I. INTRODUCTION

1.1 Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virus responsible for causing COVID-19 disease that emerged in late 2019 (Huang et al., 2020b; Zhu et al., 2020; Zou et al., 2020). The disease is mainly characterized by flu-like symptoms, including fever, cough, dyspnea, malaise, and fatigue (da Rosa et al., 2021). Due to the rapid human-to-human spread through droplets or aerosols, SARS-CoV-2 quickly caused years of pandemic with high healthcare and economic burden in multiple countries (Jayaweera et al., 2020; Menges et al., 2021; McNaughton et al., 2022; Richards et al., 2022). Although there has been rapid vaccine development and distribution, as well as implementation of preventive measures, SARS-CoV-2 still affect more than 770 million people with 6 million deaths recorded worldwide (World Health Organization, 2023).

It has been reported that SARS-CoV-2 infection frequently results in dysregulation of immune system an inflammatory response that can persist for a long period of time after initial infection (Files et al., 2021; Phetsouphanh et al., 2022; Ryan et al., 2022). Most notably, one of the key features of SARS-CoV-2 pathogenesis is altered or delayed interferon response, the front-line defense against various viral infections and clearance (Min et al., 2021). This is due to the multiple strategies equipped by SARS-CoV-2 to impair different stages within the antiviral signaling cascades, including evasion of innate immune nucleic acid sensors, alteration of interferon and/or interferon-stimulated genes (ISG) pathway, disruption of nuclear transport, and disruption of host protein production (Minkoff & tenOever, 2023; Znaidia et al., 2022).

One SARS-CoV-2 accessory protein of interest, ORF9b, has been linked to innate immune evasion mechanisms. Many recent studies have suggested that ORF9b target multiple mediators within the type I interferon signaling pathways, thereby leading to an overall suppression of host antiviral

immune response (Ayinde et al., 2022; Brandherm et al., 2021; Han et al., 2021; Gao et al., 2021; Jiang et al., 2020; Wu et al., 2021). Crucially, numerous viral genomic mutations have been documented in the ORF9b region of current SARS-CoV-2 variants of interest (VOIs) circulating worldwide, including Omicron variants XBB.1.5, XBB.1.16, and EG.5.1. Given the global prevalence of these variants, further analysis on how different mutations within the ORF9b region in these variants may potentially alter host interferon during infection is crucial.

1.2 Objective

This research aimed to characterize the anti-type I interferon activity of different SARS-CoV-2 ORF9b proteins from Wuhan Hu-1 (WH1) and Omicron subvariants, including XBB.1.5, XBB.1.16, and EG.5.1. The scope of this research methodology includes plasmid construction, cell culture, western blot, and luciferase-based type I interferon assay.

1.3 Hypothesis

Given that amino acid mutations may give rise to different ORF9b protein properties and influence its overall activity and interactions with other molecules, different mutations within the ORF9b region in different SARS-CoV-2 variants may potentially lead to an altered host type I interferon response.