A Composite Peptide Vaccine Comprised of Conserved SARS-CoV-2 and Influenza Epitopes Generated Antisera Reponses to Both Coronavirus and Influenza

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Background

- Over the last 15 years the team at **Longhorn Vaccines and Diagnostics** has developed **composite peptide vaccines that combine multiple conserved epitopes** from **one or more pathogens**, including **viruses** and **bacteria**. Our peptide vaccines are compatible with **multiple adjuvants.**
- **LHNVD-105**, a **universal influenza vaccine candidate**, which is a **multi-epitope, dual unconjugated** influenza composite peptide formulation, comprising of conserved epitopes of **HA, NA and M2e**, and t-cell epitopes delivered with the US Army's Liposomal Adjuvant (**ALFQ**), generated a **robust, durable and balanced** immune response with cross-reactive neutralizing antibodies.
- GMP manufacturing is underway and a **Phase I clinical trial of LHNVD-105** is expected to begin **in 2023**.
- The **convenience of designing and manufacturing (scaling up)** peptides and the **immunogenicity** observed in mouse studies with several peptide combinations, led us to design another composite peptide vaccine comprising of **Influenza and SARS-CoV-2** conserved epitopes, thereby **targeting multiple respiratory pathogens**.
- In this study, we have used composite peptide vaccines comprising of conserved epitopes of either **SARS-CoV-2 RNA polymerase or Spike protein**, each including conserved **Influenza (NA and M2e)** epitopes. Each composite peptide also contained a **universal tetanus T cell epitope**.
- Immunizing **outbred (ICR) mice** with the above-mentioned composite peptide vaccine, **targeting IV and SARS-CoV-2**, generated a **robust and durable immune response to both influenza and coronavirus.**

Components of the Composite Peptide Vaccines comprised of Coronavirus and Influenza epitopes

Peptide ID	Epitopes
Coronavirus Pep02	**SARS-CoV-2** RNA Polymerase (**RNA Pol.**) + tetanus **T Cell epitope**
Coronavirus Pep05	**SARS-CoV-2 RNA Pol.** + Influenza Virus (**IV**) neuraminidase (**NA**)+Matrix protein (**M2e**) + tetanus **T Cell epitope**
Coronavirus Pep11	**SARS-CoV-2** Spike protein (**SP**) + **IV NA+ M2e** + tetanus **T Cell epitope**

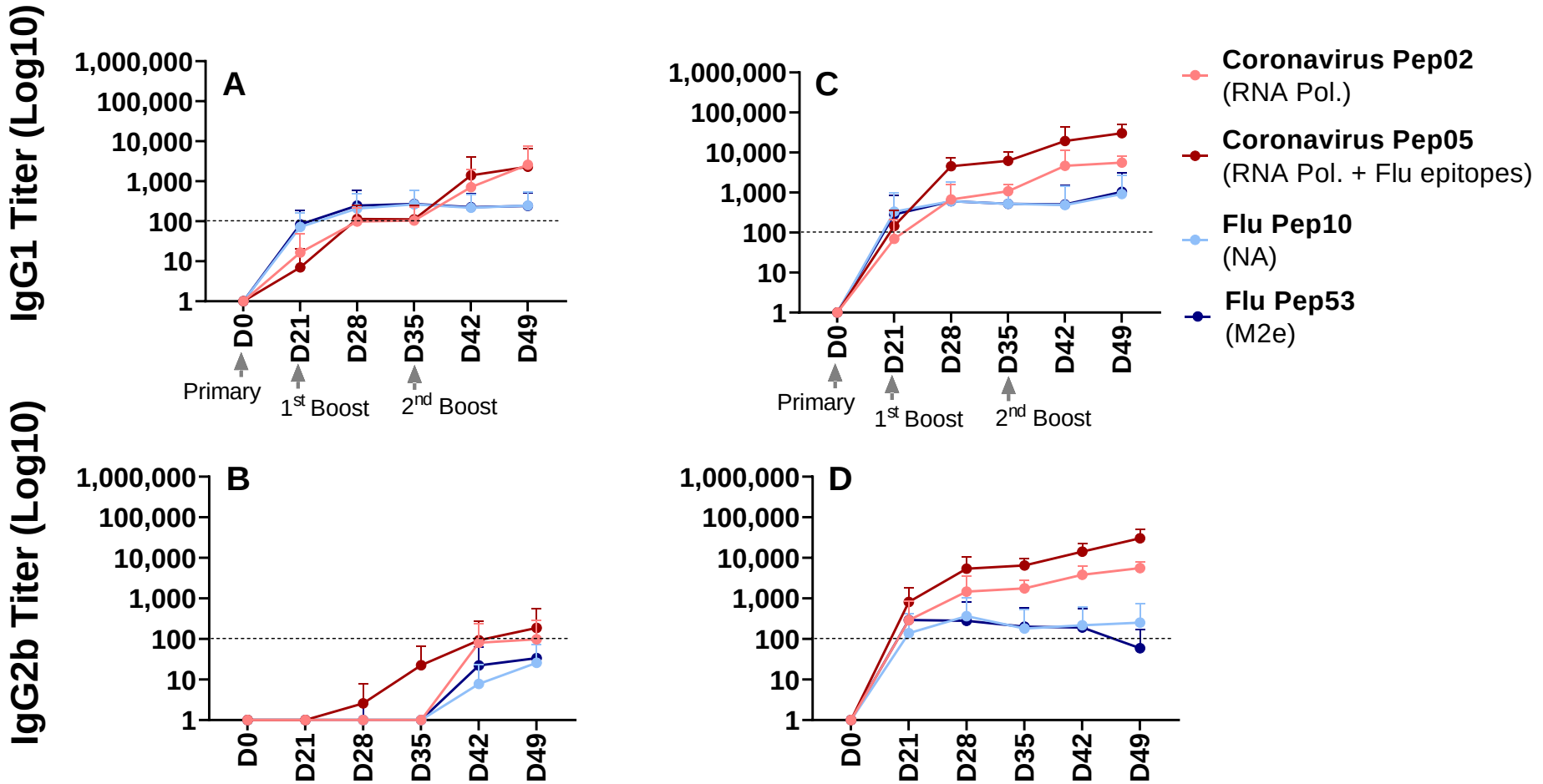
Immunization of ICR mice with Composite Peptide Vaccines

Group	Immunogen	Adjuvant	Dose	Route	Immunization days
1	**Coronavirus Pep02** Unconjugated	**AddaVax™**	**20µg**	**SQ**	**0, 21, 35**
2	**Coronavirus Pep05** Unconjugated	**AddaVax™**	**20µg**	**SQ**	**0, 21, 35**
3	**Coronavirus Pep11** Unconjugated	**AddaVax™**	**20µg**	**SQ**	**0, 21, 35**

Immune responses to SARS-CoV-2 RNA polymerase were enhanced in the presence of Influenza NA and M2e epitopes

Mice immunized with Coronavirus Pep02 (SARS-CoV-2 RNA Pol.)

Mice immunized with Coronavirus Pep05 (SARS-CoV-2 RNA Pol. + IV NA+M2e)



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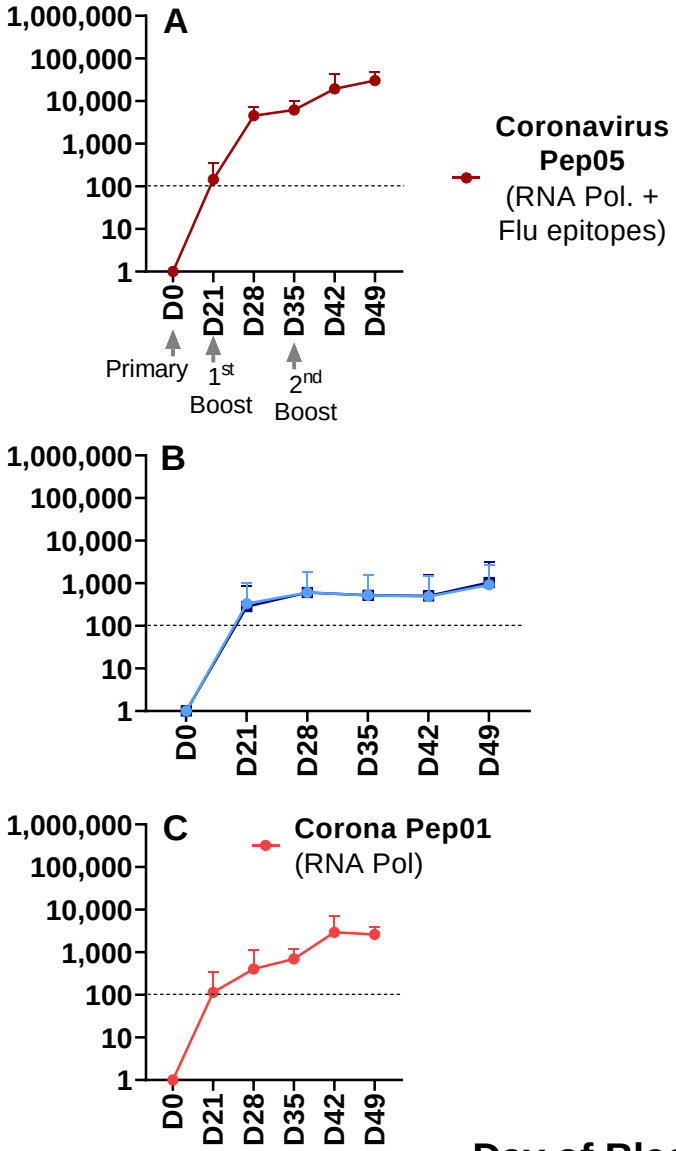
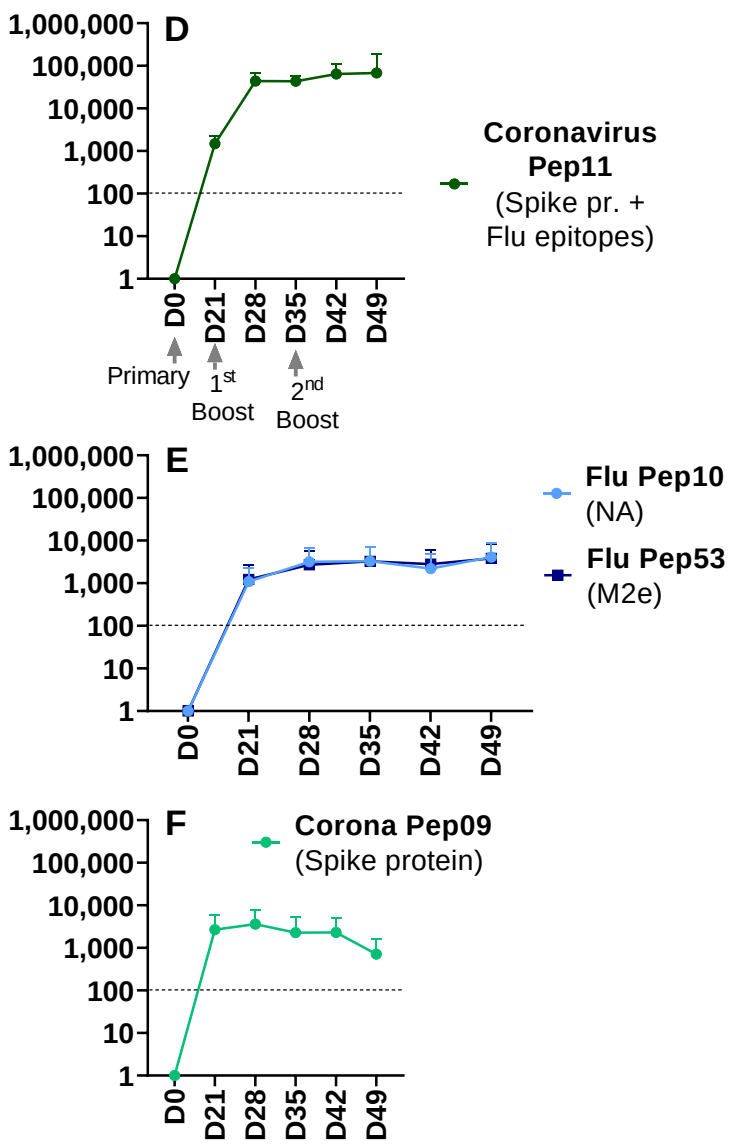
Mice immunized with Coronavirus Pep05
(SARS CoV-2 RNA Pol.+Flu NA, M2e)

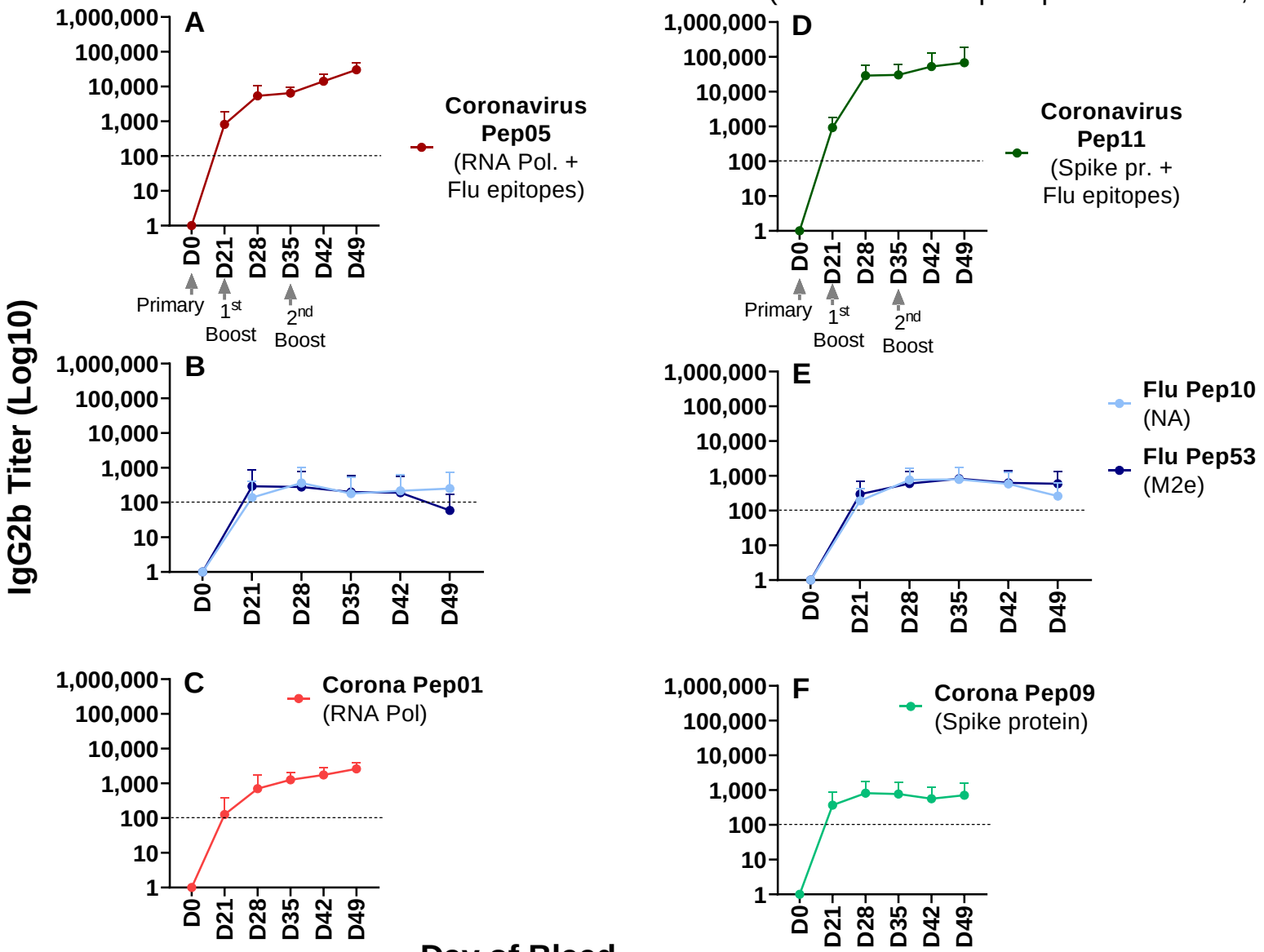


Mice immunized with Coronavirus Pep11
(SARS CoV-2 Spike protein+Flu NA, M2e)



In the presence of influenza epitopes, SARS-CoV-2 RNA polymerase and spike protein generated good IgG1 titers

Mice immunized with Coronavirus Pep05
(SARS CoV-2 RNAPol.+Flu NA, M2e)



Mice immunized with Coronavirus Pep11
(SARS CoV-2 Spike protein+Flu NA, M2e)

In the presence of influenza epitopes, SARS-CoV-2 RNA polymerase and spike protein generated good IgG2b titers

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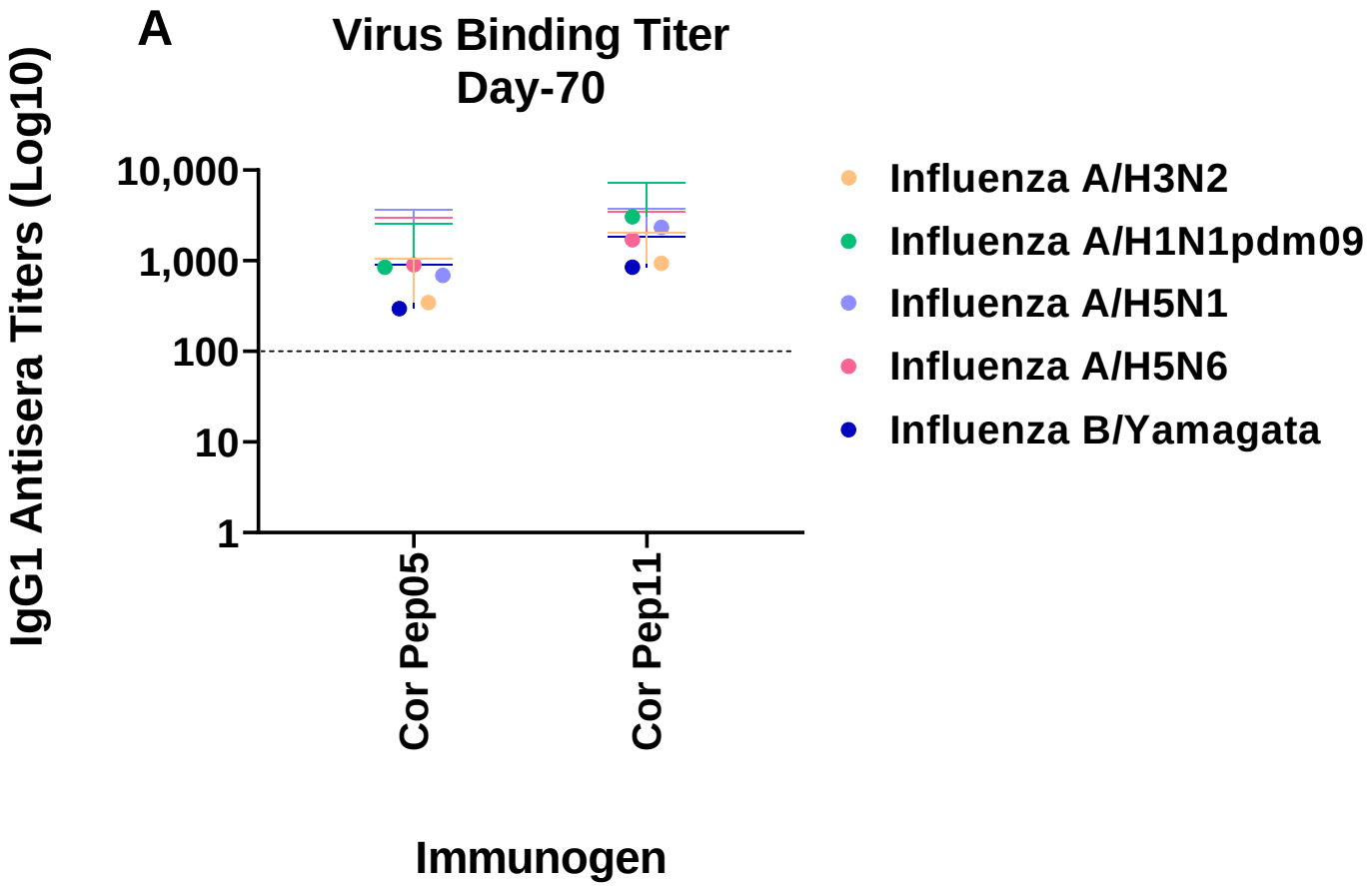
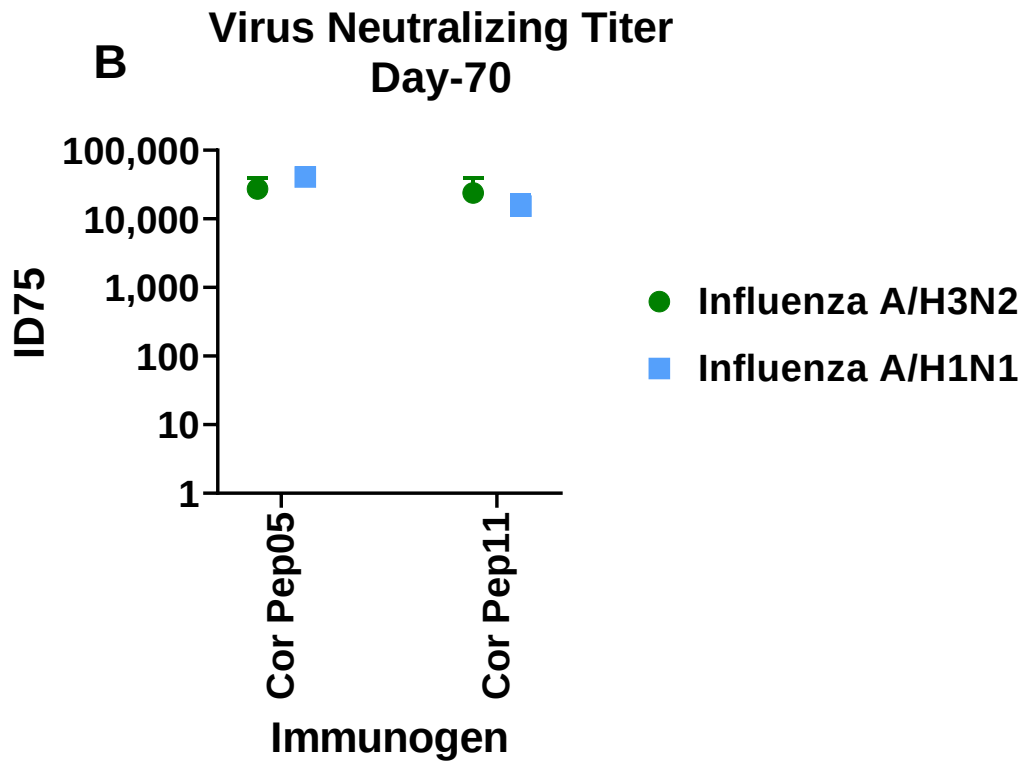
Antisera showed binding and neutralizing titers to Influenza A and B virus

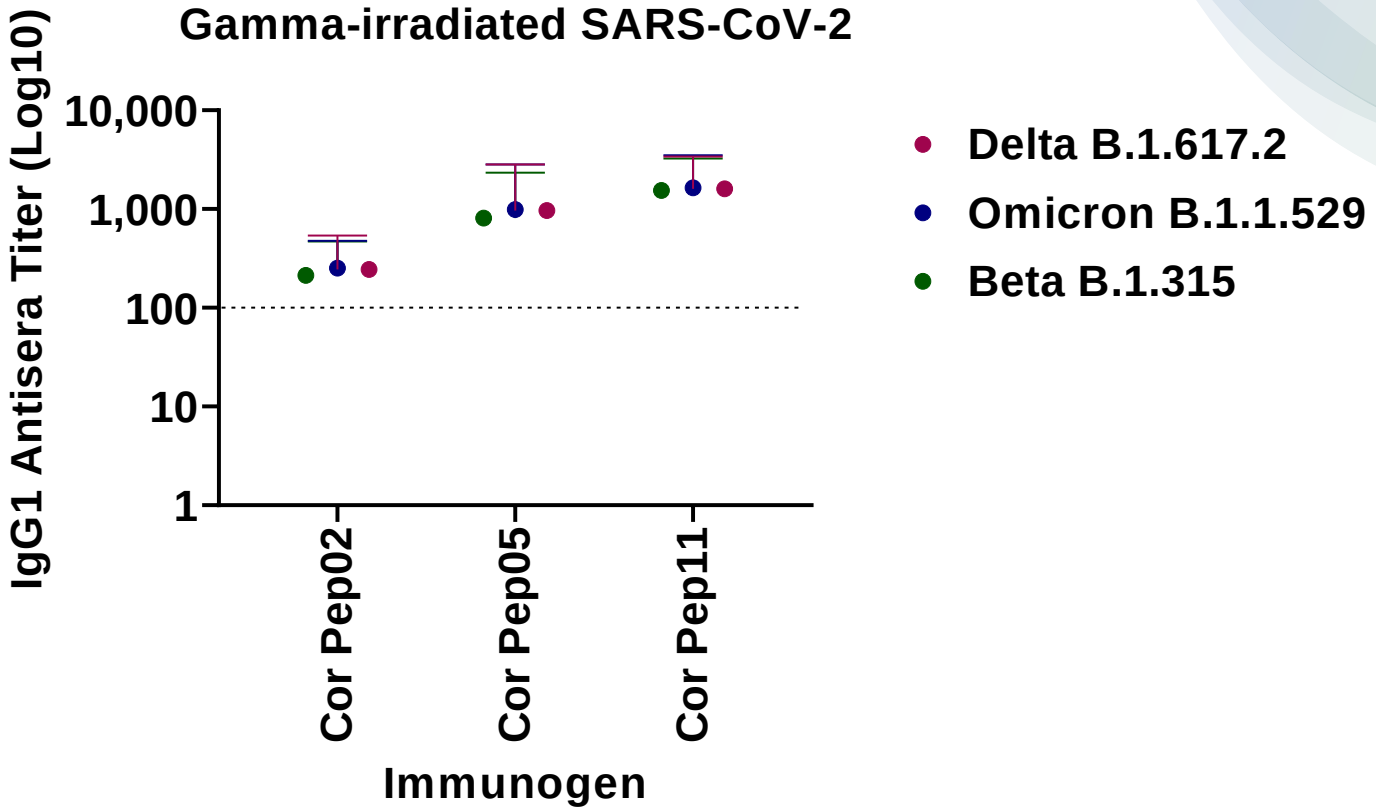




Influenza A/H3N2: Influenza A/Hong Kong/4801/2014 (H3N2)
Influenza A/H1N1: Influenza A/California/07/2009 (H1N1) pdm09
Influenza A/H5N1: BPL-inactivated Influenza A/India/NIV/2006(H5N1)-PR8-IBCDC-RG7
Influenza A/H5N6: BPL-inactivated Influenza A/Sichuan/26221/2014 (H5N6)-PR8-IDCDC-RG42A
Influenza B/Yamagata: Influenza B/Oklahoma/10/2018 (BY) (NA D197N)

Antisera showed recognition of gamma-irradiated SARS-Cov-2 variants

Day-252
Durability study



Delta B.1.617.2: Gamma-irradiated hCoV-19/USA/MD-HP05285/2021 (Lineage B.1.617.2; Delta Variant)
Omicron B.1.1.529: Gamma-irradiated hCoV-19/USA/GA-EHC-2811C/2021 (Lineage B.1.1.529; Omicron Variant)
Beta B.1.315: Gamma-irradiated hCoV-19/USA/MD-HP01542/2021 (Lineage B.1.351)

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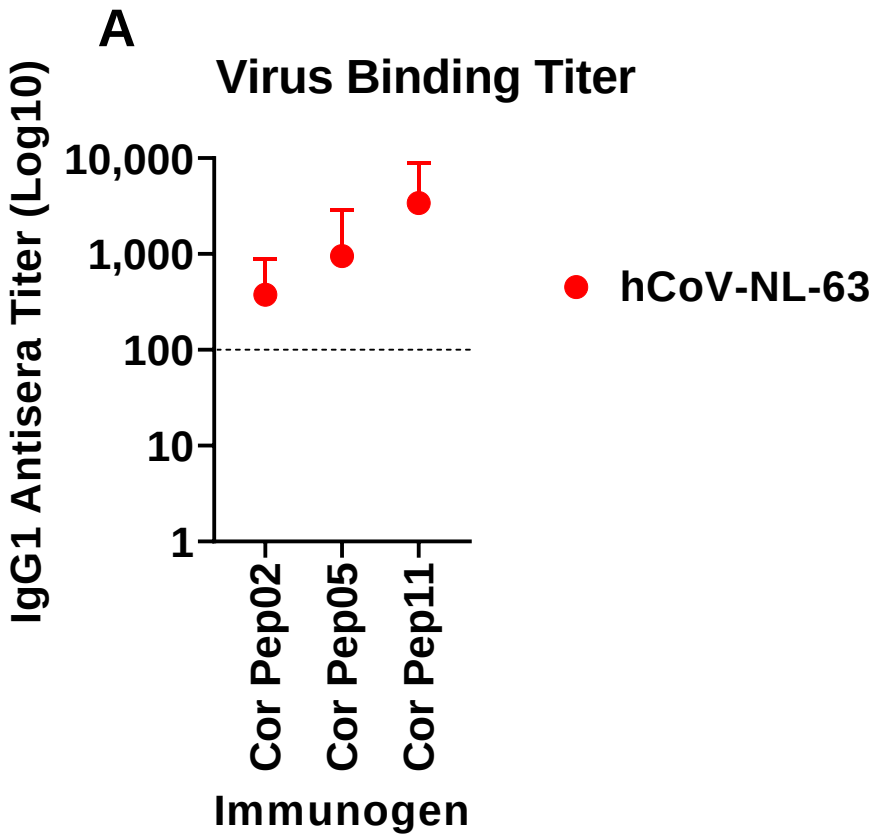
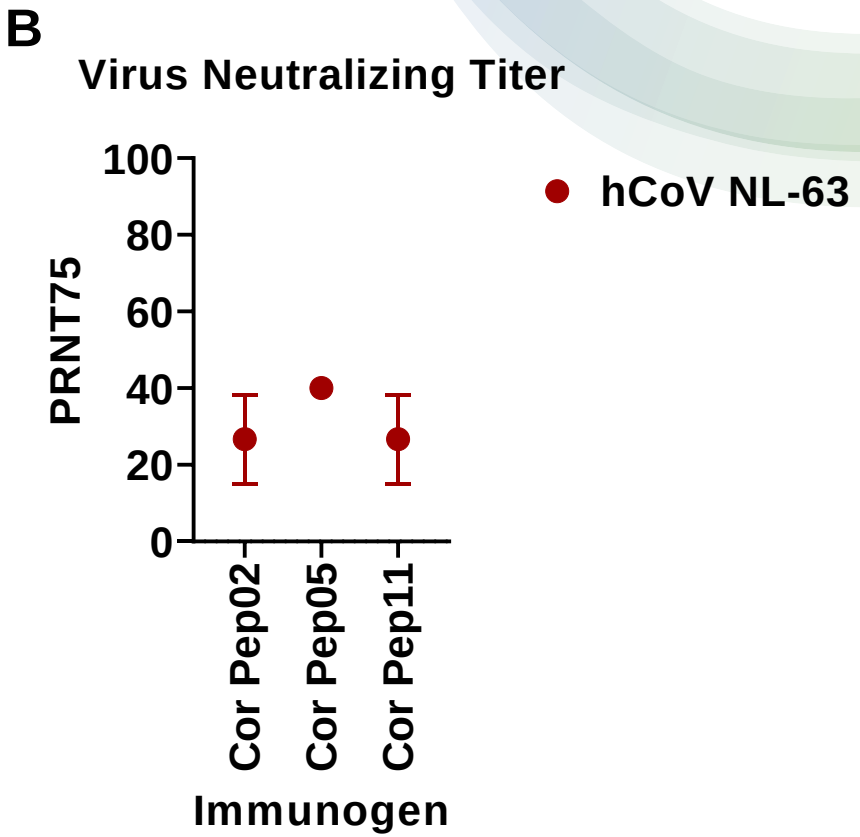
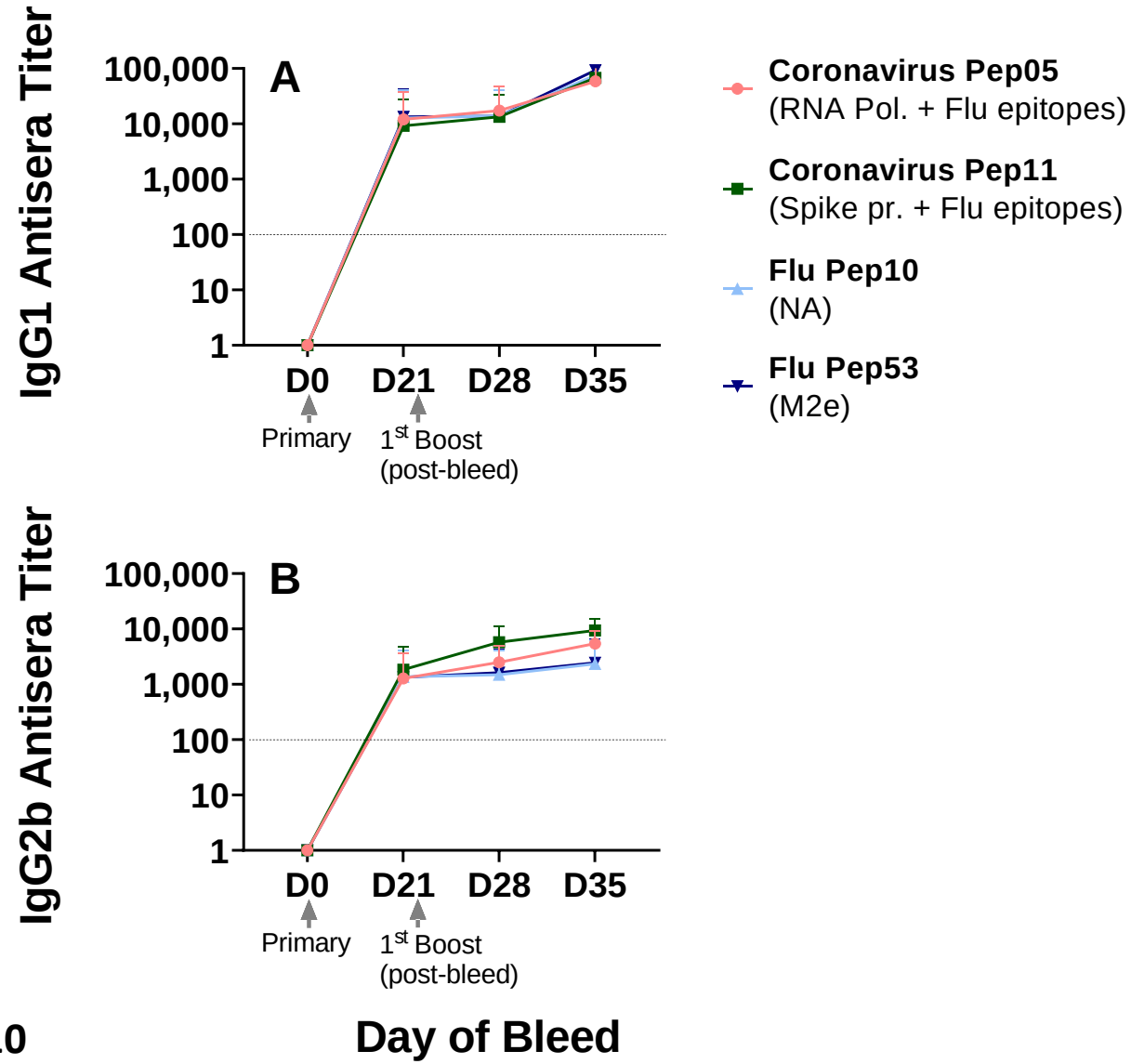
Antisera showed binding and neutralizing titers to Human Coronavirus NL-63

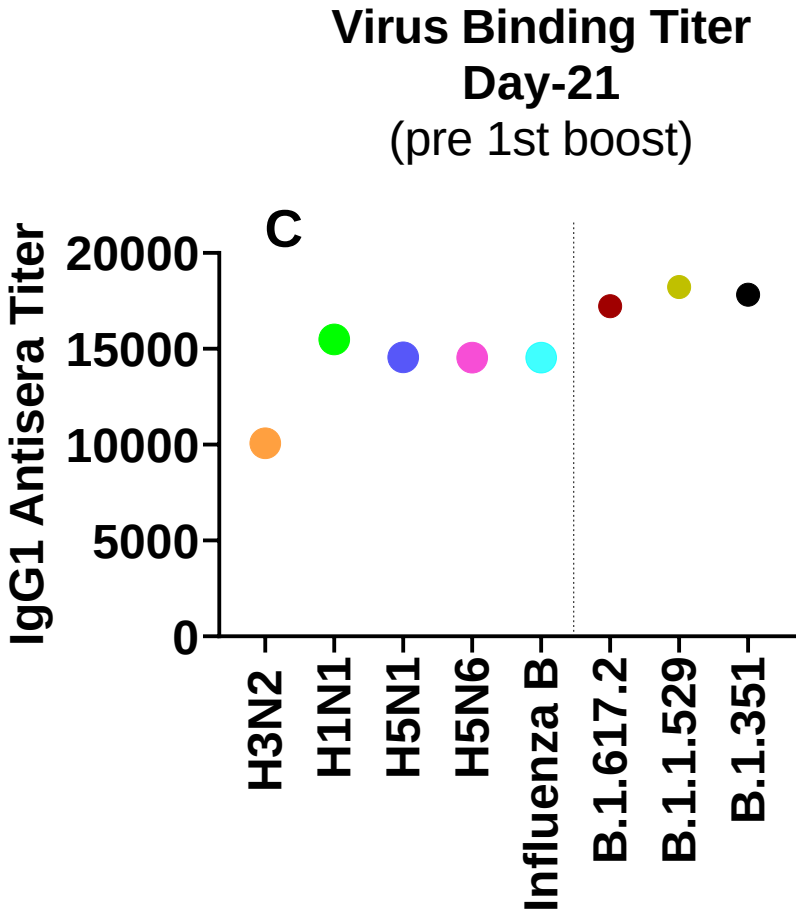
Day-266





Immune responses in mice immunized with a combination of **Coronavirus Pep05** (SARS-CoV-2 **RNA Pol.** + IV NA+M2e + T cell epitope) and **Coronavirus Pep11** (SARS-CoV-2 **SP** + IV NA+M2e + T cell epitope)





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Conclusions:

- Immunization of mice with **composite peptide vaccines** comprising of **conserved epitopes** of either **SARS-CoV-2 RNA polymerase** or **Spike protein**, each including conserved **Influenza (NA and M2e)** epitopes generated **cross-reactive and durable neutralizing antibodies to both Coronavirus and Influenza**.

- **Th1 and Th2** responses were observed (**IgG1 and IgG2b** titers) and the titers **remained steady for beyond 250 days** post primary immunization, **without additional boosts** post day-35, indicating the width and durability of immune responses.

- Combination of **SARS-CoV-2 RNA polymerase** and **Spike protein** composite peptides containing **influenza NA and M2e** epitopes and **tetanus T cell epitopes**, generated **highly enhanced immune responses** with **only primary immunization**, which increased further with a boost (**titers up to 100,000**).

- There may be an **advantage** of including **more targets** (e.g., SARS-CoV-2 RNA Pol., SP and Influenza NA, M2e, etc.) with **universal T cell epitopes** in **enhancing the immunogenicity** of composite peptide vaccines.

Outlook:

- **Further evaluation** of **combination of SARS CoV-2 RNA Pol. + SP** composite peptides with **Influenza NA+M2e** epitopes with respect to **cytokine responses and protection studies**.

- Composite **multi-epitope**, **unconjugated peptides** are a powerful vaccine platform that can be used for **multiple pathogens (viruses, bacteria)** and are **easily scalable** and **cost-effective**.

- We, at **Longhorn Vaccines and Diagnostics**, have analyzed several different **composite peptide vaccines** – separately and in combinations – against **influenza, tuberculosis, coronavirus** using **many different adjuvants with consistent results.**

Thank You!

Richard F. Schuman
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Clara J. Sei
Gerald W. Fischer
Jeff Fischer

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