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.

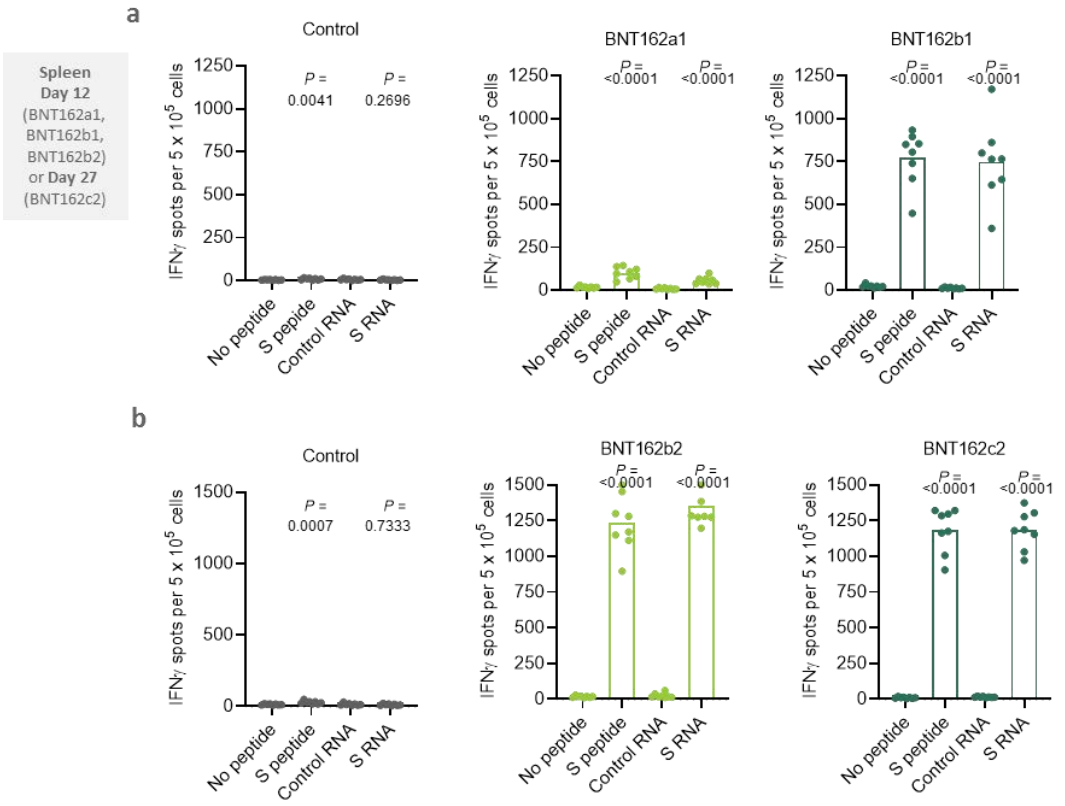

Figure 6: ELISpot analysis using splenocytes from animals treated with BNT162a1, BNT162b1, BNT162b2 or BNT162c2

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).

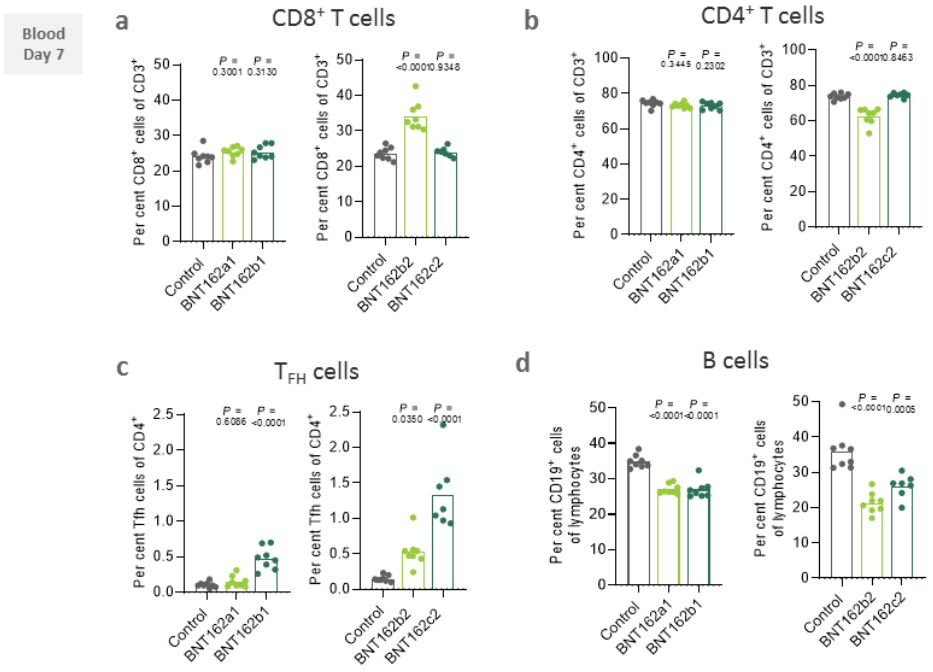

Figure 7: Analysis of lymphocyte frequencies in the blood of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

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).

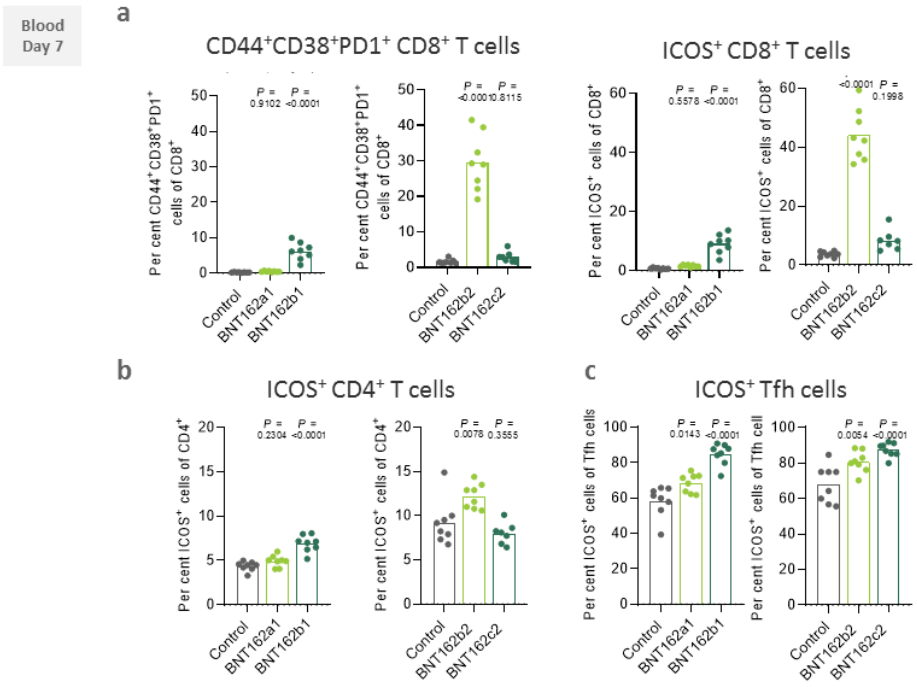

Figure 8: Analysis of T cell activation in the blood of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

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).

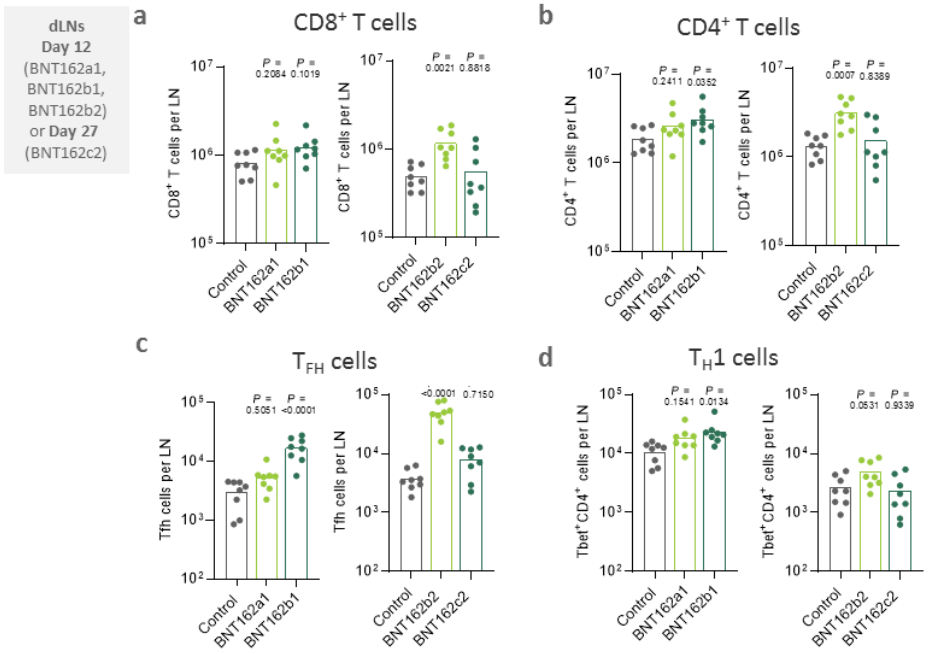

Figure 9: Analysis of T cell counts in the dLNs of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

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.

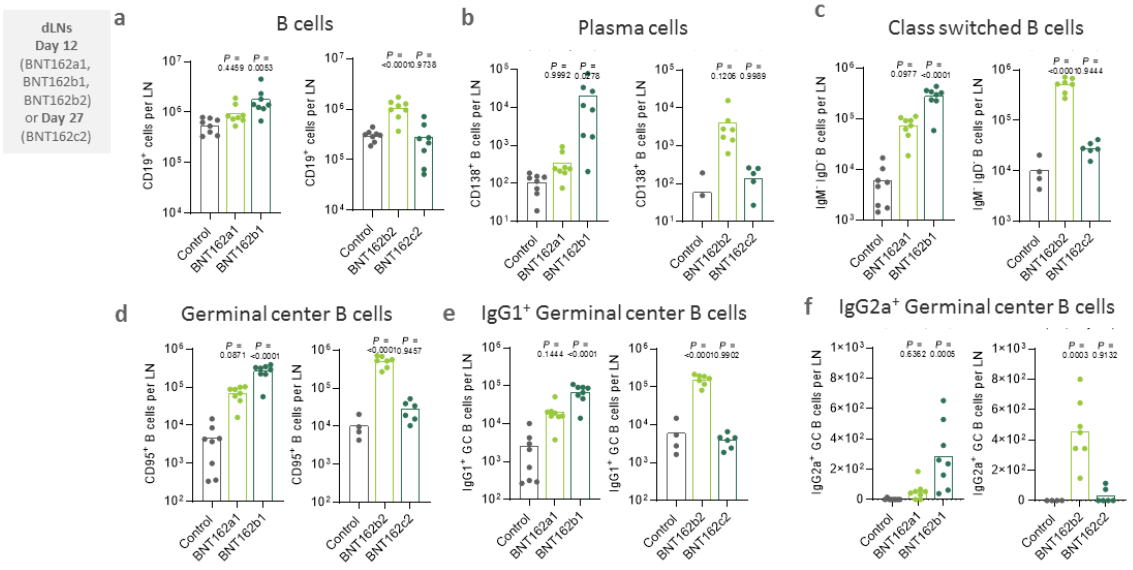

Figure 10: Analysis of B cell counts in the dLNs of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

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).

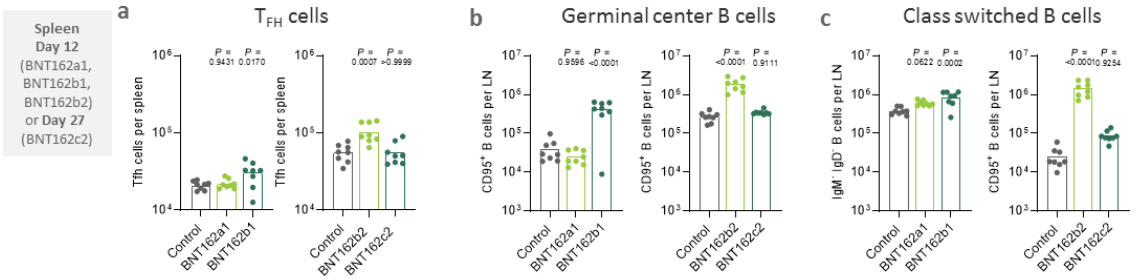

Figure 11: Analysis of T_{FH} and B cell counts in the spleen of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

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).

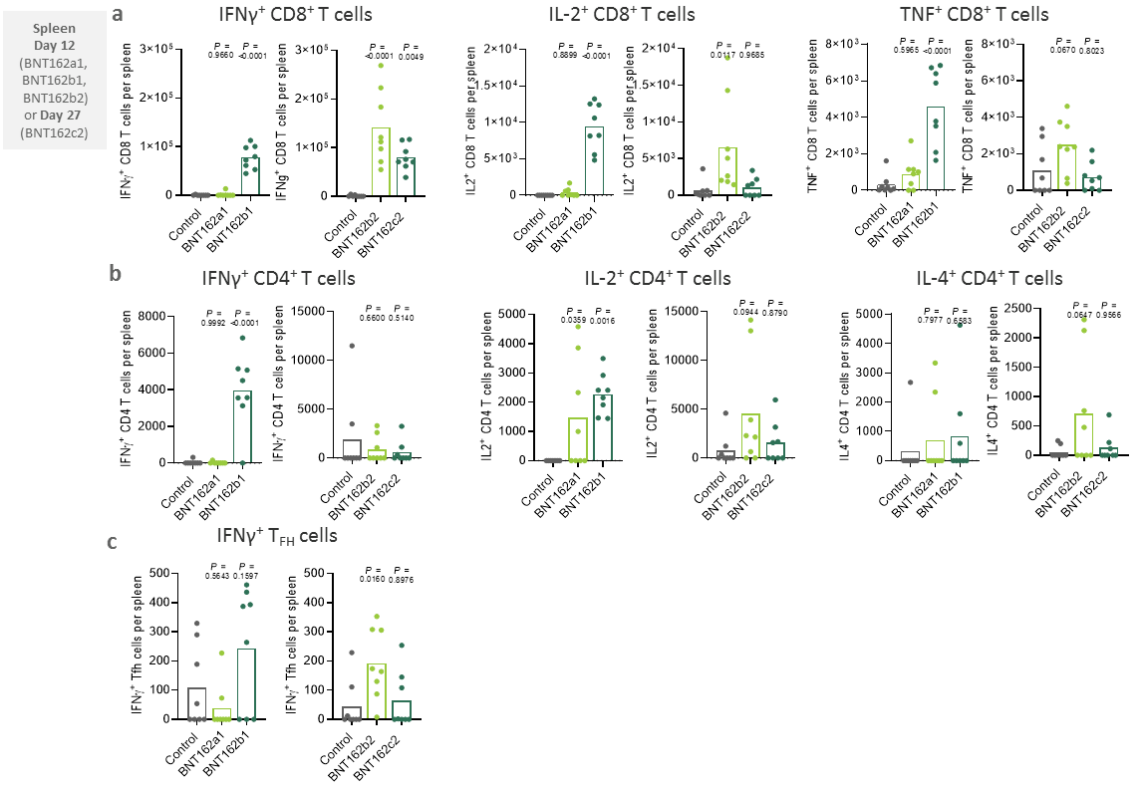

Figure 12: Quantification of cytokine secreting T cells upon S peptide restimulation in the spleen of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

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.

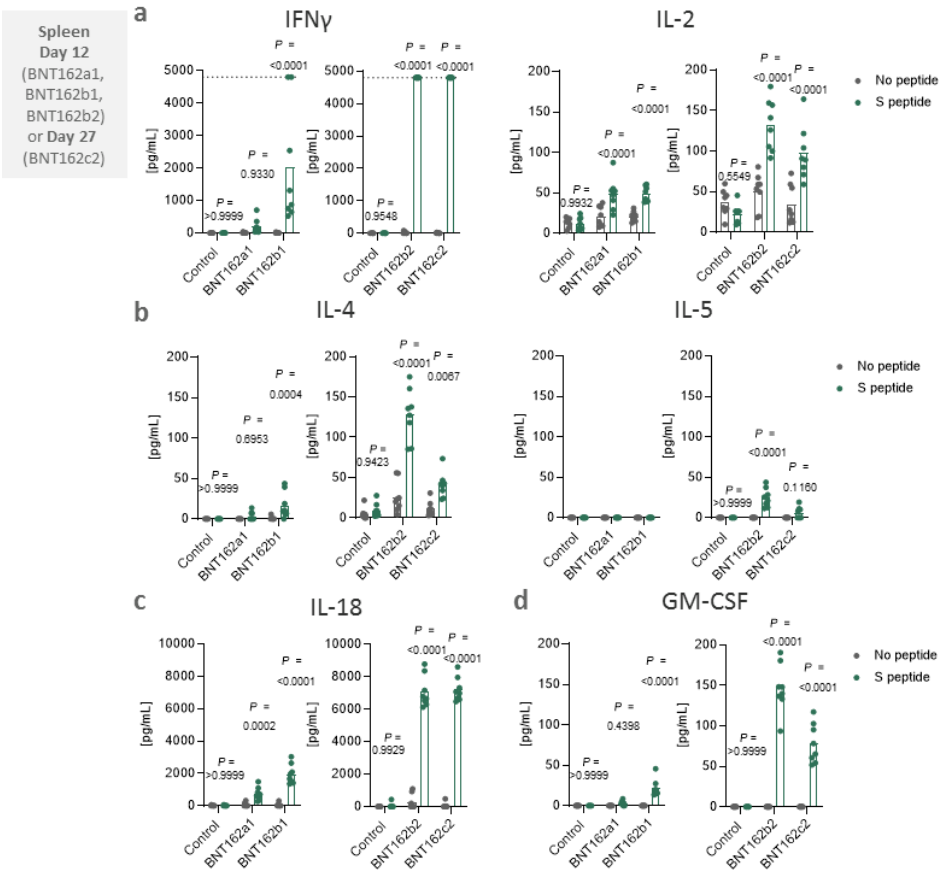

Figure 13: Quantification of cytokine secretion upon S peptide restimulation of splenocytes of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

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.

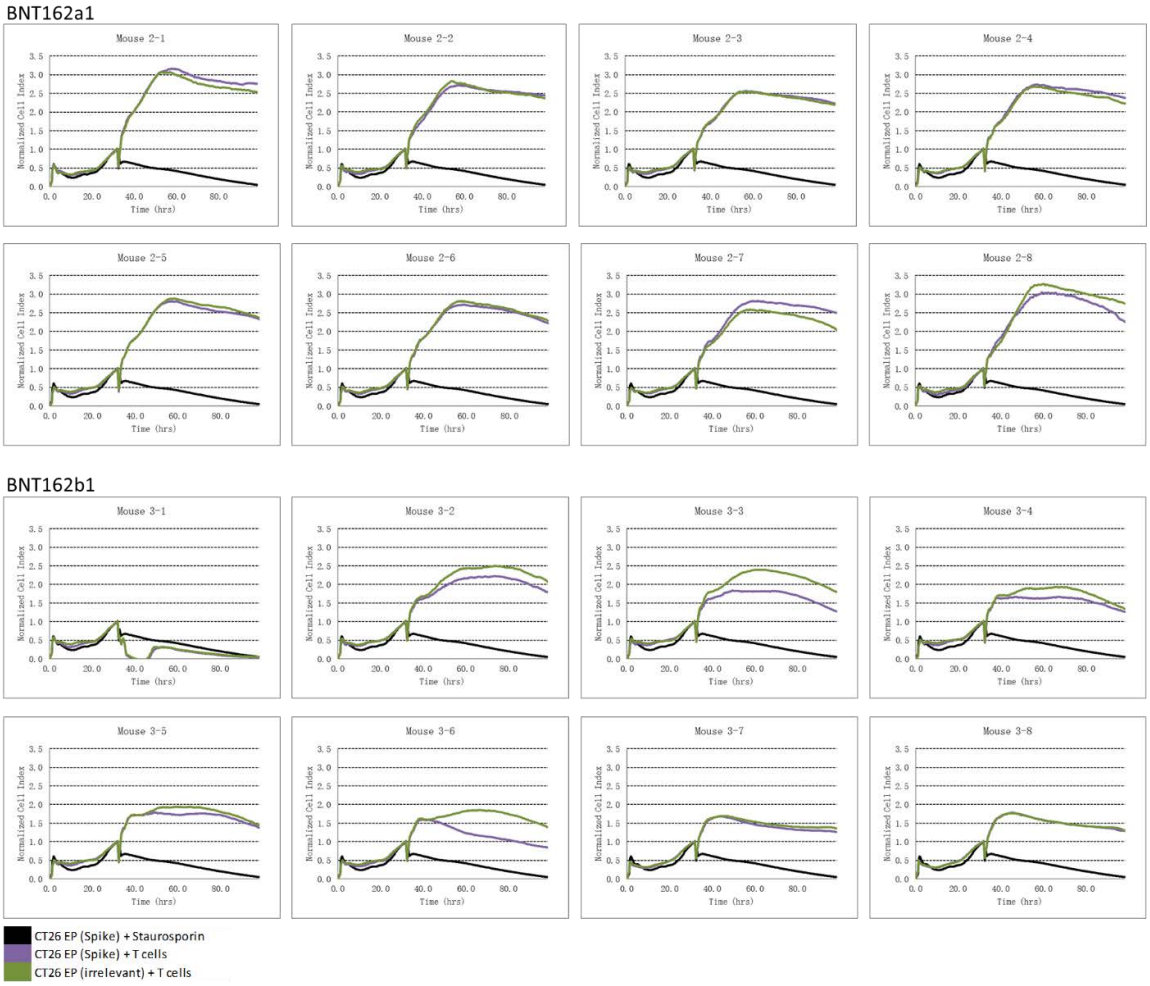

Figure 14: Cytotoxicity towards S protein expressing CT26 cells by CD8⁺ splenocytes from BNT162a1 or BNT162b1 vaccinated mice (mCorVAC#15).

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.

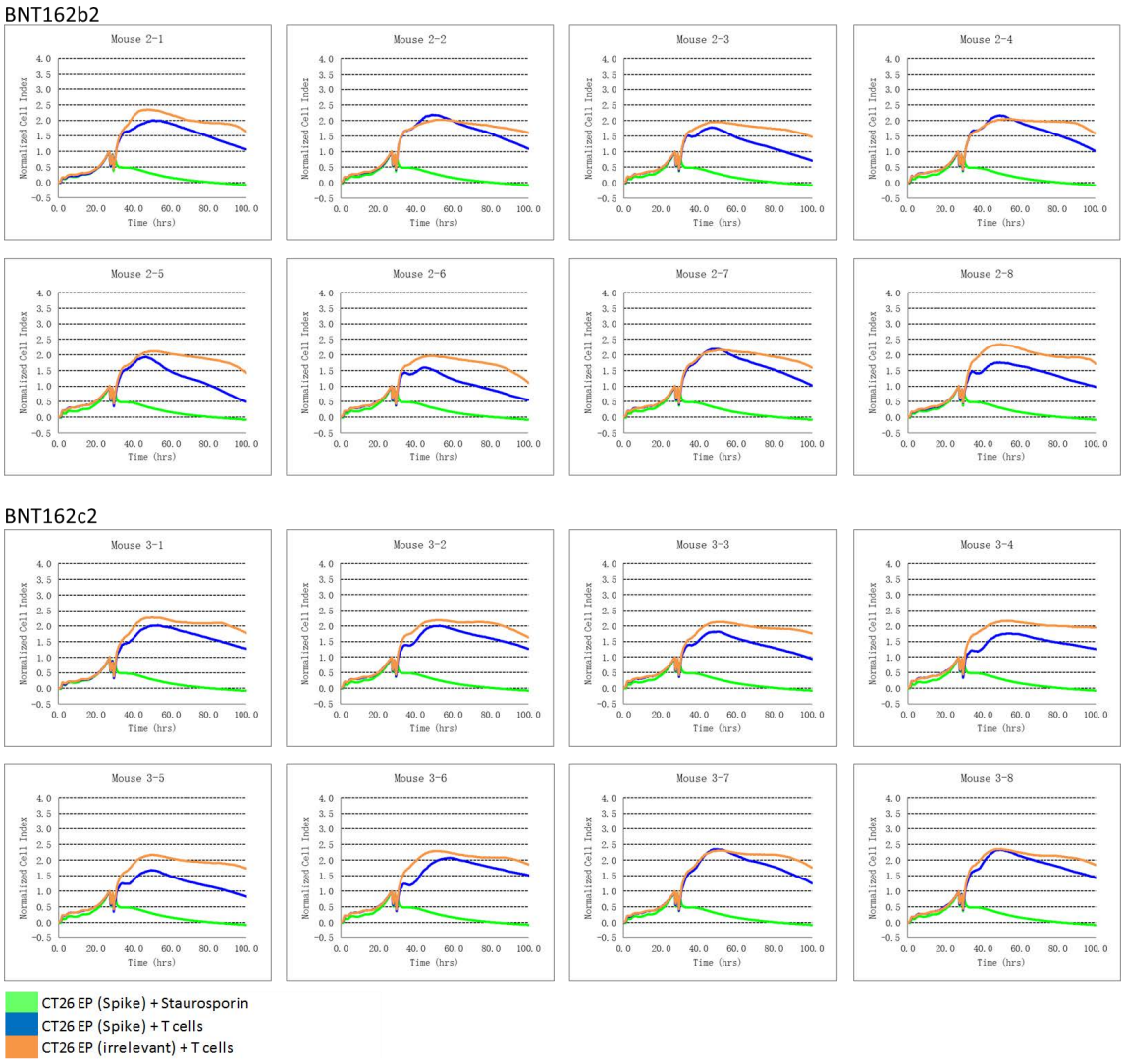

Figure 15: Cytotoxicity towards S protein expressing CT26 cells by CD8⁺ splenocytes from BNT162b2 or BNT162c2 vaccinated mice (mCorVAC#16).

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