ExoVACC: An exosome-based vaccine platform for the development of SARS-CoV-2 vaccine

Zhilin Chen[1], Joanne Lim[1], Olga Burenkova[1], Jing Zhou[1], Russell McConnell[1], Jorge Sanchez-Salazar[1], Kyriakos Economides[1], James Thornton[1], Tim Soos[1], Gaurav Gaiha[2], Sriram Sathyanarayanan[1]

[1] Codiak BioSciences, Cambridge, MA
[2] Ragon Institute of MGH, MIT and Harvard

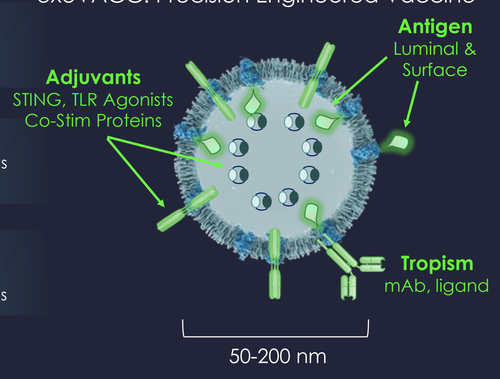
1. Introduction

Flexible antigen display
Surface and lumen
Rapid surface attachment

Diverse adjuvant combinations
Small molecules, proteins, nucleic acids
Enhanced pharmacological activity

Enhanced cell-specific tropism
Natural tropism for APCs
Modify tropism by antibodies & proteins

exoVACC: Precision Engineered Vaccine



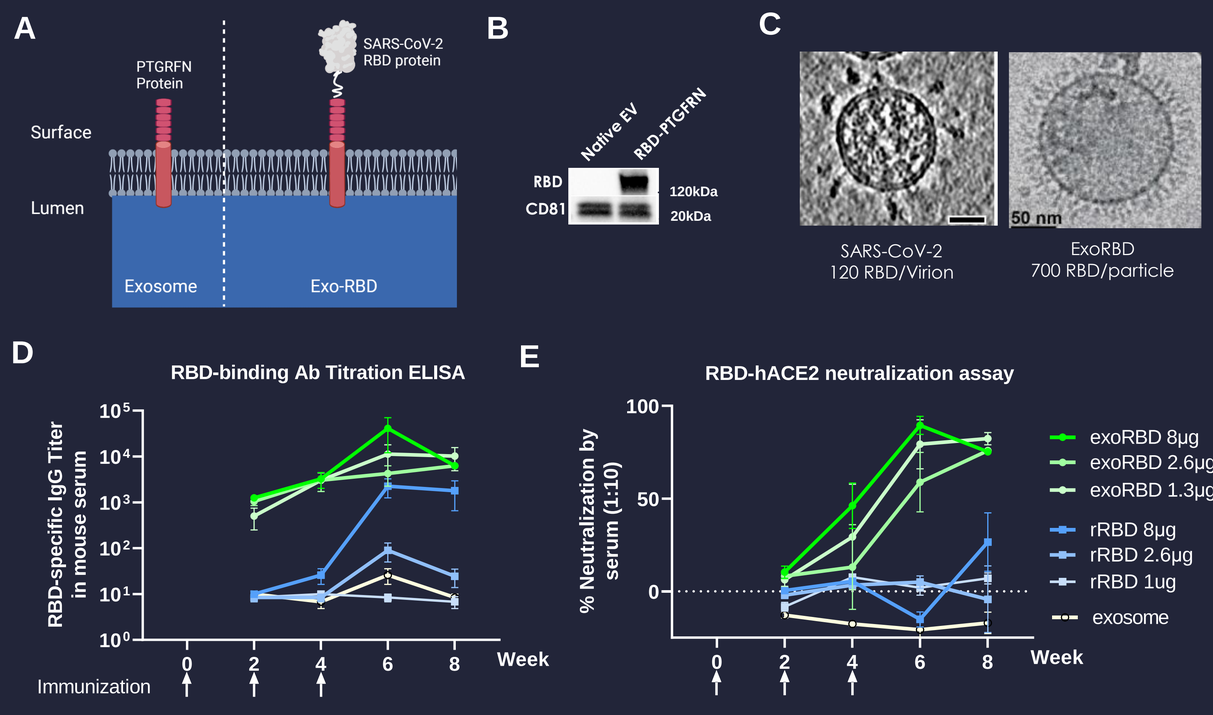
Immuno-Oncology
Tumor associated antigens
Neoantigens

Infectious disease
SARS vaccine
EBV induced PTLD
HIV

Tolerance & Autoimmunity
AAV tolerance
Autoimmunity
Neurodegeneration

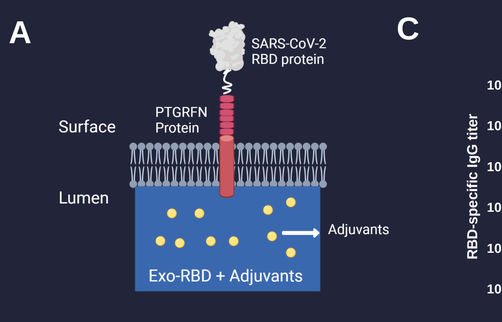
Exosomes can be engineered as a versatile vaccine platform incorporating flexible antigen display and diverse adjuvant combinations. Our exoVACC™ platform has shown excellent ability in presenting various antigens on surface/lumen and natural tropism for APCs for improved immunogenicity.

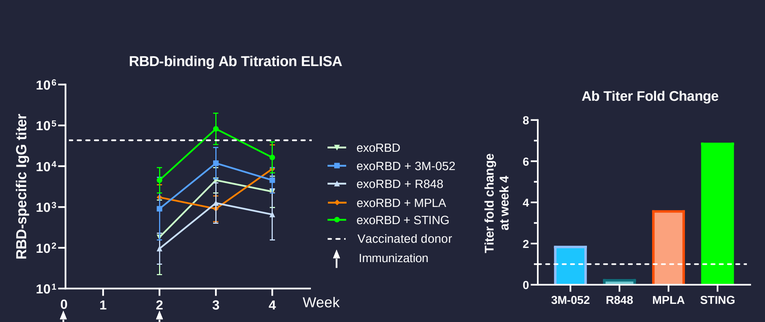
2. exoRBD, multivalent display of SARS-CoV-2 RBD molecules on exosomes, induced enhanced Ab responses in mice

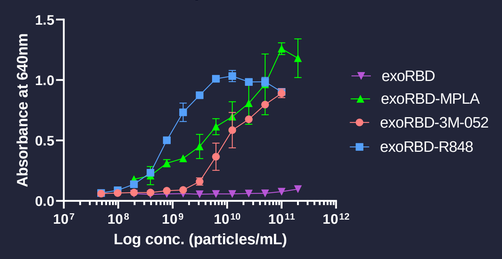


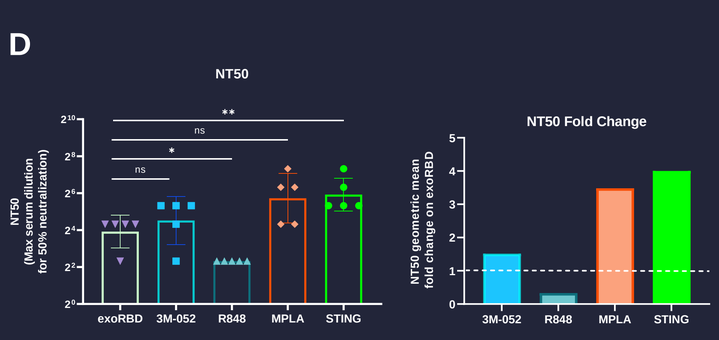
A. Cartoon depiction of native exosomes and exoRBD. SARS-CoV-2 (Wuhan strain) receptor binding domain (RBD) protein is genetically fused to the N-terminal of PTGRFN protein, a membrane protein highly expressed on exosomes. **B.** Western blotting of native exosome EV and exoRBD stained with anti-RBD Ab and anti-CD81 Ab (Control). **C.** Cryo-EM images of SARS-CoV-2 virions (Turonova et al. Science 2020) and PTGRFN exosomes. **D.** RBD-binding Ab titration ELISA results using mouse serum after 3 immunizations of exoRBD at different doses showed exoRBD induced >100x Ab titers than free RBD protein at ~1μg. **E.** RBD-hACE2 neutralization assay showed exoRBD of all doses but not rRBD induced RBD-specific neutralizing Ab.

3. Loading of adjuvants, especially STING agonist, in exoRBD strongly boosted RBD-specific neutralizing Ab responses





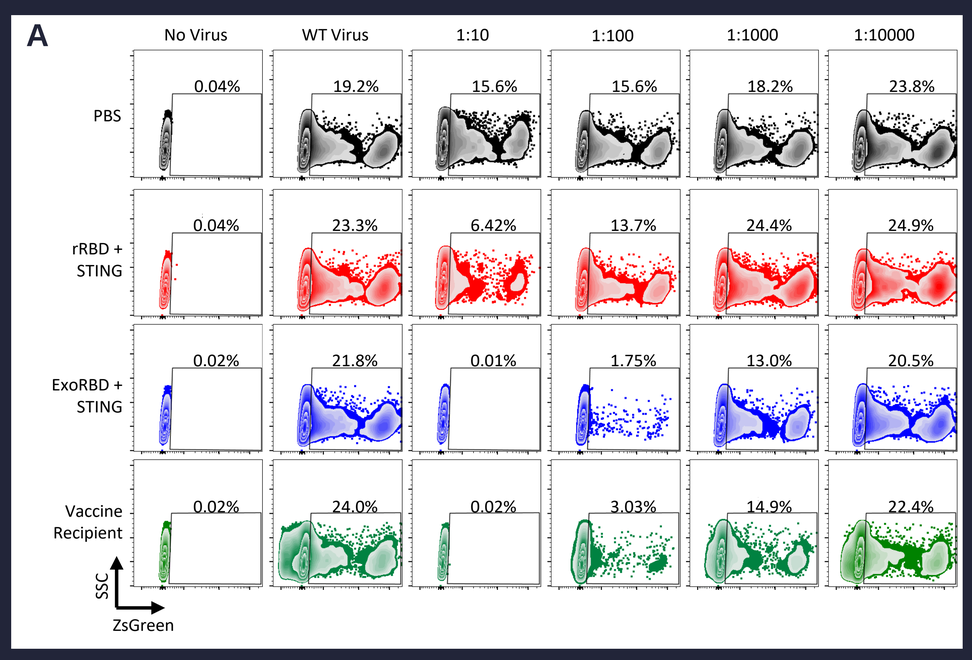




Adjuvant	3M-072	R848	MPLA	STING agonist
EC50 (p/mL)	9.52e9	8.65e8	2.25e10	-
ng/1e10 particles	947.0	92.7	391.1	43.9 (LC-MS)

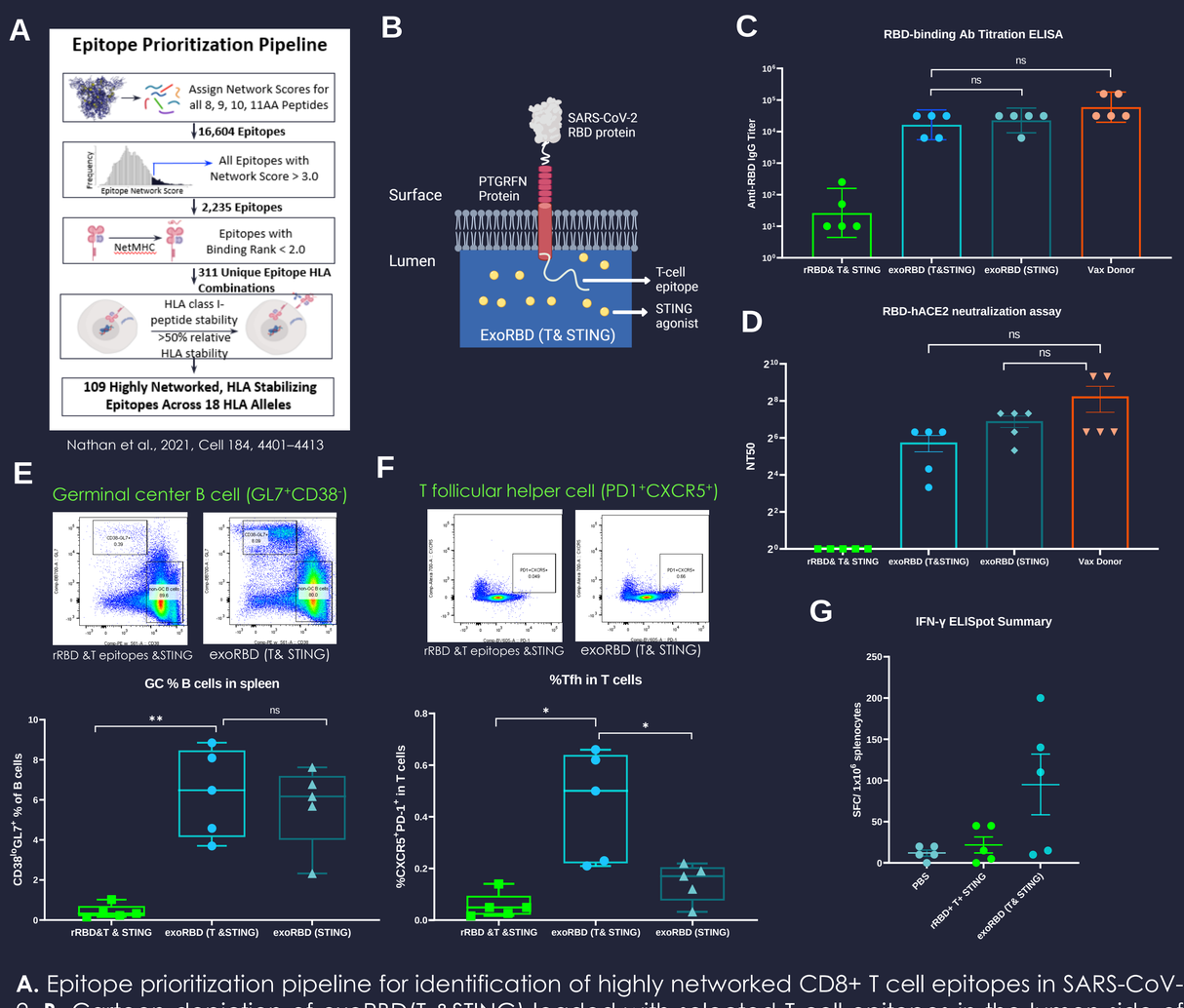
A. Cartoon depiction of exoRBD with adjuvants loaded inside. **B.** Four adjuvants (3M-052, R848, MPLA and STING agonist CDN) were loaded to separate exosomes and evaluated by RAW-Blue cell line response assay to quantitate the amounts of adjuvants loaded (results listed in the table). STING agonist loaded was quantitated by LC-MS. **C.** Two doses of exoRBD/exoRBD + adjuvant were immunized to C57BL/6 mice and mouse sera at different time points were tested for RBD-binding IgG titers. **D.** Week 4 serum was tested by RBD-hACE2 neutralization assay to determine their NT50 values and the respective fold changes.

4. exoRBD(STING) induced neutralizing Ab that can effectively block pseudotyped virus infection



A. Mouse serum after 2-dose immunizations of PBS, rRBD+STING and exoRBD (STING) as well as human serum from mRNA vaccine recipients were tested in pseudotyped virus infection assay. The results are shown by the percentages of 293T cells infected by lentiviruses pseudotyped with SARS-CoV-2 spike protein (Wuhan strain, Beta variant and Delta variant). Each row represents the infection results of pseudoviruses after incubation with selected sera of different dilution factors from 1:10 to 1:10000. **B-D.** Neutralization percentages (represented by % infection reduction) of different sera at different dilution factors are shown here for three different pseudotyped virus strains.

5. Luminal addition of T-cell epitopes to exoRBD(STING) led enhanced T-cell responses to SARS-CoV-2



A. Epitope prioritization pipeline for identification of highly networked CD8+ T cell epitopes in SARS-CoV-2. **B.** Cartoon depiction of exoRBD(T &STING) loaded with selected T cell epitopes in the lumen side of PTGRFN protein. **C-D.** RBD-binding Ab titration ELISA and RBD-hACE2 neutralization assay showed no significant differences between mouse serum induced by exoRBD(T&STING) and exoRBD(STING) as well as vaccinated human sera in binding Ab titer and neutralizing potency. **E-F.** % Germinal center B cells in total B cell and % T follicular helper cells in total CD4+ T cells in the spleens of mice immunized with rRBD& T epitopes & STING, exoRBD(T&STING) and exoRBD(STING). **G.** IFN-γ ELISpot assay using splenocytes from mice immunized with different materials restimulated by selected T-cell peptides.

6. Summary

- Multivalent display of SARS-CoV-2 RBD on exosomes induced greater RBD-specific IgG production and superior neutralization potency than rRBD, especially at lower dose.
- STING agonist loading in exoRBD strongly boosted RBD-specific Ab responses that showed excellent efficacy in inhibiting pseudotyped virus infection.
- Selected highly-networked T-cell epitopes fused to exoRBD(STING) improved their T-cell responses while maintained robust Ab responses.
- Ongoing animal challenge study using K18-hACE2 mice aims to investigate the protectiveness of exoRBD(STING) and exoRBD(T&STING) against SARS-CoV-2 infection.

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