Subject: Thesis Defense: Thomas Finley, MS BiologyDate:Tuesday, July 8, 2025 at 1:55:08 PM Eastern Daylight TimeFrom:SSB Faculty List on behalf of Diane St. GermainTo:SSB-FACULTY-LIST-L@LISTSERV.GMU.EDU

Thesis Defense Announcement To: The George Mason University Community

Candidate: Thomas Finley

Program: M.S. in Biology

Date: July 17, 2025

Time: 1:00 PM Eastern Time (US and Canada)

Location: IABR Room 1004, Science & Tech campus

and via Zoom

Join Zoom Meeting

https://gmu.zoom.us/j/96912208041?pwd=KuGtEOgSxTFIHG4dH1llbpaEyO8Skz.1

Meeting ID: 969 1220 8041

Passcode: 989350 One tap mobile +13017158592,,96912208041#,,,,*989350# US (Washington DC) +12678310333,,96912208041#,,,,*989350# US (Philadelphia)

Dial by your location +1 301 715 8592 US (Washington DC) +1 267 831 0333 US (Philadelphia)

Committee Chair: Dr. Ramin Hakami

Committee members: Dr. Lance Liotta, Dr. Yuntao Wu

Title: Assembly of the Attenuated SARS-CoV-2 \triangle ORF3-E mNG Transcomplementation System and Functional Analysis of Small Extracellular Vesicles Derived From Attenuated SARS-CoV-2-infected Cells

Abstract: Extracellular vesicles (EVs) derived from infected cells have previously been shown to modulate the immune responses of recipient cells. In this work, the biosafety level 2 (BSL-2) SARS-CoV-2 AORF3-E mNG trans-complementation system was assembled and subsequently the immunoregulatory capacity of small EVs (sEVs) harvested from cells infected with this virus was investigated. In order to allow the assembly of this attenuated virus system, which has been engineered and reported by Zhang et al., 2021, at the University of Texas Medical Branch (UTMB), we obtained the cDNA plasmids expressing various fragments of the viral genome and the engineered Vero-ORF3E virion-producer cells. Rescue of the attenuated virus was achieved following purification and ligation of the appropriate fragments of the cDNA plasmids to recover the full-length cDNA for the viral genome (FL-DNA). The purified FL-DNA was then used as a template for in-vitro transcription of the full-length RNA genome (FL-RNA). Following optimization of the electroporation parameters, FL-RNA was introduced into the producer cells and the resultant virions were propagated, concentrated, and used for production of sEVs from infected Vero cells (EXi- Δ ORF3E). The EXi- Δ ORF3E were characterized for size by both nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS), for protein markers expression by immunoblot analysis, and for functional effects by treatment of naïve recipient cells.

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