Rapid and stable mobilization of fully functional spike-specific CD8⁺ T cells preceding a mature humoral response after SARS-CoV-2 mRNA vaccination

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Rapid and stable mobilization of fully functional spike-specific CD8+ T cells preceding a mature humoral response after SARS-CoV-2 mRNA vaccination

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Abstract

SARS-CoV-2 spike mRNA vaccines¹⁻³ mediate protection from severe disease as early as 10 days post prime vaccination³, when specific antibodies are hardly detectable and still lack neutralizing activity⁴⁻⁶. Vaccine-induced T cells, especially CD8⁺ T cells, may thus be the main mediators of protection at this early stage^{7,8}. The details of antigen-specific CD8⁺ T cell induction after prime/boost vaccination, their comparison to naturally induced CD8⁺ T cell responses and their association with other arms of vaccine-induced adaptive immunity remain, however, incompletely understood. Here, we show on a single epitope level that both, a stable memory precursor pool of spike-specific CD8⁺ T cells and fully functional spike-specific effector CD8⁺ T cell populations, are vigorously mobilized as early as one week after prime vaccination when CD4⁺ T cell and spike-specific antibody responses are still weak and neutralizing antibodies are lacking. Boost vaccination after 3 weeks induced a full-fledged recall expansion generating highly differentiated CD8⁺ effector T cells, however, neither the functional capacity nor the memory precursor T cell pool was affected. Compared to natural infection, vaccine-induced early memory T cells exhibited similar frequencies and functional capacities but a different subset distribution dominated by effector memory T cells at the expense of self-renewing and multipotent central memory T cells. Our results indicate that spike-specific CD8⁺ T cells may represent the major correlate of early protection after SARS-CoV-2 mRNA/bnt162b2 prime vaccination that precede other effector arms of vaccine-induced adaptive immunity and are stably maintained after boost vaccination.

Introduction

The SARS-CoV-2 vaccines by Biontech/Pfizer (bnt162b2) and Moderna (mRNA-1273) use mRNA-based technology to express stabilized SARS-CoV-2 spike protein¹⁻³. Prime vaccination is followed by a boost 3-6 weeks later. Little is known about the precise nature of the immune response elicited by this formulation that has been licensed for the first time. The current vaccination campaign thus provides the unique opportunity to gain important insights into human CD8⁺ T cell biology in the context of prime/boost mRNA vaccination.

First data revealed that all arms of adaptive immunity such as neutralizing antibodies, virus-specific CD4⁺ T cells with TH1 polarization and IFN- γ -producing CD8⁺ T cells emerge after prime/boost vaccination^{4,5,9}. However, early kinetics, differentiation trajectories, functional dynamics and differences to natural infection have not been addressed by these initial studies. Strikingly, the onset of mRNA vaccine-mediated protection has been observed as early as 10-12 days after the first dose³. During this early phase, T cells and spike-specific antibodies are detectable^{7,8} while neutralizing antibodies first appear after boost^{4-6,10,11}. These observations point towards a key role of vaccine-induced T cells in early protection after prime vaccination.

Previous studies focused on the analysis of the overall vaccine-elicited spike-reactive T cell responses,^{4,5,7,8,12} however, by this approach, the strength, dynamics and functional capacity of spike-specific CD8⁺ T cell responses are underestimated or even blurred in contrast to analyses performed on a single epitope level⁵. In this study, we performed continuous longitudinal analyses starting at baseline of prime vaccination until 3 months after boost on a single epitope level, to track the trajectories of bnt162b2 vaccine-elicited spike-specific CD8⁺ T cell responses. Additionally, we compared the kinetics and frequencies of the spike-specific CD8⁺ T cell response with spike-specific CD4⁺ T cells, B cells and antibodies and their neutralizing activity.

Results

Bnt162b2 vaccine-elicited epitope-specific CD8⁺ T cells: early induction, vigorous Teff response and robust establishment of memory precursors

To assess the dynamics of the spike-specific CD8⁺ T cell response following bnt162b2 vaccination, we longitudinally collected PBMCs and sera in a 3 to 4-day interval from 17 health care workers (Supplementary Table 1) that have not been previously infected with SARS-CoV-2, starting before prime until d84-86 after boost (Fig. 1A). We analyzed the induction of spike-specific CD8⁺ T cells targeting A*01/S₈₆₅ and A*02/S₂₆₉ epitopes in 5 individuals each (Extended Data Fig.1). Both epitopes are not highly conserved between SARS-CoV-2 and SARS-CoV-1, MERS or the common cold coronaviruses (229E, OC43, HKU1, NL63) (Extended Data Fig. 2A). Thus the detected spike-specific CD8⁺ T cells indeed reflect a response to bnt162b2 vaccination. It is important to note that the epitopes are not affected by the sequence variations present in the variants of concern (VOC) B.1.1.7, B.1.351, P.1 and B.1.617 (Extended Data Fig. 2A). *Ex vivo* frequencies of A*01/S₈₆₅ and A*02/S₂₆₉-specific CD8⁺ T cells were rather low after vaccination (Extended Data Fig. 2B). In order to increase the detection rate and to allow subsequent comprehensive profiling we performed pMHC-I-tetramer enrichment of A*01/S₈₆₅ and A*02/S₂₆₉-specific CD8⁺ T cells (Fig. 1B). We detected a rapid and substantial induction of spike epitope-specific CD8⁺ T cells in all tested donors at d6-8 that peaked 9-12 days post prime (dpp) (Fig. 1C). The strong activation of spike epitope-specific CD8⁺ T cells was also reflected by high expression of the activation marker CD38 and the proliferation marker Ki-67 as early as d6-8 in the majority of cells (Fig. 1D/E and Extended Data Fig. 2C). Boost vaccination led to a further increase of spike epitope-specific CD8⁺ T cell frequencies that peaked 5-6 days post boost (dpb) with a subsequent slow contraction phase reaching nearly pre-boost frequencies at about d84-86 post boost (Fig. 1C). Post-boost and post-prime expansion were

accompanied by effector T (Teff) cell differentiation (high Ki-67, CD38, granzyme B, PD-1, CD39, T-BET and TOX expression (Fig. 1D and Extended Data Fig. 2D/E). However, t-SNE analysis of the concatenated expression analysis revealed that spike epitope-specific CD8⁺ Teff cells are qualitatively different at the peak expansion following boost (obtained at 5-6 dpb) compared to prime (obtained at 9-12 dpp) with a more consolidated cytotoxic effector cell phenotype (increased T-BET, TOX, CD39 expressions) post boost (Extended Data Fig. 3A). This consolidated post-boost Teff response is further supported by diffusion map analysis of the combined flow cytometry data of vaccine-elicited spike epitope-specific CD8⁺ T cells (Fig. 1E and Extended Data Fig. 3B/C). Specifically, diffusion map embedding revealed a continuous relationship of the longitudinally collected spike-specific CD8⁺ T cells following prime (depicted in reddish colors)/boost (depicted in grey shaded colors) indicating a directed trajectory of the Teff response from prime to boost vaccination (Fig. 1E and Extended Data Fig. 3C). Along the indicated trajectory, spike-specific CD8⁺ T cells exhibited highest expression of PD-1, TOX, T-BET and CD38 after boost that remained at high levels, indicating profound activation and progressing differentiation post-boost (Fig. 1E).

Next to the emergence of an effective Teff response after bnt162b2 vaccination, we also assessed the induction of a spike epitope-specific memory precursor CD8⁺ T cell (TMP) pool. TMP cells are characterized by CD127, BCL-2 and TCF-1 expression and are relevant for maintaining the CD8⁺ T cell response^{13,14}. Roughly 30% of A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells expressed CD127 after prime followed by a transient reduction and subsequent strong increase after boost (Fig.1F and Extended Data Fig. 4A). Expression dynamics of TCF-1 (Fig. 4G and Extended Data Fig. 4B) and BCL-2 (Extended Data Fig. 4C) were similar to CD127. However, the overall frequency of CD127⁺ (Fig.1F) and TCF-1⁺ (Fig.1G) spike epitope-specific CD8⁺ T

cells remained constant indicating a stable memory precursor pool of spike-specific CD8⁺ T cells induced already early after prime and being unaffected by boost vaccination. Taken together, bnt162b2 vaccination vigorously induces a lasting spike-specific CD8⁺ T cell response rapidly after prime.

Proper and conserved functional capacities of circulating spike-specific CD8⁺ T cells following prime and boost vaccination

To assess spike-specific CD8⁺ T cell function after prime/boost vaccination, we analyzed expansion capacity, cytokine production and degranulation after peptide-specific expansion (Fig. 2A/B). After two weeks of *in vitro* expansion, we detected higher frequencies of A*01/S₈₆₅ and A*02/S₂₆₉-specific CD8⁺ T cells after boost compared to prime vaccination (Fig. 2C). However, the expansion index, a measure taking the input number of virus-specific CD8⁺ T cells into account was comparable for spike epitope-specific CD8⁺ T cells after prime and boost vaccination (Fig. 2D). Thus, the increased frequencies of spike epitope-specific CD8⁺ T cells after peptide-specific expansion most probably result from the increased *ex vivo* frequencies after boost. We also assessed A*01/S₈₆₅- and A*02/S₂₆₉-specific IFN- γ and TNF production (Fig. 2E-G) and degranulation (indicated by CD107a expression; Fig. 2H) in relation to the frequency of spike epitope-specific CD8⁺ T cells after expansion as a measure of the effector function per cell. We observed proper effector capacity of circulating spike epitope-specific CD8⁺ T cells obtained as early as 6-8 dpp. Similar to the expansion capacity, cytokine production and degranulation capacity remained nearly stable after boost compared to prime. Hence, functionally competent spike-specific CD8⁺ T cells targeting different epitopes are substantially induced early after prime, and subsequent boost vaccination does not further increase their functional capacities.

Delayed appearance of circulating spike-specific CD4+ T cells, B cells and antibodies

Next, we compared kinetics and frequencies of spike-specific CD8+ T cells with spike epitope-specific CD4+ T cells, B cells and antibodies with neutralizing activity. For this, we first longitudinally assessed circulating spike-specific CD4+ T cells targeting DRB1*15:01/S₂₃₆ (Extended Data Fig. 5A) following prime and boost vaccination in 8 individuals (Supplementary Table 1). The selected DRB1*15:01/S₂₃₆ epitope is rather unique for SARS-CoV-2 in comparison to SARS-CoV-1, MERS or common cold coronaviruses (229E, OC43, HKU1, NL63) and conserved in circulating SARS-CoV-2 variants (B.1, B.1.1.7, P.1, B.1.617) except for VOC B.1.351 (Extended Data Fig. 5B). As expected for virus-specific CD4 T cells, the frequencies of DRB1*15:01/S₂₃₆-specific CD4+ T cells were lower compared to CD8 responses but detectable after pMHCII tetramer-based enrichment (Fig. 3A). At baseline (Fig. 3A) and in historic control (HC) samples (banked before August 2019) (Extended Data Fig. 5C), spike-specific CD4+ T cells were detectable with a primarily naïve phenotype (Extended Data Fig. 5C and D, respectively) reflecting the presence of antigen-unexperienced precursors. Following vaccination, the proportion of circulating spike epitope-specific CD4+ T cells with a naïve phenotype decreased suggesting vaccine-induced activation of the precursor cells (Extended Data Fig. 5D). However, compared to the vaccine-elicited CD8+ T cell response, we observed a lower mobilization of circulating spike epitope-specific CD4+ T cells indicated by a limited increase of frequencies (Fig. 3A) and a smaller percentage of activated ICOS+CD38++ or Ki-67+ subsets (Fig. 3B and Extended Data Fig. 5E) even after boost vaccination. The majority of activated (ICOS+CD38++ or Ki-67+) circulating DRB1*15:01/S₂₃₆-specific CD4+ T cells exhibited a TH1 phenotype (Fig. 3C). In line with this observation, vaccine-induced spike epitope-specific CD4+ T cell response displayed a TH1 rather than a TFH phenotype (Extended Data Fig. 5F).

Next, we assessed the kinetics of the vaccine-induced humoral response. The distribution of peripheral B cell subpopulations was stable throughout prime/boost vaccination, with the exception of a progressively slight increase in antibody-secreting cells (Extended Data Fig. 6A/B). An increase in the frequency of CD95⁺ B cells was observed shortly after boost indicating ongoing B cell activation via CD40-mediated T cell help and/or B cell receptor activation within secondary lymphoid organs (Extended Data Fig. 6B)¹⁵. In line with the appearance of activated B cells in the periphery, we observed a progressive maturation of the serum antibody response with S1-specific IgM present after prime whereas S1-specific IgG reasonably detectable after boost (Fig. 3D and Extended Data Fig. 6C) coinciding with a high neutralization capacity in SARS-CoV-2 plaque reduction assays (Fig. 3E). More precisely, SARS-CoV-2 B.1, circulating in early 2020, and SARS-CoV-2 VOC B.1.1.7, emerging in the UK in late 2020, were equally well neutralized by post-boost sera whereas the neutralization activity against SARS-CoV-2 VOC B.1.351, a variant emerging in Brazil in late 2020, was reduced approximately by a factor of 5 (Fig. 3E). Neutralization capacity of post-boost sera was clearly elevated compared to time point-matched mild infection (Extended Data Fig. 6D). To test the frequency of spike-specific B cells we applied S1 and receptor-binding domain (RBD) tetramers *ex vivo* (Fig. 3F). In line with the progressive maturation of the antibody response, S1- and RBD-specific B cells largely remained below the detection limit until the first week post boost vaccination (Fig. 3G). The delayed appearance of circulating S1-specific B cells was confirmed by polyclonal restimulation *in vitro* (Fig. 3H) showing a limited presence of class-switched B cells capable to produce S1-specific IgG before boost. S1-specific B cells were largely unswitched after prime (Fig. 3I), also reflected by S1-specific IgM production upon polyclonal restimulation *in vitro* (Extended Data Fig. 6C), and acquired a memory phenotype after boost vaccination (Fig. 3I and Extended Data Fig. 6E). In addition,

after boost vaccination, S1-specific B cells showed increased transferrin receptor (CD71) and CD95 expression (Fig 3I and Extended Data Fig. 6E) indicating their germinal center origin¹⁶. Hence, bnt162b2 vaccination efficiently elicits a protective humoral immune response, composed of both antibody secreting cells and antigen-specific memory cells that are mobilized to the periphery after boost.

Differences of circulating spike-specific CD8+ early memory CD8+ T cells after boost vaccination and natural infection

In a last set of experiments, we compared vaccine-elicited A*01/S₈₆₅ and A*02/S₂₆₉-specific early memory CD8+ T cells with time point-matched T cells induced by natural infection. Spike epitope-specific CD8+ T cell frequencies and CD127 expression, a hallmark of lasting T cells, were similar at all time points analyzed, with a slight tendency towards lower frequency of A*02/S₂₆₉-specific CD8+ T cells after natural infection (Fig. 4A and Extended Data Fig. 7A). We also observed differences in T cell memory subset distribution (Extended Data Fig. 7B) of spike-specific early memory CD8+ T cells with higher fractions of more early differentiated subsets, e.g. early differentiated (TED) and central memory T cells (TCM) after natural infection. In contrast, more effector memory 1 T cells (TEM1) were detectable after vaccination (Fig. 4B). Of note, spike-specific effector memory 2 and 3 T cells (TEM2, TEM3) and terminally differentiated effector memory T cells expressing CD45RA (TEMRA) were hardly detectable in the circulation neither after vaccination nor after natural infection (Extended Data Fig. 7C). In addition, t-SNE analysis of concatenated expression data (including differentiation, activation, anti-apoptotic marker molecules) further supports qualitative differences of spike-specific CD8+ T cells obtained from the early memory phase (>80 dpb/dps) after vaccination compared to natural infection. Indeed, we observed a higher expression of CCR7, BCL-2 and TCF-1 after natural infection, (Fig.

249 4C and Extended Data Fig. 8A) suggesting a higher capacity of homeostatic
250 maintenance of the spike-specific early memory CD8⁺ T cell population. Both, t-SNE
251 analysis and manual gating demonstrate a higher and prolonged CD38 expression on
252 spike epitope-specific CD8⁺ T cells after natural infection possibly due to different
253 dynamics of antigen recognition (Fig. 4C and Extended Data Fig. 8B). However,
254 vaccine- and natural infection-associated expansion capacity and cytokine production
255 of spike-specific CD8⁺ T cells are similar (Fig. 4D and Extended Data Fig. 8C). Hence,
256 compared to natural infection, vaccine-associated spike-specific early memory CD8⁺
257 T cell populations exhibit similar functional capacities but a different subset distribution
258 dominated by effector memory T cells at the expense of self-renewing and multipotent,
259 early differentiated subsets.

Discussion

Our comprehensive analysis of vaccine induced immunity revealed a rapid and strong induction of CD8+ T cells targeting two spike epitopes as early as one week after prime vaccination in all vaccinees at a time point when neutralizing antibodies are still lacking. Importantly, these immediately mobilized spike-specific CD8+ T cells are fully functional and able to mount a proficient recall response evoked e.g. by the boost vaccination. These findings clearly suggest a major protective role of vaccine-elicited CD8+ T cells early after first dose vaccination. In agreement with this notion, the protective effect observed for mRNA vaccines starts at 10-12 dpp^{2,3} and thus coincides with the first peak of spike-specific CD8+ T cells. A major protective capacity of virus-specific CD8 T cells was also observed in natural SARS-CoV-2 infection, since e.g. CD8+ T cells compensate for impaired humoral immunity in COVID-19 patients with hematologic cancer¹⁷.

The approved SARS-CoV-2 mRNA vaccine design includes a boost after 3-6 weeks^{2,3}. Our data demonstrate that the boost after 3 weeks hits the spike-specific CD8+ T cells at the beginning of the contraction phase of the primary response and results in a robust recall expansion of spike-specific CD8+ T cells. The generation of a fortified Teff response, however, neither leads to an improved functional capacity nor to an increased frequency of memory precursor T cells relevant for maintaining the CD8+ T cell response. These observations may indicate that a robust spike-specific CD8+ T cell response is already elicited after prime with only transient effects of boosting after 3 weeks.

In contrast to CD8+ T cells, efficient mobilization of a humoral immune response comprising neutralizing antibodies and antigen-specific memory B cells to the periphery was first detectable after boost. This is in line with previous reports^{4,11,12,18} and most probably represents maturation of the response that is initially localized in

secondary lymphoid organs (SLO)¹⁹ and subsequently released to the circulation. However, how the boost affects this SLO response requires further investigation. After boost, highly cross-neutralizing antibodies are present in the sera clearly adding a major correlate of protection on top of the early-mobilized spike-specific CD8+ T cell response.

A recent study has highlighted the important role of spike-reactive CD4+ T cells in coordinating the humoral and CD8+ T cell response⁸. Similar to CD8+ T cells, spike-reactive CD4+ T cells are also mobilized early after prime^{8,18}. We also observed an early but moderate appearance of spike-specific CD4+ T cells with a predominance of a TH1 over a TFH phenotype in the periphery. While a dominant TH1 polarization has also been reported by others⁴, spike-reactive TFH cells may also be crucial for coordinating humoral and cellular immune responses upon mRNA vaccination⁸. Further studies need to clarify whether the parallel TH1- and TFH-directed coordination is restricted by different CD4+ T cell epitopes or clonotypes, by pre-existing cross-reactive CD4+ T cells or by CD4+ T cell localization (periphery versus SLO). Unlike spike-specific CD8+ T cells, only a limited recall expansion was detectable for circulating spike-specific CD4+ T cells, supporting their coordinating role after mRNA vaccination.

Hallmark of an efficient vaccine is the induction of lasting immune memory. In parallel to the early appearance of circulating spike-specific CD8+ T cells, a stable memory precursor pool is established that develops after boost into a fully functional early memory CD8+ T cell response. Of note, the functional capacity of spike-specific early memory CD8+ T cells is similar after vaccination and natural infection up to three months post boost/symptom onset. Compared to natural infection, however, the early memory pool of spike-specific CD8+ T cells after vaccination contains a higher proportion of effector memory 1 (TEM1) T cells at the expense of central memory

(TCM) and early differentiated (TED) T cells. Of note, the latter maintain long-term memory e.g. after yellow fever virus (YFV) vaccination²⁰. In line with this, expression of CCR7, BCL-2 and TCF-1 is lower after vaccination compared to infection. These observations may hint towards a restricted self-renewal and maintenance of spike-specific CD8⁺ T cells after vaccination compared to infection. This difference may be caused by differential duration and location of antigen contact after vaccination versus infection^{21,22}. Indeed, we observed a lower CD38 expression on early memory spike-specific CD8⁺ T cells after vaccination possibly indicating limited antigen recognition compared to natural infection^{4,23}. So far, we have followed our cohort for only 3 months after boost; follow-up studies including larger cohorts of vaccinees and SARS-CoV-2 convalescent donors should comparatively assess longevity of CD8⁺ T cell immunity. In addition, our study was limited to circulating spike-specific adaptive immunity, not addressing local immunity at the viral entry site, the respiratory tract. In sum, our data clearly highlight that a robust, stable and fully functional spike-specific CD8⁺ T cell response is rapidly mobilized already after prime vaccination and is able to patrol the periphery for SARS-CoV-2 at least within the first months. These observations do not only provide insights into the protective mechanisms underlying bnt162b2 vaccination, but are also of potential relevance for the development of novel vaccination strategies against emerging pathogens and cancer.

Methods

Study cohort

17 health care workers that received a prime and boost vaccination with the mRNA vaccine bnt162b2/Comirnaty, 24 convalescent individuals following a mild course of SARS-CoV-2 infection and historic controls (sampled before August 2019) of 8 healthy individuals were recruited at the Freiburg University Medical Center, Germany. A mild course of infection was characterized by clinical symptoms without respiratory insufficiency. SARS-CoV-2 infection was confirmed by positive PCR testing from oropharyngeal swab and/or SARS-CoV-2 spike IgG positive antibody testing. Donor characteristics are summarized in Supplementary Table 1. HLA-typing was performed by next-generation sequencing and is listed in Supplementary Table 1.

Ethics

Written informed consent was obtained from all participants and the study was conducted according to federal guidelines, local ethics committee regulations (Albert-Ludwigs-Universität, Freiburg, Germany; vote #: 322/20, #21-1135 and 315/20) and the Declaration of Helsinki (1975).

PBMC isolation

Venous blood samples were collected in EDTA-anticoagulated tubes. Peripheral blood mononuclear cells (PBMCs) were isolated with lymphocyte separation medium density gradients (Pancoll separation medium, PAN Biotech GmbH; Aidenbach, Germany) and stored at -80°C. Frozen PBMCs were thawed in complete medium (RPMI 1640 supplemented with 10% fetal calf serum, 1% penicillin/streptomycin, and 1.5% HEPES buffer 1 mol/L (all additives from Thermo Scientific (Waltham, MA)) containing 50 U/mL benzonase (Sigma, Germany).

Sequence alignment

Sequence homology analyses were performed in Geneious Prime 2020.0.3 (<https://www.geneious.com/>) using Clustal Omega 1.2.2 alignment with default settings²⁴. Reference genomes of human coronaviruses 229E (NC_002645), HKU1 (NC_006577), NL63 (NC_005831), OC43 (NC_006213), MERS (NC_019843), SARS-CoV-1 (NC_004718) and SARS-CoV-2 (MN908947.3) were downloaded from NCBI database. Spike proteins of human coronaviruses were aligned according to their homology (amino acid level). Analyzed Spike SARS-CoV-2 epitopes were then mapped to the corresponding protein alignment. Correspondingly mutation analyses were performed with the spike protein of variants of concern (VOC) B.1.1.7, B.1.351, P.1 and B.1.617.

Peptides and tetramers for T cell analysis

Peptides were synthesized with an unmodified N-terminus and an amidated C-terminus with standard Fmoc chemistry and a purity of >70% (Genaxxon Bioscience, Germany). Peptide was loaded on HLA class I easYmers® (immunAware, Denmark) according to manufacturer's instructions (A*01/ S₈₆₅ LTDEMIQY and A*02/ S₂₆₉ YLQPRTFLL). SARS-CoV-2 peptide-loaded HLA class I tetramers were produced by conjugation of biotinylated peptide-loaded HLA class I easYmers® with phycoerythrin (PE)-conjugated streptavidin (Agilent, USA) according to the manufacturer's instructions. A SARS-CoV-2-specific HLA class II custom tetramer (DRB1*15:01/S₂₃₆ TRFQTLALHRSYLT) was obtained from (MBL, USA).

In vitro expansion of spike-specific CD8⁺ T cells and assessment of effector function

1.5*10⁶ PBMCs were stimulated with A*01/ S₈₆₅ or A*02/ S₂₆₉ spike-specific peptides (5 µM) and anti-CD28 mAb (0.5 µg/mL, BD) and expanded for 14 days in complete

RPMI culture medium containing rIL2 (20 IU/mL, Stemcell Technologies). Intracellular cytokine production and degranulation was assessed with spike-specific peptides (15 μ M) in the presence of anti-CD107a (H4A3, 1:100) (BD Bioscience, Germany) for 1 h at 37 °C. Afterwards, brefeldin A (GolgiPlug, 0.5 μ L/mL) and monensin (GolgiStop, 0.5 μ L/mL) (all BD Biosciences, Germany) were added for additional 5 h, followed by surface and intracellular staining. The expansion capacity was calculated based on peptide-loaded HLA class I tetramer staining as described before ²⁵.

Magnetic bead-based enrichment of spike-specific CD8+ T cells

Spike-specific CD8+ T cells were enriched as previously described ²⁶. Briefly, 1-2*10⁷ PBMCs (with an average of 15.7% CD8+ T cells) were labelled with PE-coupled peptide-loaded HLA class I tetramers for 30 min. Enrichment was then performed using anti-PE beads with MACS technology (Miltenyi Biotec, Germany) according to the manufacturer's instructions. Subsequently, enriched spike-specific CD8+ T cells were analyzed by multiparametric flow cytometry and frequencies of spike-specific CD8+ T cells were calculated as described before ²⁶. Only enriched samples with ≥ 5 spike-specific CD8 T cells were included in further analyses, resulting in a detection limit of 5*10⁻⁶.

Magnetic bead-based enrichment of spike-specific CD4+ T cells

Enrichment of spike-specific CD4+ T cells was adapted from the method described previously ²⁶. In brief, 1.5-2*10⁷ PBMCs of DRB1*15:01-positive donors were labelled with PE-coupled peptide-loaded MHC class II tetramers for 40 min. 5 μ l were taken from 1000 μ l pre-enriched sample (1:200) and used for subsequent flow cytometric staining. Subsequent enrichment was performed with anti-PE beads using MACS technology (Miltenyi Biotec, Germany) according to the manufacturer's protocol.

Enriched spike-specific CD4⁺ T cells and the pre-enriched sample were used for flow cytometric staining. The complete pre-enriched and enriched samples were recorded. Only enriched samples with ≥ 5 spike-specific CD4⁺ T cells were included in further analyses. The frequency of spike-specific CD4⁺ T cells was calculated as follows: Absolute number of spike-specific CD4⁺ T cells (enriched sample) divided by the absolute number of CD4⁺ T cells (pre-enriched sample) x 200. The detection limit as a frequency was calculated as follows: 5 spike-specific CD4⁺ T cells (enriched sample) divided by the mean number of CD4⁺ T cells (pre-enriched sample) throughout all tested donors x 200.

Multiparametric flow cytometry for T cell analysis

The following antibodies were used for multiparametric flow cytometry: anti-CCR7-PE-CF594 (150503, 1:50), anti-CCR7-BUV395 (3D12, 1:25), anti-CD4-BV786 (L200, 1:200), anti-CD8-BUV395 (RPA-T8, 1:400), anti-CD8-BUV510 (SK1, 1:100), anti-CD8-APC (SK-1, 1:200), anti-CD11a-BV510 (HI111, 1:25), anti-CD28-BV421 (CD28.2, 1:100), anti-CD38-APC-R700 (HIT2, 1:400), anti-CD38-BUV737 (HB7, 1:200), anti-CD39-BV650 (TU66, 33:1), anti-CD45RA-BUV496 (HI100, 1:800), anti-CD45RA-BUV737 (HI100, 1:200), anti-CD69-BUV395 (FN50, 1:50), anti-CD107a-APC (H4A3, 1:100), anti-CD127-BUV737 (HIL-7R-M21, 1:50), anti-CD127-BV421 (HIL-7R-M21, 3:100), anti-EOMES-PerCP-eF710 (WD1928, 1:50), anti-Granzyme B-PE-CF594 (GB11, 1:100), anti-ICOS-BV711 (DX29, 1:100), anti-IFN- γ -FITC (25723.11, 1:8), anti-IL-21-PE (3A3-N2.1, 1:25), anti-PD-1-BV605 (EH12.1, 1:50), anti-PD-1-PE-Cy7 (EH12.2H7, 1:200), anti-PD-1-BV786 (EH12.1, 1013122, 3:100), anti-T-BET-PE-CF594 (O4-46,93533305, 3:100), anti-TNF-PE-Cy7 (Mab11, 1:400) (BD Biosciences, Germany), anti-BCL-2-BV421 (100, 1:200), anti-CCR7-BV785 (G043H7, 1:50), anti-CD4-AlexaFluor700 (RPA-T4, 300526, 1:200), anti-CD25-BV650 (BC96, 1:33), anti-

CD57-BV605 (QA17A04, 1:100), anti-CD127-BV605 (A019D5, 3:100), anti-CXCR3-PerCP-Cy5.5 (G025H7, 1:33), anti-CXCR3-BV510 (G025H7, 3:100), anti-CXCR5-BV421 (J252D4, 1:100), anti-IL-2-PerCP-Cy5.5 (MQ1-17H12, 1:100), anti-Ki-67-BV711 (Ki-67, 1:200), anti-Ki-67-PE-Cy7 (Ki-67, 1:200) (BioLegend, UK), anti-TCF-1-AlexaFluor488 (C63D9, 1:100) (Cell Signaling, Germany), anti-CD14-APC-eFluor780 (61D3, 1:400), anti-CD19-APC-eFluor780 (HIB19, 1:400), anti-CD27-FITC (0323, 1:100), anti-KLRG1-BV711 (13F12F2, 1:50), anti-T-BET-PE-Cy7 (4B10, 1:200), anti-TOX-eFluor660 (TRX10, 1:100) (Thermo Fisher, Germany), anti-CD45RA-PerCP-Cy5.5 (HI100, 3:100) (Invitrogen, Germany). For live/dead discrimination a fixable Viability Dye (APC-eFluor780 1:200, 1:400) (Thermo Fisher, Germany) or ViaProbe (7-AAD, 1:33) (BD Biosciences, Germany)) was used. FoxP3/Transcription Factor Staining Buffer Set (Thermo Fisher, Germany) and Fixation/Permeabilization Solution Kit (BD Biosciences, Germany) were used according to the manufacturer's protocol to stain for intranuclear and cytoplasmic molecules, respectively. After fixation of cells in 2 % paraformaldehyde (PFA, Sigma, Germany), analyses were performed on FACSCanto II, LSRFortessa (BD, Germany) or CytoFLEX (Beckman Coulter). Data were analyzed with FlowJo 10 (Treestar, USA).

Dimensional reduction of multiparametric flow cytometry data

Dimensionality reduction of multiparametric flow cytometry data was done with R version 4.0.2 using the Bioconductor (release (3.11)) CATALYST package²³. The analyses were performed on gated virus-specific CD8⁺ T cells including the markers CD69, CD45RA, BCL-2, PD1, CD25, Ki-67, TCF-1, Eomes, CCR7, Tbet, TOX, CD38. For Figure 4 t-SNE analysis the markers CD69, Eomes and Ki-67 were excluded. Downsampling of cells to 100 or 200 cells (t-SNE Figure 4 and Extended data 3, diffusion maps Figure 1 and Extended data 3) was performed before dimensionality

reduction to facilitate the visualization of different samples. Marker intensities were transformed by arcsinh (inverse hyperbolic sine) with a cofactor of 150. Dimensionality reduction on the transformed data was achieved by t-SNE and diffusion map visualization.

S1- and RBD-tetramerization for B cell analysis

A biotinylated form of recombinant S1 and RBD proteins (BioLegend, UK) were tetramerized by addition of PE-conjugated or BV421-conjugated streptavidin (BioLegend, UK) and used for B cell tetramer staining assays. Briefly, the Streptavidin-PE or Streptavidin-BV21 were added in an amount that equals 1/5 of the monomer substrate amount. The Streptavidin was added in 5 equal portions to the monomer and incubated each time at 4°C for 20 min on a shaker. The tetramers were filled up to 100 µl with 0.1% BSA in PBS and stored at 4°C.

Multiparametric flow cytometry for B cell analysis

Phenotype of vaccinated subjects' PBMCs was determined by flow cytometry (Cytek Aurora, Cytek) with the following antibodies: anti-CD20-BV510 (2H7, 1:80), anti-IgM-BV605 (MHM-88, 1:200), anti-CD71-FITC (CY1G4, 1:1000), anti-CD95-PE-Dazzle594 (DX2, 1:50), anti-CD24-FITC (ML5, 1:100), anti-CD38-PE-Cy7 (HB-7, 1:300), anti-BAFF-R-AF647 (11C1, 1:100), anti-CD19-APC-Cy7 (HIB19, 1:150) (BioLegend, UK); anti-IgG-BV650 (G18-145, 1:600), anti-CD27-BV786 (L128, 1:100), anti-CD69-BV480 (FN50, 1:200) (BD Biosciences, Germany); anti-IgA-PerCP (polyclonal, 1:200) (Jackson ImmunoResearch, UK); anti-CD3-Super Bright 436 (OKT3, 1:200), anti-CD33-Super Bright 436 (WM-53, 1:50), anti-IgD-PerCP-eFluor 710 (IA6-2, 1:200) (Invitrogen, Germany). Dead cell exclusion was performed by Zombie NIR Fixable Viability Kit (Biolegend, UK, 1:800).

487 *In vitro PBMCs activation and ELISA*

488 PBMCs of vaccinated subjects and patients with a history of SARS-CoV-2 infection
489 were plated at 0.5×10^6 cells/ml and polyclonally stimulated for 9 days with thiol-modified
490 CpG (0.25 μ M, TCGTCGTTTTGTCGTTTTGTCGTT) and hIL-2 (100 ng/ml,
491 Immunotools). At day 9, the supernatants of the *in vitro* culture were cleared from
492 debris by centrifugation and used to determine the presence of SARS-CoV-2 Spike-
493 specific IgG antibodies (Anti-SARS-CoV-2-QuantiVac-ELISA (IgG), Euroimmun)
494 according to the manufacturer's instructions. To detect S1 specific IgM, supernatant of
495 the *in vitro* culture and serum of vaccinated subjects was incubated on a S1 pre-coated
496 plate (Anti-SARS-COV-2, Euroimmun). Bound IgM was detected with alkaline
497 phosphatase-conjugated anti-human IgM (Jackson ImmunoResearch), and developed
498 with p-nitrophenyl phosphate (Sigma-Aldrich) in DEA buffer.

499

500 *Serum IgG determination*

501 SARS-CoV-2-specific antibodies were determined by Anti-SARS-CoV-2-QuantiVac-
502 ELISA (IgG) from Euroimmun detecting anti-SARS-CoV-2 Spike IgG (anti-SARS-CoV-
503 2 S IgG; <35,2 BAU/ml: negative, $\geq 35,2$ BAU/ml: positive) according to the
504 manufacturer's instructions.

505

506 *Neutralization assay*

507 Samples of vaccinated and convalescent individuals were tested in a plaque reduction
508 neutralization assay. Briefly, VeroE6 cells were seeded in 12-well plates at a density
509 of 2.8×10^5 cells/well 24 h prior to infection. Serum samples were diluted at ratios of
510 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024 in 50 μ L PBS total volume. For each
511 sample, one negative control was included (PBS without serum). Diluted sera and
512 negative controls were subsequently mixed with 90 plaque forming units (PFU) of

513 authentic SARS-CoV-2 (either B.1, B.1.1.7 or B.1.351 variant) in 50 μ L PBS (1600
514 PFU/mL) resulting in final sera dilution ratios of 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024,
515 and 1:2048. Following incubation at RT for 1 h, 400 μ L PBS was added to each sample
516 and the mixture was subsequently used to infect VeroE6 cells. After 1.5 h of incubation
517 at RT, inoculum was removed and the cells were overlaid with 0.6% Oxoid-agar in
518 DMEM, 20 mM HEPES (pH 7.4), 0.1% NaHCO₃, 1% BSA and 0.01% DEAE-Dextran.
519 Cells were fixed 72 h post-infection using 4% formaldehyde for 30 minutes and stained
520 with 1% crystal violet upon removal of the agar overlay. PFU were counted manually.
521 Plaques counted for serum-treated wells were compared to the average number of
522 plaques in the untreated negative controls, which were set to 100%. The plaque-
523 reduction neutralization titer 50 (PRNT₅₀) was calculated using a linear regression
524 model in GraphPad Prism 9 (GraphPad Prism Software, USA).

525

526 *Statistics*

527 Statistical analysis was performed with GraphPad Prism 9 (GraphPad Prism Software,
528 USA). Statistical significance was assessed by 2way ANOVA including Tukey's
529 multiple comparisons test and Spearman correlation. (*P<0.05; **P<0.01; ***P<0.001;
530 ****P<0.0001).

Data availability statement

Raw data is available upon reasonable request. Further supporting data are available from the corresponding authors upon reasonable request. All requests for raw and analyzed data and materials will be reviewed by the corresponding authors to verify if the request is subject to any intellectual property or confidentiality obligations. Patient-related data not included in the paper were generated as part of clinical examination and may be subject to patient confidentiality. Any data and materials that can be shared will be released via a Material Transfer Agreement. Source data are provided with this paper.

Reference	viral sequences	SARS-CoV-2
https://www.ncbi.nlm.nih.gov/nuccore/MN908947 ,		(MN908947.3)
https://www.ncbi.nlm.nih.gov/nuccore/NC_002645 ,	229E	(NC_002645)
https://www.ncbi.nlm.nih.gov/nuccore/NC_006577 ,	HKU1	(NC_006577)
https://www.ncbi.nlm.nih.gov/nuccore/NC_005831 ,	NL63	(NC_005831)
https://www.ncbi.nlm.nih.gov/nuccore/NC_006213 ,	OC43	(NC_006213)
https://www.ncbi.nlm.nih.gov/nuccore/NC_019843 ,	MERS	(NC_019843)
https://www.ncbi.nlm.nih.gov/nuccore/NC_004718 ,	SARS-CoV-1	(NC_004718)

https://www.ncbi.nlm.nih.gov/nuccore/NC_004718) were downloaded from the NCBI database (<https://www.ncbi.nlm.nih.gov/>).

Code availability statement

R code to reproduce the analyses of multiparametric flow-cytometry data is available at https://github.com/sagar161286/SARSCoV2_specific_CD8_Tcells.

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Authors Contributions

V.O., H.L., J.K. and I.S. planned, performed and analyzed experiments with the help of K.C., S.G., I.J., J.S., K.W., S.M., B.C., K.B., J.F., F.T., A.J., K.Z. S. and O.S.. H.L., J.L.M., B.B., C.W. and S.R. were responsible for donor recruitment. F.E. performed four-digit HLA typing by next-generation sequencing. T.B., G.K., M.S., M.R., R.T., C.N.-H and M.H. designed the study and contributed to experimental design and planning. V.O., H.L., J.K., I.S., R.T., M.H. and C.N.-H. interpreted data and wrote the manuscript. M.H., C.N.-H., R.T. are shared last authors.

583 Declaration of interest

584 The authors have nothing to declare.

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649

Figure 1: Bnt162b2 vaccine-elicited epitope-specific CD8⁺ T cells: early induction, vigorous Teff response and robust establishment of memory precursors

(A) Timeline showing blood and serum collection before and after prime and boost vaccination. **(B)** Dot plots showing A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells ex vivo after pMHC tetramer-based enrichment at BL, before and after boost vaccination. **(C)** The calculated ex vivo frequency is indicated at BL, dpp and dpb for A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells. **(D)** % of CD38, Ki-67 and T-BET^{hi} expressing A*01/S₈₆₅- and A*02/S₂₆₉-specific non-naïve CD8⁺ T cells. **(E)** Diffusion map showing flow cytometry data for A*02/S₂₆₉-specific CD8⁺ T cells in relation to dpp (shades of red) and dpb (shades of grey) in one individual. CD38, T-BET, TOX and BCL-2 expression levels are plotted on the diffusion map (color-code: blue, low expression; red, high expression). **(F-G)** Exemplary histograms depicting the expression levels of CD127 and TCF-1 in A*02/S₂₆₉- (green) specific, naïve (grey) and bulk (black) CD8⁺ T cells 14-16 and 55-59 dpb. Calculated ex vivo frequencies of non-naïve spike-specific CD8⁺ T cells expressing CD127 or TCF-1 for A*01/S₈₆₅- and A*02/S₂₆₉- specific CD8⁺ T cells. BL: baseline; dpp: days post prime; dpb: days post boost.

Figure 2: Proper and conserved functional capacities of circulating spike-specific CD8⁺ T cells following prime and boost vaccination

(A) Workflow depicting peptide-specific *in vitro* expansion of CD8⁺ T cells. (B) Dot plots showing A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells after *in vitro* expansion. (C, D) Frequency and expansion capacity of the respective spike-specific CD8⁺ T cells after *in vitro* expansion. (E-H) Dot plots showing IFN-γ- and TNF-, IFN-γ /TNF- and CD107a-expressing CD8⁺ T cells after *in vitro* expansion. % of CD8⁺ T cells producing the respective cytokines in relation to the frequency of A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells was determined. Dot plot show samples 5-6 dpb. BL: baseline; dpp: days post prime; dpb: days post boost.

Figure 3: Delayed appearance of circulating spike-specific CD4⁺ T cells, B cells and antibodies

(A) Dot plots showing DRB1*15:01/S₂₃₆-specific CD4⁺ T cells *ex vivo* after pMHCII tetramer-based enrichment at BL and after prime/boost. The bar graph shows the calculated *ex vivo* frequency of DRB1*15:01/S₂₃₆-specific CD4⁺ T cells. (B) ICOS⁺CD38⁺⁺ and Ki-67 expression within non-naïve, DRB1*15:01/S₂₃₆-specific CD4⁺ T cells (C) ICOS⁺CD38⁺⁺ and Ki-67-expressing non-naïve DRB1*15:01/S₂₃₆-specific CD4⁺ T cells on 9-2 dpp within the population of TFH (CXCR5+PD-1⁺) and TH1 (CXCR5-CXCR3⁺) cells. (D) Anti-SARS-CoV-2 Spike IgG at BL and after prime and boost vaccination (<35,2 BAU/ml: negative, ≥35,2 BAU/ml: positive). (E) Antibody neutralization activity is depicted as PRNT₅₀ at BL, dpp and dpb vaccination for the SARS-CoV-2 ancestral variant B.1 and the VOC B.1.1.7 and B.1.351 at 13-16 and 28-30 dpb when the highest neutralization titer was detected with the ancestral B.1 variant. Numbers indicate median value (F) Dot plots showing double tetramer positive B cells for S1 and RBD epitope. (G) % of spike-specific B cells depicted at BL, dpp and dpb as well as in natural infection for S1 and RBD epitope. (H) PBMC from BL, dpp and dpb as well as samples after natural infection were stimulated *in vitro* for nine days with CpG and IL-2. Secreted anti-SARS-CoV-2 Spike IgG was detected in the supernatant by ELISA. (I) Donut plots representing co-expression of CD27/IgG (upper) and CD71/CD95 (lower) in concatenated analysis of S1-specific B cells populations (N indicated the number of individual files concatenated) after boost vaccination. BL: baseline, dpp: days post prime, dpb: days post boost; TFH follicular helper T cells; TH1 T helper cells; PRNT50: plaque-reduction neutralization titer 50; BAU: antibody units; RU: arbitrary units. Bar charts show the median with IQR.

Figure 4: Differences of circulating spike-specific early memory CD8⁺ T cells after boost vaccination and natural infection

(A) Calculated frequency of A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells after boost vaccination or natural SARS-CoV-2 infection ex vivo after pMHC tetramer-based enrichment. (B) Distribution of CD8⁺ T cell memory subsets, TED, TCM and TEM1, for CD8⁺ T cells targeting A*01/S₈₆₅- and A*02/S₂₆₉-epitopes. (C) t-SNE representation of flow cytometry data, depicting spike-specific CD8⁺ T cells more than 80 dpb and dps of natural infection (grey: natural infection, black: vaccinees). BCL-2, TCF-1, CCR7 and CD38 expression levels are plotted on the t-SNE (color-code: blue, low expression; red, high expression). (D) Frequency of the respective spike-specific CD8⁺ T cells after *in vitro* expansion. % of IFN- γ - and TNF-producing as well as IFN- γ and TNF co-producing CD8⁺ T cells in relation to the frequency of A*02/S₂₆₉-specific CD8⁺ T cells after *in vitro* expansion. Bar charts show the median with IQR. A*01/S₈₆₅ n=5 vaccinees longitudinally and n=10 naturally infected patients cross-sectional/longitudinally; A*02/S₂₆₉ n=5 vaccinees longitudinally and n=12 naturally infected patients cross-sectional. 2way ANOVA including Tukey's multiple comparisons test were performed. dpb: days post boost; dps: days post symptoms; TED: early differentiated, TCM: central memory T cells and TEM1: effector memory T cells 1; t-SNE: t-distributed stochastic neighbor embedding.

Extended Data Figure 1:

Gating strategy of flow cytometry data. A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells were identified via pMHC tetramer-based enrichment used for further analyses.

ex vivo spike-specific CD8⁺ T cells (without enrichment)

Extended Data Figure 2:

(A) Comparison of epitope sequences with amino acid sequences of SARS-CoV-1/2, MERS and common cold coronaviruses amino acid sequences (upper panel) and with circulating SARS-CoV-2 variants of concern (VOC) (lower panel), respectively for A*01/S₈₆₅ and A*02/S₂₆₉ CD8⁺ T cell epitopes. (B) Dot plots showing A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells *ex vivo* without pMHC tetramer-based enrichment. (C) Exemplary histograms (13-15 dpp) depicting the expression levels of CD38, Ki-67, T-BET, GRZB, PD-1 and CXCR3 in A*01/S₈₆₅- (blue) and A*02/S₂₆₉- (green) specific, naïve (grey) an bulk (black) CD8⁺ T cells. (D) % of granzyme B, PD-1 and CXCR3 expressing A*01/S₈₆₅- and A*02/S₂₆₉-specific non-naïve CD8⁺ T cells. (E) Exemplary histograms (5-6 dpb) depicting the expression levels of CD39 and TOX in A*01/S₈₆₅- (blue) and A*02/S₂₆₉- (green) specific, naïve (grey) an bulk (black) CD8⁺ T cells. % expressing A*01/S₈₆₅- and A*02/S₂₆₉-specific non-naïve CD8⁺ T cells is shown on the ride side. BL: baseline; dpp: days post prime; dpb: days post boost; GRZB: granzyme B.

Extended Data Figure 3:

(A) t-SNE representation of flow cytometry data comparing spike-specific CD8⁺ T cells after prime and boost vaccination of 7 individuals after prime (4 A*01/S₈₆₅, 3 A*02/S₂₆₉) and 6 individuals after boost (3 A*01/S₈₆₅, 3 A*02/S₂₆₉). Expression levels of TOX, PD-1 and T-BET are indicated (color-code: blue, low expression; red, high expression). **(B)** Diffusion map showing flow cytometry data for A*01/S₈₆₅-specific CD8⁺ T cells at dpp (shades of red) and dpb (shades of grey) with CD38, T-BET, TOX and BCL-2 expression levels plotted on the diffusion map (color-code: blue, low expression; red, high expression). **(C)** Diffusion map showing flow cytometry data for A*01/S₈₆₅- and A*02/S₂₆₉-specific CD8⁺ T cells at dpp (shades of red) and dpb (shades of grey) for two individuals each. dpp: days post prime; dpb: days post boost; t-SNE: t-distributed stochastic neighbor embedding.

Extended Data Figure 4:

(A-B) % of A*01/S₈₆₅- and A*02/S₂₆₉-specific non-naïve CD8+ T cells expressing CD127 and TCF-1 were determined. Correlation of indicated marker with dpb is depicted on the right. **(C)** Exemplary histograms depicting the expression levels of BCL-2 on A*02/S₂₆₉- (green) specific CD8+ T cells as well as on naïve CD8+ T cells (grey) and CD8+ bulk (black) 14-16 and 55-59 dpb. nMFI (normalized to naïve CD8+ T cells) of A*01/S₈₆₅- and A*02/S₂₆₉-specific non-naïve CD8+ T cells expressing BCL-2 was determined. Correlation of nMFI BCL-2 with dpb is depicted on the right. For **(A)** A*01/S₈₆₅ n=23 for A*02/S₂₆₉ n=25 in **(B, C)** (A*01/S₈₆₅ n=24 for A*02/S₂₆₉ n=25) Spearman correlation was performed. BL: baseline; dpp: days post prime; dpb: days post boost; nMFI: normalized Median Fluorescent Intensity.

Extended Data Figure 5:

(A) Gating strategy of flow cytometry data. DRB1*15:01/S₂₃₆-specific CD4⁺ T cells were identified via pMHCII tetramer-based enrichment used in further analyses. (B) Comparison of DRB1*15:01/S₂₃₆ epitope sequence with amino acid sequences of SARS-CoV-1/2, MERS and common cold coronaviruses (left) and of circulating SARS-CoV-2 variants of concern (VOC) (right). (C) Number of responses (left) and the calculated *ex vivo* frequencies (middle) of DRB1*15:01/S₂₃₆-specific CD4⁺ T cells in historic controls (HC, n=8). % naïve within total DRB1*15:01/S₂₃₆-specific CD4⁺ T cells in HC (right). (D) % naïve of total DRB1*15:01/S₂₃₆-specific CD4⁺ T cells. (E) Representative dot plots of ICOS+CD38⁺⁺ and Ki-67⁺ expression (grey: bulk, black: DRB1*15:01/S₂₃₆-specific CD4⁺ T cells). (F) % CXCR5-CXCR3⁺ TH1 cells and of CXCR5+PD-1⁺ TFH within non-naïve DRB1*15:01/S₂₃₆-specific CD4⁺ T cells with representative dot plots (grey: bulk, black: DRB1*15:01/S₂₃₆-specific CD4⁺ T cells). BL: baseline, dpp: days post prime, dpb: days post boost; TFH follicular helper T cells; TH1 T helper cells 1. Bar charts show the median with IQR.

Extended Data Figure 6:

(A) Gating strategy of flow cytometry data for different B cell subpopulations. (B) % of CD19⁺, naïve, switched memory, ASCs, CD69⁺aN and CD95⁺ aN cells within bulk B cells was determined at BL, post prime/boost. Non-linear fit was calculated in red. (C) Detection of spike IgM in serum at BL, post prime/boost by ELISA. Level of secreted IgM was determined in supernatant of PBMC from BL, post prime/boost and after natural infection after *in vitro* stimulation for nine days with CpG and IL-2. (D) Antibody neutralization capacity is depicted as PRNT₅₀ at different time points post symptom onset in natural SARS-CoV-2 infection with SARS-CoV-2 ancestral variant B.1. Numbers indicate median value (E) Dot plots representing co-expression of IgD/IgM (upper), IgG/CD27 (middle) and CD95/CD71 (lower) on concatenated files of S1-specific B cells at the indicated time points. ASCs: antibody-secreting cells; sw memory: switched memory; aN: activated naïve; BL: baseline; dpp: days post prime; dpb: days post boost; dps: days post symptoms; O.D.: optical density; PRNT₅₀: plaque-reduction neutralization titer 50. Bar charts show the median with IQR.

Extended Data Figure 7:

(A) % of CD127 expressing spike-specific non-naïve CD8+ T cells. **(B)** Gating strategy of memory CD8+ T cell populations among A*01/S₈₆₅- (blue) (28dpb) and A*02/S₂₆₉- (green) (98 dps) specific CD8+ T cells, (grey: bulk CD8+ T cells). **(C)** Distribution of spike-specific CD8+ T-cell memory subsets, TEM2, TEM3 and TEMRA. Bar charts depict the median with IQR. dpb: days post boost; dps: days post symptoms; TEM2: effector memory T cells 2; TEM3: effector memory T cells 3, TEMRA terminally differentiated effector memory cells re-expressing CD45RA.

Extended Data Figure 8:

(A) % of TCF-1 expressing and nMFI (normalized to naïve CD8+ T cells) of BCL-2 respective spike-specific non-naïve CD8+ T cells. **(B)** % of CD38 expressing spike-specific non-naïve CD8+ T cells. **(C)** Spike-specific CD8+ T cell expansion index of A*01/S₈₆₅-specific CD8+ T cells after *in vitro* expansion. % of IFN- γ - and TNF-producing as well as IFN- γ and TNF co-producing CD8+ T cells in relation to the frequency of A*01/S₈₆₅-specific CD8+ T cells after *in vitro* expansion. Bar charts show the median with IQR. A*01/S₈₆₅ n=5 vaccinees longitudinally and n=10 naturally infected patients cross-sectional/longitudinally. A*02/S₂₆₉ n=5 vaccinees longitudinally and n=12 naturally infected patients cross-sectional. 2way ANOVA including Tukey's multiple comparisons test were performed. dpp: days post prime; dps: days post symptoms; t-SNE: t-distributed stochastic neighbor embedding.

Supplementary Table 1: Donors' characteristics

Donor ID	Sex	Age	HLA Type	Cohort	Natural infection	Tested Epitopes	Serum S1 IgG1 assay	Neutralizing Antibodies (Plaque reduction assay)
V1	f	45	A*02:01, A*33:03, B*44:03, B*58:01, DRB1*07:01	vaccinated	-	CD8 T cells: A*02/S269-277 B cells: S1, RBD	yes	yes
V2	f	44	A*01:01, A*11:01, B*15:17, B*35:01, DRB1*01:03, DRB1*13:02	vaccinated	-	CD8 T cells: A*01/S865-873 B cells: S1, RBD	yes	yes
V3	f	30	A*01:01, A*02:01, B*07:02, B*37:01, DRB1*05:01, DRB1*11:03	vaccinated	-	CD8 T cells: A*01/S865-873 A*02/S269-277 B cells: S1, RBD	yes	yes
V4	m	25	A*02:01, A*29:02, B*45:01, B*51:01, DRB1*04:01, DRB1*13:01	vaccinated	-	CD8 T cells: A*02/S269-277 B cells: S1, RBD	yes	yes
V5	m	47	A*01:01, A*11:01, B*08:01, B*15:01, DRB1*01:01, DRB1*03:01	vaccinated	-	CD8 T cells: A*01/S865-873 B cells: S1, RBD	yes	yes
V6	m	62	A*01:01, A*02:01, B*08:01, B*15:01, DRB1*08:01, DRB1*11:01	vaccinated	-	CD8 T cells: A*01/S865-873 A*02/S269-277 B cells: S1, RBD	yes	yes
V7 / HC 1	f	36	A*01:01, A*03:01, B*07:02, B*57:01, DRB1*07:01, DRB1*15:01	vaccinated / historic control	-	CD8 T cells: A*01/S865-873 CD4: DRB1*15:01/ S236-250 B cells: S1, RBD	yes	yes
V8	m	31	A*02:01, B*15:01, B*51:01, DRB1*01:01, DRB1*11:01	vaccinated	-	CD8 T cells: A*02/S269-277 B cells: S1, RBD	yes	yes
V9	m	41	A*01:01, A*03:01, B*08:01, B*35:01, DRB1*01:01, DRB1*03:01	vaccinated	-	B cells: S1, RBD	no	yes
V10	m	39	A*02:01, A*23:01, B*13:02, B*58:01, DRB1*07:01, DRB1*13:01	vaccinated	-	B cells: S1, RBD	no	yes
V11 / HC2	m	33	A*02:01, A*03:01, B*07:02, DRB1*15:01	vaccinated / historic control	-	CD4 T cells: DRB1*15:01/ S236-250	no	yes
V12 / HC3	m	41	A*01:01, A*02:01, B*08:01, B*40:01, DRB1*14:02, DRB1*15:01	vaccinated / historic control	-	CD4 T cells: DRB1*15:01/ S236-250	no	yes
V13	f	37	A*02:01, A*68:01, B*15:01, B*40:01, DRB1*13:01, DRB1*15:01	vaccinated	-	CD4 T cells: DRB1*15:01/ S236-250	no	yes
V14	m	35	A*01:01, A*26:01, B*07:02, B*27:05, DRB1*01:01, DRB1*15:01	vaccinated	-	CD4 T cells: DRB1*15:01/ S236-250	no	yes
V15	m	35	A*01:01, A*24:02, B*07:02, B*27:05, DRB1*15:01, DRB1*16:01	vaccinated	-	CD4 T cells: DRB1*15:01/ S236-250	no	yes
V16/HC4	f	60	A*02:01, B*07:02, B*44:02, DRB1*04:01, DRB1*15:01	vaccinated / historic control	-	CD4 T cells: DRB1*15:01/ S236-250	no	yes
V17	m	40	A*02:01, A*03:01, B*07:02, B*57:01, DRB1*07:01, DRB1*15:01	vaccinated	-	CD4 T cells: DRB1*15:01/ S236-250	no	yes
HC5	m	33	A*24:02, A*29:02, B*07:02, B*44:03, DRB1*14:54, DRB1*15:01	historic control	-	CD4 T cells: DRB1*15:01/ S236-250	no	no
HC6	m	33	A*02:01, A*03:01, B*07:02, DRB1*04:01, DRB1*15:01	historic control	-	CD4 T cells: DRB1*15:01/ S236-250	no	no
HC7	m	30	A*01:01, A*03:01, B*08:01, B*35:03, DRB1*03:01, DRB1*15:01	historic control	-	CD4 T cells: DRB1*15:01/ S236-250	no	no
HC8	f	35	A*02:01, A*26:01, B*07:02, B*38:01, DRB1*13:01, DRB1*15:01	historic control	-	CD4 T cells: DRB1*15:01/ S236-250	no	no
I1	f	86	A*02:01, A*31:01, B*13:02, B*40:01, DRB1*04:01, DRB1*15:01	acute	mild infection	CD8 T cells: A*02/S269-277	yes	no
I2	m	63	A*02:01, B*44:02, B*58:01, DRB1*03:01, DRB1*13:01	acute	mild infection	CD8 T cells: A*02/S269-277	yes	no
I3	m	69	A*01:01, A*25:01, B*08:01, B*18:01, DRB1*04:01, DRB1*15:01	acute / resolved	mild infection	CD8 T cells: A*01/S865-873	yes	yes
I4	f	39	A*01:01, A*30:02, B*18:01, B*41:01, DRB1*07:01, DRB1*07:11	acute / resolved	mild infection	CD8 T cells: A*01/S865-873	yes	yes
I5	m	34	A*01:01, A*03:01, B*08:01, DRB1*03:01	acute / resolved	mild infection	CD8 T cells: A*01/S865-873	yes	yes
I6	m	57	A*02:01, A*24:02, B*44:02, B*57:01, DRB1*07:01, DRB1*13:01	acute / resolved	mild infection	CD8 T cells: A*02/S269-277	yes	no
I7	m	37	A*01:01, A*29:02, B*07:02, B*40:01, DRB1*01:03, DRB1*11:01	resolved	mild infection	CD8 T cells: A*01/S865-873	yes	yes
I8	m	36	A*01:01, A*03:01, B*37:01, B*40:01, DRB1*13:02, DRB1*15:01	resolved	mild infection	CD8 T cells: A*01/S865-873	yes	yes
I9	f	46	A*02:01, A*11:01, B*07:02, B*49:01, DRB1*04:01, DRB1*13:02	resolved	mild infection	CD8 T cells: A*02/S269-277	no	no
I10	m	30	A*01:01, A*02:01, B*15:01, B*51:01, DRB1*11:01, DRB1*13:01	resolved	mild infection	CD8 T cells: A*01/S865-873 A*02/S269-277	yes	yes
I11	m	29	A*01:01, A*31:01, B*39:01, B*51:01, DRB1*01:01, DRB1*12:01	resolved	mild infection	CD8 T cells: A*01/S865-873	yes	no
I12	m	56	A*01:01, A*03:01, B*07:02, B*08:01, DRB1*03:01, DRB1*15:01	resolved	mild infection	CD8 T cells: A*01/S865-873	yes	yes
I13	m	38	A*02:01, A*31:01, B*07:02, B*15:01, DRB1*12:01, DRB1*15:01	resolved	mild infection	CD8 T cells: A*02/S269-277	yes	no
I14	m	27	A*02:01, A*31:01, B*07:02, B*15:01, DRB1*12:01, DRB1*15:01	resolved	mild infection	CD8 T cells: A*02/S269-277	yes	no
I15	m	22	A*02:01, B*15:01, B*40:01, DRB1*08:01, DRB1*13:02	resolved	mild infection	CD8 T cells: A*02/S269-277	yes	no
I16	f	43	A*02:01, A*31:01, B*27:02, B*39:01, DRB1*12:01, DRB1*16:01	resolved	mild infection	CD8 T cells: A*02/S269-277	yes	no
I17	f	32	A*02:01, B*18:01, B*38:01, DRB1*07:01, DRB1*13:01	resolved	mild infection	CD8 T cells: A*02/S269-277	yes	no
I18	f	42	A*01:01, A*32:01, B*07:02, B*40:02, DRB1*07:01, DRB1*13:01	resolved	mild infection	CD8 T cells: A*01/S865-873	yes	yes
I19	f	32	A*02:01, A*03:01, B*40:01, B*44:02, DRB1*11:04, DRB1*13:01	resolved	mild infection	CD8 T cells: A*02/S269-277	yes	yes
I20	f	62	A*02:01, A*32:01, B*27:05, B*51:01, DRB1*11:01, DRB1*15:01	resolved	mild infection	CD8 T cells: A*02/S269-277	yes	yes
I21	f	48	A*02:01, A*03:01, B*07:02, B*13:02, DRB1*07:01, DRB1*13:05	resolved	mild infection	CD8 T cells: A*02/S269-277	yes	yes
I22	f	36	A*01:01, A*03:01, B*07:02, B*08:01, DRB1*03:01, DRB1*04:01	resolved	mild infection	CD8 T cells: A*01/S865-873	yes	no

Figures

Figure 1

Bnt162b2 vaccine-elicited epitope-specific CD8⁺ T cells: early induction, vigorous Teff response and robust establishment of memory precursors (A) Timeline showing blood and serum collection before and after prime and boost vaccination. (B) Dot plots showing A*01/S865- and A*02/S269-specific CD8⁺ T

cells ex vivo after pMHC tetramer-based enrichment at BL, before and after boost vaccination. (C) The calculated ex vivo frequency is indicated at BL, dpp and dpb for A*01/S865- and A*02/S269-specific CD8+ T cells. (D) % of CD38, Ki-67 and T-BET^{hi} expressing A*01/S865- and A*02/S269-specific non-naïve CD8+ T cells. (E) Diffusion map showing flow cytometry data for A*02/S269-specific CD8+ T cells in relation to dpp (shades of red) and dpb (shades of grey) in one individual. CD38, T-BET, TOX and BCL-2 expression levels are plotted on the diffusion map (color-code: blue, low expression; red, high expression). (F-G) Exemplary histograms depicting the expression levels of CD127 and TCF-1 in A*02/S269- (green) specific, naïve (grey) and bulk (black) CD8+ T cells 14-16 and 55-59 dpb. Calculated ex vivo frequencies of non-naïve spike-specific CD8+ T cells expressing CD127 or TCF-1 for A*01/S865- and A*02/S269-specific CD8+ T cells. BL: baseline; dpp: days post prime; dpb: days post boost.

Figure 2

Proper and conserved functional capacities of circulating spike-specific CD8⁺ T cells following prime and boost vaccination (A) Workflow depicting peptide-specific in vitro expansion of CD8⁺ T cells. (B) Dot plots showing A*01/S865- and A*02/S269-specific CD8⁺ T cells after in vitro expansion. (C, D) Frequency and expansion capacity of the respective spike-specific CD8⁺ T cells after in vitro expansion. (E-H) Dot plots showing IFN- γ - and TNF-, IFN- γ /TNF- and CD107a-expressing CD8⁺ T cells after in vitro expansion. % of CD8⁺ T cells producing the respective cytokines in relation to the frequency of A*01/S865- and

A*02/S269-specific CD8+ T cells was determined. Dot plot show samples 5-6 dpb. BL: baseline; dpp: days post prime; dpb: days post boost.

Figure 3

Delayed appearance of circulating spike-specific CD4+ T cells, B cells and antibodies (A) Dot plots showing DRB1*15:01/ S236-specific CD4+ T cells *ex vivo* after pMHCII tetramer-based enrichment at BL and after prime/ boost. The bar graph shows the calculated *ex vivo* frequency of DRB1*15:01/S236-

specific CD4⁺ T cells. (B) ICOS⁺CD38⁺⁺ and Ki-67 expression within non-naïve, DRB1*15:01/S236-specific CD4⁺ T cells (C) ICOS⁺CD38⁺⁺ and Ki-67-expressing non-naïve DRB1*15:01/S236-specific CD4⁺ T cells on 9-2 dpp within the population of TFH (CXCR5⁺PD-1⁺) and TH1 (CXCR5⁺CXCR3⁺) cells. (D) Anti-SARS-CoV-2 Spike IgG at BL and after prime and boost vaccination (<35,2 BAU/ml: negative, ≥35,2 BAU/ml: positive). (E) Antibody neutralization activity is depicted as PRNT50 at BL, dpp and dpb vaccination for the SARS-CoV-2 ancestral variant B.1 and the VOC B.1.1.7 and B.1.351 at 13-16 and 28-30 dpb when the highest neutralization titer was detected with the ancestral B.1 variant. Numbers indicate median value (F) Dot plots showing double tetramer positive B cells for S1 and RBD epitope. (G) % of spike-specific B cells depicted at BL, dpp and dpb as well as in natural infection for S1 and RBD epitope. (H) PBMC from BL, dpp and dpb as well as samples after natural infection were stimulated in vitro for nine days with CpG and IL-2. Secreted anti-SARS-CoV-2 Spike IgG was detected in the supernatant by ELISA. (I) Donut plots representing co-expression of CD27/IgG (upper) and CD71/CD95 (lower) in concatenated analysis of S1-specific B cells populations (N indicated the number of individual files concatenated) after boost vaccination. BL: baseline, dpp: days post prime, dpb: days post boost; TFH follicular helper T cells; TH1 T helper cells 1; PRNT50: plaque-reduction neutralization titer 50; BAU: antibody units; RU: arbitrary units. Bar charts show the median with IQR.

Figure 4

Differences of circulating spike-specific early memory CD8⁺ T cells after boost vaccination and natural infection (A) Calculated frequency of A*01/S865- and A*02/S269-specific CD8⁺ T cells after boost vaccination or natural SARS-CoV-2 infection ex vivo after pMHC tetramer-based enrichment. (B) Distribution of CD8⁺ T cell memory subsets, TED, TCM and TEM1, for CD8⁺ T cells targeting A*01/S865- and A*02/S269-epitopes. (C) t-SNE representation of flow cytometry data, depicting spike-specific CD8⁺ T cells more than 80 dpb and dps of natural infection (grey: natural infection, black: vaccines). BCL-2, TCF-1, CCR7 and CD38 expression levels are plotted on the t-SNE (color-code: blue, low expression; red, high

expression). (D) Frequency of the respective spike-specific CD8⁺ T cells after in vitro expansion. % of IFN- γ - and TNF-producing as well as IFN- γ and TNF co-producing CD8⁺ T cells in relation to the frequency of A*02/S269-specific CD8⁺ T cells after in vitro expansion. Bar charts show the median with IQR. A*01/S865 n=5 vaccinees longitudinally and n=10 naturally infected patients cross-sectional/longitudinally; A*02/S269 n=5 vaccinees longitudinally and n=12 naturally infected patients cross-sectional. 2way ANOVA including Tukey's multiple comparisons test were performed. dpb: days post boost; dps: days post symptoms; TED: early differentiated, TCM: central memory T cells and TEM1: effector memory T cells 1; t-SNE: t-distributed stochastic neighbor embedding.