

Supplemental Data for:

Antibody-dependent complement responses toward SARS-CoV-2 receptor-binding domain immobilized on “pseudovirus-like” nanoparticles.

Hanmant Gaikwad ^{1,2,3,#}, Yue Li ^{1,2,#}, Guankui Wang ^{1,2,3}, Ronghui Li ², Shaodong Dai ², Cody Rester ⁴, Ross Kedl ⁴, Laura Saba ², Nirmal K. Banda ⁵, Robert I. Scheinman ^{2,3}, Casey Patrick², Krishna M.G. Mallela², S. Moein Moghimi, ^{2,3,6,7}, and Dmitri Simberg ^{1,2,3,*}

¹*Translational Bio-Nanosciences Laboratory*, ²*Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO, 80045, USA*

³*Colorado Center for Nanomedicine and Nanosafety, University of Colorado Anschutz Medical Campus, Aurora, CO, 80045, USA*

⁴*Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO, 80045, USA*

⁵*Division of Rheumatology, School of Medicine, University of Colorado Anschutz Medical Campus, 1775 Aurora Court, Aurora, CO, 80045, USA*

⁶*School of Pharmacy, King George VI Building, Newcastle University, Newcastle upon Tyne NE1 7RU, UK*

⁷*Translational and Clinical Research Institute, Framlington Place, Newcastle University, Newcastle upon Tyne NE2 4HH, UK*

equal contribution

*corresponding author

Dmitri Simberg, dmitri.simberg@cuanschutz.edu

Table S1: Differences in levels of anti-RBD antibody (log base 2 transformed) measured with validated immunoassay among groups of subjects; (NC=naïve; CCP =convalescent; VAC=vaccinated).

contrast	estimate	SE	df	t.ratio	p.value
CCP - NC	3.3	0.67	28	5.00	0.0001
CCP - VAC	-0.7	0.68	28	-1.09	0.5251
NC - VAC	-4.1	0.67	28	-6.13	<0.0001

Table S2: Differences in levels of C3 between groups of subjects; (NC=naïve; CCP =convalescent; VAC=vaccinated). The average C3 levels across technical replicates within an individual and particle type was first calculated and then the difference across nanoparticles within an individual was calculated. The difference values (i.e., C3 binding $\mu\text{g}/\text{mg}$) were used as outcome variables in a 1-way ANOVA to examine differences between groups of subjects, i.e., vaccinated, convalescent, and healthy donors.

contrast	estimate	SE	df	t.ratio	p.value
CCP - NC	10.7	6.47	28	1.66	0.2382
CCP - VAC	-11.7	6.62	28	-1.77	0.1996
NC - VAC	-22.4	6.47	28	-3.47	0.0047

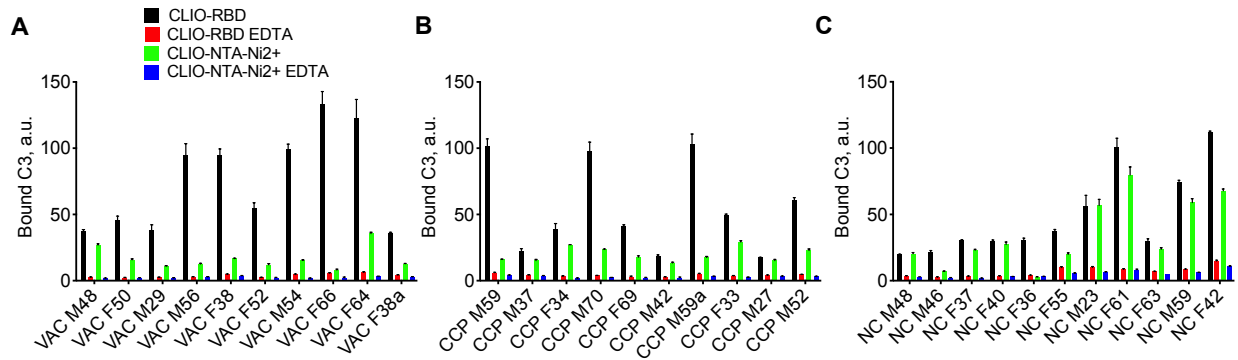
Table S3: Differences in IgG and IgM binding to CLIO-RBD between groups.

Differences in IgG binding among groups of subjects

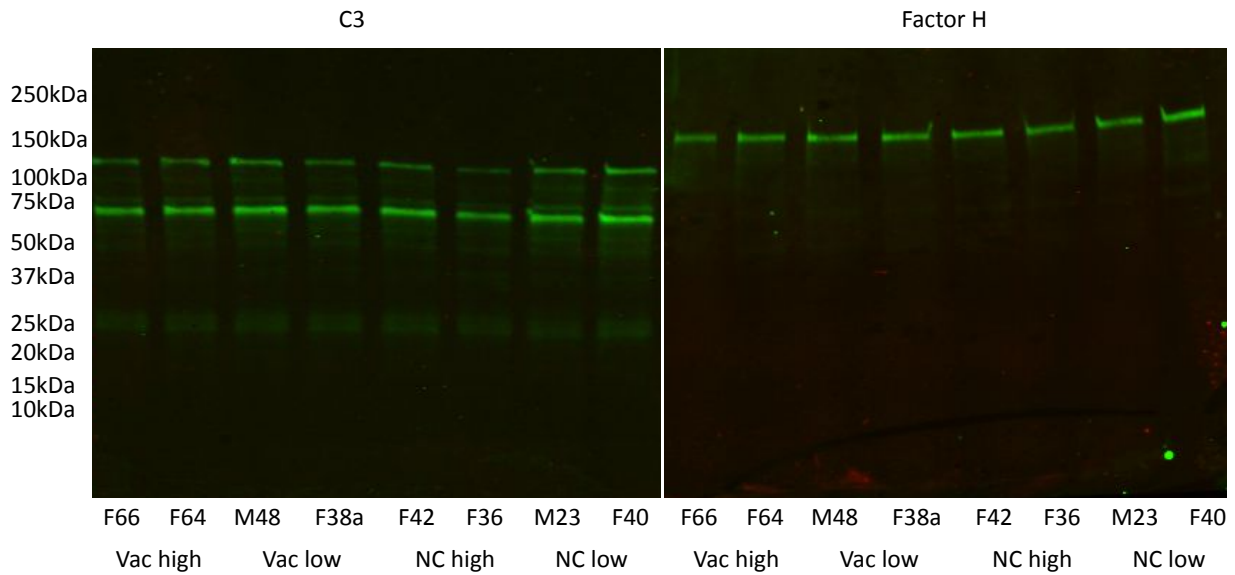
contrast	estimate	SE	df	t.ratio	p.value
CCP - NC	19.5	12.88	28	1.51	0.2998
CCP - VAC	-22.3	13.18	28	-1.69	0.2260
NC - VAC	-41.8	12.88	28	-3.25	0.0083

Differences in IgM binding among groups of subjects

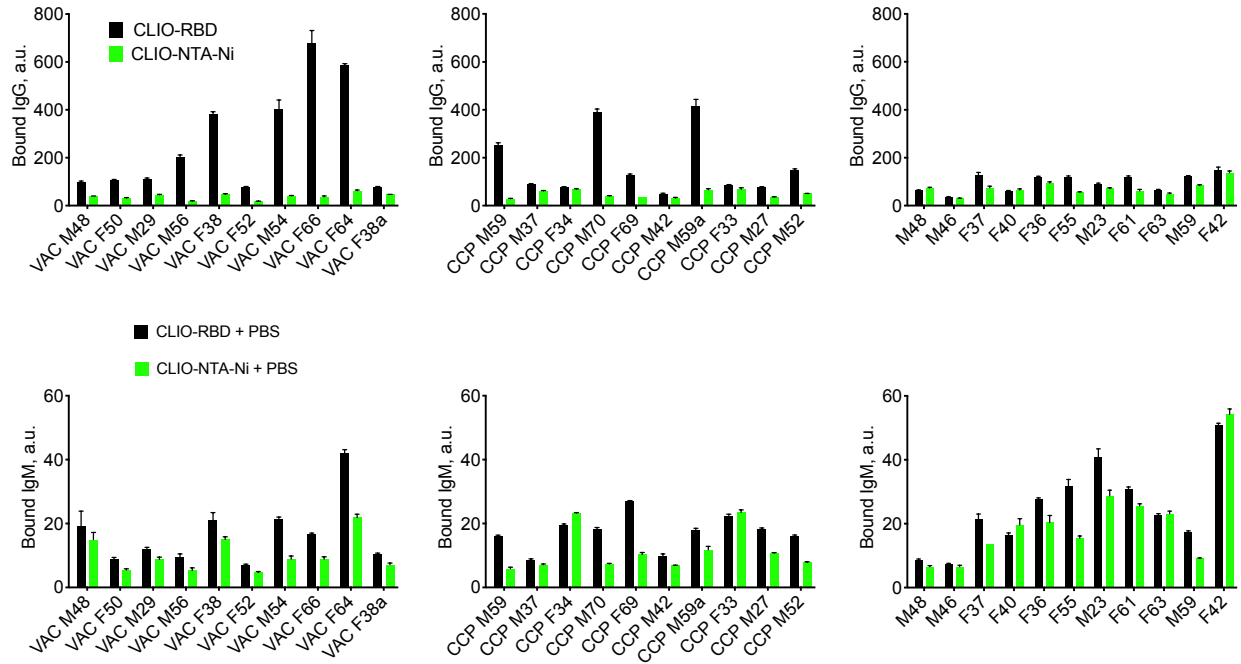
contrast	estimate	SE	df	t.ratio	p.value
CCP - NC	0.6	1.32	28	0.43	0.9037
CCP - VAC	-0.4	1.35	28	-0.28	0.9572
NC - VAC	-0.9	1.32	28	-0.72	0.7550



Supplemental Fig. S1. Raw values of C3 bound to CLIO-RBD and CLIO-NTA-Ni²⁺ with and without 10mM EDTA (total complement inhibitor). Data are 3 technical replicates \pm SD.



Supplemental Fig. S2. Levels of total C3 and Factor H in serum from 4 vaccinated and 4 naïve (NC) donors. There was no correlation with C3 deposition on the particles.



Supplemental Fig. S3. Raw values of IgG and IgM bound to CLIO-RBD and CLIO-Ni²⁺. Data are 3 technical replicates \pm SD.