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Article

A COVID-19 peptide vaccine for the induction of SARS-CoV-2 T cell immunity

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T cell immunity is central for the control of viral infections. CoVac-1 is a peptide-based vaccine candidate, composed of SARS-CoV-2 T cell epitopes derived from various viral proteins[1,2], combined with the Toll-like receptor 1/2 agonist XS15 emulsified in Montanide ISA51 VG, aiming to induce profound SARS-CoV-2 T cell immunity to combat COVID-19. We conducted a phase I open-label trial, recruiting 36 participants aged 18 to 80 years, who received one single subcutaneous CoVac-1 vaccination. The primary endpoint was safety analysed until day 56. Immunogenicity in terms of CoVac-1-induced T-cell response was analysed as main secondary endpoint until day 28 and in the follow-up until month 3. No serious adverse events and no grade 4 adverse events were observed. Expected local granuloma formation was observed in all study subjects, while systemic reactogenicity was absent or mild. SARS-CoV-2-specific T cell responses targeting multiple vaccine peptides were induced in all study participants, mediated by multifunctional T-helper 1 $CD4^+$ and $CD8^+$ T cells. CoVac-1-induced interferon-γ T cell responses persisted in the follow-up analyses and surpassed those detected after SARS-CoV-2 infection as well as after vaccination with approved vaccines. Furthermore, vaccine-induced T- cell responses were unaffected by current SARS-CoV-2 variants of concern (VOC). Together, CoVac-1 showed a favourable safety profile and induced broad, potent and VOC-independent T- cell responses, supporting the presently ongoing evaluation in a phase II trial for patients with B cell/antibody deficiency.

The Coronavirus Disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is linked to the death of millions of people[3]. As predominantly individuals with medical comorbidities are affected severely[4], vaccines inducing long-lasting immunity, particularly in those high-risk populations, are needed[5–7].

CoVac-1 is a multi-peptide-based vaccine candidate designed to induce, upon one single vaccination, a broad and long-lasting SARS-CoV-2 T-cell immunity resembling that acquired by natural infection, which is not affected by evolving viral variants of concern (VOC). Thus, CoVac-1 is composed of multiple SARS-CoV-2 human leukocyte antigen (HLA)-DR T-cell epitopes derived from various viral proteins (spike, nucleocapsid, membrane, envelope, open reading frame (ORF) 8) that were proven to be (i) frequently and HLA-independently recognized by T cells in COVID-19 convalescents, (ii) of pathophysiological relevance for T-cell immunity to combat COVID-19, and (iii) to mediate long-term immunity after infection[1,2]. CoVac-1 vaccine peptides are adjuvanted with the novel toll-like receptor (TLR) 1/2 agonist XS15 emulsified in Montanide™ ISA51 VG, which endorse activation and maturation of antigen presenting cells and prevent vaccine peptides from immediate degradation, enabling the induction of a potent T-cell response[8–10].

T cells play an important role for COVID-19 outcome and maintenance of SARS-CoV-2 immunity, even in absence of humoral immune responses[1,11–19]. Thus, the induction of SARS-CoV-2 T-cell immunity is a central goal for vaccine development and of particular importance

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for patients with congenital or acquired B-cell deficiencies. The latter comprise cancer patients with disease or treatment-related immunoglobulin deficiency, who develop only limited humoral immunity after infection or vaccination and persist with a high risk for a severe course of COVID-19[20–22].

We here report the results of the open-label first-in-human Phase I trial recruiting adults aged 18-80 years, to evaluate safety, reactogenicity, and immunogenicity of CoVac-1.

Results

Participants

From November 28th, 2020 to January 15th, 2021, 12 healthy adults were enrolled in Part I (age group 18-55 years) including sentinel dosing in the first participant. From March 24th, 2021 to April 1st, 2021, 24 adults were enrolled in Part II (age group 56-80 years). 33% and 50% of Part I and II participants, respectively, were female. The median participant age was 38.0 (range 23-50) and 62.0 (range 56-70) years for Part I and II, respectively. All participants received one dose of CoVac-1 on day 1 and were available for immunogenicity and safety analyses until day 28 (follow-up until month 3) and day 56, respectively (Extended Data Fig. 1). No major protocol violations occurred. Analyses of follow-up safety and long-term immunogenicity data (until month 6) are ongoing. Demographic and clinical characteristics of the participants are provided in Table 1.

Safety and reactogenicity

Data regarding solicited and unsolicited adverse events (AEs) were available for all participants from diary cards (for 28 days after vaccination) and safety visits (until day 56). No participant discontinued the trial because of an AE. No serious AEs and no grade 4 AEs were reported. Reactogenicity in terms of solicited AEs occurred in all participants (Fig. 1). Events were mild to moderate (grade 1 to 2) in 81% of participants. All participants showed expected formation of a granuloma/induration at the injection site, which persisted beyond day 56. Severe AEs (grade 3) comprised local erythema in 19%, accompanied by severe swelling in 6% of all participants. Grade 3 AEs resolved within 2 days (median, range 1-7). 22% of participants reported localized inguinal lymphadenopathy. Local skin ulceration at the vaccination site was reported by 25% of participants, with two participants in Part II showing a grade 2 ulceration. Ulcerations in terms of small skin defects occurred between day 28 and day 56 and healed within 20 days (median, range 15-23) until day 56, none requiring any surgical intervention or drug treatment. No difference of local solicited AEs was observed between Part I and Part II participants (Extended Data Table 1). No fever or other inflammatory systemic solicited AEs were reported. Other systemic solicited AEs occurred in 39% of all participants with no differences observed between Part I and II participants (Extended Data Table 1). All reported systemic solicited AEs were mild, with transient fatigue being reported by 31% of participants.

No clinically relevant changes in laboratory values were reported. In 31% of participants, acute phase reaction with elevated C-reactive protein was observed.

58 unsolicited AEs occurred which were predominantly mild (81%, Extended Data Table 2). Viral re-activations (Varicella-Zoster and Herpes Simplex virus) were reported by two participants (≤ grade 2) in Part II of the trial.

Until day 56, no SARS-CoV-2 infection or immune-mediated medical condition was observed in any participant.

Immunogenicity

Immunogenicity of CoVac-1 was determined in terms of $CD4^+$ and $CD8^+$ T-cell responses to the six SARS-CoV-2 HLA-DR vaccine T-cell epitopes as well as to embedded HLA class I-binding peptides (Supplementary Table S1) using interferon (IFN)-γ enzyme-linked immunospot (ELISPOT) assays. T-cell responses were assessed in all participants at baseline (day 1), on day 7, day 14, and day 28, as well as in the follow-up period on day 56 and month 3 after vaccination. None of the participants showed preexisting SARS-CoV-2 T-cell responses *ex vivo* at baseline. Vaccine-induced IFN-γ T-cell responses were observed in 100% of participants in Part I and Part II on day 28, showing a ≥ 200-fold and ≥ 100-fold increase (median calculated spot counts 2 (day 1) to 450 (day 28) and 2 (day 1) to 325 (day 28)) from baseline, respectively (Fig. 2a). Vaccine-induced T-cell responses targeted multiple CoVac-1 peptides with median 5/6 peptides recognized by participants' T cells on day 28 (Fig. 2b, Extended Data Fig. 2). The CoVac-1 peptide P6_ORF8 derived from the ORF8 of SARS-CoV-2 showed most frequently induced T-cell responses after vaccination (97%), followed by P5_mem and P4_env (both 94%), P3_spi (89%), P1_nuc (61%), and P2_nuc (58%, Extended Data Fig. 2). CoVac-1-induced T-cell responses persisted in the follow-up analyses until month 3 in all participants. Intensity of IFN-γ T-cell response decreased *ex vivo* in Part I participants over time, but equivalent expandability of CoVac-1-induced T cells was observed in both, Part I and Part II participants, at month 3 compared to day 28 post vaccination (Extended Data Fig. 3a). Intensity of CoVac-1-induced IFN-γ T-cell responses in participants of Part I and Part II at day 28 and day 56 (pCoVs (n = 24), median 488 and 319 calculated spot counts, respectively) was up to 39 times higher compared to T-cell responses against CoVac-1 vaccine peptides (median 13) as well as to previously described SARS-CoV-2-specific (median 29) and cross-reactive (median 35) T-cell epitopes[1,2] in age-matched human COVID-19 convalescents (HCs) collected 16-52 days after positive SARS-CoV-2 real-time polymerase chain reaction (PCR, Fig. 2c, Supplementary Table S2). Titration with decreasing peptide concentrations (2.5 µg/mL to 0.1 ng/mL) revealed detection of CoVac-1 peptides by vaccine-induced T cells down to 1 ng/mL (10 ng/mL 5/5 pCoVs, 1 ng/mL 3/5 pCoVs). This was lower than the detection limits of SARS-CoV-2-specific T cells in HCs for CoVac-1 vaccine peptides (10 ng/mL 4/5 HCs, 1 ng/mL 0/5 HCs), SARS-CoV-2-specific (10 ng/mL 5/5 HCs, 1 ng/mL 0/5 HCs), and cross-reactive T-cell epitopes (10 ng/mL 2/5 HCs, 1 ng/mL 0/5 HCs; Extended Data Fig. 3b). Intensity of CoVac-1-induced IFN-γ T-cell responses (pCoVs, median 488 calculated spot counts) exceeded spike-specific T-cell responses induced by mRNA- (median 141) adenoviral vector-based (median 24) as well as heterologous vaccination (median 98) assessed 18-42 days after the second vaccination (Extended Data Fig. 3c, Supplementary Table S3).

In vitro expansion of CoVac-1-specific T cells revealed preexisting low-frequency T-cell responses to single vaccine peptides at baseline in 61% of participants that could be boosted at least 2-fold by CoVac-1, as observed on day 28 in all but one participant (Extended Data Fig. 4).

CoVac-1-induced $CD4^+$ T cells displayed a multifunctional T helper 1 (Th1) phenotype with positivity for IFN-γ, tumor necrosis factor (TNF), interleukin-2 (IL-2), and CD107a (Fig. 2d). Magnitude of CoVac-1-induced $CD4^+$ T-cell responses did not differ between Part I and Part II participants and was up to 40 times higher compared to SARS-CoV-2-specific $CD4^+$ T-cell responses of HCs (0.42% vs. 0.01% (median positive samples) CoVac-1-specific IFN-γ^+CD4^+ T cells Part II participants vs. HCs respectively, Fig. 2d, Extended Data Fig. 5a). Frequency of functional $CD4^+$ T cells was increased up to 40-fold after *in vitro* expansion (17.9% vs. 0.44% (median positive samples) CoVac-1-specific TNF^+CD4^+ T cells Part I participants) reaching up to 15 times higher levels compared to expanded CoVac-1-specific T cells of HCs (18.6% vs. 1.23% (median positive samples) CoVac-1-specific TNF^+CD4^+ T cells, Part II participants vs. HCs, respectively), indicating potent expandability of CoVac-1-induced T cells upon SARS-CoV-2 exposure (Extended Data Fig. 5b, c).

Vaccine-induced $CD8^+$ T-cell responses, identified after *in vitro* expansion by tetramer staining and IFN-γ ELISPOT assay with HLA-matched, CoVac-1-embedded, HLA class I peptides (Supplementary Table S1)

were detected in 78% and 80% of participants in Part I and 100% and 95% of participants in Part II with matching HLA allotypes, respectively (Extended Data Fig. 6a, b). CoVac-1-induced $CD8^+$ T cells showed a polyfunctional phenotype reflected by IFN-γ, TNF, IL-2, and CD107a production/expression (Extended Data Fig. 6c).

No relevant differences were observed for immunogenicity parameters between Part I and Part II participants except for the frequency of IL-2 positive CoVac-1-specific $CD4^+$ T cells following 12-day *in vitro* expansion at day 28, which was increased in Part II participants, and for the expandability of CoVac-1 induced T cells at the follow-up time points (day 56 and month 3), which was decreased in Part II compared to Part I participants (Extended Data Table 3).

In addition to T-cell responses, the induction of low-concentration SARS-CoV-2 anti-spike IgG antibodies could be observed in two participants on day 28 (Extended Data Fig. 3d).

Impact of SARS-CoV-2 variants on CoVac-1

The impact of SARS-CoV-2 VOC declared by the World Health Organization as of 1st October, 2021 (B.1.1.7-Alpha, B.1.351-Beta, P.1-Gamma, B.1.617.2-Delta) on CoVac-1 was analyzed comparing CoVac-1 peptides with the corresponding mutated regions of the respective source proteins described for each VOC (Supplementary Table S4). 50% vaccine peptides sequences were not affected by any variant-defining or associated mutation[23–26] (Supplementary Table S4). None of the mutations of P.1-Gamma and B.1.617.2-Delta affect CoVac-1 vaccine peptides. Variant B.1.1.7-Alpha comprises two mutations affecting P2_nuc and P6_ORF8 with a single amino acid change, respectively. Two mutations of B.1.351-Beta affect P3_spi with either one or two amino acid changes (Fig. 3a).

T-cell responses to peptide pools comprising the B.1.1.7 and B.1.351 mutated peptides P2_nuc, P3_spi, and P6_ORF8 were detectable in 100% of Part I and Part II participants with CoVac-1-induced T-cell responses to P2_nuc, P3_spi, and P6_ORF8 wild-type (WT) peptides (Fig. 3b). Albeit intensity of T-cell responses to single peptide variants (P3_spi and P6_ORF8) was reduced compared to WT peptides, intensity of CoVac-1-induced T-cell responses targeting the variant peptide pools was unaffected and at least 10-fold higher than T-cell responses to WT and variant peptide pools observed in HCs (median calculated spot counts 288 pCoVs B.1.1.7, 485 pCoVs B1.351, 13 HCs WT, 14 HCs B1.1.7, 12 HCs B.1.351, Fig. 3c, Extended Data Fig. 7).

Discussion

Our Phase I trial shows that the CoVac-1 vaccine candidate has a favorable safety profile and induces potent T-cell responses after one single vaccination. Local granuloma formation was observed in all study subjects representing an expected and intended local reaction after Montanide-based vaccination[9,27], which enables continuous local stimulation of SARS-CoV-2-specific T cells required for induction of long-lasting T-cell responses without systemic inflammation. Follow-up data until month 3 after vaccination showed persistence of T-cell responses, which is in line with previous experience with XS15-adjuvanted peptide vaccinations[8] and data in SARS-CoV-1 convalescents, where T-cell immunity persisted for up to 17 years[16]. CoVac-1-induced Th1 $CD4^+$ T-cell responses were complemented by multifunctional $CD8^+$ T cells, counteracting the theoretical risk of vaccine-associated enhanced respiratory disease, which has been associated with a T helper 2 (Th2)-driven immune response[28]. The phenotype of CoVac-1-induced T cells resembles that acquired upon natural infection[1,2,11,16], but with higher magnitude compared to the SARS-CoV-2 T-cell responses in HCs as well as compared to spike-specific T-cell responses induced by mRNA-, vector-based and heterologous vaccination[5–7,29], substantiating the profound T-cell immunity induced by CoVac-1. This is further supported by the high diversity of CoVac-1-induced T cells that target multiple vaccine peptides from different viral proteins, which is central for effective anti-viral defense[1,30–32]. These broad T-cell responses induced by CoVac-1 remain unaffected by current SARS-CoV-2 VOC, which were associated with loss of neutralizing antibody capacity in COVID-19 convalescents and after vaccination[33–35].

In single participants, despite negative results in sequential SARS-CoV-2 PCRs, induction of SARS-CoV-2 anti-spike IgG antibodies was documented after vaccination. This might be due to CoVac-1-induced profound $CD4^+$ T-cell responses, which not only stimulate B cells upon virus encounter, but also may boost preexisting cross-reactive SARS-CoV-2 antibodies, which were reported in 3-15% of unexposed individuals[36].

T cell-mediated immunity and in particular $CD4^+$ T cells are indispensable for the generation of protective antibody responses, reinforcement of $CD8^+$ T-cell responses[37–39] as well as direct killing of virus-infected cells[40,41]. The relevance of anti-viral T-cell responses during acute infection and for long-term immunity was also proven for specifically SARS-CoV-2[1,2,13–19]. Moreover, cases of asymptomatic SARS-CoV-2 exposure, as well as reports from patients with congenital B-cell deficiency document cellular immune responses without seroconversion, providing evidence for T-cell immunity in disease control even in the absence of neutralizing antibodies[14,42]. Accordingly, CoVac-1 may well serve as a (complementary) vaccine to induce T-cell immunity, particularly in elderly and immunocompromised individuals with impaired ability to mount sufficient immune responses after SARS-CoV-2 vaccination with currently approved vaccines[20,21].

Limitations of our trial include the small sample size, low ethnic diversity, as well as the not equivalent time points of sample collection in the comparison of vaccine-induced and infection-induced SARS-CoV-2 T-cells.

In conclusion, the safety and immunogenicity results of this trial indicate that CoVac-1 is a promising multi-peptide vaccine candidate for induction of profound SARS-CoV-2 T-cell immunity, which builds the basis for a presently ongoing Phase II study evaluating CoVac-1 in patients with congenital or acquired B-cell defects, including cancer patients after B-cell-depleting therapy and disease-related immunoglobulin deficiency (NCT04954469).

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-04232-5.

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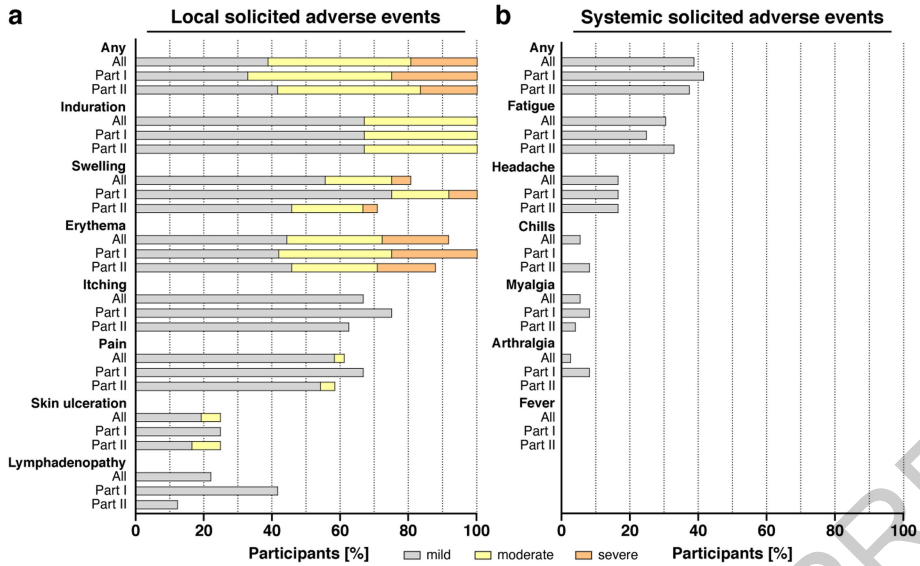

Fig. 1 | Local and systemic solicited adverse events. Related (a) local and (b) systemic solicited AEs within 56 days after vaccination. Severity was graded as mild (grade 1), moderate (grade 2), or severe (grade 3) based on the definition provided in the methods section. Healthy adults of 18-55 years were included in Part I, and participants of 56-80 years were included in Part II.

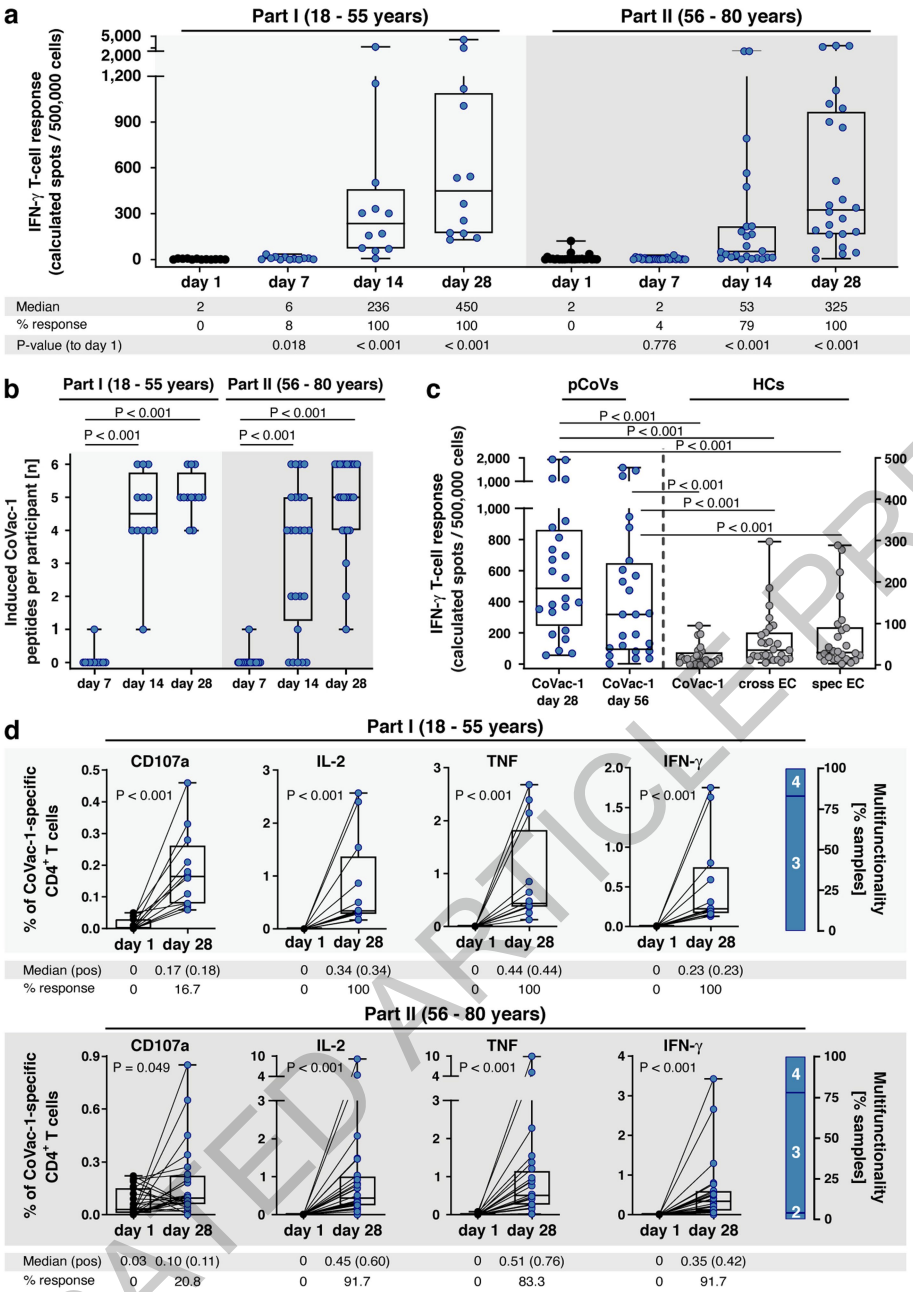

Fig. 2 | CoVac-1-induced T-cell responses. (a-c) CoVac-1-induced T-cell responses assessed *ex vivo* by IFN-γ ELISPOT assays using PBMCs from study participants of Part I (n = 12) and II (n = 24) collected before vaccination (day 1) and at different time points after vaccination (day 7, day 14, day 28 and day 56) or from human convalescents (HCs). (a) The intensity of T-cell responses is depicted as cumulative calculated spot counts (mean spot count of technical replicates normalized to 500,000 cells minus the respective negative control). (b) Number of CoVac-1 T-cell epitopes (n = 6) per participant that elicited a vaccine-induced T-cell response. (c) Intensities of CoVac-1-induced IFN-γ T-cell responses assessed *ex vivo* in Part I and Part II study participants (pCoVs, n = 24, day 28, day 56, left y-axis) compared to T-cell responses detected in HCs (right y-axis) against CoVac-1 vaccine peptides and previously published[1,2] SARS-CoV-2-specific (spec) and cross-reactive (cross) T-cell epitope compositions (ECs, CoVac-1 n = 24, cross EC n = 27, spec EC n = 26). (d) Frequencies of functional CoVac-1-induced $CD4^+$ T cells in study participants prior to vaccination (day 1) and at day 28 following vaccination using *ex vivo* intracellular cytokine (IFN-γ, TNF, IL-2) and surface marker staining (CD107a). The right graph displays the proportion of samples revealing difunctional (2), trifunctional (3), or tetrafunctional (4) T cells. (a-d) Box plots or combined box-line plots show median with 25th or 75th percentiles, and min/max whiskers. (a, b, d) two-sided

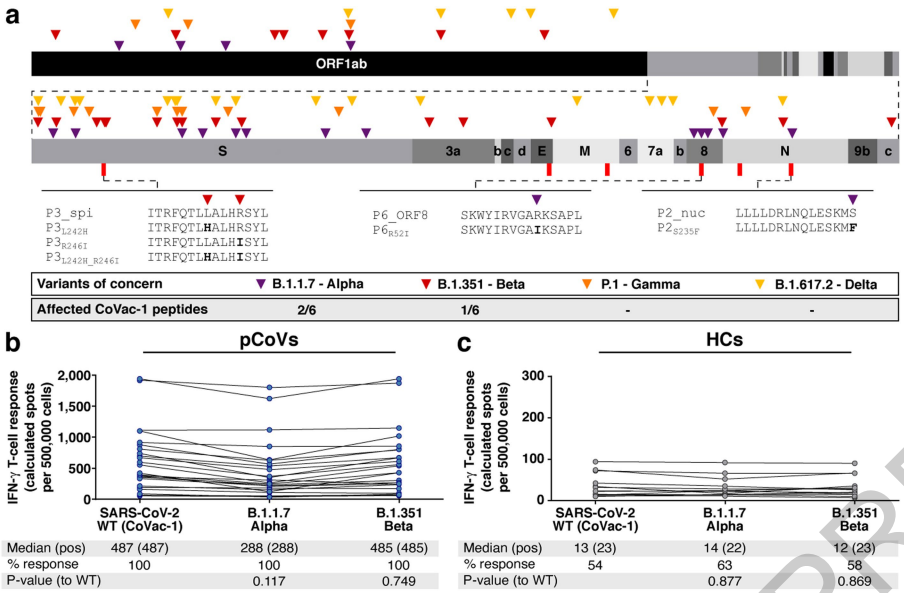

Fig. 3 | Role of SARS-CoV-2 variants of concern (VOC) on CoVac-1 peptides and immunogenicity. (a) Color-coded mutations described for VOC are shown together with corresponding affected CoVac-1 peptides. (b,c) Intensities of T-cell responses (calculated spot counts) to CoVac-1 peptides as well as to the corresponding peptide pools comprising the CoVac-1-affecting mutations of B.1.1.7 and B.1.351 were assessed *ex vivo* by IFN-γ ELISPOT assays using PBMCs from (b) study participants of Part I (n = 12) and Part II (n = 24) collected on day 28 after vaccination (pCoVs) or from (c) human COVID-19 convalescents (HCs). (b,c) two-sided Mann-Whitney U-test. no, number; pos, positive.

Table 1 | Participants' characteristics

Characteristics	All	Part I	Part II
Participants – n	36	12	24
Age – years			
Median	59.5	38.0	62.0
Range	23–70	23–50	56–70
Mean (SD)	54.8 (12.9)	38.7 (8.2)	62.8 (4.1)
Sex – n (%)			
Female	16 (44)	4 (33)	12 (50)
Male	20 (56)	8 (67)	12 (50)
Race – n (%)			
White	36 (100)	12 (100)	24 (100)
Other	0 (0)	0 (0)	0 (0)
Body-mass index (BMI)*			
Median	24.4	24.9	24.4
Range	18.5–30.1	20.1–30.1	18.5–29.3
Relevant preexisting disease† – n (%)			
Hypertension	6 (16.7)	0 (0)	6 (25)
Previous malignant disease	1 (2.8)	0 (0)	1 (4.2)
Mild psoriasis	1 (2.8)	0 (0)	1 (4.2)

Abbreviations: n, number; SD, standard deviation.
*BMI (weight in kg/m^2). Assessment was done at the time of screening
†Relevant preexisting disease includes conditions with increased risk of severe COVID-19 and with higher risk for CoVac-1 side-effects.

Methods

Trial design and oversight

The Phase I trial (ClinicalTrials.gov Identifier: NCT04546841) was designed by and conducted at the Clinical Collaboration Unit (CCU) Translational Immunology, University Hospital Tübingen, Germany. Men as well as nonpregnant women aged 18 to 55 years, without any relevant preexisting conditions and adults aged 56 to 80 years with stable medical conditions were included in Part I and II of the study, respectively. A detailed description of the inclusion and exclusion criteria can be found in the Supplementary Information. Health status was based on medical history and clinical laboratory values, vital signs, and physical examination at screening. Participants with proven history of SARS-CoV-2 infection (real-time polymerase chain reaction (PCR) or antibody test) were excluded. Prior to enrollment, all participants provided their written informed consent. As safety measure, sentinel dosing of the first participant treated in Part I was conducted with a follow-up period of 28 days after vaccination followed by a sponsor safety assessment prior to proceeding with the vaccination of further study participants. Safety assessment of the sentinel dosing participant is described in detail in the Supplementary Information. The trial was open-label without control arm.

The trial was funded by the Ministry of Science, Research and the Arts Baden-Württemberg, Germany. The trial was approved by the Ethics Committee, University Tübingen (537/2020AMG1) and the Paul Ehrlich Institute and performed in accordance with the International Council for Harmonization Good Clinical Practice guidelines.

Safety assessment to proceed to Part II was performed by an independent data safety monitoring board (DSMB).

Trial vaccine and adjuvant

CoVac-1, developed and produced by the Good Manufacturing Practices (GMP) Peptide Laboratory of the Department of Immunology, University Tübingen, is a peptide-based vaccine comprising six HLA-DR-restricted SARS-CoV-2 peptides (Supplementary Table S1) derived from various SARS-CoV-2 proteins (spike, nucleocapsid, membrane, envelope, and ORF 8) and the adjuvant lipopeptide synthetic TLR1/2 ligand XS15[8] (manufactured by Bachem AG, Bubendorf, Switzerland) emulsified in Montanide™ ISA51 VG[9] (manufactured by Seppic, Paris, France). CoVac-1 peptides represent dominant SARS-CoV-2 T-cell epitopes (peptide-specific T-cell responses detected in >50% and up to 100% of convalescents after SARS-CoV-2 infection) validated in human convalescents after SARS-CoV-2 infection to mediate long-term immunity[1,2]. CoVac-1 peptides were predicted and validated to bind to multiple HLA-DR molecules (promiscuous binding)[1], which is important to enable HLA-independent induction of T-cell responses by CoVac-1[1,2,43].

CoVac-1 HLA-DR T-cell epitopes contain embedded HLA class I sequences for induction of both, $CD4^+$ and $CD8^+$ T-cell responses (Supplementary Table S1). CoVac-1 peptides were selected from viral non-surface proteins and their subunits or - in case of the spike protein-derived T-cell epitope P3_spi - from buried/hidden amino acid sequences, which are not accessible for antibodies in their conformational state. The linear 15-amino acid peptides are characterized by a free N-terminal amino group and a free C-terminal carboxy group. All amino acid residues are in the L-configuration and not chemically modified at any position. Synthetic peptides were manufactured by established solid phase peptide synthesis procedures using Fmoc chemistry[44,45].

The novel adjuvant XS15 hydrochloride is a water-soluble synthetic linear, 9-amino acid peptide with a palmitoylated N-terminus (Pam_3Cys-GDPKHPKSF)[8]. Acting as a TLR1/2 ligand, XS15 strongly activates antigen-presenting cells[8] and enables the induction of strong *ex vivo* $CD8^+$ and Th1 $CD4^+$ responses to viral peptides, including SARS-CoV-2 T-cell epitopes, in preliminary *in vivo* analyses in a human volunteer upon a single subcutaneous injection of XS15 mixed to uncoupled viral peptides in a water-in-oil emulsion with Montanide™ ISA51 VG[8,10]. This is the first report of the adjuvant XS15 being used in a human clinical trial. Montanide™ ISA51 VG is a mixture of a highly purified mineral oil (Drakeol 6VR) and a surfactant (Mannide monooleate). When mixed with an aqueous phase in a 50/50 ratio, it forms a water-in-oil emulsion. Such a Montanide-based water-in-oil emulsion has been used as vaccine adjuvant in multiple clinical trials[9,27], to build a depot at the vaccination site thereby preventing vaccine peptides from immediate degradation and thus enhancing the immune response.

CoVac-1 peptides (250 µg/peptide) and XS15 (50 µg) are prepared as a water-oil emulsion 1:1 with Montanide™ ISA51 VG to yield an injectable volume of 500 µL. Each participant received one subcutaneous injection of the CoVac-1 vaccine at the lower abdomen on day 1.

The dosage of CoVac-1 vaccine peptides was determined based on results from various clinical trials evaluating peptide vaccines[44,46–51] (including dose-finding studies for viral T-cell epitopes), which showed significantly stronger immune responses to 250-500 µg versus 100 µg peptide dose, without significantly higher immune responses in the 1,000 µg versus 500 µg dose group[47]. Similar T-cell responses were induced with 250 µg and 500 µg peptide doses. Regarding safety, even doses up to 30 mg per peptide did not raise any concerns[48]. Based on these data, the dose of 250 µg per peptide was used for CoVac-1 vaccine peptides.

The dosage of the TLR1/2 agonist XS15 was determined based on *in vitro* analyses of immune cell activation by TLR1/2. In these assays, 10 µg/mL XS15 was shown to be the most efficient dose for the stimulation of immune cells. Considering the formation of a granuloma after subcutaneous injection of XS15 emulsified in Montanide™ ISA51 VG, which leads to a size-dependent decrease of XS15 concentration[8], 50 µg XS15 were selected to achieve the desired dosage of 10 µg/mL at the vaccination site. In a toxicity study in mice, 50 µg XS15 in Montanide™ ISA51 VG, applied subcutaneously, did not reveal any local or systemic toxicity beyond the long known and expected local toxicity of Montanide[9,27]. For a more detailed description of the dosage rationale for the vaccine peptides and the adjuvant please refer to the Supplementary Information.

Safety assessment

Primary safety outcomes reflect the nature, frequency, and severity of solicited AEs until day 56 after vaccination. The documentation was facilitated by use of a volunteer diary (for 28 day after vaccination) and graded by the investigators according to a modified Common Terminology Criteria for Adverse Events (CTCAE) V5.0 grading scale (Supplementary Table S5). In addition, the number and percentage of participants with unsolicited events until day 56 were reported (documented according to CTCAE V5.0). Safety assessment included clinically significant changes in laboratory values (hematology and blood chemistry), serious adverse events (SAE), and adverse events of special interest (AESI), which included desired induration/granuloma formation, SARS-CoV-2 infection, COVID-19 manifestations, and immune-mediated medical conditions (Supplementary Table S6 and S7).

Immunogenicity assessment

Secondary outcome was the induction of CoVac-1-specific T-cell responses to at least one of the CoVac-1 vaccine peptides evaluated on day 7, day 14, and day 28 by IFN-γ ELISPOT assay *ex vivo* and after *in vitro* T-cell expansion (baseline day 1, prior to vaccination). Follow-up analyses of CoVac-1 induced T-cell responses were performed and on day 56 and month 3 after vaccination. The 12-day *in vitro* expansion of peptide-specific T cells was performed to enable detection of low-frequent, vaccine-induced, and preexisting SARS-CoV-2-specific T cells, as well as to prove the expandability of CoVac-1-induced T cells, which is of central importance for potent T-cell response upon SARS-CoV-2 exposure. In this regard, the characterization of

vaccine-induced CD8$^+$ T cells was performed after 12-day *in vitro* expansion, due to the low frequency of peptide-specific CD8$^+$ T cells observed *ex vivo* (Supplementary Table S8). PBMCs were pulsed with CoVac-1 peptides (5 µg/mL per peptide) and cultured for 12 days adding 20 U/mL interleukin-2 (IL-2, Novartis) on days 3, 5, and 7. For IFN-γ ELISPOT (*ex vivo* or after *in vitro* expansion) cells were stimulated with 1 µg/mL of HLA class I or 2.5 µg/mL of HLA-DR peptides and analyzed in technical replicates. T-cell responses were considered positive if the mean spot count was ≥ 3-fold higher than the mean spot count of the negative control and defined as CoVac-1-induced if the mean spot count post vaccination was ≥ 2-fold higher than the respective spot count on day 1. CoVac-1-induced T-cell responses were further characterized using tetramer (5 µg/mL), cell surface marker, and intracellular cytokine staining (ICS). For ICS, cells were stimulated with 10 µg/mL per peptide. The gating strategy applied for the analyses of flow cytometry-acquired data is provided in Supplementary Fig. S1. Immunogenicity results were compared with HCs with PCR-confirmed SARS-CoV-2 infection and healthy volunteers vaccinated with an approved mRNA- or vector-based vaccine or received heterologous vaccination (Supplementary Table S2 and S3). All assays were conducted in a blinded fashion and are described in detail in the Supplementary Information.

Statistical analysis

The sample size calculation (36 participants) of the trial was based on the assumption that incidence of SAE associated with administration of CoVac-1 does not exceed a predetermined rate of 5%. Safety data are displayed by counting the respective AE that has occurred at least once in a patient. The highest grading of this AE is indicated. Data are displayed as mean ± SD, box plots as median with 25% or 75% quantiles and min/max whiskers. Continuous data were tested for distribution and individual groups were tested by use of Fisher's exact test, unpaired Mann-Whitney-U-test or paired Wilcoxon signed rank test, all performed as two-sided tests. No adjustment for multiple testing was done. Details regarding the statistical analysis plan and sample size calculation are provided in the Supplementary Information and the protocol.

Reporting summary

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

Data Availability

Data supporting the findings of this study including the study protocol and the statistical analysis plan are supplied as source data with this manuscript. Further data, including de-identified participant data are available after final completion of the trial report and are shared according to data sharing guidelines upon reasonable request to the corresponding author J.S.W.

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Author contributions J.S.H., H.-G.R., H.R.S., and J.S.W. were involved in the design of the overall study and strategy. C.M., H.-G.R., I.F., and M.W.L. provided feedback on the study design. T.B., C.T., A.N., Y.M., M.Ro., J.B., J.Ri., and M.W. performed the immunogenicity analyses. J.S.H., M.M., J.Re., S.J., L.A., L.M.W, S.H., A.P., J.K., E.J., R.K., and J.S.W. conducted patient data and sample collection as well as medical evaluation and analysis. J.S.H., M.M., J.Re., S.J., H.R.S., and J.S.W. collected data as study investigators. C.M. and I.F. developed the statistical design and oversaw the data analysis. M.D. and M.Ri. conducted GMP production of CoVac-1. J.S.H., T.B., C.T., M.W.L., H.R.S., and J.S.W. drafted the manuscript. All authors supported the review of the manuscript.

Competing interests A.N., T.B., H.-G.R., and J.S.W. are listed as inventors on a patent related to the SARS-CoV-2 T cell epitopes included in CoVac-1. H.-G.R. is listed as inventor on a patent related to the adjuvant XS15 included in CoVac-1. The other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-021-04232-5.

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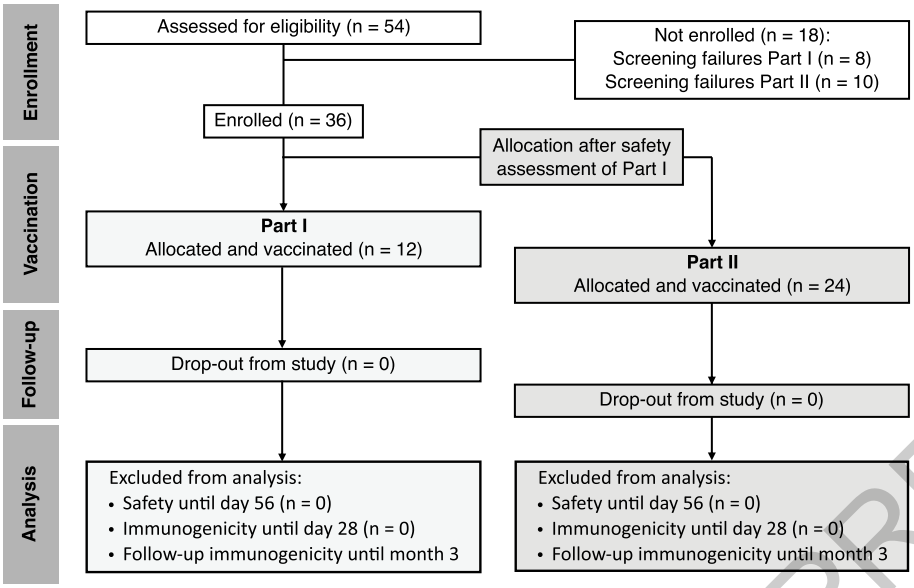
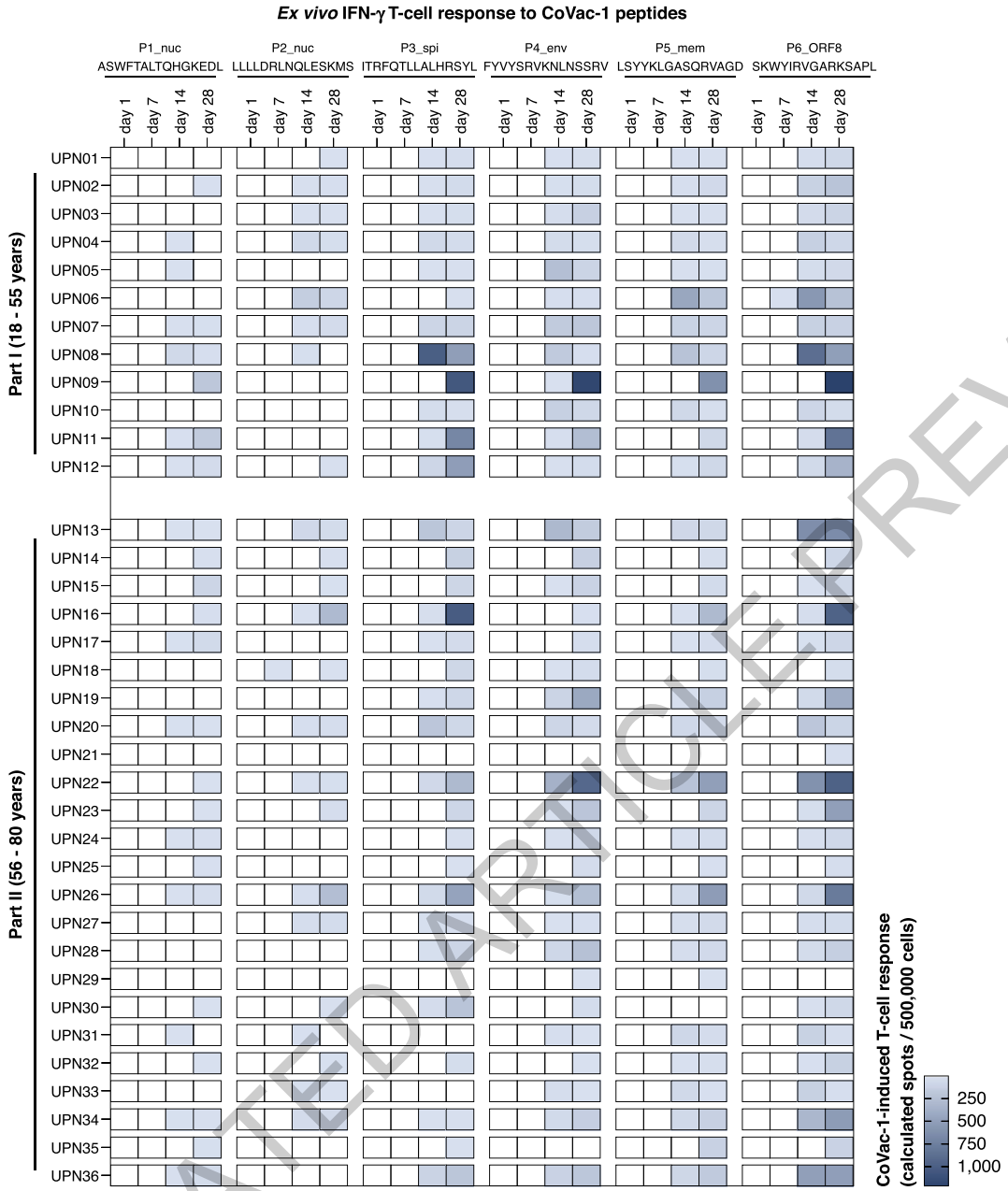
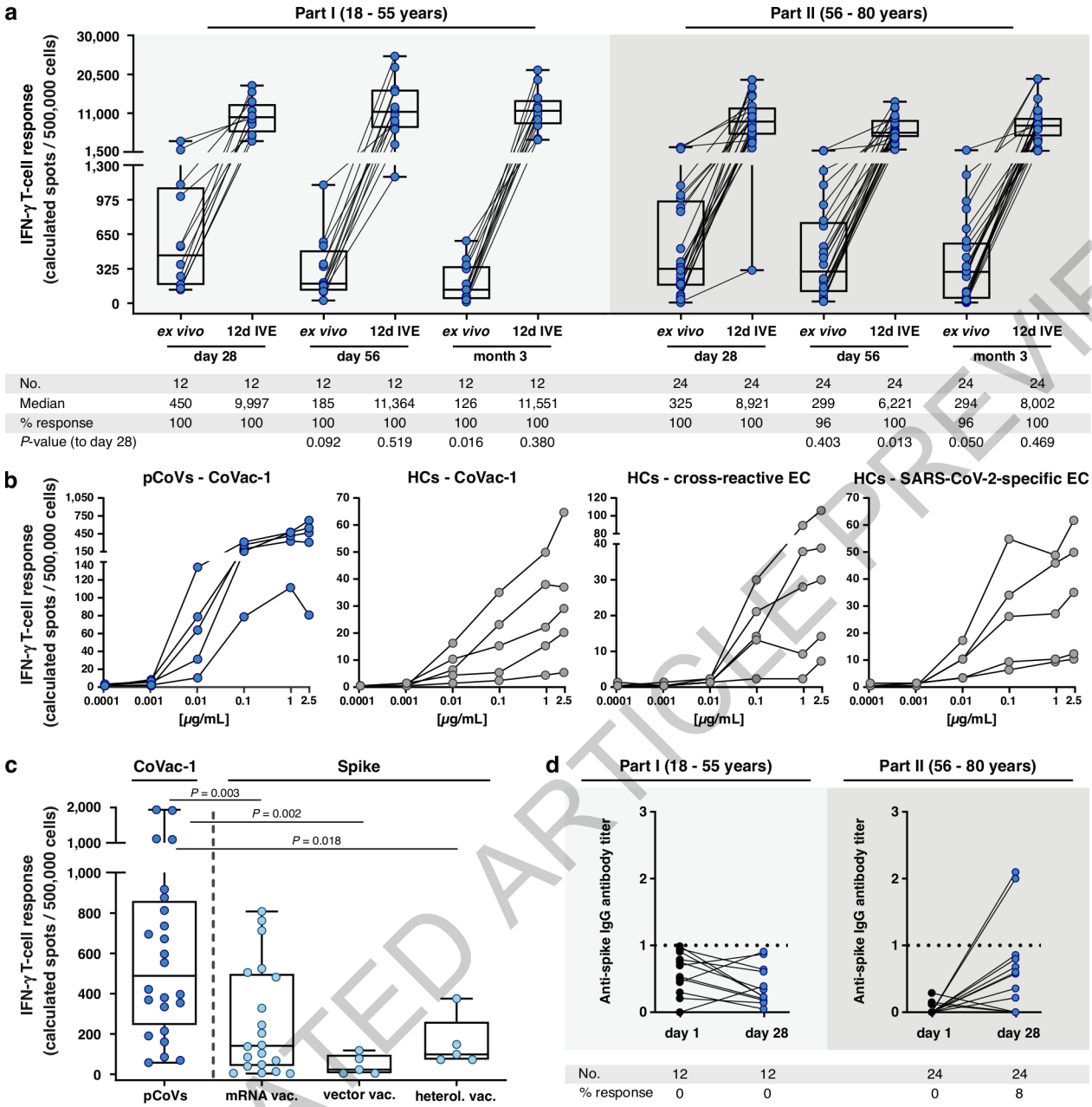
Peer review information*Nature* thanks Antonio Bertoletti and Julie Ledgerwood for their contribution to the peer review of this work. Peer review reports are available.



Extended Data Fig. 1 | Consort flow diagram of the trial. The 18 participants who were not enrolled did not meet the inclusion criteria at screening. All 36 enrolled participants received one dose of the CoVac-1 vaccine. Safety oversight to proceed to Part II was performed by an independent safety monitoring committee and approved by the Paul Ehrlich Institute and the local Ethics Committee after an interim safety and immunogenicity analysis of study participants included in Part I on day 28 after vaccine administration. n, number.



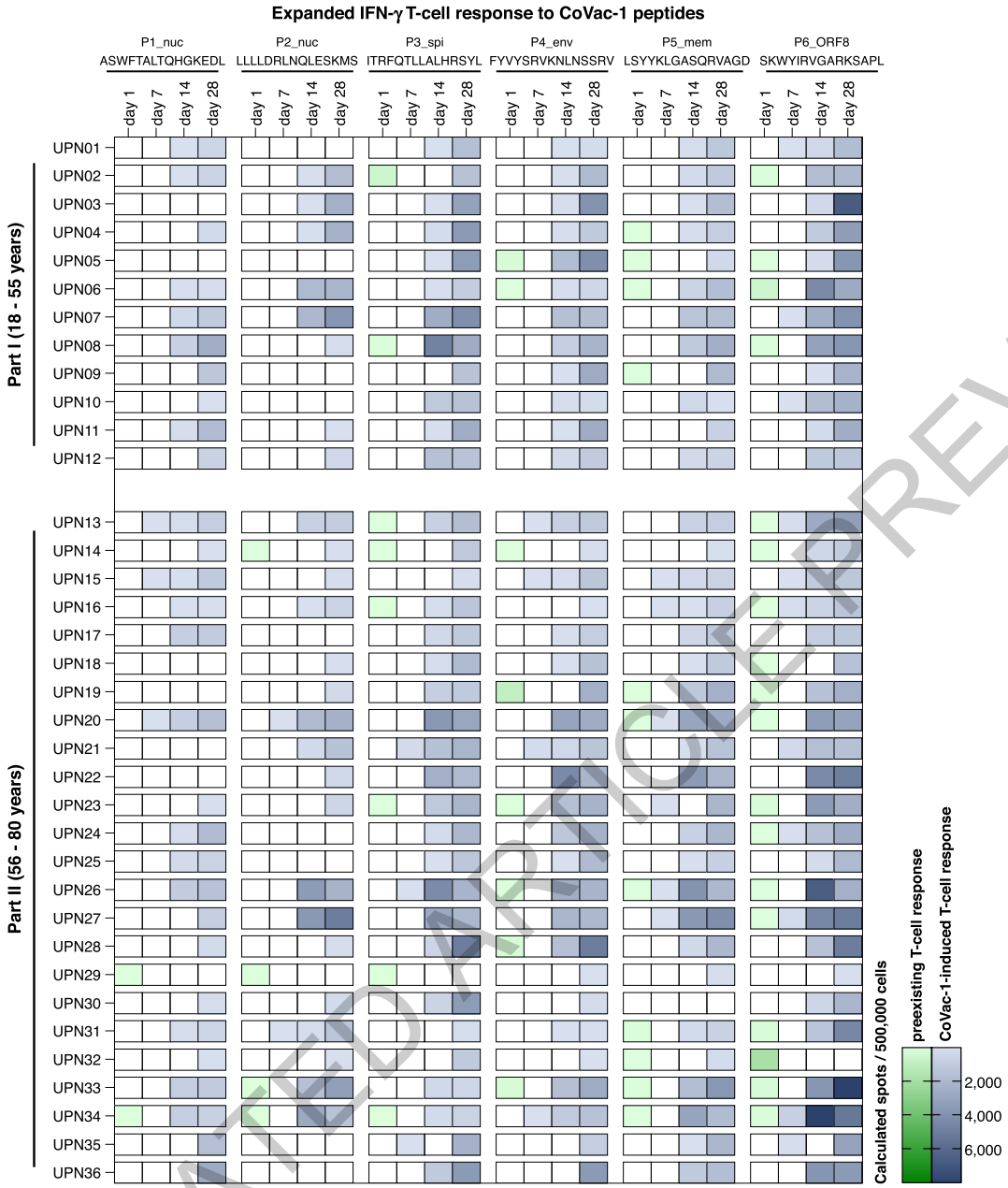
Extended Data Fig. 2 | Intensities of CoVac-1-induced T-cell responses *ex vivo* assessed in IFN-γ ELISPOT assays. Heatmap of CoVac-1-induced T-cell response intensities (calculated spots per 500,000 cells, color gradient blue) to single CoVac-1 peptides (nuc, nucleocapsid; spi, spike; env, envelope; mem, membrane; ORF, open reading frame) in *ex vivo* IFN-γ ELISPOT assays using PBMCs from study participants (uniform participant number, UPN) of Part I (n = 12) and Part II (n = 24) before vaccination (day 1) and at different time points after vaccination (day 7, day 14, day 28).



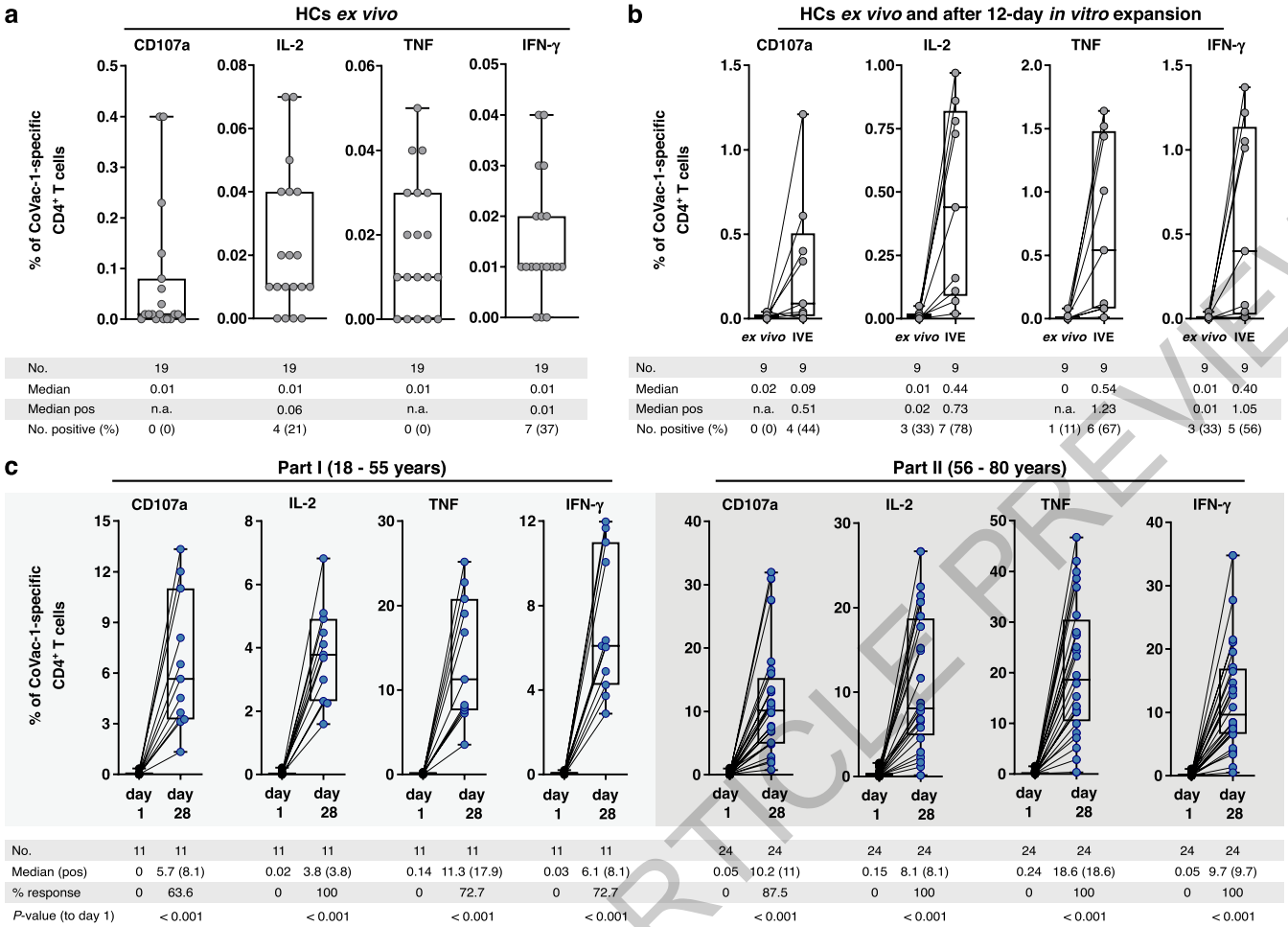
No.	12	12	12	12	12	12	24	24	24	24	24	24
Median	450	9,997	185	11,364	126	11,551	325	8,921	299	6,221	294	8,002
% response	100	100	100	100	100	100	100	100	96	100	96	100
P-value (to day 28)			0.092	0.519	0.016	0.380			0.403	0.013	0.050	0.469

No.	12	12	24	24
% response	0	0	0	8

Extended Data Fig. 3 | Characterization of CoVac-1-induced immune responses. (a) CoVac-1-induced long-term T-cell responses assessed *ex vivo* or after 12-day *in vitro* expansion (IVE) in study participants of Part I and II at day 56 and month 3 after vaccination (compared to day 28) using IFN-γ ELISPOT assays. Intensity of T-cell responses is depicted as calculated spot counts (mean spot count of technical replicates normalized to 500,000 cells minus the respective normalized negative control). (b) Peptide titration in *ex vivo* IFN-γ ELISPOT assays using PBMCs from study participants (pCoVs, n = 5, day 28) or from human COVID-19 convalescent donors (HCs, n = 5) with decreasing peptide concentrations (2.5 μg/mL to 0.1 ng/mL) of CoVac-1 (panel 1 and 2) or SARS-CoV-2 cross-reactive (panel 3) and SARS-CoV-2 specific (panel 4) epitope compositions (ECs). (c) Intensities of CoVac-1-induced IFN-γ T-cell responses assessed *ex vivo* in study participants of Part I and Part II (pCoVs, n = 24, day 28) compared to spike-specific T-cell responses in healthy immunized donors after second vaccination with approved mRNA vaccines (n = 20), vector-based vaccines (n = 5), or heterologous vaccination (n = 5). (d) Anti-spike IgG antibody titers assessed on day 1 prior to vaccination and on day 28 after vaccination. Values < 0.1 were set to zero and values ≥ 1.0 were considered positive. (a,c) Box plots or combined box-line plots show median with 25th or 75th percentiles, and min/max whiskers. (a) two-sided Wilcoxon signed-rank test, (c) two-sided Mann-Whitney U-test. no, number.



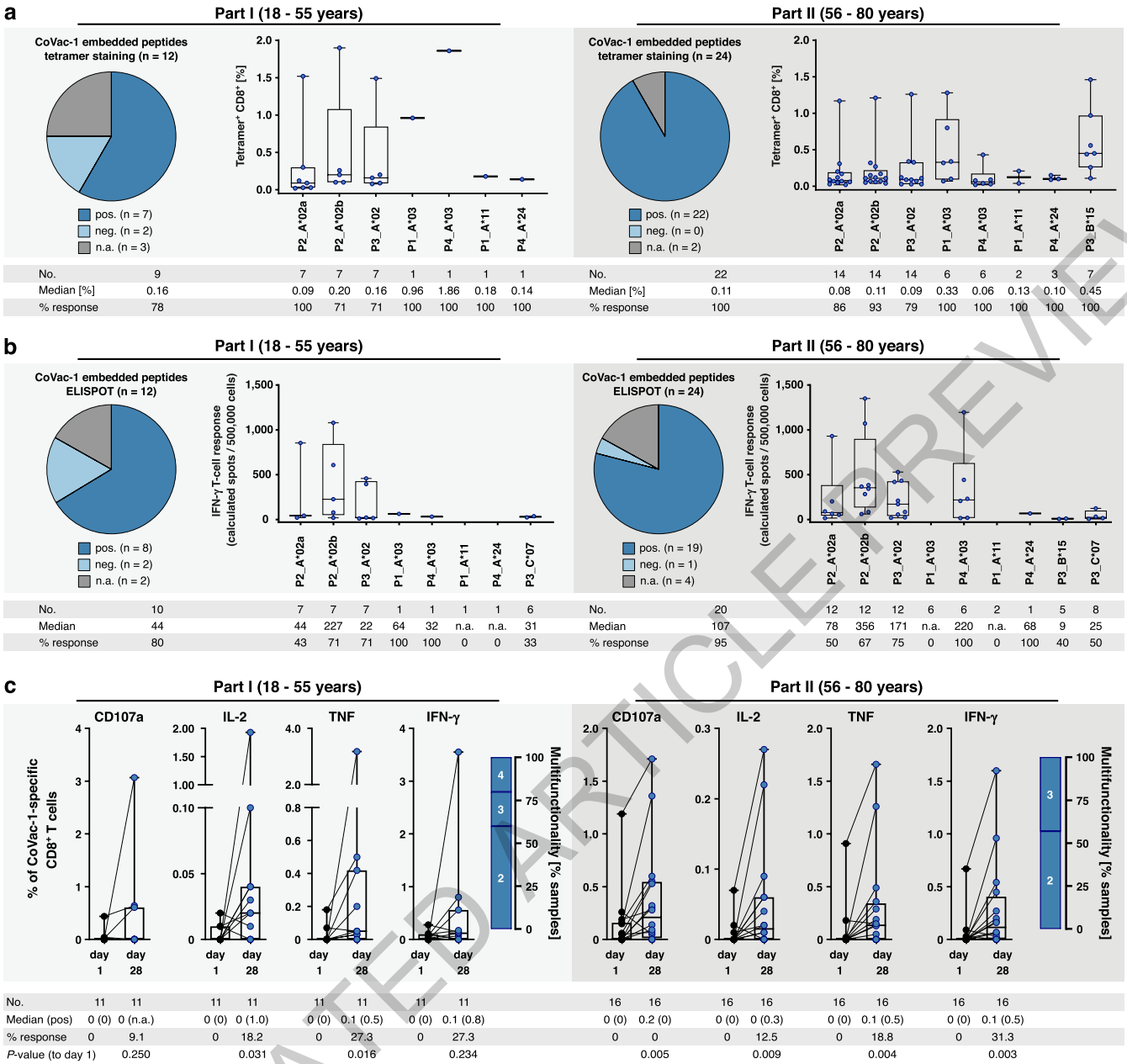
Extended Data Fig. 4 | Intensities of CoVac-1-induced T-cell responses assessed in IFN-γ ELISPOT assays after 12-day *in vitro* expansion. Heatmap of preexisting (color gradient green) or CoVac-1-induced (color gradient blue) T-cell response intensities (calculated spots per 500,000 cells) to single CoVac-1 peptides (nuc, nucleocapsid; spi, spike; env, envelope; mem, membrane; ORF, open reading frame) in IFN-γ ELISPOT assays after 12-day *in vitro* expansion of PBMCs from study participants (uniform participant number, UPN) of Part I (n = 12) and Part II (n = 24) before vaccination (day 1) and at different time points after vaccination (day 7, day 14, day 28).



No.	19		19		19		19		9	9	9	9	9	9	9	9
Median	0.01		0.01		0.01		0.01		0.02	0.09	0.01	0.44	0	0.54	0.01	0.40
Median pos	n.a.		0.06		n.a.		0.01		n.a.	0.51	0.02	0.73	n.a.	1.23	0.01	1.05
No. positive (%)	0 (0)		4 (21)		0 (0)		7 (37)		0 (0)	4 (44)	3 (33)	7 (78)	1 (11)	6 (67)	3 (33)	5 (56)

No.	11	11	11	11	11	11	11	11	24	24	24	24	24	24	24	24
Median (pos)	0	5.7 (8.1)	0.02	3.8 (3.8)	0.14	11.3 (17.9)	0.03	6.1 (8.1)	0.05	10.2 (11)	0.15	8.1 (8.1)	0.24	18.6 (18.6)	0.05	9.7 (9.7)
% response	0	63.6	0	100	0	72.7	0	72.7	0	87.5	0	100	0	100	0	100
P-value (to day 1)		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001

Extended Data Fig. 5 | CoVac-1-induced CD4⁺ T-cell responses in human COVID-19 convalescents and study participants. (a-c) Frequencies of CoVac-1-specific CD4⁺ T cells in (a) human convalescent samples (HCs) after SARS-CoV-2 infection analyzed *ex vivo* (n = 19) and (b) after 12-day *in vitro* expansion (n = 9), and (c) in study participants of Part I (n = 11) and Part II (n = 24) after 12-day *in vitro* expansion of PBMCs collected prior to vaccination (day 1) or on day 28 following vaccine administration. Functionality of CD4⁺ T cells was assessed for upregulation of the degranulation marker CD107a and production of the T helper 1 (Th1) cytokines (IFN-γ, TNF, and IL 2). (a-c) Box plots or combined box-line plots display median with 25th or 75th percentiles, and min/max whiskers, two-sided Wilcoxon signed-rank test, n.a., not applicable; no, number; pos, positive.

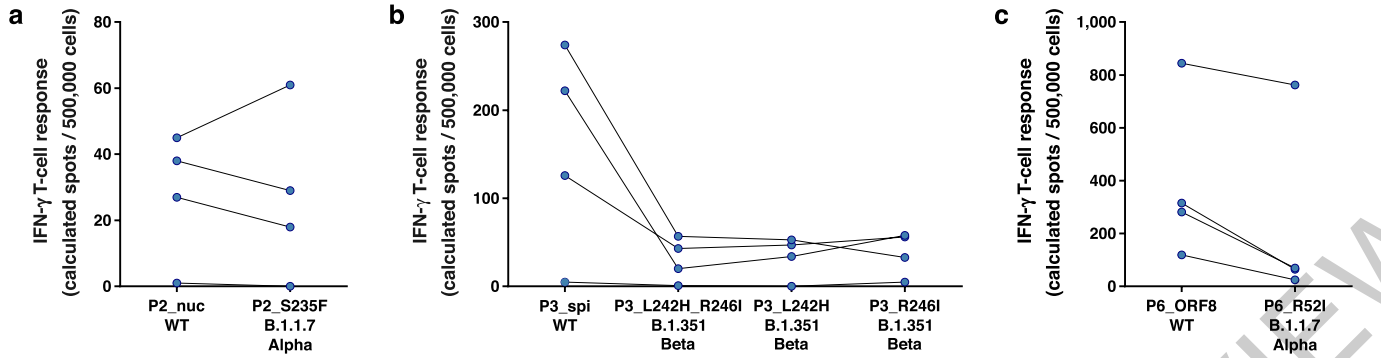


No.	9	7	7	7	1	1	1	1	No.	22	14	14	14	6	6	2	3	7
Median [%]	0.16	0.09	0.20	0.16	0.96	1.86	0.18	0.14	Median [%]	0.11	0.08	0.11	0.09	0.33	0.06	0.13	0.10	0.45
% response	78	100	71	71	100	100	100	100	% response	100	86	93	79	100	100	100	100	100

No.	10	7	7	7	1	1	1	1	6	No.	20	12	12	12	6	6	2	1	5	8
Median	44	44	227	22	64	32	n.a.	n.a.	31	Median	107	78	356	171	n.a.	220	n.a.	68	9	25
% response	80	43	71	71	100	100	0	0	33	% response	95	50	67	75	0	100	0	100	40	50

No.	11	11	11	11	11	11	11	11		16	16	16	16	16	16	16	16	
Median (pos)	0 (0)	0 (n.a.)	0 (0)	0 (1.0)	0 (0)	0.1 (0.5)	0 (0)	0.1 (0.8)		0 (0)	0.2 (0)	0 (0)	0 (0.3)	0 (0)	0.1 (0.5)	0 (0)	0.1 (0.5)	
% response	0	9.1	0	18.2	0	27.3	0	27.3		0	0	0	12.5	0	18.8	0	31.3	
P-value (to day 1)		0.250		0.031		0.016		0.234			0.005		0.009		0.004		0.003	

Extended Data Fig. 6 | CoVac-1-induced CD8$^+$ T-cell responses to HLA class I-restricted CoVac-1-embedded peptides and CoVac-1 peptides. T-cell responses to HLA class I-restricted SARS-CoV-2 peptides embedded within the CoVac-1 vaccine peptides (matching the HLA allotype of the respective participant) were assessed by (a) tetramer staining and (b) IFN-γ ELISPOT assays after *in vitro* expansion of PBMCs from study participants (Part I and II) obtained on day 28 after vaccination. Pie charts display number of samples with (a) specific T cells or (b) IFN-γ T-cell responses to CoVac-1-embedded peptides (pos, positive; neg, negative; n.a., not assessed). Dots represent frequencies of peptide-specific T cells shown for individual donors with detected T-cell responses only. (c) Frequencies of functional CoVac-1-induced CD4$^+$ T cells in study participants prior to vaccination (day 1) and at day 28 following vaccination using intracellular cytokine (IFN-γ, TNF, and IL-2) and surface marker staining (CD107a). The right graph displays the proportion of samples revealing difunctional (2), trifunctional (3), or tetrafunctional (4) T cell responses. (a-c) Box plots or combined box-line plots show median with 25th or 75th percentiles, and min/max whiskers, two-sided Wilcoxon signed-rank test. no, number; pos, positive.



Extended Data Fig. 7 | Vaccine-induced IFN-γ T-cell response to CoVac-1 peptides affected by mutations of SARS-CoV-2 variants of concern (VOC). CoVac-1-induced T-cell response to the single wild-type (WT) CoVac-1 peptides (P2_nuc (nucleocapsid), P3_spi (spike), P6_ORF8 (open reading frame 8)) in comparison to corresponding peptides comprising mutations of B.1.1.7-Alpha and B.1.351-Beta VOC were assessed by *ex vivo* IFN-γ ELISPOT assay for (a) P2_nuc, (b) P3_spi, and (c) P6_ORF8 using PBMCs from study participants (n = 4) collected on day 28 after CoVac-1 vaccination.

Extended Data Table 1 | Local and systemic solicited AEs compared between Part I and II

Local AEs	Severity	All participants (n = 36)	Part I (n = 12)	Part II (n = 24)	*P*-value*
Any local event, n (%)	Mild	14 (38.9)	4 (33.3)	10 (41.7)	0.899
	Moderate	15 (41.7)	5 (41.7)	10 (41.7)	
	Severe	7 (19.4)	3 (25)	4 (16.7)	
Induration, n (%)	Mild	24 (66.7)	8 (66.7)	16 (66.7)	1.000
	Moderate	12 (33.3)	4 (33.3)	8 (33.3)	
Swelling, n (%)	Mild	20 (55.6)	9 (75)	11 (45.8)	0.802
	Moderate	7 (19.4)	2 (16.7)	5 (20.8)	
	Severe	2 (5.6)	1 (8.3)	1 (4.2)	
Erythema, n (%)	Mild	16 (44.4)	5 (41.7)	11 (45.8)	0.806
	Moderate	10 (27.8)	4 (33.3)	6 (25)	
	Severe	7 (19.4)	3 (25)	4 (16.7)	
Itching, n (%)	Mild	24 (66.7)	9 (75)	15 (62.5)	0.709
	Moderate	-	-	-	
Pain, n (%)	Mild	21 (58.3)	8 (66.7)	13 (54.2)	0.721
	Moderate	1 (2.8)	-	1 (4.2)	
Skin ulceration, n (%)	Mild	7 (19.4)	3 (25)	4 (16.7)	0.664
	Moderate	2 (5.6)	-	2 (8.3)	
Lymphadenopathy, n (%)	Mild	8 (22.2)	5 (41.7)	3 (12.5)	0.086
	Moderate	-	-	-	

Systemic AEs	Severity	All participants (n = 36)	Part I (n = 12)	Part II (n = 24)	*P*-value*
Any systemic event, n (%)	Mild	14 (38.9)	5 (41.7)	9 (37.5)	1.000
	Moderate	-	-	-	
Fatigue, n (%)	Mild	11 (30.6)	3 (25)	8 (33.3)	0.715
	Moderate	-	-	-	
Headache, n (%)	Mild	6 (16.7)	2 (16.7)	4 (16.7)	1.000
	Moderate	-	-	-	
Chills, n (%)	Mild	2 (5.6)	-	2 (8.3)	0.543
	Moderate	-	-	-	
Myalgia, n (%)	Mild	2 (5.6)	1 (8.3)	1 (4.2)	1.000
	Moderate	-	-	-	
Arthralgia, n (%)	Mild	1 (2.8)	1 (8.3)	-	0.333
	Moderate	-	-	-	
Fever, n (%)	Mild	-	-	-	-
	Moderate	-	-	-	

Related local and systemic solicited adverse events (AEs) assessed up to 56 days after vaccination. Severity was graded as mild (grade 1), moderate (grade 2), or severe (grade 3) based on the definition provided in the methods section. * *P*-values were calculated for the comparison of Part I and Part II using two-sided Fisher's Exact test. n, number.

Extended Data Table 2 | Unsolicited AEs classified according to CTCAE V5.0

CTCAE	Severity	All participants (n = 36)		Part I (n = 12)		Part II (n = 24)	
		Not related to vaccine	Related to vaccine	Not related to vaccine	Related to vaccine	Not related to vaccine	Related to vaccine
Any event	Mild	46	1	17	-	29	1
	Moderate	9	1	4	-	5	1
	Severe	1	-	1	-	-	-
Fatigue	Mild	7	-	1	-	6	-
	Moderate	-	-	-	-	-	-
Headache	Mild	11	-	4	-	7	-
	Moderate	-	-	-	-	-	-
Dysesthesia	Mild	1	-	-	-	1	-
	Moderate	-	-	-	-	-	-
Paresthesia	Mild	1	-	-	-	1	-
	Moderate	-	-	-	-	-	-
Nausea	Mild	5	-	3	-	2	-
	Moderate	-	-	-	-	-	-
Muscle cramp	Mild	2	-	2	-	-	-
	Moderate	-	-	-	-	-	-
Sore throat	Mild	2	-	1	-	1	-
	Moderate	-	-	-	-	-	-
Dizziness	Mild	2	-	1	-	1	-
	Moderate	-	-	-	-	-	-
Ear pain	Mild	1	-	1	-	-	-
	Moderate	-	-	-	-	-	-
Sinusitis	Mild	-	-	-	-	-	-
	Moderate	1	-	1	-	-	-
Diarrhea	Mild	2	-	1	-	1	-
	Moderate	-	-	-	-	-	-
Bloating	Mild	1	-	1	-	-	-
	Moderate	-	-	-	-	-	-
Pain in extremity	Mild	2	-	-	-	2	-
	Moderate	-	-	-	-	-	-
Aphtae oral	Mild	1	-	-	-	1	-
	Moderate	-	-	-	-	-	-
Hypertension	Mild	2	-	2	-	-	-
	Moderate	3	-	3	-	-	-
	Severe	1	-	1	-	-	-
Herpes simplex reactivation	Mild	-	1	-	-	-	1
	Moderate	-	-	-	-	-	-
Laceration left hand	Mild	-	-	-	-	-	-
	Moderate	1	-	-	-	1	-
Back pain	Mild	1	-	-	-	1	-
	Moderate	-	-	-	-	-	-
Retinopathy	Mild	-	-	-	-	-	-
	Moderate	2	-	-	-	2	-
Hot flashes	Mild	1	-	-	-	1	-
	Moderate	-	-	-	-	-	-
Joint effusion	Mild	-	-	-	-	-	-
	Moderate	1	-	-	-	1	-
Abdominal pain	Mild	2	-	-	-	2	-
	Moderate	-	-	-	-	-	-
Renal colic	Mild	1	-	-	-	1	-
	Moderate	-	-	-	-	-	-
Shingles	Mild	-	-	-	-	-	-
	Moderate	-	1	-	-	-	1
Allergic reaction	Mild	1	-	-	-	1	-
	Moderate	-	-	-	-	-	-
Suspicious skin lesion	Mild	-	-	-	-	-	-
	Moderate	1	-	-	-	1	-

Severity and relationship were judged by the investigator until day 56. AE, adverse event; CTCAE, common terminology criteria for adverse events; n, number.

Extended Data Table 3 | Comparison of immunogenicity between Part I and Part II

Characteristics of T-cell response	Part	Mean	SD	*P*-value*
IFN-γ ELISPOT day 7 - *ex vivo* [calculated spot count] - (Fig. 2a)	I	8.33	10.12	0.123
	II	4.83	7.20	
IFN-γ ELISPOT day 14 - *ex vivo* [calculated spot count] - (Fig. 2a)	I	454.50	665.36	0.084
	II	234.38	373.25	
IFN-γ ELISPOT day 28 - *ex vivo* [calculated spot count] - (Fig. 2a)	I	887.42	1,183	0.481
	II	657.08	795.38	
Induced CoVac-1 peptides day 7 [n] - (Fig. 2b)	I	0.08	0.29	0.612
	II	0.04	0.20	
Induced CoVac-1 peptides day 14 [n] - (Fig. 2b)	I	4.50	1.38	0.110
	II	3.21	2.19	
Induced CoVac-1 peptides day 28 [n] - (Fig. 2b)	I	5.08	0.67	0.901
	II	4.88	1.36	
$CD107a^+$ $CD4^+$ T cells day 28 - *ex vivo* [%] - (Fig. 2d)	I	0.18	0.12	0.253
	II	0.18	0.21	
$IL\text{-}2^+$ $CD4^+$ T cells day 28 - *ex vivo* [%] - (Fig. 2d)	I	0.82	0.87	0.907
	II	1.12	1.95	
TNF^+ $CD4^+$ T cells day 28 - *ex vivo* [%] - (Fig. 2d)	I	0.93	0.92	1.000
	II	1.23	2.14	
IFN-γ^+ $CD4^+$ T cells day 28 - *ex vivo* [%] - (Fig. 2d)	I	0.53	0.58	0.724
	II	0.59	0.82	
IFN-γ ELISPOT day 56 - *ex vivo* [calculated spot count] - (Extended Data Fig. 3a)	I	321.75	303.82	0.639
	II	501.63	545.8	
IFN-γ ELISPOT month 3 - *ex vivo* [calculated spot count] - (Extended Data Fig. 3a)	I	192.31	185.71	0.365
	II	398.09	468.93	
IFN-γ ELISPOT day 28 - 12d IVE [calculated spot count] - (Extended Data Fig. 3a)	I	10,299	4,214	0.546
	II	9,440	5,137	
IFN-γ ELISPOT day 56 - 12d IVE [calculated spot count] - (Extended Data Fig. 3a)	I	12,049	7,093	0.019
	II	6,985	3,075	
IFN-γ ELISPOT month 3 - 12d IVE [calculated spot count] - (Extended Data Fig. 3a)	I	11,650	5,109	0.032
	II	8,521	4,425	
$CD107a^+$ $CD4^+$ T cells day 28 - 12d IVE [%] - (Extended Data Fig. 5c)	I	6.59	4.02	0.131
	II	11.33	8.65	
$IL\text{-}2^+$ $CD4^+$ T cells day 28 - 12d IVE [%] - (Extended Data Fig. 5c)	I	3.82	1.51	0.007
	II	10.92	7.85	
TNF^+ $CD4^+$ T cells day 28 - 12d IVE [%] - (Extended Data Fig. 5c)	I	13.67	7.44	0.136
	II	20.82	13.28	
IFN-γ^+ $CD4^+$ T cells day 28 - 12d IVE [%] - (Extended Data Fig. 5c)	I	7.18	3.37	0.065
	II	12.06	8.47	
$CD107a^+$ $CD8^+$ T cells day 28 - 12d IVE [%] - (Extended Data Fig. 6c)	I	0.39	0.92	0.148
	II	0.38	0.50	
$IL\text{-}2^+$ $CD8^+$ T cells day 28 - 12d IVE [%] - (Extended Data Fig. 6c)	I	0.20	0.57	0.724
	II	0.05	0.08	
TNF^+ $CD8^+$ T cells day 28 - 12d IVE [%] - (Extended Data Fig. 6c)	I	0.41	0.94	1.000
	II	0.30	0.48	
IFN-γ^+ $CD8^+$ T cells day 28 - 12d IVE [%] - (Extended Data Fig. 6c)	I	0.49	1.05	0.881
	II	0.28	0.44	

* *P*-values were calculated for the comparison of Part I and Part II using two-sided Mann-Whitney U-test. 12d IVE, 12-day *in vitro* expansion; n, number; SD, standard deviation.

nature portfolio

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
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☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
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☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	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	Patient diary for the first 28 days after vaccination and regular visits at the trial site. Data on reactogenicity and immunogenicity were collected in an electronic case report form. Additional data on explorative endpoints was provided via electronic sheets.
Data analysis	GraphPad Prism 9.2.0, FlowJo software version 10.7.1 and SAS 9.4.

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
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Data supporting the findings of this study including the study protocol and the statistical analysis plan are supplied as source data with this manuscript. Further data, including de-identified participant data are available after final completion of the trial report and are shared according to data sharing guidelines upon reasonable request to the corresponding author.

Field-specific reporting

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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	The sample size calculation of 36 participants for the trial was based on the stopping rule (occurrence of a vaccine-related serious adverse event (SAE)) and determined to show that the incidence of SAE associated with administration of CoVac-1 does not exceed a predetermined rate of 5% (= P1). Safety of the CoVac-1 vaccine should be shown if no SAE (= P0) occurs in the study population. An evaluable sample size of 33 achieves 81.6% of power to detect a difference (P1 - P0) of 0.0499 using a one-sided exact test based on the binomial distribution with a target significance level of 0.05 (sample size determination using PASS). These results assume that the population proportion under the null hypothesis (P0) is ≤ 0.0001. Assuming a drop-out rate of 7.5% (percentage of subjects that are expected to be lost at random during the course of the study and for whom no response data concerning existence of SAE will be collected, i.e. will be treated as "missing") the total number of 36 subjects should be enrolled in the study to achieve the threshold of 33 evaluable subjects. In total 36 subjects were included in the study.
Data exclusions	Safety and immunogenicity data were available until day 56 and month 3 after vaccination, respectively. No data were excluded from the analyses. Samples analyzed are indicated in the respective figure caption.
Replication	This is a report of an ongoing clinical trial. So far no attempt to replicate was performed.
Randomization	There was no randomization in this clinical trial as there was only one treatment arm without a control arm.
Blinding	There was no blinding performed in this clinical trial, because all participants received the CoVac-1 vaccine.

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	Flow cytometry: APC/Cy7 anti-human CD4 (clone RPA-T4, Cat# 300518, BioLegend), PE/Cy7 anti-human CD8 (clone SFCI21Thy2D3, Cat# 737661, Beckman Coulter), Pacific Blue anti-human TNF (clone MAb11, Cat# 502920, BioLegend), FITC anti-human CD107a (clone H4A3, Cat# 328606, BioLegend), APC anti-human IL-2 (clone MQ1-17H12, Cat# 500309, BioLegend), and PE anti-human IFN-γ monoclonal antibodies (clone B27, Cat# 506507, BioLegend). ELISPOT: anti-IFN-γ antibody (clone 1-D1K, Cat# 3420-3-1000, MabTech), anti-IFN-γ biotinylated detection antibody (clone 7-B6-1, Cat# 3420-6-1000 MabTech).
Validation	All antibodies were purchased from the above stated companies. Validation data / citations can be found on the suppliers' website. APC/Cy7 anti-human CD4: https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd4-antibody-1933 PE/Cy7 anti-human CD8: https://www.beckman.de/search#q=737661&t=coveo-tab-techdocs&f:@category=[Certificates%20of%20Analysis]&f:@itemnumber=[737661] Pacific Blue anti-human TNF: https://www.biolegend.com/en-us/products/pacific-blue-anti-human-tnf-alpha-antibody-4149 FITC anti-human CD107a: https://www.biolegend.com/en-us/products/fitc-anti-human-cd107a-lamp-1-antibody-4966 APC anti-human IL-2: https://www.biolegend.com/en-us/products/apc-anti-human-il-2-antibody-1348 PE anti-human IFN-γ: https://www.biolegend.com/en-us/products/pe-anti-human-ifn-gamma-antibody-1536 anti-IFN-γ antibody: https://www.mabtech.com/products/anti-human-ifn-gamma-antibody-1-d1k-purified-3420-3

anti-IFN-γ biotinylated detection antibody: https://www.mabtech.com/products/anti-human-ifn-gamma-antibody-7-b6-1-biotinylated-3420-6#tabs-min-2

Human research participants

Policy information about studies involving human research participants

Population characteristics	Eligible participants were men or women aged 18-55 (Part I) or 56-80 years (Part II). In Part I, participants were free of clinically significant health problems. In Part II, participants with stable medical history were enrolled. Participants had to refrain from blood donations during the course of the study and be willing to minimize body fluid transmission to others for 7 days after vaccination. All participants had to adhere to adequate contraception methods until three months after vaccination. Exclusion criteria comprised: pregnant or lactating females, participation in another clinical trial, treatment with immunosuppressive drugs, prior or current infection with SARS-CoV-2 (proven serologically or by PCR), known previous anaphylactic reaction to any component or hypersensitivity to any component of the CoVac-1 vaccine, relevant CNS pathology or other neurological disease, positivity for HIV or active hepatitis, lymphocyte count ≤1.000/µl, blood donation within 30 days, or administration of immunoglobulins or blood products within 120 days prior to study inclusion, diabetes type II, chronic lung disease requiring drug treatment, increased liver enzymes (≥2.5 x upper limit of normal), renal failure (GFR<60ml/min/1.73m2), serious cardiovascular disease, sickle cell anemia, obesity (defined by age adjusted body mass index), or preexisting auto-immune disease except for Hashimoto thyroiditis and mild psoriasis.
Recruitment	Participants were recruited at the University Hospital Tübingen. Information on the clinical trial was provided via press release (electronic and paper based). A selection bias is not assumed. Recruited participants were screened for eligibility. First the Part I of the trial was completed (including sentinel dosing of the first participant) and after review of reactogenicity and immunogenicity by the data safety monitoring board and approval by the regulatory authorities (Paul Ehrlich Institute and local ethic committee), Part II of the trial was initiated.
Ethics oversight	The trial was approved by the Ethics Committee, University Tübingen (537/2020AMG1) and the Paul Ehrlich Institute and performed in accordance with the International Council for Harmonization Good Clinical Practice guideline. A second approval was obtained prior to recruiting in Part II of the clinical trial.

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	ClinicalTrials.gov: NCT04546841
Study protocol	The study protocol is provided with the submission of the manuscript.
Data collection	Participants were recruited from November 28th, 2020 to January 15th, 2021. Data were collected at screening (up to 7 days before vaccination), day 1 (vaccination, baseline), day 7, day 14, day 28, day 56 and month 3. Both reactogenicity and immunogenicity were analyzed at indicated time points by outpatients visits at the University Hospital Tübingen. In addition, participants reported on reactogenicity until day 28 by paper-based diary.
Outcomes	In this report, safety as the primary endpoint is presented until day 56. Primary safety outcomes reflect the nature, frequency, and severity of solicited adverse events (AEs) until day 56 after vaccination. In addition, the number and percentage of participants with unsolicited events until day 56 were reported. The secondary endpoint immunogenicity is reported by the induction of CoVac-1-specific T-cell responses. Furthermore, explorative endpoints such as characteristics of T-cell responses were analyzed.

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	PBMCs were incubated with 10 µg/mL of peptide, 10 µg/mL Brefeldin A (Sigma-Aldrich), and a 1:500 dilution of GolgiStop (BD) for 12 - 16 h. Staining was performed using Cytofix/Cytoperm solution (BD), APC/Cy7 anti-human CD4 (BioLegend), PE/Cy7 anti-human CD8 (Beckman Coulter), Pacific Blue anti-human TNF, FITC anti-human CD107a, APC anti-human IL-2, and PE

	anti-human IFN-γ monoclonal antibodies (BioLegend). PMA (5 µg/mL) and ionomycin (1 µM, Sigma-Aldrich) served as positive control. Viable cells were determined using Aqua live/dead (Invitrogen).
Instrument	FACS Canto II cytometer (BD)
Software	FlowJo software version 10.7.1 (BD)
Cell population abundance	No cell sorting was performed prior to functional experiments.
Gating strategy	Viable cells were determined using Aqua live/dead (Invitrogen).

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