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BRIEF COMMUNICATION

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Induction of robust cellular and humoral immunity against SARS-CoV-2 after a third dose of BNT162b2 vaccine in previously unresponsive older adults

Addi J. Romero-Olmedo[1,5], Axel Ronald Schulz[2,5], Svenja Hochstätter[3], Dennis Das Gupta[1], Iiris Virta[2], Heike Hirseland[2], Daniel Staudenraus[1], Bärbel Camara[1], Carina Münch[3], Véronique Hefter[3], Siddhesh Sapre[3], Verena Krähling[3], Helena Müller-Kräuter[3], Marek Widera[4], Henrik E. Mei[2,6], Christian Keller[3,6] and Michael Lohoff[1,6] ✉

Here we compared SARS-CoV-2-specific antibody and T-cell responses between older adults (>80 years old, *n* = 51) and a younger control group (20–53 years old, *n* = 46) after receiving two doses of BNT162b2. We found that responses in older adults were generally lower, and we identified 10% low-/non-responders. After receiving a third vaccination with BNT162b2, 4 out of 5 low-/non-responders showed antibody and T-cell responses similar to those of responders after two vaccinations.

Vaccination protects against fatal courses of SARS-CoV-2 infection, also in older adults[1,2]. Induction of neutralizing serum antibodies was observed after two intramuscular applications of the BNT162b2 mRNA COVID-19 vaccine in people >80 years of age[3]. However, recent outbreaks among older vaccinees[4] and antibody responses inferior to those observed in younger vaccinees[3] have prompted discussion on the necessity of a third vaccination.

Here we compared vaccine-induced humoral and cellular immune responses to SARS-CoV-2 in 51 individuals aged >80 years (older adults) and in 46 control individuals (young) (45 individuals aged 20–44 years (Table 1 and Supplementary Table 1) plus one 53-year-old woman, not included in the mean age calculation in Table 1, but included in all figures, accordingly. All participants were randomly recruited, COVID-19-naïve and not acutely ill; they were vaccinated twice, at day 0 and day 21, with BNT162b2 in a vaccination centre (older adults) or a doctor's practice (young) in Marburg, Germany, March–May 2021. Analysis of spike-specific IgG, neutralization capacity against SARS-CoV-2 and SARS-CoV-2-reactive CD4 T cells (positive for the markers CD40L and IFNγ) in peripheral blood revealed strong induction of humoral and cellular immunity in response to vaccination (Fig. 1a–c). Notably, the neutralization capacity after two BNT162b2 doses increased even against the delta variant (B.1.617.2), although reactivity towards the wild type (B.1) was higher (Fig. 1b). This finding confirms previous data on antibodies[3]. As for spike-specific CD4 T cells, our data vary from this report[3], as young and older groups demonstrated a further increase (10-fold average) between first and second dose (Fig. 1c, day 21 versus day 35). This was previously not noted[3], potentially due to the different method used for their quantification (FluoroSpot), as compared to the herein applied multi-parameter flow cytometry (Extended Data Fig. 1).

Several important differences were noted between young and old vaccinees' immune responses. First, the overall antibody and CD4 T-cell response was lower in older vaccinees at a high level of significance (Fig. 1a–c, day 35), as also shown by the stimulation index (Extended Data Fig. 2a). Second, while responses were comparable across young donors, substantial heterogeneity was observed in older donors, both in antibody and CD4 T-cell responses, whereby several older adults showed scarce or even no reaction. Third, some older adults had high frequencies of responding CD4 T cells before vaccination (Fig. 1c, day 0), probably reflecting cross-reactive activities gained during previous encounters with other coronaviruses, as demonstrated before[5,6].

By combining results for antibodies and CD4 T cells for each vaccinee (Fig. 2a,b), we identified five older adults retaining very low levels of specific serum IgG together with almost an absence of spike-reactive CD4 T cells (Fig. 2b, red triangles). Remarkably, these older adults were potentially not protected by the previous two doses of the vaccine. Among them, donor #31 received methotrexate for rheumatoid arthritis, while no history of immune modulating medication or disease was evident in the remaining four (Supplementary Table 1). Additionally, no evident difference between responders and low-/non-responders was identified by age and the Charlson comorbidity index (CCI; Extended Data Fig. 3a,b and Supplementary Table 1). Furthermore, there was no significant difference ($P = 0.15$ by Fisher's exact test) in the distribution of male and female participants between groups (Table 1).

No similar non-responder was found in the young cohort (Fig. 2a). Notably, our local authorities (Regional Council of Giessen, Hesse, Germany) informed us about breakthrough infections in several retirement homes. These infections occurred between 1 and 3 months after the second vaccination with BNT162b2 and 5 out of 45 infected residents >80 years succumbed to infection.

[1]Institute of Medical Microbiology and Hospital Hygiene, Philipps-University Marburg, Marburg, Germany. [2]Deutsches Rheumaforschungszentrum Berlin, Leibniz Institute, Berlin, Germany. [3]Institute of Virology, Philipps-University, Marburg, Germany. [4]Institute of Medical Virology, University Hospital, Goethe University, Frankfurt, Germany. [5]These authors contributed equally: Addi J. Romero-Olmedo and Axel Ronald Schulz. [6]These authors contributed equally: Henrik E. Mei, Christian Keller, Michael Lohoff. ✉e-mail: lohoff@med.uni-marburg.de

Table 1 | Donor characteristics

Group	Total donors	Sex	Age range	Age mean ± s.d.	Low-/non-responders, $n = 5$	Age range	Age mean ± s.d.
Older adults	$n = 51$	female: 31 (61%)	80–97 years	84.1 ± 3.8	female: 1 (20%)	81–95 years	85.2 ± 5.7
		male: 20 (39%)			male: 4 (80%)		
Young	$n = 46$	female: 29 (63%)	20–53 years	30.5 ± 5.9	female: 0	N/A	N/A
		male: 17 (37%)			male: 0		

N/A, not applicable.

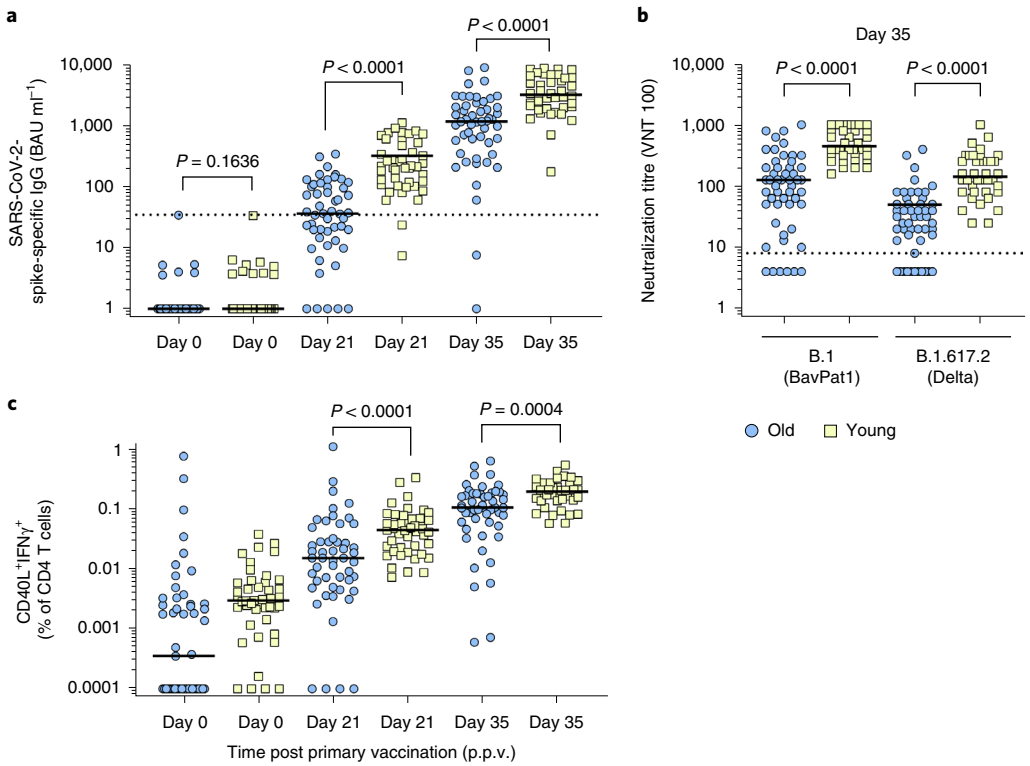

Fig. 1 | Humoral and cellular SARS-CoV-2 immunity in >80- and 20–53-year-old study participants vaccinated with the BNT162b2 vaccine. **a**–**c**, SARS-CoV-2-spike-specific serum IgG antibody titres (**a**), serum titres of 100% virus neutralization (VNT 100) for SARS-CoV-2 wild type (B.1) or its delta variant (B.617.2) (**b**) and percentages of SARS-CoV-2-spike-specific CD4 T cells (**c**) were analyzed in 51 donors aged >80 years (blue symbols) and 46 donors aged 20–53 years (yellow symbols) before (day 0; **a**,**c**), 21 d after the first (**a**,**c**) and 14 d after the second BNT162b2 (Pfizer-Biontech) vaccination (day 35; **a**–**c**). Each symbol represents one donor. Horizontal lines indicate medians, dotted lines indicate the cut-off for antibody positivity at 35.2 BAU ml^{-1} (**a**) and 8 (reciprocal titre) for VNT (**b**). A cut-off value for determining reactive T cells could be considered at 0.01% as shown previously[6] (**c**). *P* values determined by two-tailed Mann–Whitney test.

Thus, the lethality of these breakthrough infections is remarkably similar to the frequency of low-/non-responders in our older-adults study cohort. No COVID-19 infections were recorded in our cohorts until August 2021.

Aiming to enhance SARS-CoV-2 immunity, all 5 low-/non-responders received a third dose of the BNT162b2 mRNA vaccine during week 16 after the first dose. At that day, blood analyses demonstrated absence of specific immunity to SARS-CoV-2 (Fig. 2c–e). The third vaccination was well tolerated. Most importantly, 2 weeks later, 4 out of 5 vaccinees, including donor #31, demonstrated robust spike-specific T-cell and antibody responses comparable with those detectable in responders after two-dose vaccination (Fig. 2c–e and Extended Data Figs. 1 and 2b). In donor #54, a healthy man without obvious morbidities, SARS-CoV-2-specific immunity also increased, although only to low levels. He was meanwhile vaccinated a fourth time, again with BNT162b2, which unfortunately still yielded an insufficient response (46.97 BAU ml^{-1}).

Our data show that older adults initially hardly responding to two-dose vaccination can mount a virus-specific adaptive immune response after a third BNT162b2 dose. While the reason for primary unresponsiveness in our older-adults cohort remains unclear, BNT162b2 unresponsiveness in older adults is not fixed, and can be overcome by repeated vaccination. To confirm overall intact adaptive immune competence in low-/non-responders, we tested for antibody and CD4 T-cell reactivity towards control pathogens unrelated to SARS-CoV-2. Measles virus (MV)- or varicella-zoster virus (VZV)-specific IgG did not differ between low-/non-responsive donors and all other aged donors at baseline (day 0, Extended Data

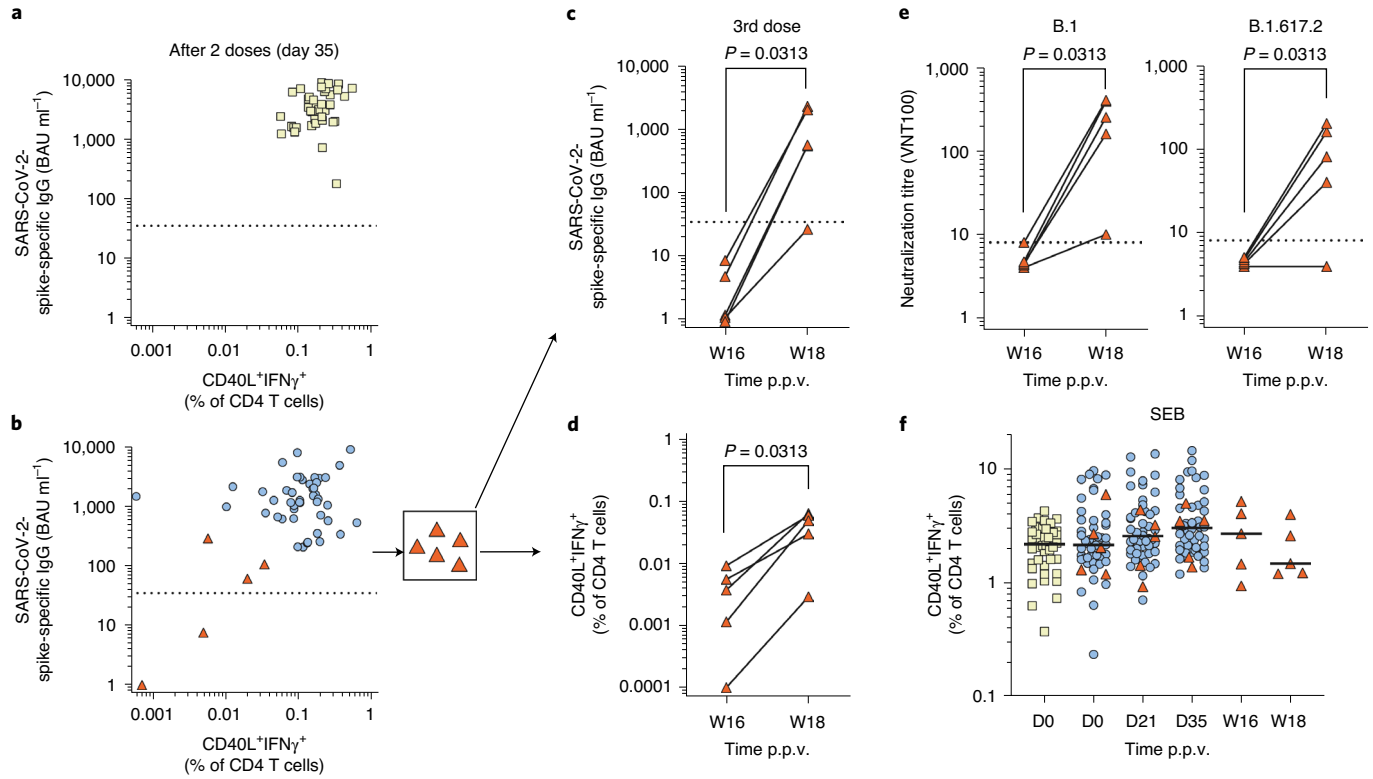

Fig. 2 | Humoral and cellular SARS-CoV-2 immunity in >80-year-old initial low-/non-responders is rescued after a third dose of the BNT162b2 mRNA vaccine. a–**e**, Combined presentation per person of the SARS-CoV-2-spike-specific serum IgG antibody and percentages of SARS-CoV-2-spike-specific CD4 T cells at day 35 in young (**a**) and aged donors (**b**). Five individuals (red triangles) mounted only low or no specific antibody and T-cell responses (**b**), and were vaccinated a third time in week 16 (W16) after the first dose (**c**–**e**). SARS-CoV-2-spike-specific serum IgG antibody titre (**c**), percentages of SARS-CoV-2-spike-specific CD4 T cells (**d**) and serum titre of 100% virus neutralization (VNT 100) for SARS-CoV-2 wild type (B.1) or its delta variant (B.1.617.2) (**e**) were measured in week 18 (W18). (**f**) T-cell response to SEB of the young and older participants measured at the indicated days or weeks after the first dose. Each dot, square and triangle represents one donor. Blue dots: aged responders; red triangles: initial low-/non-responders to the SARS-CoV-2 spike glycoprotein; yellow squares: young donors. Horizontal lines indicate medians, dotted lines indicate the cut-off for antibody positivity at 35.2 BAU ml^{-1} for SARS-CoV-2-spike-specific IgG (**a**,**c**) and 8 (reciprocal titre) for VNT (**e**). *P* values determined by one-tailed Wilcoxon matched-pairs signed-rank test (**c**–**e**).

Fig. 4). Additionally, the T-cell responsiveness towards staphylococcal enterotoxin B (SEB) was comparable to that seen in the other older vaccinees throughout the observation period (Fig. 2f and Extended Data Fig. 1). A similar response was observed in the young cohort (exemplified by their data on day 0), or in older adults (>80 years) who have recovered from COVID-19 infection several months before (Extended Data Fig. 1). These results demonstrate that the kinetics of T-cell activation in our assay conditions are similar throughout participants and evaluated timepoints. Furthermore, even if our cohort is small, we demonstrate that initial unresponsiveness to vaccination is not indicative of an overall lack of immune competence; consequently, older adults who previously did not respond or responded poorly to vaccination, will probably benefit from repeated vaccination with BNT162b2. Accordingly, in very recent studies of patients after allogeneic hematopoietic stem-cell transplantation or on haemodialysis, only a subgroup of vaccinees reacted by an increase in antibody levels after a third vaccination, while T-cell reactivity was not analysed[7,8]. A third vaccination was recently shown to increase protection against COVID-19 in people >60 years[9]. This effect probably combines overcoming of the herein described primary non-responsiveness, and a booster effect in primary responders whose antibody titre may have gradually declined.

Overall, we show lower immune responses against SARS-CoV-2 in aged versus young vaccinees, a finding which is also reflected in the antibody neutralization capacity against the SARS-CoV-2 delta variant. Nevertheless, 90% of individuals aged >80 years established adaptive SARS-CoV-2-specific immunity after receiving two doses of the BNT162b2 mRNA vaccine. However, low-/non-responders can be identified. Therefore, our data are suggestive of the importance of routine screening for spike-specific immunity in this population at risk, to assess the extent of immunity after two doses of BNT162b2. Screening should be unbiased and not limited to conditions of immunodeficiency or targeted immunosuppression. Should such tests reveal lack of specific immunity, re-vaccination should be considered.

Methods

Our research complies with all relevant ethical regulations. The study of patients with COVID-19 and vaccinations against COVID-19 was approved by the ethics committee of the medical faculty of the Philipps University Marburg (study number 40/21-12032021) and participants gave written informed consent according to the Consensus-based Clinical Case Reporting Guideline (CARE) guidelines and in compliance with the Declaration of Helsinki principles.

Study participants. Blood samples were obtained from older adults aged >80 years by venipuncture before and at additional timepoints indicated in Fig. 1 after primary and secondary vaccination by injection of Tozinameran (BNT162b2 vaccine, Comirnaty) in the deltoid muscle, at a vaccination centre in Marburg, Germany (Extended Data Table 1). On the basis of the lack of appropriate response to routine vaccination, the vaccination centre decided to vaccinate 5 individuals a third time with BNT162b2 vaccine 16 weeks after day 0, and blood samples were again obtained immediately before and 2 weeks after the third vaccination.

Analyses were performed between March and May 2021 and in August 2021 for third vaccination candidates. In May 2021 the study also obtained samples from unvaccinated elderlies >80 years of age living in a retirement home who have recovered from COVID-19 after an outbreak with SARS-CoV-2 variant B.1.221 in January 2021. All donors provided informed consent to participate in the study. Charlson comorbidity index[10] was calculated according to https://www.mdcalc.com/charlson-comorbidity-index-cci#evidence.

Sample processing and clinical lab. Blood serum was isolated from Serum Separator Clot Activator tubes (Greiner Bio-One, Germany) according to the manufacturer's instructions and stored at −80 °C until analysis.

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh heparinized whole blood by density gradient centrifugation over Pancoll human (Pan Biotech) after dilution with an equal volume of PBS at room temperature. PBMCs were washed twice (500 × *g*, 10 min, 4 °C) in cold PBS supplemented with 0.2% BSA, counted manually, and resuspended in RPMI 1640 media (Gibco, Life Technologies) supplemented with penicillin, streptomycin and 10% human AB serum (all from Sigma) at 5×10^6 cells per ml.

Assessment of antigen-specific T cells. Antigen-reactive T-cell responses were analysed using a protocol based on previous work[11]. A detailed protocol is given in the Supplementary Information. Briefly, 5×10^6 PBMCs were stimulated with either SARS-CoV-2 spike protein peptide mix (wild type, Miltenyi Biotec), SEB (0.7 µg ml^{-1}, kindly provided by Prof. Bernhard Fleischer, Bernhard Nocht Institute of Tropical Medicine, Hamburg, Germany), or with an equal volume of water as a control, in the presence of anti-CD28 (5 µg ml^{-1}) and monensin (1 µg ml^{-1}) for 12 h. Brefeldin A (1 µg ml^{-1}) was added 2 h after the start of the stimulation. The stimulation was stopped by adding 2 nM EDTA. Dead-cell labelling was performed by resuspending the cell pellet in 500 µl PBS supplemented with 1:1,000 amine reactive Zombie Aqua Fixable Viability dye (Biolegend), and PBMCs were fixed for 20 min using 2% formaldehyde solution (Thermo Scientific). Thereafter, cells were stained with a cocktail of antibodies as detailed in the supplementary section. Acquisition was performed on a MACSQuant 16 flow cytometer (Miltenyi Biotec).

FlowJo version 10 (BD) and OMIQ.ai were used for analysing flow cytometry data. Flow cytometry standard files underwent quality control and, where applicable, anomaly removal by FlowAI[12].

Quantification of SARS-CoV-2-specific antibodies. Serum antibodies against the recombinantly expressed S1 subunit of the SARS-CoV-2 spike protein were quantified using the Anti-SARS-CoV-2-QuantiVac-ELISA run on the automated EuroLab Workstation (Euroimmun, Germany), following the manufacturer's protocol. Sera exceeding the detection range of the assay were pre-diluted 1:10 or 1:100 and measured again. Results obtained in relative units per ml were converted into binding antibody units (BAU) per ml by multiplication with the factor 3.2, according to the manufacturer's instructions. Results in BAU ml^{-1} were calibrated against the 'First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136)'. The lower cut-off for this assay is at 35.2 BAU ml^{-1}.

SARS-CoV-2 neutralization tests (VNT 100). Human sera were heat-inactivated for 30 min at 56 °C and diluted in a two-fold dilution series in 96-well cell culture plates (1:4 to 1:512). One hundred plaque-forming units (PFU) of SARS-CoV-2 were added in the same volume to the serum dilutions. The following SARS-CoV-2 virus isolates were used: German isolate BavPat1/2020; European Virus Archive Global #026 V-03883 (Genbank: MZ558051.1) and the Delta variant, B.1.617.2. The sequence of the viruses was confirmed. Following incubation at 37 °C for 1 h, approximately 20,000 Vero C1008 cells (ATCC, Cat. no. CRL-1586, RRID: CVCL_0574) were added. Plates were then incubated at 37 °C with 5% CO_2, and cytopathic effects were evaluated at day 4 or day 6 (Delta variant) post infection. Neutralization was defined as the absence of cytopathic effects in the serum dilutions. The reciprocal neutralization titre was calculated from the highest serum dilution without cytopathic effects as a geometric mean based on three replicates. The lower detection limit of the assay is 8 (reciprocal titre), corresponding to the first dilution of the respective serum. Two positive controls were used as inter-assay neutralization standards and quality control for each test. Neutralization assays were performed in the BSL-4 laboratory of the Institute of Virology at Philipps University Marburg, Germany.

Virus-specific antibodies. IgG antibodies against measles and VZV were quantified using the commercial Siemens Enzygnost IgG ELISA kit that was run on an automated Siemens BEPIII system. Values were quantified using the alpha method, according to the manufacturer's instructions. The cut-off for VZV-specific IgG was 50 mIU ml^{-1}, and 150 mIU ml^{-1} for measles-specific IgG.

Statistical analysis and reproducibility. Prism version 9 (GraphPad software) was used to display data, and perform descriptive statistics and significance testing.

To determine differences between the two cohorts (older adults vs young), a two-tailed Mann–Whitney test was applied to evaluate the level of significance. For assessing donor-specific responses over time (5 low-/non-responders), the one-tailed Wilcoxon matched-pairs signed-rank test was used. To test for differences in age, CCI and sex between responders and low-/non-responders, we applied the unpaired two-tailed *t*-test, the Wilcoxon rank-sum test with continuity correction and the Fisher's exact test, respectively. No statistical method was used to predetermine sample size.

Assessment of T cells after antigen-specific stimulation was performed in a total of 29 independent batches, with up to 30 donor samples per batch. Data of a SEB-stimulated control sample included in each batch showed consistent results across all batches. Quantification of SARS-CoV-2-specific antibodies was run according to clinical routine standards, including all required calibrators and controls. Data from 5 vaccinees were excluded, on the basis of their decision to decline study participation, death (not related to the study) or previous COVID-19 infection. Only age-group assignment was available to the investigators during experiments and outcome assessment. Moreover, the experiments and analyses were performed in three independent laboratories. The experiments were not randomized regarding age of the vaccinees.

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

Data availability statement

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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Author contributions

A.J.R.-O. and A.R.S. organized and performed experiments, and analysed data. A.J.R.-O., A.R.S., D.D.G., C.K. and S.H. created the figures. D.D.G., C.K., S.H. and V.H. collected blood. I.V. and H.H. performed flow cytometry analysis. D.S., B.C., A.J.R.-O. and S.H. prepared cells. S.H., C.M., C.K. and S.S. were involved in antibody analyses. V.K., H.M.-K. and M.W. performed virus neutralization assays. H.E.M., C.K. and M.L.

designed the study and recruited the study population. H.E.M., C.K., A.J.R.-O., A.R.S., D.D.G. and M.L. critically discussed the data. M.L. wrote the manuscript. H.E.M., C.K., A.J.R.-O. and A.R.S. revised the manuscript.

Competing interests

The funders had no role in the design or conduct of the study, or in the decision to submit the manuscript for publication. The authors declare no competing interests.

Additional information

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Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41564-021-01046-z.

Correspondence and requests for materials should be addressed to Michael Lohoff.

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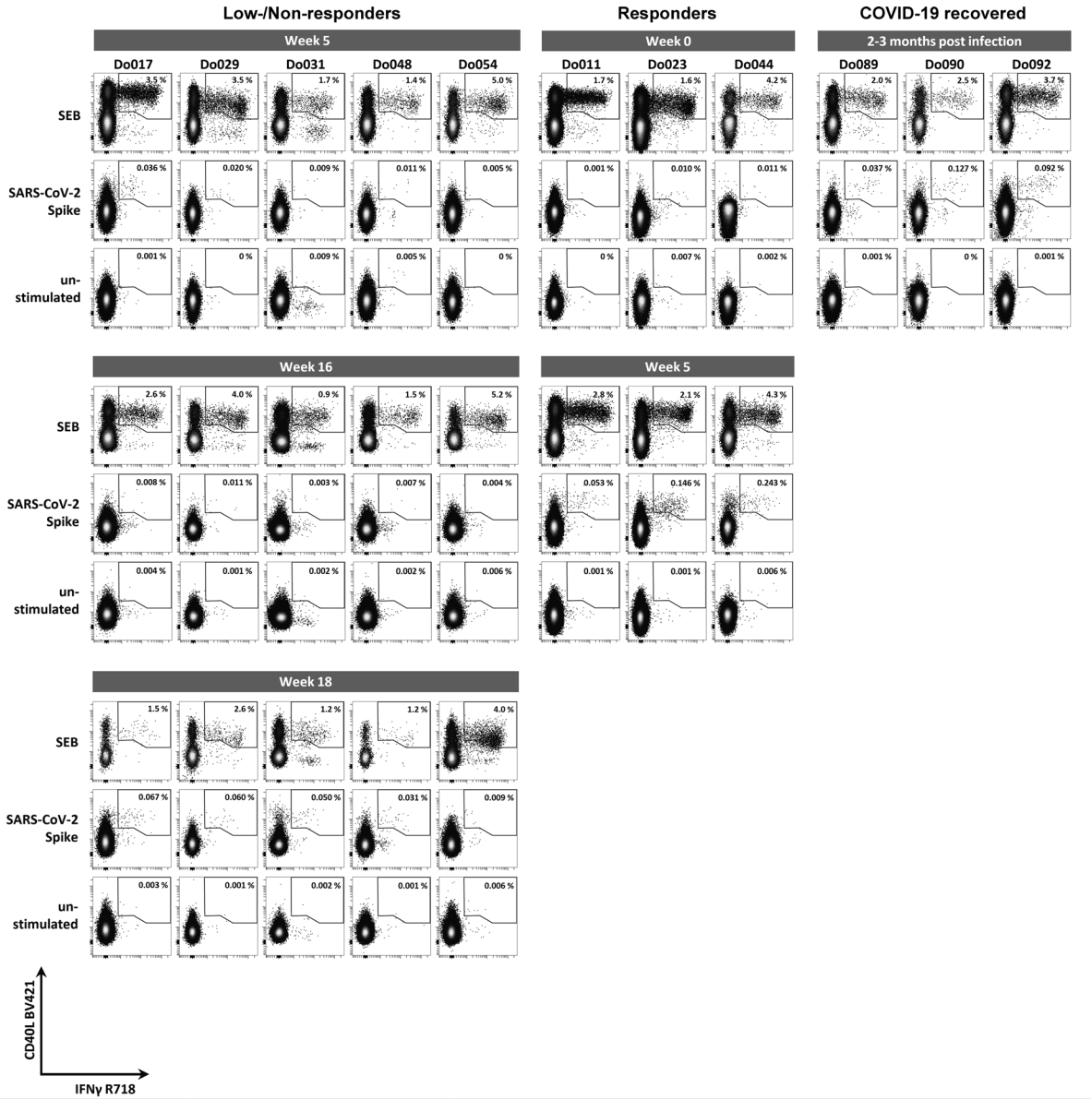
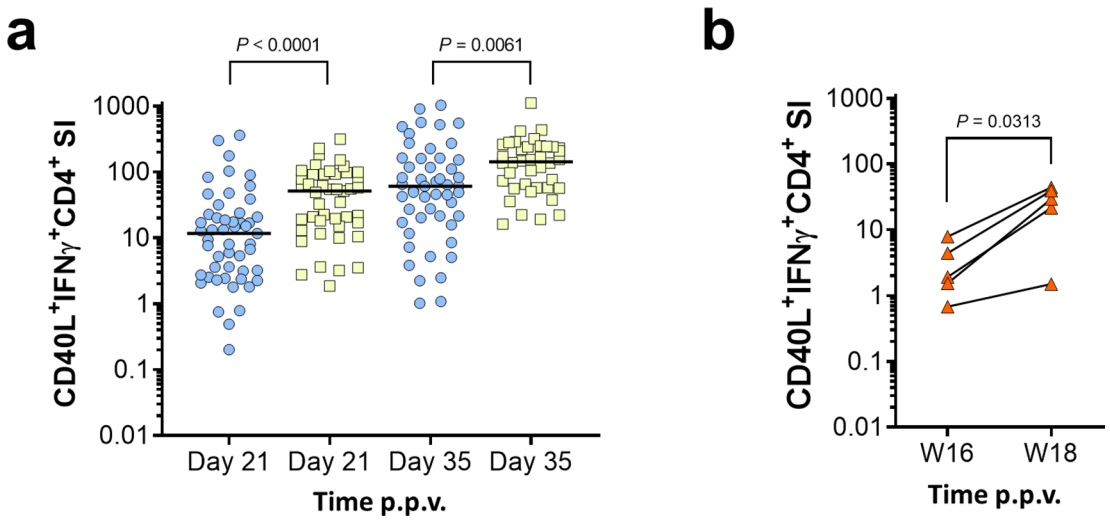
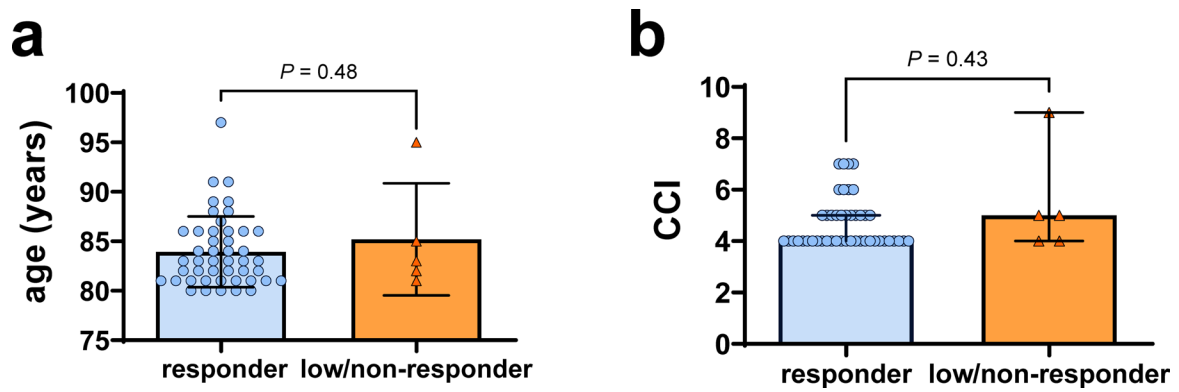


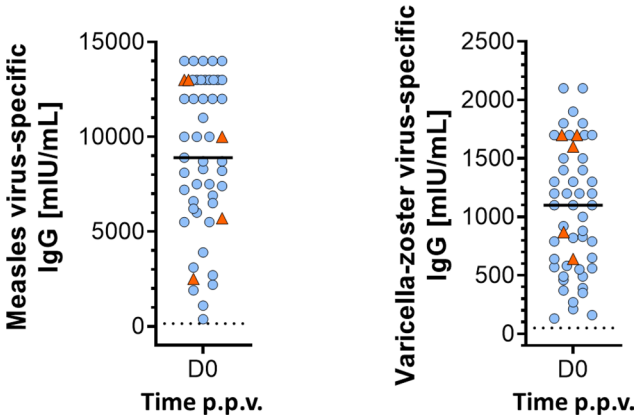
Extended Data Fig. 1 | Multi-parametric flow cytometry of T cells from individuals who received the BNT162b12 vaccine or overcame COVID-19. Peripheral blood CD4 T cell responses to SARS-CoV2 spike peptide pool, SEB, and control conditions (unstimulated) of representative responders and the five low/non-responders to two-dose BNT162b2 vaccination, and of reconvalescent COVID-19 patients were analyzed by flow cytometry at the indicated time points. All plots show data of gated CD4 T cells also stained for CD40L and IFNγ as markers of activated T cells. The frequency of CD40L/IFNγ co-expressing cells among total CD4 T cells is indicated in each plot.



Extended Data Fig. 2 | Stimulation index for SARS-CoV-2-spike specific T cells. (a) Percentages of SARS-CoV-2 spike-specific CD4 T cells were analyzed in 51 donors aged >80 years (blue symbols) and 45 donors aged 20–42 plus one 53 years old (yellow symbols) 21 d after the first and 14 d after the second BNT162b2 (Pfizer-Biontech) vaccination (day 35). (b) Five low-/non-responders (red triangles) received a third BNT162b2 dose 16 weeks (W16) after the first vaccination and T cell responses were analyzed in week 18 (W18). Stimulation index (SI) was obtained by dividing the percentages of spike-reactive T cells and those activated under control conditions. Each symbol represents one donor. Horizontal lines indicate medians. P values determined by two-tailed Mann-Whitney test (a) or one-tailed Wilcoxon matched-pairs signed rank test (b). Time p.p.v., Time post primary vaccination.



Extended Data Fig. 3 | Age and Comorbidity in BNT162b2 responders and low-/non-responders. (a) Age distribution of responders (n = 46) and low/non-responders (n = 5). Mean ± SD; P = 0.48 by unpaired two-tailed t test. (b) Distribution of Charlson comorbidity index (CCI) scores in responders (n = 46) and low/non-responders (n = 5). Median ± 95% confidence interval; P = 0.43 by Wilcoxon rank sum test with continuity correction. Each symbol represents one donor.



Extended Data Fig. 4 | Humoral immunity to measles virus and varicella-zoster virus in older adults. Antibody titers towards measles virus (MV) and varicella-zoster virus (VZV) in the elderly cohort (51 participants as in Fig. 1) determined at day 0 (before receiving the first dose of the BNT162b2 mRNA vaccine). Each symbol represents one donor. Blue dots indicate aged responders, red triangles low/non-responders to the SARS-CoV-2 spike glycoprotein. Horizontal lines indicate medians, dashed lines indicate the cutoff for antibody positivity at 150 mIU / mL for MV-specific and 50 mIU / mL for VZV-specific IgG. Time p.p.v., Time post primary vaccination.

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Corresponding author(s): Prof. Dr. Michael Lohoff

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☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	The acquisition of flow cytometry data was controlled using MACSQuantify 2.13.1 (Miltenyi Biotec).
Data analysis	FlowJo version 10 (BD, Ashland, OR) and OMIQ.ai (Santa Clara, CA) were used for analyzing flow cytometry data. Flow cytometry standard (FCS) files underwent quality control and, where applicable, anomaly removal by FlowAI. For serum IgG, results in BAU / mL are calibrated against the "First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136)". The lower cutoff for this assay is at 35.2 BAU / mL. Prism version 9 (GraphPad software, San Diego, CA) was used to display data, and perform descriptive statistics and significance testing. Analyses using OMIQ.ai (https://app.omiq.ai) were performed between March and November 2021.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Original data are available from the authors upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	102 participants from which: 56 elderly (>80 years old) were selected based on accessibility at the vaccination center that we could follow up and who accepted to join the study. We planned to have at least the same donor number as controls (20-44 years old). 46 individuals accepted the invitation to participate. Statistical differences between the elderly and young groups showed to be highly significant and therefore we were confident on the sample size.
Data exclusions	Data from vaccinees who later decided to decline, or due to death (not related to the study), or had previous COVID-19 infection was excluded. We primarily excluded vaccinees with suspected infection on day 0.
Replication	T cell assay: All T cell measurements were performed once. Stimulation experiments and subsequent flow cytometry were performed in a total of 29 independent batches, with up to 30 donor samples per batch. Data of a control sample included in each batch showed consistent results across all batches. Antibody assays: Antibody tests for spike-specific IgG were run in our DIN EN 15189-accredited laboratory in a quality-managed environment. Sera were tested in one replicate; sera reactive above the upper threshold of the test were repeated in 1:10 or 1:100 dilutions; all replications in dilution were successful.
Randomization	This is an observational study.
Blinding	Donors received vaccinations open-label as part of routine healthcare. Blood sampling, processing and stimulation were performed in one lab, antibody measurements in a second independent lab, and cytometric analyses in a third independent lab. Only age group assignment (young or old), but not the respective names, were available at the time of analysis. Samples were analyzed and data were evaluated before joint analyses.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Brilliant Violet 421 conjugated anti-human CD40L (Biolegend, 310824, 24-31, 1:100),
Brilliant Violet 510 conjugated anti-human CD19 (Biolegend, 302242, HIB19, 1:50),
Brilliant Violet 510 conjugated anti-human CD123 (Biolegend, 306022, 6H6, 1:50),
Brilliant Violet 510 conjugated anti-human CD33 (Biolegend, 303422, WM53, 1:400),
Brilliant Violet 570 conjugated anti-human CD8a (Biolegend, 301038, RPA-T8, 1:100),
PE/Cy7 conjugated anti-human CD4 (Biolegend, 300512, RPA-T4, 1:400),
BD Horizon R718 conjugated anti-human IFN-γ (BD Biosciences, 566959, B27, 1:100),
purified anti-human CD28 (Biolegend, 302943, CD28.2, 1:500)

Validation

Antibody clones were selected based on validation data shown on the vendors´ websites. Working dilutions of antibodies were optimized based on maximum staining index. Spillover compensation was optimized, and the specificity of the stainings were confirmed using single antibody stainings, FMO controls, and unstained control cells.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

State the source of each cell line used.

Authentication

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.

Mycoplasma contamination

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.

Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Old Donors: 80-97 years old. Gender: 33 Female and 23 male.

Population characteristics	Young Donors: 20-44 and one 53 years old. Gender: 29 Female and 17 male.
Recruitment	The Aged vaccinees were recruted in the vaccination center in Marburg, recovered elderly (donors 89, 90 and 92) in two retired homes with known previous corona outbreak and the young vaccinees were recruited in the doctor`s office of a general practicioner in Cölbe. Donors were approached and selected by the medical doctors involved in the study according to combinations of a priori defined inclusion criteria, that is, age, history or no history of COVID-19, and being scheduled for vaccination with BNT162b2. We are not aware of potential bias related to the recruitment. All donors provided informed consent to participate in the study.
Ethics oversight	Ethics committee of the medical faculty of the Philipps-University Marburg (study number 40/21-12032021).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*

Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	PBMC were isolated from fresh heparinized whole blood by density gradient centrifugation over Pancoll human (Pan Biotech, Aidenbach, Germany) after dilution with an equal volume of PBS at room temperature. PBMC were washed twice (500 x g, 10 min, 4 °C) in cold PBS supplemented with 0.2% BSA, counted manually, and resuspended in RPMI 1640 media (Gibco, Life Technologies, Carlsbad, CA) supplemented with penicillin, streptomycin, and 10% human AB serum (all Sigma, St. Louis, MO) at 5 x 106 cells / mL. 500 µL media containing 5,000,000 PBMC were transferred into 12 mL round-bottom tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) and stimulated with either SARS-CoV-2 spike protein peptide mix (wildtype, Miltenyi Biotec), SEB (0.7 µg / mL, kindly provided by Prof. Bernhard Fleischer, Bernhard Nocht Institute of Tropical Medicine, Hamburg, Germany), or with an equal volume of water as a control, in the presence of anti-CD28 (5 µg / mL) and monensin (1 µg / mL) for 12 hours under humid conditions in a 5 % CO2 atmosphere. Brefeldin A (1 µg / mL) was added 2 hours after the start of the stimulation. The stimulation was stopped by adding 2 nM EDTA. PBMC were harvested, transferred to a new 15 mL centrifuge tube, washed with 10 mL PBS / 0.2 % BSA, and pelleted for 10 min at 490 x g, at 4 °C. Dead cell labeling was performed by resuspending the cell pellet in 500 µL PBS supplemented with 1:1000 amine reactive Zombie Aqua™ Fixable Viability dye (Biolegend), incubated for 20 min in the dark at room temperature. PBS / 0.2 % BSA was added to quench the remaining reactive dye. After washing with 2 mL PBS / 0.2 % BSA, and pelleting for 10 min, at 490 x g, at 4 °C, PBMC were fixed for 20 minutes using 2% formaldehyde solution (Thermo Scientific, Germany) in the dark, washed with 2 mL PBS / 0.2 % BSA, and pelleted for 10 min at 490 x g, at 4 °C, and resuspended in 2 mL PBS / 0.2 % BSA. PBMC were spun down (700 x g, 10 min, 4 °C), supernatants were aspirated, cell pellets resuspended in 200 µL PBS / 0.2 % BSA and transferred into a V-bottom-96 well plate (Sarstedt), and centrifuged (700 x g, 5 min, 4 °C). After discarding the supernatants, pellets were resuspended in 50µL Brilliant Staining Buffer (Biolegend) supplemented with antibodies including anti-CD4-PECy7 for the detection of CD4 T cells, anti-CD8-BV570, and anti-CD19/123/33-BV510 (all from Biolegend) for the exclusion of CD8 T cells, monocytes and other myeloid cells, B cells, basophils, and plasmacytoid dendritic cells; anti-CD27-StarBright Blue 700 (Bio-Rad, Hercules, CA), and anti-CD45RA-PerCp (Miltenyi), and incubated 30 minutes in the dark, at room temperature. Afterwards, cells were washed once with 200 µL PBS / 0.2% BSA, and centrifuged (700 x g, 5 min, 4 °C). Cell pellets were then washed twice in 200µL permeabilization buffer (diluted using Millipore water from 10 x concentrated stock buffer, ThermoFisher, Waltham, MA), and finally resuspended in 50 µL permeabilization buffer supplemented with antibodies targeting CD40L (conjugated to BV421, Biolegend), and intracellular molecules including anti-IFNg-R718 (BD Biosciences, San Jose, CA), and incubated for 30 minutes at room temperature in the dark. Then, cells were washed once in 200µL permeabilization buffer, once in PBS / 0.2% BSA, and stored at 4 °C in PBS / 0.2 % BSA until acquisition.
Instrument	MACSQuant 16 flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany).
Software	The acquisition of flow cytometry data was controlled using MACSQuantify 2.13.1 (Miltenyi Biotec). FlowJo version 10.7 (BD, Ashland, OR) and OMIQ.ai (Santa Clara, CA) were used for analyzing flow cytometry data. Flow cytometry standard (FCS) files underwent quality control and, where applicable, anomaly removal by FlowAI.

Cell population abundance	Antigen-reactive CD4 T cells (ART) were defined as CD40L+ IFNg+ expressing cells after spike peptide stimulation, and their frequencies were determined among total CD4 T cells. Abundances of this population ranged between 0.0001% and 1%.
Gating strategy	Data of live CD4+ T lymphocytes were gated according to FSC and SSC parameters and their CD4+CD8-CD19-CD123-CD33-LD-Aqualow/- phenotype. Cell aggregates were removed by gating according to FSC-H/FSC-A and SSC-H/SSC-A parameters. Antigen-reactive CD4 T cells (ART) were defined as CD40L+ IFNg+ expressing cells after spike peptide stimulation, and their frequencies were determined among total CD4 T cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
Volume censoring	*Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings	*Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*
Effect(s) tested	*Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	*Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*
Correction	*Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity	*Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*
Graph analysis	*Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*
Multivariate modeling and predictive analysis	*Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*