

1 **Ex vivo detection of SARS-CoV-2-specific CD8+ T cells: rapid induction, prolonged**
2 **contraction, and formation of functional memory**

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13 immunological memory

14

1 Abstract:

2 CD8+ T cells are critical for the elimination and long-lasting protection of many viral
3 infections, but their role in the current SARS-CoV-2 pandemic is unclear. Emerging data
4 indicates that SARS-CoV-2-specific CD8+ T cells are detectable in the majority of individuals
5 recovering from SARS-CoV-2 infection. However, optimal virus-specific epitopes, the role of
6 pre-existing heterologous immunity as well as their kinetics and differentiation program
7 during disease control have not been defined in detail. Here, we show that both pre-existing
8 and newly induced SARS-CoV-2-specific CD8+ T-cell responses are potentially important
9 determinants of immune protection in mild SARS-CoV-2 infection. In particular, our results
10 can be summarized as follows: First, immunodominant SARS-CoV-2-specific CD8+ T-cell
11 epitopes are targeted in the majority of individuals with convalescent SARS-CoV-2 infection.
12 Second, MHC class I tetramer analyses revealed the emergence of phenotypically diverse
13 and functionally competent pre-existing and newly induced SARS-CoV-2-specific memory
14 CD8+ T cells that showed similar characteristics compared to influenza-specific CD8+ T
15 cells. Third, SARS-CoV-2-specific CD8+ T-cell responses are more robustly detectable than
16 antibodies against the SARS-CoV-2-spike protein. This was confirmed in a longitudinal
17 analysis of acute-resolving infection that demonstrated rapid induction of the SARS-CoV-2-
18 specific CD8+ T cells within a week followed by a prolonged contraction phase that outlasted
19 the waning humoral immune response indicating that CD8+ T-cell responses might serve as
20 a more precise correlate of antiviral immunity than antibody measurements after
21 convalescence. Collectively, these data provide new insights into the fine specificity,
22 heterogeneity, and dynamics of SARS-CoV-2-specific memory CD8+ T cells, potentially
23 informing the rational development of a protective vaccine against SARS-CoV-2.

24

1 Introduction:

2 Infections with the newly emerging coronavirus – severe acute respiratory syndrome
3 coronavirus-2 (SARS-CoV-2) – cause the global outbreak of coronavirus disease 2019
4 (COVID-19)¹. First cases occurred in December 2019 and as of mid-August 2020, roughly
5 20.3 million cases and 740,000 deaths have been documented. The clinical course of SARS-
6 CoV-2 infections is highly variable and ranges from asymptomatic infections over mild
7 courses with fever and cough to severe pneumonia and acute respiratory distress
8 syndrome². Identification of the determinants of immune protection is a prerequisite for the
9 development of vaccines and therapeutic interventions.

10 Early data have indicated that SARS-CoV-2-specific CD8+ T cells are detectable in up to
11 70% of convalescent individuals targeting different viral proteins^{3, 4, 5, 6, 7, 8}. However, these
12 studies did not define individual immunodominant SARS-CoV-2-specific CD8+ T-cell
13 epitopes, a pre-requisite for the *ex vivo* characterization of SARS-CoV-2-specific CD8+ T
14 cells. Interestingly, in 20-50% of unexposed individuals, CD8+ T cells responding to SARS-
15 CoV-2 peptide pools have also been observed^{3, 5, 7, 9, 10} indicating pre-existing virus-specific
16 CD8+ T-cell response most likely due to exposure to “common cold” coronaviruses. All in all,
17 currently, very little information is available about the abundance, phenotype, functional
18 capacity and fate of pre-existing and newly induced SARS-CoV-2-specific CD8+ T-cell
19 responses during the natural course of SARS-CoV-2 infection.

20 In this study, we therefore performed a high-resolution analysis of SARS-CoV-2-specific
21 CD8+ T-cell responses by defining a set of novel optimal immunodominant SARS-CoV-2-
22 specific CD8+ T-cell epitopes enabling *ex vivo* comparison of pre-existing and newly induced
23 SARS-CoV-2-specific CD8+ T cells applying peptide-loaded MHC-I-tetramer technology. By
24 these analyses, we observed a rapid induction, prolonged contraction and versatile
25 emergence of heterogeneous and functionally competent pre-existing and newly induced
26 memory CD8+ T-cell responses in individuals with a mild course of SARS-CoV-2 infection
27 that were more robust compared to the accompanied SARS-CoV-2 antibody response
28 targeting spike that are frequently used to monitor SARS-CoV-2 infections.

1 Results

2 *Definition of novel dominant virus-specific CD8+ T-cell epitopes in convalescent SARS-CoV-* 3 *2-infected patients*

4 We predicted SARS-CoV-2-derived 8-, 9- or 10-mer peptides with high affinity for 10 HLA
5 class I alleles that are common in most populations world-wide (Extended Data Fig. 1). We
6 selected 5 epitope candidate peptides for each of the following HLA alleles: A*01:01,
7 A*02:01, A*03:01, A*11:01, A*24:02 as well as B*07:02, B*08:01, B*15:01, and B*40:01 and
8 8 epitope candidate peptides for B*44:02/03 (Table 1). In addition, we also included all 13
9 described SARS-CoV-1-specific CD8+ T cell epitopes that display 100% homology in SARS-
10 CoV-2⁸ (Table 1). Next, we tested these 66 epitope peptides in 26 individuals with
11 convalescent mild SARS-CoV-2 infection (Extended Data Table 1) in peptide-specific cell
12 cultures. Importantly, we could detect SARS-CoV-2-specific CD8+ T-cell responses in 23/26
13 (88.4%) of the individuals, targeting a median of 4 epitopes (range 1-12) (Fig. 1A). Of note,
14 the HLA-A*02:01-restricted epitopes that had been pre-described for SARS-CoV-1 and that
15 are completely conserved in SARS-CoV-2 (Table 1) were only rarely targeted in our cohort
16 (Fig. 1B, Table 1). However, 33/53 (62.3%) SARS-CoV-2-specific epitope candidates
17 predicted in our study could be confirmed (Table 1, depicted in bold). The strongest
18 responses were observed for epitopes A*01/ORF3a₂₀₇₋₂₁₅, A*02/ORF3a₁₃₉₋₁₄₇, and B*07/N₁₀₅₋
19 ₁₁₃ with a median of 8.3%, 8.4%, and 62.6% of CD8+ T cells producing IFN- γ after peptide-
20 specific culture, respectively (Fig. 1B/C). Taking the protein length into account, we observed
21 a relative overrepresentation of nucleocapsid- and ORF3a-specific CD8+ T-cell responses
22 (Fig. 1D). Despite the superior immunogenicity reflected by the relative overrepresentation of
23 nucleocapsid and ORF3a, the absolute majority of detected responses (57/110 [51.2%])
24 targeted ORF1ab (Fig. 1D). The T-cell epitopes were restricted by both HLA types, HLA-A
25 and HLA-B to a similar extent (Fig. 1E). Interestingly, we were able to detect SARS-CoV-2-
26 specific CD8+ T-cell responses in all nine convalescent individuals that were seronegative for
27 anti-SARS-CoV-2 S antibodies (n=6) or had borderline test results (n=3) (Fig. 1F). Next, we
28 set out to determine whether the identified SARS-CoV-2-specific CD8+ T cell epitopes are

1 unique to SARS-CoV-2-exposed individuals. For this analysis, we tested a cohort of 25
2 healthy volunteers with comparable characteristics regarding gender and age compared to
3 our SARS-CoV-2 cohort. Blood samples were obtained before August 2019 and thus prior to
4 a possible exposure to SARS-CoV-2 (Extended Data Table 1) and tested for the presence of
5 virus-specific CD8⁺ T cells in the same way as the individuals with convalescent SARS-CoV-
6 2 infection. We observed only very low virus-specific IFN- γ and TNF CD8⁺ T-cell responses
7 in 6 individuals (5 individuals with a single response and 1 individual with 5 responses)
8 (Fig.1G, Table 1, Extended Data Fig.2A) and TNF without IFN- γ responses in additional 4
9 individuals (Extended Data Fig.2A/C). The only epitope that was targeted by IFN- γ secreting
10 CD8⁺ T cells in more than one SARS-CoV-2-naïve individual was epitope B*07/N₁₀₅₋₁₁₃
11 (Extended Data Fig.2A). Of note, this is the SARS-CoV-2-specific epitope in our study with
12 the highest conservation between SARS-CoV-2 and “common cold” corona viruses
13 (Extended Data Fig.2B, Extended Data Table 2). In summary, these results reveal that the
14 majority of identified SARS-CoV-2-specific CD8⁺ T-cell epitopes that were dominantly
15 targeted in convalescent individuals with mild SARS-CoV-2 infection, show little evidence for
16 cross-recognition in SARS-CoV-2-naïve individuals.

17

18 *Phenotypic memory characteristics of ex vivo detectable HLA-A and HLA-B-restricted*
19 *SARS-CoV-2-specific CD8⁺ T cells*

20 To evaluate the phenotypic characteristics of SARS-CoV-2-specific memory CD8⁺ T-cell
21 populations, by using a set of novel MHC I tetramers we analyzed *ex vivo* SARS-CoV-2-
22 specific CD8⁺ T cells targeting six immunodominant epitopes (A*01/ORF3a₂₀₇₋₂₁₅,
23 A*01/ORF1ab₄₁₆₃₋₄₁₇₂, A*02/ORF3a₁₃₉₋₁₄₇, B*07/N₁₀₅₋₁₁₃, B*44:03/N₃₂₂₋₃₃₀, B*44:03/ORF1ab₃₉₄₆₋
24 ₃₉₅₄) in comparison to influenza (FLU)-specific CD8⁺ T cells (A*02/Flu-M1₅₈₋₆₆) in a cohort of
25 18 convalescent individuals following a mild course of infection. In order to increase the
26 detection rate and to allow subsequent in-depth phenotypic analysis, we performed peptide-
27 loaded MHC I tetramer-based enrichment (Fig. 2A). Remarkably, we could detect SARS-
28 CoV-2-specific CD8⁺ T cells *ex vivo* in nearly all tested convalescent individuals (Fig. 2B).

1 The *ex vivo* frequencies of SARS-CoV-2-specific CD8+ T cells targeting A*01/ORF3a₂₀₇₋₂₁₅;
2 A*01/ORF1ab₄₁₆₃₋₄₁₇₂; A*02/ORF3a₁₃₉₋₁₄₇; B*44:03/N₃₂₂₋₃₃₀ and B*44:03/ORF1ab₃₉₄₆₋₃₉₅₄ were
3 similar (Fig. 2B). CD8+ T cells targeting B*07/N₁₀₅₋₁₁₃ were present in slightly higher
4 frequencies compared to other SARS-CoV-2-specific CD8+ T-cell populations reaching
5 levels of A*02/Flu-M1₅₈₋₆₆-specific CD8+ T cells (Fig. 2B). This probably reflects heterologous
6 stimulation of pre-existing B*07/N₁₀₅₋₁₁₃-specific CD8+ T cells (Extended Data Fig.2A). SARS-
7 CoV-2-specific CD8+ T-cell populations in convalescent individuals were composed of naïve
8 (T_{naive}), central memory (T_{CM}), effector memory 1 (T_{EM1}), effector memory 2 (T_{EM2}), effector
9 memory 3 (T_{EM3}) and terminally differentiated effector memory expressing RA (T_{EMRA}) T-cell
10 subsets irrespective of the targeted epitope (Extended Data Fig. 3A/B). The presence of an
11 only minor T_{naive} subset fraction among all tested SARS-CoV-2-specific CD8+ T cells
12 supports that these cells have been efficiently primed during the infection. In comparison to
13 HLA-B-restricted SARS-CoV-2-specific CD8+ T cells, HLA-A restricted virus-specific CD8+ T
14 cells showed a shift towards the early differentiated T_{CM} and T_{EM1} subset (Extended Data Fig.
15 3B). Similar results were obtained by applying the CX₃CR1-based definition of memory T-cell
16 subsets (Extended Data Fig. 3C). To more comprehensively compare the phenotypes of the
17 different SARS-CoV-2-specific CD8+ T cells we performed t-distributed stochastic neighbor
18 embedding (t-SNE) of all analyzed virus-specific CD8+ T cells from the tested convalescent
19 individuals (Fig. 2C). Topographical clustering of SARS-CoV-2-specific CD8+ T cells
20 separated these cells according to their HLA restriction (left panel) dominating the respective
21 differences associated with the targeted viral proteins (right panel). This was further
22 supported by multidimensional scaling (MDS) analysis (Fig. 2C). HLA-A-restricted SARS-
23 CoV-2-specific CD8+ T cells were characterized by a cluster of markers including CD38, PD-
24 1 and TOX that are associated with antigen recognition as well as CD28 and TCF-1 labelling
25 less differentiated cells (Fig. 2D). In contrast, HLA-B-restricted SARS-CoV-2-specific CD8+ T
26 cells cluster based on CD45RA, CD57, KLRG1, CD25, CX₃CR1 and high T-BET expression
27 probably reflecting a more terminally differentiated effector cell state (Fig. 2D and Extended
28 Data Fig. 3D). Of note, FLU-A*02/M1₅₈-specific CD8+ T cells showed differences to HLA-A

1 and B-restricted SARS-CoV-2-specific CD8⁺ T cells (Fig. 2C/D). In particular, FLU
2 A*02/M1₅₈₋₆₆-compared to SARS-CoV-2-specific CD8⁺ T cells expressed higher levels of
3 CD127 and BCL2, both important factors for the homeostatic maintenance of memory T cells
4 while the T-cell memory-associated transcription factors TCF-1 and FOXO1 were similarly
5 expressed (Fig. 2E, Extended Data Fig. 4A). The reduced BCL-2 expression of SARS-CoV-
6 2-specific CD8⁺ T cells was most prominent among the early differentiated T_{CM} and T_{EM1}
7 subsets that have the highest BCL-2 expression among memory T-cell subsets in general
8 (Extended Data Fig. 4B). Importantly, BCL-2 expression of SARS-CoV-2-specific CD8⁺ T
9 cells correlated with the days after onset of symptoms (Fig. 2F). Thus, SARS-CoV-2-specific
10 CD8⁺ T cells are most probably within the dynamic process of establishing a *bona fide* long-
11 lasting memory compartment. In summary, circulating SARS-CoV-2-specific CD8⁺ T cells
12 are frequently detectable *ex vivo* in convalescent individuals and are composed of different
13 *bona fide* memory subsets with an additional layer of phenotypic heterogeneity based on the
14 HLA restriction.

15

16 *Similar vigorous functional capacity of pre-existing and newly induced SARS-CoV-2-specific*
17 *memory T cells*

18 Next, we assessed the functional capacity of SARS-CoV-2-specific compared to FLU-specific
19 memory CD8⁺ T cells *in vitro* (Fig. 3A). As shown in Fig. 3B after two weeks of *in vitro*
20 expansion, we detected comparable frequencies of SARS-CoV-2 B*07/N₁₀₅₋₁₁₃- and FLU
21 A*02/M1₅₈₋₆₆-specific CD8⁺ T cells that were higher compared to the other tested SARS-
22 CoV-2-specific CD8⁺ T cells (left panel). However, when analyzing the expansion index, a
23 measure taking the input number of virus-specific CD8⁺ T cells into account, we observed
24 comparable *in vitro* expansion capacities of the analyzed SARS-CoV-2- and FLU-specific
25 CD8⁺ T cells (Fig. 3B, right panel). Thus, the increased frequencies of SARS-CoV-2
26 B*07/N₁₀₅-specific CD8⁺ T cells after peptide-specific CD8⁺ T-cell expansion most probably
27 reflect a higher *ex vivo* frequency of these cells. We also analyzed cytokine production (IFN- γ
28 and TNF) and degranulation as indicated by CD107a expression in relation to the frequency

1 of virus-specific CD8⁺ T cells after expansion in order to have an approximation for the
2 effector functions of SARS-CoV-2-specific CD8⁺ T cells. As shown in Fig. 3C, irrespective of
3 the targeted epitope, the cytokine production and degranulation capacity of SARS-CoV-2-
4 specific CD8⁺ T cells is similar to A*02/Flu-M1₅₈₋₆₆-specific CD8⁺ T cells. In a next set of
5 experiments, we addressed the question whether SARS-CoV-2 B*07/N₁₀₅₋₁₁₃-specific
6 memory CD8⁺ T-cell responses observed in SARS-CoV-2 convalescent individuals differ
7 compared to “common cold” corona viruses-exposed individuals. For this, we analyzed
8 SARS-CoV-2 B*07/N₁₀₅₋₁₁₃-specific CD8⁺ T cells in historical samples (banked before August
9 2019) of six B*07:02 positive individuals (Fig. 3D). We detected SARS-CoV-2 B*07/N₁₀₅₋₁₁₃-
10 specific CD8⁺ T cells *ex vivo* in three out of six historic controls, however, at lower
11 frequencies compared to SARS-CoV-2 convalescent individuals (Fig. 3D) indicating a
12 heterologous boost expansion in the latter cohort. As depicted in Fig. 3E, the
13 CD45RA/CCR7/CD27-based T-cell subset distribution revealed a slight shift towards the
14 further differentiated T_{EM3} subset in SARS-CoV-2 convalescent individuals again supporting
15 heterologous stimulation. However, we did not observe differences in expansion and
16 cytokine production of SARS-CoV-2 B*07/N₁₀₅₋₁₁₃-specific CD8⁺ T-cell population in SARS-
17 CoV-2 convalescent individuals compared to historic controls (Fig. 3F). Taken together,
18 these observations suggest that pre-existing and newly induced SARS-CoV-2-specific CD8⁺
19 T cells establish a functionally competent *bona fide* memory response similar to FLU-specific
20 CD8⁺ T cells.

21

22 *Rapid expansion and prolonged contraction of newly induced SARS-CoV-2-specific CD8⁺ T* 23 *cells*

24 We had the unique opportunity to longitudinally follow the SARS-CoV-2-specific CD8⁺ T-cell
25 response before, during and after SARS-CoV-2 infection in an HLA-B*44:03⁺ individual with a
26 defined infection event (Fig. 4A). As depicted in Fig. 4B and Extended Data Fig. 5A, SARS-
27 CoV-2 B*44:03/N₃₂₂₋₃₃₀⁻ and SARS-CoV-2 B*44:03/ORF1ab₃₉₄₆₋₃₉₅₄⁻ specific CD8⁺ T cells
28 were clearly expanded as early as 7 days post infection together with symptom onset.

1 Importantly, both T-cell populations were not detectable prior to the SARS-CoV-2 infection
2 clearly indicating novel priming (Fig. 4B and Extended Data Fig. 5A). The kinetics of both T
3 cell responses were similar and the contraction phase lasted at least 70 days with SARS-
4 CoV-2-specific CD8⁺ T cells still detectable at significant frequencies (approx. 1×10^{-5}) 109
5 days post infection. Interestingly, the serum anti-SARS-CoV-2 S protein antibody titer fell
6 below the upper detection limit at 84 days post infection (Fig. 4C) while the virus-specific
7 CD8⁺ T cells remained detectable at this exact time point and also at later follow-up time
8 points. Next, we performed deep profiling of SARS-CoV-2-specific CD8⁺ T cells including T-
9 cell differentiation and activation markers, transcription factors, inhibitory receptors and pro-
10 survival factors by using flow and mass cytometry to more comprehensively understand the
11 T-cell phenotype and differentiation program during the course of infection. Diffusion map
12 embedding combining flow cytometry data of SARS-CoV-2 B*44:03/N₃₂₂₋₃₃₀⁻ and SARS-CoV-
13 2 B*44:03/ORF1ab₃₉₄₆₋₃₉₅₄⁻ specific CD8⁺ T cells indicated a continuous relationship between
14 all SARS-CoV-2-specific CD8⁺ T cells longitudinally collected during and after infection, with
15 cells from early time points after infection and those from late time points at opposing ends,
16 reflecting a dynamic differentiation of the virus-specific CD8⁺ T-cell response (Fig. 4D,
17 Extended Data Fig. 5B/C/D). SARS-CoV-2-specific CD8⁺ T cells collected at later time points
18 compared to earlier time points post infection clustered more closely within the diffusion map
19 suggesting a higher degree of similarity and likely establishment of a steady state at the
20 memory phase of the T-cell response (Fig. 4D, Extended Data Fig. 4C). Based on the
21 linearity of the differentiation program suggested by the diffusion map analysis, we performed
22 single-cell trajectory detection using wanderlust analysis¹⁷ of CyTOF data to understand the
23 differentiation trajectories in more detail (Extended Data Fig. 6A). This analysis showed that
24 a small fraction of virus-specific T cells identified after one week of infection with a CD28⁺
25 TCF-1⁺ CD127⁺ CD45RA⁺ phenotype may represent the precursor population of the large
26 pool of effector cells (Extended Data Fig. 6A). As indicated by these wanderlust (Extended
27 Data Fig. 6A) and diffusion map (Fig. 4D) analyses, Phenotyping by Accelerated Refined
28 Community-partitioning (PARC) of mass cytometry data confirmed a significant shift of

1 SARS-CoV-2-specific CD8⁺ T cells from an early effector state characterized by a high
2 expression of the activation markers CD38, CD39 or PD-1 together with Ki-67 towards a T_{EM}
3 differentiation program with high expression of CD45RA, CX₃CR1, KLRG1 and CD57 with
4 little involvement of T_{CM} cells (Fig. 4E, Extended Data Fig. 6B-D). These changes were also
5 apparent on non-MHC I tetramer⁺ CD8⁺ T cells (Extended Data Fig. 6B/E) suggesting broad
6 activation of virus-specific responses targeting other epitopes. Within a time-span of more
7 than 100 days post infection, we did not detect major changes in the *in vitro* functional
8 capacity (expansion, cytokine production and degranulation) of both SARS-CoV-2
9 B*44:03/N₃₂₂₋₃₃₀⁻ and SARS-CoV-2 B*44:03/ORF1ab₃₉₄₆₋₃₉₅₄⁻ specific CD8⁺ T-cell populations
10 (Fig. 4F-H). Together, these findings suggest an ongoing efficient control of or protection
11 from SARS-CoV-2 infection by virus-specific CD8⁺ T cells even at late time points, when
12 antibodies may already have waned.

1 Discussion

2 Here, we have defined a set of immunodominant CD8+ T-cell epitopes that were targeted in
3 the majority of tested convalescent individuals of a Caucasian cohort after a mild course of
4 SARS-CoV-2 infection. This even exceeds the previously reported high detection rate of T-
5 cell responses in up to 70% of convalescent individuals in different cohorts using peptide
6 pools for T-cell stimulation^{3, 4, 5, 6, 7}, most likely owing to our more specific approach. These
7 analyzed cohorts of convalescent individuals comprised citizen of the UK⁷, Sweden⁴,
8 Singapore⁵ and California/USA³. Thus, a SARS-CoV-2-specific CD8+ T-cell memory is
9 robustly induced on a global population level. This observation gains further relevance when
10 taking into account that we could detect SARS-CoV-2-specific CD8+ T cells in individuals
11 being seronegative for anti-SARS-CoV-2 spike antibodies indicating a higher sensitivity for
12 detecting SARS-CoV-2-specific T cells compared to antibodies to prove a recent SARS-CoV-
13 2 infection. A faster waning of the antibody response is also suggested by our longitudinal
14 analysis of an individual with a mild disease course before, during and after SARS-CoV-2
15 infection where T-cell but not antibody responses were still detectable long after clinical
16 resolution of infection. In addition, this is in line with previous reports in the context of SARS-
17 CoV-1 infection reporting long-lasting detection of virus-specific CD8+ T cells in contrast to
18 antibodies^{18, 19}. Altogether, our findings indicate that SARS-CoV-2-specific CD8+ T cells
19 represent a major determinant of immune protection on an individual as well as population
20 level.

21 The set of CD8+ T-cell epitopes defined in this study is mainly composed of epitopes that
22 have not been described to date and for which there is in most cases no evidence for pre-
23 existing immunity as assessed by testing historic control samples collected before August
24 2019. The individuals included into our historic control cohort had no history of SARS-CoV-1
25 exposure that potentially impacts the virus-specific CD8+ T-cell response towards SARS-
26 CoV-2 due to high sequence homology within T-cell epitopes⁸ and long-living cross-reactive
27 virus-specific CD8+ T cells^{5, 19}. The here-identified SARS-CoV-2-specific CD8+ T-cell
28 responses target different structural and non-structural proteins with a specific focus against

1 ORF1ab, in agreement with its protein length. However, taking the protein length into
2 account, we observed a relative dominance of the N protein and ORF3a as targets. In line
3 with Grifoni *et al.*, this finding emphasizes the broad recognition of SARS-CoV-2 by virus-
4 specific CD8+ T cells and extends other previous studies restricted to structural proteins^{3, 4, 6}
5 and omitting ORF1ab⁷. Thus, the SARS-CoV-2-specific CD8+ T-cell response interferes with
6 different stages of the viral lifecycle, e.g. by targeting ORF1ab products necessary for the
7 early viral replication and transcription²⁰. Clearly, our approach to define optimal CD8+ T-cell
8 epitopes based on a *in silico* prediction has the limitation that it does not completely cover
9 the entire viral genome as it is the case in studies that have used overlapping peptides^{3, 7, 9}.
10 However, an advantage of this approach is the definition of exact, single and optimal CD8+
11 T-cell epitopes including HLA restriction. With this, our data revealed that there is no clear
12 dominance of HLA-A or B-restricted epitopes that are targeted by SARS-CoV-2-specific
13 CD8+ T cells indicating an evenly broad and robust induction of an antiviral CD8+ T-cell
14 response among individuals.
15 Importantly, the definition of optimal epitopes also allowed a comparative *ex vivo* detection
16 and characterization of SARS-CoV-2-specific CD8+ T cells after peptide-loaded MHC I
17 tetramer-based enrichment. The highest frequency *ex vivo* was detectable for B*07/N₁₀₅₋₁₁₃-
18 specific CD8+ T cells which was also in agreement with their strong peptide-specific
19 expansion. Indeed, B*07/N₁₀₅₋₁₁₃-specific CD8+ T cells were similarly frequent as A*02/Flu-
20 M1₅₈₋₆₆-specific CD8+ T cells. Since the sequence homology of the B*07/N₁₀₅₋₁₁₃ epitope
21 among the corona viruses including “common cold” corona viruses is high and since we also
22 identified pre-existing B*07/N₁₀₅₋₁₁₃-specific CD8+ T cells in historic controls, the higher
23 frequency of these CD8+ T cells in SARS-CoV-2 convalescent individuals most probably
24 reflects heterologous boosting. Of note, in the study by Peng *et al.*⁷, this epitope was also
25 included in the dominant overlapping peptide pool showing CD8+ T-cell responses in 11 out
26 of 42 individuals. Interestingly, 10 out of these 11 individuals expressed HLA-B*07:02.
27 However, we did not observe clear phenotypical and functional differences between the
28 potentially pre-existing B*07/N₁₀₅₋₁₁₃-specific CD8+ T cells and other newly induced SARS-

1 CoV-2-specific CD8⁺ T cells either targeting structural or non-structural proteins. One reason
2 for this may be a rapid and strong induction also of newly induced SARS-CoV-2-specific
3 CD8⁺ T cells that we have detected in one individual followed longitudinally during mild
4 SARS-CoV-2 infection. Furthermore, the phenotypic and functional characteristics of SARS-
5 CoV-2-specific CD8⁺ T cells were quite similar to the immunodominant A*02/Flu-M1₅₈₋₆₆-
6 specific CD8⁺ T cells representing classical, fully functional memory T cells²¹. The examined
7 lower BCL-2 expression of SARS-CoV-2- compared to FLU-specific CD8⁺ T cells is most
8 probably due to the fact that the SARS-CoV-2-specific CD8⁺ T-cell memory response was
9 not in a resting steady state. This hypothesis is corroborated by our findings that BCL-2
10 expression increased with time after SARS-CoV-2 infection and that the contraction phase of
11 SARS-CoV-2-specific CD8⁺ T cells was apparently prolonged in the individual that we
12 longitudinally followed during SARS-CoV-2 infection. Taken together, these comprehensive
13 analyses revealed that a fully functional immune response is generated by both, pre-existing
14 and newly induced virus-specific CD8⁺ T cells, irrespective of the targeted viral protein and
15 the HLA restriction. The established functionally competent SARS-CoV-2-specific CD8⁺ T-
16 cell memory is composed of heterogeneous subsets, e.g. T_{CM}, T_{EM1}, T_{EM2} and T_{EM3}, required
17 for a flexible response upon re-infection. Future studies have to evaluate whether differences
18 in pre-existing and newly induced SARS-CoV-2-specific CD8⁺ T-cell responses are linked to
19 different courses of infection. However, with this study, we now established the experimental
20 tools for high resolution *ex vivo* analyses of SARS-CoV-2-specific CD8⁺ T cells required to
21 answer the question about a potential rheostat action of virus-specific CD8⁺ T cells in SARS-
22 CoV-2 pathogenesis versus protection.

23

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12

13 Author contributions

14 I.S., J.K., V.O. and K.W. planned, performed and analyzed experiments with the help of S.,
15 F.D. and O.S. L.S. and S.K. performed and analyzed CyTOF data with the assistance of M.
16 S. L. I.S., J.K., V.O., K.W., L.S. and S.K. contributed equally to this work. A.D., H.L., B.Bi.,
17 D.B., S.R., C.F.W., A. N., D.D. were responsible for patient recruitment. H.M. and A.S.
18 provided barcoding reagents for CyTOF analysis. S.L.-L. and D.P. provided influenza
19 M1₅₈/A*02 tetramers. F.E. performed four-digit HLA-typing by next generation sequencing.
20 M.P. and D.H. performed antibody testing. M.S. and G.K. provided virological expertise and
21 contributed to data interpretation. B.B. designed and supervised CyTOF analysis. T.B., R.T.,
22 M.H. and C.N.H. designed the study and contributed to experimental design and planning.
23 I.S., J.K., V.O., R.T., M.H. and C.N.H. interpreted data and wrote the manuscript. C.N.H.,
24 M.H., R.T., B.B. and T.B. are shared last authors.

25

26 Declaration of interest

27 The authors have nothing to declare.

28

29

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17

1 Methods

2 *Study Cohort*

3 A total of 26 COVID-19 convalescent individuals following a mild course of SARS-CoV-2
4 infection and 25 age and sex-matched historic controls (collected before August 2019) were
5 recruited at the Freiburg University Medical Center, Germany. Mild course of infection was
6 defined as clinical symptoms without signs of respiratory insufficiency. Patient characteristics
7 are summarized in Extended Data Table I. SARS-CoV-2 infection was confirmed by positive
8 PCR testing from oropharyngeal swab and/or SARS-CoV-2 spike IgG positive antibody
9 testing in the presence of typical symptoms. Peptide-loaded major histocompatibility complex
10 I (MHC I) tetramer-based magnetic bead enrichment of virus-specific CD8+ T cells was
11 performed with samples from 18 SARS-CoV-2 convalescent individuals and 6 historic
12 controls. HLA-typing was performed by next-generation sequencing. Influenza-specific CD8+
13 T-cell characterization was performed in 5 SARS-CoV-2 convalescent individuals. Written
14 informed consent was obtained from all participants and the study was conducted according
15 to federal guidelines, local ethics committee regulations (Albert-Ludwigs-Universität,
16 Freiburg, Germany; vote #: 322/20) and the Declaration of Helsinki (1975).

17

18 *PBMC isolation*

19 Venous blood samples were collected in EDTA-anticoagulated tubes. Peripheral blood
20 mononuclear cells (PBMCs) were isolated with lymphocyte separation medium density
21 gradients (Pancoll separation medium, PAN Biotech GmbH; Aidenbach, Germany) and
22 resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 1%
23 penicillin/streptomycin, and 1.5% HEPES buffer 1 mol/L (complete medium; all additives from
24 Thermo Scientific (Waltham, MA)) and stored at -80°C until used.

25

26 *Prediction of SARS-CoV-2-specific CD8+ T-cell epitopes*

27 The entire viral amino acid sequence of SARS-CoV-2 (GenBank: MN908947.3) was
28 analyzed for *in silico* peptide binding with ANN 4.0 on the Immune Epitope Database

1 website¹¹. The five best 8-,9- or 10-mer peptides calculated for the HLA alleles A*01:01,
2 A*02:01, A*03:01, A*11:01, A*24:02, B*07:02, B*08:01, B*15:01, B*40:01, and B*44:02/03
3 were selected and synthesized for further analysis. Additionally, 13 epitopes that were
4 predicted by Grifoni et al. with high sequence similarity to SARS-CoV-1 were included,
5 summarized in Table 1⁸.

6

7 *Sequence Alignment*

8 Sequence homology analyses were performed in Geneious Prime 2020.0.3
9 (<https://www.geneious.com/>) using Clustal Omega 1.2.2 alignment with default settings¹².
10 Reference genomes of human coronaviruses were downloaded from NCBI database 229E
11 (NC_002645), HKU1 (NC_006577), NL63 (NC_005831), OC43 (NC_006213), MERS
12 (NC_019843) and SARS-CoV-1 (NC_004718). Proteins of human coronaviruses were
13 aligned according to their homology (amino acid level) only if the protein of interest has a
14 homolog in the respective coronavirus. Confirmed SARS-CoV-2 epitopes were then mapped
15 to the corresponding protein alignment, summarized in Extended Data Table II.

16

17 *Peptides and tetramers*

18 Peptides were synthesized with an unmodified N-terminus and an amidated C-terminus with
19 standard Fmoc chemistry and a purity of >70% (Genaxxon Bioscience, Ulm, Germany). HLA
20 class I easYmers® (immunAware, Copenhagen, Denmark) were loaded with peptide
21 according to manufacturer's instructions (A*01/ORF3a₂₀₇₋₂₁₅, A*01/ORF1ab₄₁₆₃₋₄₁₇₂,
22 A*02/ORF3a₁₃₉₋₁₄₇, B*07/N₁₀₅₋₁₁₃) or ordered as peptide-loaded monomers (B*44:03/N₃₂₂₋₃₃₀,
23 B*44:03/ORF1ab₃₉₄₆₋₃₉₅₄). SARS-CoV-2 peptide-loaded HLA class I tetramers were
24 generated by conjugation of biotinylated peptide-loaded HLA class I easYmers® with
25 phycoerythrin (PE)-conjugated streptavidin (Agilent, Santa Clara, US) according to the
26 manufacturer's instructions. Influenza-specific HLA-A*02/M1₅₈₋₆₆ (GILGFVFTL) tetramers
27 were generated as described previously¹³.

28

1 *In vitro expansion of virus-specific CD8⁺ T-cells and assessment of effector function*

2 PBMCs ($1-2 \times 10^6$) were stimulated with epitope-specific peptides (5 μ M) and anti-CD28 mAb
3 (0.5 μ g/mL, BD) and expanded for 14 days in complete RPMI culture medium containing rIL2
4 (20 IU/mL, Miltenyi Biotec). The expansion factor was calculated based on peptide-loaded
5 HLA class I tetramer staining as described before¹⁴. Cytokine production and degranulation
6 were assessed 5 hours after restimulation with epitope-specific peptides as previously
7 described¹⁴.

8

9 *Magnetic bead-based enrichment of antigen-specific CD8 T cells*

10 Enrichment of virus-specific CD8⁺ T cells was performed as described before¹⁵. Briefly,
11 $2-3 \times 10^7$ peripheral blood mononuclear cells (PBMCs) were labelled for 30 min with PE-
12 coupled peptide-loaded HLA class I tetramers. Subsequent enrichment was performed with
13 anti-PE beads using MACS technology (Miltenyi Biotec, Germany) according to the
14 manufacturer's protocol. Enriched SARS-CoV-2-specific CD8⁺ T cells were used for
15 multiparametric flow cytometry analysis. Frequencies of virus-specific CD8⁺ T cells were
16 calculated as described previously¹⁵.

17

18 *Multiparametric flow cytometry*

19 The following antibodies were used for multiparametric flow cytometry: anti-CCR7-PE-CF594
20 (150503, 1:50), anti-CCR7-BUV395 (3D12, 1:50), anti-CCR7-BV421 (150503, 1:33), anti-
21 CD4-BV786 (L200, 1:200), anti-CD8-BUV496 (SK1, 1:100), anti-CD8-BUV510 (SK1, 1:100),
22 anti-CD8-APC (SK-1, 1:200), anti-CD27-BV605 (L128, 1:200), anti-CD28-BV421 (CD28.2,
23 1:100), anti-CD28-BV711 (CD28.2, 1:100), anti-CD45RA-BV786 (HI100, 1:800), anti-
24 CD45RA-BUV737 (HI100, 1:200), anti-CD69-BUV395 (FN50, 1:50), anti-CD107a-APC
25 (H4A3, 1:100), anti-CD127-BV510 (HIL-7R-M21, 1:25), anti-EOMES-PerCP-eF710
26 (WD1928, 1:50), anti-IFN- γ -FITC (25723.11, 1:8), anti-IL-21-PE (3A3-N2.1, 1:25), anti-PD-1-
27 BV786 (EH12.1, 1:33), anti-TNF-PE-Cy7 (Mab11, 1:400) (BD Biosciences, Germany). Anti-
28 BCL-2-BV421 (100, 1:200), anti-CD25-BV650 (BC96, 1:33), anti-CD38-BV650 (HB-7, 1:400),

1 anti-CD57-BV605 (QA17A04, 1:100), anti-CX₃CR1-APC-eFluor660 (2A9-1, 1:50), anti-
2 CXCR3-PerCP-Cy5.5 (G025H7, 1:33), anti-IL-2-PerCP-Cy5.5 (MQ1-17H12, 1:100), anti-
3 IL17A-BV605 (BL168, 1:100), anti-PD-1-PE-Cy7 (EH12.2H7, 1:200), anti-rabbit-PE-CF594
4 (Poly4064, 1:200) anti-CD45RA-BV510 (HI100, 1:200), (BioLegend, UK), anti-FOXO1-pure
5 (C29H4, 1:33), anti-TCF1-AlexaFluor488 (C63D9, 1:100) (Cell Signaling, Germany), anti-
6 CD14-APC-eFluor780 (61D3, 1:400), anti-CD19-APC-eFluor780 (HIB19, 1:400), anti-CD27-
7 FITC (0323, 1:100), anti-KLRG1-BV711 (13F12F2, 1:50), anti-T-BET-PE-Cy7 (4B10, 1:200),
8 anti-TOX-eFluor660 (TRX10, 1:100) (eBioscience, Germany). A fixable Viability Dye (APC-
9 eFluor780 1:200, 1:400) (eBioscience, Germany) or ViaProbe (7-AAD, 1:33) (BD
10 Biosciences, Germany)) was used for live/dead discrimination. FoxP3/Transcription Factor
11 Staining Buffer Set (eBioscience, Germany) and Fixation/Permeabilization Solution Kit (BD
12 Biosciences, Germany) were applied according to the manufacturer's instructions to stain for
13 intranuclear and cytoplasmic molecules, respectively. Fixation of cells in 2%
14 paraformaldehyde (PFA, Sigma, Germany) was followed by subsequent analyses on
15 FACSCanto II, LSRFortessa (BD, Germany) or CytoFLEX (Beckman Coulter). Data analyses
16 were performed with FlowJo 10 (Treestar, USA).

17

18 *Dimensionality reduction of multiparametric flow cytometry data*

19 The visualization of multiparametric flow cytometry data was done with R version 4.0.2 using
20 the Bioconductor (version: Release (3.11)) CATALYST package (Crowell H, Zanotelli V,
21 Chevrier S, Robinson M (2020). CATALYST: Cytometry dATa anALYSIS Tools. R package
22 version 1.12.2, <https://github.com/HelenaLC/CATALYST>). The analyses were performed on
23 gated virus-specific CD8⁺ T cells for two panels separately. Analysis of panel 1 (transcription
24 factors) included the markers CD45RA, CCR7, CD27, CD28, BCL-2, TCF-1, CD69, CD38,
25 PD-1, EOMES, T-BET and TOX. Analysis of panel 2 (surface markers) was performed on
26 CCR7, CD45RA, CD27, CD28, CD25, CD127, CD57, KLRG1, CXCR3, PD-1, CX₃CR1 and
27 FOXO-1. Down sampling of cells to the number of cells present in the sample with the fewest
28 cells was performed prior to dimensionality reduction in order to facilitate the visualization of

1 different samples. Marker intensities were transformed by arcsinh (inverse hyperbolic sine)
2 with a cofactor of 150. Dimensionality reduction on the transformed data was achieved by t-
3 distributed stochastic neighbor embedding (t-SNE), multidimensional scaling (MDS) and
4 Diffusion Map visualization.

5

6 *Mass cytometry*

7 Mass cytometry reagents were obtained from Fluidigm or generated by custom conjugation
8 to isotope-loaded polymers using MAXPAR X8 conjugation kit (Fluidigm). Mass cytometry
9 antibodies used are shown in SI-Table 2. Mass cytometry tetramers were generated by
10 tetramerization of pMHC monomers with Streptavidin conjugated to Eu¹⁵¹ using Lightning link
11 conjugation kit (Expedon, Inc.) Sample barcoding was performed using anti-β2M barcodes,
12 cells were then pooled and staining was performed as previously described¹⁶. Briefly, the
13 single-cell suspension was pelleted, incubated with 20 μM Lanthanum-139 (Trace Sciences)-
14 loaded maleimido-mono-amine-DOTA (Macrocyclics) in PBS for 10 min at RT for live/dead
15 discrimination (LD). Cells were washed in staining buffer and resuspended in staining buffer
16 containing tetramers, incubated for 30min at RT and washed twice. Cells were then
17 resuspended in surface antibody cocktail, incubated for 30 min at RT, washed twice in
18 staining buffer, pre-fixed with PFA 1.6%, washed, then fixed and permeabilized using FoxP3
19 staining buffer set (eBioscience) and stained intracellularly for 60 min at RT. Cells were
20 further washed twice before fixation in 1.6% PFA (Electron Microscopy Sciences) solution
21 containing 125 nM Iridium intercalator overnight at 4°C. Prior to data acquisition on a CyTOF
22 Helios (Fluidigm), cells were washed twice in PBS and once in CAS. Mass cytometry data
23 was analyzed after debarcoding and bead-based normalization. For analysis of mass
24 cytometric data samples were first gated on Iridium intercalator positive, live, single
25 CD45+CD3+CD8+ T cells using FlowJo (v10.6). CD8+ T cells were then exported for
26 analysis in Omiq (Omiq, Inc.). Virus-specific CD8+ T cells were identified by manual gating. A
27 workflow including dimension reduction using optSNE, PARC clustering analysis and
28 Wishbone trajectory analysis was implemented in Omiq. Clustering and dimension reduction

1 analysis were performed based on CD45RA, CD45RO, CCR7, CD28, CD127, CD16, CD25,
2 CD26, CD38, CD39, CD56, CD57, CD69, CD103, CD161, CCR6, CCR9, CXCR3, CXCR5,
3 CXCR6, CX3CR1, CRTH2, TCF-1, TOX, TIGIT, T-BET, EOMES, KLRG1 and PD-1. Further
4 analysis and heatmap visualization was performed using R (v4.0) (<https://www.r-project.org>).

5

6 *SARS-CoV-2 spike IgG antibody determination*

7 SARS-CoV-2 spike IgG antibodies were determined by the Euroimmune assay as described
8 in the product instructions.

9

10 *Statistics*

11 Statistical analysis was performed with GraphPad Prism 8 (USA). Statistical significance was
12 assessed by Kruskal-Wallis testing including Dunn's multiple comparisons test and
13 Spearman correlation. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

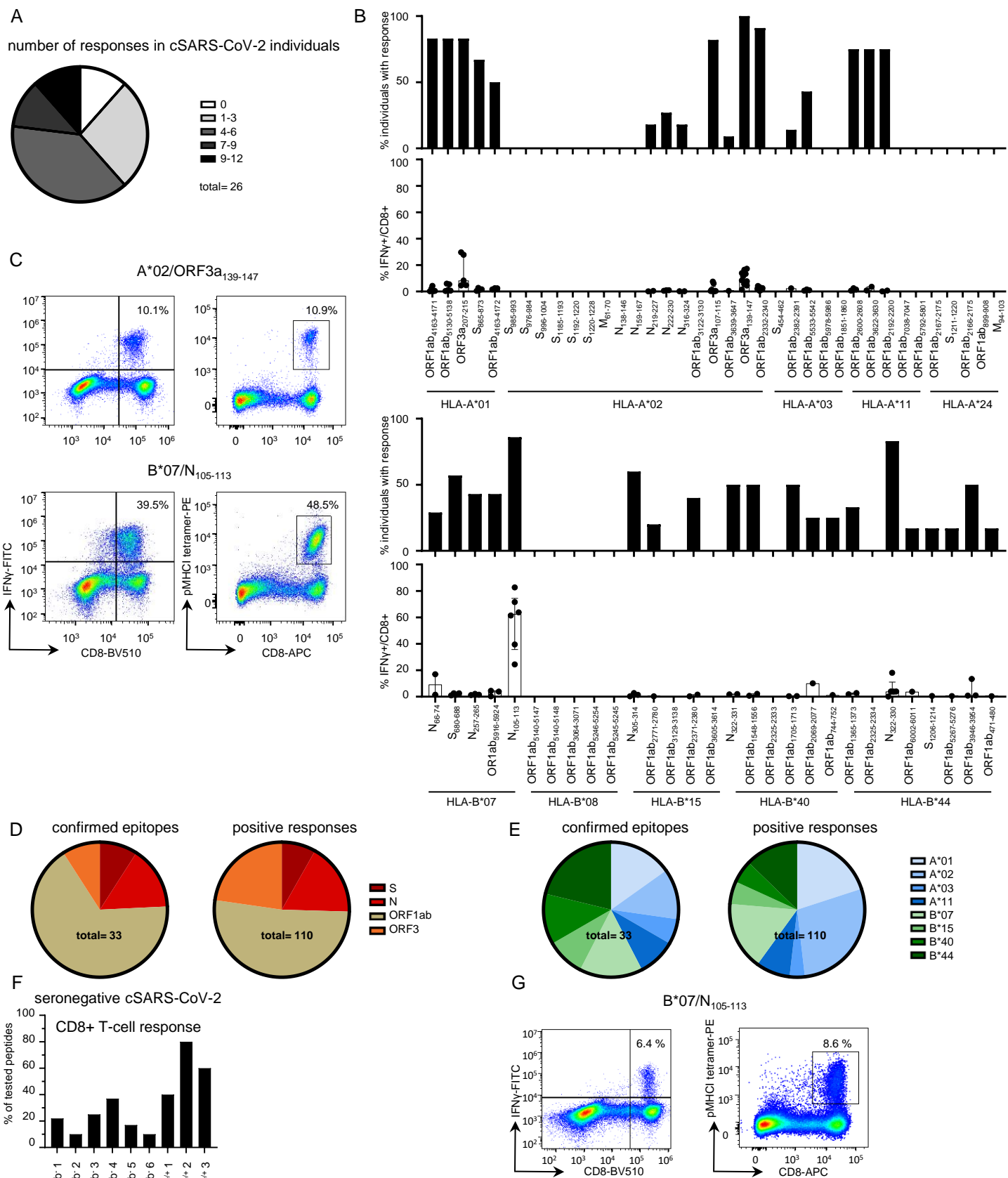


Figure 1: Definition of dominant SARS-CoV-2-specific CD8+ T-cell epitopes in convalescent SARS-CoV-2 individuals
(A) Pie chart illustrating the number of epitopes recognized per tested individual. **(B)** % of convalescent SARS-CoV-2 individuals with positive response towards HLA-A- and HLA-B- restricted SARS-CoV-2 peptides as well as the strength of individual responses as % IFN- γ + of CD8+ T cells. **(C)** Representative dot plots showing pMHC I-tetramer stainings and IFN γ production of A*02/ORF3a₁₃₉₋₁₄₇ and B*07/N₁₀₅₋₁₁₃-specific CD8+ T cells after 14-days *in vitro* expansion. Numbers refer to the respective percentage of pMHC I-tetramer+ and IFN- γ + cells among CD8+ T cells. Confirmed epitopes and total positive responses are depicted regarding their location within the SARS-CoV-2 genome **(D)** and according to their HLA restriction **(E)**. **(F)** CD8+ T-cell responses in SARS-CoV-2 antibody seronegative or borderline positive convalescent patients as percentage of responses out of all peptides tested matching the patient's HLA alleles. **(G)** Exemplary dot plots showing a pMHC I-tetramer staining and IFN γ production of HLA-B*07/N₁₀₅₋₁₁₃-specific CD8+ T cells from a historic control after 14-days *in vitro* expansion. Numbers refer to the respective percentage of pMHC I-tetramer+ and IFN- γ + cells among CD8+ T cells. Bar charts show the median with IQR.

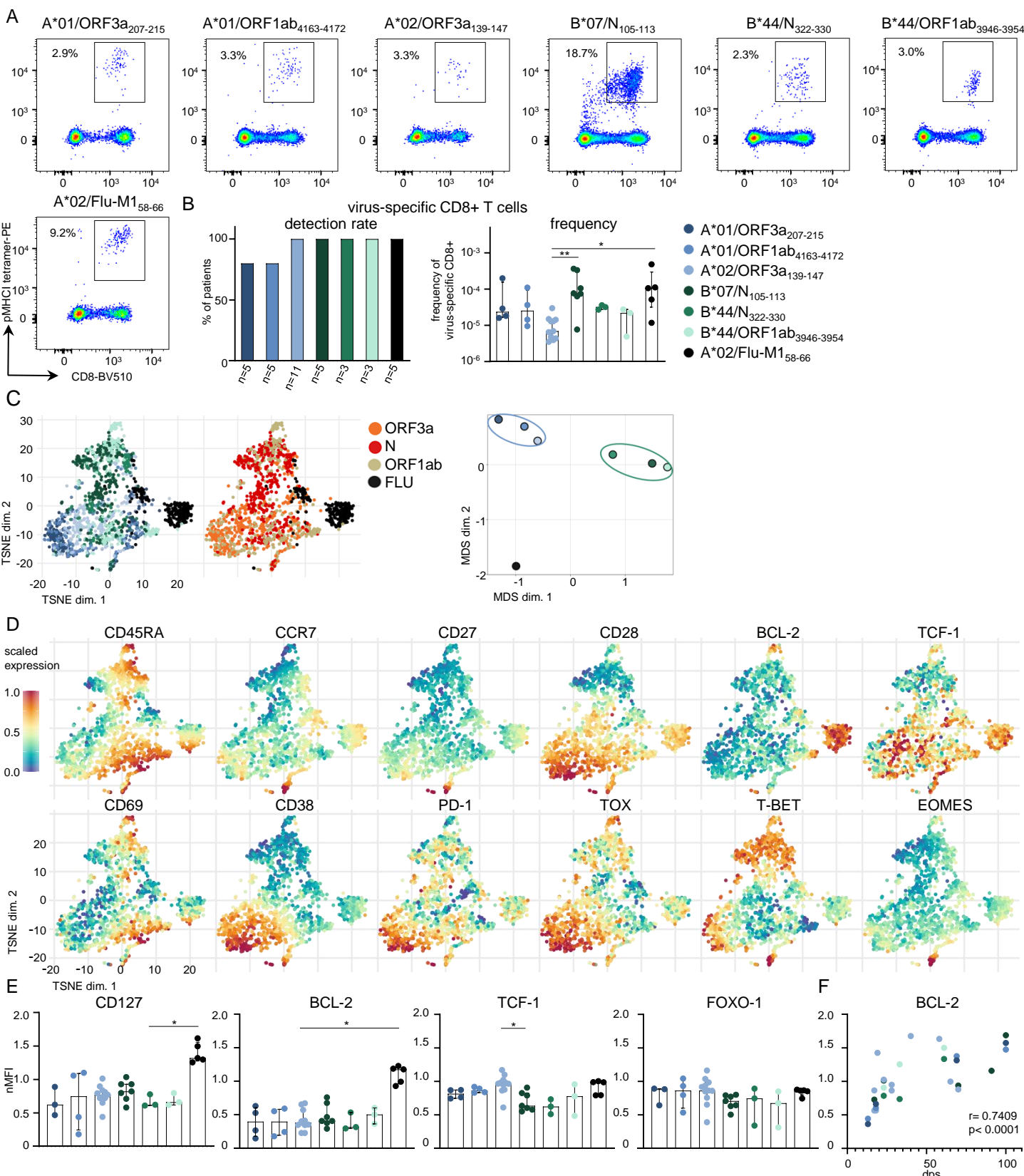


Figure 2: Phenotypic differences of ex vivo detectable virus-specific CD8+ T cells in SARS-CoV-2 convalescent individuals

(A) Representative dot plots showing A*01/ORF3a₂₀₇₋₂₁₅, A*01/ORF1ab₄₁₆₃₋₄₁₇₂, A*02/ORF3a₁₃₉₋₁₄₇, B*07/N₁₀₅₋₁₁₃, B*44/N₃₂₂₋₃₃₀, B*44/ORF1ab₃₉₄₆₋₃₉₅₄ and A*02/Flu-M1₅₈₋₆₆, specific CD8+ T cells ex vivo after pMHCII tetramer-based enrichment. **(B)** The detection rate (left) and frequency (right) of epitope-specific CD8+ T cells was determined. **(C)** t-distributed stochastic neighbor embedding (t-SNE) representation of flow cytometry data, which were derived from 18 SARS-CoV-2 convalescent individuals, comparing SARS-CoV-2-specific CD8+ T cells by their HLA restriction (left) and by their targeted viral proteins (middle). Multidimensional scaling (MDS) analysis comparing the similarity of HLA-A and HLA-B-restricted SARS-CoV-2-specific epitopes (right). **(D)** Expression levels of CD45RA, CCR7, CD27, CD28, BCL-2, TCF-1, CD69, CD38, PD-1, TOX, T-BET and EOMES are plotted on the t-SNE plot. Expression levels are color-coded: blue, low expression; red, high expression. **(E)** Mean fluorescence intensity of CD127, BCL-2, TCF-1 and FOXO-1 of virus-specific CD8+ T cells normalized to mean fluorescence intensity of naïve CD8+ T cells (nMFI). **(F)** Correlation of BCL-2 expression with date post symptom onset (dps). Bar charts show the median with IQR. Statistical significance was assessed by Kruskal-Wallis test including Dunn's multiple comparisons test and Spearman correlation. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001)

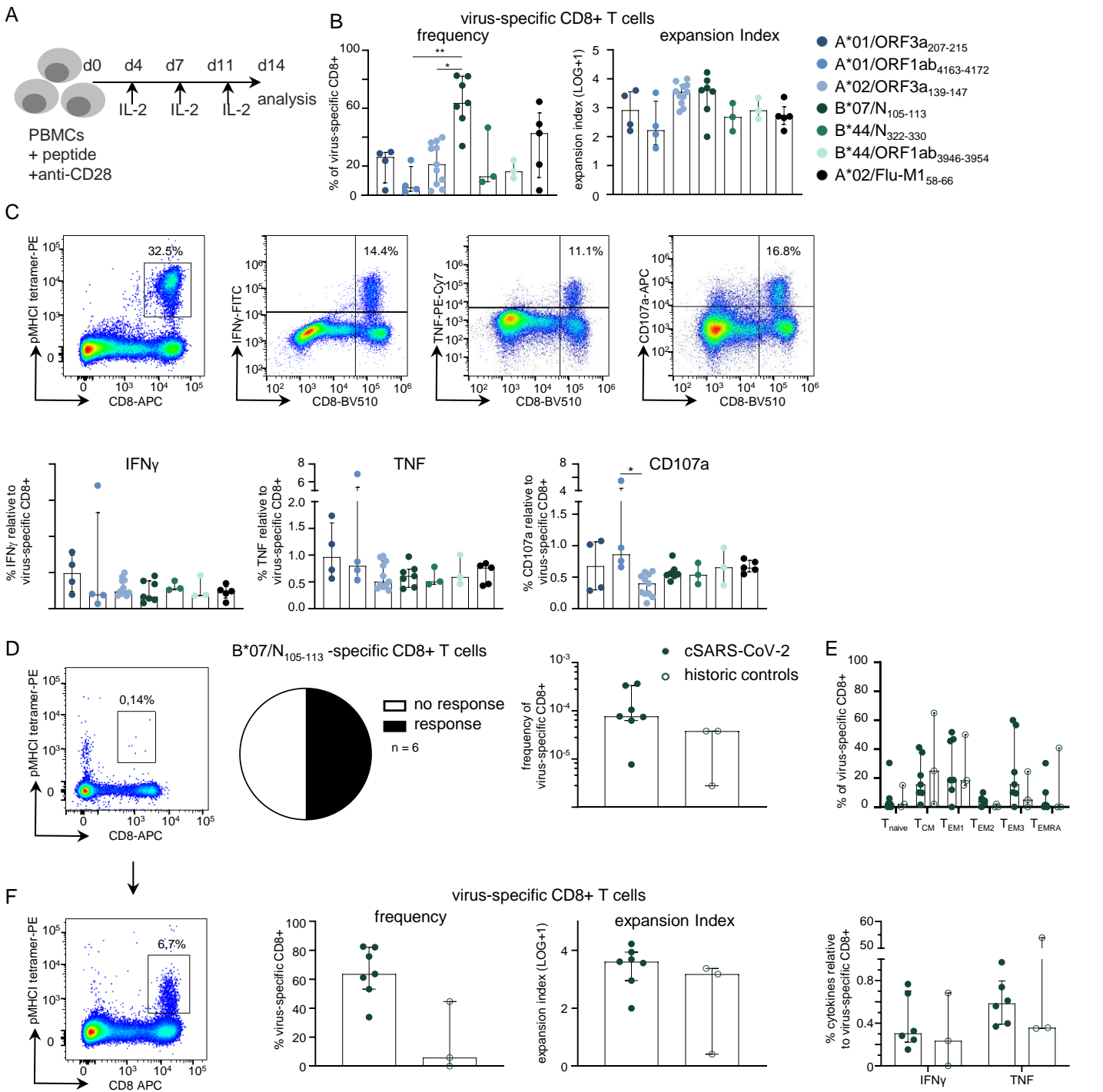


Figure 3: Similar vigorous functional capacity of pre-existing and newly induced SARS-CoV-2-specific memory CD8+ T cells

(A) Workflow illustrating the experimental set-up for the peptide-specific *in vitro* expansion of CD8+ T cells. 1.5×10^6 PBMCs were stimulated with SARS-CoV-2-specific peptides and anti-CD28 mAb and expanded for 14 days in the presence of IL-2. **(B)** After 14 days of *in vitro* expansion the % of virus-specific CD8+ T cells (left) and the expansion index (right) of the respective epitope-specific CD8+ T cells were calculated. **(C)** Representative dot plots showing SARS-CoV-2-specific CD8+ T cells, as well as IFN- γ -, TNF and CD107a-producing CD8+ T cells after 14-days *in vitro* expansion (top). The percentage of IFN- γ -, TNF- and CD107a-producing CD8+ T cells in relation to the frequency of epitope-specific CD8+ T cells was determined after 14-days *in vitro* expansion (bottom).

(D) Representative dot plot showing virus-specific CD8+ T cells ex vivo after B*07/N₁₀₅₋₁₁₃ tetramer-based enrichment (left), pie chart depicting the number of positive responses of patients tested (middle) and frequency of B*07/N₁₀₅₋₁₁₃-specific CD8+ T cells in historic controls in comparison to convalescent SARS-CoV-2 individuals (cSARS-CoV-2) (right).

(E) Distribution of T-cell memory subsets, T_{naive}, T_{CM}, T_{EM1}, T_{EM2}, T_{EM3} and T_{EMRA} of B*07/N₁₀₅₋₁₁₃-specific CD8+ T cells in historic controls compared to SARS-CoV2 convalescent individuals. **(F)** Representative dot plot showing virus-specific CD8+ T cells after 14 days *in vitro* expansion (left). The frequency and expansion index (middle) of virus-specific CD8+ T cells was determined. Expression of IFN- γ and TNF in percentage relative to the frequency of epitope-specific CD8+ T cells is shown. Bar charts show the median with IQR. Statistical significance was assessed by Kruskal-Wallis testing including Dunn's multiple comparisons test. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001)

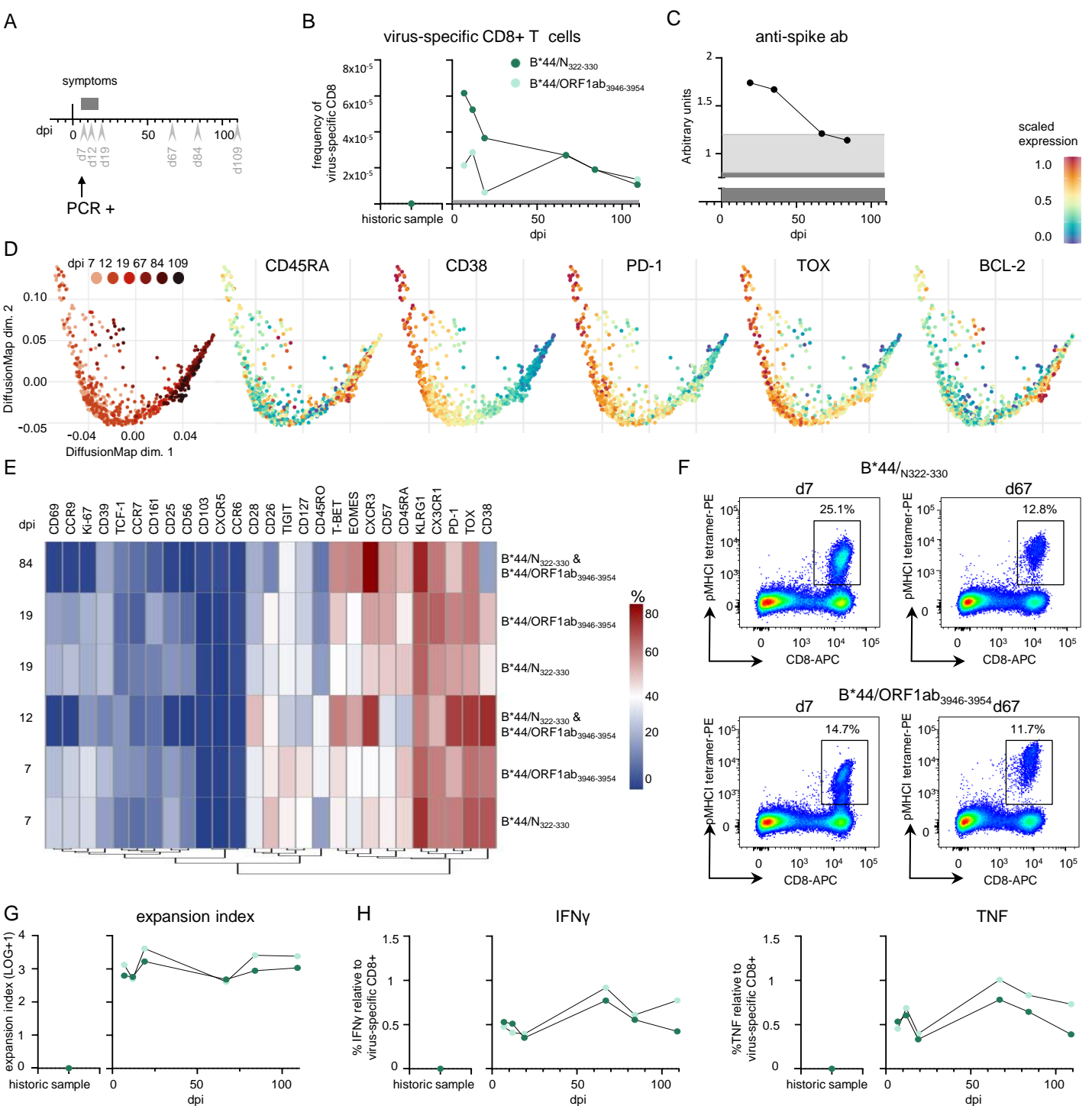


Figure 4: Rapid expansion and prolonged contraction of newly induced SARS-CoV-2-specific CD8+ T cells

(A) Timeline depicting the longitudinal sampling for the SARS-CoV-2-infected patient analyzed. Bleed dates (gray arrow heads), symptoms (dark grey bar) and positive PCR testing are shown at days post infection (dpi). **(B)** The frequency of B*44/N₃₂₂₋₃₃₀- and B*44/ORF1ab₃₉₄₆₋₃₉₅₄-specific T cells in the patient is indicated at dpi together with a historic sample. Grey line indicates detection threshold **(C)** Timeline depicting the anti-spike antibody response in arbitrary units at dpi. Light grey and dark grey background color indicate the area below the upper and lower detection limit, respectively **(D)** Diffusion map representation of flow cytometry data, which were derived from longitudinal analysis, demonstrating the diffusion of B*44/N₃₂₂₋₃₃₀- and B*44/ORF1ab₃₉₄₆₋₃₉₅₄-specific T cells in relation to dpi which is distinguished by a color gradient from light (early time points) to dark red (late time points) color. Protein expression levels are plotted on the diffusion map. **(E)** The dynamic expression profile of SARS-CoV-2-specific CD8+ T cells is visualized in a heatmap. Heatmap coloring represents % of virus-specific CD8+ T cells expressing a given marker; blue, low expression; red, high expression. **(F)** Dot plots showing pMHC1-tetramer stainings of B*44/N₃₂₂₋₃₃₀- and B*44/ORF1ab₃₉₄₆₋₃₉₅₄-specific CD8+ T cells after 14-days *in vitro* expansion at different time points post infection. Numbers refer to the respective percentage of pMHC1-tetramer+ cells among CD8+ T cells. Expansion index of virus-specific CD8+ T cells **(G)** and expression of IFN γ and TNF **(H)** in percentage relative to the frequency of epitope-specific CD8+ T cells after 14-days *in vitro* expansion at dpi and historic sample.

Table 1: SARS-CoV-2-derived peptides used for analyses of SARS-CoV-2-specific CD8+ T cell responses. Peptides in bold indicate epitopes confirmed in this study.

HLA class I restriction	Peptide Name	Protein	Aa start	Aa end	Sequence	IC ₅₀ [nM]	Comment	Responses in resolvers	Responses in controls
HLA-A*01:01	A*01/ORF1ab ₄₁₆₃₋₄₁₇₁	ORF1ab	4163	4171	CTDDNALAY	2,2		5/6	0/5
HLA-A*01:01	A*01/ORF1ab ₅₁₃₀₋₅₁₃₈	ORF1ab	5130	5138	DTDFVNEFY	2,8		5/6	0/5
HLA-A*01:01	A*01/ORF3a ₂₀₇₋₂₁₅	ORF3a	207	215	FTSDYQQLY	3,2		5/6	0/5
HLA-A*01:01	A*01/S ₈₆₅₋₈₇₃	S	865	873	LTDEMIQAQY	3,4		4/6	0/5
HLA-A*01:01	A*01/ORF1ab ₄₁₆₃₋₄₁₇₂	ORF1ab	4163	4172	CTDDNALAYY	5,3		3/6	0/5
HLA-A*02:01	A*02/S ₉₈₅₋₉₉₃	S	985	993	ALNTLVKQL	5,7	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/S ₉₇₆₋₉₈₄	S	976	984	VLNDILSRL	33,6	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/S ₉₉₆₋₁₀₀₄	S	996	1004	LITGRQLSK	5,7	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/S ₁₁₈₅₋₁₁₉₃	S	1185	1193	RLNEVAKLN	940,9	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/S ₁₁₉₂₋₁₂₂₀	S	1192	1200	NLNESLIDL	177,3	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/S ₁₂₂₀₋₁₂₂₈	S	1220	1228	FIAGLIAIV	10,3	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/M ₆₁₋₇₀	M	61	70	TLACFVLA AV	20,3	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/N ₁₃₈₋₁₄₆	N	138	146	ALNTPKDH I	6841,2	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/N ₁₅₉₋₁₆₇	N	159	167	LQLPQGTTL	3114,5	SARS-CoV-1*	0/11	0/9
HLA-A*02:01	A*02/N ₂₁₉₋₂₂₇	N	219	227	LALLLLDRL	4107,6	SARS-CoV-1*	2/11	0/9
HLA-A*02:01	A*02/N ₂₂₂₋₂₃₀	N	222	230	LLLDRLNLQ	14,8	SARS-CoV-1*	3/11	0/9
HLA-A*02:01	A*02/N ₃₁₆₋₃₂₄	N	316	324	GMSRIGMEV	50,6	SARS-CoV-1*	2/11	0/9
HLA-A*02:01	A*02/ORF1ab ₃₁₂₂₋₃₁₃₀	ORF1ab	3122	3130	FLAHIQWMV	2,5		0/11	0/9
HLA-A*02:01	A*02/ORF3a ₁₀₇₋₁₁₅	ORF3a	107	115	YLVALVYFL	2,7		9/11	1/09
HLA-A*02:01	A*02/ORF1ab ₃₆₃₉₋₃₆₄₇	ORF1ab	3639	3647	FLLPSLATV	2,8		1/11	0/9
HLA-A*02:01	A*02/ORF3a ₁₃₉₋₁₄₇	ORF3a	139	147	LLYDANYFL	3,1		11/11	0/9
HLA-A*02:01	A*02/ORF1ab ₂₃₃₂₋₂₃₄₀	ORF1ab	2332	2340	ILFTRFFYV	3,2		10/11	0/9
HLA-A*03:01	A*03/S ₄₅₄₋₄₆₂	S	454	462	RLFRKSNLK	5,9		0/7	0/5
HLA-A*03:01	A*03/ORF1ab ₂₃₈₂₋₂₃₉₁	ORF1ab	2382	2391	RMVFFASFY	6,1		1/7	0/5
HLA-A*03:01	A*03/ORF1ab ₅₅₃₃₋₅₅₄₂	ORF1ab	5533	5542	VVYRGTTTYK	7,2		3/7	0/5
HLA-A*03:01	A*03/ORF1ab ₅₉₇₈₋₅₉₈₆	ORF1ab	5978	5986	RLISMGGFK	8,7		0/7	0/5
HLA-A*03:01	A*03/ORF1ab ₁₈₅₁₋₁₈₆₀	ORF1ab	1851	1860	ALLTKSSEYK	8,8		0/7	0/5
HLA-A*11:01	A*11/ORF1ab ₂₆₀₀₋₂₆₀₈	ORF1ab	2600	2608	STFNVPM EK	4,3		3/4	0/5
HLA-A*11:01	A*11/ORF1ab ₃₆₂₂₋₃₆₃₀	ORF1ab	3622	3630	SAFAMMFVK	5,2		3/4	0/5
HLA-A*11:01	A*11/ORF1ab ₂₁₉₂₋₂₂₀₀	ORF1ab	2192	2200	ASMPPTIAK	5,3		3/4	1/5
HLA-A*11:01	A*11/ORF1ab ₇₀₃₈₋₇₀₄₇	ORF1ab	7038	7047	SSYSLFDMSK	5,8		0/4	0/5
HLA-A*11:01	A*11/ORF1ab ₅₇₉₂₋₅₈₀₁	ORF1ab	5792	5801	SAQCQKMFYK	5,8		0/4	0/5
HLA-A*24:02	A*24/ORF1ab ₂₁₆₇₋₂₁₇₅	ORF1ab	2167	2175	NYMPYFFTL	6,8		0/3	0/5
HLA-A*24:02	A*24/S ₁₂₁₁₋₁₂₂₀	S	1211	1220	KWPWYIWLGF	9,0		0/3	1/5
HLA-A*24:02	A*24/ORF1ab ₂₁₆₆₋₂₁₇₅	ORF1ab	2166	2175	TNYMPYFFTL	9,3		0/3	0/5
HLA-A*24:02	A*24/ORF1ab ₈₉₉₋₉₀₈	ORF1ab	899	908	EWSMATYYLF	10,5		0/3	0/5
HLA-A*24:02	A*24/M ₉₄₋₁₀₃	M	94	103	SYFIASFRLF	10,7		0/3	0/5
HLA-B*07:02	B*07/N ₆₆₋₇₄	N	66	74	FPRGGVPI	3,8		2/7	0/5
HLA-B*07:02	B*07/S ₆₈₀₋₆₈₈	S	680	688	SPRRARSVA	4,2		4/7	0/5
HLA-B*07:02	B*07/N ₂₅₇₋₂₆₅	N	257	265	KPRQKRTAT	4,4		3/7	0/5
HLA-B*07:02	B*07/ORF1ab ₅₉₁₆₋₅₉₂₄	ORF1ab	5916	5924	IPRRNVATL	5,1		3/7	0/5
HLA-B*07:02	B*07/N ₁₀₅₋₁₁₃	N	105	113	SPRWYFYFL	6,3		6/7	2/5
HLA-B*08:01	B*08/ORF1ab ₅₁₄₀₋₅₁₄₇	ORF1ab	5140	5147	YLRKHFSM	3,6		0/3	0/4
HLA-B*08:01	B*08/ORF1ab ₅₁₄₀₋₅₁₄₈	ORF1ab	5140	5148	YLRKHFSMM	3,6		0/3	0/4
HLA-B*08:01	B*08/ORF1ab ₃₀₆₄₋₃₀₇₁	ORF1ab	3064	3071	FMRFRRAF	4,8		0/3	0/4
HLA-B*08:01	B*08/ORF1ab ₅₂₄₆₋₅₂₅₄	ORF1ab	5246	5254	LMIERFVSL	5,2		0/3	0/4
HLA-B*08:01	B*08/ORF1ab ₅₂₄₅₋₅₂₅₄	ORF1ab	5245	5254	TLMIERFVSL	6,2		0/3	0/4
HLA-B*15:01	B*15/N ₃₀₅₋₃₁₄	N	305	314	AQFAPSASAF	3,6		3/5	0/5
HLA-B*15:01	B*15/ORF1ab ₂₇₇₁₋₂₇₈₀	ORF1	2771	2780	KQLIKVTLVF	3,9		1/5	0/5
HLA-B*15:01	B*15/ORF1ab ₃₁₂₉₋₃₁₃₈	ORF1ab	3129	3138	MVMFTPLVPF	4,3		0/5	0/5
HLA-B*15:01	B*15/ORF1ab ₂₃₇₁₋₂₃₈₀	ORF1ab	2371	2380	LVQMAPISAM	4,4		2/5	0/5
HLA-B*15:01	B*15/ORF1ab ₃₆₀₅₋₃₆₁₄	ORF1ab	3605	3614	FLYENAFLPF	5,0		0/5	0/5
HLA-B*40:01	B*40/N ₃₂₂₋₃₃₁	N	322	331	MEVTPSGTWL	28,6	SARS-CoV-1*	2/4	0/4
HLA-B*40:01	B*40/ORF1ab ₁₅₄₈₋₁₅₅₆	ORF1ab	1548	1556	GEVITFDNL	4,4		2/4	1/4
HLA-B*40:01	B*40/ORF1ab ₂₃₂₅₋₂₃₃₃	ORF1ab	2325	2333	AEWFLAYIL	4,9		0/4	1/4
HLA-B*40:01	B*40/ORF1ab ₁₇₀₅₋₁₇₁₃	ORF1ab	1705	1713	GEAANFCAL	4,9		2/4	1/4
HLA-B*40:01	B*40/ORF1ab ₂₀₆₉₋₂₀₇₇	ORF1ab	2069	2077	TEVVGDIIIL	5,8		1/4	1/4
HLA-B*40:01	B*40/ORF1ab ₇₄₄₋₇₅₂	ORF1ab	744	752	GETLPTEVL	7,1		1/4	0/4
HLA-B*44:02/03	B*44/ORF1ab ₁₃₆₅₋₁₃₇₃	orf1ab	1365	1373	QEILGTVSW	5.8/20.2		2/6	0/8
HLA-B*44:02/03	B*44/ORF1ab ₂₃₂₅₋₂₃₃₄	orf1ab	2325	2334	AEWFLAYILF	6.5/14.2		0/6	0/8
HLA-B*44:02/03	B*44/N ₃₂₂₋₃₃₀	N	322	330	MEVTPSGTW	11.5/40.5		5/6	0/8
HLA-B*44:02/03	B*44/ORF1ab ₆₀₀₂₋₆₀₁₁	ORF1ab	6002	6011	EEAIRHVRW	12.3/28.7		1/8	0/8
HLA-B*44:02/03	B*44/S ₁₂₀₆₋₁₂₁₄	S	1206	1214	YEYIKHPWP	12.9/38.8		1/8	0/8
HLA-B*44:02/03	B*44/ORF1ab ₅₂₆₇₋₅₂₇₆	ORF1ab	5267	5276	QEYADVHFLY	13.4/10.7		1/8	0/8
HLA-B*44:02/03	B*44/ORF1ab ₃₉₄₆₋₃₉₅₄	ORF1ab	3946	3954	SEFSSLPYSY	18.3/13.0		3/8	0/8
HLA-B*44:02/03	B*44/ORF1ab ₄₇₁₋₄₈₀	ORF1ab	471	480	EEAIIILASF	17.3/19.7		1/8	0/8

*prescribed in SARS-CoV-1 infection and 100% homology between SARS-CoV-1 and 2