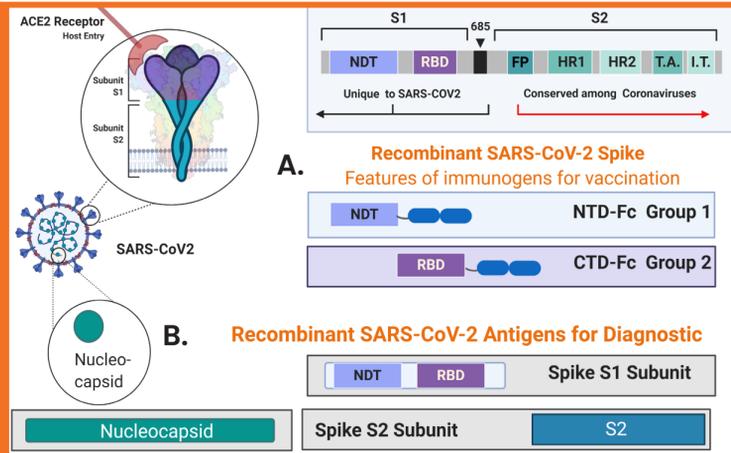


# Investigation of SARS-CoV-2 Proteins as Potential Vaccine Targets and Development of Companion Diagnostics for use in Nonhuman Primates.

Lindsey Moser<sup>1</sup>, Keith Mansfield<sup>2</sup>, Monika Burns<sup>2</sup>, Lori Martin<sup>2</sup>, Daise Cunha<sup>3</sup>, Jessica Fischer<sup>3</sup>, David Rancour<sup>4</sup>, Fritz Schomburg<sup>4</sup>, Kimberly Luke<sup>1\*</sup>.

1. Intuitive Biosciences, Madison WI USA \*corresponding author, 2. Novartis Institutes for Biomedical Research, Cambridge, MA USA, 3. Covance Greenfield Laboratories, Greenfield, IN USA, 4. Lytic Solutions, Madison WI USA

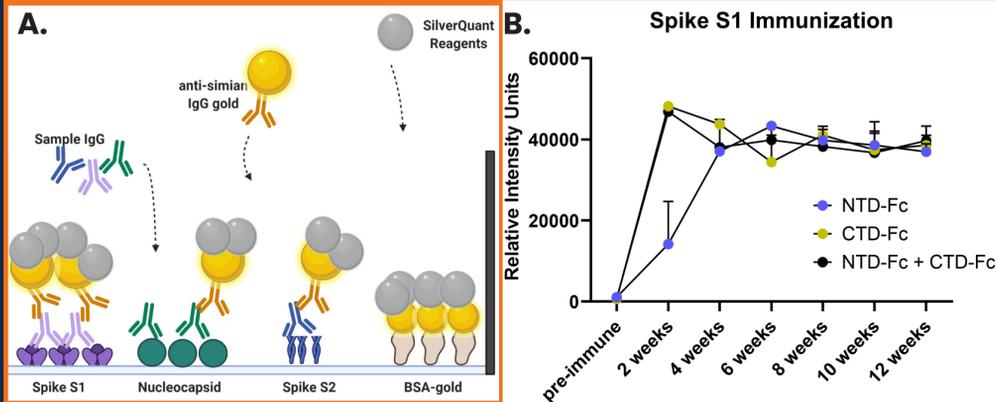
## Select Spike Subunits for Vaccination and SARS-CoV-2 Specific Antigens for a Diagnostic Serology Test



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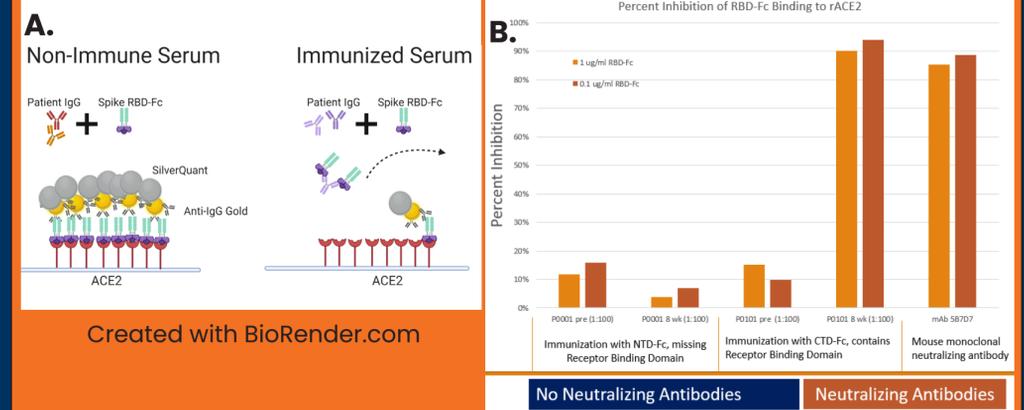
**Figure 1.** The S1 subunit of the structural protein Spike binds to host ACE2 receptor and facilitates entry. This makes it an excellent target for vaccines to disrupt that interaction and prevent entry. **A.** For immunization, the NTD= N-terminal Domain and the CTD= C-Terminal Domain of Spike S1 subunit were selected. The CTD-Fc contains the Receptor Binding Domain (RBD). **B.** For the diagnostic, the S1 subunit of Spike and the full recombinant Nucleocapsid protein were selected as specific antigens. Spike S2 subunit is used as a confirmatory antigen.

## Evaluate Antibody Response with CSA: SARS-CoV-2 Serology Assay



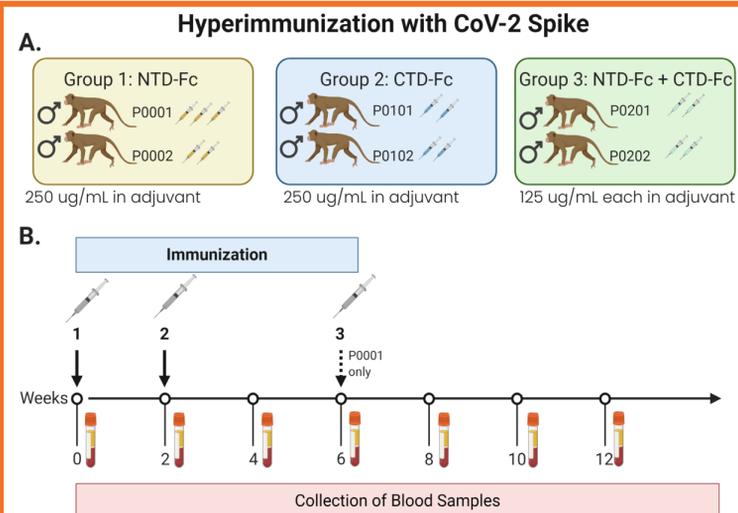
**Figure 3. A. Schematic illustrating the CSA: SARS-CoV-2 assay steps.** Antigen Spike S1 and Nucleocapsid are used for sensitivity, and Spike S2 is used for confirmation. Specific antibodies in serum are detected with SilverQuant™ reagents. **B. Serum samples from 3 vaccine groups** (NTD-Fc in blue, CTD-Fc in yellow, and NTD-Fc + CTD-Fc in black) were analyzed with the CSA:SARS-CoV-2 assay over 12 weeks, with serum collected every 2 weeks. Relative Intensity Units are shown on the y-axis and time post-immunization is shown on the x-axis. A robust antibody response was detected as early as 2 weeks following the first immunization. Only the NTD-Fc vaccinated animals had a slower response to vaccination.

## CTD-Fc Vaccination Results in Neutralizing Antibodies



**Figure 5. A. SARS-CoV-2 Neutralizing Antibody Assay.** Recombinant purified ACE2 was immobilized on the surface of the CSA plates. Recombinant spike Receptor Binding Domain (RBD-Fc) was incubated with test serum. If neutralizing antibodies are present these antibodies will compete with ACE2 for binding to RBD-Fc and washed away. Only RBD-Fc bound to ACE2 on the surface is detected in this assay. A decrease in signal indicates presence of neutralizing antibodies. **B. Results from Immunized Cynos.** Serum from P0001 (NTD-Fc immunized) did not show any change in ACE2-RBD inhibition from 0 to 8 weeks post immunization. Serum from P0101 (CTD-Fc immunized) resulted in >90% inhibition of ACE2 binding to RBD at 8 weeks post immunization similar to a known mouse monoclonal neutralizing antibody.

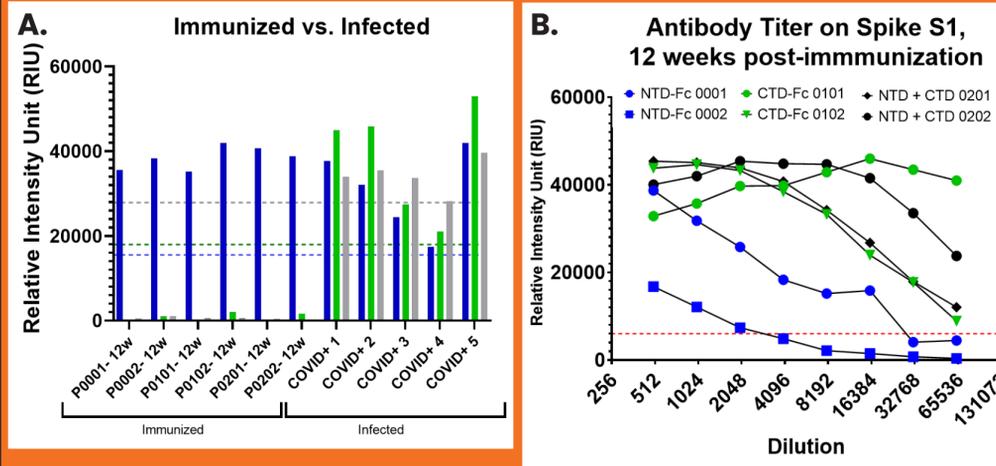
## Hyperimmunization Schedule with Spike Protein Subunits to Generate Antibody Response in NHP



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**Figure 2. A.** Six male cynomolgus macaques were immunized with one of 3 Spike antigen formulations. Each group contained 2 randomly assigned animals. All 3 groups were immunized twice; animal P0001 received 3 total doses. **B.** Blood draws were performed every 2 weeks over the duration of the study, and processed for serum.

## Antibody Profile Distinguishes Infected from Vaccinated and High Antibody Titers Generated



**Figure 4. A.** Samples from NHP 12 weeks post-vaccination (immunized) were compared to 5 confirmed human COVID-19 patients by screening on the CSA: SARS-CoV-2 assay. Relative Intensity Units are graphed on the y-axis, and the groups of immunized NHP or recovered human samples are shown on the x-axis. Dotted lines represent the cut-off value for each antigen. Spike S1 (blue), Nucleocapsid (green), Spike S2 (grey). **B.** Serial dilutions of serum were screened on the CSA: SARS-CoV-2 assay to determine antibody titers at 12 weeks. The lowest dilution at which the serum remains above the cut-off value is the titer.

## Conclusions and Future Directions:

- Immunization with Spike Receptor Binding Domain (CTD-Fc) produced a robust antibody response with a duration longer than 20 weeks (data not shown, see link below).
- Use of a multiplex serological assay like the CSA: SARS-CoV-2 test can distinguish between immunized/vaccinated and infected individuals.
- Immunization with Spike Receptor Binding Domain (CTD-Fc) results in a neutralizing antibody response, which generates antibodies that can prevent Spike binding to the host receptor ACE2.
- Further studies with CTD-Fc immunization of macaques will examine dosing schedule and maternal transfer of antibodies.

For detailed Materials and Method and for additional data for this study, please [click here to follow the link](#) to more information or scan the QR code to the right.

