Introduction: Despite the widespread use and efficacy of vaccines, the mechanisms undermining how and why certain vaccines work remain poorly understood with the majority of vaccine design of the past being largely empirical (Shen-Orr S. and Furman D., 2013). Recent advances in technology have allowed for a systems level approach to understanding the mechanisms underlying vaccine-induced protection. Most notably, the application of systems vaccinology has leveraged multiomics both pre and post vaccination to strengthen rational design approaches (Raeven R., et al, 2019). At the patient level, this has shed insights into the complex biological variability to vaccination of individuals within a population. This has driven a new emphasis towards identifying baseline correlates of positive (strong and robust immunity) vaccine responses (Tsang J., 2015). Within the context of Influenza, vaccination remains the most effective measure at preventing flu and its complications. One of the most pertinent challenges facing Influenza vaccination is the evolutionary arms race between vaccine development and antigenic drift (Janssens Y. et al., 2022). As such, annual Influenza vaccinations are based primarily on what is predicted to be the dominant strains that season. However, even in cases where the seasonal vaccine matches the circulating influenza strains, the highly variable nature of the vaccination response leaves some individuals unprotected despite receiving the vaccine (Tsang J., 2015). Previous studies from the Davis group at Stanford have identified a robust list of pre-vaccination cytokines in patient sera which correlate to a higher antibody response (e.i. stronger immunity) from a longitudinal cohort study across 5 flu seasons (n = 664). Of the cytokines screened, the interferon-β (IFNβ) cytokine was identified as one of the most abundant proteins in the sera of individuals who had a robust post-vaccine immune response. IFNβ is a type 1 interferon signaling protein that binds to the heterodimeric cytokine receptor comprised of IFNAR1 and IFNAR2 subunits (de Weerd N., et al., 2007). In this study, patient-derived spleen organoids were used as a model system to analyze the effects of IFNβ on antibody production in response to vaccination. Thus, the central question this analysis seeks to address is as follows**: What is the impact of interferon–β on antibody production when exposed to flu vaccination?**

Methods:

Luminex Immunoassay: 4 patient derived spleen organoids were seeded and cultured in a sterile BSL2 environment. The organoids were named as follows with the identifying number being the patient from which they were derived: sp43, sp44, sp46, and sp54. The cells were split into 4 conditions. The first of which was the negative control wherein the cells were not exposed to any vaccine or IFNβ. The second condition was the addition of IFNβ alone. The third condition was the addition of IFNβ with the 2023-24 Fluzone Quadrivalent inactivated influenza vaccine (IIV). As per the United States Public Health Service (USPHS) requirements, the Fluzone High-Dose Quadrivalent is formulated to contain the HA protein of the following four influenza strains: A/Victoria/4897/2022 IVR-238 (H1N1), A/Darwin/9/2021 SAN-010 (H3N2), B/Phuket/3073/2013 (B Yamagata lineage), and B/Michigan/01/2021 (a

B/Austria/1359417/2021-like virus, B Victoria lineage). The fourth condition was the addition of IFNβ with the 2023-24 FluMist Quadrivalent live, attenuated influenza vaccine (LAIV). FluMist Quadrivalent contains four influenza strains as per the recommendation of USPHS : an A/H1N1 strain, an A/H3N2 strain and two B strains from both the B/Yamagata/16/88 and the B/Victoria/2/87 lineages. The supernatant was collected from the organoids periodically (t = 3 days, 7 days, and 10 days) and stored at 4℃. All samples were then sent to the Human Immune Monitoring Center (HMIC) at Stanford and analyzed.

Data Analysis: Standard error of the mean (SEM) was used as the statistic to quantify noise and plotted as the error bars for the graphs of the results section. SEM measures the sample variance and hence the variability of sample means around the true population mean. In this case, we want to capture the mean fluorescent intensity from a population of 4 distinct spleen organoids. By using the SEM, we are representing the variability of the sample means around the true population mean while accounting for the fact that the data comes from different samples. If we were to have plotted the standard deviation for the error bars, we would have shown the total variability including within-donor and between-donor variability. For example, had we performed the experiment as a triplicate with the data being collected from the same organoid donor, the standard deviation would have been a more appropriate statistic to report.

Results:

We compared IgG antibody abundance in spleen organoids across each of the four treatment groups for three different strains of the influenza virus. Furthermore, for each strain, we assessed antibody counts in organoids treated with IIV and LAIV. The following figures show the results for each strain:

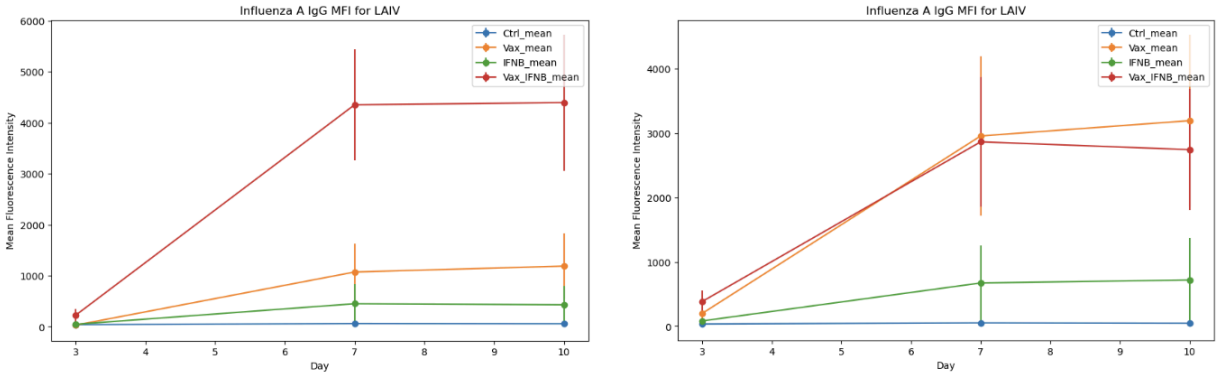

Figure 1. IgG abundance against *Influenza-A*

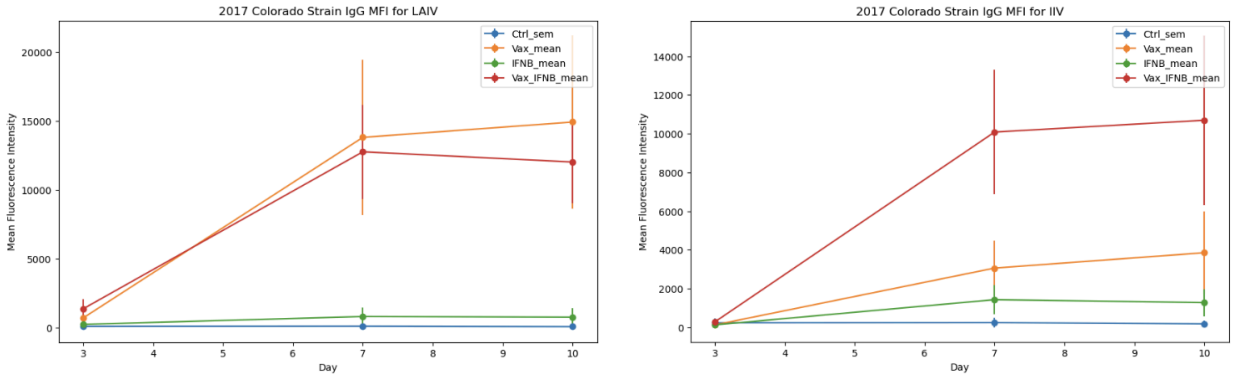

Figure 2. IgG abundance against Influenza Strain *B/Colorado/06/2017/B/Victoria/2019*

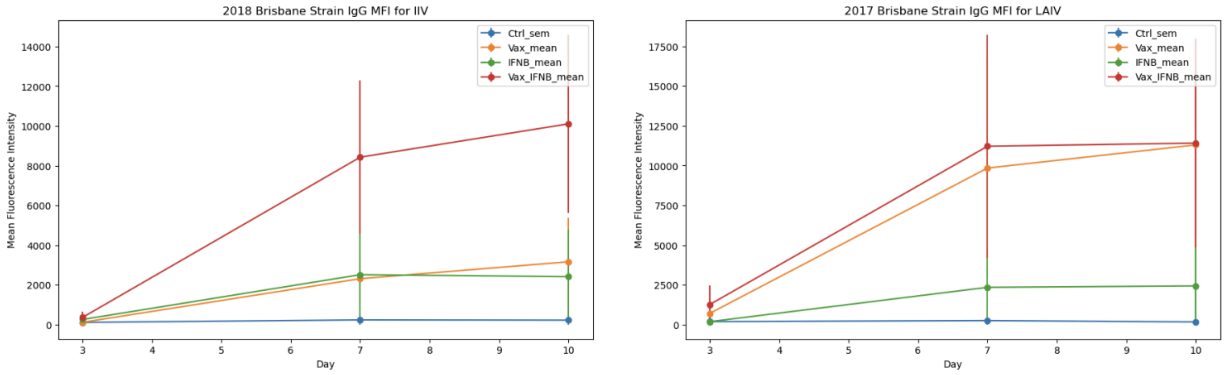

Figure 3. IgG abundance against Influenza Strain *A/Brisbane/02/2018(H1N1)2019*

Key Findings: Across all three strains, we found that antibody counts were highest in the samples treated with both the vaccine and IFNβ. Furthermore, addition of IFNβ to the vaccine treatment seems to have a greater influence on antibody count for samples treated with the IIV; samples treated with LAIV show similar antibody counts regardless of whether IFNβ was administered alongside the vaccine. However, a more detailed statistical analysis is necessary to determine the significance of the effect of IFNβ treatment on antibody abundance.

Discussion: The findings from this preliminary study seem to suggest that the addition of IFNβ seems to cause an increase in antibody production. In context of type I interferon signaling, IFNβ binds to the heterodimeric cytokine receptor with an IFNAR1 and IFNAR2 subunit (de Weerd N., et al., 2007). Interesting next steps would include the demonstration of IFNβ signaling dependence on high antibody titers post-vaccination. To address this, two CRISPR knock outs were generated to delete the IFNAR1 and IFNAR2 subunits with 35% and 75% efficiency, respectively (see appendix). Future directions include optimization of the guide RNAs, and ultimately knockout efficiency, for the IFNAR1 subunit and the generation of a double knockout

of IFNAR1 and IFNAR2 to totally diminish IFNβ signaling. A double knockout is necessary to show IFNβ dependence as IFNAR1 and IFNAR2 may be able to form homodimeric receptors with some IFNβ recognition ability. Additionally, replicates of the experiments reported in '*results*' would allow for greater confidence in the findings and overall significance of this study.

Citations:

1. de Weerd, N. A., Samarajiwa, S. A., & Hertzog, P. J. (2007). Type I interferon receptors: Biochemistry and biological functions. *Journal of Biological Chemistry*, *282*(28), 20053–20057. https://doi.org/10.1074/jbc.r700006200
2. Raeven, R. H., van Riet, E., Meiring, H. D., Metz, B., & Kersten, G. F. (2018). Systems vaccinology and big data in the Vaccine Development Chain. *Immunology*, *156*(1), 33–46. https://doi.org/10.1111/imm.13012
3. Shen-Orr, S. S., & Furman, D. (2013a). Variability in the immune system: Of vaccine responses and Immune States. *Current Opinion in Immunology*, *25*(4), 542–547. https://doi.org/10.1016/j.coi.2013.07.009
4. Shen-Orr, S. S., & Furman, D. (2013b). Variability in the immune system: Of vaccine responses and Immune States. *Current Opinion in Immunology*, *25*(4), 542–547. https://doi.org/10.1016/j.coi.2013.07.009
5. Tsang, J. S. (2015). Utilizing population variation, vaccination, and systems biology to study human immunology. *Trends in Immunology*, *36*(8), 479–493. https://doi.org/10.1016/j.it.2015.06.005

Appendix:

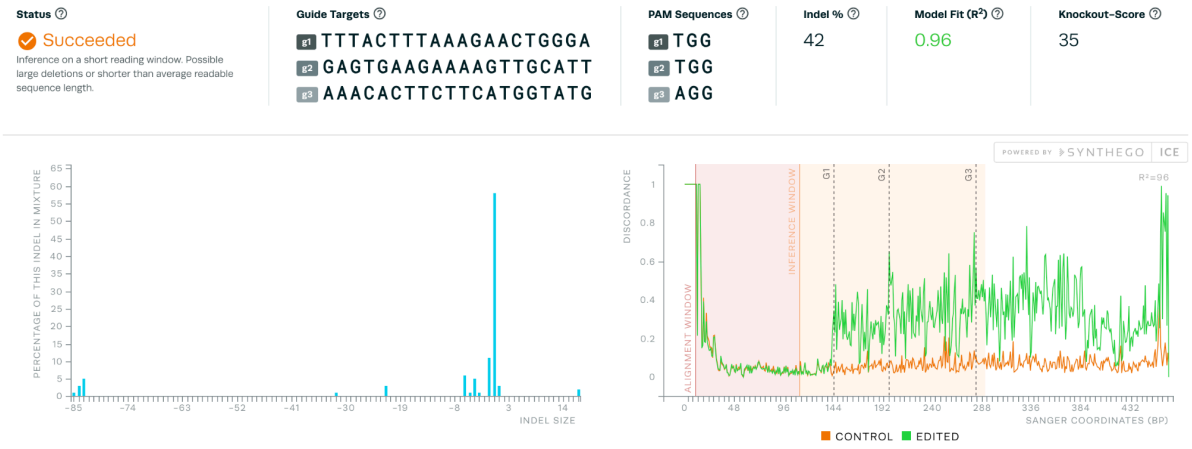

Figure 4. CRISPR Knockout Efficiency of IFNAR1 Results. Three guide RNAs were used to generate the knockouts of an isolated B cell population derived from a spleen organoid. The full analysis report can be accessed online.

Figure 5. CRISPR Knockout Efficiency of IFNAR2 Results. Three guide RNAs were used to generate the knockouts of an isolated B cell population derived from a spleen organoid. The full analysis report can be accessed here.