Mucosal Delivery of Cross Protective Influenza Vaccine Antigens

Susan Park Ochsner [1,2], Weizhong Li [1], Xiaoping Zhu [1]
[1] Division of Immunology, College of Veterinary Medicine, [2] Department of Cell Biology and Molecular Genetics
University of Maryland, College Park, 20742, USA

Abstract

Based on the ability of neonatal Fc receptor (FcRn) to transcytose Fc fusion proteins across mucosal epithelial cells, we have constructed Fc fusion proteins that consist of Influenza A viral antigens, specifically nucleoprotein (NP) and hemagglutinin (HA), which are fused to the Fc portion of mouse IgG. These vaccine antigens will be used to mucosally immunize mice, which are targeted for FcRn-mediated uptake across respiratory mucosa. We will then analyze their mucosal and systemic immune responses. Finally, the immunized mice will be challenged with antigenically similar or dissimilar viruses to evaluate the efficacy of protection or cross protection. Our work has the potential to contribute to the development of a universal mucosal vaccine platform that can offer protection to a broad range of influenza virus strains.

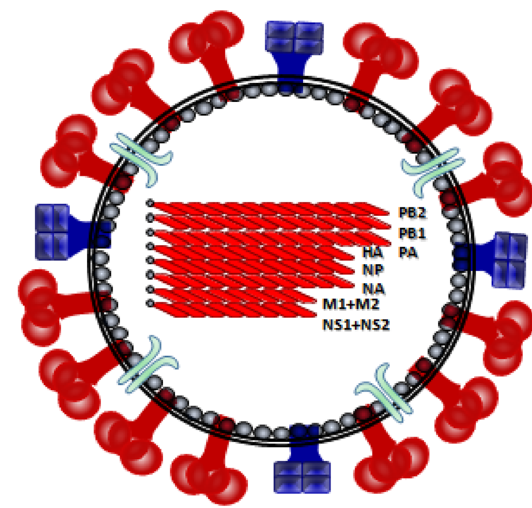
Background

Influenza viruses are a continuous global health concern, and the immune protection that is induced by current vaccines is closely strain-specific, which requires that the vaccines be updated each year. Because the availability of strain-matched vaccines usually lags behind viral antigenic changes, it is a major goal to broaden the range of influenza vaccine efficacy by using conserved influenza virus antigens in order to induce cross protective immunity against both antigenically similar and dissimilar viruses. Since influenza virus initiates its infection at mucosal surfaces, it is important to induce cross-protective and long-lasting mucosal immunity. Generally, it is difficult to induce an effective mucosal immune response from mucosal vaccines, but we have recently found that the mucosal delivery of vaccine antigens by the FcRn can engender strong immune responses against mucosal infections [1-2]. We propose that immunization with the conserved viral proteins as part of an Fc fusion protein will result in cross-reactivity and cross-protection against different strains of influenza virus.

Figure 1: Criteria for a universal Influenza vaccine

- Induces both humoral and cellular immune responses
- Gives long-lasting and cross-strain protection

Major antigens:
- HA-HA2
- NP
- matrix 1 (M1)
- matrix 2 (M2)



Proposed model of FcRn-mediated mucosal vaccine delivery

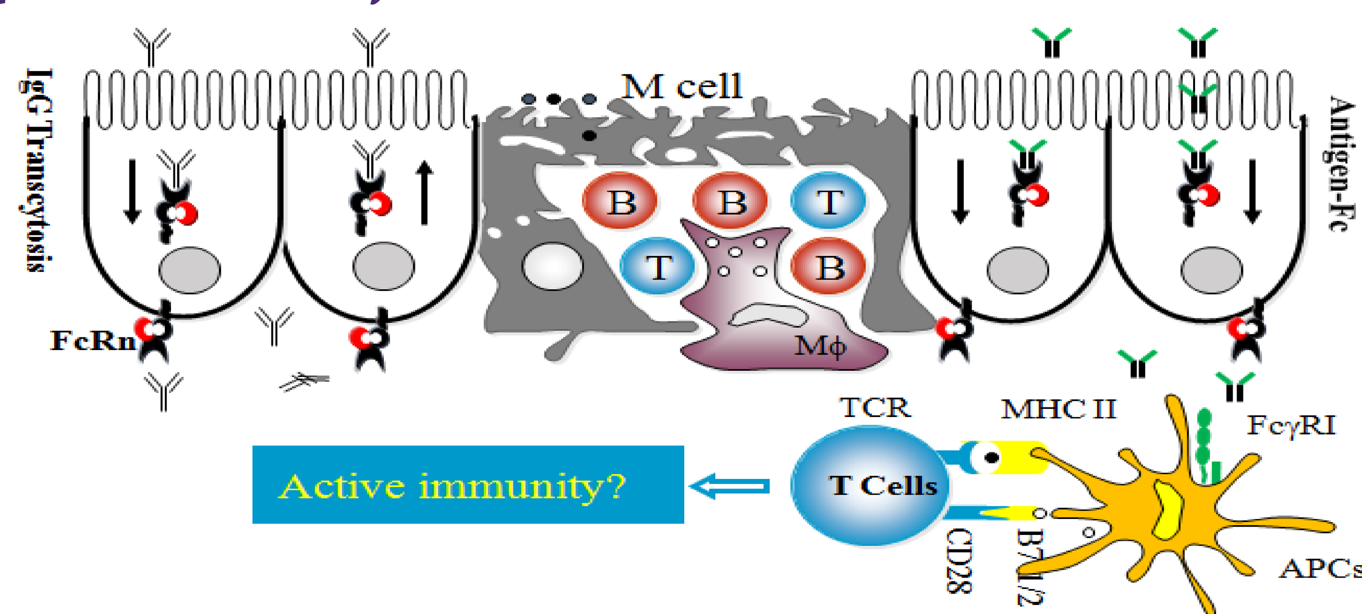
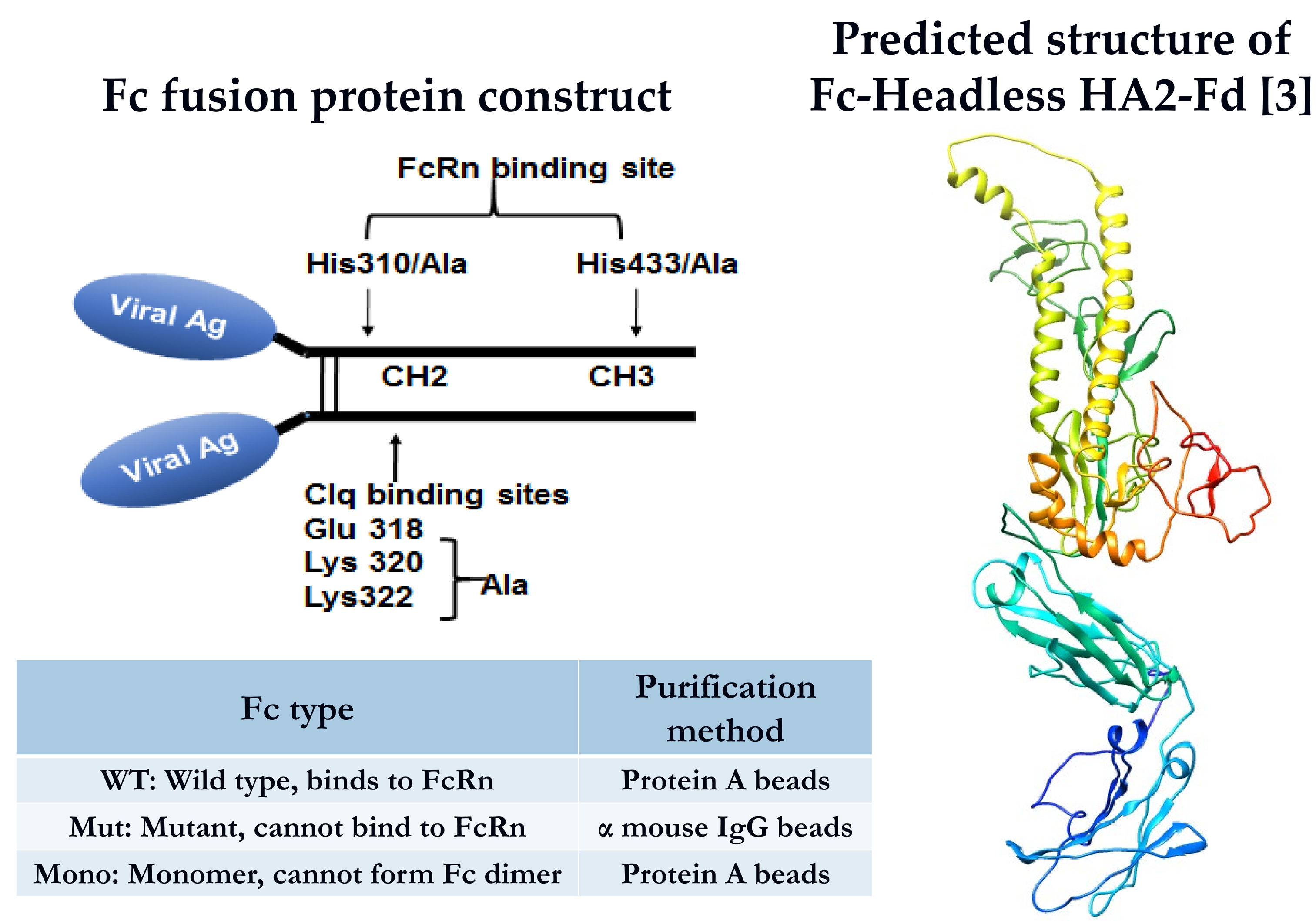


Figure 2: Strategy for construction of Fc-fused influenza vaccine antigens



Fc type	Purification method
WT: Wild type, binds to FcRn	Protein A beads
Mut: Mutant, cannot bind to FcRn	α mouse IgG beads
Mono: Monomer, cannot form Fc dimer	Protein A beads

Figure 3: Production of Fc-fused vaccine antigens

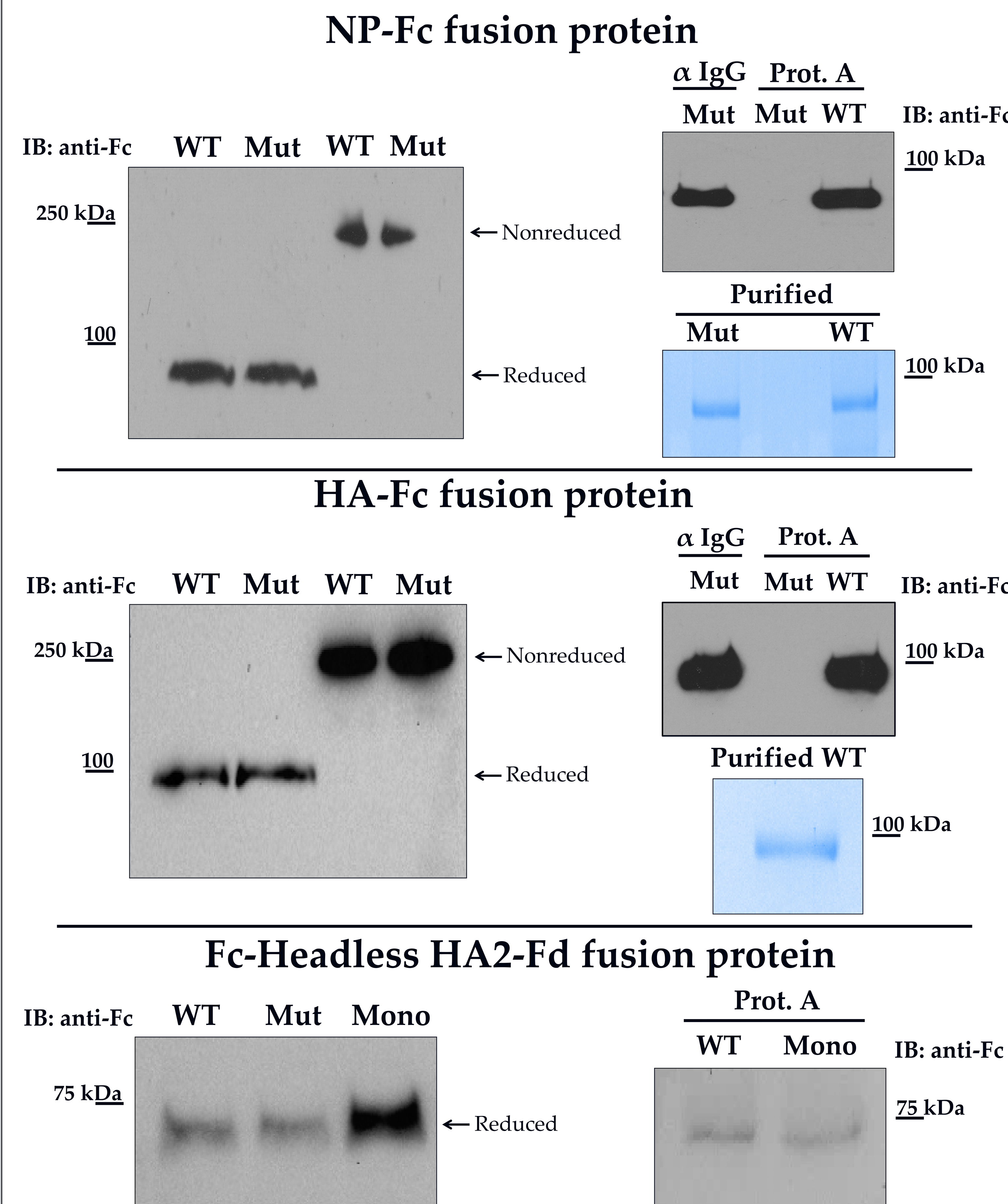
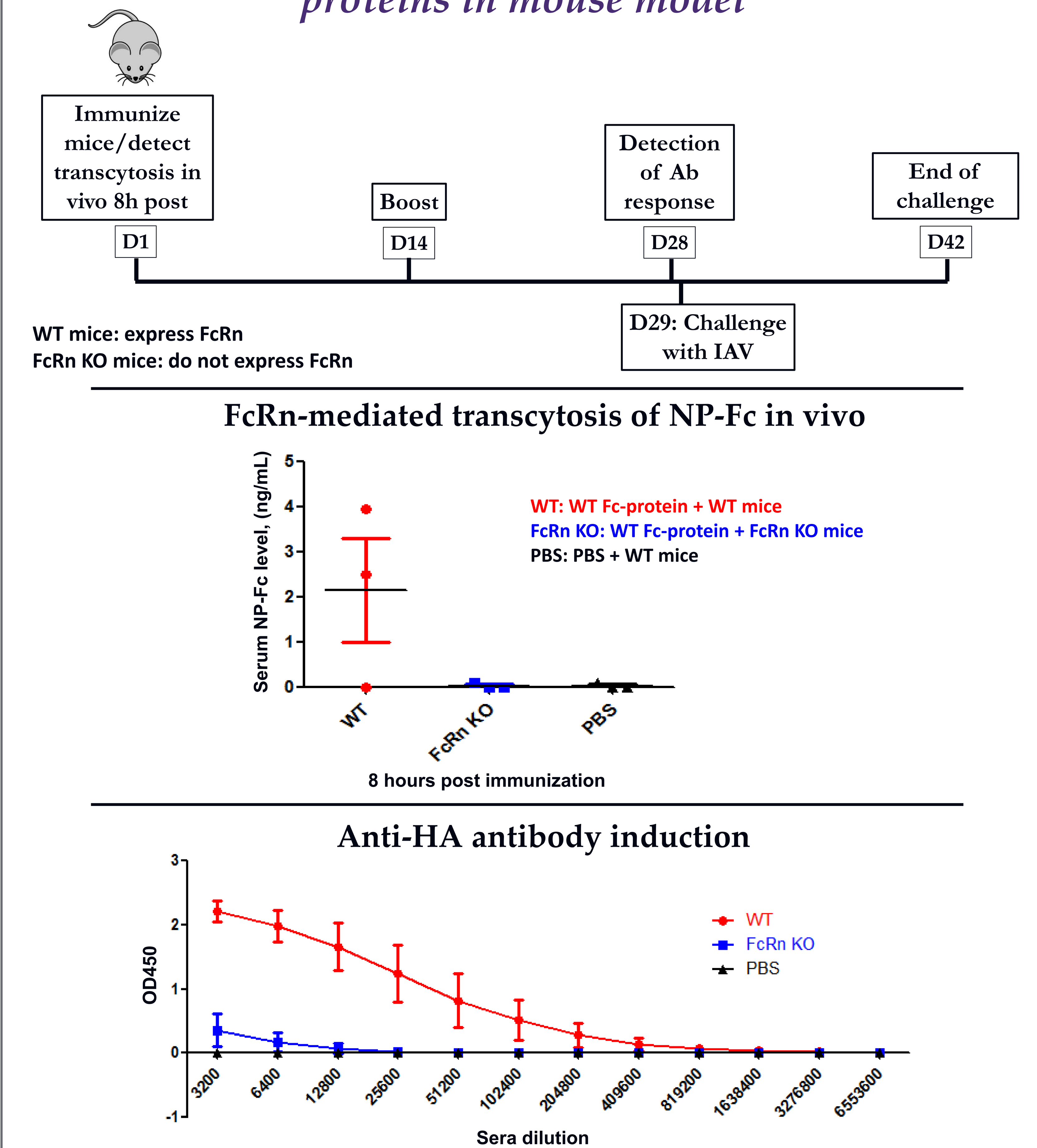


Figure 4: Mucosal immunization with Fc fusion proteins in mouse model



Summary

- Secretion of CHO PR8 HA-Fc, Fc-Headless HA2-Fd, and NP-Fc fusion proteins is detectable by Western Blot and interaction with FcRn is strongly indicated by Protein A pull down assay
- Immunization with NP-Fc fusion protein results in high levels of fusion protein in serum, demonstrating transcytosis across the lung epithelium
- Immunization with HA-Fc fusion protein results in high antibody titers in WT mice and mice are currently undergoing IAV challenge to determine level of protection offered by vaccination
- Future work will extensively characterize the immune response from HA-Fc vaccination, and further develop NP-Fc and Fc-Headless HA2-Fd vaccination trials in mouse model

References

1. Ye L et al. (2011) *Nat Biotechnol* 29(2):158-163.
2. Li L et al. (2011) *JVI* 05441-11.
3. Kelley LA et al. (2015) *Nature Protocols* 10, 845-858

Acknowledgements

This work is supported by the National Institute of Health R01 and R21 and T32 Virology Training grants