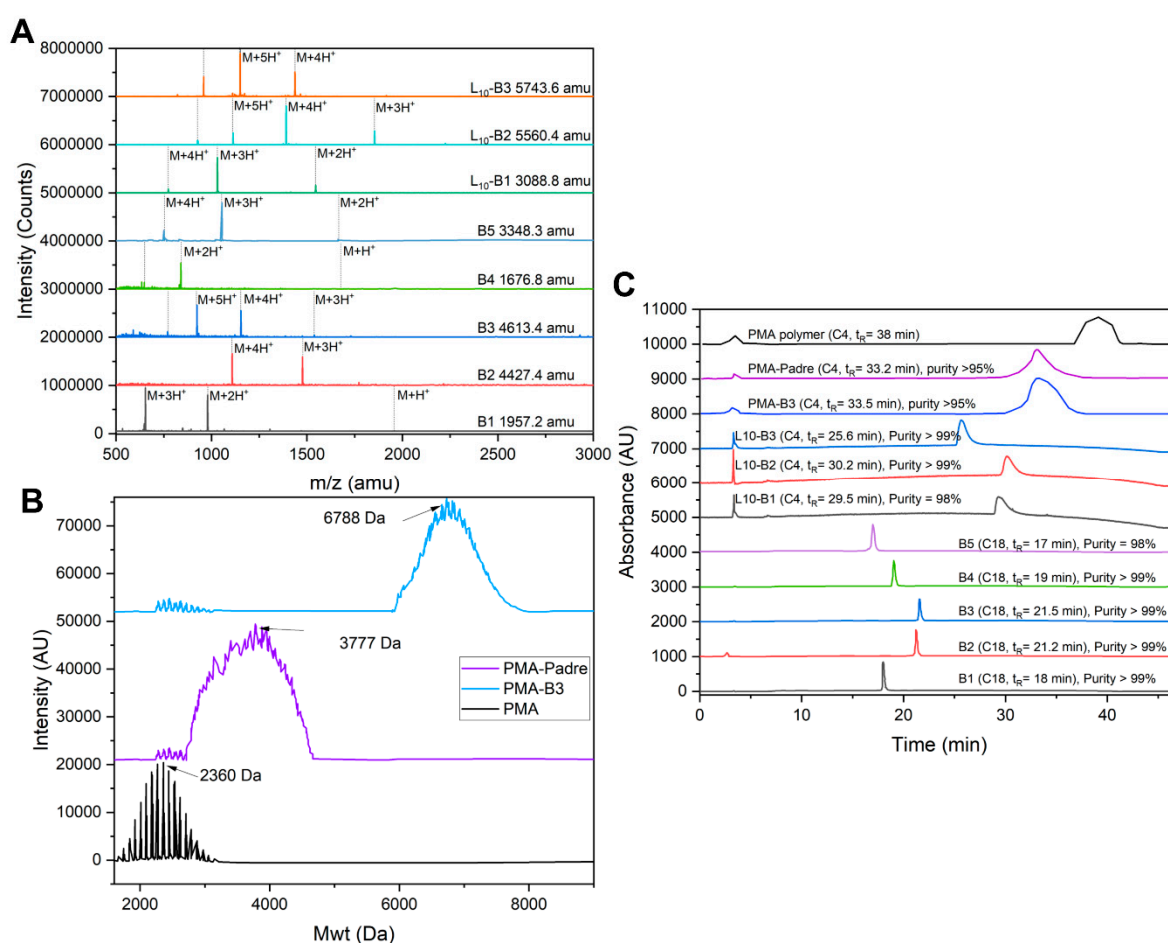


Supplementary Materials:

# Peptide-based Vaccine against SARS-CoV-2: Peptide Antigen Discovery and Screening of Adjuvant Systems

Ahmed O. Shalash, Armira Azuar, Harrison Y. R. Madge, Naphak Modhiran, Alberto A. Amarilla, Benjamin Liang, Alexander A. Khromykh, Waleed M. Hussein, Keith J. Chappell, Daniel Watterson, Paul R. Young, Mariusz Skwarczynski, and Istvan Toth



**Figure S1.** Characterization results of synthesized peptides and peptide conjugates by MS-ESI (A), MALDI-ToF (B), and RP-HPLC (C).

```

320          330          340          350
RV QPTESIVRFP NITNLCPFGE VFNATRFASV

          360          370          380          390          400
YAWNRKRISN CVADYSVLYN SASFSTFKCY GVSPTKLNDL CFTNVYADSF
          -----B5 (S366-S395) -----
          410          420          430          440          450
VIRGDEVQRQI APGQTGKIAD YNYKLPDDFT GCVIAWNSNN LDSKVGGNYN
          -----
          460          470          480          490          500
YLYRLFRKSN LKPFERDIST EIIYQAGSTPC NGVEGFNCYF PLQSYGFQPT
-----B3 (S444-S483) -----B2 (S469-S508) -----
          510          520          530          540          550
NGVGYQPYRV VVLSFELLHA PATVCGPKKS TNLVKNKCVN FNFNGLTGTG
-----
          560          570          580          590          600
VLTESNKKFL PFQQFGRDIA DTDAVRDPQ TLEILDITPC SFGGVSVITP
          --B4 (S559-S572) ---
          610          620          630          640
GTNTSNQVAV LYQDVNCTEV PVAIHADQLT PTWRVYSTGS
          --- B1 (S623-S639) ---

```

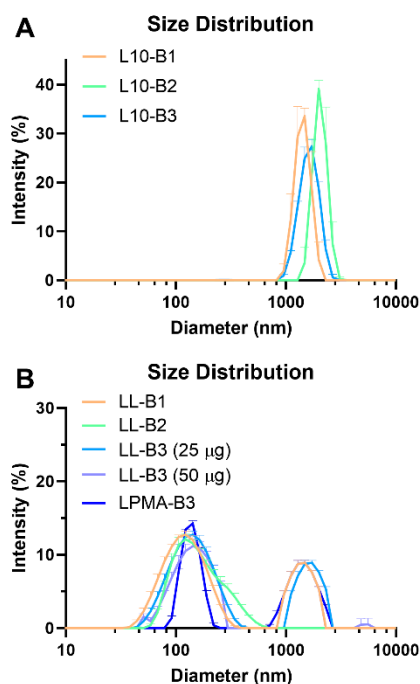
**Figure S2.** Peptide antigen sequences B1-B5 (bolded) and their locations on the original Wuhan-1 strain SARS-CoV-2 spike protein sequence with RBD amino acid sequence coloured in blue. The italicized sequence “*STEIYQAGSTPCNGV*” is a part of both B2 and B3 sequences.

**Table S1.** Secondary structural content of screened and candidate peptide B-cell epitopes.

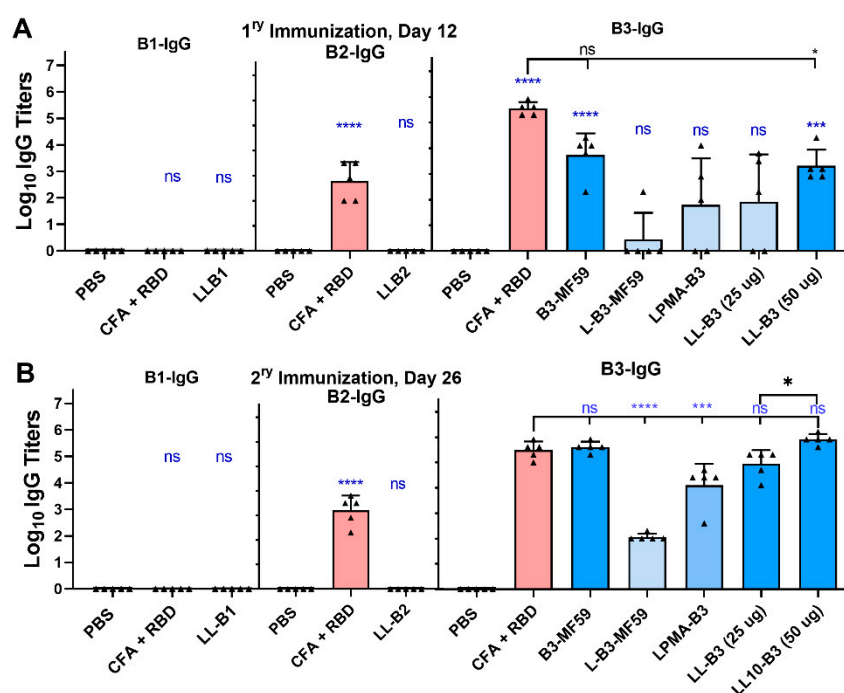
Peptide	Experimental			<i>i-tasser predicted</i> <sup>a</sup>			Native (Cryo-EM) <sup>a</sup>		
	Helix %	Strand %	Random Coil %	Helix %	Strand %	Random Coil %	Helix %	Strand %	Random Coil %
<i>B1</i>	28.5	2.5	69.0	47.0	0.0	53.0	--	--	--
<i>B2</i>	18.0	20.5	61.5	7.5	22.5	70.0	0.0	10.0	90.0
<i>B3</i>	29.0	0.0	71.0	59.0	0.0	41.0	0.0	10.0	90.0
<i>B4</i>	27.0	0.0	73.0	43.0	0.0	57.0	0.0	17.5	82.5
<i>B5</i>	0.0	59.5	40.5	10.0	20.0	70.0	13.0	13.0	74.0
<i>L10-B1</i>	18.0	82.0	0.0	35.0	23.5	41.5	Non-native		
<i>L10-B2</i>	95.0	0.0	5.0	22.5	0.0	77.5	Non-native		
<i>L10-B3</i>	61.0	23.0	16.0	59.0	0.0	41.0	Non-native		

<sup>a</sup> Predicted percentage results were calculated by summing the amino acid residues of a specific secondary structure, then dividing that by the total number of residues in the peptide [1,70].

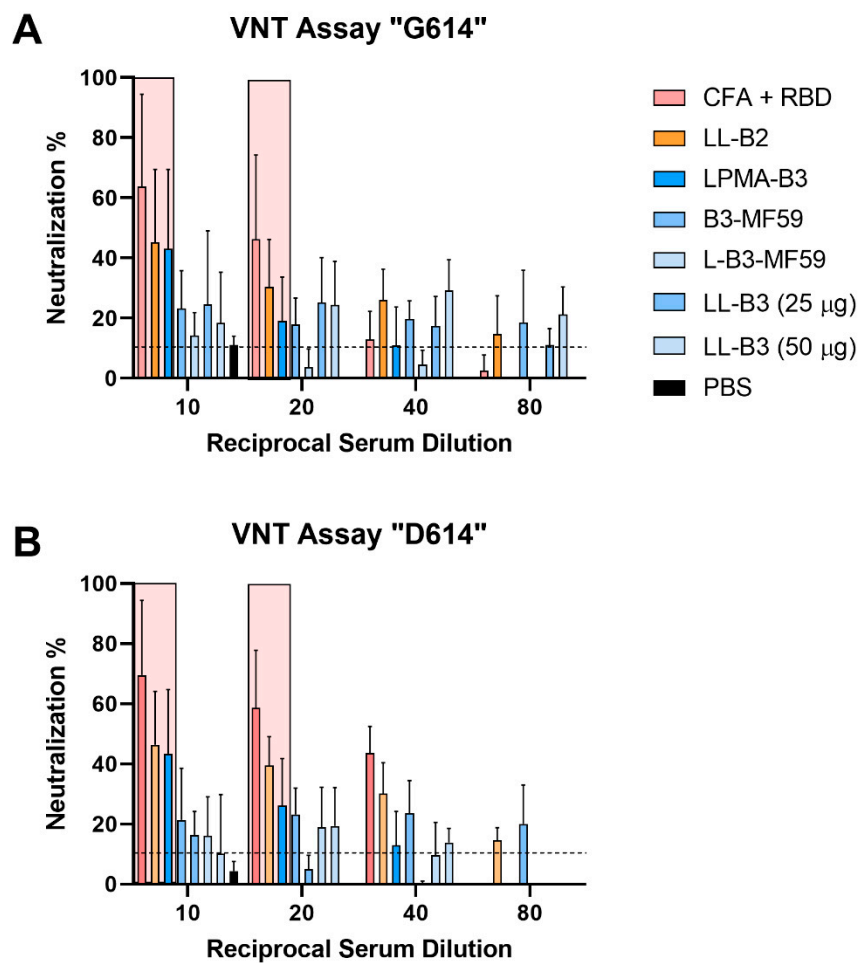
The L10-conjugated peptide antigens were self-assembled in PBS, resulting in micro-particles (Figure S2). L10-B1, L10-B2, and L10-B3 self-assembled into particles with sizes of:  $1.42 \pm 0.12 \mu\text{m}$ ,  $1.32 \pm 0.08 \mu\text{m}$ , and  $1.38 \pm 0.17 \mu\text{m}$ , respectively, with relatively small polydispersity indices (PDIs) for aggregating, self-assembled particles ( $\text{PDI} = 0.302 \pm 0.072$ ,  $0.265 \pm 0.078$ , and  $0.398 \pm 0.022$ , respectively).



**Figure S3.** Size distribution of (A) self-assembled peptide antigens, and (B) liposomal formulations of polyleucine-, or PMA- conjugated peptides.



**Figure S4.** Immunogenicity results from the peptide adjuvant screening study following immunization of C57BL/6 mice. Anti-peptide IgG titers in the serum after primary immunization (A), and anti-peptide IgG titers against self-peptide antigens B1–B3 after secondary immunization (B). Error bars represent standard deviation. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparison test. (ns) non-significant,  $p > 0.05$ ; (\*)  $p < 0.05$ ; (\*\*)  $p < 0.01$ ; (\*\*\*)  $p < 0.001$ , and (\*\*\*\*)  $p < 0.0001$ .



**Figure S5.** Live virus neutralization assays of mouse serum against the original SARS-CoV-2 Wuhan strain (G614) (A), and the D614 variant (B). Red boxes highlight the most promising neutralizing sera.