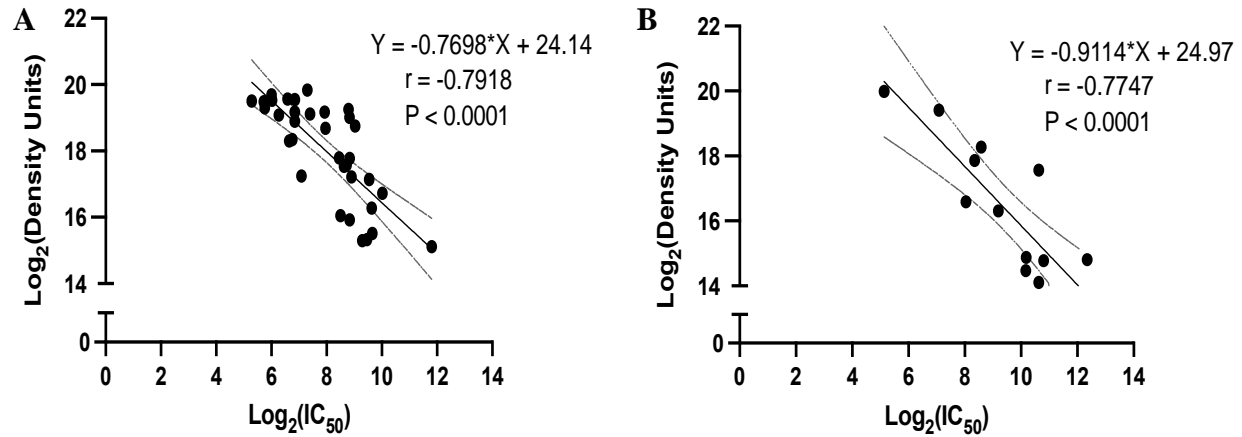


## **Supplementary Materials**

This material has been provided to give readers additional information about the work.

Supplement to: Douglas F. Lake, Alexa J. Roeder, et. Al. Third COVID-19 Vaccine Dose Boosts Neutralizing Antibodies in Poor Responders

**Supplementary Figure S1 – Regression analysis of LFA density values and microneutralization IC<sub>50</sub> values.**



**Supplementary Figure S1.** Comparison between LFA density units and IC<sub>50</sub> values obtained using **A**) convalescent sera isolated from 36 recovered COVID-19 patients and **B**) vaccinated sera from 13 individuals. Neutralization was tested on LFA using 6  $\mu$ L serum and a dilution series was performed by authentic SARS-CoV-2 FRNT assay to obtain IC<sub>50</sub> values. To calculate IC<sub>50</sub>, data were analyzed in GraphPad Prism 9.0 using methods described by Ferrara and Temperton. Density values and IC<sub>50</sub> values were Log<sub>2</sub>-transformed and analyzed using a simple linear regression and nonparametric Spearman correlation with two-tailed P value and a 95% confidence interval (CI). Regression analysis with 95% CI boundaries is indicated in solid black and grey dotted lines. Spearman's rho and two-tailed P value are labeled.

**Supplementary Figure S2 – NAb LFA Tests with Density Values, IC50s, NAb Titers, and Percent Neutralization**

	VAC-93	VAC-81	VAC-77	VAC-82	VAC-74	VAC-99
<b>TLD</b>	1,007,231	765,297	477,787	295,032	156,481	14,212
<b>IC<sub>50</sub></b>	43.8	62.6	115.4	215.9	492.2	11,103.9
<b>NAb Titer</b>	≤ 40	≥40, <80	≥80, <160	≥160, <320	≥320, >640	≥640
<b>% Neut</b>	0	19	49	69	84	99

**Supplementary Figure S2.** LFA densitometer images with corresponding Test Line Density (TLD), IC<sub>50</sub> value, reciprocal NAb titer, and percent neutralization. TLD is reported as unmodified, discrete values. IC<sub>50</sub> values are derived from the regression equation shown in Supplementary **Figure S1**. Reciprocal NAb titer is derived from IC<sub>50</sub>, such that titer is equal to the last dilution factor (DF) (DF = 20\*2<sup>-n</sup>) for which IC<sub>50</sub> is ≥ the lower threshold range for a given titer. For example, IC<sub>50</sub>=43.8 is classified as an NAb titer of 40. Further, a hypothetical IC<sub>50</sub>=158 would classify as NAb titer ≥80 and <160. Percent neutralization was calculated using the equation: 1-(TLD/LoD) when LoD=942,481 as described in **Table 1** legend.