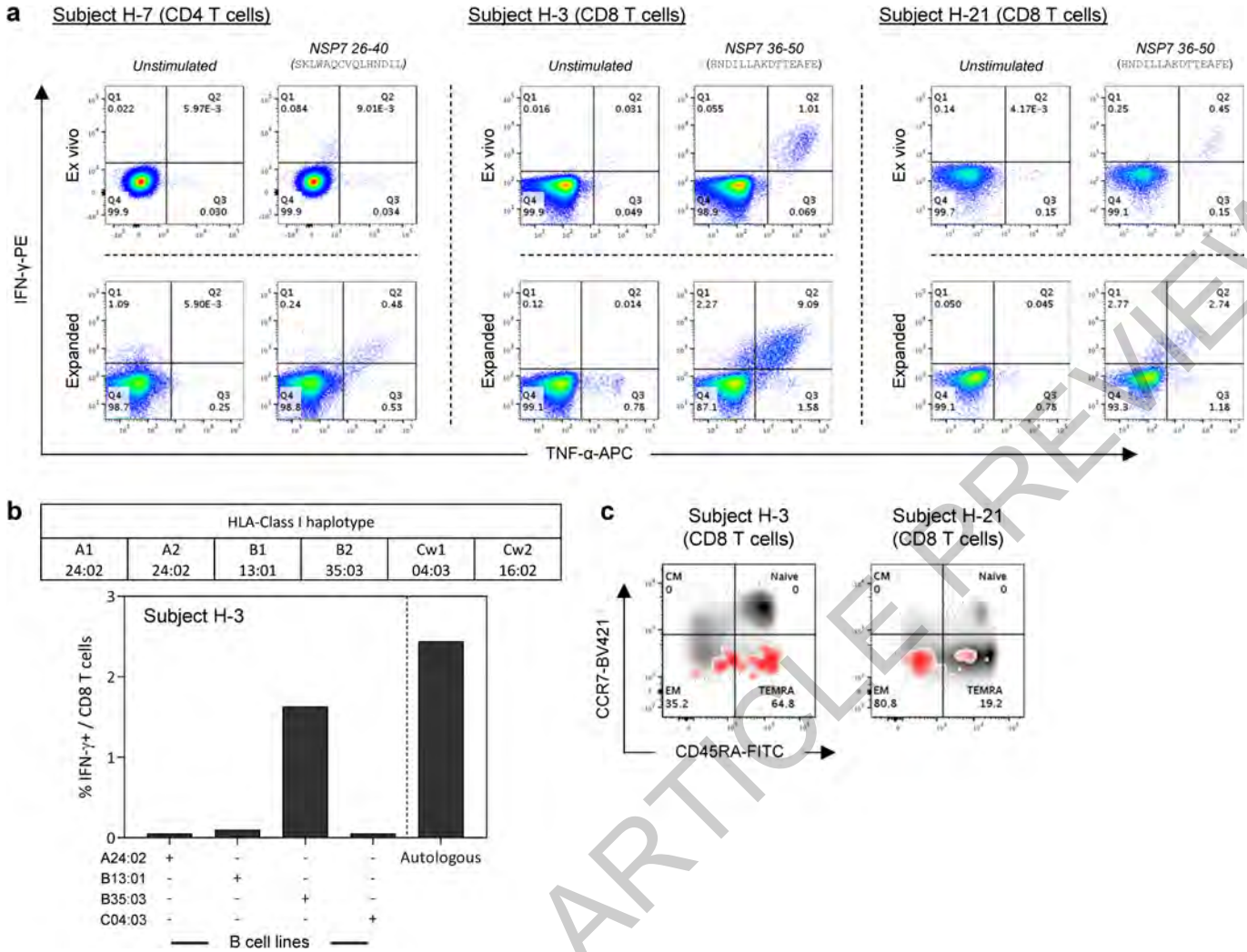
Extended Data Fig. 6 | Analysis of SARS-CoV-1 NP response. PBMC of subject S-20 were expanded for 10 days and the frequency of T cells specific for NP-1 peptide pool were analyzed by intracellular cytokine staining for IFN-γ and TNF-α. Dot plots show CD8 and CD4 T cells producing IFN-γ and/or TNF-α in response to stimulation with the NP-1 peptide pool.



Extended Data Fig. 7 | Dominance of SARS-CoV-2 NP, NSP7 and NSP13 responses in COVID-19 or SARS recovered donors and in unexposed individuals. PBMC from the respective subjects were stimulated with SARS-CoV-2 peptide pools as described in Figure 1. The composition of the SARS-CoV-2 response is shown as a percentage of the total detected response in each subject group (NP-1 = light blue; NP-2 = dark blue; NSP7 = orange; NSP13-1 = light red; NSP13-2 = red; NSP13-3 = dark red). The proportion of subjects with NSP dominant responses are illustrated in the pie charts.



Extended Data Fig. 8 | Identification of SARS-CoV-2 epitopes in SARS-CoV-1/2 unexposed donors. a, Longitudinal analysis of SARS-CoV-2 NP 101-120 response in subject H-2. PBMC collected at the stated time points were stimulated with peptides spanning NP 101-120 and assayed by IFN-γ ELISpot. The frequencies of IFN-γ-SFU are shown. **b**, 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 on the right. Activating peptides are indicated in red and neighboring peptides in black. **c**, PBMC from subject H-3 and H-21 were stimulated with the NSP7 36-50 peptide from SARS-CoV-2, MERS-CoV, OC43, HKU1, NL63 and 229E and analyzed *ex vivo* by IFN-γ ELISpot. A NSP7 36-50 T cell line expanded from subject H-3 was also tested with the corresponding peptides of other coronaviruses by IFN-γ ELISpot. Amino acid sequences of the various peptides are shown in the table.



Extended Data Fig. 9 | Characterization of SARS-CoV-2 NSP7-specific T cell responses in three SARS-CoV-1/2 unexposed donors. a, Dot plots shows the frequency of IFN-γ and/or TNF-α producing CD8 or CD4 T cells specific to the SARS-CoV-2 peptides directly *ex vivo* and after a 10-day expansion in 3 unexposed donors. **b**, The HLA-class I haplotype of subject H-3 is shown in the table. HLA-restriction of the NSP7 36-50 specific T cells from the subject was deduced by co-culturing the T cells with NSP7 36-50 peptide pulsed EBV-transformed B cell lines that shares the indicated HLA-Class I molecule (+). Activation of the NSP7 36-50 specific T cells by autologous cells was achieved by the direct addition of the peptide and used as the positive control. **c**, The memory phenotype of CD8 T cells specific for NSP7 36-50 in subjects H-3 and H-21 were analyzed *ex vivo* and shown in the dot plots. The frequencies of naïve, effector memory, central memory and terminally differentiated NSP7 36-50 specific CD8 T cells (red) are shown and density plots were overlaid on the total CD8 T cells (grey).

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Extended Data Table 1 | Donor Characteristics

	COVID-19 recovered	SARS recovered	SARS-CoV-1/2 unexposed
Number	36	23	37
Median age in years (range)	42 (27-78)	49 (21-67)	39 (28-63)
Gender			
Male	72% (26/36)	26% (6/23)	62% (23/37)
Female	28% (10/36)	74% (17/23)	38% (14/37)
Residence			
Singapore	100%	100%	100%
Ethnicity			
Chinese	38.9% (14/36)	43.5% (10/23)	62.2% (23/37)
Caucasian	27.8% (10/36)	0% (0/23)	16.2% (6/37)
Indian	25.0% (9/36)	21.7% (5/23)	8.1% (3/37)
Bangladeshi	5.6% (2/36)	0% (0/23)	0% (0/37)
Japanese	2.8% (1/36)	0% (0/23)	0% (0/37)
Malay	0% (0/36)	30.4% (7/23)	13.5% (5/37)
Ceylonese	0% (0/36)	4.3% (1/23)	0% (0/37)
*Disease Severity			
Mild	72.2% (26/36)	73.9% (17/23)	N/A
Moderate	13.9% (5/36)	13% (3/23)	N/A
Severe	13.9% (5/36)	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
[23]SARS-CoV-2 NP Ig positivity	100%	100%	0%
[23]SARS-CoV-2 RBD Ig positivity	100%	0%	0%
Time since PCR negativity	2-28 days	17 years	N/A

*Definition of disease severity:
Mild: with or without CXR changes; not requiring oxygen supplement
Moderate: oxygen supplement less than 50%
Severe: oxygen supplement 50% or more or high flow oxygen or intubation

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