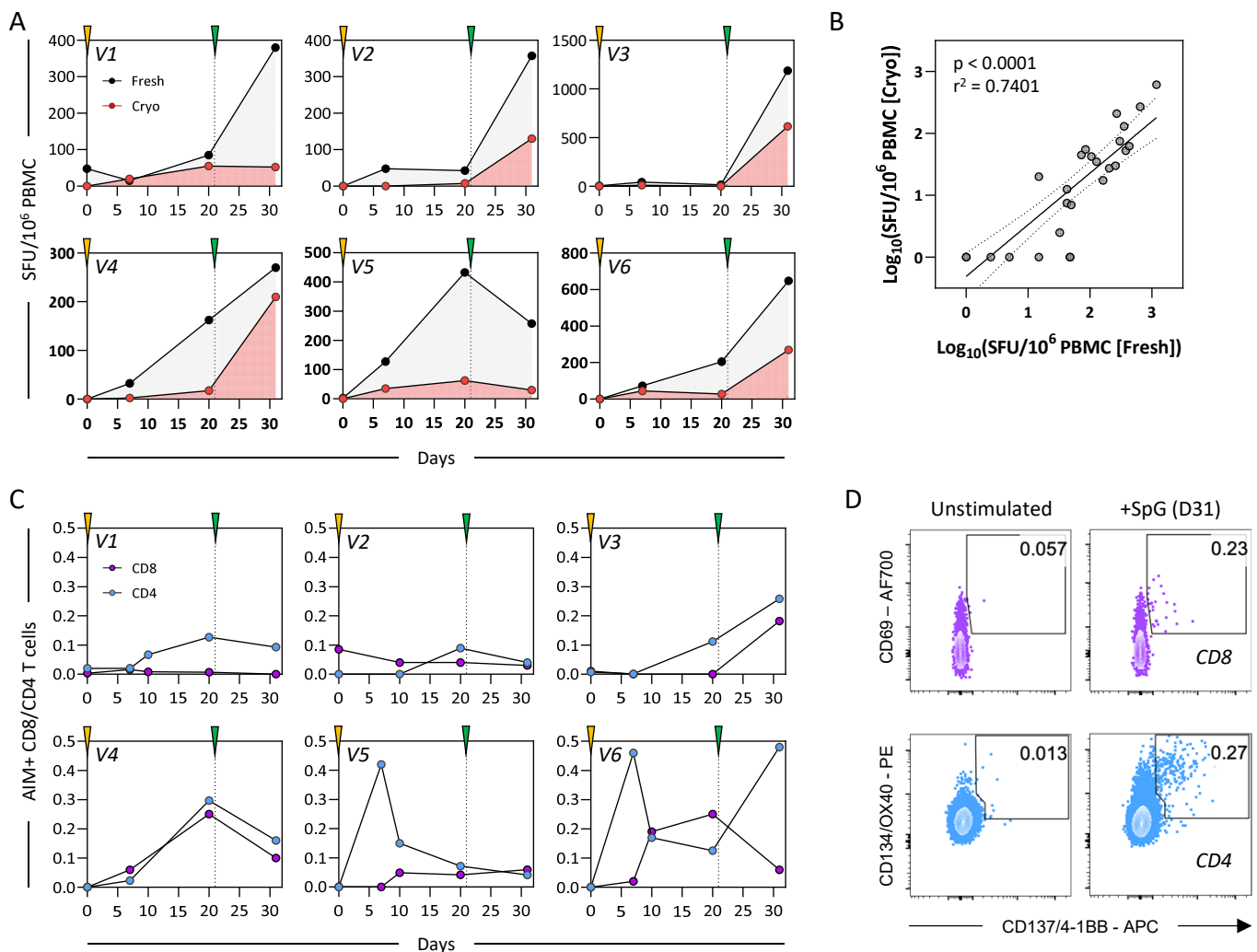
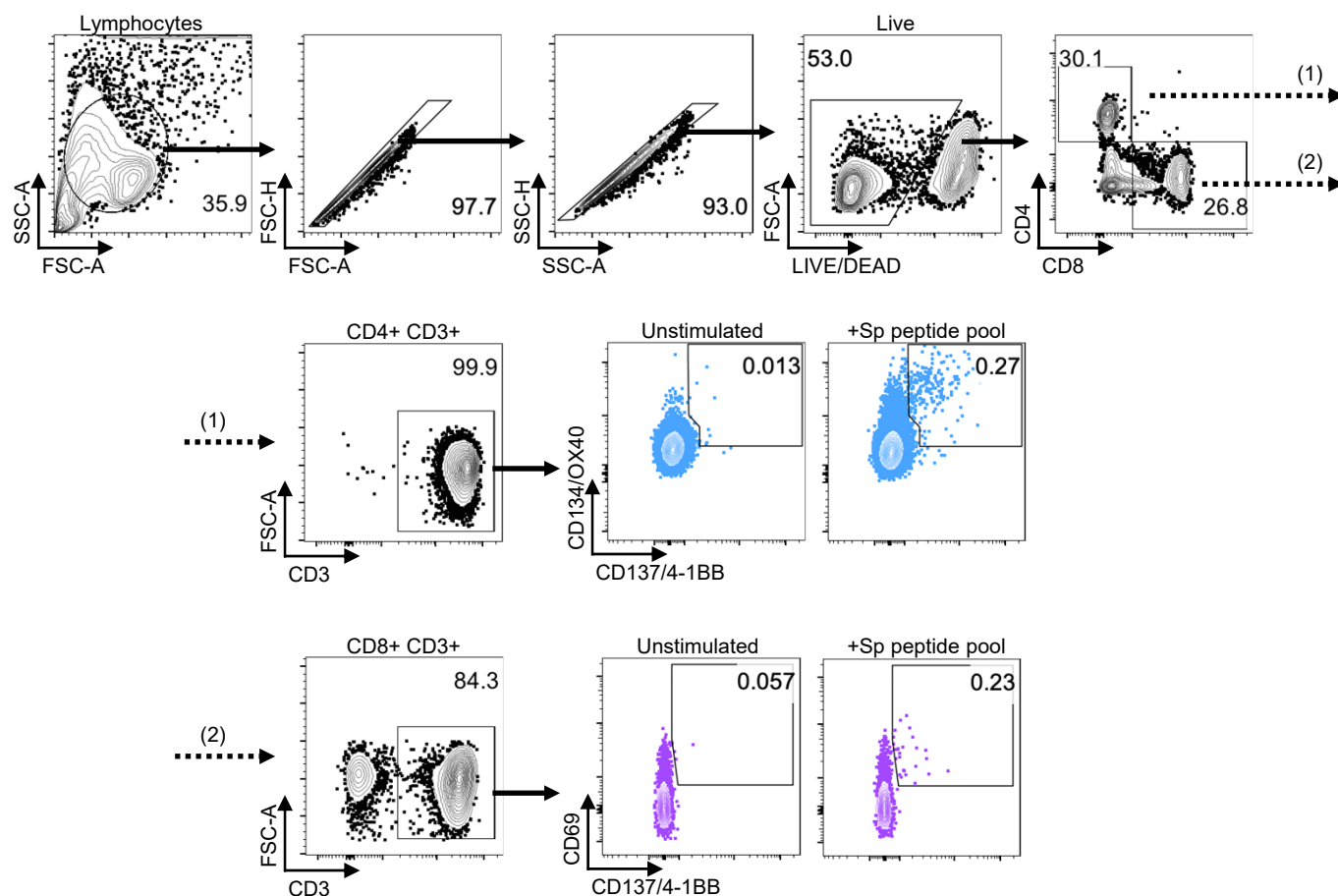
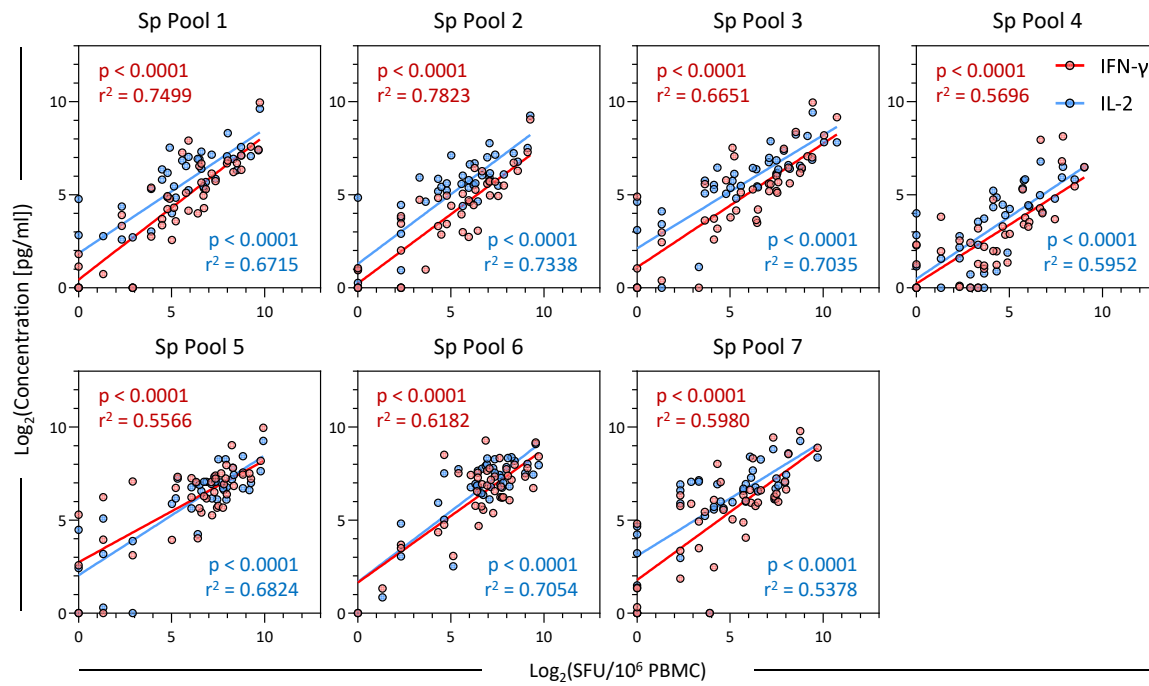




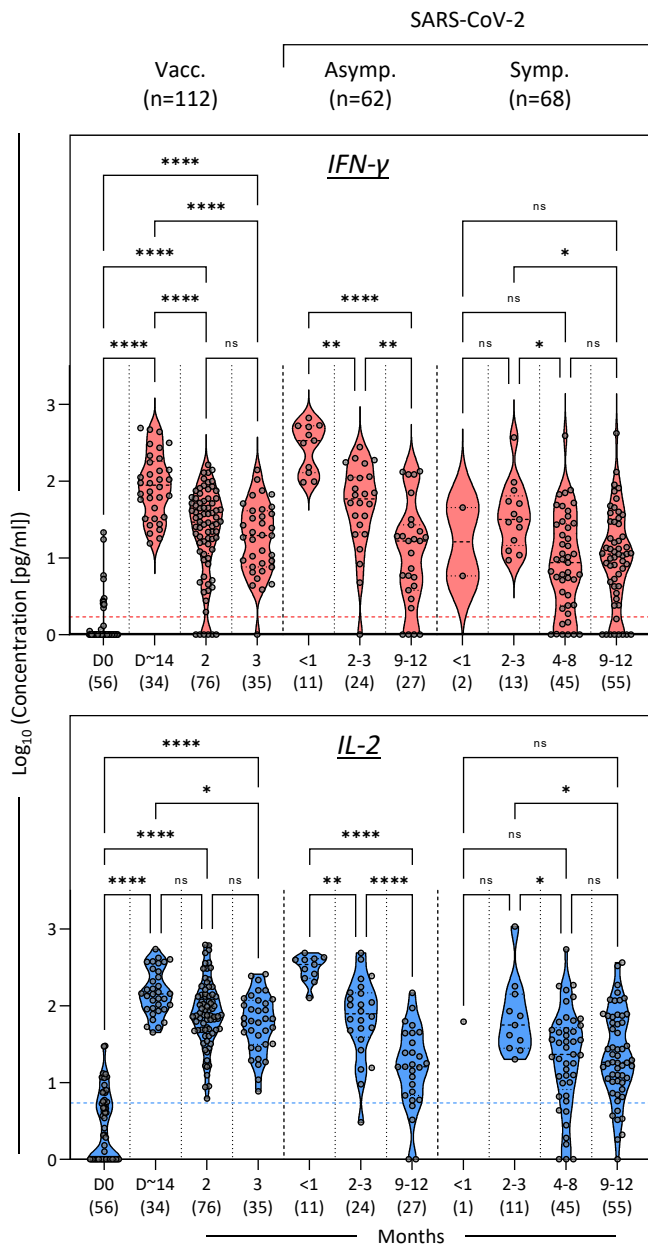
Supplementary Figure 1. Longitudinal response dynamics of ELISPOT assay performed using fresh and cryopreserved PBMCs. **A)** Standard IFN- γ ELISPOT assays using SpG peptide pool were performed in parallel using freshly isolated (black) or cryopreserved PBMCs (red) obtained from vaccinated individuals at different time points post vaccination ($n=6$; 24 samples). **B)** Linear regression analysis of IFN- γ SFU/ 10^6 PBMCs quantified using freshly isolated or cryopreserved PBMCs shows a strong positive correlation. Dotted lines denote the 95% confidence interval. **C)** The frequency of Spike-specific CD4 and CD8 T cells 10 days after the second dose of BNT162b2 were quantified from cryopreserved PBMC samples through the induction of activation markers (CD69, 4-1BB and/or OX40) after SpG peptide pool stimulation ($n=6$; 27 samples). **D)** Flow cytometry bivariate dot plots showing AIM+ CD8 and CD4 T cells frequencies after SpG peptide pool stimulation in a representative vaccinee 10 days after the boost dose.



Supplementary Figure 2. Representative gating strategy for AIM+ CD4+ (CD134/OX40+ CD137/4-1BB+; blue) and CD8+ (CD69+ CD137/4-1BB+; purple) T cells in PBMCs with and without peptide stimulation. Frequencies of gated populations relative to parent are affixed into the contour plots.



Supplementary Figure 3. Linear regression analysis of the concentration of IFN- γ and IL-2 in Spike peptide pool 1-7 stimulated whole blood and the corresponding frequency of total Spike-specific T cells quantified by IFN- γ ELISPOT in vaccinated individuals (V1-V6). Data from multiple time points (Days 0-41) after vaccination were used for this analysis.



Supplementary Figure 4. SARS-CoV-2 spike specific T cells in the whole blood of vaccinated and infected individuals. A) SARS-CoV-2 Spike-specific T cell response in vaccinated individuals (n=112; 201 samples), asymptomatic (n=62; 62 samples) and symptomatic (n=68; 115 samples) COVID-19 patients were longitudinally quantified by measuring IFN- γ (red) and IL-2 (blue) secretion in whole blood after SpG peptide pool stimulation. Dashed lines denote the detection cut-off for the measured cytokines. The number of samples analysed at each time point were indicated in parentheses. Significant differences in each group were analysed by one-way ANOVA and the adjusted p-value (adjusted for multiple comparison) are shown. ns = not significant $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$.

SpG peptide pool				Sp-1 peptide pool				Sp-2 peptide pool				Sp-3 peptide pool				Sp-4 peptide pool				Sp-5 peptide pool				Sp-6 peptide pool				Sp-7 peptide pool				NP peptide pool			
No	Peptide	Sequence	aa	No	Peptide	Sequence	aa	No	Peptide	Sequence	aa	No	Peptide	Sequence	aa	No	Peptide	Sequence	aa	No	Peptide	Sequence	aa	No	Peptide	Sequence	aa	No	Peptide	Sequence	aa				
1	SP_21	IKRWGITLIDSKTQ	101-115	1	SP_1	MFVFLVLPLVSSQC	1-15	1	SP_35	VSQFLMDLEGKQCN	171-185	1	SP_68	CPFGEYFNATRFASV	336-350	1	SP_101	NGCVGYQPIRVVVLSF	501-515	1	SP_140	THSLGAEANSVAYSNW	696-710	1	SP_178	WTFGACAGLQIPFAM	886-900	1	SP_216	ITAPALCHDQGAHFPI	1076-1090	1	NP_42	SPARMANGQDAALA	206-220
2	SP_22	FGITLDSKTQSLIV	106-120	2	SP_2	VLLPVLSCQCNLTI	6-20	2	SP_36	LMDEGQQGFANLH	176-190	2	SP_69	VFNATRFASVYANNR	341-355	2	SP_102	QPIRVNVSFSLHUA	506-520	2	SP_141	AENSVAYSNSIATP	701-715	2	SP_179	GAALQPPAMQANVH	891-905	2	SP_217	ICHQKAGHPRFCVF	1081-1095	2	NP_43	KAKCQDAALLALLD	211-225
3	SP_34	CTFEVYSQPFMDLE	166-180	3	SP_3	VSSQCNLITRTQLP	11-25	3	SP_37	QDQGNFKNLREFEV	181-195	3	SP_70	RFASVYANWKRTSN	346-360	3	SP_103	NVLSEFELLHAPATVC	511-525	3	SP_142	AYSNASTAIPTNETI	706-720	3	SP_180	IFPAMQMYARENGCIG	886-910	3	SP_218	KAHFPRFCVYVSNCTI	1086-1100	3	NP_44	DAALALLILDRILNDL	216-230
4	SP_35	VSQPFMDLEGKQCN	171-185	4	SP_4	VNLITRTQLPPAYTN	16-30	4	SP_38	FNKNLRFVFNKIDCVI	186-200	4	SP_71	YAMNKRISNCVADY	351-365	4	SP_104	ELLHAPATVCQPKKS	516-530	4	SP_143	INFTISVTTEILPVS	716-730	4	SP_181	QMYAKFNQGVTHQNV	901-915	4	SP_219	REGVYVSNGTWNTFV	1091-1105	4	NP_45	LLLLDRILNQLKESMS	221-235
5	SP_48	TRFQTLTLLAHLRSYLT	236-250	5	SP_5	RTQLPPAYTNFSTRC	21-35	5	SP_39	EFVKNIDQYFKIYS	191-205	5	SP_72	KRISNCVADYSVLNV	356-370	5	SP_105	PATVCQPKKSNTNLVK	521-535	5	SP_144	INFTISVTTEILPVS	716-730	5	SP_182	FNQIGVTVQNLVYENQ	906-920	5	SP_220	VSNGTWNTFVTRNFY	1096-1110	5	NP_46	RLNLQESKMSQCGQ	226-240
6	SP_49	LLAHLRSYLTPODSS	241-255	6	SP_6	PATYNSTRFGVYVDP	26-40	6	SP_40	NIDQYFKIYSKHTPI	196-210	6	SP_73	CVADYSVLNSASF5	361-375	6	SP_106	CPKKSNTNLVKKNVCN	526-540	6	SP_145	SVTTEILPVSMKTS	721-735	6	SP_183	VTQNLVYENQKLIAN	911-925	6	SP_221	HWFTVQRNFYEPQII	1101-1115	6	NP_47	ESKMSGAGQQOQQQT	231-245
7	SP_60	RSYLTPRODSSGATA	246-260	7	SP_7	SFTKQYVFNQVYVDP	31-45	7	SP_41	FKIYSKHTPIVNB	201-215	7	SP_74	SVLNSASF5PFCB	366-380	7	SP_107	TNLVKKNVFNPNK	531-545	7	SP_146	LLVYENQKLIANFSA	916-930	7	SP_184	LVYENQKLIANFSA	916-930	7	NP_48	CGGQZQZQZQVTVNKS	236-250				
8	SP_59	CALDPLSETKCTLKS	186-200	8	SP_8	VYVFNQVYVRSVLSH	36-50	8	SP_42	HTPIVNBVLVRLVQ	206-220	8	SP_75	SASF5PFCBQYVPT	371-385	8	SP_108	NKNVFNPNKLTGTC	536-550	8	SP_147	HTKTSQVCTWYVTC	731-745	8	SP_185	KLJANFNSATGATQ	921-935	8	NP_49	CGGQZQZQZQVTVNKS	241-255				
9	SP_60	LESTKCTLKSFYVEK	206-310	9	SP_9	KVFRSVLHSTQDLF	41-55	9	SP_43	NLVDRDPQSFALEP	211-225	9	SP_76	TFKCYCVSPKTLNDL	376-390	9	SP_109	FNPNGLTCTGVLTES	541-555	9	SP_148	VQCTWYVCTDSTES	736-750	9	SP_186	QFNSATCKIQDSL55	926-940	9	SP_224	TDTNFVSGACQDVNI	1116-1130	9	NP_50	VTKSAEASAKPKRG	246-260
10	SP_61	CTLKSFYVEKGIYQT	301-315	10	SP_10	SVLHSTQDLFLPFFS	46-60	10	SP_44	LPGQSFALEPVLDP	216-230	10	SP_77	CVSPKTLNDLCTFNV	381-395	10	SP_110	LTCTVLTESNKKFL	546-560	10	SP_149	YICDSTECNSLLQ	741-755	10	SP_187	ICKIQDSL55SATSL	931-945	10	SP_225	VFSGNCQDVICGVNI	1121-1135	10	NP_51	AAEASCKPRGKRTAT	251-265
11	SP_62	FTVEKGIYQTYNFRV	306-320	11	SP_11	TQDLFLPFFSNFTV	51-65	11	SP_45	SALEPVLDPICGNI	221-235	11	SP_78	KLNDLCTNNVADSF	386-400	11	SP_111	VLTESNKKFLPFQGF	551-565	11	SP_150	STECNSLLQYSCFC	746-760	11	SP_188	DSL55SATSLGKLQD	936-950	11	NP_52	KKPRKRTATKAYNV	256-270				
12	SP_63	GIYQTSNFRVQPTFS	311-325	12	SP_12	LPFFSNMTWFHAIH	56-70	12	SP_46	LVDLPICGINTTRFQT	226-240	12	SP_79	CFNNVADSFVIRGD	391-405	12	SP_112	NKKFLPFQGFQDZTA	556-570	12	SP_151	NLLQYSCFQTLNR	751-765	12	SP_189	TASALCKLQDVVQNN	941-955	12	NP_53	KRTATKAYNVQAFQ	261-275				
13	SP_75	SASF5PFCBQYVPT	371-385	13	SP_13	NWTFHAIHVSCTNC	61-75	13	SP_47	ICGINTTRFQTLALH	231-245	13	SP_80	VADSFVIRGDZARQI	396-410	13	SP_113	PFQGFQDZATDTTA	561-575	13	SP_152	YSCFQTLNRALTCI	756-770	13	SP_190	KLQDVLVQNNQALN	946-960	13	NP_54	KAYNVQAFQGRCPQ	266-280				
14	SP_76	TFKCYCVSPKTLNDL	376-390	14	SP_14	HAHVSCTNCKTKRFD	66-80	14	SP_48	TRFQTLTLLAHLRSYLT	236-250	14	SP_81	VIRGDZARQIAPQTI	401-415	14	SP_114	CRQIADDTADVDRPD	566-580	14	SP_153	TLQNLRAKLTCAVEDQ	761-775	14	SP_191	VVQNNQALNLTWKQ	951-965	14	NP_55	TOAFGRGRCFQGTQA	271-285				
15	SP_85	YNNKLPODFTCGVIA	421-435	15	SP_15	SGTNCNKRFPNPLVF	71-85	15	SP_49	LLAHLRSYLTPODSS	241-255	15	SP_82	EVIRQIAPQTIQKAD	406-420	15	SP_115	DTTADVADPQTLLEL	571-585	15	SP_154	ALTCLGAEVQKQDTE	766-780	15	SP_192	AQALNTLVKQLSSNF	956-970	15	NP_56	RRGFQTCQNFQDQE	276-290				
16	SP_86	WNSNLDKSGVGNVY	436-450	16	SP_16	TKRFNPNPLVPNDQV	76-90	16	SP_50	RSYLTPODSSSQWTA	246-260	16	SP_83	APQTIQKADIYNNKL	411-425	16	SP_116	VRDPQTLLEIDTTPC	576-590	16	SP_155	AVEQKQDTEVFAQV	771-785	16	SP_193	TLVKQLSSNFSAISS	961-975	16	NP_57	QTCQNFQDQELLRCQ	281-295				
17	SP_89	LDSKVGQNNVLYRL	441-455	17	SP_17	NPLVLPNDQVYFAST	81-95	17	SP_51	PCDSSSQWTAAGAY	251-265	17	SP_84	GKIADIYNNKLPODFT	416-430	17	SP_117	LTLELIDTIPCSFGGV	581-595	17	SP_156	KNQTEVFAQVQKIVY	776-790	17	SP_194	LSNFSAISSVSLNDI	966-980	17	NP_58	FQDQELIRQGTIDYKH	286-300				
18	SP_90	GQNNVLYRLFRKSN	446-460	18	SP_18	FNQGVYFASTKSNL	86-100	18	SP_52	SGWTAAGAYNNVYL	256-270	18	SP_85	YNNKLPODFTCGVIA	421-435	18	SP_118	DLTPCFSFGVSYVTP	586-600	18	SP_157	VFAPQVQKIVKTPPIK	781-795	18	SP_195	GAISSVSLNDLSLQD	971-985	18	NP_59	LIRQGTIDYHWFQDIA	291-305				
19	SP_91	YLYRLFRKSNLKIFQ	451-465	19	SP_19	YFASTKSNLIRQWT	91-105	19	SP_53	CAANNVYCVLPQRTI	261-275	19	SP_86	PODFTCGVIAWNSN	426-440	19	SP_119	SFGGVSYVTPCTNFS	591-605	19	SP_158	QKIVKTPPIKDFQDGF	786-800	19	SP_196	VLNDTLSRDKVFAE	976-990	19	NP_60	TDYHWFQDIAAPS	296-310				
20	SP_92	FRKSNLKFPERDLSI	456-470	20	SP_20	EKSNIRQWLGFTCLL	96-110	20	SP_54	YCVGLQPTFLTKLYN	266-280	20	SP_87	GCVIAMWNSNLDKVC	431-445	20	SP_120	SVLTPCTNITSNQAV	596-610	20	SP_159	TPPIKDFGCFNFSQI	791-805	20	SP_197	LSRLDKVFAEQVQDR	981-995	20	NP_61	WQIQAQAPASAFSAF	301-315				
21	SP_93	IKLKFPERDLSIYQA	461-475	21	SP_21	IKRWGITLIDSKTQ	101-115	21	SP_55	QPTFLTKYENMGITD	271-285	21	SP_88	WNSNLDKSGVGNVY	436-450	21	SP_121	CTNITSNQAVLVNCTV	601-615	21	SP_160	DFGCFNFSQILPQPS	796-810	21	SP_198	VQAEIRLITRQLRSLQ	986-1000	21	NP_62	QFAPASAFQMSRIT	306-320				
22	SP_106	GPKKSTNLVKNNVCN	526-540	22	SP_22	FGITLDSKTQSLTIV	106-120	22	SP_56	LLKYNENMGITDAVD	276-290	22	SP_89	LDSKVGQNNVLYRL	441-455	22	SP_122	NQAVLVQDVNCTVE	606-620	22	SP_161	NFSQILPDPSPKPSKR	801-815	22	SP_199	VQIDRLITRQLQSLQ	991-1005	22	NP_63	ASAFQMSRITQMEVT	311-325				
23	SP_107	TNLVKNKCVNMFNG	531-545	23	SP_23	DSKTQSLTIVNNATN	111-125	23	SP_57	ENGTITDAVDCAIDP	281-295	23	SP_90	GQNNVLYRLFRKSN	446-460	23	SP_123	LYQDVNCTVEVPAIH	611-625	23	SP_162	LPDPSKPSKRSFIED	806-820	23	SP_200	LITRQLQSLQTYTQ	1096-1100	23	NP_64	QMSRITQMEVTSQTM	316-330				
24	SP_109	FNPNGLTCTGVLTES	541-555	24	SP_24	SLLTVNATVNNATN	116-130	24	SP_58	TDAVDCAIDPLSETH	286-300	24	SP_91	YLYRLFRKSNLKIFQE	451-465	24	SP_124	NCTVEVPAIHQDLT	616-630	24	SP_163	KPSKRSFIEDLFK	811-825	24	SP_201	LOSQTYTQTLIRA	1001-1015	24	NP_65	QMEVTSQTMITYTC	321-335				
25	SP_110	LTGLTGVLTESNKKFL	546-560	25	SP_25	NATNWNKVCYFQF	121-135	25	SP_59	CALDPLSETKCTLKS	291-305	25	SP_92	FRKSNLKFPERDLSI	456-470	25	SP_125	VPVIAHQDLTPMWR	621-635	25	SP_164	SFIEDLFLFNKYLTV	816-830	25	SP_202	TYVQDLTIRAAEIRA	1006-1020	25	NP_66	PCSTNLTYTCAIKLKL	326-340				
26	SP_130	RAGCLIGAEHVNNSY	646-660	26	SP_26	VVIXKVECFQCNDFP	126-140	26	SP_60	LESTKCTLKSFYVEK	206-310	26	SP_93	IKLKFPERDLSIYQA	461-475	26	SP_126	ADQTLPTMWRVYSTCS	626-640	26	SP_165	LLKNAVTLADAGFK	821-835	26	SP_203	QLIRAAEIRASANLA	1011-1025	26	NP_67	LYTTCAGLKLDKDPN	331-345				
27	SP_131	IGAEHVNNSYECDDP	651-665	27	SP_27	CEFGQCNDFLGVVY	131-145	27	SP_61	CTLKSFYVEKGIYQT	301-315	27	SP_94	RDISTEYQAGCTPC	466-480	27	SP_127	PTMWRVYSTCSMVFQ	631-645	27	SP_166	VTADAGFKIYQYQDC	826-840	27	SP_204	AEIRAAEIRASATKS	1016-1030	27	NP_68	ATLKDOKDPNPDQV	336-350				
28	SP_138	SVASQSIAYTMSLG	686-700	28	SP_28	CNDPFLGYVYHKNK	136-150	28	SP_62	FTVEKGIYQTYNFRV	306-320	28	SP_95	EIYQAGCTPCSGVFC	471-485	28	SP_128	YSTCSMVFQTRAGCL	636-650	28	SP_167	AGFKIYQYQDCLQDIA	831-845	28	SP_205	SANLAATKMSKCVLG	1021-1035	28	NP_69	DKDPNFKDQVILLNK	341-355				
29	SP_139	SIAATMSLGAENV	691-705	29	SP_29	LCQYHKNKSNWSES	141-155	29	SP_63	GIYQTSNFRVQPTFS	311-325	29	SP_96	GSTQACNPGVGFNCF	476-490	29	SP_129	NVFTIRAGCLIGAEH	641-655	29	SP_168	IQDCLQDCLQAROLI	836-850	29	SP_206	ATMKSQKLVQSKRV	1026-1040	29	NP_70	FNQVILLNKHIDFA	346-360				
30	SP_140	THSLGAEANSVAYSNW	696-710	30	SP_30	HKNKSNWSEFFRYR	146-160	30	SP_64	SNFRVQPTFSIRFPI	316-330	30	SP_97	NGVGFNCFNFPFLQSY	481-495	30	SP_130	RAGCLIGAEHVNNSY	646-660	30	SP_169	LQDIAAROLICAKFI	841-855	30	SP_207	ECLVQSKRVDFQCA	1031-1045	30	NP_71	ILLNKHIDFAETPTFP	351-365				
31	SP_150	STECNSLLQYSCFC	746-760	31	SP_31	SNMSEFRVSYSAANN	151-165	31	SP_65	OPTESIVRPNFTNL	321-335	31	SP_98	FNCFYVPLQSYCFQPT	486-500	31	SP_131	IGAEHVNNSYECDDP	651-665	31															