Development and Characterization of Recombinant Vesicular Stomatitis Virus (rVSV)-based Bivalent Vaccine Against COVID-19 Delta Variant and Influenza Virus

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Acknowledgements

Summary

COVID-19 and influenza are both highly contagious respiratory diseases with a wide range of severe symptoms and cause great disease burdens globally. It has become very urgent and important to develop a bivalent vaccine that is able to target these two infectious diseases simultaneously.

In this study, we generated several rVSV bivalent vaccine candidates that co-expressed SARS-CoV2 Delta variant spike protein (SP) or RBD and four copies of highly conserved influenza M2 ectodomain (M2e) fused with a DC-targeting/activation domain derived from EBOV GP (EboGPΔM) based on our previously established novel vaccine platform. Here, we characterized the expression of SARS-CoV-2 Delta variant spike protein (SP) or RBD and influenza M2 ectodomains of these bivalent vaccine candidates and their abilities to induce immune responses against SARS-CoV-2 SP, especially Delta SP, and influenza M2e.

Our results have shown that immunization with these bivalent rVSV vaccines induced 1) efficient humoral and cell-mediated immune responses against both SARS-CoV-2 and influenza M2e protein; 2) high levels of neutralizing antibodies that protected cells against SARS-CoV-2 Delta and other SP-pseudovirus infections in cell culture; 3) efficiently protected hamsters/mice from SARS-CoV-2 Delta infection and the lethal challenge of H1N1 and H3N2 influenza viruses.

Overall, this study provide convincing evidence for the high efficacy of the bivalent vaccine to prevent SARS-CoV-2 Delta variants and influenza infections.

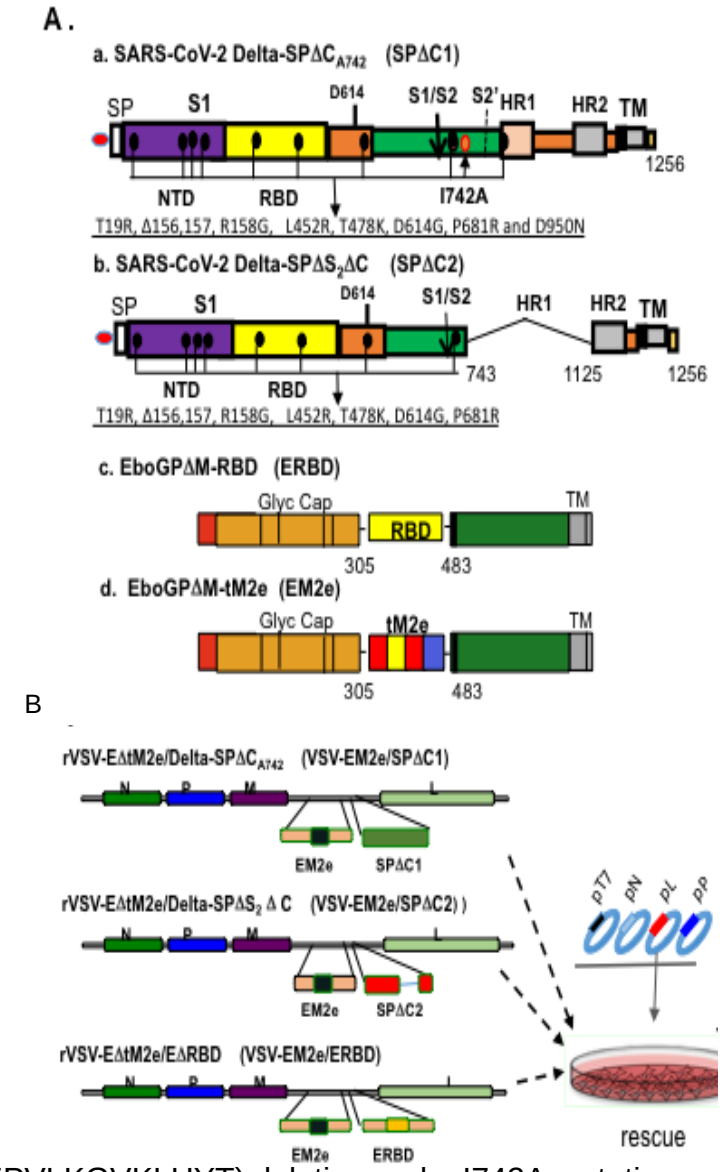

Fig.1. Generation of rVSV-based vaccines expressing both the conserved M2 ectodomain (M2e) of influenza and SARS-CoV-2 Delta spike protein. A) a,SARS-CoV-2 Delta-SPΔC$_{A742}$ (SPΔC1), containing a C-terminal 17 aa (DEDDSEPVLKGVKLHYT) deletion and a I742A mutation as indicated. B,Delta SPΔC2, containing the C-terminal 17 aa deletion and another 381 aa deletion in S2 domain. c,d, EboGPΔM-RBD or EboGPΔM-tM2e, the RBD of SARS-CoV-2 or four copies of influenza virus M2 ectodomain (24 aa) polypeptide was used to replace the MLD domain in EboGP. B) Schematic diagram of VSV-EM2e/SPΔC1, VSV-EM2e/SPΔC2 and VSV-EM2e/ERBD and the virus rescuing procedures.

The bivalent VSV vaccine candidates induced strong anti-SARS-CoV-2 RBD and anti-influenza M2 antibodies in mice

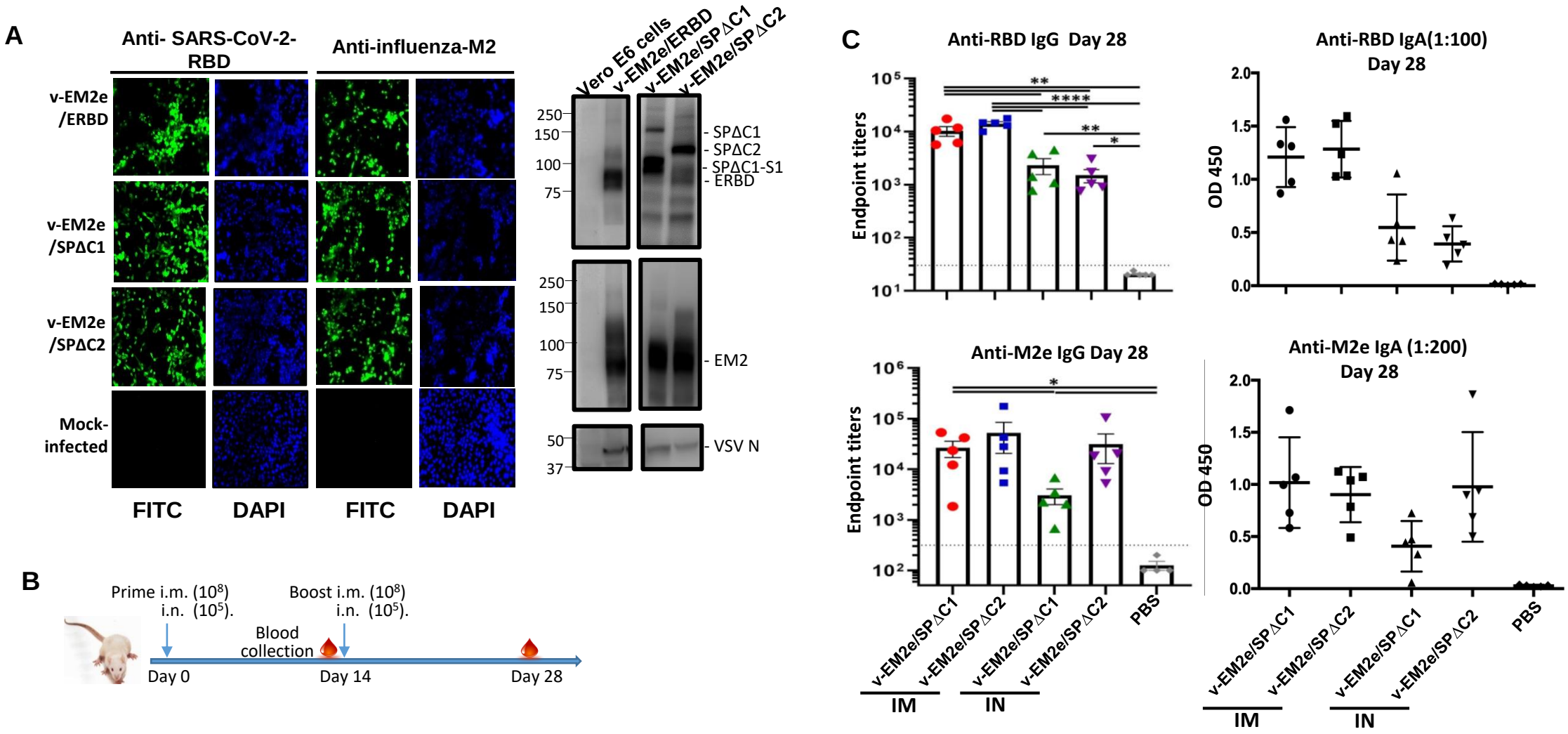

Fig. 2. A) The expressions of V-EM2e/SPΔC1, V-EM2e/SPΔC2 or V-EM2e/ERBD in infected VeroE6 cells were assessed by Immunofluorescenceassay (left) and WB (right). **B)** Schematic of the bivalent rVSV vaccine candidates immunization protocol in mouse. Balb/c mice were immunized with V-EM2e/SPΔC1, V-EM2e/SPΔC2 or V-EM2e/ERBD via intramuscular (IM) or intranasal (IN) routes, as indicated. **C**)The mice sera were measured for anti-SARS-CoV-2 RBD or anti-M2e IgG and IgA antibody levels.

The neutralization antibodies and T-cell cytokine response induced by bivalent VSV vaccine candidates

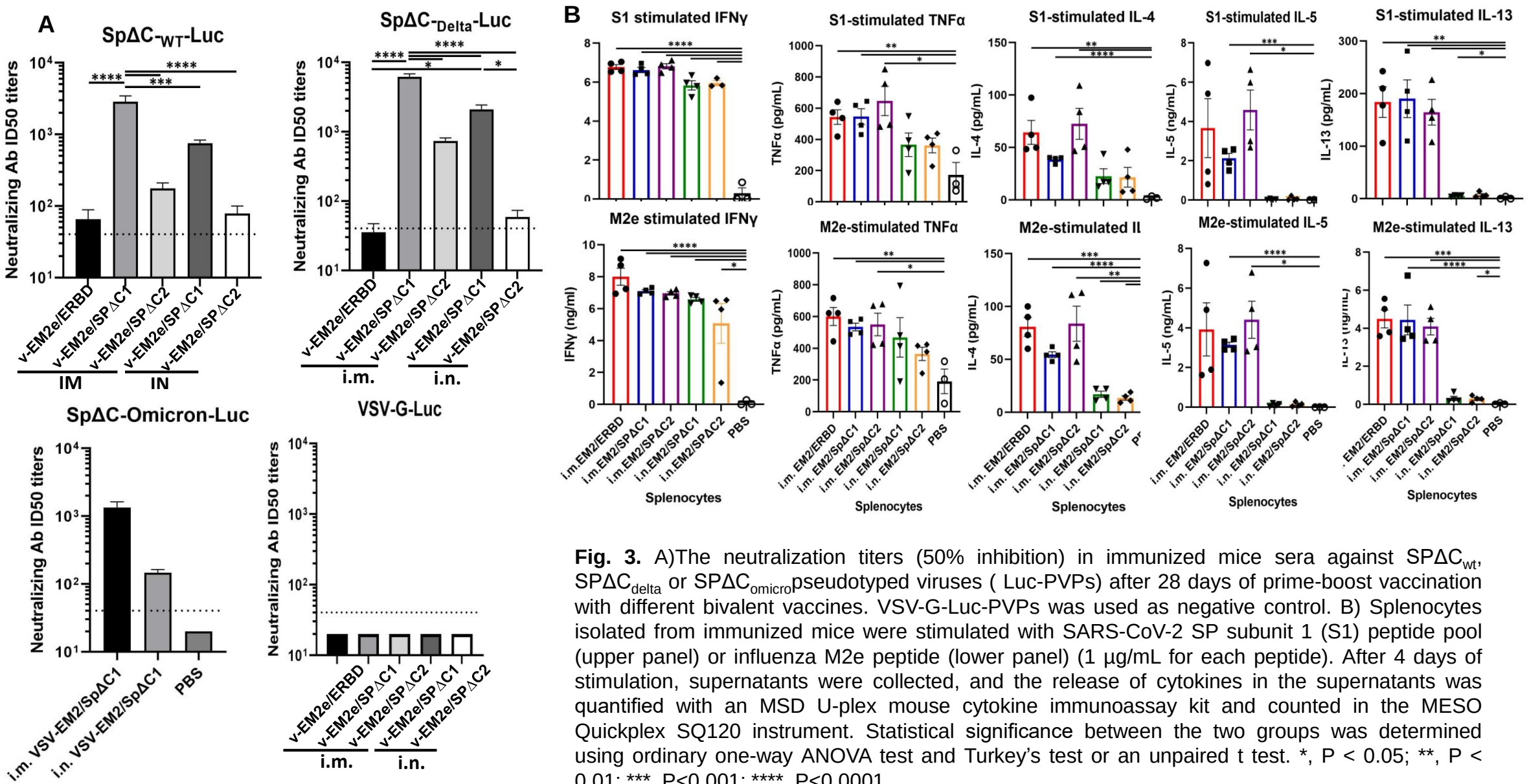

Fig. 3. A)The neutralization titers (50% inhibition) in immunized mice sera against $SP\Delta C_{wt}$, $SP\Delta C_{delta}$ or $SP\Delta C_{omicro}$pseudotyped viruses (Luc-PVPs) after 28 days of prime-boost vaccination with different bivalent vaccines. VSV-G-Luc-PVPs was used as negative control. B) Splenocytes isolated from immunized mice were stimulated with SARS-CoV-2 SP subunit 1 (S1) peptide pool (upper panel) or influenza M2e peptide (lower panel) (1 μg/mL for each peptide). After 4 days of stimulation, supernatants were collected, and the release of cytokines in the supernatants was quantified with an MSD U-plex mouse cytokine immunoassay kit and counted in the MESO Quickplex SQ120 instrument. Statistical significance between the two groups was determined using ordinary one-way ANOVA test and Turkey's test or an unpaired t test. *, P < 0.05; **, P < 0.01; ***, P<0.001; ****, P<0.0001.

V-EM2/SPΔC1 and V-EM2/SPΔC2 provided protection against SARS-CoV-2 Delta/influenza virus infection in Syrian Hamsters or BALB/c mice

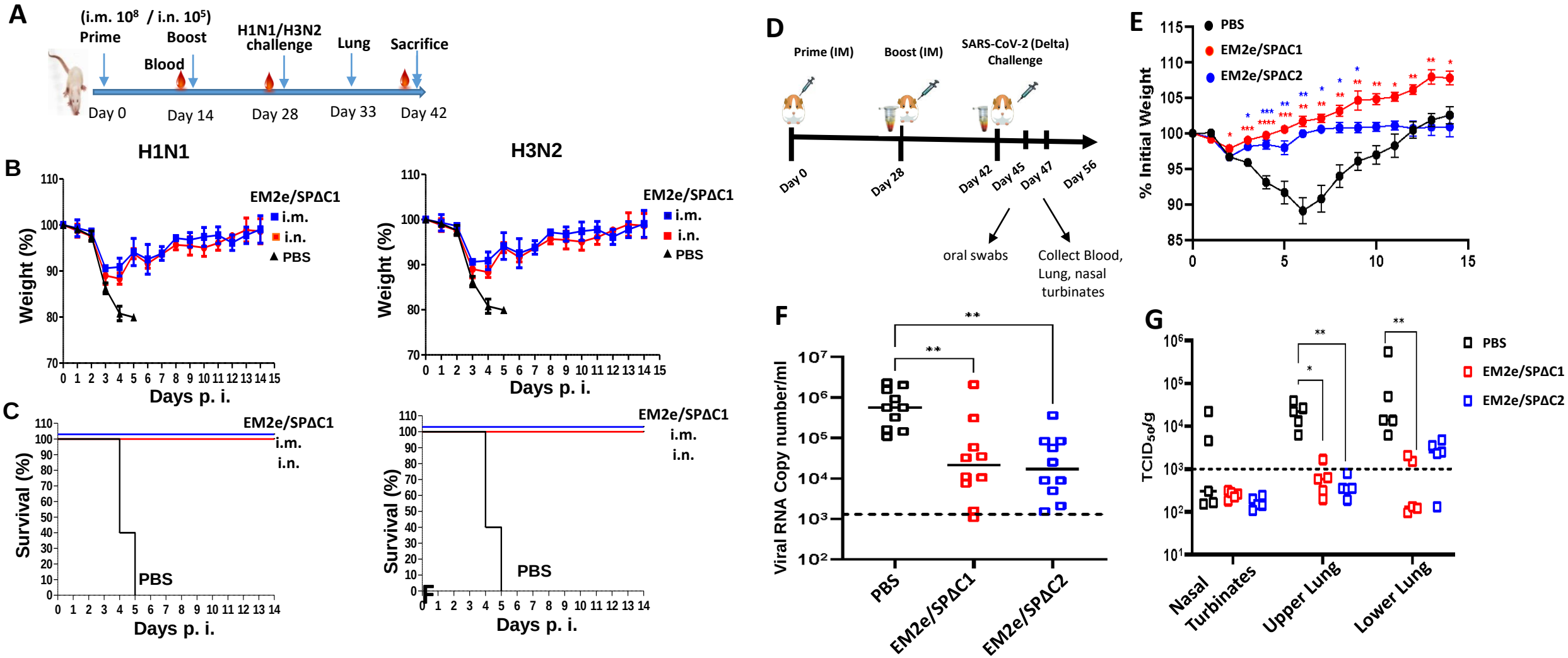

Fig.4. A,D) Schematic of the bivalent VSV vaccine candidate immunization and SARS-CoV-2 Delta variant/influenza virus challenge protocols. B,C, E) Weight loss or survive rates of immunized animals following infection with SARS-CoV-2 Delta variant or H1N1/H3N2. F) Viral RNA levels in oral swabs on day 3 following infection with SARS-CoV-2 Delta variant. G) SARS-CoV-2 Delta virus titers in nasal turbinates and lungs tissues on day 5 following infection. * = p <0.05, ** = p <0.01, *** = p<0.001, **** = p<0.0001.