

SUPPLEMENTAL MATERIAL

METHODS - NEUTRALIZATION ASSAY

Serum neutralization assays were performed as previously described by Crawford et al using luciferase-expressing lentiviral particles pseudotyped for the SARS-CoV-2 spike protein (SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike-Pseudotyped Lentiviral Kit, BEI Resources, NR-52948) and HEK-293T cells over-expressing the ACE2 receptor (HEK-293T-hACE2, BEI Resources, NR-52511) (34).

Pseudovirus preparation – Pseudotyped lentivirus was generated in HEK-293T cells transfected simultaneously with the following: helper plasmids encoding for Gag and Pol, Tat1b, and Rev1b; lentiviral backbone plasmid encoding for Luc2 and ZsGreen; and plasmid encoding the Wuhan-Hu-1 strain of SARS-CoV-2 Spike glycoprotein (Lentiviral Kit, BEI Resources, NR-52948). After 72 hrs, the culture supernatant containing pseudovirus was harvested, syringe-filtered (0.45 µm), and stored at -80° C.

Neutralization assay - HEK-293T-hACE2 cells (80,000 cells/well; BEI Resources, NR-52511) were cultured in white-walled microplates for 24 hr. Pseudovirus entry was assessed from luciferase activity using a GloMax Discover luminometer (Promega Corp., GM3000) and Bright-Glo reagent (Promega, E2610). Initially, to determine optimum conditions for the assay, the signal generated by various pseudovirus dilutions was assessed in the absence of serum (**Fig. S3**). Negative controls i.e., supernatants from HEK-293T cells transfected with carrier DNA only (Promega, E4881) (data not shown), or with a lentiviral plasmid lacking the Spike glycoprotein, yielded RLU values less than 0.2% of the undiluted pseudovirus (**Fig. S3**). For the neutralization assay itself, a 10x-dilution of pseudovirus stock (plus 5 µg/mL polybrene, EMD Millipore Corp., TR-2003-G) which yielded an RLU of 25,000 was used. Seven-point serial serum dilutions were incubated with pseudovirus for 1hr at 37°C in a separate sterile plate.

The serum-pseudovirus mixtures were then incubated with HEK-293T- hACE2 cells for 48 hr at 37°C, followed by lysis with Bright-Glo reagent. A potent neutralizing Spike RBD IgG antibody (BEI Resources, NR 53795) was used as a positive control in each run (**Fig. S4**).

Experimental Design and Data Analysis - Neutralization assays were performed pre-vaccination and at days 14 and 42 post 1st injection of vaccine. Individual serum samples (50 μ L / subject) were pooled with 7 subjects per pool in both COVID and control groups. On days 14 and 42, data points for both groups are the mean \pm SE of 3 pools of the same 21 COVID and 21 control subjects. In contrast, the pre-vaccination timepoint was a single pool for each group.

Maximal pseudovirus uptake by HEK cells in the absence of serum was taken as 100% of control. IC₅₀ i.e., 50% inhibition of pseudovirus entry, was calculated using sigmoidal 4 factor polynomial, non-linear regression (GraphPad Prism version 9).

ACKNOWLEDGEMENTS

The following reagents were obtained through BEI Resources, NIAID, NIH: Monoclonal Anti-SARS Coronavirus Recombinant Human IgG1, Clone CR3022 (produced in *Nicotiana benthamiana*), NR-52392; SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike-pseudotyped Lentiviral Kit, NR-52948; Human Embryonic Kidney Cells (HEK-293T) Expressing Human Angiotensin-Converting Enzyme 2, HEK-293T-hACE2 Cell Line, NR-52511; Monoclonal Anti-SARS-Related Coronavirus 2 Spike Glycoprotein RBD-mFc Fusion Protein (produced in vitro), NR-53795.

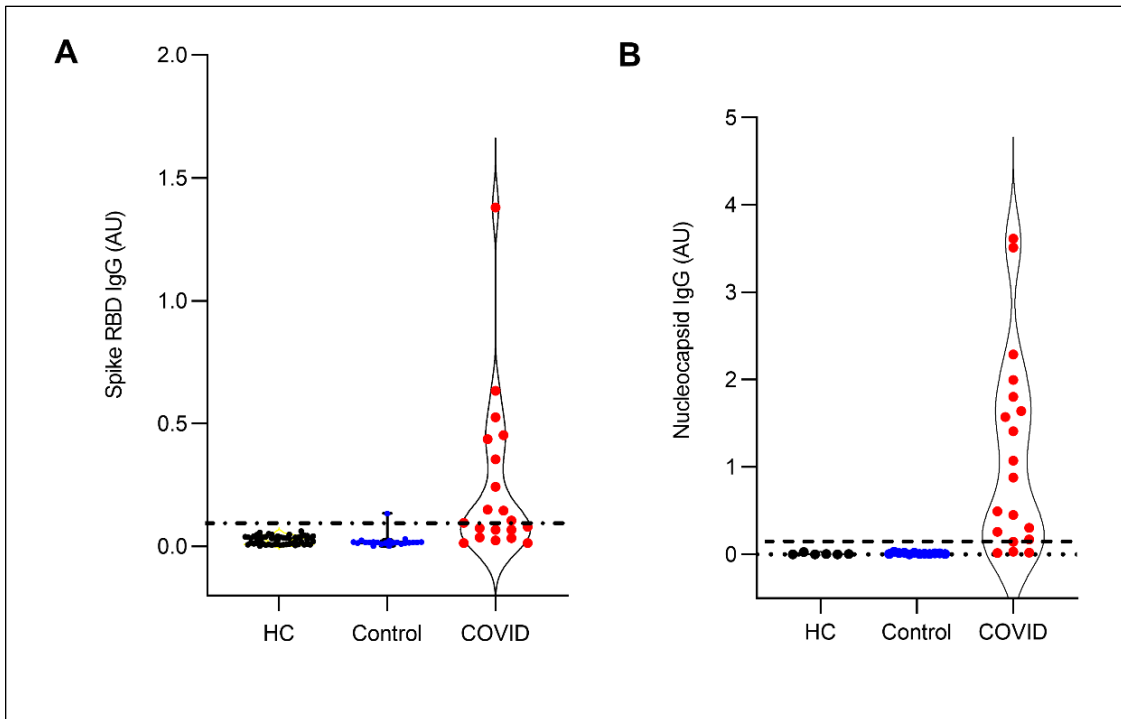


Figure S1. Violin plots showing pre-vaccination serum Spike RBD IgG antibody (panel A) and nucleocapsid IgG antibody (panel B) in individual COVID (●, n=22) and control (NC●, n=22) subjects. Spike RBD IgG ($p < 0.01$) and nucleocapsid IgG ($p < 0.003$) antibody levels were significantly higher in the COVID compared to control groups prior to vaccination. Historical control samples (HC-●; n=57) were archived prior to the COVID-19 pandemic i.e., 2008. Horizontal dashed line demarcates 4 standard deviations from the mean of the historical controls.

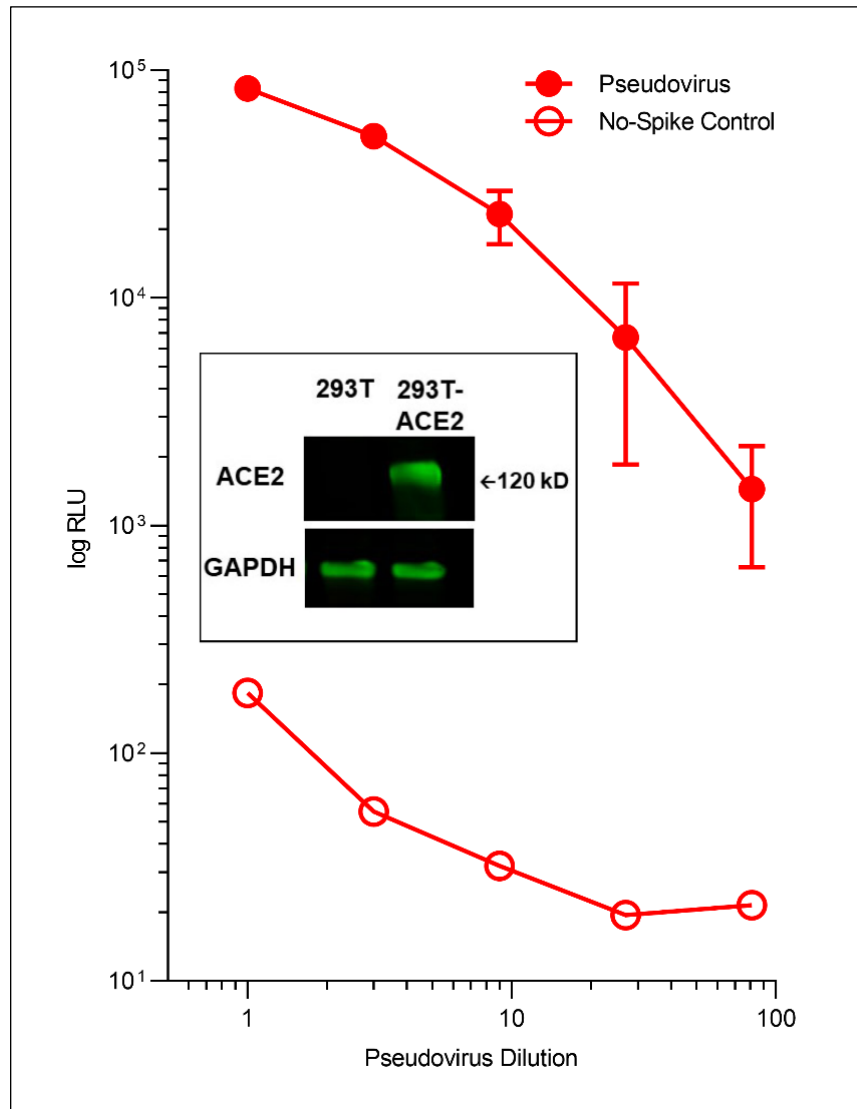


Figure S3. Uptake of pseudotyped SARS-CoV-2 lentivirus into HEK293T-ACE2 cells. Intact pseudotyped lentivirus (●) uptake shown as luciferase activity on the Y axis (relative light units - RLU) was assessed in the absence of serum. Pseudovirus dilutions are shown on the X axis. Lentivirus lacking SARS-CoV-2 Spike protein (○) was used as an inactive, negative control. Data are Group mean ± 1 SE from multiple wells (1 experiment representative of 3). Note that intact pseudovirus diluted 10X from stock generated a signal 500-fold greater than that produced by the inactive pseudovirus. Inset - Western blot showing marked increase in ACE2 receptor expression in 293T-ACE2 vs native 293T cells.

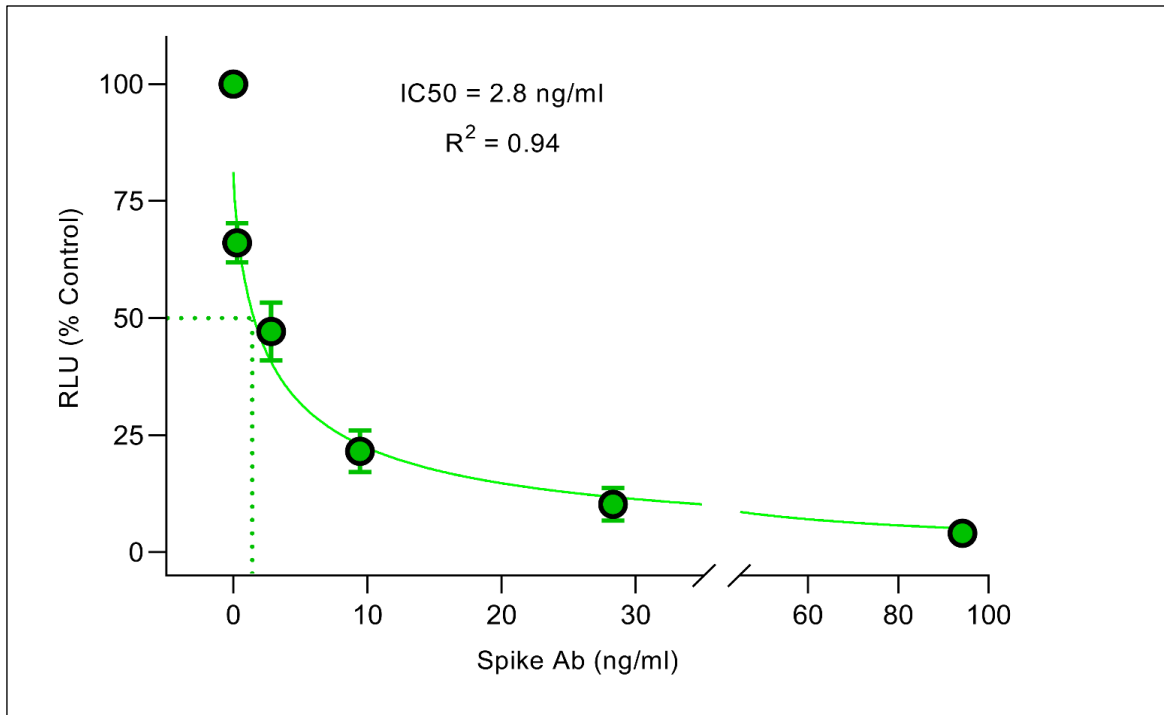


Figure S4. Effect of neutralizing Spike IgG antibody on pseudotyped SARS-CoV-2 lentivirus entry into HEK293T-ACE2 cells. Note that positive control Spike RBD neutralizing antibody (BEI Resources, NR-53795) strongly inhibited pseudovirus uptake (IC₅₀=2.8 ng/mL). Data are mean ± 1 SE of 4 experiments.