

## Article

# High-Content Imaging-Based Assay for SARS-CoV-2-Neutralizing Antibodies

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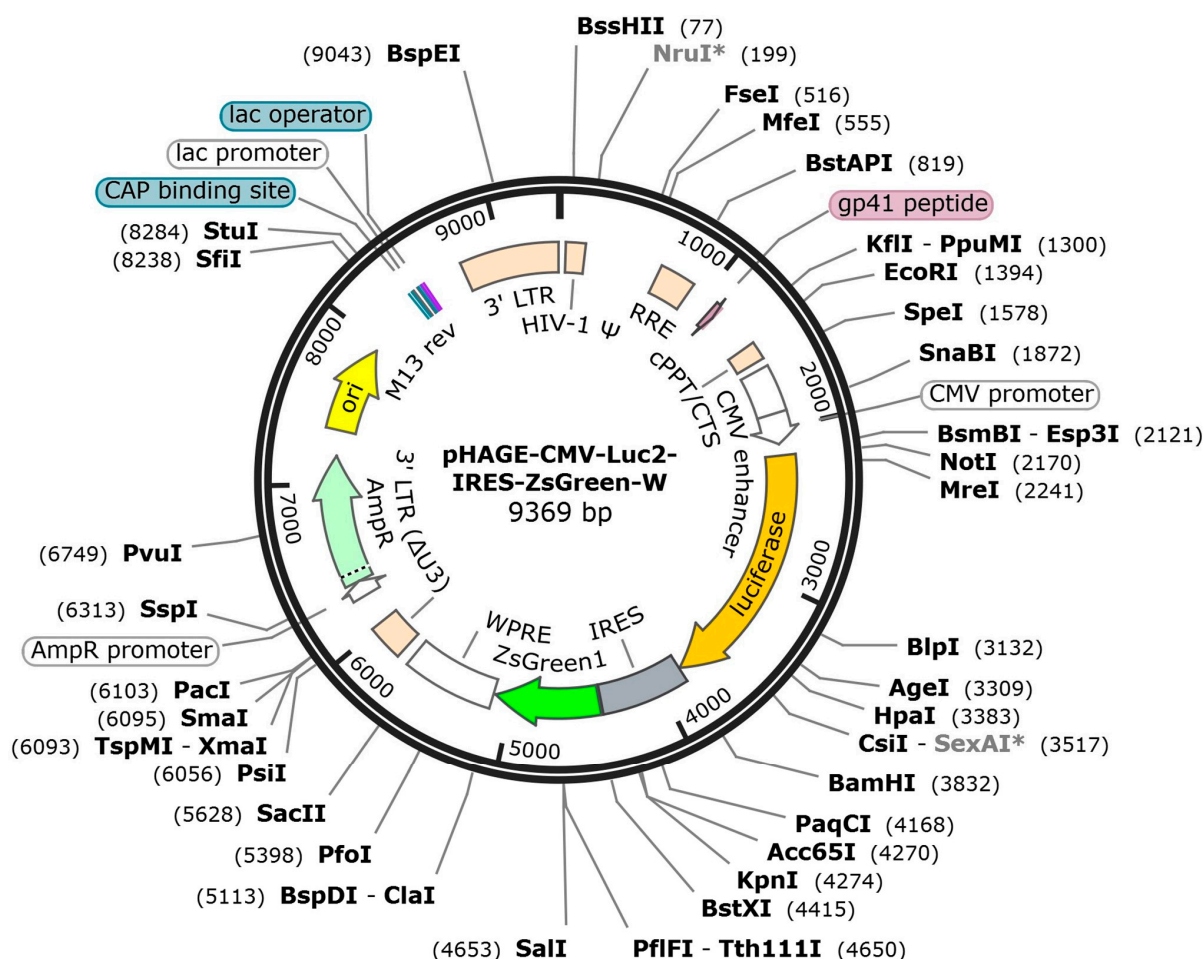


Figure S1. pHAGE-CMV-Luc2-IRES-ZsGreen-W vector map designed using SnapGene version 7.1.0.

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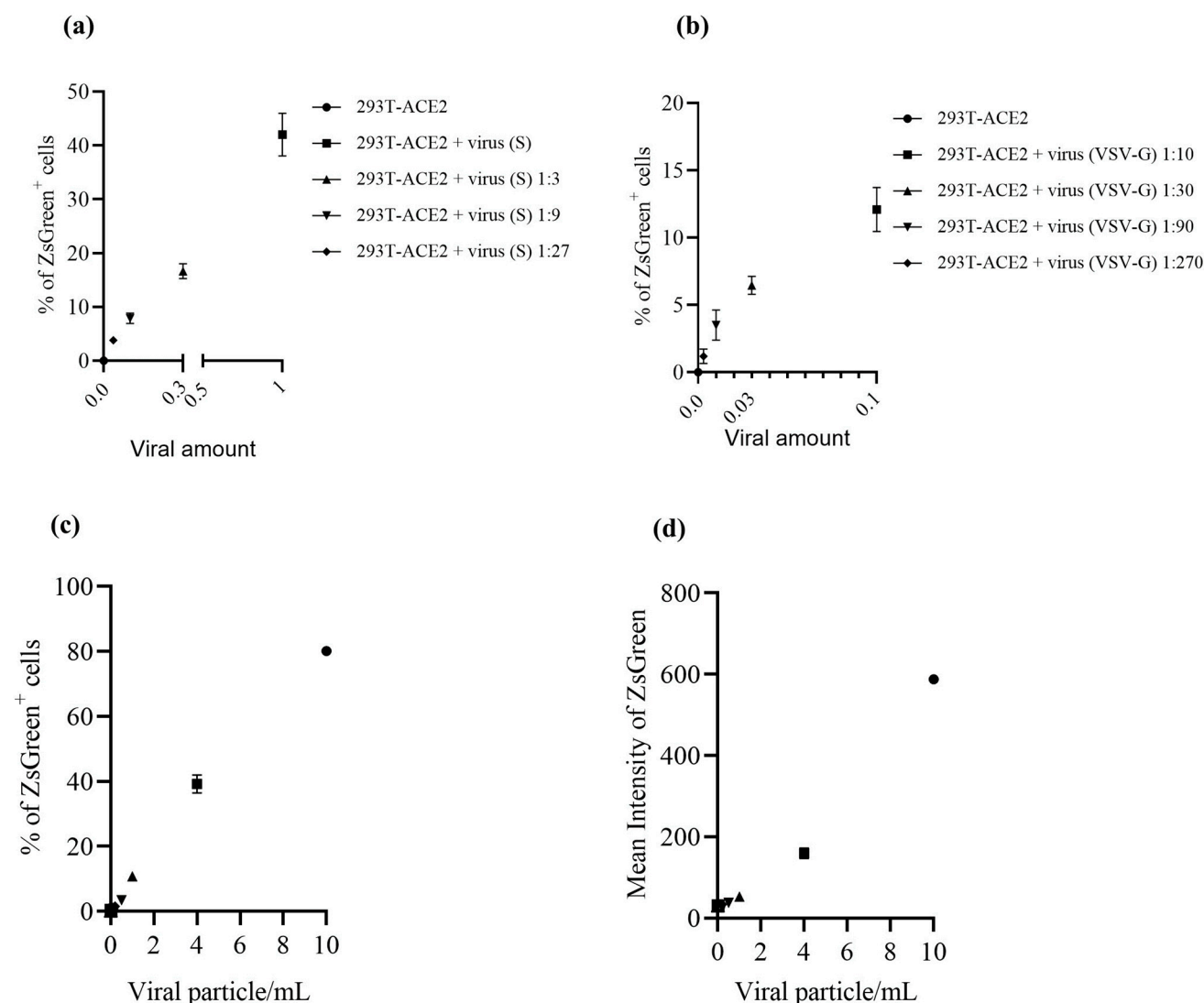
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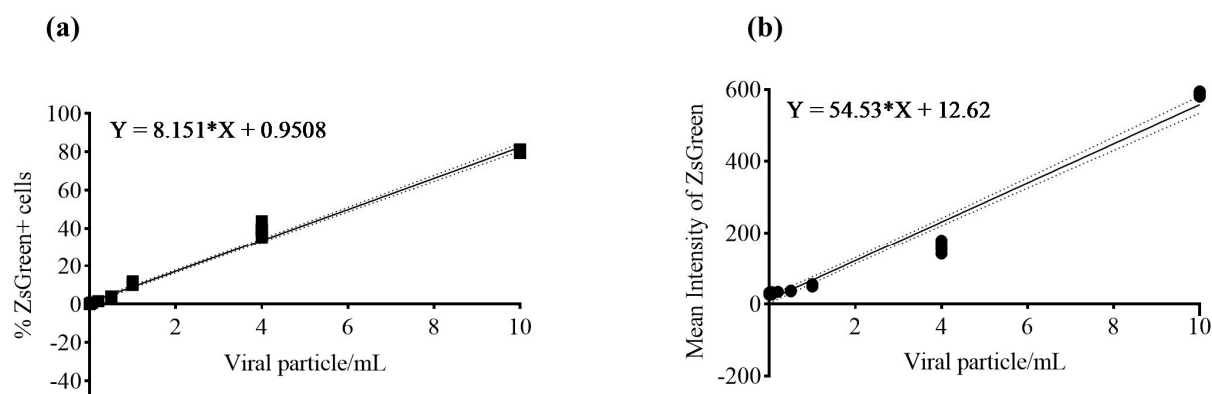
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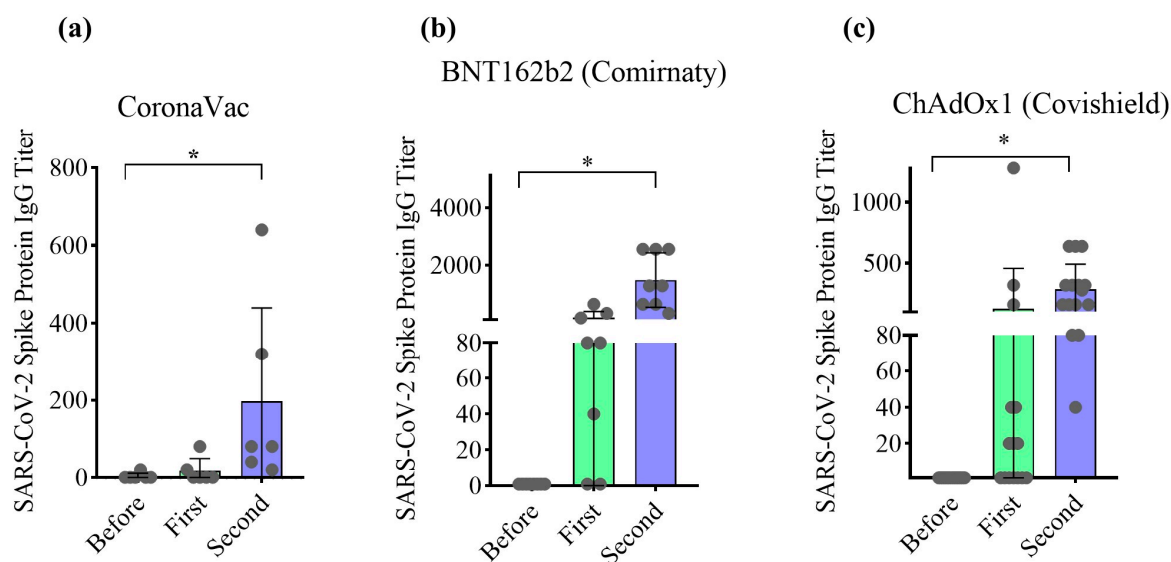
**Figure S2.** pHAGE-CMV-Luc2-IRES-ZsGreen-W vector sequence



**Figure S3.** Linear regression related to Figure 2. **(a)** Linear regression of Figure 2A. For each group the slope calculated were, respectively:  $42.02 \pm 2.294$ ;  $55.47 \pm 2.667$ ;  $78.77 \pm 5.603$ ;  $103.5 \pm 7.257$  **(b)** Linear regression of Figure 2B. For each group the slope calculated were, respectively:  $120.9 \pm 9.468$ ;  $215.3 \pm 12.95$ ;  $350.0 \pm 65.34$ ;  $398.9 \pm 102.3$  **(c)** Linear regression of Figure 2H. For each viral particle/mL the slope  $\pm$  standard error was, respectively:  $8.014 \pm 0.02756$ ;  $9.798 \pm 0.2824$ ;  $10.75 \pm 0.2928$ ;  $6.972 \pm 0.2619$ ;  $7.620 \pm 0.3153$ ;  $12.12 \pm 0.5653$ ;  $20.05 \pm 1.343$ . **(d)** Linear regression of Figure 2I. For each viral particle/mL the slope  $\pm$  standard error was, respectively:  $58.77 \pm 0.2284$ ;  $39.86 \pm 1.346$ ;  $52.67 \pm 0.9739$ ;  $75.10 \pm 1.013$ ;  $167.7 \pm 1.673$ ;  $513.0 \pm 15.07$ ;  $1510 \pm 21.98$ ; vertical line.

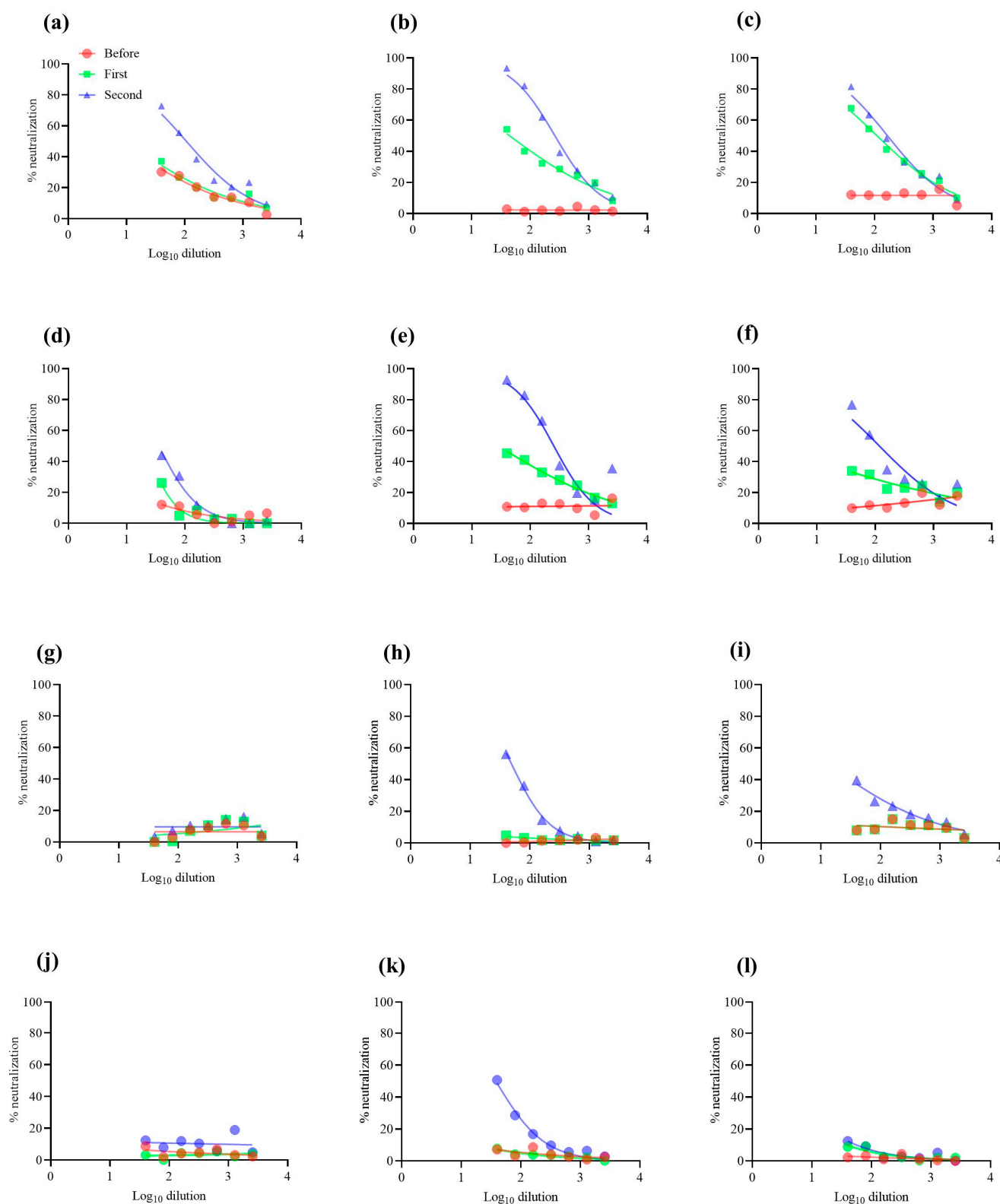


**Figure S4.** Correlation curve and equation were determined for (a) ZsGreen<sup>+</sup> cells (95% CI from 0.9775 to 0.9993,  $p < 0.0001$ ) and the (b) average fluorescence intensity (95% CI from 0.9244 to 0.9976,  $p < 0.0001$ ), based on the results showed on Figure 2H and 2I, respectively.

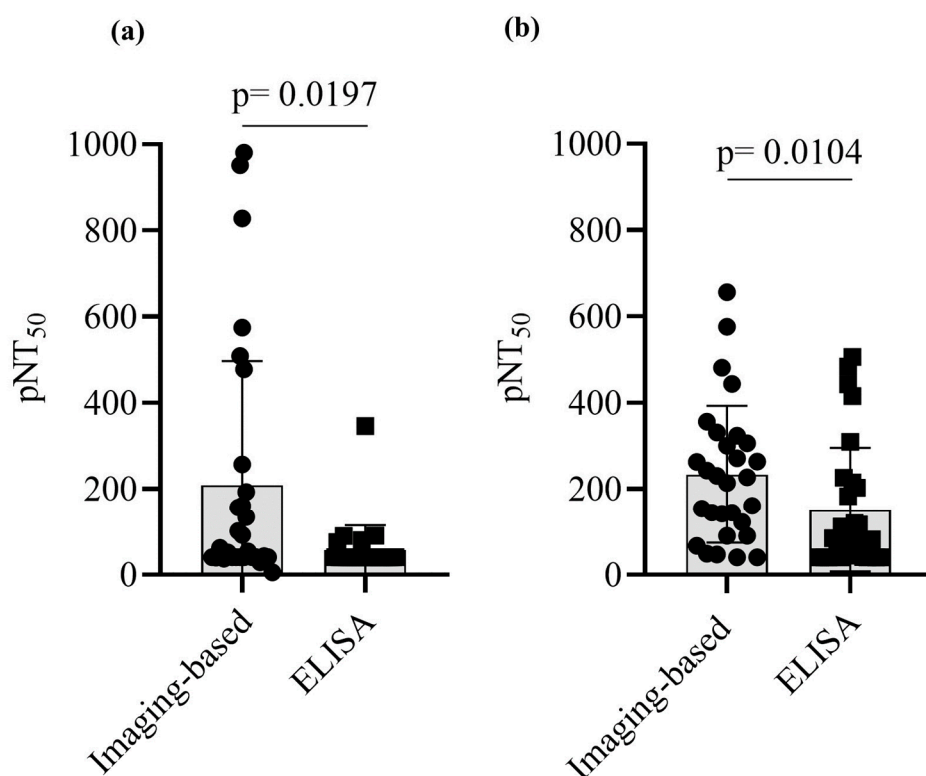


**Figure S5.** IgG titers were measured in serum samples by indirect ELISA using Spike protein as antigen. (a) Volunteers immunized with CoronaVac ( $n = 7$ ). (b) Volunteers immunized with Comirnaty ( $n = 8$ ). (c) Volunteers immunized with Covishield ( $n = 15$ ). Values were considered statistically significant when  $p < 0.05$  (\*) by Friedman test, followed by Dunn's multiple comparisons test, calculated by GraphPad Prism, version 8.0. Data are shown as mean  $\pm$  SD.





**Figure S6:** Non-linear regression curves related to Figure 6. (a-c) Pseudovirus wild type, (d-f) Delta, (g-i) Omicron B.1.529, (j-l) Omicron BA4/5.



**Figure S7.** Comparison between pNT<sub>50</sub> values obtained from imaging-based neutralization analysis **(a)** and by commercial ELISA kit. **(a)** pNT<sub>50</sub> values obtained by imaging-based or ELISA methods after the first dose of CoronaVac, Comirnaty and Covishield. **(b)** pNT<sub>50</sub> values obtained by imaging-based or ELISA methods after the second dose of CoronaVac, Comirnaty and Covishield. Graphs represent pooled data from all three vaccines following the administration of one or two doses. \* $p < 0.05$ , obtained by Mann Whitney test. calculated by GraphPad Prism, version 8.0. Data are shown as mean  $\pm$  SEM.