

## **Yeast-expressed Recombinant SARS-CoV-2 Receptor Binding Domain, RBD203-N1 as a COVID-19 Protein Vaccine Candidate**

Wen-Hsiang Chen<sup>a</sup>, Jeroen Pollet<sup>a</sup>, Ulrich Strych<sup>a</sup>, Jungsoon Lee<sup>a</sup>, Zhuyun Liu<sup>a</sup>, Rakhi Tyagi Kundu<sup>a</sup>, Leroy Versteeg<sup>a</sup>, Maria Jose Villar<sup>a</sup>, Rakesh Adhikari<sup>a</sup>, Junfei Wei<sup>a</sup>, Cristina Poveda<sup>a</sup>, Brian Keegan<sup>a</sup>, Aaron Oakley Bailey<sup>b</sup>, Yi-Lin Chen<sup>a</sup>, Portia M. Gillespie<sup>a</sup>, Jason T. Kimata<sup>c</sup>, Bin Zhan<sup>a</sup>, Peter J. Hotez<sup>acde</sup>, Maria Elena Bottazzi<sup>acdf\*</sup>

<sup>a</sup> Departments of Pediatrics; Texas Children's Hospital Center for Vaccine Development; National School of Tropical Medicine; Baylor College of Medicine, Houston, TX, USA

<sup>b</sup> Mass Spectrometry Facility, Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX, USA

<sup>c</sup> Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA

<sup>d</sup> Department of Biology, Baylor University, Waco, TX, USA

<sup>e</sup> James A. Baker III Institute for Public Policy, Rice University, Houston, TX, USA

### **SUPPLEMENTARY DATA**

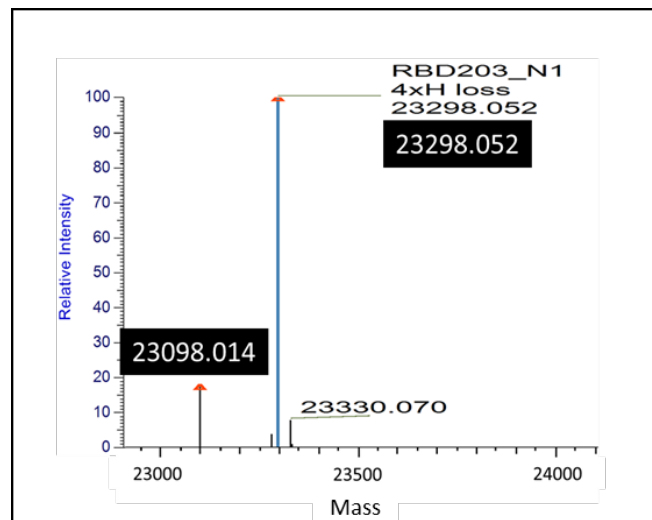
#### **Supplementary Method**

##### **Intact Mass Analysis of Deglycosylated Proteins**

RBD203-N1 or RBD219-N1C1 were treated with PNGase-F (NEB) following the vendor's manual to remove N-linked glycans. The deglycosylated proteins were then analyzed by micro-flow reversed-phase LC-MS using a 45-min gradient with an Ultimate 3000 UHPLC system coupled to Orbitrap Fusion mass spectrometer (ThermoFisher Scientific), operated at a resolution setting of 240,000 (at m/z 200). The data were analyzed using the Xtract deconvolution algorithm in Biopharma Finder v4.0 (ThermoFisher Scientific).

**Supplementary Table 1.** Study group and formulation information. N1C1: RBD219-N1C1; 203-N1: RBD203-N1.

Group Label	Group Size	Mouse #	Antigen	Antigen Dose ( $\mu\text{g}$ )	Adjuvant Molecule	Adjuvant Dose ( $\mu\text{g}$ )	Route	Volume (mL)
1	5	1-5	None	-	Alhydrogel <sup>®</sup>	200	IM	0.05
2	5	6-10	None	-	Alhydrogel <sup>®</sup> +CpG	200 + 20	IM	0.05
3	8	11-18	N1C1	25	Alhydrogel <sup>®</sup>	200	IM	0.05
4	8	19-26	203-N1	25	Alhydrogel <sup>®</sup>	200	IM	0.05
5	8	27-34	N1C1	7	Alhydrogel <sup>®</sup>	200	IM	0.05
6	8	35-42	N1C1	7	Alhydrogel <sup>®</sup> +CpG	200 + 20	IM	0.05
7	8	43-50	203-N1	7	Alhydrogel <sup>®</sup>	200	IM	0.05



**Supplementary Figure 1.** Intact mass spectrometry for deglycosylated RBD203-N1. This result indicated two major species matching the sequences of (1) RBD203-N1<sup>a</sup> with additional EAEF amino acid residues at the N-terminus (23,098 Da), and (2) RBD203-N1<sup>b</sup> with additional EAEAEF amino acid residues at the N-terminus (23,298 Da). 82-85% of the RBD203-N1 is the EAEAEF variant while 15-18% is the EAEF variant.