

Supplementary Table S1. Non-allergenic vaccine constructs against *C. difficile*.

Serial no.	Construct	Length	antigenic	stability	GRAVY	Molecular weight
1	>C1 EAAAKMAENSNIDDIKAPLL AALGAADLALATVNELITNL RERAEETRRSRVEESRARLTK LQEDLPEQLTELREKFTAEE LKAAEGYLEAATSELVERGE AALERLRSQQSFEEVSARAE GYVDQAVELTQEALGTVAS QVEGRAAKLVGIELEAAAK AKFVAAWTLKAAAGGGSG AGQQSRIHCTRLAGGGGSA KFVAAWTLKAAAGGGSRGV KGTGTQASFLGGGSHEYG AEALERAGAKFVAAWTLKA AAGGGS	260	1.02	unstable	-0.25	26957.14
2	>C2 EAAAKGIINTLQKYCRVR GGRCVLSCLPKEEQIGKC STRGRKCCRRKKEAAKA KFVAAWTLKAAAGGGSRG VKGTGTQASFLGGGSAKF VAAWTLKAAAGGGSGAG QQSRIHCTRLAGGGGSHEY GAEALERAGAKFVAAWTL KAAAGGGS	155	1.36	stable	-0.20	15607.91
3	>C3 EAAAKMAENPNIDDLAPL LAALGAADLALATVNDLIA NLRERAEETRAETRTRVEER RARLTKFQEDLPEQFIELRDK FTTEELRKAAEGYLEAATNR YNELVERGEAALQRLRSQTA FEDASARAEGYVDQAVELT QEALGTVASQTRAVGERAA KLVGIELEAAAKAKFVAAW TLKAAAGGGSRGVKGTGT QASFLGGGSAKFVAAWTLK AAGGGSGAGQQSRIHCTR	269	1.00	stable	-0.29	28075.36

	LAGGGGSHYGAERALERAG AKFVAAWTLKAAAGGGS					
4	>C4 EAAAKMAENSNIDDIKAPLL AALGAADLALATVNELITNL RERAEETRRSRVEESRARLTK LQEDLPEQLTELREKFTAEL RKAAEGYLEAATSELVERGE AALERLRSQQSFEEVSARAE GYVDQAVELTQEALGTVAS QVEGRAAKLVGIELEAAAK AKFVAAWTLKAAAGGGS KFVAAWTLKAAAGGSRGV KGTGTGTQASFLGGGSHYGA ERALERAGGAGQQSRIHCT RLAGGGGSHYGAERALERA GAKFVAAWTLKAAAGGGS	272	0.99	unstable	-0.276	28241.49
5	>C5 EAAAKMAQVINTNSLSLLTQ NNLNKSQSSLSSAIERLSSGL RINSAKDDAAGQAIANRFTS NIKGLTQASRNANDGISIAQ TTEGALNEINNNLQRVRELS VQATNGTNSDSLKSIQDEI QQRLEEIDRVSNQTQFNGVK VLSQDNQMKIQVGANDGET ITIDLQKIDVKSLGLDGFNVE AAAKAKFVAAWTLKAAAG GGSRGVKGTGTGTQASFLGGG SAKFVAAWTLKAAAGGGSG AGQQSRIHCTRLAGGGGSH EYGAERALERAGAKFVAAWT LKAAAGGGS	285	1.10	stable	-0.32	29350.54
6	>C6 EAAAKMAENPNIDDLAPL LAALGAADLALATVNDLIA	269	1.00	stable	-0.29	28075.36

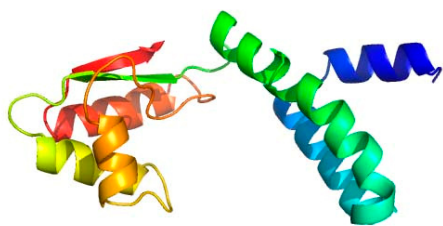
	NLRERAEEETRAETRTRVEER RARLTKFQEDLPEQFIELRDK FTTEELRKAAEGYLEAATNR YNELVERGEAALQRLRSQTA FEDASARAEGYVDQAVELT QEALGTVASQTRAVGERAA KLVGIELEAAAKAKFVAAW TLKAAAGGGSAGQQSRIH CTRLAGGGS AKFVAAWTL KAAAGGGSRGVKGTTGTQA SFLGGGSHEYGAELERAG AKFVAAWTLKAAAGGGS					
7	>C7 EAAAKMAENSNIDDIKAPLL AALGAADLALATVNELITNL RERAEEETRRSRVEESRARLTK LQEDLPEQLTELREKFTAEL RKAAEGYLEAATSELVERGE AALERLRSQQSFEEVSARAE GYVDQAVELTQEALGTVAS QVEGRAAKLVGIELEAAAK AKFVAAWTLKAAAGGGSR GVKGTTGTQASFLGGGSHEY GAELERAGGAGQQSRIHC TRLAGGGSHEYGAELER AGAKFVAAWTLKAAAGGGS	255	0.95	unstable	-0.33	26653.65

Supplementary Table S2. 3D structure statistics of the vaccine construct, using various tools.

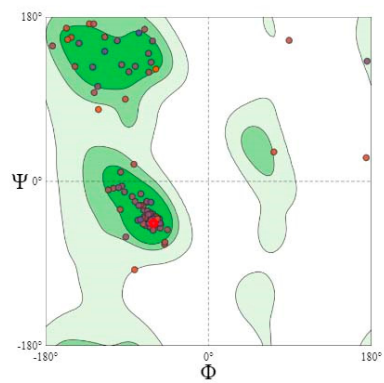
Tool	MolProbity score	Ramachandran favoured residues (%)	Ramachandran outliers (%)	QMEANDisCo Global
I-Tasser	4.55	44.32	30.68	0.32± 0.07
Alpha Fold	3.58	62.75	23.53	0.35 ± 0.07
Swiss-Model	2.27	89.60	3.20	0.64 ± 0.07

Supplementary Table S3. HLA and TLR receptor interaction statistics with the designed vaccine construct. Non-bonded contacts can involve attractive forces, such as van der Waals interactions and hydrophobic interactions, or repulsive forces, such as steric clashes. No disulphide bond was detected in any interaction.

Serial no.	PDB ID	Complex	ClusPro lowest docking score with C2	ΔG (kcal mol⁻¹)	K_d (M) at °C	Non-bonded interactions	No. of hydrogen bonds in main chains	Salt bridges
1.	3OX8	HLA-A-vaccine	-814.8	-10.5	2.1e-08	158	10	3
2.	4JQX	HLA-B-vaccine	-878.6	-14.8	1.3e-11	149	12	3
3.	3FXI	TLR-4-vaccine	-770.4	-17.1	2.9e-13	226	19	6
4.	4GIQ (control)	Rank-RankL	-878.4	-11.6	2.9e-09	162	12	4



A



B

Supplementary Figure S1(A) 3D structure of vaccine construct using Swiss-Model (B) Ramachandran plot of the model.