

ARTICLE

Design of a multi-epitope vaccine against SARS-CoV-2: immunoinformatic and computational methods

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***Corresponding Author**

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SUPPLEMENTARY MATERIALS

Supplementary Materials SM 1: Primary sequence of the final vaccine construct

MTPQNITDLCAEYHNTQIHTLNDKIFSYTESLAGKREMAIITFKNGATFQVEVPGSQHIDSQKKAIER
MKDTLRIAYLTEAKVEKLCVWNNKTPHAIAAISMAN**EAAAK**WTAGAAAY**AA**FTISVTTEI**AA**RLD
PPEAEV**AA**VVFLHVTVV**AA**LLMGCVAET**AA**PYRVVLSF**AA**IAIPTNFTI**AA**FVFLVLLPL**AA**WT
FGAGAAL**AA**IPNFTISV**AA**LPFNDGVYF**AA**HVSGTNGTK**AA**TLADAGFIK**AA**GVIYFASTEK**GPG**
PGFQTLALHRSYLT**PG**QSIAYTMSLGAENS**GPGPG**SIIAYTMSLGAENSV**GPGPG**IAYTMSLGAEN
SVAY**GPGPG**NFRVQPTESIVRFPN**GPGPG**IIAYTMSLGAENSV**GPGPG**VVLSFELLHAPATVC**GPGPG**QIPF
AMQMAYRFNGI**GPGPG**SQSIAYTMSLGAEN**GPGPG**IRAAEIRASANLAAT**GPGPG**RAAEIRASANLAATK

Bold Text = Adjuvant

EAAAK, **AA**, **GPGPG** = Linkers

9 mer epitopes = CTL epitopes

15 mer epitopes = HTL and IFN- γ inducing epitopes

Supplementary Materials SM 2: Ramachandran plot statistics of the vaccine structure

Plot statistics

Residues in most favoured regions [A, B, L]	347	86.1%
Residues in additional allowed regions [a,b,l,p]	46	11.4%
Residues in generously allowed regions [\sim a, \sim b, \sim l, \sim p]	4	1.0%
Residues in disallowed regions	6	1.5%
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Number of non-glycine and non-proline residues	403	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	52	
Number of proline residues	37	

Total number of residues	494	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions

Supplementary material SM 3: Physico-chemical properties of the final multi-epitope vaccine construct

Number of amino acids: 494

Molecular weight: 51520.82

Theoretical pI: 6.46

Amino acid composition:

Ala (A)	82	16.6%
Arg (R)	13	2.6%
Asn (N)	21	4.3%
Asp (D)	7	1.4%
Cys (C)	4	0.8%
Gln (Q)	11	2.2%
Glu (E)	22	4.5%
Gly (G)	52	10.5%
His (H)	8	1.6%
Ile (I)	33	6.7%
Leu (L)	32	6.5%
Lys (K)	14	2.8%
Met (M)	12	2.4%
Phe (F)	21	4.3%
Pro (P)	37	7.5%
Ser (S)	28	5.7%
Thr (T)	36	7.3%
Trp (W)	3	0.6%

Tyr (Y) 30 6.1%

Val (V) 28 5.7%

Pyl (O) 0 0.0%

Sec (U) 0 0.0%

(B) 0 0.0%

(Z) 0 0.0%

(X) 0 0.0%

Total number of negatively charged residues (Asp + Glu): 29

Total number of positively charged residues (Arg + Lys): 27

Atomic composition:

Carbon C 2344

Hydrogen H 3586

Nitrogen N 598

Oxygen O 679

Sulfur S 16

Formula: C2344H3586N598O679S16

Total number of atoms: 7223

Extinction coefficients:

Extinction coefficients are in units of M⁻¹ cm⁻¹, at 280 nm measured in water.

Ext. coefficient 61450

Abs 0.1% (=1 g/l) 1.193, assuming all pairs of Cys residues form cystines

Ext. coefficient 61200

Abs 0.1% (=1 g/l) 1.188, assuming all Cys residues are reduced

Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).

>20 hours (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 21.64

This classifies the protein as stable.

Aliphatic index: 84.35

Grand average of hydropathicity (GRAVY): 0.213

SUPPLEMENTARY TABLES

Supplementary Table S1: Antigenic and Physiochemical properties of the target spike glycoprotein. The parameters were predicted by ExPASy Proparam tool and VaxiJen v2.0 server.

PDB ID	Antigenicity (Threshold, 0.4)	Molecular Weight	Instability Index	Aliphatic Index	Theoretical pI	No. of Amino acids	Extinction Co-Efficient	Estimated half-life:	Gravy
6VSB	0.4512	142274.61	31.58	81.58	6.14	1288	146845	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	-0.163
6VXX	0.4587	141410.94	31.26	83.32	6.09	1281	138825	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	-0.139
6X2A	0.4559	140666.66	32.08	81.47	6.09	1273	148210	100 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	-0.181
6XR8	0.4612	144793.24	33.38	82.73	6.22	1310	159960	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	-0.103
6ZB4	0.4517	139612.06	31.29	83.61	6.45	1259	142835	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	-0.145
6ZGE	0.4587	142150.01	32.63	84.51	6.04	1287	137335	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	-0.110

7KDH	0.4501	142307.69	31.17	81.80	6.19	1288	146845	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).	-0.160
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Supplementary Table S2: Predicted CTL epitopes. The epitopes were predicted by NetCTL 1.2 server.

PDB ID	Residue number	Epitope	Supertypes	Combined Score	Antigenicity	Allergenicity	Immunogenicity
6VXX	884	LTDEMIAQY	A1	3.6616	0.1043	No	0.02
6VXX	277	WTAGAAAYY	A1	3.1128	0.6306	No	0.15259
6VXX	765	STECNLLL	A1	2.3492	0.4871	No	-0.20478
6VXX	179	YSSANNCTF	A1	1.9531	-0.1036	No	-0.04954
6VXX	181	SANNCTFEY	A1	1.8739	-0.0924	No	0.13273
6VXX	280	GAAAYYVGY	A1	1.2194	0.6604	No	0.09963
6VXX	376	RISNCVADY	A1	1.2032	-0.5375	No	-0.02787
6VXX	834	RSFIEDLLF	A1	0.8441	-0.5782	No	0.27446
6VXX	391	ASFSTFKCY	A1	0.8085	0.2795	No	-0.19397
6VXX	1231	GSGRENLYF	A1	0.7939	1.0034	No	0.14297
6VXX	647	QLTPTWRVY	A1	0.7887	1.2119	No	0.31555
6VXX	102	VLPFNDGVY	A1	0.7675	0.4642	No	0.1815
6VXX	1114	FVSNGTHWF	A1	0.7622	0.0807	No	0.16605
6VXX	995	VLNDILSRL	A2	1.354	-0.8524	No	0.03
6VXX	1019	RLQSLQTYV	A2	1.2727	-0.2167	No	-0.29331
6VXX	11	ALLSLVSLI	A2	1.247	0.6491	No	-0.28811
6VXX	737	FTISVTTEI	A2	1.1808	0.8535	No	0.04473

6VXX	128	TLDSKTQSL	A2	1.0777	1.0685	No	-0.52715
6VXX	22	LLMGCVAET	A2	1.0322	0.4298	No	0.05498
6VXX	1079	VVFLHVTVV	A2	1.0304	1.5122	No	0.1278
6VXX	840	LLFNKVTLA	A2	1.0091	0.615	No	-0.11337
6VXX	1067	HLMSFPQSA	A2	0.9793	-0.0681	No	-0.31433
6VXX	901	ITSGWTFGA	A2	0.9541	0.4577	No	0.35124
6VXX	1002	RLDPPEAEV	A2	0.9436	0.4496	No	0.17101
6VXX	221	KIYSKHTPI	A2	0.9329	0.7455	No	-0.32094
6VXX	876	GLTVLPPLL	A2	0.9214	0.6621	No	0.01706
6VXX	81	VTWFHAIHV	A2	0.9144	0.5426	No	0.38925
6VXX	934	VLYENQKLI	A2	0.9028	0.4361	No	-0.20427
6VXX	805	KQIYKTPPI	A2	0.8755	0.2705	No	-0.14982
6VXX	531	VLSFELLHA	A2	0.8218	1.0776	No	0.1607
6VXX	524	YQPYRVVVL	A2	0.8143	0.5964	No	0.1409
6VXX	307	AVDCALDPL	A2	0.8102	0.6604	No	-0.00715
6VXX	413	NVYADSFVI	A2	0.7994	-0.5617	No	0.02696
6VXX	13	LSLVSLLSV	A2	0.7702	1.1681	No	-0.23962
6VXX	753	TSVDCTMYI	A2	0.7648	0.7328	No	-0.1306
6VXX	977	ALNTLVKQL	A2	0.7518	-0.5716	No	-0.18466
6VXX	473	RLFRKSNLK	A3	1.7563	-0.2829	No	-0.28759
6VXX	108	GVYFASTEK	A3	1.4615	0.7112	No	0.09023
6VXX	60	KVFRSSVLH	A3	1.3419	-0.6913	No	-0.19839
6VXX	846	TLADAGFIK	A3	1.2451	0.5781	No	0.28158
6VXX	376	RISNCVADY	A3	1.1734	-0.5375	No	-0.02787
6VXX	88	HVSGTNGTK	A3	1.1575	1.0956	No	0.06339
6VXX	776	GSFCTQLNR	A3	1.0783	0.9306	No	-0.10063
6VXX	289	LQPRTFLLK	A3	1.0511	0.0371	No	0.18064
6VXX	428	QIAPGQTGK	A3	1.0483	1.8297	No	-0.02194
6VXX	975	AQALNTLVK	A3	1.0252	-0.2279	No	0.04654

6VXX	569	GVLTESNKK	A3	0.9827	0.8797	No	-0.15423
6VXX	548	KSTNLVKNK	A3	0.9809	0.8596	No	-0.15163
6VXX	477	KSNLKPFER	A3	0.9792	0.949	No	-0.0764
6VXX	994	SVLNDILSR	A3	0.9477	-0.7223	No	0.03075
6VXX	391	ASFSTFKCY	A3	0.8899	0.2795	No	-0.19397
6VXX	1266	VLLSTFLGH	A3	0.8461	-0.2415	No	-0.01163
6VXX	181	SANNCTFEY	A3	0.8377	-0.0924	No	0.13273
6VXX	206	KNLREFVFK	A3	0.7504	-0.5146	No	0.35942
PDB ID	Residue number	Epitope	Supertypes	Combined Score	Antigenicity	Allergenicity	Immunogenicity
6VSB	865	LTDEMIAQY	A1	3.6616	0.1043	NO	0.02757
6VSB	258	WTAGAAAYY	A1	3.1128	0.6306	NO	0.15259
6VSB	746	STECNLLL	A1	2.3492	0.4871	NO	-0.20478
6VSB	160	YSSANNCTF	A1	1.9531	-0.1036	NO	-0.04954
6VSB	162	SANNCTFEY	A1	1.8739	-0.0924	NO	0.13273
6VSB	261	GAAAYYVGY	A1	1.2194	0.6604	NO	0.09963
6VSB	357	RISNCVADY	A1	1.2032	-0.5375	NO	-0.02787
6VSB	815	RSFIEDLLF	A1	0.8441	-0.5782	NO	0.27446
6VSB	372	ASFSTFKCY	A1	0.8085	0.2795	NO	-0.19397
6VSB	628	QLTPTWRVY	A1	0.7887	1.2119	NO	0.31555
6VSB	83	VLPFNDGVY	A1	0.7675	0.4642	NO	0.1815
6VSB	1095	FVSNNGTHWF	A1	0.7622	0.0807	NO	0.16605
6VSB	976	VLNDILSRL	A2	1.354	-0.8524	NO	0.03
6VSB	1000	RLQSLQTYV	A2	1.2727	-0.2167	NO	-0.29331
6VSB	2	FVFLVLLPL	A2	1.1947	0.8601	NO	0.04076
6VSB	718	FTISVTTEI	A2	1.1808	0.8535	NO	0.04473
6VSB	1236	FLGRSLEVL	A2	1.1019	0.6113	NO	0.00016
6VSB	109	TLDSKTQSL	A2	1.0777	1.0685	NO	-0.52715
6VSB	1060	VVFLHVITYV	A2	1.0304	1.5122	NO	0.1278
6VSB	821	LLFNKVTLA	A2	1.0091	0.6150	NO	-0.11337
6VSB	1048	HLMSFPQSA	A2	0.9793	-0.0681	NO	-0.31433
6VSB	882	ITSGWTFGA	A2	0.9541	0.4577	NO	0.35124
6VSB	983	RLDPPEAEV	A2	0.9436	0.4496	NO	0.17101

6VSB	202	KIYSKHTPI	A2	0.9329	0.7455	NO	-0.32094
6VSB	857	GLTVLPPLL	A2	0.9214	0.6621	NO	0.01706
6VSB	62	VTWFHAIHV	A2	0.9144	0.5426	NO	0.38925
6VSB	915	VLYENQKLI	A2	0.9028	0.4361	NO	-0.20427
6VSB	786	KQIYKTPPI	A2	0.8755	0.2705	NO	-0.14982
6VSB	512	VLSFELLHA	A2	0.8218	1.0776	NO	0.1607
6VSB	505	YQPYRVVVL	A2	0.8143	0.5964	NO	0.1409
6VSB	288	AVDCALDPL	A2	0.8102	0.6604	NO	-0.00715
6VSB	394	NVYADSFVI	A2	0.7994	-0.5617	NO	0.02696
6VSB	734	TSVDCTMYI	A2	0.7648	0.7328	NO	-0.1306
6VSB	958	ALNTLVKQL	A2	0.7518	-0.5716	NO	-0.18466
6VSB	454	RLFRKSNLK	A3	1.7563	-0.2829	NO	-0.28759
6VSB	89	GVYFASTEK	A3	1.4615	0.7112	NO	0.09023
6VSB	41	KVFRSSVLH	A3	1.3419	-0.6913	NO	-0.19839
6VSB	1231	VLLSTFLGR	A3	1.2481	0.1933	NO	-0.01163
6VSB	827	TLADAGFIK	A3	1.2451	0.5781	NO	0.28158
6VSB	357	RISNCVADY	A3	1.1734	-0.5375	NO	-0.02787
6VSB	69	HVSGTNGTK	A3	1.1575	1.0956	NO	0.06339
6VSB	757	GSFCTQLNR	A3	1.0783	0.9306	NO	-0.10063
6VSB	270	LQPRTFLK	A3	1.0511	0.0371	NO	0.18064
6VSB	409	QIAPGQTGK	A3	1.0483	1.8297	NO	-0.02194
6VSB	956	AQALNTLVK	A3	1.0252	-0.2279	NO	0.04654
6VSB	550	GVLTESNKK	A3	0.9827	0.8797	NO	-0.15423
6VSB	529	KSTNLVKNK	A3	0.9809	0.8596	NO	-0.15163
6VSB	458	KSNLKPFER	A3	0.9792	-0.6565	NO	-0.0764
6VSB	975	SVLNDILSR	A3	0.9477	-0.7223	NO	0.03075
6VSB	372	ASFSTFKCY	A3	0.8899	0.2795	NO	-0.19397
6VSB	162	SANNCTFEY	A3	0.8377	-0.0924	NO	0.13273
6VSB	187	KNLREFVFK	A3	0.7504	-0.5146	NO	0.35942
PDB ID	Residue number	Epitope	Supertypes	Combined Score	Antigenicity	Allergenicity	Immunogenicity
6X2A	850	LTDEMIAQY	A1	3.6616	0.1043	NO	0.02757
6X2A	243	WTAGAAAYY	A1	3.1128	0.6306	NO	0.15259
6X2A	731	STECSNLLL	A1	2.3492	0.4871	NO	-0.20478

6X2A	145	YSSANNCTF	A1	1.9531	-0.1036	NO	-0.04954
6X2A	147	SANNCTFEY	A1	1.8739	-0.0924	NO	0.13273
6X2A	246	GAAAYYVGY	A1	1.2194	0.6604	NO	0.09963
6X2A	342	RISNCVADY	A1	1.2032	-0.5375	NO	-0.02787
6X2A	800	RSFIEDLLF	A1	0.8441	-0.5782	NO	0.27446
6X2A	357	ASFSTFKCY	A1	0.8085	0.2795	NO	-0.19397
6X2A	613	QLTPTWRVY	A1	0.7887	1.2119	NO	0.31555
6X2A	68	VLPFNDGVY	A1	0.7675	0.4642	NO	0.1815
6X2A	1080	FVSNGTHWF	A1	0.7622	0.0807	NO	0.16605
6X2A	961	VLNDILSRL	A2	1.354	-0.8524	NO	0.03
6X2A	985	RLQSLQTYV	A2	1.2727	-0.2167	NO	-0.29331
6X2A	703	FTISVTTEI	A2	1.1808	0.8535	NO	0.04473
6X2A	1221	FLGRSLEVL	A2	1.1019	0.6113	NO	0.00016
6X2A	94	TLDSKTQSL	A2	1.0777	1.0685	NO	-0.52715
6X2A	1045	VVFLHVITYV	A2	1.0304	1.5122	NO	0.1278
6X2A	806	LLFNKVTLA	A2	1.0091	0.615	NO	-0.11337
6X2A	1033	HLMSFPQSA	A2	0.9793	-0.0681	NO	-0.31433
6X2A	867	ITSGWTFGA	A2	0.9541	0.4577	NO	0.35124
6X2A	968	RLDPPEAEV	A2	0.9436	0.4496	NO	0.17101
6X2A	187	KIYSKHTPI	A2	0.9329	0.7455	NO	-0.32094
6X2A	842	GLTVLPPLL	A2	0.9148	0.6621	NO	0.01706
6X2A	47	VTWFHAIHV	A2	0.9144	0.5426	NO	0.38925
6X2A	900	VLYENQKLI	A2	0.9028	0.4361	NO	-0.20427
6X2A	771	KQIYKTPPI	A2	0.8755	0.2705	NO	-0.14982
6X2A	497	VLSFELLHA	A2	0.8218	1.0776	NO	0.1607
6X2A	490	YQPYRVVVL	A2	0.8143	0.5964	NO	0.1409
6X2A	273	AVDCALDPL	A2	0.8102	0.6604	NO	-0.00715
6X2A	379	NVYADSFVI	A2	0.7994	-0.5617	NO	0.02696
6X2A	719	TSVDCTMYI	A2	0.7648	0.7328	NO	-0.1306
6X2A	943	ALNTLVKQL	A2	0.7518	-0.5716	NO	-0.18466
6X2A	492	PYRVVLSF	A24	1.8786	1.0281	NO	0.03138
6X2A	154	EYVSQPFLM	A24	1.7025	0.2605	NO	-0.18399
6X2A	297	IYQTSNFRV	A24	1.6294	0.3109	NO	-0.03669

6X2A	152	TFEYVSQPF	A24	1.6168	0.6641	NO	-0.19099
6X2A	1214	EWVLLSTFL	A24	1.3414	0.4215	NO	-0.06313
6X2A	435	NYLYRLFRK	A24	1.3274	-0.8611	NO	0.16168
6X2A	129	YYHKNNKSW	A24	1.2214	0.4536	NO	-0.49755
6X2A	1122	VYDPLQPEL	A24	1.1735	0.4525	NO	-0.07466
6X2A	697	IAIPTNFTI	A24	1.0788	0.7052	NO	0.18523
6X2A	63	RFDNPVLPF	A24	1.0633	0.1828	NO	0.01291
6X2A	740	QYGSFCTQL	A24	1.0275	1.2906	NO	-0.12714
6X2A	1086	HWFVTQRNF	A24	0.9755	0.7460	NO	0.0482
6X2A	490	YQPYRVVVL	A24	0.9003	0.5964	NO	0.1409
6X2A	22	YYPDKVFRS	A24	0.8245	-0.3237	NO	-0.02338
6X2A	145	YSSANNCTF	A24	0.8021	-0.1036	NO	-0.04954
PDB ID	Residue number	Epitope	Supertypes	Combined Score	Antigenicity	Allergenicity	Immunogenicity
6XR8	865	LTDEMIAQY	A1	3.6616	0.1043	NO	0.02757
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6XR8	746	STECSNLLL	A1	2.3492	0.4871	NO	-0.20478
6XR8	160	YSSANNCTF	A1	1.9531	-0.1036	NO	-0.04954
6XR8	162	SANNCTFEY	A1	1.8739	-0.0924	NO	0.13273
6XR8	261	GAAAYYVGY	A1	1.2194	0.6604	NO	0.09963
6XR8	357	RISNCVADY	A1	1.2032	-0.5375	NO	-0.02787
6XR8	815	RSFIEDLLF	A1	0.8441	-0.5782	NO	0.27446
6XR8	1264	VLKGVKLHY	A1	0.8253	1.2378	NO	-0.18916
6XR8	372	ASFSTFKCY	A1	0.8085	0.2795	NO	-0.19397
6XR8	628	QLTPTWRVY	A1	0.7887	1.2119	NO	0.31555
6XR8	83	VLPFNDGVY	A1	0.7675	0.4642	NO	0.1815
6XR8	1095	FVSNNGTHWF	A1	0.7622	0.0807	NO	0.16605
6XR8	258	WTAGAAAYY	A26	2.0048	0.6306	NO	0.15259
6XR8	1095	FVSNNGTHWF	A26	1.1411	0.0807	NO	0.16605
6XR8	1220	FIAGLIAIV	A26	1.0908	0.3162	NO	0.27206
6XR8	1147	SFKEELDKY	A26	1.0689	-1.3338	NO	0.01053
6XR8	865	LTDEMIAQY	A26	1.0671	0.1043	NO	0.02757
6XR8	554	ESNKKFLPF	A26	1.0271	1.0278	NO	-0.33474
6XR8	2	FVFLVLLPL	A26	1.0076	0.8601	NO	0.04076

6XR8	718	FTISVTTEI	A26	1.0014	0.8535	NO	0.04473
6XR8	886	WTFGAGAAL	A26	0.9302	0.4918	NO	0.19798
6XR8	372	ASFSTFKCY	A26	0.879	0.2795	NO	-0.19397
6XR8	215	DLPQGFSAL	A26	0.862	0.5622	NO	-0.09372
6XR8	603	NTSNQVAVL	A26	0.7916	0.4987	NO	-0.07701
6XR8	1067	YVPAQEKNF	A26	0.7664	0.8731	NO	-0.16856
PDB ID	Residue number	Epitope	Supertypes	Combined Score	Antigenicity	Allergenicity	Immunogenicity
6ZB4	865	LTDEMIAQY	A1	3.6616	0.1043	NO	0.02757
6ZB4	258	WTAGAAAYY	A1	3.1128	0.6306	NO	0.15259
6ZB4	746	STECNLLL	A1	2.3492	0.4871	NO	-0.20478
6ZB4	160	YSSANNCTF	A1	1.9531	-0.1036	NO	-0.04954
6ZB4	162	SANNCTFEY	A1	1.8739	-0.0924	NO	0.13273
6ZB4	261	GAAAYYVGY	A1	1.2194	0.6604	NO	0.09963
6ZB4	357	RISNCVADY	A1	1.2032	-0.5375	NO	-0.02787
6ZB4	815	RSFIEDLLF	A1	0.8441	-0.5782	NO	0.27446
6ZB4	372	ASFSTFKCY	A1	0.8085	0.2795	NO	-0.19397
6ZB4	628	QLTPTWRVY	A1	0.7887	1.2119	NO	0.31555
6ZB4	83	VLPFNDGVY	A1	0.7675	0.4642	NO	0.1815
6ZB4	1095	FVSNGTHWF	A1	0.7622	0.0807	NO	0.16605
6ZB4	714	IPTNFTISV	B7	1.5427	0.882	NO	0.17229
6ZB4	24	LPPAYTNSF	B7	1.5189	0.3775	NO	-0.03341
6ZB4	680	SPRRAASVA	B7	1.4453	0.7227	NO	0.02831
6ZB4	951	VVNQNAQAL	B7	1.1258	0.4749	NO	-0.16303
6ZB4	630	TPTWRVYST	B7	1.0987	0.4605	NO	0.22497
6ZB4	1014	RAAEIRASA	B7	1.0923	0.2752	NO	0.22813
6ZB4	1218	VPRGSPGSG	B7	1.0706	-0.1116	NO	-0.1887
6ZB4	84	LPFNDGVYF	B7	1.0427	0.5593	NO	0.11767
6ZB4	411	APGQTGKIA	B7	0.9845	1.2002	NO	-0.1401

6ZB4	892	AALQIPFAM	B7	0.9235	0.7747	NO	0.12066
6ZB4	1052	FPQSAPHGV	B7	0.8956	-0.0058	NO	-0.12931
6ZB4	462	KPFERDIST	B7	0.8646	-0.4114	NO	0.22569
6ZB4	262	AAAYYVGYL	B7	0.8439	0.4605	NO	0.07068
6ZB4	478	TPCNGVEGF	B7	0.8026	-0.3772	NO	0.15215
6ZB4	250	TPGDSSSGW	B7	0.7709	0.133	NO	-0.40333
6ZB4	811	KPSKRSFIE	B7	0.7689	0.2768	NO	-0.19947
PDB ID	Residue number	Epitope	Supertypes	Combined Score	Antigenicity	Allergenicity	Immunogenicity
6ZGE	896	LTDEMIAQY	A1	3.6616	0.1043	NO	0.02757
6ZGE	289	WTAGAAAYY	A1	3.1128	0.6306	NO	0.15259
6ZGE	777	STECNLLL	A1	2.3492	0.4871	NO	-0.20478
6ZGE	191	YSSANNCTF	A1	1.9531	-0.1036	NO	-0.04954
6ZGE	193	SANNCTFEY	A1	1.8739	-0.0924	NO	0.13273
6ZGE	292	GAAAYYVGY	A1	1.2194	0.6604	NO	0.09963
6ZGE	388	RISNCVADY	A1	1.2032	-0.5375	NO	-0.02787
6ZGE	846	RSFIEDLLF	A1	0.8441	-0.5782	NO	0.27446
6ZGE	403	ASFSTFKCY	A1	0.8085	0.2795	NO	-0.19397
6ZGE	659	QLTPTWRVY	A1	0.7887	1.2119	NO	0.31555
6ZGE	114	VLPFNDGVY	A1	0.7675	0.4642	NO	0.1815
6ZGE	1126	FVSNGTHWF	A1	0.7622	0.0807	NO	0.16605
6ZGE	487	FRKSNLKPF	B8	1.395	0.628	NO	-0.44169
6ZGE	536	YQPYRVVVL	B8	1.1348	0.5964	NO	0.1409
6ZGE	233	KIYSKHTPI	B8	1.124	0.7455	NO	-0.32094
6ZGE	217	FKNLREFVF	B8	1.106	0.0523	NO	0.25431
6ZGE	158	VIKVCEFQF	B8	0.9429	-0.1189	NO	0.04441
6ZGE	264	INTRFQTL	B8	0.8988	0.3934	NO	0.16778
6ZGE	373	FNATRFASV	B8	0.8751	0.5609	NO	0.14872

6ZGE	790	FCTQLNRAL	B8	0.8545	0.5159	NO	-0.05431
6ZGE	33	FVFLVLLPL	B8	0.8252	0.8601	NO	0.04076
6ZGE	482	YLYRLFRKS	B8	0.7972	-0.6217	NO	0.06796
6ZGE	1065	LGQSKRVDF	B8	0.7535	2.0590	NO	-0.31755
PDB ID	Residue number	Epitope	Supertypes	Combined Score	Antigenicity	Allergenicity	Immunogenicity
7KDH	865	LTDEMIAQY	A1	3.6616	0.1043	No	0.02757
7KDH	258	WTAGAAAYY	A1	3.1128	0.6306	No	0.15259
7KDH	746	STECNLLL	A1	2.3492	0.4871	No	-0.20478
7KDH	160	YSSANNCTF	A1	1.9531	-0.1036	No	-0.04954
7KDH	162	SANNCTFEY	A1	1.8739	-0.0924	No	0.13273
7KDH	261	GAAAYYVGY	A1	1.2194	0.6604	No	0.09963
7KDH	357	RISNCVADY	A1	1.2032	-0.5375	No	-0.02787
7KDH	815	RSFIEDLLF	A1	0.8441	-0.5782	No	0.27446
7KDH	372	ASFSTFKCY	A1	0.8085	0.2795	No	-0.19397
7KDH	628	QLTPTWRVY	A1	0.7887	1.2119	No	0.31555
7KDH	83	VLPFNDGVY	A1	0.7675	0.4642	No	0.1815
7KDH	1095	FVSNNGTHWF	A1	0.7622	0.0807	No	0.16605
7KDH	456	FRKSNLKPF	B27	1.3086	0.628	No	-0.44169
7KDH	413	GQTGKIADY	B27	1.0811	1.4019	No	0.00796
7KDH	20	TRTQLPPAY	B27	1.0554	1.2923	No	-0.1117
7KDH	327	VRFPNITNL	B27	1.0199	1.1141	No	0.1748
7KDH	846	ARDLICAQK	B27	0.9787	0.8157	No	0.04023
7KDH	77	KRFDNPVLP	B27	0.9728	0.2834	No	0.07194
7KDH	1204	GKYEQSGGY	B27	0.9663	0.1691	No	-0.10117
7KDH	814	KRSFIEDLL	B27	0.9131	-0.6422	No	0.30019
7KDH	956	AQALNTLVK	B27	0.8298	-0.2279	No	0.04654
7KDH	443	SKVGGNYNY	B27	0.7843	1.1856	No	0.06751
7KDH	557	KKFLPFQQF	B27	0.7794	0.1647	No	-0.0392
7KDH	1106	QRNFYEPQI	B27	0.7763	0.3296	No	0.12931
7KDH	764	NRALTGIAV	B27	0.7752	0.5302	No	0.20642

Supplementary Table S3: Predicted HTL epitopes. The epitopes were predicted by NetMHC 2 pan 3.2 server.

Epitope	PDB ID	Position	Antigenicity	Allergenicity
FQTLALHRSYLTPG	6VSB	238	0.5789	no
NFRVQPTESIVRFPN	6VSB	317	0.4307	no
NATRFASVYAWNRKR	6VSB	343	0.4017	yes
ASQSIIAYTMSLGAE	6VSB	688	0.5336	yes
SQSIIAYTMSLGAEN	6VSB	689	0.6141	yes
QSIIAYTMSLGAENS	6VSB	690	0.5728	no
IIAYTMSLGAENSV	6VSB	692	0.5426	yes
IAYTMSLGAENSVAY	6VSB	693	0.7072	yes
Epitope	PDB ID	Position	Antigenicity	Allergenicity
INITRFQTLALHRS	6VXX	252	0.411	NO
FQTLALHRSYLTPG	6VXX	257	0.5789	No
VVLSFELLHAPATV	6VXX	529	0.8083	No
VVLSFELLHAPATVC	6VXX	530	0.8618	No
SIIAYTMSLGAENSV	6VXX	710	0.5691	No
IAYTMSLGAENSVAY	6VXX	742	0.7072	No
Epitope	PDB ID	Position	Antigenicity	Allergenicity
NFRVQPTESIVRFPN	6X2A	302	0.4307	No
VVLSFELLHAPATV	6X2A	495	0.8083	No
VVLSFELLHAPATVC	6X2A	496	0.8618	no
Epitope	PDB ID	Position	Antigenicity	Allergenicity
NFRVQPTESIVRFPN	6XR8	317	0.4307	no
NATRFASVYAWNRKR	6XR8	343	0.4062	yes
TRFASVYAWNRKRIS	6XR8	345	0.4963	yes
ASQSIIAYTMSLGAE	6XR8	688	0.5336	yes

SQSIIAYTMSLGAEN	6XR8	689	0.6141	No
QSIIAYTMSLGAENS	6XR8	690	0.5728	no
SIIAYTMSLGAENSV	6XR8	691	0.5691	yes
IIAYTMSLGAENSVA	6XR8	692	0.5426	No
IAYTMSLGAENSVAY	6XR8	693	0.7072	yes
Epitope	PDB ID	Position	Antigenicity	Allergenicity
VVLSFELLHAPATVC	6ZB4	511	0.8618	No
VLSFELLHAPATVCG	6ZB4	512	0.4784	No
QIPFAMQMAYRFNGI	6ZB4	895	0.9573	No
Epitope	PDB ID	Position	Antigenicity	Allergenicity
AYYVGYLQPRTFLLK	6ZGE	295	0.4269	No
ASQSIIAYTMSLGAE	6ZGE	719	0.5336	Yes
SQSIIAYTMSLGAEN	6ZGE	720	0.6141	No
QSIIAYTMSLGAENS	6ZGE	721	0.5728	No
IIAYTMSLGAENSVA	6ZGE	723	0.5426	Yes
Epitope	PDB ID	Position	Antigenicity	Allergenicity
FLPFFSNVTWFHAIH	7KDH	55	0.48	no
FSNVTWFHAIHVSGT	7KDH	59	0.7533	no
SNVTWFHAIHVSGTN	7KDH	60	0.7067	yes
NVTWFHAIHVSGTNG	7KDH	61	0.7869	No
IRAAEIRASANLAAT	7KDH	1013	0.6785	yes
RAAEIRASANLAATK	7KDH	1031	0.5709	no

Supplementary Table S4: IFN- γ epitope prediction for the top HTL epitopes

S.N	Epitope Name	Sequence	Method	Results	Score
1	Epitope-1	FQTLALHRSYLTPG	SVM	Positive	0.17464486
2	Epitope-2	QSIIAYTMSLGAENS	MERCI	Positive	1
3	Epitope-3	SIIAYTMSLGAENSV	MERCI	Positive	1
4	Epitope-4	IAYTMSLGAENSVAY	MERCI	Positive	1
5	Epitope-5	NFRVQPTESIVRFPN	MERCI	Positive	5
6	Epitope-6	IIAYTMSLGAENSV	MERCI	Positive	1
7	Epitope-7	VVLSFELLHAPATVC	SVM	Positive	0.1764
8	Epitope-8	QIPFAMQMAYRFNGI	SVM	Positive	0.2388
9	Epitope-9	SQSIIAYTMSLGAEN	SVM	Positive	0.051
10	Epitope-10	IRAAEIRASANLAAT	SVM	Positive	0.60811745
11	Epitope-11	RAAEIRASANLAATK	SVM	Positive	0.21113838

Supplementary Table S5: Overlapping HTL and CTL epitopes sequences.

Epitope sequences	HLA class I supertypes (Combined score) and alleles (Percentile rank)	HLA class II alleles (Binding score%)
FQTLALHRSYLTPG	A2 (0.7546), A3 (1.0535), B8 (1.4274), B62 (1.3630), HLA-A*02:02 (0.24), HLA-A*80:01 (0.33), HLA-B*08:01(0.4), HLA-B*15:01 (1.4), HLA-A*29:02 (0.63), HLA-A*02:03 (0.64), HLA-B*08:03 (1.54), HLA-A*02:19 (1.12)	DRB1*08:01 (1.40), DRB1*10:01 (0.70), DRB1*11:01 (1.40), DRB1*12:01 (1.40), DRB1*13:01 (1.80), DRB1*14:01 (1.50), DRB1*15:01 (0.40)
QSIHAYTMSLGAENS	A2 (1.3674), A26 (1.1502), B7 (0.7513), B62 (0.9574), HLA-A*25:01 (0.38), HLA-A*68:23 (0.68), HLA-A*02:17 (0.715), HLA-A*02:02 (0.77), HLA-B*39:01 (0.8), HLA-B*54:01 (0.9), HLA-B*08:01 (1.0), HLA-A*26:02 (1.195), HLA-A*26:01 (1.25), HLA-A*02:01 (1.4), HLA-A*02:03 (1.45), HLA-A*32:01 (1.5), HLA-A*69:01 (1.75)	DRB1*01:01 (1.0), DRB1*04:01 (1.0), DRB1*10:01(0.30)
SIHAYTMSLGAENSV	A2 (1.3680), A26 (1.1508), B7 (0.7519), B62 (0.9581), HLA-A*25:01 (0.38), HLA-A*69:01 (0.59), HLA-A*68:23 (0.68), HLA-A*02:17 (0.715), HLA-A*02:02 (0.77), HLA-B*39:01 (0.8), HLA-B*54:01 (0.9), HLA-B*08:01 (1.0), HLA-A*26:02 (1.195), HLA-A*26:01 (1.25), HLA-A*02:01 (1.4), HLA-A*02:03 (1.45), HLA-A*32:01 (1.5), HLA-A*68:02 (1.5), HLA-B*57:01 (1.7), HLA-A*69:01 (1.75),	DRB1*01:01 (0.50), DRB1*04:01 (0.70), DRB1*07:01 (1.60), DRB1*10:01 (0.12)
IAYTMSLGAENSVAY	B62 (1.2841), HLA-B*35:01 (0.3), HLA-A*69:01 (0.59), HLA-A*68:23 (0.68), HLA-B*15:01 (0.8), HLA-B*54:01 (0.9), HLA-A*30:02 (1.305), HLA-A*68:02 (1.5), HLA-B*57:01 (1.7), HLA-A*02:16 (1.8), HLA-A*02:50 (1.9)	DRB1*01:01 (0.701), DRB1*04:01 (1.30), DRB1*10:01 (0.17)
NFRVQPTESIVRFPN	B7 (0.9927), B27(0.8540), HLA-B*58:02 (0.38), HLA-C*06:02 (0.58), HLA-B*18:01 (0.6), HLA-B*53:01 (0.7), HLA-B*35:03 (0.805), HLA-B*39:01 (1.06), HLA-B*08:03 (1.245), HLA-B*15:03 (1.4), HLA-B*27:05 (1.5), HLA-B*83:01 (1.505), HLA-B*35:01 (1.7), HLA-B*40:01 (1.8)	DRB1*01:01 (1.90), DRB1*04:01 (0.90)
IIAYTMSLGAENSVVA	HLA-A*69:01 (0.59), HLA-A*68:23 (0.68), HLA-B*54:01 (0.9), HLA-A*68:02 (1.5), HLA-B*57:01 (1.7), HLA-A*02:16 (1.8), HLA-A*02:50 (1.9)	DRB1*01:01 0.40), DRB1*04:01 (0.6), DRB1*07:01 (2), DRB1*10:01 (0.80)
VVLSFELLHAPATVC	A2 (0.7895), HLA-B*40:02 (0.18), HLA-A*69:01 (0.225), HLA-A*02:19 (0.25), HLA-A*80:01 (0.37), HLA-B*15:42 (0.4), HLA-A*02:16 (0.535), HLA-A*02:03 (0.825), HLA-B*45:01 (1.105), HLA-B*18:01 (1.205), HLA-A*02:11 (1.345), HLA-A*29:02 (1.495), HLA-B*14:02 (1.555)	DRB1*01:01 (0.40), DRB1*04:01 (0.6), DRB1*10:01 (0.50)
QIPFAMQMAYRFNGI	A2(0.7998), B8(0.7829), B58(1.6030), B62 (0.9065), HLA-B*40:02 (0.18), HLA-A*69:01(0.225), HLA-A*02:19 (0.25), HLA-A*80:01 (0.37), HLA-B*15:42 (0.4), HLA-A*02:16 (0.535), HLA-A*02:03 (0.825), HLA-B*45:01 (1.105), HLA-B*18:01 (1.205), HLA-A*02:11 (1.345), HLA-B*54:01 (1.5), HLA-A*02:12 (1.53), HLA-B*14:02 (1.55)	DRB1*01:01(1.0), DRB1*10:01 (0.90), DRB1*14:01 (0.80), DRB1*15:01 (1.15)

SQSIAYTMSLGAEN	A2 (1.3688), A26(1.151), B7(0.7526), B62(1.0372), HLA-A*25:01 (0.38), HLA-B*40:13 (0.44), HLA-A*32:07 (0.495), HLA-B*39:01 (0.5), HLA-A*68:23 (0.68), HLA-A*02:17 (0.715), HLA-A*02:01 (1.4), HLA-B*15:01 (1.4), HLA-A*26:01 (1.25)	DRB1*01:01 (1.10), DRB1*07:01 (2)
IRAAEIRASANLAAT	B7 (1.0878), B44 (1.8004), HLA-B*40:01 (0.115), HLA-B*44:03 (0.195), HLA-B*44:02 (0.22), HLA-B*40:02 (0.355), HLA-B*07:02 (0.77), HLA-B*14:02 (1.0), HLA-B*45:01 (1.275), HLA-B*73:01 (1.45)	DRB1*04:01 (1.40), DRB1*08:01 (1.20), DRB1*13:02 (1.90)
RAAEIRASANLAATK	A3 (1.2429), B7 (1.0763), B44 (1.8005), HLA-B*40:01 (0.115), HLA-B*44:03 (0.195), HLA-B*44:02 (0.22), HLA-B*40:02 (0.355), HLA-B*07:02 (0.7), HLA-A*03:01 (0.9), HLA-B*45:01 (1.275), HLA-B*73:01 (1.44)	DRB1*04:01 (0.70), DRB1*08:01 (0.90), DRB1*13:02 (1.30)

Supplementary Table S6: Allergenicity and Toxicity profile of selected epitope included in the vaccine construct.

Epitope sequence	Allergenicity		Toxicity
	AllerTOP v.2.0	AllergenFP v.1.0	ToxinPred
WTAGAAAYY	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
FTISVTTEI	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
RLDPPEAEV	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
VVFLHVITYV	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
LLMGCVAET	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
PYRVVLSF	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
IAIPTNFTI	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
FVFLVLLPL	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
WTFGAGAAL	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
IPTNFTISV	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
LPFNDGVYF	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
HVSGTNGTK	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
TLADAGFIK	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
GVYFASTEK	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
FQTLALHRSYLTPG	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
QSIIAYTMSLGAENS	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
SIIAYTMSLGAENSV	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
IAYTMSLGAENSVAY	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
NFRVQPTESIVRFPN	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
IIAYTMSLGAENSVA	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
VVLSFELLHAPATVC	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
QIPFAMQMAYRFNGI	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin

SQIIAYTMSLGAEN	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
IRAAEIRASANLAAT	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin
RAAEIRASANLAATK	NON-ALLERGEN	NON-ALLERGEN	Non-Toxin

Supplementary Table S7: Refined best vaccine structures. The Refinement was performed by using Galaxy refine web server

Model	GDT-HA	RMSD	MolProbity	Clash score	Poor rotamers	Rama favored
Initial	1.0000	0.000	3.318	100.1	1.9	86.4
MODEL 1	0.9393	0.434	2.357	21.6	0.8	90.7
MODEL 2	0.9433	0.439	2.369	21.3	0.8	90.0
MODEL 3	0.9357	0.450	2.379	22.1	0.8	90.2
MODEL 4	0.9388	0.433	2.423	25.0	0.8	90.4
MODEL 5	0.9337	0.441	2.366	21.4	0.6	90.2

Supplementary Table S8: Cluster scores of docked vaccine TLR2 complex

Cluster (Docked complexes)	Representative	Weighted Score
0	Center	-1012.7
	Lowest Energy	-1012.7
1	Center	-775.8
	Lowest Energy	-886.0
2	Center	-872.1
	Lowest Energy	-1031.0
3	Center	-847.5
	Lowest Energy	-889.9
4	Center	-772.9
	Lowest Energy	-898.1
5	Center	-827.4
	Lowest Energy	-867.8
6	Center	-976.1
	Lowest Energy	-976.1
7	Center	-753.4
	Lowest Energy	-1055.1
8	Center	-827.8
	Lowest Energy	-859.6
9	Center	-937.5

Cluster (Docked complexes)	Representative	Weighted Score
	Lowest Energy	-937.5
10	Center	-847.5
	Lowest Energy	-847.5
11	Center	-879.8
	Lowest Energy	-879.8
12	Center	-879.6
	Lowest Energy	-879.6
13	Center	-863.7
	Lowest Energy	-863.7
14	Center	-848.0
	Lowest Energy	-860.2
15	Center	-773.5
	Lowest Energy	-834.3
16	Center	-863.5
	Lowest Energy	-863.5
17	Center	-932.7
	Lowest Energy	-932.7
18	Center	-894.8
	Lowest Energy	-894.8
19	Center	-893.2

Cluster (Docked complexes)	Representative	Weighted Score
	Lowest Energy	-893.2
20	Center	-860.8
	Lowest Energy	-860.8
21	Center	-789.5
	Lowest Energy	-837.3
22	Center	-839.0
	Lowest Energy	-839.0
23	Center	-797.1
	Lowest Energy	-797.1
24	Center	-766.5
	Lowest Energy	-830.7
25	Center	-912.3
	Lowest Energy	-912.3
26	Center	-755.2
	Lowest Energy	-791.4
27	Center	-840.7
	Lowest Energy	-840.7
28	Center	-757.4
	Lowest Energy	-761.4

Supplementary Table S9: Cluster scores of docked vaccine TLR4 complex

Cluster (Docked complexes)	Representative	Weighted Score
0	Center	-921.0
	Lowest Energy	-953.7
1	Center	-897.2
	Lowest Energy	-953.0
2	Center	-831.3
	Lowest Energy	-998.3
3	Center	-902.8
	Lowest Energy	-972.8
4	Center	-855.7
	Lowest Energy	-958.5
5	Center	-993.4
	Lowest Energy	-1010.5
6	Center	-864.3
	Lowest Energy	-906.9
7	Center	-910.9
	Lowest Energy	-910.9
8	Center	-995.4
	Lowest Energy	-995.4
9	Center	-826.5

Cluster (Docked complexes)	Representative	Weighted Score
	Lowest Energy	-919.5
10	Center	-977.7
	Lowest Energy	-997.0
11	Center	-864.9
	Lowest Energy	-880.0
12	Center	-838.8
	Lowest Energy	-870.3
13	Center	-868.7
	Lowest Energy	-985.4
14	Center	-845.0
	Lowest Energy	-1009.4
15	Center	-1048.7
	Lowest Energy	-1066.6
16	Center	-935.5
	Lowest Energy	-962.0
17	Center	-908.0
	Lowest Energy	-908.0
18	Center	-952.1
	Lowest Energy	-1037.5
19	Center	-816.3

Cluster (Docked complexes)	Representative	Weighted Score
	Lowest Energy	-923.8
20	Center	-871.2
	Lowest Energy	-1056.9
21	Center	-1010.1
	Lowest Energy	-1094.1
22	Center	-910.1
	Lowest Energy	-953.1
23	Center	-890.9
	Lowest Energy	-1008.7
24	Center	-865.0
	Lowest Energy	-964.3
25	Center	-825.7
	Lowest Energy	-910.6
26	Center	-841.9
	Lowest Energy	-910.4
27	Center	-1034.3
	Lowest Energy	-1034.3
28	Center	-935.2
	Lowest Energy	-1129.7
29	Center	-898.3

Cluster (Docked complexes)	Representative	Weighted Score
	Lowest Energy	-948.6

Figure S2: Phylogenetic relationship of seven spike glycoproteins of SARS-CoV-2 included in the study. The tree was constructed through the Maximum likelihood method with 1000 bootstrap replicates by using MEGA-X

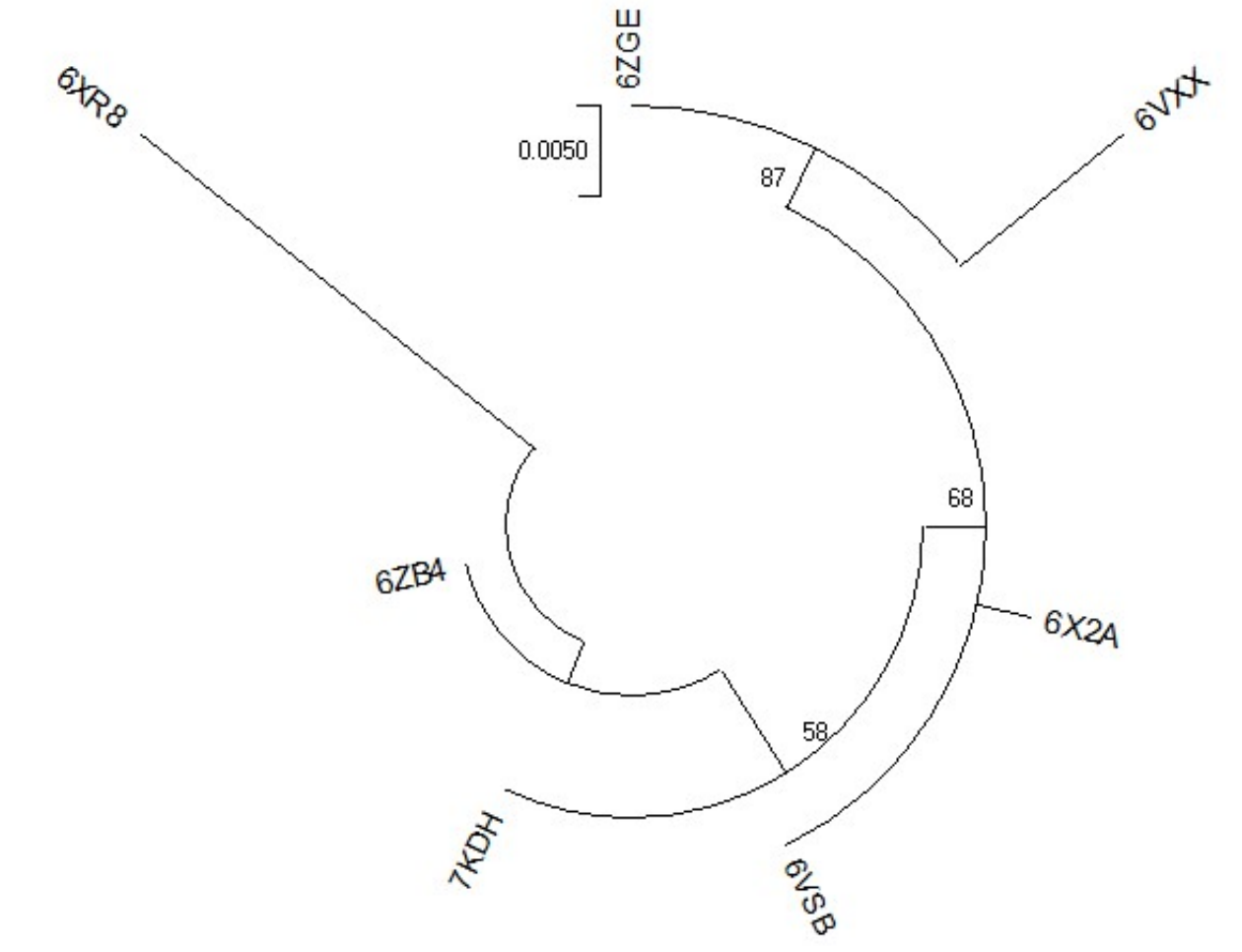


Figure S3: Antigenic and immunogenic assessment of cytotoxic T-lymphocyte epitopes. The bar plot represents the antigenicity and immunogenicity score of each epitope.

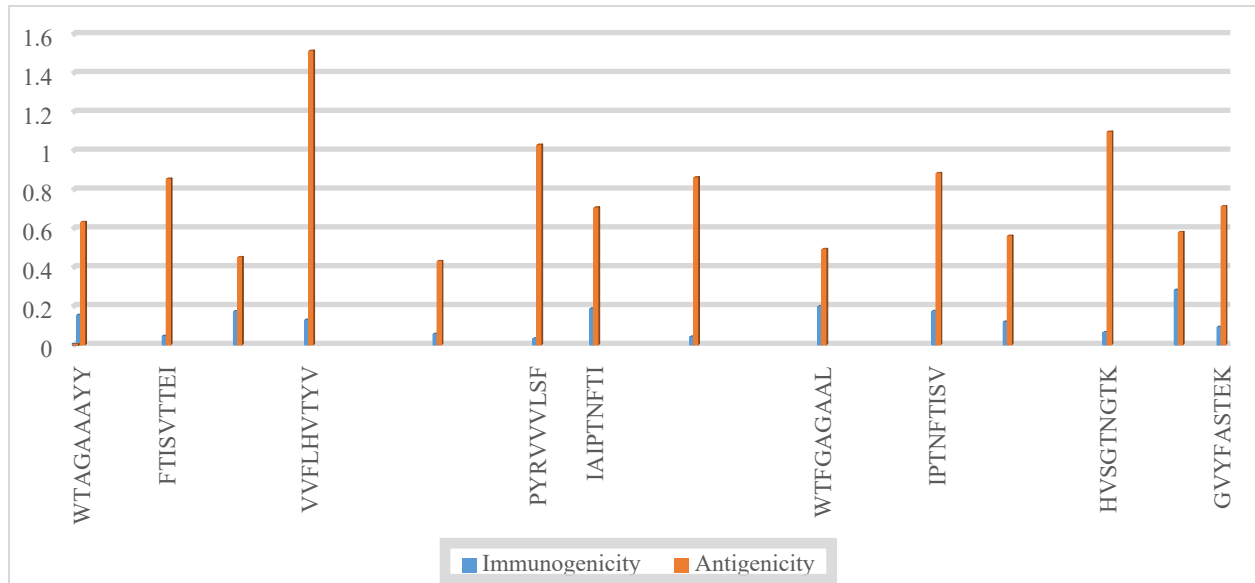


Figure S4: Allergenicity assessment of the final multi-epitope vaccine. The vaccine was found to be non-allergen by (A) AllerTOP v2.0 and (B) Allergen FP v1.0 sever

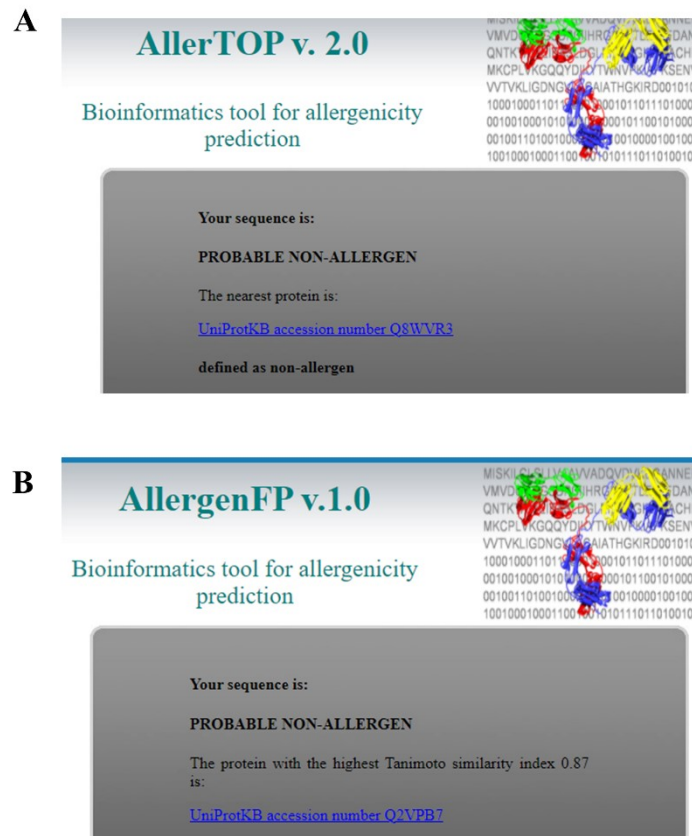


Figure S5: Signal Peptide prediction in the vaccine constructs

Measure Position Value

max. C 19 0.107

max. Y 70 0.106

max. S 60 0.111

mean S 1-69 0.097

D 1-69 0.101 0.450 NO

Name=vaccine SP='NO' D=0.101 D-cutoff=0.450 Networks=SignalP-noTM

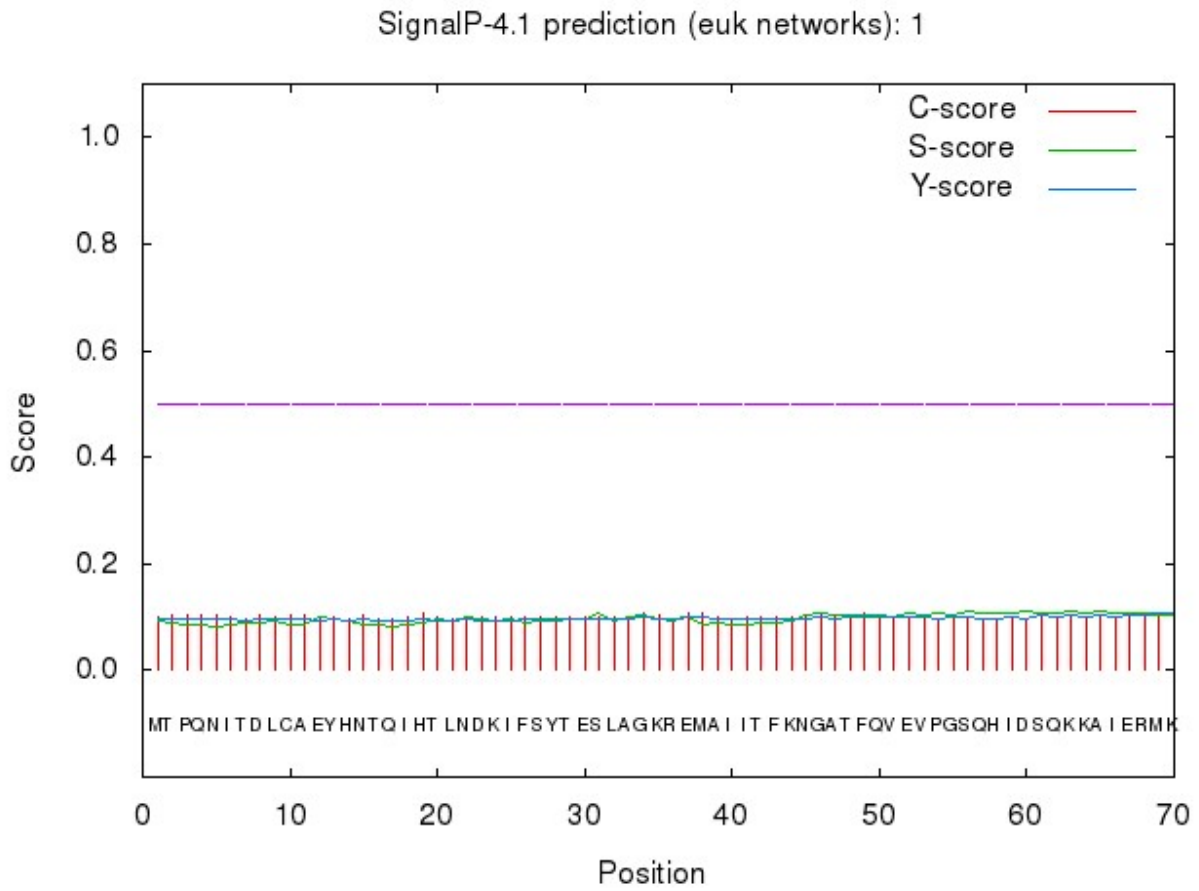


Figure S6: B Cell Epitopes

Continuous Epitopes, predicted by ElliPro and visualized using PyMol. Epitopes are shown in green color and the vaccine is shown in hot red color

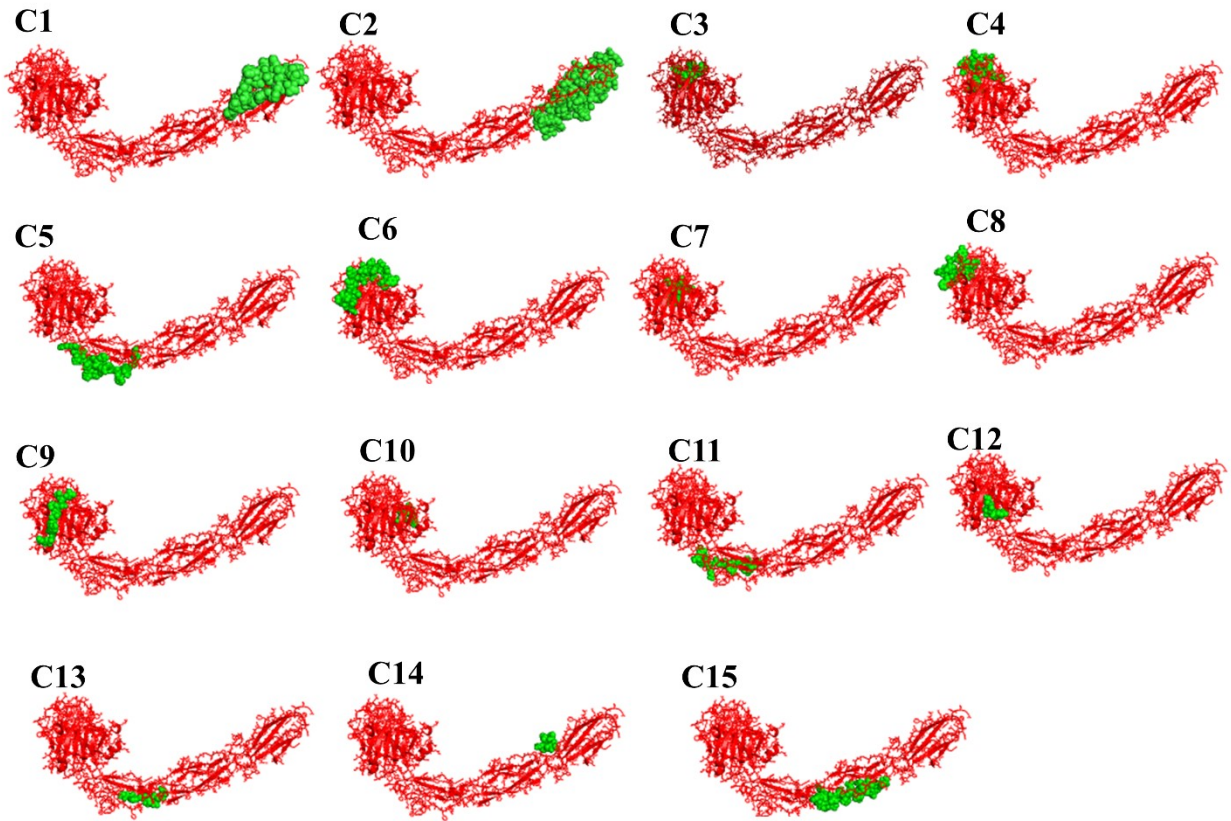


Figure S7: B Cell Epitopes

Discontinuous Epitopes predicted by ElliPro and visualized using PyMol. Epitopes are shown in blue color and the vaccine is shown in red color

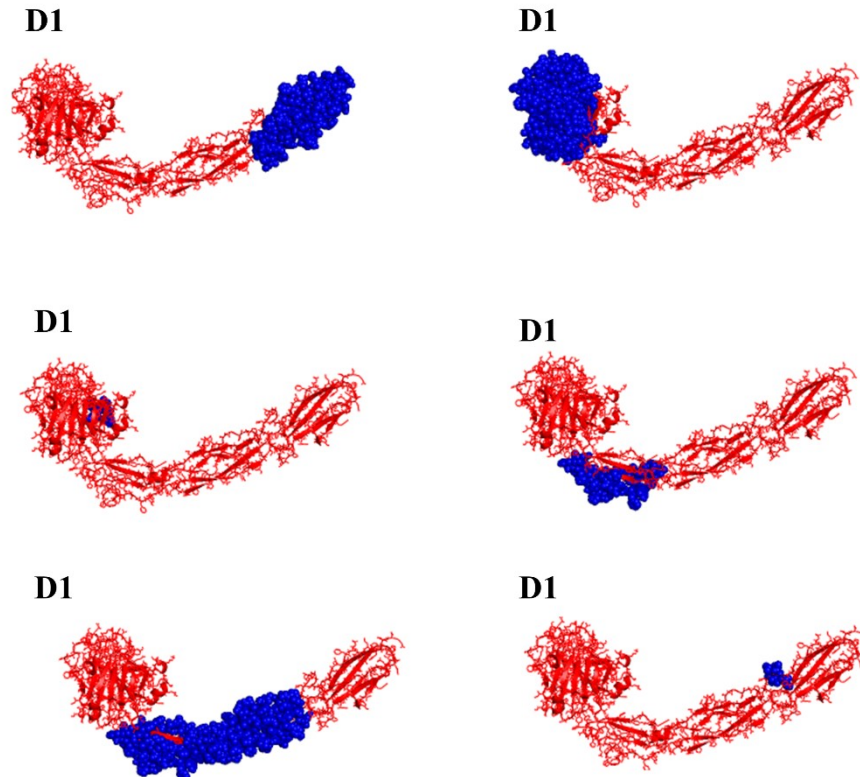


Figure S8: (A) changes in Structure conformations of vaccine (vaccine-TLR-2) between 0 and 50 ns, and (B) changes in Structure conformations of Chain B of TLR-2 (vaccine-TLR-2) between 0 and 50 ns.

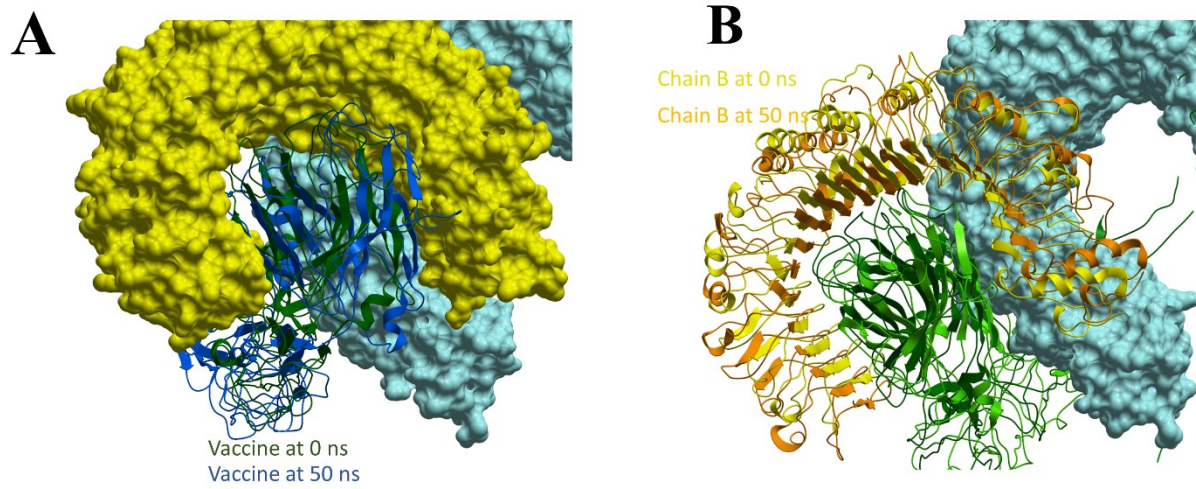


Figure S9: In silico immune simulation of vaccine construct. (A) Plasma B lymphocytes count sub-divided per isotype (IgM, IgG1, and IgG2). (B) B lymphocytes population per entity-state, (C) CD4 T-helper lymphocytes count. The plot shows total and memory counts, (D) CD4 T-regulatory lymphocytes count, (E) CD8 T-cytotoxic lymphocytes count, (F) Natural Killer cells (total count), (G) Macrophages. Total count, internalized, presenting on MHC class-II, active and resting macrophages, (H) Epithelial cells. Total count is broken down to active, pathogen-infected, and presenting on class-I MHC molecule, (I) Dendritic cells. DC can present antigenic peptides on both MHC class-I and class-II molecules. The curves show the total number broken down into active, resting, internalized, and presenting the ag.

