BioNTech SE
An der Goldgrube 12
55131 Mainz, Germany
Phone: +49 (0)6131 9084-0
Telefax: +49 (0)6131 9084-390

R&D STUDY REPORT No. R-20-0112

CHARACTERIZING THE IMMUNOPHENOTYPE IN SPLEEN AND LYMPH NODE OF MICE TREATED WITH SARS-COV-2 VACCINE CANDIDATES

Version 01
Date: 13 AUG 2020

Reported by (b) (6)

Test item: BNT162a1, BNT162b1, BNT162b2, BNT162c2
Key words: Covid-19, SARS-CoV-2, Vaccine, BALB/c mice, immunophenotyping

This R&D report consists of 105 pages.

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LIST OF ABBREVIATIONS

Ab	Antibody
CP	Cytoplasmic domain
dLNs	Draining lymph nodes
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
FM	Fluorescence minus
FP	Fusion peptide
GC	Germinal center
HR1, HR2	Heptad repeats 1 and 2
i.m.	Intramuscular
IFNγ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
IL	Iliac
IN	Inguinal
LD	LiveDead viability dye
LN	Lymph node
LNPs	Lipid nanoparticles
Lot	Lot number
MM	Master mix
modRNA	Nucleoside modified mRNA
NEAA	Non-essential amino acids
PO	Popliteal
RBD	Receptor binding domain
RBM	Receptor binding motif
S	Spike protein
saRNA	Self-amplifying RNA
SP	Signal peptide
T_{FH}	Follicular helper T cells
T_H	T helper cells
TM	Transmembrane domain
TNF	Tumor necrosis factor
uRNA	Non-modified uridine-containing mRNA

RESPONSIBILITIES

Person responsible for the study:	(b) (6)	27 Aug 2020
	(b) (6) BioNTech RNA Pharmaceuticals	Date
Author:	(b) (6)	27 Aug 2020
	(b) (6) BioNTech RNA Pharmaceuticals	Date
Reviewer:	(b) (6)	27 AUG 2020
	BioNTech RNA Pharmaceuticals	Date
QA representative	(b) (6)	27AUG2020
	BioNTech SE	Date

Meaning of the signatures:

Person responsible for the study: I am responsible for the content of the R&D report and confirm that it represents an accurate record of the results. This study was performed according to the SOPs and methods as well as the rules and regulations described in the report.

Author: I am the author of this document.

Reviewer: I reviewed the R&D report and confirm that this document complies with the scientific and technical standards and requirements.

QA representative: I confirm that this document complies with the relevant quality assurance requirements.

1 SUMMARY

BioNTech is developing RNA-based vaccines designed to protect against the novel coronavirus disease that emerged in 2019 (COVID-19). The project involves testing three RNA platforms, which are under development at BioNTech, with the surface or spike (S) protein of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as the viral antigen.

In the present study, T- and B-cell responses as well as the ability of $CD8^+$ T cells to kill viral antigen-presenting cells induced by four clinical SARS-CoV-2 vaccine candidates were characterized.

The study was divided into two parts, with the first part characterizing the vaccine candidates BNT162a1 and BNT162b1, and the second part characterizing BNT162b2 and BNT162c2. For each part, eight BALB/c mice per group were vaccinated with 5 µg of RNA encapsulated in lipid nanoparticles (LNPs) or buffer control on day 0 by intramuscular injection. T and B cells were analyzed seven days after vaccination in the blood. Serum for optional determination of SARS-CoV-2 specific IgG responses was stored, spleen and the draining lymph nodes (dLNs) were analyzed after 12 days (BNT162a1, BNT162b1 and BNT162b2), or 27 days (BNT162c2). Splenocytes were used for IFNγ ELISpot assay and xCELLigence cytotoxicity assay, and cell suspensions prepared from dLNs and spleen were analyzed by flow cytometry. Cytokines produced by restimulated dLN and spleen cells were analyzed by ProcartaPlex cytokine multiplex assay.

IFNγ ELISpot revealed a strong S protein specific T-cell responses particularly in BNT162b2, BNT162b1 and BNT162c2 and to a lesser extent in BNT162a1 treated groups. In line, $CD8^+$ and $CD4^+$ T cells in dLNs were significantly increased after BNT162b2 treatment, the former already detectable at day 7 in the blood. A trend for increased T cell numbers was detected in the BNT162b1 and BNT162a1 groups. Particular BNT162b1 and BNT162b2 treatment resulted in T cell activation (CD44, CD38, PD1 and ICOS expression of T cells in blood) and antigen specific secretion of cytokines by splenocytes. In those groups, a predominant T_H1 phenotype was detected with increased numbers of T-bet^+ $CD4^+$ T cells, high secretion of T_H1 type cytokines (IFNγ, IL-2, TNF) and low secretion of T_H2 type cytokines (IL-4, IL-5). In all analyzed compartments BNT162b1, BNT162b2 and BNT162c2 treatment mediated the increase and activation of T_{FH} cells, a cell type known for its crucial support of B cell responses. B cell numbers in dLNs were significantly elevated after BNT162b1 and BNT162b2 treatment with higher numbers of antibody producing plasma B cells, class switched and germinal center B cells essential for affinity maturation of antibodies.

Due to the prominent induction of both T and B cell responses, these results particularly support further clinical evaluation of the SARS-CoV-2 vaccine candidates BNT162b1 and BNT162b2.

(b) (6)	27 Aug 2020
BioNTech RNA Pharmaceuticals	Date

2 GENERAL INFORMATION

2.1 Sponsor and Test Facilities

Sponsor

BioNTech RNA Pharmaceuticals GmbH
An der Goldgrube 12
55131 Mainz
Germany

Test Facility

BioNTech SE
An der Goldgrube 12
55131 Mainz
Germany

2.2 Participating Pers

Responsible person: (as defined in SOP-100-024)	(b) (6) BioNTech RNA Pharmaceuticals GmbH An der Goldgrube 12 55131 Mainz
Author:	(b) (6) BioNTech RNA Pharmaceuticals GmbH
Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH
Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH

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Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH

2.3 Study Dates

Start of experiments: 06 MAY 2020

Completion of experiments: 04 JUN 2020

2.4 Guidelines and Regulations

All experiments are executed in accordance with the existing standard operating procedures and described processes from BioNTech SE. Applicable documents are listed below.

- Animal test application approval number: G18-12-100, Amendment from 24.04.2020 (approved 30.04.2020).
- SOP-010-015 Pipetten und Dispenser
- SOP-010-017 Brutschränke - Biolytics
- SOP-010-028 Vi-Cell XR
- SOP-010-045 Brutschrank HERAcell 150i
- SOP-010-047 Zentrifuge Eppendorf 5810/5810R
- SOP-010-051 Tiefkühlschränke -80°C
- SOP-010-058 Sicherheitswerkbank Klasse II
- SOP-010-086 Zentrifuge Thermo Scientific Heraeus Pico und Fresco 17
- SOP-010-099 CTL ELISPOT Reader
- SOP-010-128 FACSCelesta
- SOP-020-009 Ansetzen von Medien und Zusätzen für die Zellkultur
- SOP-030-038 Standardisierte Kultivierung von Zellen
- SOP-030-041 Auftauen von Zellen
- SOP-030-050 Elektroporation von Zellen
- SOP-030-051 Selektion mit MACS MicroBeads
- SOP-030-054 Extrazelluläre Färbung für Durchflusszytometrie
- SOP-030-071 Abtöten von Mäusen
- SOP-030-072 Fixiergriff und Ohrmarkierung bei Mäusen
- SOP-030-073 Betäubung bei Mäusen
- SOP-030-074 Blutentnahme bei Mäusen
- SOP-030-078 Isolierung muriner Splenozyten
- SOP-030-079 Intramuskuläre Applikation bei Mäusen
- SOP-030-110 IFNγ ELISpot (murin)
- SOP-090-013 Biological safety in laboratories
- SOP-110-022 Entsorgung von Biostoffabfällen

2.5 Changes and Deviations

This R&D study was conducted according to R&D plan P-20-0112. Table 1 summarizes all changes and deviations to the R&D plan.

Table 1: Changes and deviations to R&D study plan

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
mCorVAC#15	xCELLigence cytotoxicity assay	Duplicates for all samples	Duplicates only for some samples	Not enough cells per mouse	Some samples were assayed in singlicates
mCorVAC#15	Flow cytometry	Functional and phenotypic T cell analysis, dLN: 2×10^6 cells/well	Functional and phenotypic T cell analysis, dLN: 1×10^6 cells/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#15	Flow cytometry	Phenotypic T cell analysis, SP: 2×10^6/well	Phenotypic T cell analysis, SP: 4×10^6/well	Improve the quality of the results	None.
mCorVAC#15	Flow cytometry	B cell analysis, dLN, SP: 2×10^6/well	B cell analysis, dLN: 2.5×10^5/well. SP: 1×10^6/well	Not enough cells per mouse	Lower sample numbers for this assay
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mCorVAC#15, mCorVAC#16	Flow cytometry	Analysis of SP and dLNs on day 12 (day 27)	Analysis of blood 7 days after immunization	Gain further information on the T and B cell immunophenotype early after immunization	Blood phenotyping data available for 7 days after immunization.
mCorVAC#15, mCorVAC#16	ELISpot	Use MultiScreenHTS plates (Millipore)	Precoated ELISpot plates were used (Mabtech)	bring in line with previous mCorVac experiments	None.
mCorVAC#15, mCorVAC#16	Flow cytometry, cytokine multiplex assay	Restimulation with S peptide mixes at 0.1 µg/mL per peptide	Restimulation with S peptide mixes at 0.2 µg/mL per peptide (mCorVAC#15 and mCorVAC#16) and at 0.5 µg/mL per peptide (flow cytometry, mCorVAC#16)	Increase peptide concentration to increase sensitivity and pick up low responses	Direct quantitative comparison of cytokine profiles derived from multiplex assay of mCorVac#15 (BNT162a1 and BNT162b1) and mCorVac#16 (BNT162b2 and BNT162c2) no longer advisable
mCorVAC#15, mCorVAC#16	Flow cytometry (functional T cell analysis), cytokine	dLN: All samples	dLN: Not all samples included in assay	Not enough cells per mouse	Lower sample numbers for this assay

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
	multiplex analysis				
mCorVAC#15, mCorVAC#16	Flow cytometry	Brefeldin A and GolgiStop in medium were added to a final concentration of 10 µg/mL brefeldin A and a dilution of GolgiStop of 1:500.	GolgiStop and GolgiPlug in medium were added to a final dilution of GolgiStop of 1:1,500 and GolgiPlug of 1:1,000.	GolgiPlug contains brefeldin A and replaced brefeldin A solution. Dilution of GolgiStop was lowered as this lower dilution was found to be sufficient for its purpose.	None.
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mCorVAC#15, mCorVAC#16	Cytokine multiplex assay	dLN: 5×10^5/well	dLN: 4×10^5/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#16	Flow cytometry	Phenotypic T cell analysis, dLN: 2×10^6 cells/well.	Phenotypic T cell analysis, dLN: 1.5×10^6 cells/well	Not enough cells per mouse.	Lower sample numbers for this assay
mCorVAC#16	Flow cytometry	Phenotypic T cell analysis, SP: 2×10^6/well	Phenotypic T cell analysis, SP: 4×10^6/well	Improve the quality of the results.	None.
mCorVAC#16	Flow cytometry	B cell analysis, dLN, SP: 2×10^6/well	B cell analysis, dLN: 2.5×10^5/well. SP: 1×10^6/well	Not enough cells per mouse.	Lower sample numbers for this assay
mCorVAC#16	xCELLigence cytotoxicity assay	Using CT26 cells electroporated	S RNA electroporated CT26 cells were loaded with S peptide mix after electroporation	Improve the quality of the results	None.

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
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mCorVAC#15, mCorVAC#16	Flow cytometry (functional	dLN: All samples	dLN: Not all samples included in assay	Not enough cells per mouse	Lower sample numbers for this assay

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mCorVAC#15, mCorVAC#16	Cytokine multiplex assay	dLN: 5×10^5/well	dLN: 4×10^5/well	Not enough cells per mouse	Lower sample numbers for this assay

2.6 Documentation and Archive

Study plans and reports are stored and archived according to SOP-100-003 Archiving of Paper-Based Documents.

Raw data and evaluated data are saved at:

- P:\BioNTechRNA\RN9391R00_CoV-VAC\04_Preclinic\00_Pharmacology\mCorVac#15_modRNA_uRNA_V5_dLN_SP

- P:\BioNTechRNA\RN9391R00_CoV-VAC\04_Preclinic\00_Pharmacology\mCorVac#16_saRNAV9_modRNAV9_dLN_SP
- Lab book #1934, page 16-80

3 INTRODUCTION

3.1 Background

In December 2019, an outbreak of pneumonia of unknown cause in Wuhan, Hubei province in China, started. The disease spread rapidly and in January 2020, the agent was identified. By July 27th 2020, infection with the novel Coronavirus SARS-CoV-2 was confirmed in approximately 16,100,000 people with more than 640,000 casualties[1]. A vaccine is urgently needed against the elicited coronavirus disease 19 (COVID-19) and BioNTech decided to initiate a rapid vaccine project based on the surface or spike (S) protein of the virus as the viral antigen. The S protein is a trimer and during viral egress, the precursor protein is cleaved into S1 and S2 (Figure 1). While the S1 domain recognizes the host receptor, the S2 domain is essential for membrane fusion of the viral envelope and the endosomal membrane. To initiate membrane fusion, the S2 domain undergoes a conformational change within the central helix domain.

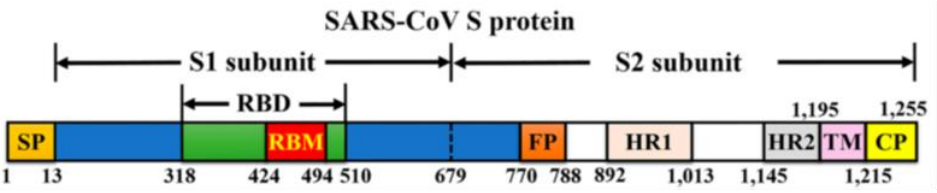

Figure 1: Schematic overview of the S protein structure of the SARS-CoV S protein

The sequence within the S1 subunit consists of the signal peptide (SP) and the receptor binding domain (RBD) with its receptor binding motif (RBM). The S2 subunit contains the fusion peptide (FP) for membrane fusion, heptad repeats (HR1 and HR2), the transmembrane domain (TM) and a cytoplasmic domain (CP). Source: modified from Song et al. 2019.

Based on these features, the S protein is the target of neutralizing antibodies that bind predominantly the receptor-binding domain (RBD) of the S protein.

The development of *in vitro* transcribed RNA as an active platform for the use in infectious disease vaccines is based on the extensive knowledge of the company in RNA technology, which has been gained over the last decade. The core innovation is based on *in vivo* delivery of a pharmacologically optimized, antigen-coding RNA vaccine to induce robust neutralizing antibodies and concomitant T-cell responses to achieve protective immunization with minimal vaccine doses (Vogel et al. 2018, Pardi et al. 2017, Moyo et al. 2019).

At BioNTech, there are three different RNA platforms under development, which are non-modified uridine containing mRNA (uRNA), nucleoside modified mRNA (modRNA) and self-amplifying RNA (saRNA). It is unknown today which RNA vaccine

[1] Coronavirus disease (COVID-2019) situation report 189, World Health Organization; https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

platform performs best in terms of activation and duration of a potent immune response. Initial studies in mice demonstrated the induction of T-cell responses as well as SARS-CoV-2-specific (neutralizing) IgG antibodies with vaccine candidates of all platforms. Four of these candidates are currently tested in clinical trials (Table 2).

The BNT162 vaccine candidate RNA is encapsulated into lipid nanoparticles (LNPs), which protect the RNA from degradation and enable transfection of host cells after intramuscular (i.m.) injection. For all of the BNT162 vaccine candidates, the same LNP formulation is used.

Table 2: Clinical stage SARS-CoV-2 vaccine candidates developed at BioNTech

ID	RNA platform	Antigen
BNT162a1	uRNA	RBD of S1S2 protein (V5)
BNT162b1	modRNA	RBD of S1S2 protein (V5)
BNT162b2	modRNA	S1S2 full-length protein, sequence variant (V9)
BNT162c2	saRNA	S1S2 full-length protein, sequence variant (V9)

This report covers a mouse study characterizing the immunophenotype in the blood, spleen and lymph nodes of mice treated with these four SARS-CoV-2 vaccine candidates.

3.2 Objectives

The objective of this study was to further characterize the four clinical SARS-CoV-2 vaccine candidates to support fast clinical development and approval. In particular, the goal of this study was to:

- Characterize T- and B-cell responses in the spleen, lymph nodes and blood. Analysis included a thorough phenotypic and functional (cytokine secretion on the cellular level) characterization of cells by ELISPot and flow cytometry, and definition of the cytokine profile by multiplex protein quantitation. In particular, the subtype of SARS-CoV-2-specific $CD4^+$ T cells (T_H1, T_H2, T_{FH}) and the abundance of plasma and germinal center (GC) B cells were of interest. Characterize changes in the myeloid cell compartment.
- Determine the ability of $CD8^+$ T cells to kill cells presenting the vaccine-encoded antigen.
- Collect serum of mice to determine (neutralizing) antibody responses (collection was performed, analysis of samples may be performed in the future, if required).

3.3 Study Design

The study was separated into two parts characterizing the vaccine candidates BNT162a1 and BNT162b1 (mCorVac#15, Figure 2) and BNT162b2 and BNT162c2 (mCorVac#16, Figure 3). Each part compared the effects of vaccinated mice to a

control group receiving buffer only. Eight BALB/c mice per group were vaccinated once (day 0) and blood analyzed 7 days later. Serum and tissues were analyzed 12 days later. Since T-cell responses of mice vaccinated with saRNA (BNT162c2) take longer to develop, the analysis time point for serum and tissues was postponed to day 27 after vaccination.

Blood, spleen and draining lymph nodes (dLNs) were harvested from mice. Figure 4 shows an overview of the subsequent analytical methods including sample allocation to the respective assays.

- Serum was obtained from blood and stored frozen for optional determination of SARS-CoV-2 specific IgG responses.
- Splenocytes were tested for recognition of an S protein-specific peptide mix or S RNA-electroporated CT26 cells by secretion of IFNγ (IFNγ ELISpot assay).
- A fraction of splenocytes (N=3 only for the control group, N=8 for treatment groups) was restimulated overnight with an S protein-specific peptide mix and recombinant IL-2, and isolated $CD8^+$ T cells were challenged on the next day for killing of S RNA-electroporated CT26 colon carcinoma cells (xCELLigence cytotoxicity assay).
- Splenocytes and dLN (popliteal, iliac and inguinal, pooled) cells were analyzed for T- ($CD4^+$ T_H1, T_H2, T_{FH}, $CD8^+$ T cells) and B-cell phenotype (GC, plasma, memory B cells), T-cell cytokine secretion after restimulation with an S protein-specific peptide mix, and myeloid cell subsets (flow cytometry).
- dLN and spleen cells were restimulated for 48 h with an S protein-specific peptide mix to analyze T-cell secreted cytokines in the supernatant (ProcartaPlex cytokine multiplex assay).

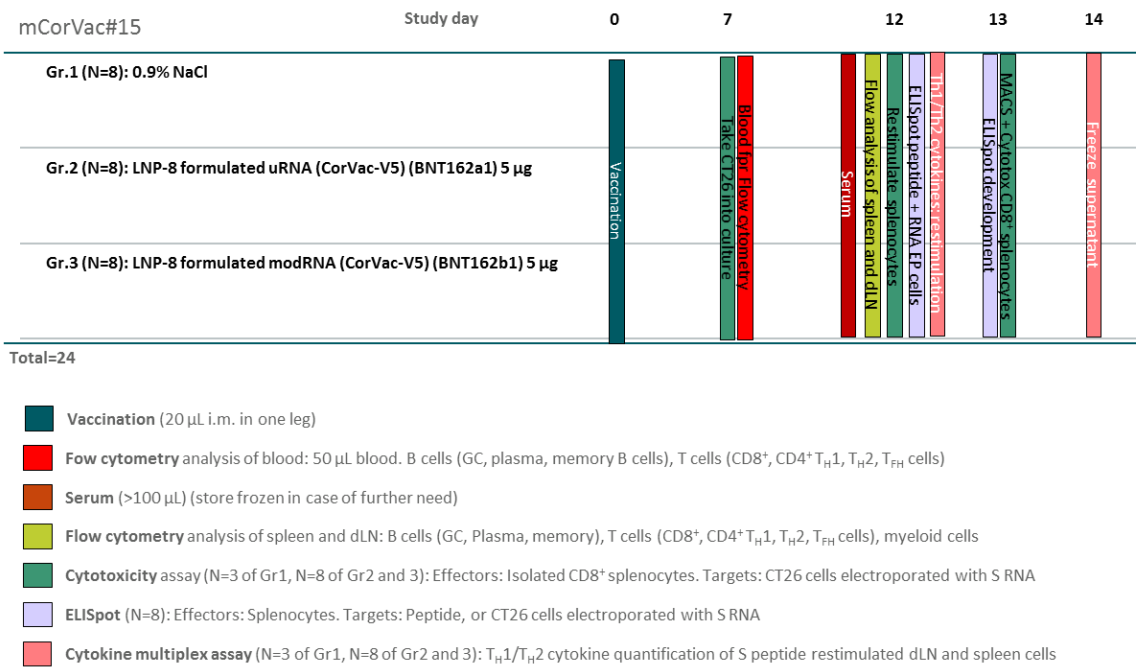

Figure 2: Workflow of part 1 of the study (mCorVac#15)

dLN, draining lymph node. GC, germinal center. Gr, group. N, number of mice per group. T_H, T helper cells. T_{FH}, follicular T helper cells.

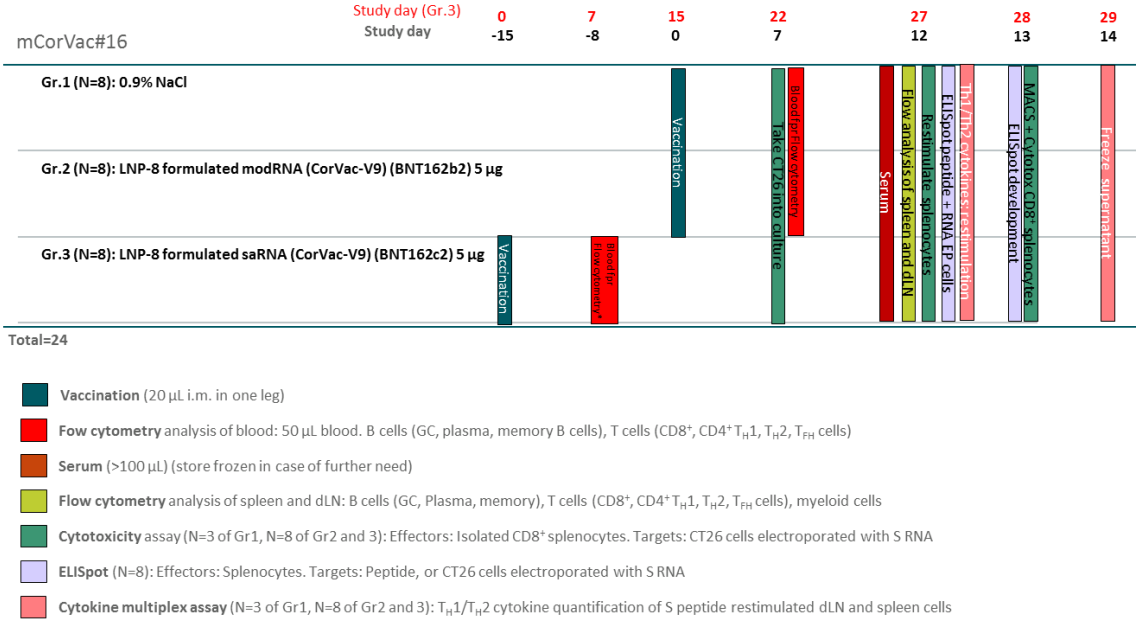

Figure 3: Workflow of part 2 of the study (mCorVac#16)

Study dates for group 3 are depicted in red. dLN, draining lymph node. GC, germinal center. Gr, group. N, number of mice per group. T_H, T helper cells. T_{FH}, follicular T helper cells.

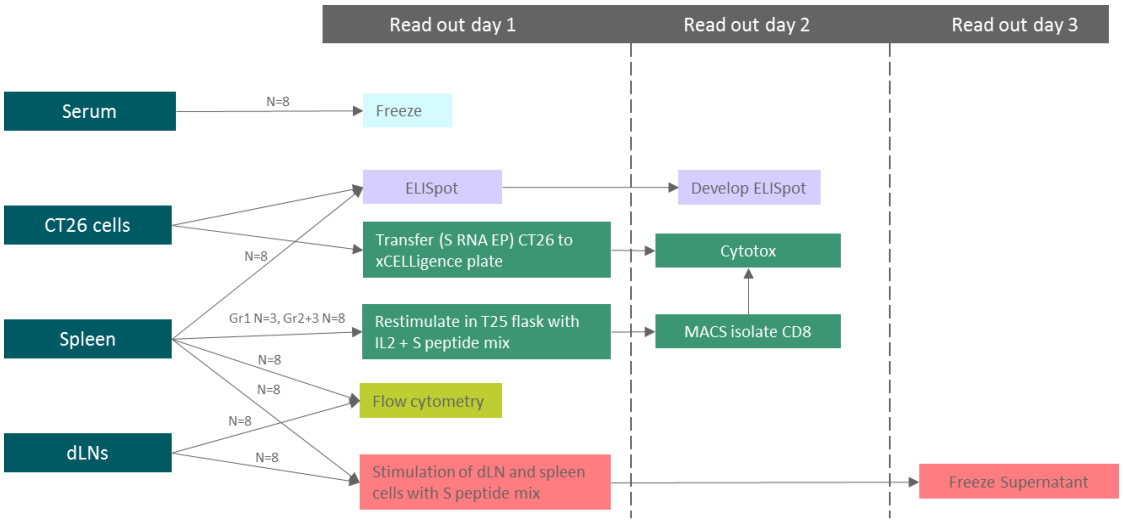

Figure 4: Analysis and assay overview

Schematic depiction of sample allocation to different analysis methods and their timing during analysis days 1 to 3. dLN, draining lymph node. EP, electroporated.

4 MATERIALS AND METHODS

4.1 Test Item

BNT162a1 (ATM): For CoAs see Appendix 9.6

- RNA batch: RNA-SK200305-01
- Polymun batch RBL063.3 LNP with the lot: CoVVAC/090320
- Dilution buffer: 0.9% NaCl
- Concentration: 0.5 mg/mL

BNT162b1 (ATM): For CoAs see Appendix 9.6.

- RNA batch: RNA-RF200304-03
- Polymun batch RBP020.3 LNP with the lot: CoVVAC/100320
- Dilution buffer: 0.9% NaCl
- Concentration: 0.5 mg/mL

BNT162b2 (ATM): For CoAs see Appendix 9.8.

- RNA batch: RNA-RF200321-06
- Polymun batch RBP20.2 LNP with the lot: CoVVAC/270320
- Dilution buffer: 0.9% NaCl
- Concentration: 0.5 mg/mL

BNT162c2 (ATM): For CoAs see Appendix 9.9.

- RNA batch: RNA-RF200310-01
- Polymun batch RBS004.2 LNP with the lot: CoVVAC/170320
- Dilution buffer: 0.9% NaCl
- Concentration: 0.3 mg/mL

Test items are diluted to 0.25 mg/mL with sterile 0.9% NaCl before administration.

4.2 Control Item

- 0.9% NaCl

4.3 Test System

- 48 female BALB/c mice with approximately nine weeks of age at study start

4.4 Materials

For antibodies used in flow cytometry, refer to Section 4.5.11.

Table 3: Materials

Product name	Application/specification	Article no.	Working dilution	Provider
15 mL/50 mL tube	Conical bottom, PP, 30/115 MM, CELLSTAR®	188271/ 227261	N/A	Greiner Bio-One GmbH
2 mL tube	CRYO.S, round bottom	122278	N/A	Greiner Bio-One GmbH
2-Mercaptoethanol	50 mM	31350-010	N/A	Gibco
4mL Sample Cup	Cell counting	NC9756824	N/A	Beckman Coulter GmbH
8-channel manifold	Polypropylene	BR704526-1EA	N/A	Sigma-Aldrich Chemie GmbH
96-well Microplate	Clear round bottom TC-treated microplate, with lid, sterile	3799	N/A	Corning Holding GmbH
ACK lysis buffer	Flow cytometry (blood)	A10492-01	1x	Gibco
Ammonium chloride	NH_4Cl	A0988,5000	N/A	AppliChem GmbH
Brilliant Stain Buffer	Flow cytometry	563794	N/A	BD Bioscience
Brilliant Stain Buffer Plus	Flow cytometry	566385	N/A	BD Biosciences
Capillary pipettes	minicaps®, blood sampling, 4 µL/ 10 µL, not heparinized	9000104/ 9000110	N/A	Hirschmann Laborgeräte GmbH & Co.KG
Cell culture flask 250 ML, 75 cm^2	Cell culture	658175	N/A	Greiner Bio-One GmbH
CD8a (Ly-2) MicroBeads	CD8 T cell purification	130-117-044	N/A	Miltenyi Biotec
Collagenase D	Lymphnode preparation	11088866001	1 mg/ml	Merck KGaA
Combitips advanced®	Biopur®, 50 mL	0030089693	N/A	Eppendorf Vertrieb Deutschland GmbH
Concanavalin A	from Canavalia ensiformis (Jack bean, 5mg),Type IV-S, lyophilized	C0412-5MG	2 µg/mL	Sigma-Aldrich Chemie GmbH
Dimethyl sulfoxide	Cell culture	A3672,0100	N/A	AppliChem GmbH

Product name	Application/specification	Article no.	Working dilution	Provider
DPBS	No calcium, no magnesium	14190-094	1 ×	Thermo Fisher Scientific
Easystrainer 70 µm	For 50 mL tubes	542070	N/A	Greiner Bio-One GmbH
Electroporation cuvette	Electroporation	732-1137	N/A	VWR International GmbH
E-Plate VIEW 96 PET	xCelligence	300600910	N/A	ACEA Biosciences
Eppendorf safe-lock tubes	0.5 mL/ 1.5 mL/ 2.0 mL/ 5.0 mL, Eppendorf Quality™	0030121023/ 0030120086/ 0030120094/ 0030119401	N/A	Eppendorf Vertrieb Deutschland GmbH
Ethylenediaminetetraacetic acid solution	EDTA	03690-100ML	N/A	Sigma-Aldrich Chemie GmbH
Fetal Bovine Serum	Non-USA origin, sterile-filtered	F7524	N/A	Sigma-Aldrich Chemie GmbH
Filtration unit for medium flasks	High Performance, PES, 0.45 µm, 1000 mL	514-0301	N/A	VWR International GmbH
FoxP3/Transcription Factor Staining Buffer Set	Flow cytometry	00-5523-00	N/A	Thermo Fisher Scientific
GolgiStop	Flow cytometry (Restimulation)	554724	1:1,500	BD Biosciences
GolgiPlug	Flow cytometry (Restimulation)	555029	1:1,000	BD Biosciences
Heparin Tubes	Flow cytometry (Blood)	20.1309	N/A	Sarstedt AG & Co.
HEPES	1 M	15630-056	N/A	Gibco
Insulin syringes	BD Micro-Fine™+, 30 G, 0.3 mL	324826	N/A	Becton Dickinson GmbH
Ionomycin, 10 µg/µL	Flow cytometry (Restimulation)	I9657	1 µg/mL	Sigma
Isoflurane	Anesthesia	9714675	N/A	Piramal Critical Care
Isotonic saline	Injection solution	06173569	N/A	Fresenius Kabi Deutschland GmbH
LS columns	CD8 T cell purification	130-042-401	N/A	Miltenyi Biotec

Product name	Application/specification	Article no.	Working dilution	Provider
MEM Non-Essential Amino Acids Solution (100X)	Cell culture	11140-035	1X	Gibco
Mouse IFN-γ ELISpot^{PLUS} kit	Kit for enumeration of cells secreting mouse IFN-γ	3321-4APT-2	N/A	Mabtech
PBS dry substance	No calcium, no magnesium	L182-10	N/A	Merck KGaA
Penicillin-Streptomycin	10,000 U/mL	15140-122	N/A	Gibco
PepMix™ against RBD	ELISpot	N/A (customized)	0.0625mg per peptide/ vial	JPT
Pipette tips	ep Dualfilter T.I.P.S.®, PCR clean und sterile, 0.1–10 µL/2–100 µL/50–1000 µL/50–1250 µL/0.1–5 mL	0030077512/ 0030077547/ 0030077555/ 0030077792/ 0030077750/ 0030078616	N/A	Eppendorf Vertrieb Deutschland GmbH
PMA, 1 µg/µL	Flow cytometry (Restimulation)	P1585	0.5 µg/mL	Sigma
Potassium bicarbonate	$KHCO_3$	A2375,1000	N/A	AppliChem GmbH
ProcartaPlex mouse T_H1/T_H2 cytokine 11-plex kit	Cytokine multiplex assay Lot. No. 232634-004	EPX110-20820-901	N/A	Thermo Fisher Scientific
Proleukin S	Cell culture	N/A	100 U/ml	Clinigen
Reservoir	25 mL, 100 mL	613-1174/613-1171	N/A	VWR International GmbH
RotiHistofix	Flow cytometry	P087.1	2%	Roth
Round bottom 5-mL tubes	Flow cytometry (blood)	10579511	N/A	Thermo Fisher Scientific
RPMI 1640 Medium	GlutaMAX™ Supplement	61870-010	N/A	Gibco
Serological pipettes	5 mL, 10 mL, 25 mL, 50 mL	606180/607180/601180/768180	N/A	Greiner Bio-One GmbH
Serum Tubes	Serum preparation	20.1344	N/A	Sarstedt AG & Co.
Single-use syringe	Injekt® Solo 5 mL	4606051V	N/A	B. Braun Melsungen AG

Product name	Application/specification	Article no.	Working dilution	Provider
Sodium acide (10%)	Flow cytometry (blood)	13553.00100	0.01%	Morphisto
Sodium Pyruvate	100 mM	11360-039	N/A	Gibco
StemPro™ Accutase™ Cell dissociation reagent	Cell culture	A1110501	N/A	Gibco
Sterile filters	0.45 µm	514-4123	N/A	VWR International
Vi-CELL™ XR Quad Pak	For Vi-CELL™ XR Cell Viability Analyzer	383722	N/A	Beckman Coulter GmbH
X-VIVO 15, serum-free	Electroporation	BE02-060Q	N/A	Lonza Group Ltd

Table 4: Peptide pool for restimulation of splenocytes and dLN cells for ELISpot assays, flow cytometry and cytokine multiplex assay

S protein-specific peptides	
Name	**Sequence**
2019-nCoV S.wt With a total of 315 overlapping peptides (15mers overlapping by 11 amino acids) GenBank: QHD43416.1 Batch: 43000LHB-1 and 43000LHB-2	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQ DLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWI FGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEF RVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPI NLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAA AYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTS NFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLY NSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQA GSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCG PKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRD PQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPT WRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRA RSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCT MYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTP PIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAA RDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTY VTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHG VVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYE PQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVD LGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLG FIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHY T

Table 5: RNAs used for CT26 electroporation

Name	Sequence (open reading frame)
S RNA (Full construct name: JR206_mRNA_pST4_m1ψ_wtsec_SARS-CoV-2-S (Sino CDS) CΔ19)	atgtttgtgttcctggtgctgctgccactggtgtccagccagtgtgtgaacctgaccaccaggacccaacttcctcctgcctaca ccaactccttcaccaggggagtctactaccctgacaaggtgttcaggtcctctgtgctgcacagcacccaggacctgttcctg ccattcttcagcaatgtgacctggttccatgccatccatgtgtctggcaccaatggcaccaagaggtttgacaaccctgtgctgc cattcaatgatggagtctactttgccagcacagagaagagcaacatcatcaggggctggatttttggcaccaccctggacag caagacccagtccctgctgattgtgaacaatgccaccaatgtggtgattaaggtgtgtgagttccagttctgtaatgacccattc ctgggagtctactaccacaagaacaacaagtcctggatggagtctgagttcagggtctactcctctgccaacaactgtaccttt gaatatgtgagccaaccattcctgatggacttggagggcaagcagggcaacttcaagaacctgagggagtttgtgttcaag aacattgatggctacttcaagatttacagcaaacacacaccaatcaacctggtgagggacctgccacagggcttctctgcctt ggaaccactggtggacctgccaattggcatcaacatcaccaggttccagaccctgctggctctgcacaggtcctacctgaca cctggagactcctcctctggctggacagcaggagcagcagcctactatgtgggctacctccaaccaaggaccttcctgctga aatacaatgagaatggcaccatcacagatgctgtggactgtgccctggacccactgtctgagaccaagtgtaccctgaaatc cttcacagtggagaagggcatctaccagaccagcaacttcagggtccaaccaacagagagcattgtgaggtttccaaacat caccaacctgtgtccatttggagaggtgttcaatgccaccaggtttgcctctgtctatgcctggaacaggaagaggattagca actgtgtggctgactactctgtgctctacaactctgcctccttcagcaccttcaagtgttatggagtgagcccaaccaaactgaa tgacctgtgtttcaccaatgtctatgctgactcctttgtgattaggggagatgaggtgagacagattgcccctggacaaacagg caagattgctgactacaactacaaactgcctgatgacttcacaggctgtgtgattgcctggaacagcaacaacctggacagc aaggtgggaggcaactacaactacctctacagactgttcaggaagagcaacctgaaaccatttgagagggacatcagca cagagatttaccaggctggcagcacaccatgtaatggagtggagggcttcaactgttactttccacctccaatcctatggcttcc aaccaaccaatggagtgggctaccaaccatacagggtggtggtgctgtcctttgaactgctccatgcccctgccacagtgtgt ggaccaaagaagagcaccaacctggtgaagaacaagtgtgtgaacttcaacttcaatggactgacaggcacaggagtgc tgacagagagcaacaagaagttcctgccattccaacagtttggcagggacattgctgacaccacagatgctgtgagggac ccacagaccttggagattctggacatcacaccatgttcctttggaggagtgtctgtgattacacctggcaccaacaccagcaa ccaggtggctgtgctctaccaggatgtgaactgtactgaggtgcctgtggctatccatgctgaccaacttacaccaacctgga gggtctacagcacaggcagcaatgtgttccagaccagggctggctgtctgattggagcagagcatgtgaacaactcctatg agtgtgacatcccaattggagcaggcatctgtgcctcctaccagacccagaccaacagcccaaggagggcaaggtctgtg gcaagccagagcatcattgcctacacaatgagtctgggagcagagaactctgtggcttacagcaacaacagcattgccatc ccaaccaacttcaccatctctgtgaccacagagattctgcctgtgagtatgaccaagacctctgtggactgtacaatgtatatct gtggagacagcacagagtgtagcaacctgctgctccaatatggctccttctgtacccaacttaacagggctctgacaggcatt gctgtggaacaggacaagaacacccaggaggtgtttgcccaggtgaagcagatttacaagacacctccaatcaaggactt tggaggcttcaacttcagccagattctgcctgacccaagcaagccaagcaagaggtccttcattgaggacctgctgttcaac aaggtgaccctggctgatgctggcttcatcaagcaatatggagactgtctgggagacattgctgccagggacctgatttgtgc ccagaagttcaatggactgacagtgctgcctccactgctgacagatgagatgattgcccaatacacctctgccctgctggctg gcaccatcacctctggctggacctttggagcaggagcagccctccaaatcccatttgctatgcagatggcttacaggttcaat ggcattggagtgacccagaatgtgctctatgagaaccagaaactgattgccaaccagttcaactctgccattggcaagattc aggactccctgtccagcacagcctctgccctgggcaaactccaagatgtggtgaaccagaatgcccaggctctgaacacc ctggtgaagcaactttccagcaactttggagccatctcctctgtgctgaatgacatcctgagcagactggacaaggtggaggc tgaggtccagattgacagactgattacaggcagactccaatccctccaaacctatgtgacccaacaacttatcagggctgct gagattagggcatctgccaacctggctgccaccaagatgagtgagtgtgtgctgggacaaagcaagagggtggacttctgt ggcaagggctaccacctgatgagttttccacagtctgcccctcatggagtggtgttcctgcatgtgacctatgtgcctgcccag gagaagaacttcaccacagcccctgccatctgccatgatggcaaggctcactttccaagggagggagtgtttgtgagcaatg gcacccactggtttgtgacccagaggaacttctatgaaccacagattatcaccacagacaacaccttttgtgtctggcaactgt gatgtggtgattggcattgtgaacaacacagtctatgacccactccaacctgaactggactccttcaaggaggaactggaca aatacttcaagaaccacaccagccctgatgtggacctgggagacatctctggcatcaatgcctctgtggtgaacatccagaa ggagattgacagactgaatgaggtggctaagaacctgaatgagtccctgattgacctccaagaactgggcaaatatgaac aatacatcaagtggccatggtacatctggctgggcttcattgctggactgattgccattgtgatggtgaccataatgctgtgttgta tgacctcctgttgttcctgtctgaaaggctgttgttcctgtggctcctgttgttgatga
Irrelevant RNA (Batch: RNA-KG200106-06c)	atgggcgccatggcccctagaacattgctcctgctgctggccgctgccctggcccctacacagacaagagctggacctggc ggctctggaggaggcggctccggaggcggaggatccggtggtggcggcagcggcggcgtgatcgtggctgtgctgggcg tgctgggagccatggccatcattggagccgtggtggccttcgtgatgaagcggagaagaaacaccggcggcaagggcgg cgattacgctctggctcctggcagccagtccagcgagatgagcctgagagactgcaaggcctagtaa

Table 6: Software

Product name	Application	Provider
BD FACSDiva software version 9.1 and 8.0.1.1	Flow cytometry	BD Biosciences
Excel	Raw data	Microsoft Corp.
FlowJo software version 10.6	Flow cytometry	FlowJo LLC, BD Biosciences
GraphPad Prism software version 8	Statistical analysis	GraphPad Software Inc.
ImmunoCapture 7.0.7.0	ELISpot assay	Cellular Technology Ltd
ImmunoSpot® analysis software version 57.0.17.0	ELISpot assay	Cellular Technology Ltd
ProcartaPley Analyst software version 1	Cytokine multiplex assay	Thermo Fisher Scientific
RTCA Data analysis software	xCELLigence cytotoxicity assay	ACEA Biosciences
xCELLigence RTCA Software Pro	xCELLigence cytotoxicity assay	ACEA Biosciences

Table 7: Machines

Product name	Application	Provider
BD Symphony A3	Flow cytometry	BD Biosciences
BD Celesta	Flow cytometry	BD Biosciences
Bioplex200 system	Cytokine multiplex assay	Bio-Rad
Centrifuges	Centrifugation	Eppendorf
CTL ELISPOT reader ImmunoSpot® S6 Core Analyzer	ELISpot assay	Cellular Technology Limited
Electroporation system	Electroporation	BTX
Vi-CELL XR	Cell counting	Beckman Coulter GmbH
xCELLigence RTCA MP	xCELLigence cytotoxicity assay	ACEA Biosciences

4.5 Methods

4.5.1 Animal Care

4.5.1.1 General Information

BALB/c mice were delivered at the age of at least six weeks. Delivered mice were used for experiments after approximately one week of acclimatization. All experiments and protocols were approved by the local authorities (local welfare committee), conducted according to the FELASA recommendations and in compliance with the German animal welfare act and Directive 2010/63/EU. Only animals with an unobjectionable health status were selected for testing procedures.

All animals were registered upon arrival in the lab animal colony management system PyRAT (Scionics Computer Innovation GmbH, Dresden, Germany) and tracked until death. Each cage was labelled with a cage card indicating the mouse strain, gender, date of birth and number of animals per cage. At the start of an experiment additional information was added such as the project and license number, the start of the experiment and details on interventions. Where necessary for identification, animals were arbitrarily numbered with earmarks.

4.5.1.2 Housing Condition and Husbandry

Mice were housed at BioNTech SE's animal facility under barrier and SPF conditions (An der Goldgrube 12, 55131 Mainz) in individually ventilated cages (Sealsafe GM500 IVC Green Line, TECNIPLAST, Hohenpeißenberg, Germany; 500 cm²) with a maximum of five animals per cage. The temperature and relative humidity in the cages and animal unit was kept at 20-24°C and 45-55%, respectively, and the air change (AC) rate in the cages at 75 AC/h. The cages with dust-free bedding made of debarked chopped aspen wood (Abedd LAB & VET Service GmbH, Vienna, Austria, product code: LTE E-001) and additional nesting material were changed weekly. Autoclaved ssniff M-Z food (sniff Spezialdiäten GmbH, Soest, Germany; product code: V1124) and autoclaved water (tap water) were provided *ad libitum* and changed at least once weekly. All materials were autoclaved prior to use.

4.5.2 Animal Monitoring

Routine animal monitoring was carried out daily and included inspection for dead animals and control of food and water supplies. Each animal's health was closely assessed at least once weekly and the results documented in health monitoring sheets. The general physical condition was assessed with regard to the following parameters:

- Body weight change
- Macroscopic assessment of activity level/ behavior

- Macroscopic assessment of general discomfort: drop in body temperature determined by touch and by visual inspection of ears and paws. Ears and paws appear pink in a healthy mouse, white in a mouse with discomfort indicated by reduced blood circulation
- Macroscopic assessment of fur condition and appearance of eyes, inspection of body cavities/ fluids
- Macroscopic assessment of irregularities in breathing ability
- Indication of pain
- Macroscopic assessment for signs of automutilation and or fighting

4.5.3 Animal Treatment

4.5.3.1 Treatment Schedule, Route of Administration, and Dose

The test compounds were administered i.m. once at a dose of 5 µg (see Figure 2 and Figure 3). The control group was treated with buffer only.

4.5.3.2 Immunization

For immunization, prior anesthesia by inhalation of 2.5% isoflurane in oxygen, the injection site (hind leg) was shaved. Buffer or dissolved test item was applied i.m. into the *musculus gastrocnemius* in a volume of 20 µL. After immunization and a short recovery phase from anesthesia, the animals were observed for any immediate signs of discomfort following the immunization procedure.

4.5.3.3 Blood Sampling via the Retro-Orbital Venous Plexus or *Vena Facialis*

Blood was sampled via the retro-orbital venous plexus according to SOP-030-074. In short, mice were anesthetized by inhalation of 2.5% isoflurane in oxygen and held tightly. A thin glass capillary (29 G) was inserted gently through the retro-orbital sinus membrane and blood was collected into either Serum tubes (serum preparation) or Heparin tubes (flow cytometry analysis). After careful removal of the glass capillary, the restraining grip was loosened. Alternatively, blood collection was performed via the *vena facialis* according to SOP-030-074. In short, without prior anesthesia, mice were held tightly and using a lancet, the *vena facialis* was punctured in a precise and short movement. Blood was collected into either Serum tubes (serum preparation) or Heparin tubes (flow cytometry analysis), and the restraining grip was loosened. Blood samples were centrifuged at 10,000 x g and ambient temperature for 5 min and serum transferred to a pre-labeled 0.5 mL reagent tube, to be stored at -20°C.

4.5.4 Endpoint of Experiment / Termination Criteria

Animals were euthanized in accordance with §4 of the German animal welfare act and the recommendation of GV-SOLAS by cervical dislocation or by exposure to carbon dioxide. Additionally, termination criteria applied according to the specification within the respective animal test approval as listed below. Body weight losses exceeding 20%, or a high severity level in any of the parameters found in Section 4.5.2 were on their own sufficient reason for immediate euthanasia.

4.5.4.1 Dissection of Animals and Organ Collection

Following euthanasia, mice were disinfected with 70% ethanol and the dissection was performed starting with an abdominal incision. The spleen and dLNs (popliteal (PO), iliac (IL) and inguinal (IN), see Figure 5) were collected, pooled and stored in PBS on ice for subsequent single cell preparations.

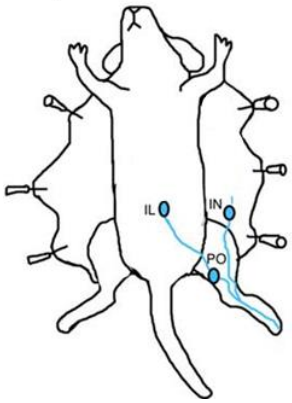

Figure 5: Draining lymph nodes resection for subsequent analysis

Depicted are the predicted draining lymph nodes after i.m. injection into the gastrocnemius muscle used for further analysis. Figure adopted according to **Harrell et al. 2008**. IL, iliac. IN, inguinal. PO, popliteal.

4.5.5 Preparation of Splenocyte Single Cell Suspensions

Single cell suspensions from collected spleens were prepared according to SOP-030-078. To this end, spleens were squeezed through 70 µm cell strainers using the plunger of a syringe to release the splenocytes into a 50 mL tube. Splenocytes were washed with an excess volume of PBS followed by centrifugation at 300 x g for 6 min at ambient temperature and discarding the supernatants. Erythrocytes were lysed with erythrocyte lysis buffer (154 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) for 5 min at ambient temperature. The reaction was stopped with an excess volume of PBS. After another washing step, cells were resuspended in DC medium (RPMI medium1640 (1x) + GlutaMAX-I [Life Technologies], 10% FBS, 1% NEAA, 1% sodium-pyruvate, 0.5% penicillin/streptomycin, 50 µM 2-mercaptoethanol), passed through a 70 µm cell strainer again, counted according to SOP-010-028, and stored at 4°C until further use.

4.5.6 Preparation of Lymph Node Single Cell Suspensions

The popliteal, iliac and inguinal dLNs (Figure 5) were stored together in a plastic tube containing 450 µL PBS at ambient temperature in the dark until single cell preparation. 50 µL collagenase D (10 mg/mL) were added to yield a final concentration of 1 mg/mL, the dLNs were thoroughly cut into pieces using forceps or scissors, and incubated for 10 min at 37°C. Cells were passed through a 70 µm cell strainer placed on a 50 mL plastic tube and minced using the plunger of a 5 mL syringe. The cell strainer is subsequently rinsed using 5 mL of PBS and the cell solution counted according to SOP-010-028.

4.5.7 RNA Electroporation

CT26 colon carcinoma cells (ATCC) were washed once with 10 mL of serum-free X-Vivo 15 medium, centrifuged (300 ×g, 6 min, ambient temperature), taken up in 1–2 mL of X-Vivo 15 medium, counted (SOP-010-028), and diluted to a concentration of 25 × 10^6 cells/mL. S Protein encoding modRNA or irrelevant modRNA (10 µg in 40 µL of X-Vivo 15 medium each) was carefully placed at the bottom of a 4 mm electroporation cuvette, topped up with 200 µL of cells (corresponding to 5 × 10^6 cells) and shortly mixed by pipetting up and down. Electroporation was then performed with a BTX™ ECM™ 830 Square Wave Electroporator applying one 300 V pulse for 15 ms. Immediately after electroporation, cells were transferred to a 15 mL tube containing 1–2 mL of DC medium, counted, and diluted to 4 × 10^5 cells/mL for the cytotoxicity assay, and 5 × 10^5 cells/mL for the IFNγ ELISpot assay (Section 4.5.8).

4.5.8 ELISpot Assay

IFNγ ELISpot assay was performed according to SOP-030-110 (with minor modifications as described below) using the mouse IFN-γ ELISpot^{PLUS} kit. Briefly, 96-well ELISpot plates were washed with PBS and blocked with serum-containing medium (DC medium) for at least 30 min at 37°C. After blocking, 100 µl of the splenocyte solution (5 x 10^5 cells) as well as 100 µl electroporated CT26 cells (5 x 10^4 cells) or 100 µl S peptide mix (final concentration per well: 0.1 µg/ml) were added yielding a final volume per well of 200 µL. No peptide or irrelevant RNA transfected cells were used as controls. Plates were incubated overnight in a 37°C humidified incubator with 5% CO_2. After approximately 18 h cells were discarded and a second biotinylated anti-mouse IFN-γ antibody incubated for 2 h at ambient temperature. The plate was then developed by addition of Streptavidin-ALP for 1 h at ambient temperature in the dark followed by addition of BCIP®/NBT substrate for 5–7 min at ambient temperature in the dark. Spots were counted on a CTL ELISPOT reader ImmunoSpot® S6 Core Analyzer according to SOP-010-099.

4.5.9 xCELLigence Cytotoxicity Assay

Preparation of targets:

Of DC medium, 50 µL per well were added to a 96-well PET E-plate to perform a blank measurement at an xCELLigence RTCA MP, Real Time Cell Analyzer. Tumor cells (50 µL of a 4 × 10^5 cells/mL suspension, corresponding to 2 × 10^4 cells) electroporated with S RNA or irrelevant RNA (10 µg each) were subsequently added to the E-plate. After allowing the cell suspension to settle down for 30 min at ambient temperature, the E-plate was transferred to the xCELLigence device and measurement was continued.

Peptide loading of targets:

In mCorVac#16, 100 µl S peptide mix (final concentration per well: 0.1 µg/ml) was added to S RNA electroporated tumor cells one hour prior T cell addition. After one hour of incubation, the medium was carefully aspirated and the wells were washed with PBS twice. Before adding the effector cells, 100 µl of DC medium was dispensed per well.

Addition of effectors:

On the same day, splenocytes were transferred to a T25 cell culture flask at a density of 1.5–2 × 10^6 cells/cm^2. S peptide mixes and recombinant IL-2 (Proleukin) were added to yield a final concentration of 0.1 µg/mL and 100 U/mL, respectively, and the cell suspension was kept at 37°C, 5% CO_2 overnight. On the day after, restimulated splenocytes were transferred to a 15 mL plastic tube, the T25 flask was rinsed with 5 mL of MACS buffer and added to the same tube. Subsequently, $CD8^+$ cells were isolated from restimulated splenocytes using CD8a (Ly-2) MACS® MicroBeads according to the manufacturer's instructions. Labeled cells were eluted from MACS LS columns, centrifuged (5 min at 460 ×g), taken up in 1–2 mL of warm (approximately 37°C) DC medium, counted (SOP-010-028) and diluted with DC medium to a concentration of 6 × 10^6 cells/mL. $CD8^+$ cells (100 µL), DC medium or Staurosporin (4 µM final concentration) were added in duplicate to the targets in the E-plate and the xCELLigence measurement was continued for at least three days. RTCA Data analysis software or xCELLigence RTCA Software Pro (both ACEA Biosciences) were used for data analysis.

4.5.10 Cytokine Multiplex Protein Quantification

Cytokine concentrations were determined in supernatants derived from *ex vivo* restimulated splenocytes and dLN cells. 5 × 10^5 splenocytes or dLN cells in 100 µL medium/well were transferred to a 96-well U-bottom plate, and 100 µL medium supplemented with S peptide mixes to a final concentration of 0.2 µg/mL/peptide/well, or cell culture medium only (negative control) were added and mixed. For each group, three samples were treated with 100 µL PMA and ionomycin to a final concentration of

0.5 µg/mL and 1 µg/mL/well, respectively (positive controls). Cells were incubated for 48 h at 37°C, 5% CO_2. Supernatants were harvested and stored at -20°C for the cytokine multiplex assay.

Cytokine concentrations in supernatants of restimulated splenocytes and dLN cells were determined from thawed cell culture supernatants using a bead-based, 11-plex Th1/Th2 mouse ProcartaPlex immunoassay according to the manufacturer's instructions. Analytes included in the assay were IFNγ, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-6, TNF, GM-CSF, and IL-18.

Fluorescence was measured with the Bioplex200 system and analyzed with ProcartaPlex Analyst 1.0 software (Thermo Fisher Scientific).

4.5.11 Flow Cytometry

All flow cytometric data were acquired on a BD Symphony A3 or BD Celesta (B cell analysis) flow cytometer using BD FACSDiva software version 9.1 or 8.0.1.1, respectively, and analyzed with FlowJo 10.6 (FlowJo LLC, BD Biosciences).

4.5.11.1 Restimulation of T cells for functional T cell analysis in the spleen and dLN

For functional analysis, splenocytes and dLN cells were *ex vivo* restimulated. 4×10^6 splenocytes and 1×10^6 (mCorVAC#15) or 2×10^6 (mCorVAC#16) dLN cells in 100 µL DC medium/well were transferred to a 96-well U-bottom plate. To each well, 50 µL medium were added, supplemented with either S peptide mixes to a final concentration of 0.2 µg/mL/peptide/well (mCorVAC#15) or 0.5 µg/mL/peptide/well (mCorVAC#16), or medium only (negative controls), and mixed. To one sample per group, 50 µL PMA and ionomycin to a final concentration of 0.5 µg/mL and 1 µg/mL/well, respectively, were added (positive controls). Three additional wells of any group were added as unstained controls.

Cells were quickly spun down (30 s, 460 × g) and incubated for 1 h at 37°C, 5% CO_2. To each well, 50 µL GolgiStop and GolgiPlug in medium were added to a final dilution of GolgiStop of 1:1,500 and GolgiPlug of 1:1,000, mixed, and cells were further incubated for 4 h at 37°C, 5% CO_2.

4.5.11.2 Functional T cell analysis in the spleen and dLN

For mouse functional T cell analysis, restimulated cells (see 4.5.11.1) were centrifuged (5 min, 300 × g) and supernatants discarded. Flow cytometry master mixes (MM) for functional T cell analysis are depicted in Table 8 and Table 9.

Cells were stained with fixable viability dye and extracellularly with antibodies against CD3, CD4, CD8α, CD44, PD-1, CD40L, CD62L and CXCR5 (mCorVAC#15, MM1a), or CD4, CD8α, CD44, CD45, PD-1, CD40L, CD62L and CXCR5 mCorVAC#16, MM1b)

in Brilliant Stain Buffer Plus diluted 1:5 in flow buffer (PBS supplemented with 2% FCS, 2 mM EDTA, 0.01% sodium azide) in a total volume of 50 µL for 30 min at 2-8 °C. After washing cells once with 200 µL flow buffer (5 min, 300 × g), cells were stained with streptavidin Brilliant Stain Buffer Plus diluted 1:5 in flow buffer (MM2). After washing cells once with 200 µL flow buffer, cells were fixed with 200 µL 2% RotiHistofix for 15 min at room temperature (mCorVAC#15) and resuspended in 200 µL Perm/Wash buffer (FoxP3/Transcription Factor Staining Buffer Set) overnight at 2-8 °C (mCorVAC#15), or overnight at 2-8 °C (mCorVAC#16). After washing cells once with 200 µL Perm/Wash buffer (5 min, 500 × g) (mCorVAC#16), permeabilized cells were intracellulary treated with 25 µL Fc block (diluted 1:50) for 10 min at 2-8 °C before IL-4, TNF, Bcl-6, IFNγ, T-bet and IL-2 antibodies (mCorVAC#15, MM3a) or IL-4, TNF, IFNγ, T-bet, IL-2 and CD3 (mCorVAC#16, MM3b) in Perm/Wash buffer in a total volume of 25 µL were added, and cells incubated for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells twice with 200 µL Perm/Wash buffer (5 min, 500 × g), cells were resuspended in 200 µL flow buffer. Fluorescence minus 10 (FM10) controls were stained for viability and with antibodies against CD3, CD8a, CD4 and CD62L (mCorVAC#15), or CD3, CD8a, CD4, CD45 and CD62L (mCorVAC#16) only.

Table 8: Flow cytometry antibody master mixes for functional T cell analysis in the spleen and dLN (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1a	mCorVAC#15							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD	563565	9204644	31.05.2022	100	0,5
BUV563	CD44	IM7	BD	741227	0119427	30.04.2021	2,500	0,1
BV421	CXCR5	L138D7	BioLegend	145512	B281252	L138D7	50	1
BV480	CD4	RM4-5	BD	565634	9016508	31.05.2020	250	0,2
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1
BV785	CD62L	MEL-14	BioLegend	104440	B272550	N/A	200	0,25
FITC	CD8	30-F11	BD	553079	6197750	31.08.2021	200	0,25
Biotin	CD40L	MR1	BD	553657	8186567	12.04.2024	100	0,5
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,000	0,05

MM1b	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV563	CD44	IM7	BD Biosciences	741227	0119427	30.04.2021	2,500	0,02

BUV737	CD45	53-6.7	BD Biosciences	564297	9030634	N/A	200	0,25
BV421	CXCR5	L138D7	BioLegend	145512	B281252	N/A	100	0,50
BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV785	CD62L	MEL-14	BioLegend	104440	B258213	N/A	200	0,25
FITC	CD8	30-F11	BD Biosciences	553079	6197750	31.08.2021	200	0,25
Biotin	CD40L	MR1	BD Biosciences	553657	8186567	12.04.2024	100	0,50
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,000	0,05

MM2	**mCorVAC#16**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
PE	Streptavidin	N/A	BioLegend	405203	B170498	N/A	200	0,25

MM3a	**mCorVAC#15**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BV711	IL-4	11B11	BD	564005	9276915	21.03.2021	100	0,5
BB700	TNF	MP6-XT22	BD	566510	0021825	31.03.2021	5,000	0,01
PE	Bcl-6	K112-91	BD	561522	9165931	30.06.2022	50	1
PE-Cy7	IFNγ	XMG1.2	eBioscience	25-731182	E07672-1632	09.2014	1,000	0,05
AF647	T-bet	4B10	biolegend	644804	B248741	N/A	5,000	0,01
APC-R700	IL-2	JES6-5H4	BD	565186	9303906	31.03.2021	5,000	0,01

MM3b	**mCorVAC#16**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD Biosciences	563565	9204644	31.05.2022	500	0,10
BV711	IL-4		BD Biosciences	564005	9276915	21.03.2021	100	0,5

BB700	TNF	MP6-XT22	BD Biosciences	566510	0021825	31.03.2021	5,000	0,01
PE-Cy7	IFNγ	XMG1.2	eBioscience	25-731182	E07672-1632	09.2014	1,000	0,05
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5,000	0,01
APC-R700	IL-2	JES6-5H4	BD Biosciences	565186	9303906	31.03.2021	5,000	0,01

4.5.11.3 Phenotypic T cell analysis in the spleen and dLN

For mouse phenotypic T cell analysis in the spleen and dLNs, 4×10^6 splenocytes and 1×10^6 (mCorVAC#15) or 1.5×10^6 (mCorVAC#16) dLN cells/well were transferred to a 96-well U bottom plate, centrifuged (3 min, 300 × g, 2–8°C) and supernatants discarded. Flow cytometry MM for phenotypic T cell analysis are depicted in Table 9.

Cells were stained with fixable viability dye and extracellularly with antibodies against CD3, CD4, CD8α, CD25, CD44, PD-1, CD62L, ICOS, CD19 and CXCR5 (mCorVAC#15, MM1a), or CD4, CD8α, CD25, CD44, CD45, PD-1, CD62L, ICOS, CD19 and CXCR5 (mCorVAC#16, MM1b) in Brilliant Stain Buffer Plus diluted 1:5 in flow buffer (PBS supplemented with 2% FCS, 2 mM EDTA, 0.01% sodium azide) in a total volume of 50 µL for 30 min at 2-8 °C. After washing cells twice with 200 µL flow buffer (5 min, 300 × g), cells were resuspended in 200 µL 2% RotiHistofix, immediately centrifuged (5 min, 300 × g) and fixed again with 200 µL Fix/Perm buffer (FoxP3/Transcription Factor Staining Buffer Set) overnight at 2-8 °C (mCorVAC#15), or for 20 min at 2-8 °C and incubated in 200 µL Perm/Wash buffer overnight at 2-8°C (mCorVAC#16). After washing cells once with 200 µL Perm/Wash buffer (5 min, 500 × g) (mCorVAC#15), permeabilized cells were intracellulary treated with 25 µL Fc block (diluted 1:50) for 10 min at room temperature before T-bet, GATA3, FoxP3 and Bcl-6 antibodies (mCorVAC#15, MM2a) or T-bet, GATA3, FoxP3 and CD3 (mCorVAC#16, MM2b) in Perm/Wash buffer in a total volume of 25 µL, and cells incubated for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells twice with 200 µL Perm/Wash buffer (5 min, 500 × g), cells were resuspended in 200 µL flow buffer. Fluorescence minus 10 (FM10) controls were stained for viability and with antibodies against CD3, CD8a, CD4, CD62L and CD19 only.

Table 9: Flow cytometry antibody master mixes for phenotypic T cell analysis in the spleen and dLN (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1a	**mCorVAC#15**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD Biosciences	565992	9204644	31.05.2022	100	0,50

BUV563	CD44	IM7	BD Biosciences	741227	119427	30.04.2021	2,500	0,02
BV421	CXCR5	L138D7	BioLegend	145512	B281252	N/A	100	0,50
BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV711	CD25*	PC61	BD Biosciences	740714	119426	30.04.2021	500	0,10
BV785	CD62L	MEL-14	BioLegend	104440	B272550	N/A	200	0,25
FITC	CD8	53-6.7	BD Biosciences	553031	9143776	31.08.2021	200	0,25
PerCPeF710	ICOS	7E.17G9	Invitrogen	46-9942-82	2029789	30.04.2021	50	1,00
AF700	CD19	6D5	BioLegend	115528	B261756	N/A	100	0,50
ef780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,000	0,05

MM1b	**mCorVAC#16**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV563	CD44	IM7	BD Biosciences	741227	0119427	30.04.2021	2,500	0,02
BUV737	CD45	53-6.7	BD Biosciences	564297	9030634	N/A	200	0,25
BV421	CXCR5	L138D7	BioLegend	145512	B281252	N/A	100	0,50
BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV711	CD25	PC61	BD Biosciences	740714	0119426	30.04.2021	500	0,10
BV785	CD62L	MEL-14	BioLegend	104440	B272550	N/A	200	0,25
FITC	CD8	30-F11	BD Biosciences	553031	9143776	31.08.2021	200	0,25
PerCPeF710	ICOS	7E.17G9	Invitrogen	46-9942-82	2029789	30.04.2021	50	1,00
AF700	CD19	6D5	BioLegend	115528	B261756	N/A	100	0,50
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,000	0,05

MM2a	mCorVAC#15							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
PE	Bcl-6		BD Biosciences	561522	9165931	30.06.2022	100	0,5
PECF594	FoxP3	MF23	BD Biosciences	562466	9276149	28.20.2021	200	0,25
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	2142972	N/A	25	2
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	100	0,5

MM2b	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD Biosciences	563565	9204644	31.05.2022	50	0,50
PE-CF594	FoxP3	MF23	BD Biosciences	562466	9276149	28.20.2021	200	0,25
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5,000	0,01

4.5.11.4 Phenotypic T cell analysis in the blood

For mouse phenotypic T cell analysis in peripheral blood, 50 µL freshly drawn blood were transferred to round bottom 5-mL tubes, washed once with 500 µL PBS (Gibco) (300 × g, 8 min) and the cell pellet was resuspended in 2 mL ACK lysing buffer (Gibco) and incubated for 3 min at room temperature. Flow cytometry master mixes (MM) for phenotypic T cell analysis are depicted in Table 10.

Cells were washed twice with 1 mL flow buffer (300 × g, 8 min) and stained with fixable viability dye and anti-CXCR5 (rat IgG2a) antibody in the presence of Fc block diluted 1:100) in flow buffer in a total volume of 50 µL for 20 min at room temperature (MM1). After washing cells twice with 1 mL flow buffer (8 min, 300 × g), cells were stained with anti-rat IgG2a biotin in flow buffer in a total volume of 50 µL for 20 min at 2-8 °C (MM2). After washing cells twice with 1 mL flow buffer (8 min, 300 × g), cells were stained extracellularly with antibodies against CD3, CD4, CD8α, CD25, CD38, CD44, PD-1, CD62L, ICOS, CD127 (4-1BB), CD19 and streptavidin (mCorVAC#15, MM3a), or CD4, CD8α, CD25, CD38, CD44, PD-1, CD62L, ICOS, CD127 (4-1BB), CD19 and treptavidin (no CD3, mCorVAC#16, MM3b) in Brilliant Stain Buffer Plu diluted 1 5 in flow buffer in a total volume of 50 µL for 20 min at 2-8 °C. After washing cells once with 1 mL flow buffer (5 min, 300 × g), cells were fixed in 200 µL 2% RotiHistofix for 15 min at room temperature (mCorVAC#15), or centrifuged immediately after mixing (5 min,

300 × g) and fixed again with 200 µL Fix/Perm buffer (FoxP3/Transcription Factor Staining Buffer Set) for 20 min at 2-8 °C (mCorVAC#16). After centrifugation (5 min, 500 × g), cells were resuspended in 200 µL Perm/Wash buffer (FoxP3/Transcription Factor Staining Buffer Set) and incubated over night at 2-8 °C. Permeabilized cells were centrifuged (5 min, 500 × g) and intracellulary treated with 25 µL Fc block (diluted 1:50) in Perm/Wash buffer for 10 min at 2-8 °C before T-bet and GATA3 antibodies (mCorVAC#15, MM4a) or CD3, FoxP3, T-bet and GATA3 antibodies (mCorVAC#16, MM4b) in Perm/Wash buffer in a total volume of 25 µL were added, and cells incubated for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells twice with 1 mL Perm/Wash buffer (5 min, 500 × g), cells were resuspended in 150 µL flow buffer. Fluorescence minus 10 (FM10) controls were stained for viability and with antibodies against CD3, CD8a, CD4, CD62L, CD19 and streptavidin only.

Table 10: Flow cytometry antibody master mixes for phenotypicT cell analysis in the blood (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
Purified	CXCR5	2G8	BD Biosciences	551961	9143926	28.02.2027	100	0,50
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1000	0,05
N/A	Fc block	2.4G2	BD	553142	0028326	31.05.2027	100	0,50

MM2								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
Biotin	IgG2a	RG7/1.30	BD Biosciences	553894	9288614	31.05.2024	100	0,50

MM3a	**mCorVAC#15**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD	563565	9204644	31.05.2022	100	0,50
BUV563	CD44	IM7	BD Biosciences	741227	0119427	30.04.2021	2,500	0,02
BUV737	CD8a	53-6.7	BD Biosciences	564297	9030634	N/A	200	0,25
BV421	Streptavidin	N/A	BD Biosciences	563259	9197684	31.12.2021	200	0,25

BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV711	CD25	PC61	BD Biosciences	740714	0119426	30.04.2021	500	0,10
BV785	CD62L	MEL-14	BioLegend	104440	B258213	N/A	200	0,25
AF488	CD38	90	BioLegend	102714	B298187	N/A	100	0,50
PerCPeF710	ICOS	7E.17G9	Invitrogen	46-9942-82	2029789	30.04.2021	50	1,00
PE	4-1BB	17B5	eBioscience	12-1371-82	E01500-1632	N/A	100	0,50
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	B2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5000	0,01
AF700	CD19	6D5	BioLegend	115528	B261756	N/A	100	0,50

MM3b	**mCorVAC#16**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV563	CD44	IM7	BD Biosciences	741227	0119427	30.04.2021	2500	0,02
BUV737	CD8a	53-6.7	BD Biosciences	564297	9030634	N/A	200	0,25
BV421	Streptavidin	N/A	BD Biosciences	563259	9197684	31.12.2021	200	0,25
BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV711	CD25	PC61	BD Biosciences	740714	0119426	30.04.2021	500	0,10
BV785	CD62L	MEL-14	BioLegend	104440	B258213	N/A	200	0,25
AF488	CD38	90	BioLegend	102714	B298187	N/A	100	0,50
PerCPeF710	ICOS	7E.17G9	Invitrogen	46-9942-82	2029789	30.04.2021	50	1,00
PE	4-1BB	17B5	eBioscience	12-1371-82	E01500-1632	N/A	100	0,50
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	B2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5000	0,01
AF700	CD19	6D5	BioLegend	115528	B261756	N/A	100	0,50

MM4a	mCorVAC#15							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
PE-CF594	FoxP3	MF23	BD Biosciences	562466	9276149	28.02.2021	200	0,25
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	B2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5,000	0,01

MM4b	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD Biosciences	563565	9204644	31.05.2022	50	0,50
PE-CF594	FoxP3	MF23	BD Biosciences	562466	9276149	28.02.2021	200	0,25
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	B2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5,000	0,01

4.5.11.5 B cell analysis in the spleen and dLN

For mouse B cell analysis in the spleen and dLNs, 1 × 10^6 splenocytes and 2.5 × 10^5 dLN cells/well were transferred to a 96-well V bottom plate, centrifuged (5 min, 300 × g, 2–8 °C) and supernatants discarded. Flow cytometry MM for B cell analysis are depicted in Table 11.

Cells were treated with Fc block (diluted 1:50) in 50 µL flow buffer for 15 min at 2-8 °C and cells were stained with fixable viability dye and extracellularly with antibodies against CD19, CD45R/B220, IgD, CD138, IgM, CD38, CD95/FAS, IgG1, IgG2a, GR-1, F4/80, CD4 and CD8a (mCorVAC#15, MM1a) in Brilliant Stain Buffer in a total volume of 50 µL for 20 min at 2-8 °C (staining volume: 100 µL); or cells were directly treated with fixable viability dye and extracellularly with antibodies against CD19, CD45R/B220, IgD, CD138, IgM, CD38, CD95/FAS, GR-1, F4/80, CD4 and CD8a (mCorVAC#16, MM1b), in Brilliant Stain Buffer in a total volume of 100 µL for 20 min at 2-8 °C (staining volume: 100 µL). In addition, cells were treated with Fc block (diluted 1:50) in 50 µL flow buffer for 15 min at 2-8 °C and stained with fixable viability dye and extracellularly with antibodies against with PD-L2, CD45R/B220, CD19, CD73, IgM, CD80, GR-1, F4/80, CD4 and CD8a in Brilliant Stain Buffer in a total volume of 50 µL (mCorVAC#15, MM3) (staining volume: 100 µL); or cells were directly treated with fixable viability dye and extracellularly with MM3 (mCorVAC#16) in Brilliant Stain Buffer in a total volume of 100 µL for 20 min at 2-8 °C (staining volume: 100 µL). After washing cells twice with 200 µL flow buffer (5 min, 400 × g, 2–8 °C), cells were fixed

with 200 µL 2% RotiHistofix and incubated over night at 2-8 °C. After washing cells once with 200 µL flow buffer (5 min, 400 × g), cells were resuspended in 100 µL flow buffer (mCorVAC#15), or stained intracellularly with antibodies against IgG1 and IgG2a (MM2) in Perm/Wash buffer in a total volume of 50 µL for 30 min at 2-8 °C (staining volume: 50 µL), before being resuspended in 100 µL flow buffer (mCorVAC#16). Fluorescence minus Fas/CD138 (FM Fas/CD138) controls were stained for MM1a and MM1b excluding CD95/FAS and CD138; fluorescence minus IgG2a (FM IgG2a) controls were stained for MM1a excluding IgG2a; fluorescence minus IgG1 (FM IgG1) controls were stained for MM1a excluding IgG1; fluorescence minus 34 (FM 34) controls were stained for MM1b excluding CD138 and CD95/FAS; fluorescence minus CD73 and CD80 (FM 73/80) controls were stained for MM3 excluding CD73 and CD80; fluorescence minus PD-L2 (FM PD-L2) controls were stained for MM3 excluding PD-L2; and fluorescence minus 35 (FM 35) controls were stained for MM3 excluding PD-L2, CD73 and CD80.

Table 11: Flow cytometry antibody master mixes for B cell analysis in the spleen and dLN (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1a	mCorVAC#15							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
FITC	FAS/CD95	Jo2	BD Biosciences	561979	8296755	30.11.2023	100	1
PE	CD38	90	Thermo Fisher	12-0381-82	2150667	25.04.2021	400	0,25
PerCPCy5.5	Gr1	RB6-8C5	BioLegend	108428	B278340	N/A	800	0,12
PerCPCy5.5	F4/80	BM8	BioLegend	123128	B276793	N/A	800	0,12
PerCPCy5.5	CD4	RM4-5	BioLegend	100540	B261856	N/A	800	0,12
PerCPCy5.5	CD8	53-6.7	BD Biosciences	551162	9098816	31.05.2023	800	0,12
PE-Cy7	IgM	R6-60.2	BD Biosciences	552867	9269114	18.07.2021	200	0,5
AF647	CD45R/B220	RA3-6B2	BioLegend	103226	B243962	N/A	1,500	0,07
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,600	0,06
BV421	IgD	11-26c.2a	BioLegend	405725	B280598	N/A	2,500	0,04
BV510	IgG1	A85-1	BD	746811	0115095	30.04.2021	200	0,5
BV605	CD138	281-2	BioLegend	142531	B299222	N/A	200	0,5
BV711	IgG2a	R19-15	BD	744533	0115092	30.04.2021	200	0.5

BV785	CD19	1D3	BD Biosciences	563333	0023948	30.06.2021	1,000	0.1

MM1b	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume /test [µL]**
FITC	FAS/CD95	Jo2	BD Biosciences	561979	8296755	30.11.2023	100	1
PE	CD38	90	Thermo Fisher	12-0381-82	2150667	25.04.2021	400	0,25
PerCPCy5.5	Gr1	RB6-8C5	BioLegend	108428	B278340	N/A	800	0,12
PerCPCy5.5	F4/80	BM8	BioLegend	123128	B276793	N/A	800	0,12
PerCPCy5.5	CD4	RM4-5	BioLegend	100540	B261856	N/A	800	0,12
PerCPCy5.5	CD8	53-6.7	BD Biosciences	551162	9098816	31.05.2023	800	0,12
PE-Cy7	IgM	R6-60.2	BD Biosciences	552867	9269114	18.07.2021	200	0,5
AF647	CD45R/B220	RA3-6B2	BioLegend	103226	B243962	N/A	1,500	0,07
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,600	0,06
BV421	IgD	11-26c.2a	BioLegend	405725	B280598	N/A	2,500	0,04
BV605	CD138	281-2	BioLegend	142531	B299222	N/A	200	0,5
BV785	CD19	1D3	BD Biosciences	563333	0023948	30.06.2021	1,000	0.1

MM2	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume /test [µL]**
BV510	IgG1	A85-1	BD	746811	0115095	30.04.2021	400	0,125
BV711	IgG2a	R19-15	BD	744533	0115092	30.04.2021	400	0,125

MM3								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume /test [µL]**
BV421	PD-L2	TY25	BD Biosciences	564245	9204505	30.11.2021	600	0.2

BV605	CD45R/B220	RA3-6B2	BioLegend	103244	B305934	N/A	800	0,12
BV786	CD19	1D3	BD Biosciences	563333	0023948	30.06.2021	1,000	0,1
PE	CD73	TY/11.8	BioLegend	127206	B267137	N/A	600	0,2
PerCPCy5.5	Gr1	RB6-8C5	BioLegend	108428	B278340	N/A	800	0,12
PerCPCy5.5	F4/80	BM8	BioLegend	123128	B276793	N/A	800	0,12
PerCPCy5.5	CD4	RM4-5	BioLegend	100540	B261856	N/A	800	0,12
PerCPCy5.5	CD8	53-6.7	BD Biosciences	551162	9098816	31.05.2023	800	0,12
PE-Cy7	IgM	R6-60.2	BD Biosciences	552867	9269114	18.07.2021	200	0,5
AF647	CD80	16-10A1	BioLegend	104718	B278896	N/A	400	0,25
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,600	0,06

4.5.11.6 Myeloid cell analysis in the spleen

For mouse myeloid cell analysis in the spleen, 2×10^6 splenocytes/well were transferred to a 96-well U bottom plate, centrifuged (3 min, 460 × g) and supernatants discarded. Flow cytometry MM for myeloid cell analysis is depicted in Table 12.

Cells were stained with Fc block and fixable viability dye in PBS in a total volume of 100 µL (MM1) for 15 min at 2-8 °C. After washing cells once with 200 µL PBS (3 min, 460 × g), cells were stained extracellularly with antibodies against CD8, CD45, BST2, CD86, XCR1, MHC class II, CD11b, PD-L1, CD103, F4/80, CD11c and GR-1 in Brilliant Stain Buffer in a total volume of 50 µL (MM2) for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells once with 200 µL PBS (3 min, 460 × g), cells were fixed with 100 µL Fix/Perm buffer (FoxP3/Transcription Factor Staining Buffer Set) for 30 min at 2-8 °C. After washing cells twice with 200 µL Perm/Wash buffer (3 min, 460 × g), cells were resuspended in 200 µL Perm/Wash buffer and incubated overnight at 2-8 °C. Permeabilized cells were centrifuged (3 min, 460 × g) and intracellularly treated with CD206 antibody in Perm/Wash buffer in a total volume of 50 µL (MM3) for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells twice with 200 µL Perm/Wash buffer (3 min, 460 × g), cells were resuspended in 200 µL flow buffer.

Table 12: Flow cytometry antibody master mixes for myeloid cell analysis in the spleen (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BV605-like	LD	N/A	ThermoFisher	L34959	1921586	N/A	800	0,06

N/A	Fc block	2.4G2	BD	553142	0028326	31.05.2027	100	0,50

MM2								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD45	30-F11	BD Biosciences	564279	9016570	N/A	100	0,50
BUV737	CD8	53-6.7	BD Biosciences	564297	9030634	N/A	100	0,50
eF450	BST2	eBio927	invitrogen	48-3172-82	2055199	N/A	100	0,50
BV510	CD86	GL-1	BioLegend	105039	B264604	N/A	100	0,50
BV650	XCR1	ZET	BioLegend	148220	B265588	N/A	100	0,50
BV786	MHC II	M5/114.15.2	BD Biosciences	742894	9333783	30.11.2020	500	0,10
FITC	CD11b	M1/70	BD Biosciences	553310	8295813	31.08.2024	200	0,25
PerCP-Cy5.5	PD-L1	10F.9G2	BioLegend	124333	B286738	N/A	100	0,50
PE	CD103	Invitrogen	12-1031-83	2054351	26.12.2021	N/A	400	0,13
PE-Dazzle594	F4/80	BM8	BioLegend	123145	B268244	N/A	100	0,50
APC	CD11c	N418	Miltenyi	130-119-802/130-102-493	5200308676/25200308676	24.3.2021/13.12.2015	100	0,50
APC-Cy7	GR-1	RB-8C5	BioLegend	108423	B209677	N/A	800	0,06

MM3								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
PE-Cy7	CD206	C068C2	BioLegend	141719	B260552	N/A	400	0,13

4.5.12 Statistical Analysis

GraphPad Prism 8 Software (La Jolla, USA) was used for statistical analysis and figure generation. The following tests were used for data analysis:

Table 13: Statistical analyses

Data set	Comparison	Statistical test
Flow cytometry, immune cell subsets	Test groups vs. control group	One-way ANOVA and Dunnett's posttest

ELISpot assay	Test groups vs. control group	Repeated measurement one-way ANOVA and Sidak's posttest
Th1/Th2 cytokines	Test groups vs. control group	Two-way ANOVA and Sidak's posttest

5 RESULTS

5.1 ELISpot assay

BALB/c mice were euthanized on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Isolated splenocytes were restimulated with S-specific overlapping peptide mixes (S peptide) or CT26 cells electroporated with RNA encoding the full-length S protein (S RNA). Recognition of S RNA transfected cells served as an additional proof for successful processing of S-specific epitopes. Cells cultivated without the presence of a peptide (No peptide) or control RNA electroporated CT26 cells (Control RNA) served as control. Statistical significance was assessed by repeated measurement one-way ANOVA and Sidak's multiple comparison post-test. Raw data can be found in Table 19.

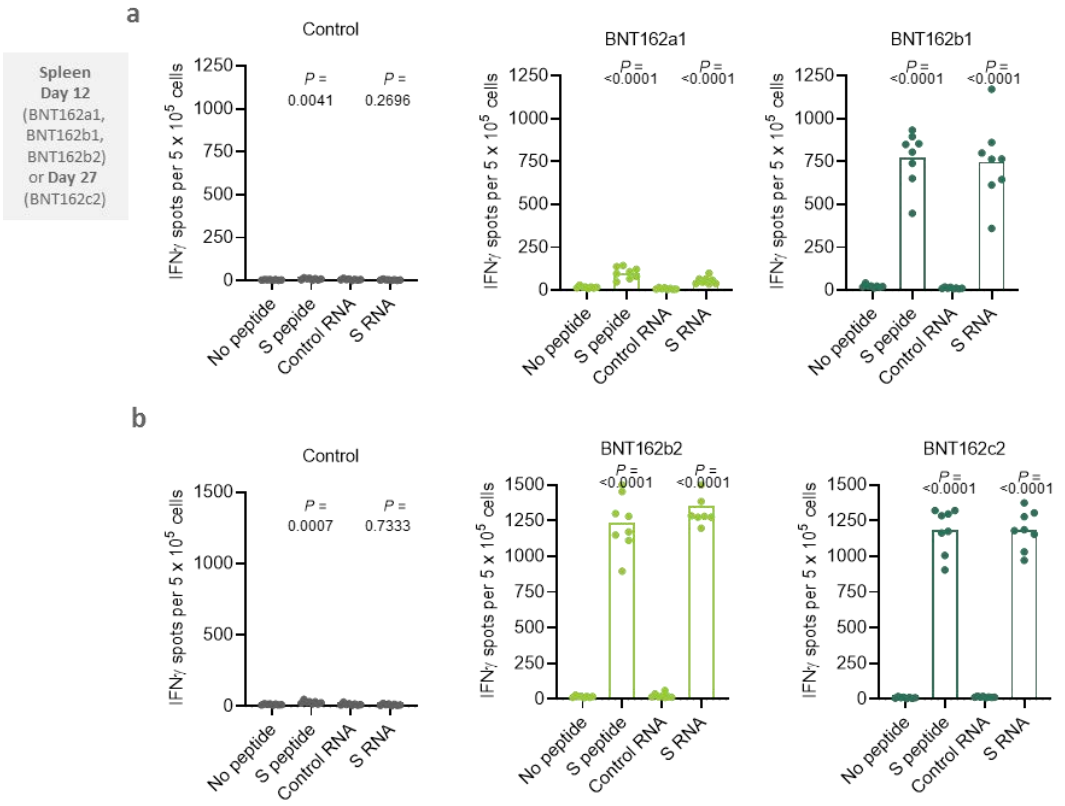

Figure 6: ELISpot analysis using splenocytes from animals treated with BNT162a1, BNT162b1, BNT162b2 or BNT162c2

ELISpot assay of splenocytes from BNT162a1 or BNT162b1 (**a**) or BNT162b2 or BNT162c2 (**b**) vaccinated mice (n=8 per group). Cells were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Splenocytes were restimulated with S-specific overlapping peptide mixes and IFNγ secretion was measured to assess T-cell responses. Mean spot counts per mouse are shown by dots; group mean values are indicated by bars. One sample in the BNT162b2 group in response to S peptide and S RNA restimulation yielded results that were too numerous to count; these values were set to 1,500.

Saturating amounts of IFNγ spots were detected in groups receiving BNT162b1, BNT162b2 or BNT162c2 after restimulation with either S peptide or S RNA. Mean spot counts were as high as 750 for BNT162b1 and exceeded 1,000 for BNT162b2 and BNT162c2. Low but significant spot counts were detected for BNT162a1, reaching a mean of 100 after S peptide restimulation and 36 after S RNA restimulation.

5.2 Flow Cytometry

Flow cytometry was applied to further characterize T- and B-cell numbers, activation status, functional profile and subtypes after vaccination in the blood, spleen and dLNs. dLNs were analyzed for functionality but are not further described in this report. Myeloid cell subsets in the spleen were analyzed but are not further described in this report. dLNs were not assayed for myeloid cell subsets due to insufficient cell numbers (for further details see Section 2.5). Statistical significance comparing the vaccinated groups to the respective control group was determined by one-way ANOVA and Dunnett's multiple comparison post-test. Raw data for analyzed immune cell subsets including tissues and subsets not described here can be found in Attachment I. Gating strategies can be found in Attachment II.

Phenotypic T- and B-cell analysis in the blood

Blood was analyzed 7 days after vaccination. The $CD8^+$ T cell percentage among $CD3^+$ T cells in the blood was significantly increased around 45% to a mean of 34% for BNT162b2 treated mice with a corresponding decrease in $CD4^+$ T cells (Figure 7a,b). No change in the percentage of $CD8^+$ or $CD4^+$ T cells among $CD3^+$ T cells was observed in any other group. A significant increase of T_{FH} cells among $CD4^+$ T cells was observed in the BNT162b1, BNT162b2 and BNT162c2 groups (Figure 7c). Highest T_{FH} levels with a mean of 1.34% were found for BNT162c2 followed by BNT162b2 (0.53%) and BNT162b1 (0.48%).

Among lymphocytes, B cell levels were significantly reduced in all groups, suggesting a redistribution from the blood into secondary lymphoid organs (Figure 7d).

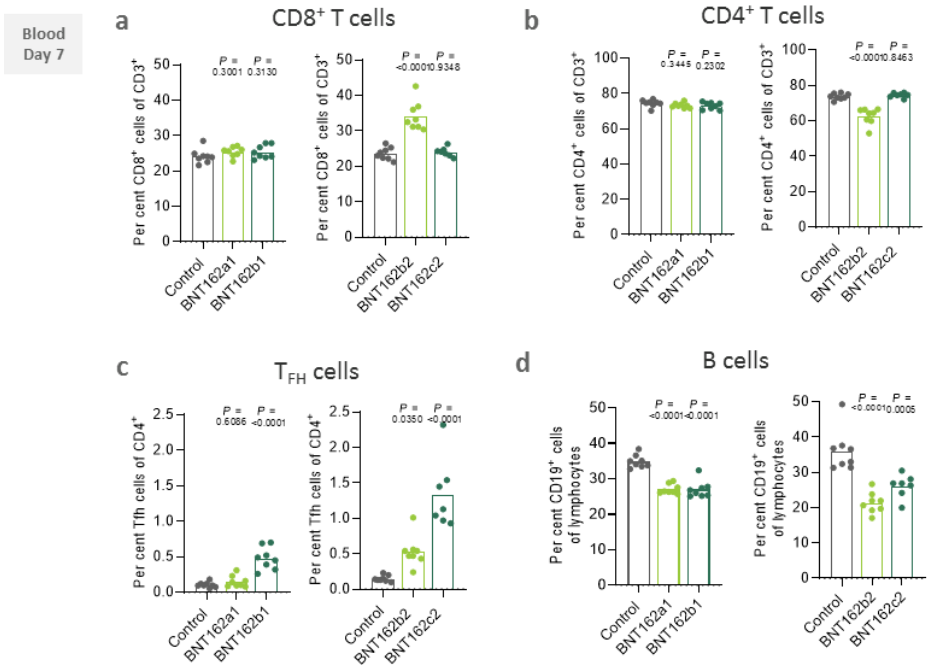

Figure 7: Analysis of lymphocyte frequencies in the blood of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of blood 7 days post BNT162a1, BNT162b1, BNT162b2 or BNT162c2 treatment (N=8 per group). Buffer treated mice served as control. For BNT162c2, the control group of mCorVAC#15 served as control (sample processing and acquisition on the same day). Cell fractions per mouse are shown by dots; group mean values are indicated by bars.

The fraction of activated T cells was particularly elevated when mice were treated with BNT162b1 or BNT162b2. In these groups, $CD8^+$ T cells significantly upregulated CD44, CD38, PD-1 as well as ICOS (Figure 8a). ICOS expression was also elevated among $CD4^+$ T cells (Figure 8b). The fraction of $ICOS^+$ T_{FH} cells was increased in all vaccinated groups but most significantly for BNT162b1, BNT162b2 and BNT162c2 (Figure 8c).

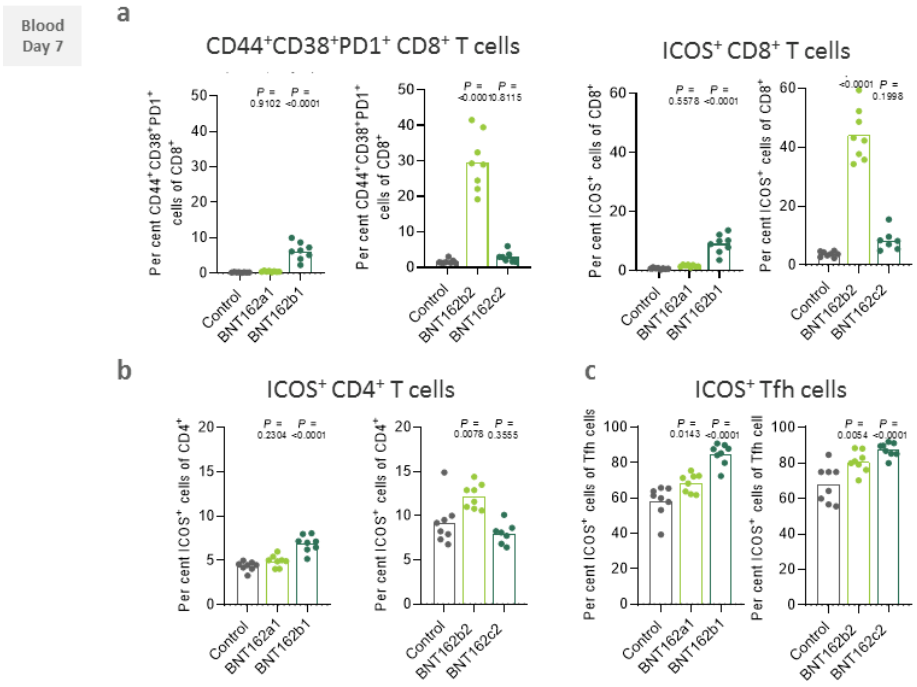

Figure 8: Analysis of T cell activation in the blood of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of blood 7 days after BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccination (N=8 per group). Buffer treated mice served as control. Cell fractions per mouse are shown by dots; group mean values are indicated by bars.

Phenotypic T- and B-cell analysis dLNs

dLNs were analyzed 12 days (BNT162a1, BNT162b1, BNT162b2) or 27 days (BNT162c2) after vaccination. As shown for the frequency among $CD3^+$ T cells in the blood (Figure 7a), $CD8^+$ T cell counts in the dLNs were significantly elevated in the BNT162b2 group (Figure 9a). $CD4^+$ T cells as well as T_{FH} cells were significantly increased in mice treated with BNT162b1 or BNT162b2 (Figure 9b,c). T_H1 T cell increase was most pronounced in the BNT162b1 (P=0.0134) and BNT162b2 (P=0.0531) groups (Figure 9d).

In line with increased T_{FH} cell counts, B cell numbers were highest in BNT162b1 (P=0.0053) and BNT162b2 (P>0.0001) vaccinated mice (Figure 10a). Among B cells, antibody secreting plasma B cells, class switched B cells and germinal center B cells crucial for affinity maturation of antibodies were significantly expanded (Figure 10b-d). In BNT162a1, BNT162b1 and BNT162b2 groups only, germinal center B cells demonstrated a class switch to IgG1 (BNT162a1, BNT162b1 and BNT162b2) or IgG2a (BNT162b1 and BNT162b2) (Figure 10e,f).

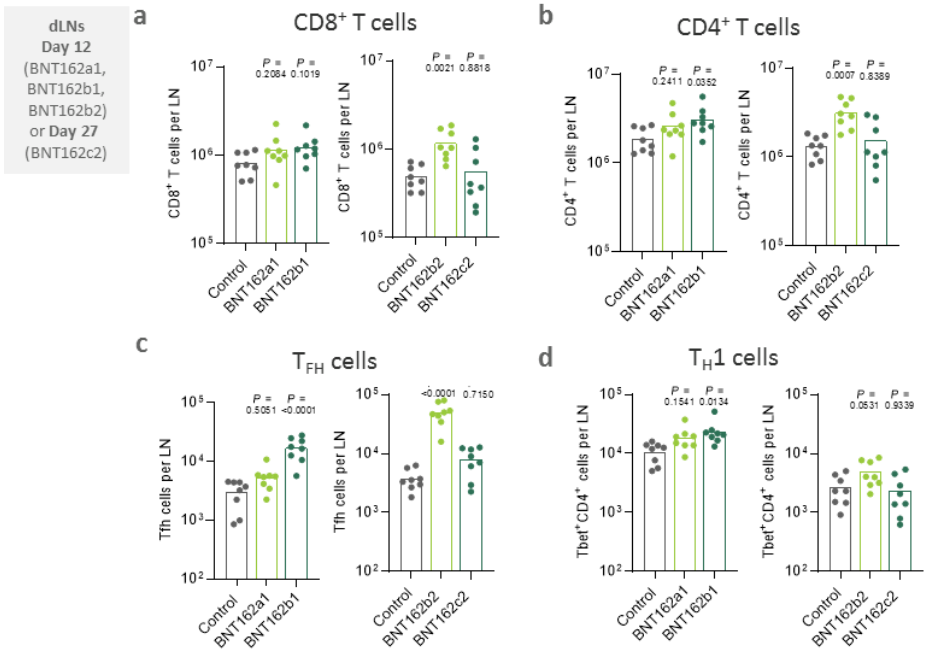

Figure 9: Analysis of T cell counts in the dLNs of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of T cells in the dLNs after BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccination (N=8 per group). Cells were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Buffer treated mice served as control. Cell counts per mouse are shown by dots; group mean values are indicated by bars.

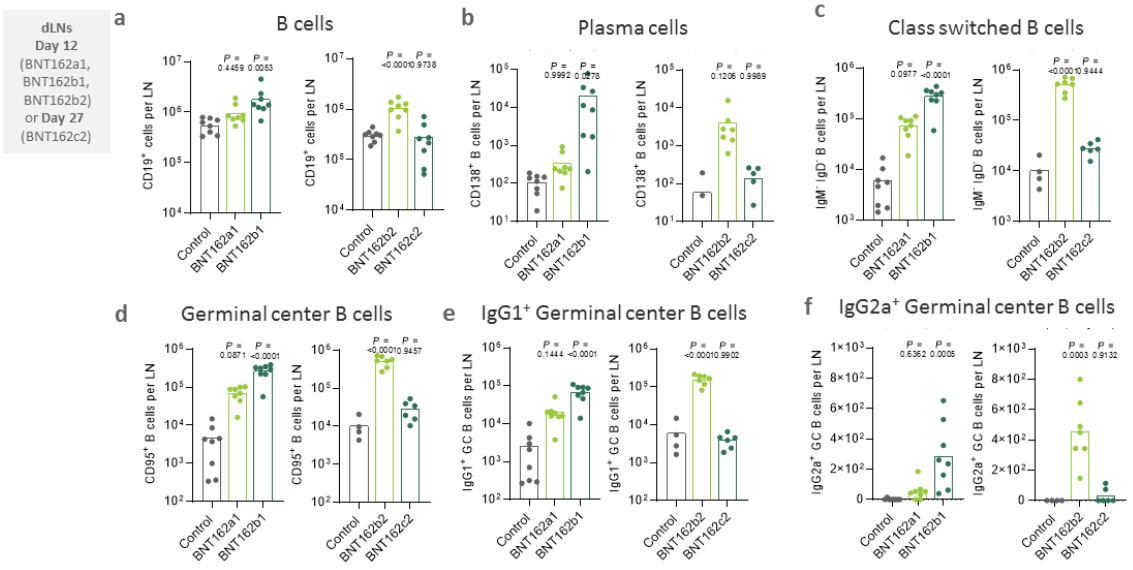

Figure 10: Analysis of B cell counts in the dLNs of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of B cells in the dLNs after BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccination (N=8 per group). Cells were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after

vaccination. Buffer treated mice served as control. Cell counts per mouse are shown by dots; group mean values are indicated by bars.

Phenotypic T- and B-cell analysis in the spleen

Analysis of T cells and B cells in the spleen revealed similar but less pronounced results compared to blood and dLNs. T_{FH} cells, germinal center B cells and class switched B cells were significantly increased upon BNT162b1 or BNT162b2 vaccination (Figure 11).

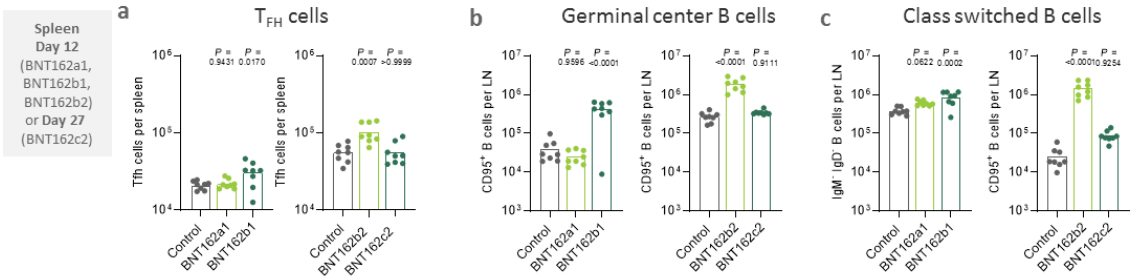

Figure 11: Analysis of T_{FH} and B cell counts in the spleen of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of T_{FH} cells (**a**), germinal center B cells (**b**) and class switched B cells (**c**) in the spleen after BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccination (N=8 per group). Cells were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Buffer treated mice served as control. Cell counts per mouse are shown by dots; group mean values are indicated by bars.

Functional T-cell analysis in the spleen

Splenocytes were analyzed by intracellular cytokine staining 12 days (BNT162a1, BNT162b1, BNT162b2) or 27 days (BNT162c2) after vaccination, to quantify antigen-specific T cells via flow cytometry. Secretion of IFNγ, IL-2 or TNF was determined in unstimulated or S peptide restimulated samples. Responses without stimulation were subtracted from S peptide stimulated samples from the same mouse and depicted for each treatment group. Cytokine responses in vaccinated animals were compared to buffer treated mice (Control) (Figure 12).

In line with ELISpot data (Figure 6), significant antigen-specific secretion of IFNγ among $CD8^+$ T cells was detectable in splenocytes of BNT162b1, BNT162b2 and BNT162c2 vaccinated animals. $CD8^+$ T cells from BNT162b1 and BNT162b2 vaccinated mice also showed significant release of IL-2 and TNF (Figure 12a). Significant numbers of $CD4^+$ T cells from BNT162b1 vaccinated mice secreted the T_H1 cytokines IFNγ and IL-2, but not the T_H2 cytokine IL-4 (Figure 12b). Although numbers were generally low and the spread between treated groups high, significant antigen-specific secretion of IFNγ among T_{FH} cells was detected in the BNT162b2 group (Figure 12c).

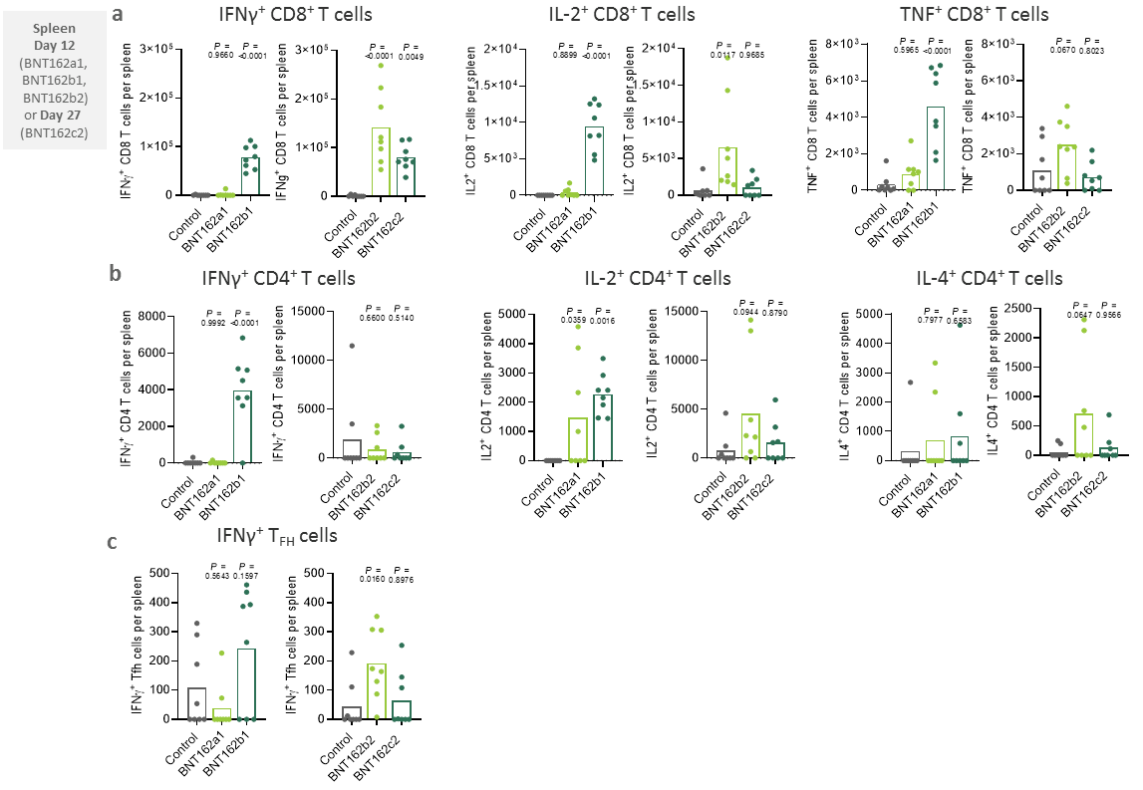

Figure 12: Quantification of cytokine secreting T cells upon S peptide restimulation in the spleen of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of cytokine secreting $CD8^+$ (**a**), $CD4^+$ (**b**) and T_{FH} cells (**c**) upon S peptide restimulation. Splenocytes of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice (N=8 per group) were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Splenocytes of buffer treated mice served as control. Cytokine positive cell counts per mouse are shown by dots; group mean values are indicated by bars. Values represent S peptide restimulated samples subtracted by unstimulated samples from the same mouse.

In summary, particularly BNT162b1, BNT162b2 and BNT162c2 vaccination mediated a potent T-cell response demonstrated by overall increased T-cell numbers, expression of molecules related to T-cell activation and the production of effector cytokines. Mainly BNT162b1 and BNT162b2 mediated a T_{FH} response in the dLNs, B cell proliferation, and the generation of significant numbers of plasma B cells and germinal center B cells undergoing Ig class switch and affinity maturation.

5.3 Cytokine Multiplex Assay

Complimentary to the analysis of cytokine secretion by IFNγ ELISpot and flow cytometry, spleen and LN cells were restimulated for 48 h with S peptide mixes or without peptide, and the release of cytokines quantified by a bead-based multiplex assay. Buffer treated animals served as control group. Unstimulated samples (cell culture medium) were compared to S peptide restimulated samples and P-values were

determined by two-way ANOVA and Sidak's multiple comparisons test. Detection ranges are provided in Table 20. Raw data including tissues and cytokines not shown in Figure 13 can be found in Table 21 to

Table 32.

Significant antigen-specific release of the T_H1 cytokines IFNγ and IL-2 was observed in the BNT162b1, BNT162b2 and BNT162c2 vaccinated groups (Figure 13a). Splenocytes from BNT162a1 treated mice mediated a significant IL-2 response and a weak IFNγ release in three of eight mice. Highest responses for both cytokines surpassing the upper limit of quantification for IFNγ were found in the BNT162b2 and BNT162c2 groups encoding the full-length S protein. Comparably weak or no secretion of the T_H2 cytokines IL-4 and IL-5 was measured (Figure 13b). Low but significant release of IL-4 and IL-5 was shown for BNT162b2 and BNT162c2. IL-4 but not IL-5 was detected in the supernatant of splenocytes from BNT162b1 vaccinated mice. Besides T_H1 cytokines, high amounts of proinflammatory IL-18 were released in the BNT162b2 and BNT162c2 vaccinated groups, and to lesser extent in the BNT162b1 and BNT162a1 vaccinated groups (Figure 13c). Additional proinflammatory cytokines were significantly elevated, such as GM-CSF (Figure 13d) or IL-6 (not shown), particularly in the BNT162b1, BNT162b2 and BNT162c2 vaccinated groups.

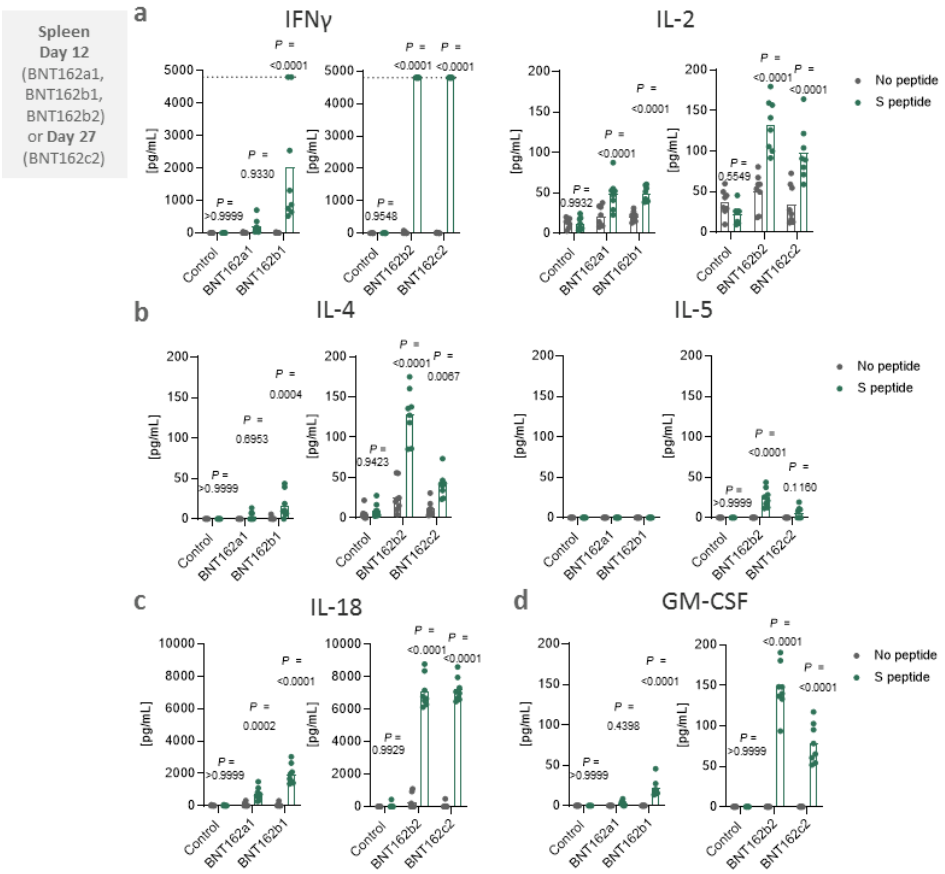

Figure 13: Quantification of cytokine secretion upon S peptide restimulation of splenocytes of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Cytokine multiplex analysis of supernatants of splenocytes upon S peptide restimulation. Splenocytes of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice (N=8 per group) were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Splenocytes of buffer treated mice (N=3) served as control. Dots indicate individual values, group mean values are indicated by bars, horizontal dotted lines indicate the upper limit of detection (ULOQ). Values below the lower limit of quantification (LLOQ) were set to zero. Values above the upper limit of quantification (ULOQ) were set to the ULOQ.

5.4 xCELLigence Cytotoxicity Assay

Isolated $CD8^+$ splenocytes were probed for their capacity to kill CT26 cells electroporated with S RNA (mCorVac#15) and additionally pulsed with S peptide mixes (mCorVac#16). $CD8^+$ T cells stimulated with CT26 cells electroporated with irrelevant RNA served as negative control. Complete tumor cell lysis was modeled by addition of Staurosporin to the S RNA electroporated or S peptide mix loaded CT26 cells. Raw data can be found in Attachment III.

In line with weak antigen-specific cytokine release (Figure 6, Figure 12, Figure 13), no relevant CT26 cell lysis was observed in the BNT162a1 group. For the BNT162b1 vaccinated group, a tendency for cell killing was observed in four out of eight mice (3-2, 3-3, 3-4 and 3-6) given that the Normalized Cell Index of CT26 cells electroporated

with irrelevant RNA was higher than for S RNA electroporated cells (Figure 14). More pronounced tumor cell lysis in eight out of eight mice was observed for splenocytes of mice vaccinated with BNT162b2 or BNT162c2, which encode the full-length S protein (Figure 15). Overall, the detected effects were rather weak and warrant further optimization of the assay. No quantitative and statistical analysis of this dataset was performed.

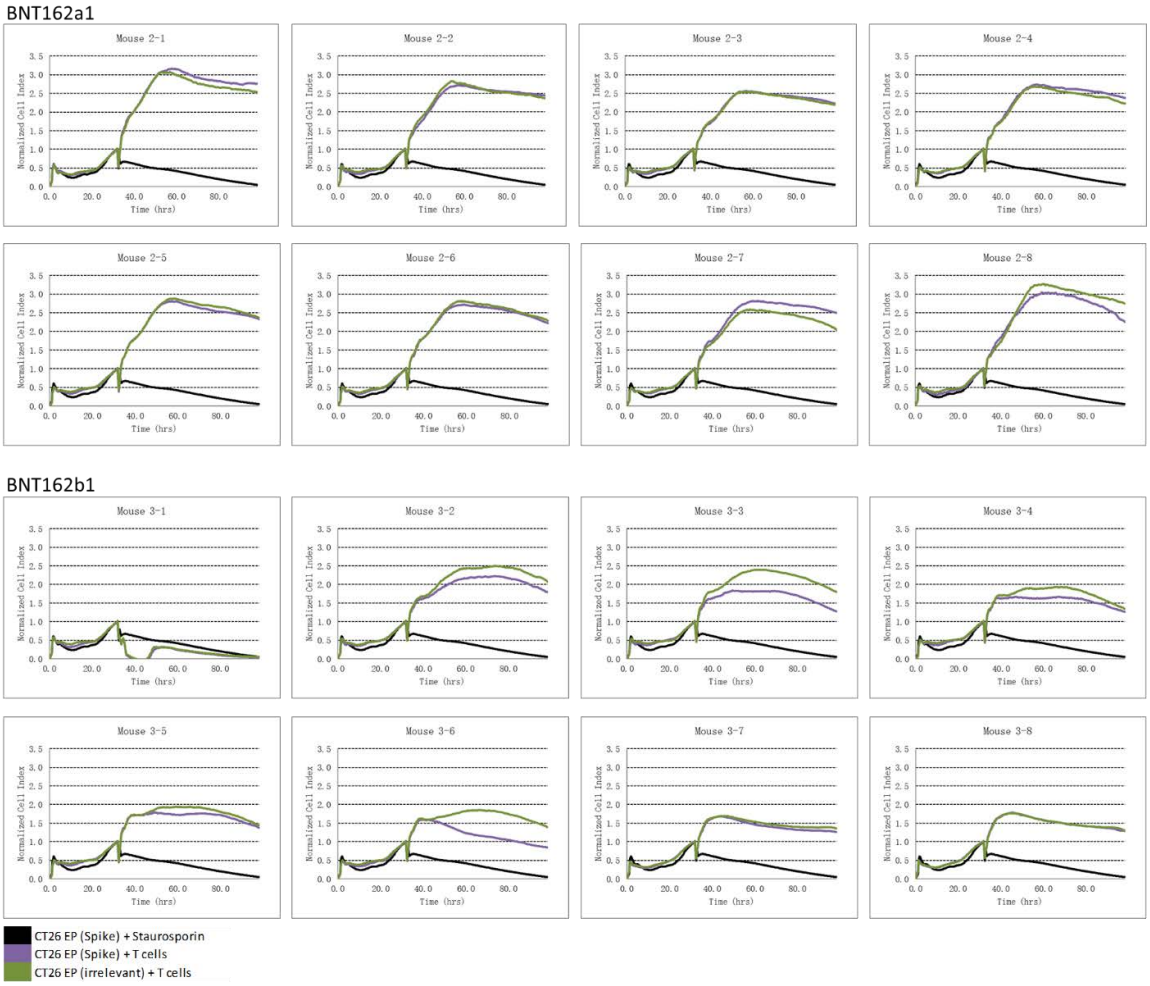

Figure 14: Cytotoxicity towards S protein expressing CT26 cells by CD8⁺ splenocytes from BNT162a1 or BNT162b1 vaccinated mice (mCorVAC#15).

Splenocytes of BNT162a1 or BNT162b1 vaccinated mice (N=8 per group) were cultured over night with S peptide and recombinant IL-2 and subsequently CD8⁺ cells were isolated via magnetic bead based separation (MACS). CT26 cells electroporated with S RNA or irrelevant RNA were cultured in xCELLigence plates for 24 h prior to addition of isolated CD8⁺ T cells. CT26 cell numbers were quantified via impedance measurement (Normalized Cell Index, higher values indicate more viable CT26 cells, normalization was performed at the time point of T cell addition). Staurosporin treatment modeled complete tumor cell lysis. CT26 cells transfected with irrelevant RNA served as negative control. Depicted is the Normalized Cell Index over time for individual mice.

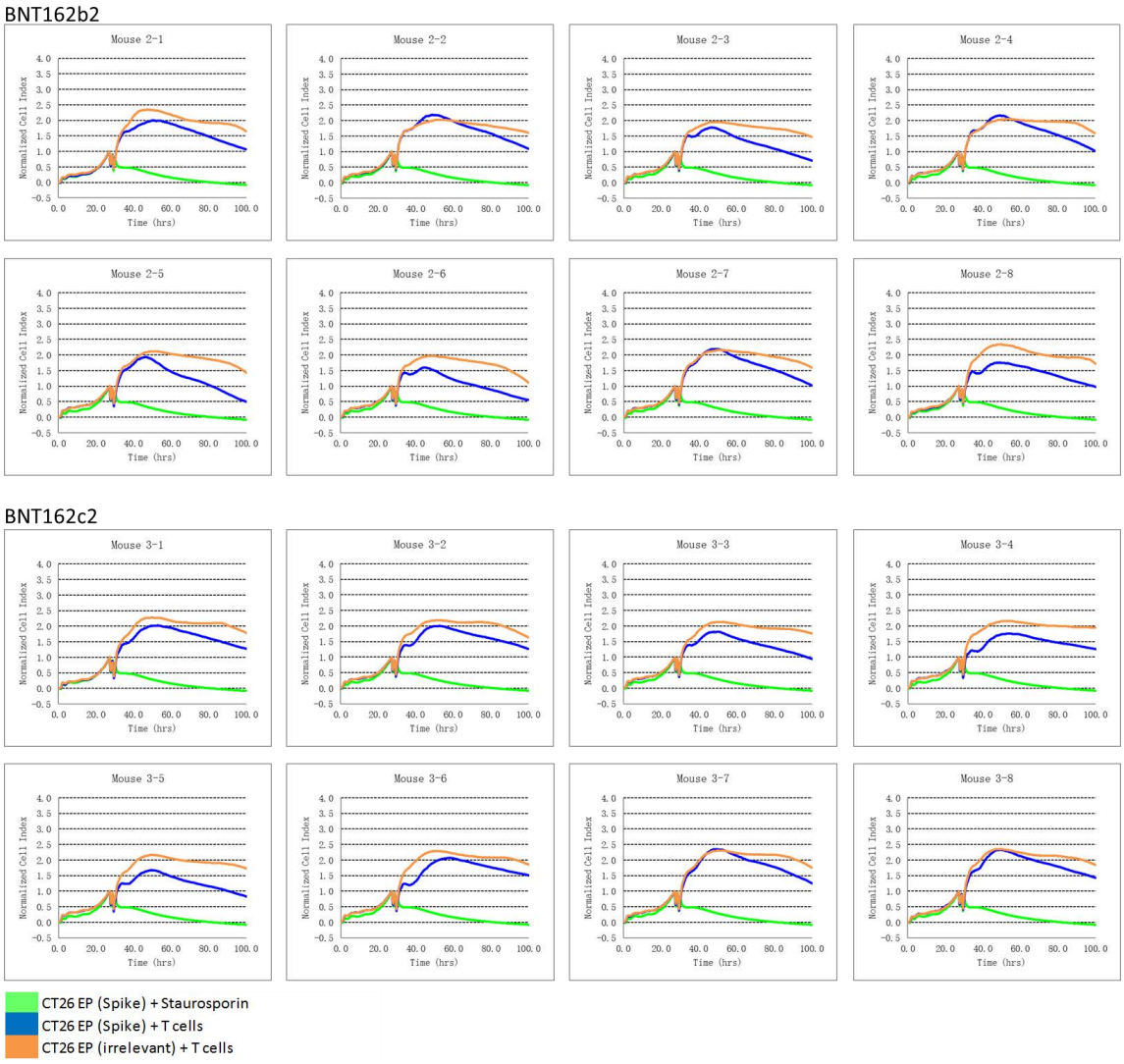

Figure 15: Cytotoxicity towards S protein expressing CT26 cells by CD8⁺ splenocytes from BNT162b2 or BNT162c2 vaccinated mice (mCorVAC#16).

Splenocytes of BNT162b2 or BNT162c2 vaccinated mice (N=8 per group) were cultured over night with S peptide and recombinant IL-2 and subsequently CD8⁺ cells were isolated via magnetic bead based separation (MACS). CT26 cells electroporated with S RNA or irrelevant RNA were cultured in xCELLigence plates for 24 h. Prior to addition of isolated CD8⁺ T cells, S RNA transfected CT26 cells were pulsed with S peptide. CT26 cell numbers were quantified via impedance measurement (Normalized Cell Index, higher values indicate more viable CT26 cells, normalization was performed at the time point of T cell addition). Staurosporin treatment modeled complete tumor cell lysis. CT26 cells transfected with irrelevant RNA served as negative control. Depicted is the Normalized Cell Index over time for individual mice.

6 CONCLUSION

This study aimed at characterizing T- and B-cell responses induced by the COVID-19 vaccine candidates BNT162a1, BNT162b2, BNT162b1 and BNT162c2 in detail.

Overall, the results of the different assay types pointed towards similar conclusions, highlighting the validity of the obtained data. IFNγ ELISpot assay, flow cytometry analysis and multiplexed quantification of cytokines suggested that particularly BNT162b1, BNT162b2 and BNT162c2 vaccination induced a potent T-cell response demonstrated by overall increased T-cell numbers, expression of molecules related to T-cell activation and the potential of T cells to produce cytokines. T-cell responses showed primarily a T_H1 phenotype with increased numbers of T-bet$^+$ CD4$^+$ T cells (mainly BNT162b1 and BNT162b2) and high secretion of T_H1 type cytokines (IFNγ, IL-2, TNF) and low secretion of T_H2 type cytokines (IL-4, IL-5). Mainly BNT162b1 and BNT162b2 mediated a T_{FH} response in the dLNs, B cell proliferation and the generation of significant numbers of antibody producing plasma B cells and germinal center B cells undergoing Ig class switch and affinity maturation.

The results of this study are in agreement with prior studies investigating the number of IFNγ specific T cells by ELISpot and IgG titers by ELISA 28 days after vaccination (R-20-0040, R-20-0042, R-20-0053, R-20-0085). Similarly to this study, responses of BNT162b1 and BNT162b2 were much stronger compared to BNT162a1 in those studies.

Since the kinetics of expression for the vaccine encoded protein of BNT162c2 differs from the other three vaccine candidates, the analysis time point was set on day 27 instead of day 12 after vaccination. It is possible that the selected time point was suboptimal and missed the peak expansion of lymphocytes. BNT162c2 induced a potent T-cell response (IFNγ ELISpot, intracellular cytokine staining by flow cytometry and multiplexed protein quantification) including the highest T_{FH} cell responses amongst all tested candidates in the blood on day 7 after treatment. However, in the dLNs on day 27 after vaccination, the impact on T_{FH} cells and B cells was weak to undetectable. Effects of BNT162c2 on both T and B cells might be stronger when analyzed at an earlier time point. Direct comparison of BNT162c2 to BNT162a1, BNT162b2 or BNT162b1 is therefore difficult and might underestimate the potential of BNT162c2.

Due to the prominent induction of both T- and B-cell responses, these results particularly support further clinical evaluation of the COVID-19 vaccine candidates BNT162b1 and BNT162b2 and warrant further evaluation of BNT162c2.

7 DOCUMENT HISTORY

First version / no change.

8 REFERENCES

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9 APPENDIX

9.1 Animal Monitoring

9.2 Animal Monitoring - Observations

Table 14: Parameters for experimental animal monitoring (single animal assessment)

The table is separated in immediate euthanasia criteria (end of experiment) and criteria, which, solitarily observed, do not lead to an immediate termination, but result in higher monitoring frequency of re-assessment. BCS, body conditioning score.

		Observation (if applicable, categorize [a]):	
Code	**Parameter**	**Renew assessment within < 24 h. Attention: evaluate accumulated parameters**	**Immediate euthanasia criteria**
1	Bodyweight [b]. Take into account BCS [c]	Body weight loss >5–10%, or BCS transition 3 to 2	Body weight loss >15-20%, or BCS 2
2	Activity	Moderate deviation from normal or unusual behavior (e.g. limited, reduced or hyperactive movements)	Immobility, very slow movements (high grade of lethargy), self-isolation
3	Appearance (condition) of fur & eyes	Fur defects/ grooming malfunction (reduced or exaggerated grooming). Moderate orbital tightening.	Distinct scruffy fur, strongly neglected grooming. Eye lids narrowed, eyes closed and sticky.
4	Body cavities & body fluids	Slight to moderate damp & sticky cavities	Clinical signs of disease (diarrhea, distinct sticky)
5	Body temperature & blood circulation ears	-	Body temperature low, ears appear white and hardly noticeable blood vessels

		Observation (if applicable, categorize [a]):	
Code	**Parameter**	**Renew assessment within < 24 h. Attention: evaluate accumulated parameters**	**Immediate euthanasia criteria**
6	Posture	Moderate deviation of normal physiological posture i.e., short pause in hunched posture	Abnormal posture, hunched, abnormally stretched (belly touches ground) or cramps
7	Reaction to stimulus [d]	Delayed reaction to unconditioned stimulus, moderate deviation from normal behavior (e.g. slight to moderate apathy)	Abnormal (distinct delayed reaction to unconditioned stimulus). Winding and enduring sound utterance ("pain"), aggressiveness to touch
8	Automutilation	-	Noticable burden, i.e. missing extremities, continuous nibbling, biting and gnawing, open wounds
9	Bites (tail, vibrissae, reproductive organs…), other wounds	Open and bleeding wounds (take care of wounds and separate from others)	Noticable burden, i.e. inflamed wounds
10	Respiration frequency	Moderate deviation of spontaneous breathing (normal respiration frequency)	High frequency, any sign of dyspnea, gasping, flat stretched posture in combination with strongly retracting flanks
11	Motor function	Weak, loose grip (cage grid)	Staggering, circular movement, missing grasp
12	Other abnormalities [e]	-	-

a Categories: NAD, no abnormality detected; +, slight; ++, moderate; +++, distinct.
b Calculate ratio bodyweight start of experiment/bodyweight monitoring day.
c According to Ullman-Culleré and Foltz 1999.
d Unconditioned = Stimulus to force a reaction e.g. normal background noise, tapping the cage and normal handling procedure e.g. tilt and turns of the cage.
e Description of abnormality (or abnormalities) on monitoring sheet.

Table 15: Record of body weights of mCorVAC#15 animals during study

						Bodyweight (grams)						
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
SBIO-15337	BIO-LO78	BALB/cJRj	f	03.03.20	1	20.4	20.3	20.5	20.6	20.3	20.8	20.6
SBIO-15337	BIO-LO79	BALB/cJRj	f	03.03.20	1	22.1	22.6	22.5	22.3	22.4	23.5	22.7
SBIO-15337	BIO-LO80	BALB/cJRj	f	03.03.20	1	20.9	21.1	20.9	20.8	20.9	21.6	21.3
SBIO-15337	BIO-LO81	BALB/cJRj	f	03.03.20	1	21.7	21.5	21.4	21.0	21.2	22.5	22.1
SBIO-15338	BIO-LO82	BALB/cJRj	f	03.03.20	1	19.6	19.8	20.2	20.4	20.7	20.5	21.2
SBIO-15338	BIO-LO83	BALB/cJRj	f	03.03.20	1	20.9	20.7	21.2	21.0	21.6	20.9	21.3
SBIO-15338	BIO-LO84	BALB/cJRj	f	03.03.20	1	19.7	19.5	19.5	19.3	19.9	20.3	19.9
SBIO-15338	BIO-LO85	BALB/cJRj	f	03.03.20	1	18.9	18.6	18.3	18.4	19.0	18.9	18.9
SBIO-15339	BIO-LO86	BALB/cJRj	f	03.03.20	2	20.9	20.6	20.9	21.2	20.8	21.1	21.2
SBIO-15339	BIO-LO87	BALB/cJRj	f	03.03.20	2	21.3	19.3	20.2	22.7	21.4	21.1	20.7
SBIO-15339	BIO-LO88	BALB/cJRj	f	03.03.20	2	23.2	20.5	21.9	22.5	22.4	22.9	22.9
SBIO-15339	BIO-LO89	BALB/cJRj	f	03.03.20	2	19.8	18.9	20.0	20.8	20.3	21.0	20.7
SBIO-15340	BIO-LO90	BALB/cJRj	f	03.03.20	2	22.5	20.9	21.3	21.7	21.6	21.7	21.6
SBIO-15340	BIO-LO91	BALB/cJRj	f	03.03.20	2	20.9	19.2	20.6	21.6	20.8	20.8	20.9
SBIO-15340	BIO-LO92	BALB/cJRj	f	03.03.20	2	21.8	21.1	21.5	22.1	21.8	21.5	22.1
SBIO-15340	BIO-LO93	BALB/cJRj	f	03.03.20	2	22.7	20.6	21.8	22.5	22.5	22.2	22.8
SBIO-15341	BIO-LO94	BALB/cJRj	f	03.03.20	3	19.3	18.2	18.9	19.0	18.9	18.9	18.9
SBIO-15341	BIO-LO95	BALB/cJRj	f	03.03.20	3	21.1	21.6	20.6	21.1	21.2	21.9	21.1
SBIO-15341	BIO-LO96	BALB/cJRj	f	03.03.20	3	20.3	19.3	20.2	20.5	20.8	20.3	20.2
SBIO-15341	BIO-LO97	BALB/cJRj	f	03.03.20	3	22.9	22.0	23.0	23.4	23.3	22.9	22.3
SBIO-15342	BIO-LO98	BALB/cJRj	f	03.03.20	3	21.1	21.0	21.7	21.7	22.6	23.1	23.3
SBIO-15342	BIO-LO99	BALB/cJRj	f	03.03.20	3	19.9	18.9	19.3	19.7	19.2	19.9	19.2
SBIO-15342	BIO-LP00	BALB/cJRj	f	03.03.20	3	22.1	21.0	22.3	22.3	20.8	22.1	21.9
SBIO-15342	BIO-LP01	BALB/cJRj	f	03.03.20	3	20.6	19.8	21.1	21.4	22.1	21.1	21.3

Table 16: Record of animal monitoring during CorVac#15 study

12: swelling of injection site muscle

						Animal Monitoring - Observations						
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
SBIO-15337	BIO-LO78	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO79	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO80	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO81	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO82	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO83	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO84	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO85	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15339	BIO-LO86	BALB/cJRj	f	03.03.20	2	NAD	3+;12+	12+	NAD	12+	NAD	NAD
SBIO-15339	BIO-LO87	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	NAD	12+	NAD	NAD
SBIO-15339	BIO-LO88	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	12+	NAD	NAD	NAD
SBIO-15339	BIO-LO89	BALB/cJRj	f	03.03.20	2	NAD	3+;12+	12+	12+	NAD	NAD	NAD
SBIO-15340	BIO-LO90	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	NAD	12+	NAD	NAD
SBIO-15340	BIO-LO91	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	NAD	12+	NAD	NAD
SBIO-15340	BIO-LO92	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	12+	12+	NAD	NAD
SBIO-15340	BIO-LO93	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	12+	NAD	NAD	NAD
SBIO-15341	BIO-LO94	BALB/cJRj	f	03.03.20	3	NAD	3+;12+	12+	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO95	BALB/cJRj	f	03.03.20	3	NAD	3+;12+	12+	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO96	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12+	NAD	NAD	NAD
SBIO-15341	BIO-LO97	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12+	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LO98	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LO99	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12+	NAD	NAD	NAD
SBIO-15342	BIO-LP00	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12++	12+	NAD	NAD
SBIO-15342	BIO-LP01	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12+	12+	NAD	NAD	NAD

Table 17: Record of body weights of CorVac#16 animals during study

n/a: not available (Treatment group 1+2: no weight measurement performed as treatment had just occurred [day 15]; Treatment group 3: Weekly weight measurement sufficient)

						Bodyweight (grams)												
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 15	Day 16	Day 17	Day 18	Day 19	Day 22
SBIO-15337	BIO-LO78	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.1	20.8	20.8	21.1	21.5	21.0
SBIO-15337	BIO-LO79	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.3	21.2	20.9	21 0	21.9	21.9
SBIO-15337	BIO-LO80	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	20.0	20.2	20.2	20 3	21.2	20.7
SBIO-15337	BIO-LO81	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	22.5	22.4	21.9	21 9	23.1	22.7
SBIO-15338	BIO-LO82	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.9	22.4	22.3	22.1	22.1	22.6
SBIO-15338	BIO-LO83	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	20.2	20.5	20.6	20.6	20.7	21.1
SBIO-15338	BIO-LO84	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	20.5	21.1	20.8	21 2	21.7	20.8
SBIO-15338	BIO-LO85	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.9	22.6	22.3	21 9	22.6	22.3
SBIO-15339	BIO-LO86	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	22.5	21.7	22.6	23 2	22.9	23.1
SBIO-15339	BIO-LO87	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	22.1	21.3	21.9	22 5	23.2	22.1
SBIO-15339	BIO-LO88	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.7	20.9	21.6	21.6	22.2	22.1
SBIO-15339	BIO-LO89	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.5	21.2	22.6	22.7	23.2	22.7
SBIO-15340	BIO-LO90	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.9	20.3	20.5	21.1	21.4	21.1
SBIO-15340	BIO-LO91	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.1	20.3	20.4	22.7	21.1	20.6
SBIO-15340	BIO-LO92	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	22.4	21.7	23.9	23 8	23.8	22.5
SBIO-15340	BIO-LO93	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	23.3	21.7	22.4	20 9	22.7	22.2
SBIO-15341	BIO-LO94	BALB/cJRj	f	03 03 20	3	21 8	20.8	21 5	22.1	21.9	22.1	22.1	n/a	23.4	n/a	n/a	n/a	22.8
SBIO-15341	BIO-LO95	BALB/cJRj	f	03 03 20	3	20 8	19.3	20 3	21.2	20.6	21.1	21.5	n/a	21.7	n/a	n/a	n/a	22.1
SBIO-15341	BIO-LO96	BALB/cJRj	f	03 03 20	3	22.4	20.1	21.4	22.5	22.1	22.2	22.1	n/a	23.3	n/a	n/a	n/a	22.8
SBIO-15341	BIO-LO97	BALB/cJRj	f	03 03 20	3	19.1	17.6	17 8	19.1	18.5	19.4	20.5	n/a	19.5	n/a	n/a	n/a	19
SBIO-15342	BIO-LO98	BALB/cJRj	f	03 03 20	3	18.7	17.2	18 0	18.6	18.5	18.8	18.2	n/a	20.1	n/a	n/a	n/a	20.6
SBIO-15342	BIO-LO99	BALB/cJRj	f	03 03 20	3	20 5	19.2	20.4	21.2	21.1	21.7	20.9	n/a	22.1	n/a	n/a	n/a	21.5
SBIO-15342	BIO-LP00	BALB/cJRj	f	03 03 20	3	19.6	17.6	19.1	19.8	19.9	19.9	19.9	n/a	22.3	n/a	n/a	n/a	22.8
SBIO-15342	BIO-LP01	BALB/cJRj	f	03 03 20	3	18.1	16.8	17.4	17.9	18.2	18.2	18.1	n/a	19.8	n/a	n/a	n/a	19

Table 18: Record of animal monitoring during CorVac#16 study

12: swelling of injection site muscle

n/a: not available (no weight measurement performed as treatment had just occurred [day 15])

						Animal Monitoring - Observations												
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 15	Day 16	Day 17	Day 18	Day 19	Day 22
SBIO-15337	BIO-LO78	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO79	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO80	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO81	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO82	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO83	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO84	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO85	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15339	BIO-LO86	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12+	12+	NAD	NAD	NAD
SBIO-15339	BIO-LO87	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12++	12+	NAD	NAD
SBIO-15339	BIO-LO88	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12+	NAD	NAD	NAD
SBIO-15339	BIO-LO89	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12+	NAD	NAD	NAD
SBIO-15340	BIO-LO90	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12+	12+	NAD	NAD
SBIO-15340	BIO-LO91	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12++	NAD	NAD	NAD
SBIO-15340	BIO-LO92	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12+	12+	NAD	NAD	NAD
SBIO-15340	BIO-LO93	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12++	12+	NAD	NAD
SBIO-15341	BIO-LO94	BALB/cJRj	f	03.03.20	3	NAD	3++;12++	3++;12++	3++;3++	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO95	BALB/cJRj	f	03.03.20	3	NAD	3++;12+++	3+;12++	3+; 12+	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO96	BALB/cJRj	f	03.03.20	3	NAD	3++;12++	3+;12++	3+;12+	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO97	BALB/cJRj	f	03.03.20	3	NAD	3++;12+++	3++;12++	3+; 12++	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LO98	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12+	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LO99	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12+	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LP00	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LP01	BALB/cJRj	f	03.03.20	3	NAD	3++;12+++	3+;12+++	3+;12+	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD

9.3 ELISpot – Raw data

Table 19: ELISpot raw data.

TNTC, too numerous to count (these values are set to 1,500 in Figure 6). Thousands were not separated by commas.

		Stimulation (well 1 | well 2)							
Group	**Mouse**	**No peptide**		**S peptide**		**Control RNA**		**S RNA**	
Control (mCorVac#15)	1	2	1	4	3	0	1	3	1
	2	3	2	2	2	6	11	2	1
	3	3	1	8	11	7	3	6	3
	4	5	6	6	4	2	4	3	4
	5	6	4	11	15	5	9	6	3
	6	6	5	9	13	5	7	4	5
	7	3	5	8	14	12	14	11	6
	8	8	4	18	15	5	6	4	1
BNT162a1	1	13	13	118	127	7	6	57	63
	2	12	9	128	148	12	7	98	101
	3	23	17	75	86	5	9	39	40
	4	14	21	51	48	5	5	38	34
	5	20	18	87	107	13	9	43	51
	6	17	23	132	156	11	22	48	84
	7	15	14	69	65	7	3	38	41
	8	18	42	96	121	13	18	64	67
BNT162b1	1	42	44	658	645	19	21	676	615
	2	11	16	456	440	21	14	399	322
	3	21	23	889	977	8	9	1124	1218
	4	26	21	871	918	11	12	779	751
	5	22	26	873	834	15	9	841	881
	6	33	16	733	746	12	12	758	842
	7	16	24	861	837	16	11	825	702
	8	17	18	837	772	9	8	628	598
Control (mCorVac#16)	1	21	9	7	57	12	11	8	11
	2	4	15	7	28	28	31	18	16
	3	13	5	12	23	11	7	6	9

		Stimulation (well 1 | well 2)							
Group	**Mouse**	**No peptide**		**S peptide**		**Control RNA**		**S RNA**	
	4	19	19	26	38	4	9	6	7
	5	8	9	19	31	8	8	7	2
	6	22	13	26	28	19	12	11	17
	7	17	15	21	24	17	20	12	13
	8	14	11	37	62	12	15	16	26
BNT162b2	1	6	14	1267	1296	13	13	1674	1628
	2	20	17	1196	1147	15	20	1281	1268
	3	17	20	1503	1404	39	37	1278	1117
	4	11	13	1311	1289	20	17	1226	1324
	5	21	21	911	881	23	12	1171	1391
	6	15	25	1126	1173	11	13	1143	1427
	7	9	14	1128	1096	15	16	1435	1334
	8	33	24	TNTC	TNTC	59	62	TNTC	TNTC
BNT162c2	1	7	6	1315	1328	9	18	1348	1263
	2	11	13	1315	1328	24	4	1222	1089
	3	7	5	1328	1267	12	14	1351	1206
	4	21	16	877	1135	24	15	1188	1173
	5	12	9	1371	1199	19	11	1504	1246
	6	6	14	1025	786	5	20	1143	1232
	7	11	15	1218	1132	21	22	1034	911
	8	4	6	1275	1054	13	6	973	1092

9.4 Cytokine multiplex analysis – Assay detection ranges

Table 20: Detection ranges of the ProcartaPlex immunoassay for mCorVAC#15 and mCorVAC#16.

Depicted are lower limt of quantification (LLOQ) and upper limit of quantification (ULOQ) for each analyte. LN, lymph node. SP, spleen.

[pg/mL]	IFNγ	IL-12p70	IL-13	IL-1β	IL-2	IL-4	IL-5	IL-6	TNFα	GM-CSF	IL-18
mCorVAC#15 (SP, LN)	1.1-4,800	1.5-409.3	2.1-8,650	1-4,350	1.2-5,250	4.8-4,950	1.9-8,000	4.7-19,500	2.8-731.2	2.4-9,950	50.5-207,000
mCorVAC#16 Plate 1 (SP)	1.1-4,800	1.5-102.3	2.1-2,162.5	1-1,087.5	1.2-1,312.5	1.2-4,950	7.8-2,000	4.7-4,875	2.8-731.2	9.7-2,487.5	202.1-51,750
mCorVAC#16 Plate 2 (LN)	1.1-4,800	1.5-102.3	2.1-8,650	1-4,350	1.2-5,250	1.2-4,950	1.9-8,000	4.7-19,500	2.8-731.2	2.4-9,950	202.1-51,750

9.5 Cytokine multiplex analysis – Raw data and calculated data

Table 21: Cytokine raw data and calculated data for mCorVAC#15, part 1 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. SP, spleen.

					IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
2	1	1	Medium	SP	187,5	1,26	1,26	11	<=0	0	24	<=0	0	17	0,28	0	754	14,82	14,82
3	1	2	Medium	SP	487	3,99	3,99	13	<=0	0	25	<=0	0	18	0,3	0	583	11,34	11,34
4	1	3	Medium	SP	25	0,24	0	10	<=0	0	21	<=0	0	17	0,28	0	152	2,85	2,85
5	1	4	Medium	SP	54	0,39	0	11	<=0	0	21,5	<=0	0	18	0,3	0	813,5	16,05	16,05
6	1	5	Medium	SP	27	0,25	0	9	<=0	0	21	<=0	0	16	0,26	0	333	6,38	6,38
7	1	6	Medium	SP	118	0,77	0	11	<=0	0	27	0,03	0	13	0,2	0	1012,5	20,24	20,24
8	1	7	Medium	SP	46,5	0,35	0	11	<=0	0	59,5	1,34	0	14	0,22	0	915	18,17	18,17
9	1	8	Medium	SP	124	0,81	0	10	<=0	0	28	0,07	0	12	0,18	0	737,5	14,48	14,48
10	2	1	Medium	SP	1249,5	14,42	14,42	20	<=0	0	44	0,70	0	19	0,32	0	1778	37,67	37,67
11	2	2	Medium	SP	165,5	1,10	1,10	14,5	<=0	0	41,5	0,60	0	15	0,24	0	539	10,45	10,45
12	2	3	Medium	SP	219	1,51	1,51	13	<=0	0	30	0,14	0	21	0,36	0	406,5	7,82	7,82
13	2	4	Medium	SP	50	0,37	0	11	<=0	0	33	0,26	0	16	0,26	0	470	9,08	9,08
14	2	5	Medium	SP	2466	40,87	40,87	30	<=0	0	123	4,11	4,11	26	0,47	0	1123	22,62	22,62
15	2	6	Medium	SP	455	3,66	3,66	12	<=0	0	62	1,44	0	16	0,26	0	730	14,32	14,32
16	2	7	Medium	SP	162,5	1,08	0	12	<=0	0	28	0,07	0	17,5	0,29	0	1605	33,53	33,53
17	2	8	Medium	SP	327	2,42	2,42	17	<=0	0	31	0,18	0	19	0,32	0	1560	32,47	32,47
18	3	1	Medium	SP	2160,5	33,01	33,01	36	<=0	0	43	0,66	0	45,5	0,91	0	1498,5	31,04	31,04
19	3	2	Medium	SP	446	3,57	3,57	15	<=0	0	33	0,26	0	18	0,30	0	1318	26,93	26,93
20	3	3	Medium	SP	380,5	2,92	2,92	14	<=0	0	48	0,86	0	20	0,34	0	755	14,84	14,84
21	3	4	Medium	SP	265	1,88	1,88	15	<=0	0	63	1,49	0	20	0,34	0	657,5	12,84	12,84
22	3	5	Medium	SP	154	1,02	0	16,5	<=0	0	98,5	3,02	3,02	18	0,30	0	1112	22,38	22,38
23	3	6	Medium	SP	128	0,84	0	12	<=0	0	46	0,78	0	15	0,24	0	1013	20,25	20,25
24	3	7	Medium	SP	77	0,52	0	11	<=0	0	26	0,00	0	16	0,26	0	1116	22,47	22,47
25	3	8	Medium	SP	347	2,61	2,61	14	<=0	0	115	3,75	3,75	18	0,30	0	902	17,90	17,90

Table 22: Cytokine raw data and calculated data for mCorVAC#15, part 2 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. SP, spleen.

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
2	1	1	Medium	SP	37	<=0	0	10	<=0	0	21	2,36	0	36	0,90	0	9	0,31	0	25	16,07	0
3	1	2	Medium	SP	48	<=0	0	10	<=0	0	27	3,34	0	30	0,72	0	12	0,42	0	48	61,82	61,82
4	1	3	Medium	SP	24	<=0	0	11	<=0	0	24,5	2,93	0	26	0,60	0	6	<=0	0	15	<=0	0
5	1	4	Medium	SP	22	<=0	0	10	<=0	0	23	2,68	0	27	0,63	0	9	0,31	0	14,5	<=0	0
6	1	5	Medium	SP	12	<=0	0	11	<=0	0	18	1,89	0	27	0,63	0	7	0,24	0	15	<=0	0
7	1	6	Medium	SP	15	<=0	0	10	<=0	0	22,5	2,60	0	25	0,58	0	7	0,24	0	22	9,40	0
8	1	7	Medium	SP	24	<=0	0	15	<=0	0	22	2,52	0	29	0,69	0	15,5	0,55	0	15	<=0	0
9	1	8	Medium	SP	25	<=0	0	11	<=0	0	22	2,52	0	33	0,81	0	9,5	0,33	0	22	9,40	0
10	2	1	Medium	SP	131,5	0,82	0	12	<=0	0	81	13 37	13,37	43	1,11	0	21	0,75	0	108	169,05	169,05
11	2	2	Medium	SP	153	1,18	0	12,5	<=0	0	70	11,20	11,20	32	0,78	0	10	0,35	0	25	16,07	0
12	2	3	Medium	SP	148	1,09	0	10	<=0	0	56,5	8,61	8,61	31	0,75	0	11	0,39	0	29	24,53	0
13	2	4	Medium	SP	164	1,37	0	12	<=0	0	55	8 33	8,33	23	0,52	0	9	0,31	0	16	<=0	0
14	2	5	Medium	SP	149	1,11	0	14,5	<=0	0	413,5	92,66	92,66	909	35,52	35,52	24	0,85	0	200,5	324,27	324,27
15	2	6	Medium	SP	44	<=0	0	16,5	<=0	0	48	7,03	7,03	32	0,78	0	11	0,39	0	44	54,22	54,22
16	2	7	Medium	SP	111	0,5	0	11	<=0	0	62	9,65	9,65	42,5	1,09	0	21	0,75	0	24	13,88	0
17	2	8	Medium	SP	111	0,5	0	10	<=0	0	78	12,77	12,77	40	1,02	0	14	0,50	0	36	38,68	0
18	3	1	Medium	SP	119	0,62	0	12	<=0	0	762	192,57	192,57	1697	77,07	77,07	27	0,96	0	189	305,29	305,29
19	3	2	Medium	SP	83,5	0,11	0	12	<=0	0	59	9,08	9,08	40	1,02	0	22	0,78	0	44	54,22	54,22
20	3	3	Medium	SP	303	4,01	0	11	<=0	0	99,5	17,13	17,13	36	0,90	0	17	0,60	0	39	44,57	0
21	3	4	Medium	SP	275	3,44	0	12,5	<=0	0	157	29,48	29,48	32	0,78	0	13	0,46	0	32	30,67	0
22	3	5	Medium	SP	385	5,72	5,72	29	0,43	0	154	28,82	28,82	33	0,81	0	13	0,46	0	26	18,22	0
23	3	6	Medium	SP	153,5	1,19	0	16,5	<=0	0	107	18,68	18,68	28	0,66	0	15	0,53	0	22	9,40	0
24	3	7	Medium	SP	77	0,03	0	11	<=0	0	45	6,48	6,48	23	0,52	0	11	0,39	0	16	<=0	0
25	3	8	Medium	SP	195	1,92	0	10,5	<=0	0	137	25,08	25,08	38	0,96	0	24	0,85	0	37	40,65	0

Table 23: Cytokine raw data and calculated data for mCorVAC#15, part 3 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control). SP, spleen.

Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
26	1	1	S peptide	SP	386,5	2,98	2,98	12	<=0	0	24	<=0	8650	19	0,32	0	610	11,88	11,88
27	1	2	S peptide	SP	75	0,51	0	13	<=0	0	39	0,50	0	16	0,26	0	955	19,01	19,01
28	1	3	S peptide	SP	178	1,19	1,19	10	<=0	0	30	0,14	0	15	0,24	0	246,5	4,69	4,69
29	1	4	S peptide	SP	35	0,29	0	11	<=0	0	19	<=0		16	0,26	0	190,5	3,60	3,60
30	1	5	S peptide	SP	203	1,38	1,38	9,5	<=0	0	25	<=0		15	0,24	0	365	7,01	7,01
31	1	6	S peptide	SP	94	0,62	0	11	<=0	0	20,5	<=0		13	0,20	0	321	6,14	6,14
32	1	7	S peptide	SP	586,5	5,09	5,09	12	<=0	0	21	<=0		11	0,16	0	885	17,54	17,54
33	1	8	S peptide	SP	318	2,34	2,34	14	<=0	0	23	<=0		13	0,20	0	1203,5	24,38	24,38
34	2	1	S peptide	SP	4966,5	149,75	149,75	35	<=0	0	266	10,75	10,75	27	0,49	0	2348	52,26	52,26
35	2	2	S peptide	SP	5474	186,09	186,09	32	<=0	0	224,5	8,78	8,78	24	0,42	0	1414	29,10	29,10
36	2	3	S peptide	SP	4423,5	117,49	117,49	30	<=0	0	336	14,14	14,14	30	0,56	0	1907	40,84	40,84
37	2	4	S peptide	SP	2160	33,00	33,00	21	<=0	0	107	3,40	3,40	17	0,28	0	1130,5	22,78	22,78
38	2	5	S peptide	SP	7059	356,73	356,73	47,5	<=0	0	682,5	31,94	31,94	32	0,60	0	2363,5	52,68	52,68
39	2	6	S peptide	SP	8699,5	707,98	707,98	62	<=0	0	1080,5	54,24	54,24	31	0,58	0	3508	87,45	87,45
40	2	7	S peptide	SP	2327	37,18	37,18	21	<=0	0	134,5	4,62	4,62	19	0,32	0	2460	55,32	55,32
41	2	8	S peptide	SP	3945	93,72	93,72	29	<=0	0	178	6,61	6,61	22	0,38	0	2176	47,70	47,70
42	3	1	S peptide	SP	12251	5435,41	4800	113	0,53	0	1666	90,58	90,58	53	1,09	1,09	2323	51,59	51,59
43	3	2	S peptide	SP	11207	2540,72	2540,72	67	<=0	0	446,5	19,64	19,64	32	0,60	0	1969,5	42,40	42,40
44	3	3	S peptide	SP	13878	55904,59	4800	123	0,67	0	2112,5	121,31	121,31	50	1,02	1,02	2650	60,65	60,65
45	3	4	S peptide	SP	8838	752,35	752,35	79,5	0,05	0	1314	68,22	68,22	34,5	0,66	0	2477	55,79	55,79
46	3	5	S peptide	SP	10020	1309,58	1309,58	77,5	0,02	0	1206	61,67	61,67	35	0,67	0	1807	38,38	38,38
47	3	6	S peptide	SP	7982	521,41	521,41	55	<=0	0	882	42,87	42,87	27	0,49	0	1849,5	39,42	39,42
48	3	7	S peptide	SP	9172,5	873,94	873,94	58,5	<=0	0	648	30,09	30,09	28	0,51	0	1861	39,70	39,70
49	3	8	S peptide	SP	8488	646,01	646,01	59	<=0	0	1148	58,21	58,21	29	0,53	0	2611	59,54	59,54
52	1	6	PMA Iono	SP	3238	65,07	65,07	338	3,83	3,83	10885	3220,14	3220,14	82	1,81	1,81	16603	5,85E+07	5250
51	1	7	PMA Iono	SP	3585	78,26	78,26	349,5	4,01	4,01	11523	4700,47	4700,47	95	2,15	2,15	17179,5	5,85E+07	5250
50	1	8	PMA Iono	SP	3246	65,35	65,35	319	3,55	3,55	10442	2593,95	2593,95	67	1,43	1,43	16984	5,85E+07	5250
60	2	5	PMA Iono	SP	4643	129,80	129,80	324	3,62	3,62	10730	2975,64	2975,64	118	2,75	2,75	15930	9,10E+04	5250
59	2	6	PMA Iono	SP	4585	126,45	126,45	371,5	4,35	4,35	13072	93260,89	8650	115,5	2,69	2,69	17962	5,85E+07	5250
58	2	7	PMA Iono	SP	5308	173,46	173,46	306,5	3,36	3,36	10521,5	2691,16	2691,16	84,5	1,87	1,87	18176,5	5,85E+07	5250
66	3	1	PMA Iono	SP	4589,5	126,71	126,71	304,5	3,33	3,33	11181	3790,84	3790,84	86	1,91	1,91	16344	1,67E+06	5250
67	3	4	PMA Iono	SP	3133	61,38	61,38	348,5	3,99	3,99	12338	9811,27	8650	105	2,41	2,41	16468,5	5,85E+07	5250
68	3	7	PMA Iono	SP	4499	121,62	121,62	334	3,77	3,77	11511	4662,32	4662,32	100,5	2,29	2,29	17556	5,85E+07	5250

Table 24: Cytokine raw data and calculated data for mCorVAC#15, part 4 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control). SP, spleen.

Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
26	1	1	S pep ide	SP	88	0,16	0	9	<=0	0	32	4,18	0	33	0,81	0	11	0,39	0	41	48,45	0
27	1	2	S pep ide	SP	252	2,99	0	10	<=0	0	85	14,17	14,17	36	0,90	0	10	0,35	0	18	<=0	0
28	1	3	S pep ide	SP	22	<=0	0	8,5	<=0	0	28	3,51	0	23	0,52	0	9	0,31	0	27	20,34	0
29	1	4	S pep ide	SP	16,5	<=0	0	10	<=0	0	17,5	1,81	0	21	0,46	0	6,5	0,22	0	15	<=0	0
30	1	5	S pep ide	SP	45,5	<=0	0	9	<=0	0	33	4 36	0	29	0,69	0	9	0,31	0	29	24,53	0
31	1	6	S pep ide	SP	23,5	<=0	0	9	<=0	0	17,5	1,81	0	20	0,43	0	8	0,27	0	19	2,11	0
32	1	7	S pep ide	SP	81	0,07	0	9	<=0	0	38	5,23	5,23	34	0,84	0	12	0,42	0	50	65,59	65,59
33	1	8	S pep ide	SP	78	0,04	0	10	<=0	0	32	4,18	0	41	1,05	0	12	0,42	0	33	32,69	0
34	2	1	S pep ide	SP	302	3,98	0	15	<=0	0	243	49,41	49,41	151	4,63	4,63	90	3,06	3,06	436,5	706,91	706,91
35	2	2	S pep ide	SP	308,5	4,12	0	33	0,59	0	172	32,85	32,85	87	2,50	0	89,5	3,05	3,05	508	822,51	822,51
36	2	3	S pep ide	SP	452	7,19	7,19	17	<=0	0	222	44,41	44,41	107	3,15	3,15	75	2,57	2,57	375,5	608,48	608,48
37	2	4	S pep ide	SP	267,5	3,30	0	10,5	<=0	0	112	19,73	19,73	56	1,51	0	26	0,92	0	170	273,80	273,8
38	2	5	S pep ide	SP	446,5	7,06	7,06	26	0,31	0	303	64,13	64,13	165	5,12	5,12	152,5	5,08	5,08	674,5	1094,22	1094,22
39	2	6	S pep ide	SP	723	13,65	13,65	44	1,05	0	397	88 29	88,29	182	5,71	5,71	264	8,61	8,61	911,5	1491,08	1491,08
40	2	7	S pep ide	SP	290,5	3,75	0	17	<=0	0	176	33,75	33,75	77	2,17	0	59	2,04	0	198,5	320,97	320,97
41	2	8	S pep ide	SP	269	3,33	0	13	<=0	0	178,5	34,32	34,32	144	4,40	4,40	87	2,96	2,96	350	567,33	567,33
42	3	1	S pep ide	SP	553	9,50	9,50	34	0,63	0	1090,5	298,53	298,53	836	32,17	32,17	850	27,38	27,38	1542	2640,12	2640,12
43	3	2	S pep ide	SP	297,5	3,89	0	22	0,16	0	184	35,57	35,57	230	7,41	7,41	497,5	15,98	15,98	1248	2084,32	2084,32
44	3	3	S pep ide	SP	1721	43,68	43,68	33	0,59	0	869	225,90	225,90	383	13,13	13,13	1383	45,75	45,75	1735,5	3029,31	3029,31
45	3	4	S pep ide	SP	1594,5	39,34	39,34	55	1,52	0	845	218,32	218,32	217,5	6,97	6,97	461,5	14,84	14,84	936	1532,98	1532,98
46	3	5	S pep ide	SP	921	18,85	18,85	17	<=0	0	552,5	130,83	130,83	224	7,20	7,20	613	19,67	19,67	1182	1964,71	1964,71
47	3	6	S pep ide	SP	429,5	6,69	6,69	17	<=0	0	285,5	59,77	59,77	182	5,71	5,71	419	13,50	13,50	864	1410,36	1410,36
48	3	7	S pep ide	SP	382,5	5,66	5,66	17	<=0	0	207,5	41,01	41,01	183,5	5,76	5,76	615,5	19,75	19,75	1009	1658,93	1658,93
49	3	8	S pep ide	SP	605	10,74	10,74	20	0,08	0	375	82 52	82,52	205,5	6,54	6,54	483	15,52	15,52	831	1354,65	1354,65
52	1	6	PMA Iono	SP	5638	283,37	283,37	4671,5	414,22	414,22	1682	515,81	515,81	4643	470,16	470,16	6211	441,29	441,29	1531,5	2619,57	2619,57
51	1	7	PMA Iono	SP	4136	161,80	161,80	5052	470,40	470,40	1556	466,69	466,69	4764	518,54	518,54	6473	501,59	501,59	1571,5	2698,16	2698,16
50	1	8	PMA Iono	SP	4102	159,57	159,57	7938,5	1092,17	1092,17	1234 5	348 31	348,31	4334,5	378,81	378,81	5295	296,02	296,02	1397,5	2362,01	2362,01
60	2	5	PMA Iono	SP	1610	39,87	39,87	6150,5	661,29	661,29	2882	1065,19	1065,19	4961	626,92	626,92	4831	245,44	245,44	1761	3082,15	3082,15
59	2	6	PMA Iono	SP	3768,5	138,70	138,70	7970	1101,46	1101,46	4470	2065,48	2065,48	5630,5	1732,84	731,2	5489	320,78	320,78	1775	3111,32	3111,32
58	2	7	PMA Iono	SP	1620,5	40,22	40,22	3044,5	219,53	219,53	2258	760,46	760,46	5527	1732,84	731,2	4976,5	260,16	260,16	1813	3191,10	3191,1
66	3	1	PMA Iono	SP	1616,5	40,09	40,09	5670	572,11	572,11	1719	530,52	530,52	4811,5	540,57	540,57	5771,5	361,79	361,79	1645	2844,76	2844,76
67	3	4	PMA Iono	SP	2902,5	92,23	92,23	4899	447,25	447,25	3504,5	1415,19	1415,19	5162	832,46	731,2	6452	496,30	496,30	1568	2691,25	2691,25
68	3	7	PMA Iono	SP	1295	29,70	29,70	4521	393,25	393,25	2322	789,75	789,75	5321	1977,29	731,2	6617	540,43	540,43	1864	3299,55	3299,55

Table 25: Cytokine raw data and calculated data for mCorVAC#15, part 5 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ LN, lymph node. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control).

					IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
53	2	1	Medium	LN	39,5	0,31	0	10	<=0	0	22	<=0		6	<=0	0	269	5,13	5,13
54	2	2	Medium	LN	44	0,33	0	10	<=0	0	22	<=0		7	0,08	0	432,5	8,34	8,34
55	2	3	Medium	LN	28	0,25	0	9	<=0	0	14	<=0		6	<=0	0	234,5	4,45	4,45
56	3	1	Medium	LN	62,5	0,43	0	9	<=0	0	16	<=0		6	<=0	0	355	6,81	6,81
57	3	3	Medium	LN	235	1,63	1,63	9	<=0	0	20	<=0		7	0,08	0	166	3,12	3,12
61	3	4	Medium	LN	68	0,47	0	9	<=0	0	14	<=0		8	0,1	0	204	3,86	3,86
62	3	5	Medium	LN	457	3,68	3,68	12	<=0	0	18	<=0		7	0,08	0	786	15,48	15,48
63	3	7	Medium	LN	57	0,40	0	9	<=0	0	17	<=0		7	0,08	0	157,5	2,95	2,95
64	3	8	Medium	LN	99	0,65	0	8,5	<=0	0	19	<=0		6	<=0	0	782	15,39	15,39
69	2	1	S peptide	LN	561,5	4,81	4,81	11	<=0	0	33	0,26	0	6	<=0	0	208,5	3,95	3,95
70	2	2	S peptide	LN	1013	10,70	10,70	14	<=0	0	118	3,88	3,88	7	0,08	0	1873,5	40,01	40,01
71	2	3	S peptide	LN	938,5	9,61	9,61	12	<=0	0	53	1,07	0	7	0,08	0	436	8,41	8,41
72	3	1	S peptide	LN	678	6,17	6,17	12	<=0	0	31	0,18	0	8	0,1	0	703	13,77	13,77
73	3	3	S peptide	LN	916	9,30	9,30	11,5	<=0	0	40	0,54	0	7	0,08	0	331	6,34	6,34
77	3	4	S peptide	LN	1924	27,51	27,51	14	<=0	0	43	0,66	0	7	0,08	0	723,5	14,19	14,19
78	3	5	S peptide	LN	1095	11,94	11,94	13,5	<=0	0	34,5	0,32	0	7	0,08	0	367	7,04	7,04
79	3	7	S peptide	LN	1686	22,46	22,46	13	<=0	0	51	0,98	0	6	<=0	0	701	13,73	13,73
80	3	8	S peptide	LN	564	4,83	4,83	10	<=0	0	19,5	<=0	0	6	<=0	0	658,5	12,86	12,86
74	2	1	PMA Iono	LN	4496,5	121,48	121,48	294	3,17	3,17	6532	662,77	662,77	59	1,24	1,24	17418	5,85E+07	5250
75	2	3	PMA Iono	LN	4808,5	139,72	139,72	406	4,88	4,88	8767	1352,13	1352,13	66	1,41	1,41	19327	5,85E+07	5250
76	2	7	PMA Iono	LN	3138,5	61,57	61,57	258	2,63	2,63	8778	1357,23	1357,23	51	1,04	1,04	16182	3,09E+05	5250
81	3	3	PMA Iono	LN	2999	56,86	56,86	232	2,25	2,25	6309,5	618,58	618,58	51	1,04	1,04	16058,5	1,54E+05	5250
65	3	4	PMA Iono	LN	3287	66,84	66,84	248	2,49	2,49	8188,5	1114,99	1114,99	57	1,19	1,19	17501	5,85E+07	5250
1	3	7	PMA Iono	LN	3505	75,08	75,08	281	2,98	2,98	7593,5	921,89	921,89	66,5	1,42	1,42	16859,50	5,85E+07	5250

Table 26: Cytokine raw data and calculated data for mCorVAC#15, part 6 of 6 (LN)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. LN, lymph node. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control).

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
53	2	1	Medium	LN	9	<=0	0	25	0,27	0	12	0,99	0	28	0,66	0	9	0,31	0	15	<=0	0
54	2	2	Medium	LN	7	<=0	0	32,5	0,57	0	15	1,43	0	21	0,46	0	7	0,24	0	14	<=0	0
55	2	3	Medium	LN	6,5	<=0	0	19	0,05	0	11	0,85	0	21	0,46	0	7	0,24	0	13	<=0	0
56	3	1	Medium	LN	7	<=0	0	10	<=0	0	12	0,99	0	22	0,49	0	7	0,24	0	15	<=0	0
57	3	3	Medium	LN	10	<=0	0	20	0,08	0	13	1,13	0	29	0,69	0	10	0,35	0	26	18,22	0
61	3	4	Medium	LN	9	<=0	0	13	<=0	0	14	1,28	0	33	0,81	0	6	<=0	0	14	<=0	0
62	3	5	Medium	LN	15	<=0	0	10	<=0	0	12	0,99	0	26	0,60	0	8	0,27	0	41,5	49,42	0
63	3	7	Medium	LN	13	<=0	0	14	<=0	0	15	1,43	0	29	0,69	0	6	<=0	0	14,5	<=0	0
64	3	8	Medium	LN	12	<=0	0	15	<=0	0	15	1,43	0	26	0,60	0	7	0,24	0	17	<=0	0
69	2	1	S pep ide	LN	12,5	<=0	0	30	0,47	0	13	1,13	0	34	0,84	0	11	0,39	0	52,5	70,27	70,27
70	2	2	S pep ide	LN	46,5	<=0	0	215	9,08	9,08	23	2,68	0	46,5	1,22	0	47	1,64	0	83,5	126,35	126,35
71	2	3	S pep ide	LN	11	<=0	0	96,5	3,38	3,38	15	1,43	0	33	0,81	0	20	0,71	0	82	123,69	123,69
72	3	1	S pep ide	LN	32	<=0	0	20	0,08	0	14	1,28	0	31	0,75	0	17	0,60	0	66,5	95,97	95,97
73	3	3	S pep ide	LN	25	<=0	0	16	<=0	0	13	1,13	0	30,5	0,74	0	17,5	0,62	0	76	113,04	113,04
77	3	4	S pep ide	LN	44,5	<=0	0	15	<=0	0	21	2 36	0	54	1,45	0	31	1,09	0	156	250,44	250,44
78	3	5	S pep ide	LN	54	<=0	0	18	0,01	0	15	1,43	0	40	1,02	0	21	0,75	0	91	139,53	139,53
79	3	7	S pep ide	LN	49,5	<=0	0	27,5	0,37	0	18,5	1,96	0	45	1,17	0	26	0,92	0	136,5	217,66	217,66
80	3	8	S pep ide	LN	20	<=0	0	14	<=0	0	15	1,43	0	26	0,60	0	11	0,39	0	52	69,33	69,33
74	2	1	PMA Iono	LN	1662	41,64	41,64	8746	1353,47	1353,47	359,5	78,50	78,50	5696	1732,84	731,2	3332,5	133,64	133,64	1848	3265,35	3265,35
75	2	3	PMA Iono	LN	1720	43,64	43,64	11982	3124,01	3124,01	383	84,61	84,61	6322,5	1732,84	731,2	3394	137,20	137,20	1879,5	3332,84	3332,84
76	2	7	PMA Iono	LN	1056,5	22,63	22,63	10201	1974,51	1974,51	569	135,52	135,52	5226	962,86	731,2	1981	68,61	68,61	1547	2649,93	2649,93
81	3	3	PMA Iono	LN	737	14,00	14,00	7712	1027,35	1027,35	257,5	52,91	52,91	4908	592,68	592,68	3682	154,80	154,80	1503	2564,06	2564,06
65	3	4	PMA Iono	LN	853	17,02	17,02	7043,5	854,66	854,66	435	98,41	98,41	5309	1469,86	731,2	5271,5	293,18	293,18	1475,5	2510,88	2510,88
1	3	7	PMA Iono	LN	943,5	19,47	19,47	7862	1069,89	1069,89	491	113,65	113,65	5508,5	1732,84	731,2	4949	257,31	257,31	1666	2887,17	2887,17

Table 27: Cytokine raw data and calculated data for mCorVAC#16, part 1 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. SP, spleen.

						IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Plate	Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
1	1	1	1	Medium	SP	98,5	2,06	2,06	13	<=0	0	35	2,39	2,39	23	1,64	1,64	971,5	59,72	59,72
1	2	1	2	Medium	SP	20	0,17	0	10	<=0	0	24	0,70	0	17	0,94	0	106	9,49	9,49
1	3	1	3	Medium	SP	76,5	1,50	1,50	13	<=0	0	41	3,25	3,25	16	0,82	0	646,5	42,56	42,56
1	4	1	4	Medium	SP	78	1,54	1,54	11	<=0	0	29	1,49	0	22	1,53	1,53	692,5	45,05	45,05
1	5	1	5	Medium	SP	26	0,30	0	9	<=0	0	23	0,53	0	18	1,06	1,06	404,5	28,98	28,98
1	6	1	6	Medium	SP	236	5,80	5,80	13	<=0	0	42	3,40	3,40	18	1,06	1,06	354	25,99	25,99
1	7	1	7	Medium	SP	259,5	6,47	6,47	13	<=0	0	28	1,34	0	18,5	1,12	1,12	426	30,24	30,24
1	8	1	8	Medium	SP	53	0,92	0	15	<=0	0	23	0,53	0	20	1,30	1,30	776	49,50	49,50
1	9	2	1	Medium	SP	44	0,71	0	10	<=0	0	24	0,70	0	15	0,69	0	245	19,22	19,22
1	10	2	2	Medium	SP	275	6,91	6,91	17	<=0	0	59	5,72	5,72	16	0,82	0	955	58,87	58,87
1	11	2	3	Medium	SP	161	3,71	3,71	26,5	0,5	0	95	10,38	10,38	19	1,18	1,18	848	53,29	53,29
1	12	2	4	Medium	SP	228	5,57	5,57	17	<=0	0	52	4,78	4,78	16	0,82	0	773	49,34	49,34
1	13	2	5	Medium	SP	1728	57,82	57,82	27	0,53	0	93	10,12	10,12	18	1,06	1,06	1119,5	67,33	67,33
1	14	2	6	Medium	SP	54	0,95	0	12	<=0	0	22	0,35	0	17	0,94	0	226,5	18,02	18,02
1	15	2	7	Medium	SP	128,5	2,84	2,84	13	<=0	0	47	4,09	4,09	15	0,69	0	953,5	58,79	58,79
1	16	2	8	Medium	SP	2259,5	80,30	80,30	27,5	0,55	0	68	6,91	6,91	20	1,30	1,30	1375,5	80,35	80,35
1	17	3	1	Medium	SP	79	1,56	1,56	10	<=0	0	27	1,18	0	18	1,06	1,06	132	11,47	11,47
1	18	3	2	Medium	SP	79	1,56	1,56	12	<=0	0	29	1,49	0	19	1,18	1,18	296	22,45	22,45
1	19	3	3	Medium	SP	84,5	1,70	1,70	11	<=0	0	25	0,86	0	23	1,64	1,64	142,5	12,24	12,24
1	20	3	4	Medium	SP	919,5	27,52	27,52	22	0,27	0	80	8,47	8,47	22	1,53	1,53	956,5	58,95	58,95
1	21	3	5	Medium	SP	322,5	8,30	8,30	16	<=0	0	60	5,86	5,86	33	2,72	2,72	869	54,39	54,39
1	22	3	6	Medium	SP	130	2,88	2,88	12,5	<=0	0	44	3,68	3,68	19,5	1,24	1,24	374	27,18	27,18
1	23	3	7	Medium	SP	108,5	2,32	2,32	12	<=0	0	32,5	2,02	0	22	1,53	1,53	189,5	15,54	15,54
1	24	3	8	Medium	SP	168	3,90	3,90	13	<=0	0	39,5	3,04	3,04	17	0,94	0	1218	72,35	72,35

Table 28: Cytokine raw data and calculated data for mCorVAC#16, part 2 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. SP, spleen.

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
1	1	1	Medium	SP	106,5	5,5	5,5	10	<=0	0	48	26,82	26,82	49	5,51	5,51	7	<=0	0	21	<=0	0
2	1	2	Medium	SP	21,5	0,8	0	11	<=0	0	18	5,99	5,99	26	2,49	0	5	<=0	0	13	<=0	0
3	1	3	Medium	SP	91	4,7	4,7	12	<=0	0	43,5	23,94	23,94	34	3,61	3,61	8	<=0	0	17	<=0	0
4	1	4	Medium	SP	83	4,2	4,2	11	<=0	0	43	23,61	23,61	44	4,90	4,90	8	<=0	0	17	<=0	0
5	1	5	Medium	SP	23	0,9	0	11	<=0	0	29	14,20	14,20	26,5	2,57	0	5,5	<=0	0	14	<=0	0
6	1	6	Medium	SP	429	22,0	22,0	19	0,2	0	129,5	73,96	73,96	41	4,52	4,52	7	<=0	0	29	114,63	0
7	1	7	Medium	SP	63	3,2	3,2	10	<=0	0	30	14,90	14,90	38	4,14	4,14	8	<=0	0	32	144,62	0
8	1	8	Medium	SP	50	2,4	2,4	9	<=0	0	185	103,35	103,35	652,5	56,48	56,48	7	<=0	0	16,5	<=0	0
9	2	1	Medium	SP	86,5	4,4	4,4	12	<=0	0	34	17,65	17,65	32	3,34	3,34	7	<=0	0	13	<=0	0
10	2	2	Medium	SP	487,5	24,9	24,9	12	<=0	0	122	69,87	69,87	77	8,68	8,68	19	4,13	0	38,5	202,86	202,86
11	2	3	Medium	SP	1087,5	55,5	55,5	12	<=0	0	297	159,65	159,65	69	7,81	7,81	14	2,36	0	29	114,63	0
12	2	4	Medium	SP	458	23,4	23,4	28	2,39	0	128	73,14	73,14	57	6,45	6,45	8	<=0	0	27	92,89	0
13	2	5	Medium	SP	1096	55,9	55,9	15	<=0	0	292,5	157,45	157,45	67	7,59	7,59	19,5	4,29	0	162	929,78	929,78
14	2	6	Medium	SP	206,5	10,7	10,7	14	<=0	0	60,5	34,59	34,59	34	3,61	3,61	6	<=0	0	14	<=0	0
15	2	7	Medium	SP	294,5	15,2	15,2	9	<=0	0	105	60,46	60,46	51,5	5,81	5,81	11	0,87	0	21,5	14,31	0
16	2	8	Medium	SP	395	20,3	20,3	12	<=0	0	163	91,86	91,86	529	47,07	47,07	17	3,48	0	198	1098,85	1098,85
17	3	1	Medium	SP	69,5	3,5	3,5	10	<=0	0	33	16,97	16,97	32	3,34	3,34	7	<=0	0	16	<=0	0
18	3	2	Medium	SP	114	5,9	5,9	13	<=0	0	39	21,00	21,00	37	4,01	4,01	7	<=0	0	17	<=0	0
19	3	3	Medium	SP	63	3,2	3,2	12	<=0	0	34,5	17,99	17,99	37	4,01	4,01	8	<=0	0	18	<=0	0
20	3	4	Medium	SP	608,5	31,0	31,0	12	<=0	0	174	97,63	97,63	64	7,25	7,25	18	3,81	0	78	479,51	479,51
21	3	5	Medium	SP	345	17,7	17,7	14	<=0	0	122	69,87	69,87	51	5,75	5,75	10	<=0	0	34	163,35	0
22	3	6	Medium	SP	219	11,3	11,3	9	<=0	0	92	53,11	53,11	36	3,88	3,88	8	<=0	0	21	<=0	0
23	3	7	Medium	SP	211,5	11,0	11,0	17	<=0	0	69,5	40,01	40,01	41	4,52	4,52	9	<=0	0	20	<=0	0
24	3	8	Medium	SP	144	7,5	7,5	14	<=0	0	47	26,18	26,18	41	4,52	4,52	8	<=0	0	23,5	48,81	0

Table 29: Cytokine raw data and calculated data for mCorVAC#16, part 3 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control). SP, spleen.

						IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Plate	Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
1	25	1	1	S peptide	SP	60,5	1,10	1,10	10	<=0	0	24	0,70	0	19	1,18	1,18	346	25,51	25,51
1	26	1	2	S peptide	SP	70	1,34	1,34	10	<=0	0	24	0,70	0	16	0,82	0	101	9,10	9,10
1	27	1	3	S peptide	SP	53	0,92	0	9,5	<=0	0	21	0,17	0	14	0,56	0	122,5	10,76	10,76
1	28	1	4	S peptide	SP	182,5	4,30	4,30	12	<=0	0	27	1,18	0	20,5	1,36	1,36	348	25,63	25,63
1	29	1	5	S peptide	SP	46	0,75	0	14	<=0	0	31	1,80	0	15,5	0,76	0	151	12,85	12,85
1	30	1	6	S peptide	SP	781,5	22,82	22,82	17,5	0,03	0	66,5	6,72	6,72	19	1,18	1,18	697	45,29	45,29
1	31	1	7	S peptide	SP	276	6,94	6,94	12	<=0	0	23	0,53	0	18	1,06	1,06	331	24,60	24,60
1	32	1	8	S peptide	SP	248,5	6,15	6,15	14	<=0	0	29	1,49	0	25	1,87	1,87	351	25,81	25,81
1	33	2	1	S peptide	SP	13171	5808,01	4800	174	7,19	7,19	3368	382,28	382,28	48	4,21	4,21	1871	105,49	105,49
1	34	2	2	S peptide	SP	14077	5808,01	4800	184,5	7,65	7,65	5418	676,64	676,64	65	5,80	5,80	3257	179,56	179,56
1	35	2	3	S peptide	SP	15533	5808,01	4800	210	8,78	8,78	4642	555,60	555,60	97	8,64	8,64	1595	91,47	91,47
1	36	2	4	S peptide	SP	13978	5808,01	4800	180	7,45	7,45	4989,5	608,07	608,07	58	5,16	5,16	2848	156,78	156,78
1	37	2	5	S peptide	SP	13461	5808,01	4800	164	6,75	6,75	3984,5	462,82	462,82	52	4,59	4,59	2266,5	125,84	125,84
1	38	2	6	S peptide	SP	13336,5	5808,01	4800	169	6,97	6,97	4404	521,10	521,10	61	5,44	5,44	2555	141,01	141,01
1	39	2	7	S peptide	SP	12383	5808,01	4800	170	7,01	7,01	5234	646,64	646,64	52	4,59	4,59	2893,5	159,27	159,27
1	40	2	8	S peptide	SP	17133	5808,01	4800	236	9,93	9,93	4725	567,90	567,90	89,5	7,99	7,99	1762	99,94	99,94
1	41	3	1	S peptide	SP	13734	5808,01	4800	121,5	4,86	4,86	505	57,29	57,29	58	5,16	5,16	1366	79,87	79,87
1	42	3	2	S peptide	SP	13464	5808,01	4800	119	4,75	4,75	653	73,40	73,40	54	4,78	4,78	1191	70,98	70,98
1	43	3	3	S peptide	SP	13090,5	5808,01	4800	112	4,44	4,44	610	68,74	68,74	59	5,25	5,25	954	58,82	58,82
1	44	3	4	S peptide	SP	15944	5808,01	4800	159	6,53	6,53	1533	168,50	168,50	76	6,80	6,80	2185	121,61	121,61
1	45	3	5	S peptide	SP	14532,5	5808,01	4800	152	6,22	6,22	2293,5	253,39	253,39	87	7,77	7,77	1549	89,14	89,14
1	46	3	6	S peptide	SP	15439	5808,01	4800	151	6,17	6,17	891	99,08	99,08	61	5,44	5,44	2981	164,08	164,08
1	47	3	7	S peptide	SP	13213,5	5808,01	4800	124	4,98	4,98	1358	149,45	149,45	58	5,16	5,16	1839,5	103,88	103,88
1	48	3	8	S peptide	SP	13278	5808,01	4800	104	4,08	4,08	414	47,28	47,28	53	4,69	4,69	1581	90,76	90,76
1	49	1	1	PMA Iono	SP	4486	201,33	201,33	350	15,01	15,01	9735	1951,80	1951,80	97	8,64	8,64	16018	5,7E+04	1312,5
1	50	1	2	PMA Iono	SP	4369,5	193,67	193,67	399,5	17,25	17,25	11225	4223,94	2162,5	108	9,58	9,58	15895	5,7E+04	1312,5
1	51	1	3	PMA Iono	SP	4254	186,26	186,26	389	16,77	16,77	10268	2346,54	2162,5	104	9,24	9,24	15356	5,7E+04	1312,5
1	52	2	1	PMA Iono	SP	4815	223,94	223,94	348	14,92	14,92	11244	4327,85	2162,5	134,5	11,80	11,80	16167	5,7E+04	1312,5
1	53	2	2	PMA Iono	SP	6032	323,14	323,14	271	11,48	11,48	11185	4031,79	2162,5	99	8,81	8,81	15750,5	5,7E+04	1312,5
1	54	2	3	PMA Iono	SP	5693	292,66	292,66	281,5	11,94	11,94	10321	2395,89	2162,5	111	9,84	9,84	13797	5,7E+04	1312,5
1	55	3	1	PMA Iono	SP	4782,5	221,64	221,64	403,5	17,43	17,43	11140	3848,56	2162,5	120	10,60	10,60	16203,5	5,7E+04	1312,5
1	56	3	2	PMA Iono	SP	5422	270,00	270,00	323	13,80	13,8	10867	3124,27	2162,5	103	9,16	9,16	15723	5,7E+04	1312,5
1	57	3	3	PMA Iono	SP	5531	278,95	278,95	261,5	11,06	11,06	9846	2022,03	2022,03	98	8,73	8,73	14422	5,7E+04	1312,5

Table 30: Cytokine raw data and calculated data for mCorVAC#16, part 4 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control). SP, spleen.

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
25	1	1	S pep ide	SP	47	2,3	2,3	9	<=0	0	41	22,31	22,31	52	5,87	5,87	7	<=0	0	15	<=0	0
26	1	2	S pep ide	SP	68	3,4	3,4	11,5	<=0	0	32	16,29	16,29	32	3,34	3,34	8	<=0	0	16	<=0	0
27	1	3	S pep ide	SP	58	2,9	2,9	10	<=0	0	37	19,67	19,67	28,5	2,86	2,86	6	<=0	0	14	<=0	0
28	1	4	S pep ide	SP	121	6,3	6,3	9	<=0	0	65,5	37,61	37,61	44	4,90	4,90	8	<=0	0	25	69,01	0
29	1	5	S pep ide	SP	320	16,5	16,5	9	<=0	0	89,5	51,68	51,68	33	3,48	3,48	7	<=0	0	16	<=0	0
30	1	6	S pep ide	SP	545	27,8	27,8	14	<=0	0	183	102,32	102,32	53	5,99	5,99	15	2,76	0	71	435,82	435,82
31	1	7	S pep ide	SP	167	8,7	8,7	10	<=0	0	50	28,08	28,08	38	4,14	4,14	7	<=0	0	30	124,92	0
32	1	8	S pep ide	SP	171	8,9	8,9	9,5	<=0	0	69	39,71	39,71	245,5	24,38	24,38	10	<=0	0	35	172,42	0
33	2	1	S pep ide	SP	3097	175,0	175,0	80,5	11,50	11,5	1939	921,39	921,39	748,5	63,72	63,72	1357	133,31	133,31	1730	6283,48	6283,48
34	2	2	S pep ide	SP	2500	135,8	135,8	178,5	25,53	25,53	1790	850,48	850,48	719	61,50	61,50	1401	136,55	136,55	2007,5	7154,03	7154,03
35	2	3	S pep ide	SP	2530	137,7	137,7	120,5	17,47	17,47	2662	1280,76	1280,76	882	73,74	73,74	2013	180,57	180,57	2382	8351,97	8351,97
36	2	4	S pep ide	SP	2212,5	118,2	118,2	321	43,79	43,79	1915,5	910,15	910,15	694	59,61	59,61	1559	148,08	148,08	1846,5	6647,80	6647,80
37	2	5	S pep ide	SP	2365,5	127,5	127,5	271	37,56	37,56	2038	969,03	969,03	601,5	52,61	52,61	840	93,57	93,57	1806	6521,00	6521,00
38	2	6	S pep ide	SP	1640	85,2	85,2	146,5	21,15	21,15	1583,5	753,58	753,58	830	69,84	69,84	1562,5	148,33	148,33	1889,5	6782,64	6782,64
39	2	7	S pep ide	SP	2880	160,3	160,3	200	28,40	28,4	2046	972,90	972,90	827	69,61	69,61	1447	139,92	139,92	1678,5	6122,76	6122,76
40	2	8	S pep ide	SP	1653	85,9	85,9	87,5	12,58	12,58	2760,5	1332,05	1332,05	2128,5	173,56	173,56	2153,5	190,53	190,53	2511	8773,84	8773,84
41	3	1	S pep ide	SP	919	46,8	46,8	24	1,52	0	620	312,22	312,22	657	56,82	56,82	460	60,65	60,65	1982	7073,58	7073,58
42	3	2	S pep ide	SP	839,5	42,7	42,7	29	2,60	0	488,5	251,16	251,16	680	58,56	58,56	509	65,21	65,21	1791	6474,08	6474,08
43	3	3	S pep ide	SP	477	24,4	24,4	133	19,26	19,26	334	177,69	177,69	551,5	48,80	48,80	368	51,71	51,71	1829,5	6594,56	6594,56
44	3	4	S pep ide	SP	787,5	40,0	40,0	40	4,74	0	786	388,32	388,32	1065	87,50	87,50	1142	117,21	117,21	2457,5	8598,20	8598,20
45	3	5	S pep ide	SP	1429	73,6	73,6	71	10,01	10,01	1561	743,11	743,11	803	67,81	67,81	860	95,18	95,18	2058	7313,69	7313,69
46	3	6	S pep ide	SP	668,5	34,0	34,0	60	8,22	8,22	624	314,07	314,07	1027	84,63	84,63	954	102,67	102,67	2260	7957,81	7957,81
47	3	7	S pep ide	SP	825	42,0	42,0	78	11,11	11,11	494	253,73	253,73	587	51,51	51,51	656	78,24	78,24	1859,5	6688,55	6688,55
48	3	8	S pep ide	SP	454	23,2	23,2	34	3,60	0	294	158,18	158,18	631,5	54,89	54,89	393	54,19	54,19	1951	6975,93	6975,93
49	1	1	PMA Iono	SP	2056	108,9	108,9	6519,5	872,65	872,65	3366	1662,56	1662,56	5721	4142,40	731,2	5495	461,34	461,34	1671	6099,37	6099,37
50	1	2	PMA Iono	SP	3480	202,3	202,3	9358,5	1609,56	1609,56	4770	2563,19	2563,19	5510,5	1256,15	731,2	5864	502,13	502,13	1411	5288,61	5288,61
51	1	3	PMA Iono	SP	1521	78,6	78,6	7790,5	1147,31	1147,31	4659	2483,32	2483,32	5649,5	1891,68	731,2	5892,5	505,47	505,47	1638	5996,47	5996,47
52	2	1	PMA Iono	SP	1759	91,8	91,8	4315,5	516,09	516,09	4106	2109,67	2109,67	5675	2216,16	731,2	6114	532,52	532,52	1684	6139,92	6139,92
53	2	2	PMA Iono	SP	1338	68,7	68,7	5758	735,82	735,82	3354	1655,73	1655,73	5067	752,98	731,2	6086	528,98	528,98	1184	4576,36	4576,36
54	2	3	PMA Iono	SP	1183	60,5	60,5	5530,5	698,08	698,08	3337	1646,08	1646,08	4612	557,74	557,74	5094	421,22	421,22	1063	4192,38	4192,38
55	3	1	PMA Iono	SP	1795,5	93,9	93,9	10589	2139,77	2000	4271	2217,20	2217,20	6012	4142,40	731,2	5849	500,39	500,39	1660,5	6066,62	6066,62
56	3	2	PMA Iono	SP	870	44,2	44,2	11204,5	2499,94	2000	3363,5	1661,14	1661,14	5047	741,29	731,2	5963	513,87	513,87	1215,5	4675,72	4675,72
57	3	3	PMA Iono	SP	1056,5	53,9	53,9	8771	1415,41	1415,41	2883	1396,74	1396,74	4732	598,42	598,42	4594	375,76	375,76	1068	4208,33	4208,33

Table 31: Cytokine raw data and calculated data for mCorVAC#16, part 5 of 6 (LN)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. LN, lymph node. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control).

						IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Plate	Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
2	1	1	2	Medium	LN	30	0,06	0	10	<=0	0	32,5	2,58	2,58	6	0,27	0	172	14,15	14,15
2	2	1	3	Medium	LN	35,5	0,23	0	9	<=0	0	32	2,52	2,52	6	0,27	0	520	33,53	33,53
2	3	1	5	Medium	LN	32	0,12	0	10	<=0	0	29	2,18	2,18	7	0,40	0	51	5,29	5,29
2	7	2	1	Medium	LN	93	1,76	1,76	9	<=0	0	31	2,41	2,41	6	0,27	0	190	15,30	15,30
2	8	2	2	Medium	LN	159	3,37	3,37	10	<=0	0	24	1,60	0	7	0,40	0	275,5	20,43	20,43
2	9	2	3	Medium	LN	194	4,20	4,20	8	<=0	0	24	1,60	0	8	0,52	0	130	11,36	11,36
2	10	2	4	Medium	LN	427	9,61	9,61	11	<=0	0	33	2,63	2,63	7	0,40	0	247	18,76	18,76
2	11	2	5	Medium	LN	86	1,58	1,58	11	<=0	0	35	2,85	2,85	7	0,40	0	503	32,66	32,66
2	12	2	6	Medium	LN	106	2,08	2,08	10	<=0	0	63,5	5,86	5,86	7	0,40	0	693,5	42,09	42,09
2	13	2	7	Medium	LN	452,5	10,20	10,20	11	<=0	0	85	8,01	8,01	8	0,52	0	373	25,86	25,86
2	14	2	8	Medium	LN	275	6,10	6,10	11	<=0	0	43	3,73	3,73	7	0,40	0	407	27,68	27,68
2	4	3	1	Medium	LN	88	1,63	1,63	10	<=0	0	27	1,95	0	7	0,40	0	247	18,76	18,76
2	5	3	2	Medium	LN	118	2,38	2,38	9	<=0	0	27	1,95	0	6	0,27	0	227	17,57	17,57
2	6	3	5	Medium	LN	3862,5	112,04	112,04	28	0,72	0	65,5	6,06	6,06	21	1,83	1,83	722	43,46	43,46
2	15	1	2	S peptide	LN	27	<=0	0	9	<=0	0	30,5	2,35	2,35	6	0,27	0	144	12,31	12,31
2	16	1	3	S peptide	LN	24	<=0	0	9	<=0	0	24,5	1,66	0	6	0,27	0	42,5	4,52	4,52
2	17	1	5	S peptide	LN	38	0,30	0	9	<=0	0	23	1,48	0	6	0,27	0	39	4,19	4,19
2	21	2	1	S peptide	LN	6019	228,22	228,22	43	1,39	0	1158	98,84	98,84	13	1,06	1,06	3419	169,20	169,20
2	22	2	2	S peptide	LN	9299	625,33	625,33	71	2,57	2,57	1738	147,12	147,12	17	1,45	1,45	3274	161,90	161,90
2	23	2	3	S peptide	LN	12071	1686,02	1686,02	80	2,94	2,94	1253	106,68	106,68	25,5	2,23	2,23	2581	128,41	128,41
2	24	2	4	S peptide	LN	6145	237,22	237,22	45	1,47	0	1326	112,68	112,68	13	1,06	1,06	3027	149,72	149,72
2	25	2	5	S peptide	LN	5060	168,77	168,77	50	1,69	1,69	2139	181,51	181,51	12	0,96	0	3253	160,86	160,86
2	26	2	6	S peptide	LN	3496	97,61	97,61	28,5	0,74	0	672	58,84	58,84	10	0,74	0	2548	126,87	126,87
2	27	2	7	S peptide	LN	4729	151,42	151,42	42	1,34	0	3101	268,62	268,62	14	1,16	1,16	2190	110,32	110,32
2	28	2	8	S peptide	LN	7910	405,40	405,40	54	1,86	1,86	1622	137,39	137,39	19	1,64	1,64	2899,5	143,55	143,55
2	18	3	1	S peptide	LN	1803,5	43,49	43,49	13	<=0	0	41	3,51	3,51	7	0,40	0	701,5	42,47	42,47
2	19	3	2	S peptide	LN	3364,5	92,70	92,70	20	0,34	0	87	8,20	8,20	8	0,52	0	553,5	35,21	35,21
2	20	3	5	S peptide	LN	4311	131,42	131,42	27	0,67	0	138	13,06	13,06	33	2,87	2,87	1182,5	64,83	64,83
2	29	1	1	PMA Iono	LN	12326	1874,34	1874,34	196	7,52	7,52	5467	527,14	527,14	37	3,20	3,20	16127	8492,61	5250
2	30	2	1	PMA Iono	LN	12488	2008,41	2008,41	333	12,79	12,79	8110	953,50	953,50	65	5,36	5,36	18009	5,2E+05	5250
2	31	2	2	PMA Iono	LN	13892	3937,75	3937,75	232	8,91	8,91	7472	830,47	830,47	57,5	4,80	4,80	17831	1,5E+05	5250
2	32	3	1	PMA Iono	LN	13092,5	2635,94	2635,94	287	11,02	11,02	7797	891,10	891,10	65	5,36	5,36	18563	1,7E+07	5250

Table 32: Cytokine raw data and calculated data for mCorVAC#16, part 6 of 6 (LN)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. LN, lymph node. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control).

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
1	1	2	Medium	LN	5	<=0	0	94	13,14	13,14	17	7,72	7,72	37,5	4,19	4,19	6	1,14	0	14	<=0	0
2	1	3	Medium	LN	6	0,23	0	67	9,43	9,43	15	6,36	6,36	32	3,57	3,57	5	<=0	0,00	13	<=0	0
3	1	5	Medium	LN	8	0,37	0	107,5	14,94	14,94	11	3,47	0	27	2,99	2,99	5	<=0	0,00	12	<=0	0
7	2	1	Medium	LN	17	0,95	0	44	6,09	6,09	15	6,36	6,36	28	3,11	3,11	7	1,50	0	18	<=0	0
8	2	2	Medium	LN	21	1,19	0	16	1,55	0	14	5,66	5,66	55	6,01	6,01	7	1,50	0	21	51,55	0
9	2	3	Medium	LN	11,5	0,61	0	12	0,77	0	17	7,72	7,72	32	3,57	3,57	6	1,14	0	22,5	70,19	0
10	2	4	Medium	LN	28	1,60	1,60	14	1,17	0	17	7,72	7,72	38	4,24	4,24	8	1,83	0	41	224,61	224,61
11	2	5	Medium	LN	33,5	1,90	1,90	39	5,33	5,33	17	7,72	7,72	47	5,20	5,20	7	1,50	0	19	19,32	0
12	2	6	Medium	LN	18,5	1,04	0	29	3,76	3,76	13	4,95	4,95	35,5	3,96	3,96	9	2,12	0	17	<=0	0,00
13	2	7	Medium	LN	23	1,31	1,31	126	17,36	17,36	16	7,04	7,04	48	5,30	5,30	16	3,78	3,78	42	231,36	231,36
14	2	8	Medium	LN	28	1,60	1,60	23	2,77	2,77	21	10,35	10,35	39	4,35	4,35	8	1,83	0	32	158,79	0
4	3	1	Medium	LN	6	0,23	0	33	4,40	4,40	11	3,47	0	25	2,74	0	5	<=0	0,00	18	<=0	0,00
5	3	2	Medium	LN	5	<=0	0,00	44	6,09	6,09	12	4,22	0	27	2,99	2,99	7	1,50	0	18	<=0	51750
6	3	5	Medium	LN	80	4,32	4,32	19	2,09	2,09	183	94,27	94,27	112	11,24	11,24	11	2,65	2,65	327	1366,33	1366,33
15	1	2	S peptide	LN	6	0,23	0	77	10,83	10,83	12	4,22	0	29	3,22	3,22	6	1,14	0	11	<=0	0,00
16	1	3	S peptide	LN	6,5	0,27	0	37	5,02	5,02	12	4,22	0	23	2,50	0	6	1,14	0	13	<=0	0,00
17	1	5	S peptide	LN	7	0,30	0	79	11,10	11,10	11	3,47	0	22,5	2,43	0	5	<=0	0,00	12	<=0	0,00
21	2	1	S peptide	LN	585	26,67	26,67	306	39,25	39,25	50,5	27,77	27,77	185	17,18	17,18	208,5	24,46	24,46	525	1945,60	1945,60
22	2	2	S peptide	LN	620	28,14	28,14	108	15,01	15,01	68,5	37,55	37,55	249	22,03	22,03	246,5	27,42	27,42	932	3012,15	3012,15
23	2	3	S peptide	LN	315	15,07	15,07	123,5	17,03	17,03	100	53,89	53,89	327,5	27,72	27,72	362	35,69	35,69	1379	4111,96	4111,96
24	2	4	S peptide	LN	722	32,50	32,50	124	17,10	17,10	51	28,04	28,04	190,5	17,61	17,61	326	33,21	33,21	566	2060,06	2060,06
25	2	5	S peptide	LN	676	30,52	30,52	838,5	98,03	98,03	41	22,40	22,40	242	21,51	21,51	333	33,69	33,69	466	1780,70	1780,70
26	2	6	S peptide	LN	374	17,63	17,63	172	23,18	23,18	35	18,92	18,92	114	11,41	11,41	176	21,79	21,79	304	1292,04	1292,04
27	2	7	S peptide	LN	388	18,23	18,23	3354,5	371,05	371,05	44	24,12	24,12	185,5	17,22	17,22	543	47,36	47,36	421	1650,50	1650,50
28	2	8	S peptide	LN	835	37,33	37,33	112	15,53	15,53	72	39,41	39,41	231,5	20,73	20,73	537	46,99	46,99	748	2542,37	2542,37
18	3	1	S peptide	LN	18	1,01	0	41	5,64	5,64	20	9,70	9,70	66	7,09	7,09	10	2,39	0	145	731,35	731,35
19	3	2	S peptide	LN	22	1,25	1,25	137	18,77	18,77	17	7,72	7,72	76	8,03	8,03	17	3,99	3,99	256	1136,68	1136,68
20	3	5	S peptide	LN	83	4,47	4,47	23	2,77	2,77	185	95,21	95,21	188	17,41	17,41	40	7,78	7,78	375	1513,77	1513,77
29	1	1	PMA Iono	LN	802	35,92	35,92	6819	837,42	837,42	951	425,96	425,96	5191,5	528,45	528,45	1086	78,50	78,50	1598	4645,82	4645,82
30	2	1	PMA Iono	LN	930	41,41	41,41	11126	1777,66	1777,66	1430	628,21	628,21	6317	924,47	731,2	4143,5	273,05	273,05	2253	6278,43	6278,43
31	2	2	PMA Iono	LN	494	22,76	22,76	7001	866,85	866,85	1107	491,32	491,32	6276	901,77	731,2	3748	241,36	241,36	2246	6260,47	6260,47
32	3	1	PMA Iono	LN	1641	73,08	73,08	6896,5	849,87	849,87	2801	1244,18	1244,18	6159,5	842,57	731,2	3033,5	190,98	190,98	2431	6740,05	6740,05

9.6 Certificates of Analysis BNT162a1

BioNTech RNA Pharmaceuticals GmbH

An der Goldgrube 12, 55131 Mainz, Germany
Tel.: +49 (0) 6131-90 84-0, Fax: +49 (0) 6131-90 84-390
info@biontech.de

BIONTECH
RNA Pharmaceuticals

Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBL063.3 (ATM batch uRNAv05)**
Lot/Batch No.:	RNA-SK200305-01
RNA length:	1261 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	06 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
Potency *In vitro* translation followed by gel electrophoresis	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6)

(b) (6)

POLYMUN SCIENTIFIC
Immunbiologische Forschung GmbH

Donaustraße 99
A-3400 Klosterneuburg, Austria
Tel.: +43-2243-25060-300
Fax: +43-2243-25060-399
E-Mail: office@polymun.com
http://www.polymun.com

Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBL063.3 LNP
Lot: CoVVAC/090320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/016)	
RNA integrity	CE (223/SOP/016)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 content	HPLC-CAD (222/SOP/044)	
ALC-0159 content	HPLC-CAD (222/SOP/044)	
DSPC content	HPLC-CAD (222/SOP/044)	
Cholesterol content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{avg})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 26.03.2020 (b) (6)

Date: 26.03.20 (b) (6)

(b) (6)

9.7 Certificates of Analysis BNT162b1

BioNTech RNA Pharmaceuticals GmbH

An der Goldgrube 12, 55131 Mainz, Germany
Tel.: +49 (0) 6131-90 84-0, Fax: +49 (0) 6131-90 84-390
info@biontech.de

Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBP020.3 (ATM batch modRNAv05)**
Lot/Batch No.:	RNA-RF200304-03
RNA length:	1262 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	05 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
Potency *In vitro* translation followed by gel electrophoresis	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Residual dsRNA Antibody-based limit test	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6)

(b) (6)

POLYMUN SCIENTIFIC
Immunbiologische Forschung GmbH

Donaustraße 99
A-3400 Klosterneuburg, Austria
Tel.: +43-2243-25060-300
Fax: +43-2243-25060-399
E-Mail: office@polymun.com
http://www.polymun.com

Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBP020.3 LNP
Lot: CoVVAC/100320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/016)	
RNA integrity	CE (223/SOP/016)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 content	HPLC-CAD (222/SOP/044)	
ALC-0159 content	HPLC-CAD (222/SOP/044)	
DSPC content	HPLC-CAD (222/SOP/044)	
Cholesterol content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{ave})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 26.03.2020 (b) (6)

Date: 26.03.2020

9.8 Certificates of Analysis BNT162b2

BioNTech RNA Pharmaceuticals GmbH

An der Goldgrube 12, 55131 Mainz, Germany
Tel.: +49 (0) 6131-90 84-0, Fax: +49 (0) 6131-90 84-390
info@biontech.de

Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBP020.2 (ATM batch modRNAv09)**
Lot/Batch No.:	RNA-RF200321-06
RNA length:	4283 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	19 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Denaturing Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
Potency *In vitro* translation followed by gel electrophoresis	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Residual dsRNA Antibody-based limit test	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6)

POLYMUN SCIENTIFIC
Immunbiologische Forschung GmbH
Donaustraße 99
A-3400 Klosterneuburg, Austria
Tel.: +43-2243-25060-300
Fax: +43-2243-25060-399
E-Mail: office@polymun.com
http://www.polymun.com

Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBP020.2LNP
Lot: CoVVAC/270320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/016)	
RNA integrity	CE (223/SOP/016)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 content	HPLC-CAD (222/SOP/044)	
ALC-0159 content	HPLC-CAD (222/SOP/044)	
DSPC content	HPLC-CAD (222/SOP/044)	
Cholesterol content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{ave})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 09.04.20 (b) (6)

Date: 09.04.20 (b) (6)

9.9 Certificates of Analysis BNT162c2

BioNTech RNA Pharmaceuticals GmbH

An der Goldgrube 12, 55131 Mainz, Germany
Tel. +49 (0) 6131-90 84-0 Fax: +49 (0) 6131-90 84-390
info@biontech.de

BIONTECH
RNA Pharmaceuticals

Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBS004.2 (ATM batch saRNAv09)**
Lot/Batch No.:	RNA-RF200310-01
RNA length:	11917 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	09 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6)

POLYMUN SCIENTIFIC
Immunbiologische Forschung GmbH

Donaustraße 99
A-3400 Klosterneuburg, Austria
Tel.: +43-2243-25060-300
Fax: +43-2243-25060-399
E-Mail: office@polymun.com
http://www.polymun.com

Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBS004.2 LNP
Lot: CoVVAC/170320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/015)	
RNA integrity	CE (223/SOP/015)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 identification and content	HPLC-CAD (222/SOP/044)	
ALC-0159 identification and content	HPLC-CAD (222/SOP/044)	
DSPC identification and content	HPLC-CAD (222/SOP/044)	
Cholesterol identification and content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{avg})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

(b) (6)

Date: 26.03.2020

Date: 26.03.2020

9.10 Statistical analysis

ELISpot – details on statistical analysis performed with GraphPad Prism 8.

Related to Figure 6

Group	Sidak's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Control (mCorVac#15)	No peptide vs. S pepide	-4.938	-8.321 to -1,554	Yes	**	0.0041
	Control RNA vs. S RNA	2.125	-1.258 to 5.508	No	ns	0.2696
BNT162a1	No peptide vs. S pepide	-82.81	-103.4 to -62.26	Yes	****	<0.0001
	Control RNA vs. S RNA	-47.13	-67.67 to -26.58	Yes	****	<0.0001
BNT162b1	No peptide vs. S pepide	-748.2	-894.1 to -602.3	Yes	****	<0.0001
	Control RNA vs. S RNA	-734.5	-880.4 to -588.6	Yes	****	<0.0001
Control (mCorVac#16)	No peptide vs. S peptide	-14.50	-22.73 to -6.270	Yes	***	0.0007
	Control RNA vs. S RNA	2.438	-5.793 to 10.67	No	ns	0.7333
BNT162b2	No peptide vs. S peptide	-1177	-1314 to -1041	Yes	****	<0.0001
	Control RNA vs. S RNA	-1311	-1448 to -1175	Yes	****	<0.0001
BNT162c2	No peptide vs. S peptide	-1174	-1293 to -1056	Yes	****	<0.0001
	Control RNA vs. S RNA	-1171	-1290 to -1053	Yes	****	<0.0001

Flow cytometry – details on statistical analysis performed with GraphPad Prism 8.

Related to Figure 7

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
$CD8^+$ T cells	Control vs. BNT162a1	-1.250	-3.386 to 0.8864	No	ns	0.3001
	Control vs. BNT162b1	-1.225	-3.361 to 0.9114	No	ns	0.3130
	Control vs. BNT162b2	-10.63	-13.87 to -7.377	Yes	****	<0.0001
	Control vs. BNT162c2	-0.4321	-3.794 to 2.930	No	ns	0.9348
$CD4^+$ T cells	Control vs. BNT162a1	1.188	-0.9863 to 3.361	No	ns	0.3445
	Control vs. BNT162b1	1.425	-0.7488 to 3.599	No	ns	0.2302
	Control vs. BNT162b2	11.06	7.473 to 14.65	Yes	****	<0.0001
	Control vs. BNT162c2	-0.7571	-4.473 to 2.959	No	ns	0.8463

T_{FH} cells	Control vs. BNT162a1	-0.04500	-0.1701 to 0.08005	No	ns	0.6086
	Control vs. BNT162b1	-0.3726	-0.4977 to -0.2476	Yes	****	<0.0001
	Control vs. BNT162b2	-0.3828	-0.7397 to -0.02579	Yes	*	0.0350
	Control vs. BNT162c2	-1.190	-1.560 to -0.8208	Yes	****	<0.0001
B cells	Control vs. BNT162a1	7.813	5.540 to 10.08	Yes	****	<0.0001
	Control vs. BNT162b1	7.900	5.628 to 10.17	Yes	****	<0.0001
	Control vs. BNT162b2	14.64	9.503 to 19.77	Yes	****	<0.0001
	Control vs. BNT162c2	9.921	4.607 to 15.24	Yes	***	0.0005

Related to Figure 8

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
$CD44^+CD38^+PD1^+$ $CD8^+$ T cells	Control vs. BNT162a1	-0.2575	-1.951 to 1.436	No	ns	0.9102
	Control vs. BNT162b1	-6.015	-7.708 to -4.322	Yes	****	<0.0001
	Control vs. BNT162b2	-28.05	-33.72 to -22.37	Yes	****	<0.0001
	Control vs. BNT162c2	-1.344	-7.222 to 4.533	No	ns	0.8115
$ICOS^+$ $CD8^+$ T cells	Control vs. BNT162a1	-0.8663	-3.073 to 1.341	No	ns	0.5578
	Control vs. BNT162b1	-8.401	-10.61 to -6.194	Yes	****	<0.0001
	Control vs. BNT162b2	-40.48	-47.07 to -33.89	Yes	****	<0.0001
	Control vs. BNT162c2	-4.713	-11.54 to 2.109	No	ns	0.1998
$ICOS^+$ $CD4^+$ T cells	Control vs. BNT162a1	-0.5650	-1.427 to 0.2973	No	ns	0.2304
	Control vs. BNT162b1	-2.551	-3.414 to -1.689	Yes	****	<0.0001
	Control vs. BNT162b2	-2.981	-5.174 to -0.7890	Yes	**	0.0078
	Control vs. BNT162c2	1.218	-1.051 to 3.488	No	ns	0.3555
$ICOS^+$ Tfh cells	Control vs. BNT162a1	-10.11	-18.24 to -1.987	Yes	*	0.0143
	Control vs. BNT162b1	-26.43	-34.55 to -18.30	Yes	****	<0.0001
	Control vs. BNT162b2	-12.49	-21.24 to -3.733	Yes	**	0.0054
	Control vs. BNT162c2	-19.20	-27.95 to -10.45	Yes	****	<0.0001

Related to Figure 9

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
$CD8^+$ T cells	Control vs. BNT162a1	-337552	-833529 to 158426	No	ns	0.2084
	Control vs. BNT162b1	-420848	-916825 to 75130	No	ns	0.1019
	Control vs. BNT162b2	-683950	-1112484 to -255415	Yes	**	0.0021
	Control vs. BNT162c2	-75551	-504086 to 352984	No	ns	0.8818
$CD4^+$ T cells	Control vs. BNT162a1	-749301	-1913693 to 415091	No	ns	0.2411
	Control vs. BNT162b1	-1246977	-2411369 to -82585	Yes	*	0.0352
	Control vs. BNT162b2	-1850559	-2886016 to -815102	Yes	***	0.0007
	Control vs. BNT162c2	-216563	-1252020 to 818895	No	ns	0.8389
T_{FH} cells	Control vs. BNT162a1	-2366	-7903 to 3171	No	ns	0.5051
	Control vs. BNT162b1	-14242	-19780 to -8705	Yes	****	<0.0001
	Control vs. BNT162b2	-46173	-60706 to -31640	Yes	****	<0.0001
	Control vs. BNT162c2	-4251	-18783 to 10282	No	ns	0.7150
T_H1 cells	Control vs. BNT162a1	-7820	-18193 to 2552	No	ns	0.1541
	Control vs. BNT162b1	-13043	-23416 to -2671	Yes	*	0.0134
	Control vs. BNT162b2	-2268	-4564 to 28.31	No	ns	0.0531
	Control vs. BNT162c2	297.1	-1999 to 2593	No	ns	0.9339

Related to Figure 10

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
B cells	Control vs. BNT162a1	-415609	-1302980 to 471763	No	ns	0.4459
	Control vs. BNT162b1	-1266693	-2154065 to -379321	Yes	**	0.0053
	Control vs. BNT162b2	-761299	-1089182 to -433417	Yes	****	<0.0001
	Control vs. BNT162c2	26360	-301523 to 354242	No	ns	0.9738
Plasma cells	Control vs. BNT162a1	-244.3	-18256 to 17768	No	ns	0.9992
	Control vs. BNT162b1	-20130	-38142 to -2117	Yes	*	0.0278

	Control vs. BNT162b2	-4115	-9265 to 1034	No	ns	0.1206
	Control vs. BNT162c2	-79.67	-5383 to 5223	No	ns	0.9989
Class switched B cells	Control vs. BNT162a1	-69656	-150840 to 11528	No	ns	0.0977
	Control vs. BNT162b1	-280116	-361300 to -198931	Yes	****	<0.0001
	Control vs. BNT162b2	-509776	-672575 to -346977	Yes	****	<0.0001
	Control vs. BNT162c2	-18446	-186106 to 149214	No	ns	0.9444
Germinal center B cells	Control vs. BNT162a1	-66066	-140887 to 8755	No	ns	0.0871
	Control vs. BNT162b1	-267264	-342085 to -192443	Yes	****	<0.0001
	Control vs. BNT162b2	-509776	-673052 to -346500	Yes	****	<0.0001
	Control vs. BNT162c2	-18261	-186412 to 149889	No	ns	0.9457
IgG1$^+$ Germinal center B cells	Control vs. BNT162a1	-17771	-40867 to 5325	No	ns	0.1444
	Control vs. BNT162b1	-67436	-90532 to -44340	Yes	****	<0.0001
	Control vs. BNT162b2	-152191	-198806 to -105575	Yes	****	<0.0001
	Control vs. BNT162c2	2178	-45830 to 50185	No	ns	0.9902
IgG2a$^+$ Germinal center B cells	Control vs. BNT162a1	-52.50	-205.8 to 100.8	No	ns	0.6362
	Control vs. BNT162b1	-285.0	-438.3 to -131.7	Yes	***	0.0005
	Control vs. BNT162b2	-459.6	-678.2 to -240.9	Yes	***	0.0003
	Control vs. BNT162c2	-31.33	-256.5 to 193.9	No	ns	0.9132

Related to Figure 11

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
T_{FH} cells	Control vs. BNT162a1	-943.6	-8833 to 6946	No	ns	0.9431
	Control vs. BNT162b1	-9561	-17451 to -1671	Yes	*	0.0170
	Control vs. BNT162b2	-47817	-74515 to -21120	Yes	***	0.0007
	Control vs. BNT162c2	35.88	-26661 to 26733	No	ns	>0.9999
Germinal center B cells	Control vs. BNT162a1	14390	-129166 to 157946	No	ns	0.9596
	Control vs. BNT162b1	-382477	-526032 to -238921	Yes	****	<0.0001
	Control vs. BNT162b2	-1601371	-2093312 to -1109430	Yes	****	<0.0001

	Control vs. BNT162c2	-74428	-566369 to 417514	No	ns	0.9111
Class switched B cells	Control vs. BNT162a1	-228955	-468753 to 10843	No	ns	0.0622
	Control vs. BNT162b1	-476506	-716304 to -236708	Yes	***	0.0002
	Control vs. BNT162b2	-1445088	-1887202 to -1002973	Yes	****	<0.0001
	Control vs. BNT162c2	-60963	-503077 to 381151	No	ns	0.9254

Related to Figure 12

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IFNγ$^+$ CD8$^+$ T cells	Control vs. BNT162a1	-1560	-18564 to 15444	No	ns	0.9660
	Control vs. BNT162b1	-77611	-94615 to -60607	Yes	****	<0.0001
	Control vs. BNT162b2	-140659	-195692 to -85625	Yes	****	<0.0001
	Control vs. BNT162c2	-79418	-134451 to -24384	Yes	**	0.0049
IL-2$^+$ CD8$^+$ T cells	Control vs. BNT162a1	-382.5	-2638 to 1873	No	ns	0.8899
	Control vs. BNT162b1	-9429	-11684 to -7174	Yes	****	<0.0001
	Control vs. BNT162b2	-5903	-10507 to -1298	Yes	*	0.0117
	Control vs. BNT162c2	-406.1	-5010 to 4198	No	ns	0.9685
TNF$^+$ CD8$^+$ T cells	Control vs. BNT162a1	-591.5	-2201 to 1018	No	ns	0.5965
	Control vs. BNT162b1	-4292	-5901 to -2683	Yes	****	<0.0001
	Control vs. BNT162b2	-1403	-2898 to 91.18	No	ns	0.0670
	Control vs. BNT162c2	351.1	-1143 to 1846	No	ns	0.8023
IFNγ$^+$ CD4$^+$ T cells	Control vs. BNT162a1	19.00	-1350 to 1388	No	ns	0.9992
	Control vs. BNT162b1	-3941	-5310 to -2572	Yes	****	<0.0001
	Control vs. BNT162b2	995.9	-2046 to 4038	No	ns	0.6600
	Control vs. BNT162c2	1282	-1760 to 4324	No	ns	0.5140
IL-2$^+$ CD4$^+$ T cells	Control vs. BNT162a1	-1472	-2850 to -92.56	Yes	*	0.0359
	Control vs. BNT162b1	-2276	-3655 to -897.3	Yes	**	0.0016
	Control vs. BNT162b2	-3755	-8093 to 583.6	No	ns	0.0944
	Control vs. BNT162c2	-774.8	-5113 to 3564	No	ns	0.8790

IL-4$^+$ CD4$^+$ T cells	Control vs. BNT162a1	-377.1	-1961 to 1207	No	ns	0.7977
	Control vs. BNT162b1	-520.3	-2104 to 1064	No	ns	0.6583
	Control vs. BNT162b2	-654.0	-1345 to 36.86	No	ns	0.0647
	Control vs. BNT162c2	-71.88	-762.7 to 619.0	No	ns	0.9566
IFNγ$^+$ T_{FH} cells	Control vs. BNT162a1	70.25	-110.7 to 251.2	No	ns	0.5643
	Control vs. BNT162b1	-134.9	-315.8 to 46.04	No	ns	0.1597
	Control vs. BNT162b2	-147.3	-267.6 to -26.93	Yes	*	0.0160
	Control vs. BNT162c2	-19.63	-139.9 to 100.7	No	ns	0.8976

Multiplex protein quantification – details on statistical analysis performed with GraphPad Prism 8.

Related to Figure 13

Group	Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IFNγ	Control (mCorVac#15)	-0,9662	-969,8 to 967,8	No	ns	>0,9999
	BNT162a1	-202,2	-1171 to 766,6	No	ns	0,9330
	BNT162b1	-2025	-2994 to -1056	Yes	****	<0,0001
	Control	-3,160	-20,63 to 14,31	No	ns	0,9548
	BNT162b2	-4780	-4798 to -4763	Yes	****	<0,0001
	BNT162c2	-4794	-4811 to -4776	Yes	****	<0,0001
IL-2	Control	1,260	-12,20 to 14,72	No	ns	0,9932
	BNT162a1	-27,52	-40,98 to -14,06	Yes	****	<0,0001
	BNT162b1	-27,35	-40,81 to -13,90	Yes	****	<0,0001
	Control	14,00	-15,79 to 43,79	No	ns	0,5549
	BNT162b2	-81,77	-111,6 to -51,98	Yes	****	<0,0001
	BNT162c2	-63,07	-92,86 to -33,28	Yes	****	<0,0001
IL-4	Control	0,000	-9,014 to 9,014	No	ns	>0,9999
	BNT162a1	-3,488	-12,50 to 5,526	No	ns	0,6953
	BNT162b1	-16,09	-25,11 to -7,079	Yes	***	0,0004
	Control	-4,335	-26,27 to 17,60	No	ns	0,9423

	BNT162b2	-101,9	-123,9 to -79,98	Yes	****	<0,0001
	BNT162c2	-29,45	-51,39 to -7,513	Yes	**	0,0067
IL-5	n/a	n/a	n/a	n/a	n/a	n/a
	n/a	n/a	n/a	n/a	n/a	n/a
	n/a	n/a	n/a	n/a	n/a	n/a
	Control	3,553e-015	-7,207 to 7,207	No	ns	>0,9999
	BNT162b2	-24,75	-31,95 to -17,54	Yes	****	<0,0001
	BNT162c2	-6,075	-13,28 to 1,132	No	ns	0,1160
IL-18	Control	-0,4713	-355,8 to 354,9	No	ns	>0,9999
	BNT162a1	-667,2	-1023 to -311,9	Yes	***	0,0002
	BNT162b1	-1914	-2270 to -1559	Yes	****	<0,0001
	Control	-54,48	-627,7 to 518,7	No	ns	0,9929
	BNT162b2	-6801	-7374 to -6228	Yes	****	<0,0001
	BNT162c2	-7150	-7723 to -6576	Yes	****	<0,0001
GM-CSF	Control	0,000	-5,859 to 5,859	No	ns	>0,9999
	BNT162a1	-3,166	-9,025 to 2,693	No	ns	0,4398
	BNT162b1	-21,55	-27,41 to -15,69	Yes	****	<0,0001
	Control	-2,842e-014	-20,39 to 20,39	No	ns	>0,9999
	BNT162b2	-146,4	-166,8 to -126,0	Yes	****	<0,0001
	BNT162c2	-78,13	-98,53 to -57,74	Yes	****	<0,0001
n/a measured values < lower limit of quantification						

9.11 List of attachments

Attachment I includes the following raw data sets:

- Attachment_001_CorVac#15_phenotypic_analysis_blood_Freq_Report
- Attachment_002_CorVac#16_phenotypic_analysis_blood_Freq_Report
- Attachment_003_CorVav#15_phenotypic_analysis_Spleen_Freq_Report
- Attachment_004_CorVac#16_phenotypic_analysis_Spleen_Freq_Report
- Attachment_005_CorVav#15_phenotypic_analysis_LN_Freq_Report
- Attachment_006_CorVav#16_phenotypic_analysis_LN_Freq_Report
- Attachment_007_CorVac#15_phenotypic_analysis_Spleen_Counts_Report
- Attachment_008_CorVac#16_phenotypic_analysis_Spleen_Counts_Report
- Attachment_009_CorVav#15_phenotypic_analysis_LN_Counts_Report

- Attachment_010_CorVav#16_phenotypic_analysis_LN_Counts_Report
- Attachment_011_CorVac#15_phenotypic_analysis_Spleen_gMFI_Report
- Attachment_012_CorVac#16_phenotypic_analysis_Spleen_gMFI_Report
- Attachment_013_CorVac#15_phenotypic_analysis_LN_gMFI_Report
- Attachment_014_CorVav#16_phenotypic_analysis_LN_gMFI_Report
- Attachment_015_CorVac#15_myeloid_Spleen_Freq_Report
- Attachment_016_CorVac#16_myeloid_Spleen_Freq_Report
- Attachment_017_CorVac#15_myeloid_Spleen_Counts_Report
- Attachment_018_CorVac#16_myeloid_Spleen_Counts_Report
- Attachment_019_CorVac#15_functional_analysis_Spleen_Freq_Report
- Attachment_020_CorVac#16_functional_analysis_Spleen_Freq_Report
- Attachment_021_CorVac#15_functional_analysis_LN_Freq_Report
- Attachment_022_CorVac#16_functional_analysis_LN_Freq_Report
- Attachment_023_CorVac#15_functional_analysis_Spleen_Counts_Report
- Attachment_024_CorVac#16_functional_analysis_Spleen_Counts_Report
- Attachment_025_CorVac#15_functional_analysis_LN_Counts_Report
- Attachment_026_CorVac#16_functional_analysis_LN_Counts_Report
- Attachment_027_CorVav#15_B-cell_Spleen_Freq_Report
- Attachment_028_CorVav#16_B-cell_Spleen_Freq_Report
- Attachment_029_CorVav#15_B-cell_LN_Freq_Report
- Attachment_030_CorVav#16_B-cell_LN_Freq_Report
- Attachment_031_CorVac#15_B-cell_Spleen_Counts_Report
- Attachment_032_CorVac#16_B-cell_Spleen_Counts_Report
- Attachment_033_CorVac#15_B-cell_LN_Counts_Report
- Attachment_034_CorVac#16_B-cell_LN_Counts_Report
- Attachment_035_CorVac#15_memB-cell_Spleen_Counts_Report
- Attachment_036_CorVac#16_memB-cell_Spleen_Counts_Report
- Attachment_037_CorVac#15_memB-cell_LN_Counts_Report
- Attachment_038_CorVac#16_memB-cell_LN_Counts_Report

Attachment II includes all gating strategies used for the analysis of flow cytometry data

Attachment III includes the following raw data sets:

- Attachment_039_CorVac#15_xCelligence_Report
- Attachment_040_CorVac#16_xCelligence_Report