Role of S309-CAR-NK cells in Neutralizing SARS-CoV 2

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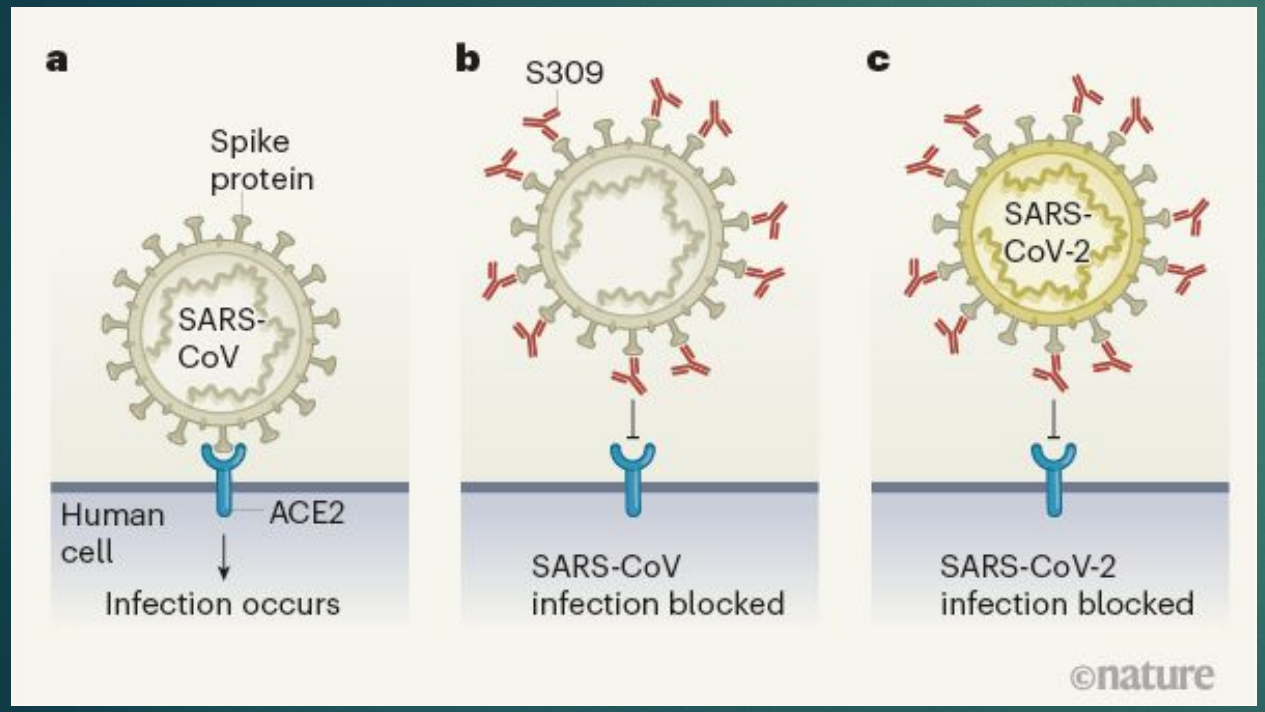
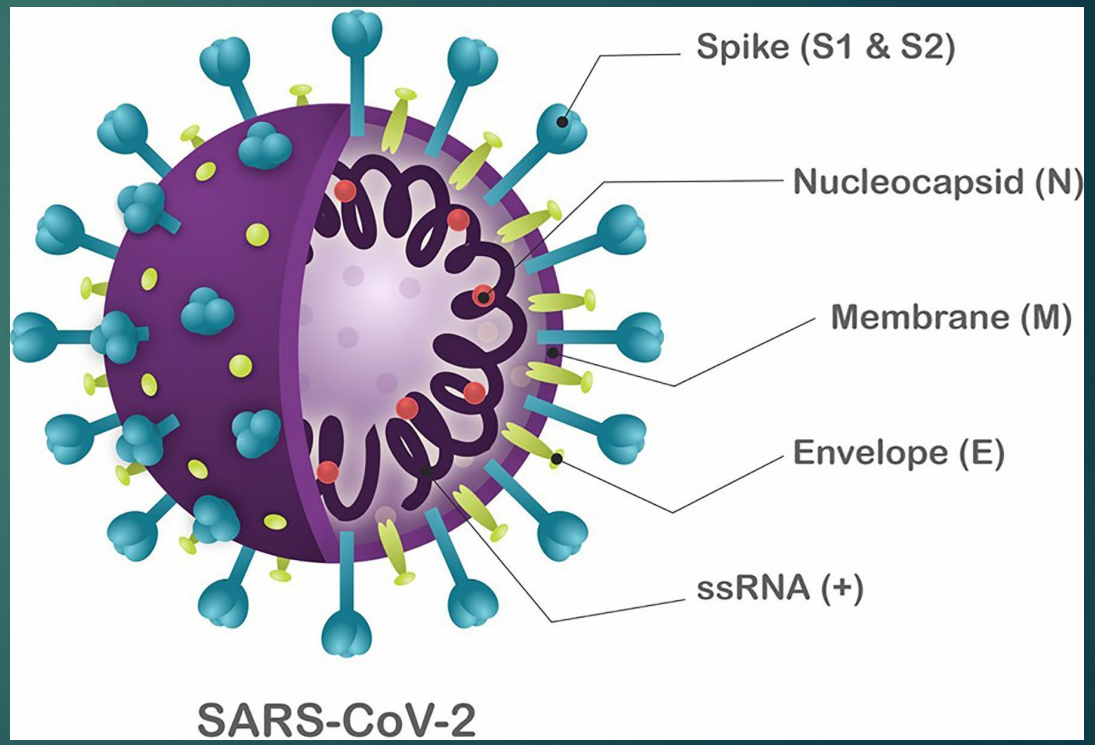
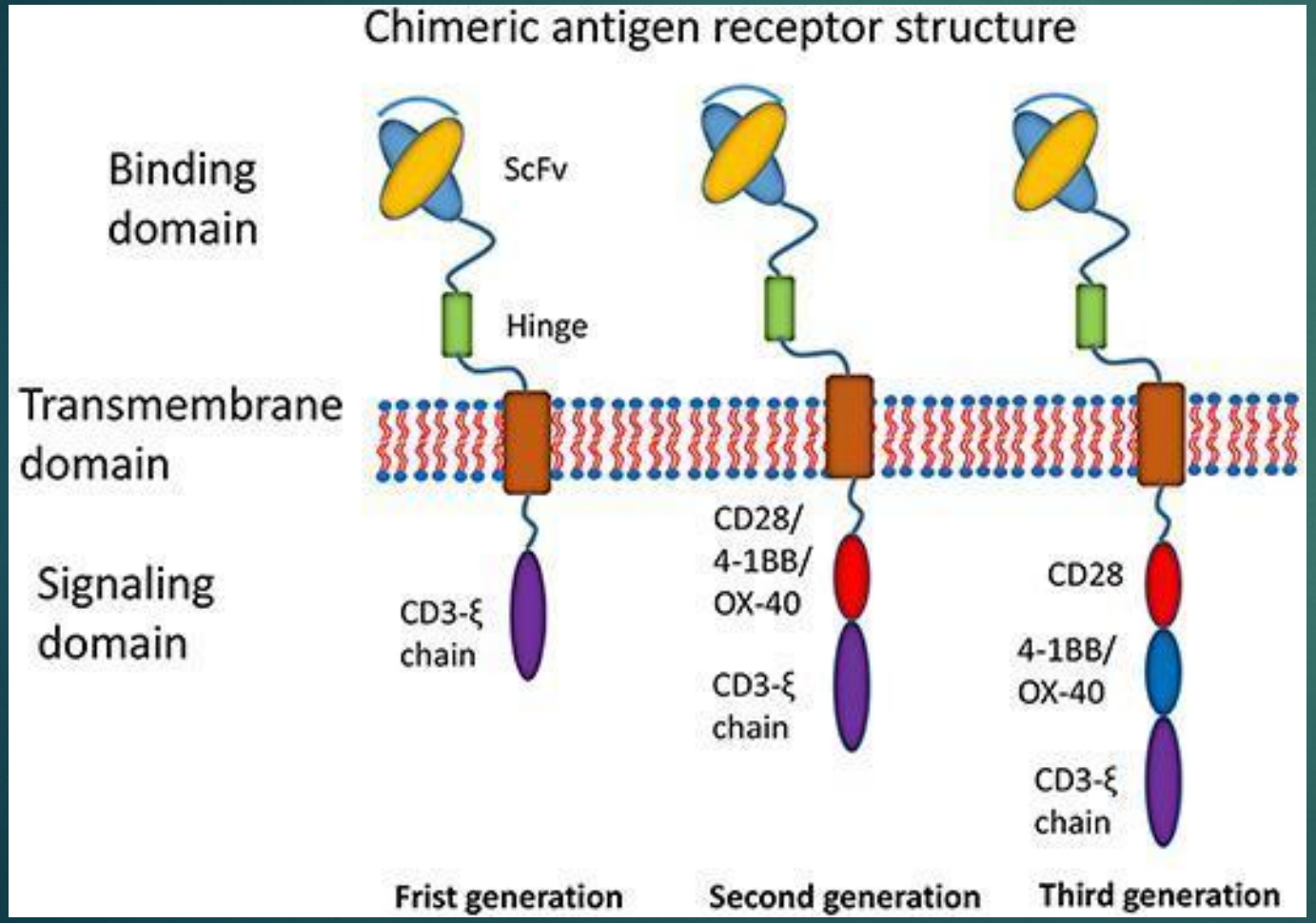
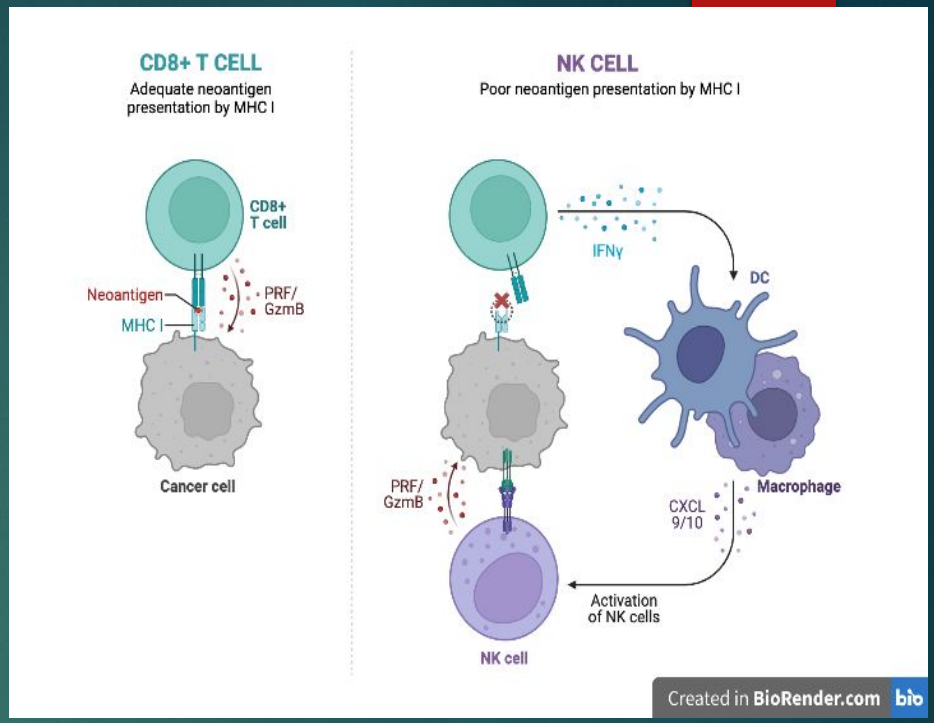
SARS-CoV-2

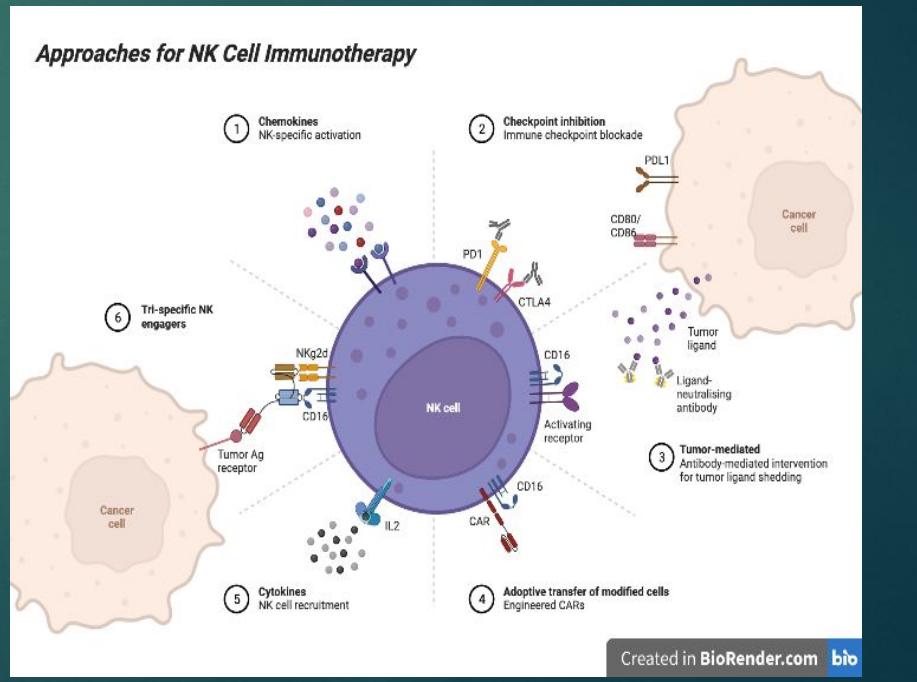

a
Spike protein
SARS-CoV
Human cell
ACE2
Infection occurs
b
S309
SARS-CoV infection blocked
c
SARS-CoV-2
SARS-CoV-2 infection blocked
©nature


Spike (S1 & S2)
Nucleocapsid (N)
Membrane (M)
Envelope (E)
ssRNA (+)
SARS-CoV-2

Role of NK Cells







Hypothesis

S309-CAR-NK cells can bind to Omicron subvariant XBB.1.5 Spike protein; therefore, neutralizing the pseudoviral SARS-CoV2 XBB.1.5 particles *in vitro* and can be used as a potential therapeutic for immunocompromised COVID patients.

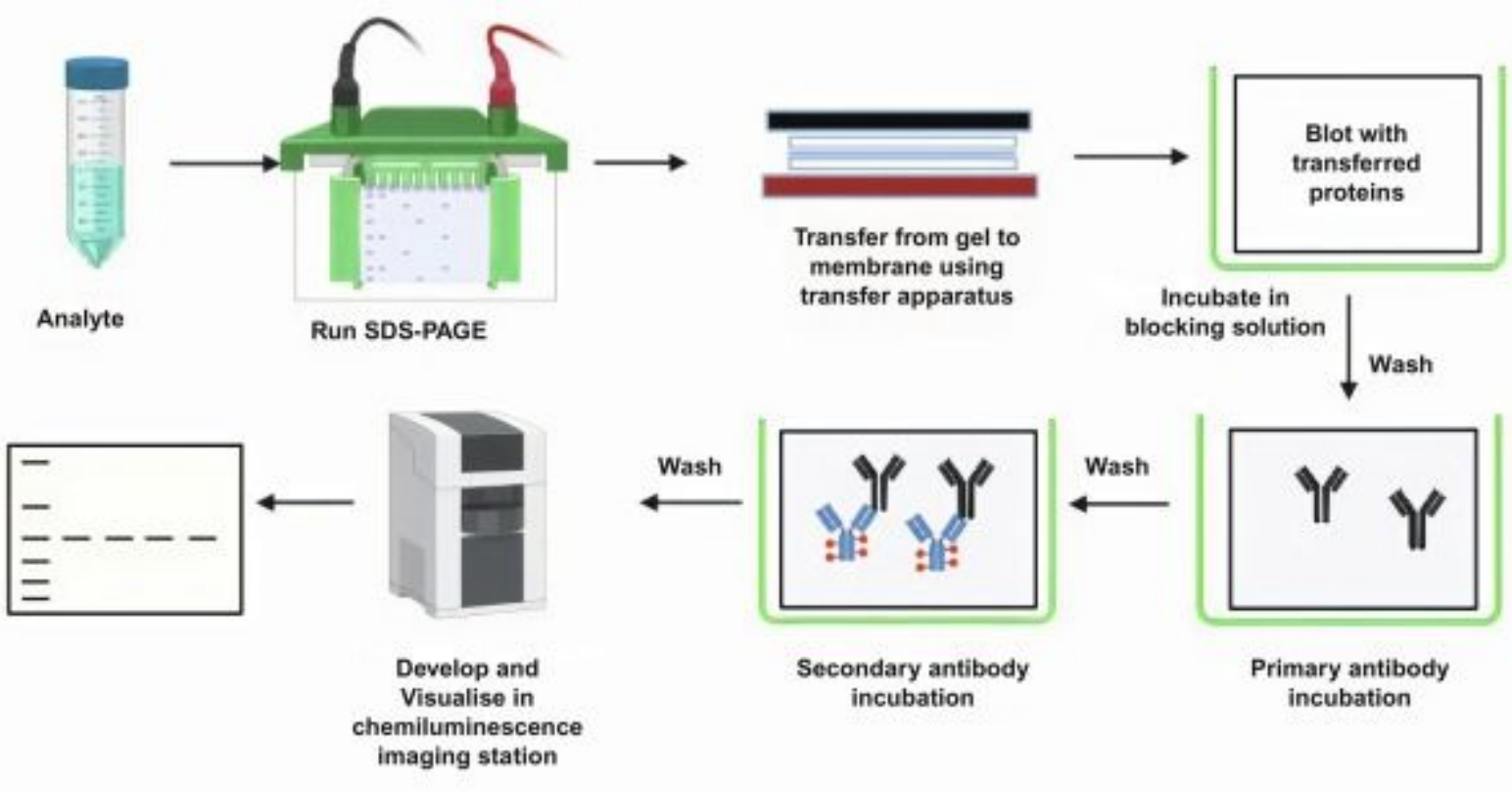
Materials

Materials for Flow Cytometry

- NK92MI cells (purchased from ATCC, catalog number CRL-2408) & genetically modified S309-CAR-NK92MI cells used for flow cytometry are maintained by Liu Lab.
- -PE anti-His Tag (Clone: J095G46, BioLegend, Catalog number 362603)
- -Alexa Fluor 647 anti-human CD56 (Clone: HCD56, BiolLegend, Calalog number 318314)
- -FITC anti-human CD3 (Clone: HIT3A, BioLegend, Catalog number 300306)
- -Alexa Fluor 488 goat anti-rabbit IgG (H+L) (Invitrogen, catalog number A11034)
- -SARS-CoV-2 Spike Trimer Protein, His Tag (Biosystem Acro, catalog number SPN-C522T)

Materials for Western Blot and SDS Page

- -Purified anti-CD247 (TCRζ) (Clone: 6B10.2, Biolegend, catalog number 644102)
- -Pierce BCA Protein Kit (Thermo Scientific, Catalog number 23225)
- -10x Tris/Glycine Buffer (Bio-Rad, catalog number 161-0771)
- -10x Tris/Glycine/SDS Buffer (Bio-Rad, catalog number 161-0772)
- -Anti-mouse IgG HRP- linked Antibody (Cell Signaling Technology, catalog number, 7076S)
- -SuperSignal WestFemto Maximum Sensitivity Substrate (Thermal scientific, catalog number 349095)
- -B-Actin Rabbit mAb (HRP Conjugate Cell Signaling, catalog number 5125)
- -Non-fat dry milk (Cell Signaling, Catalog number 9999)
- -Phosphate buffer saline powder (Sigma, Catalog number P13813)
- -Tween20 (Sigma, Catalog number P1379)
- -10% Mini-PROTEAN TGX Precast Gels (Bio-Rad, Catalog number 4561034)
- Precision Plus Protein Dual Color Standards (Bio-Rad, Catalog number 1610374)

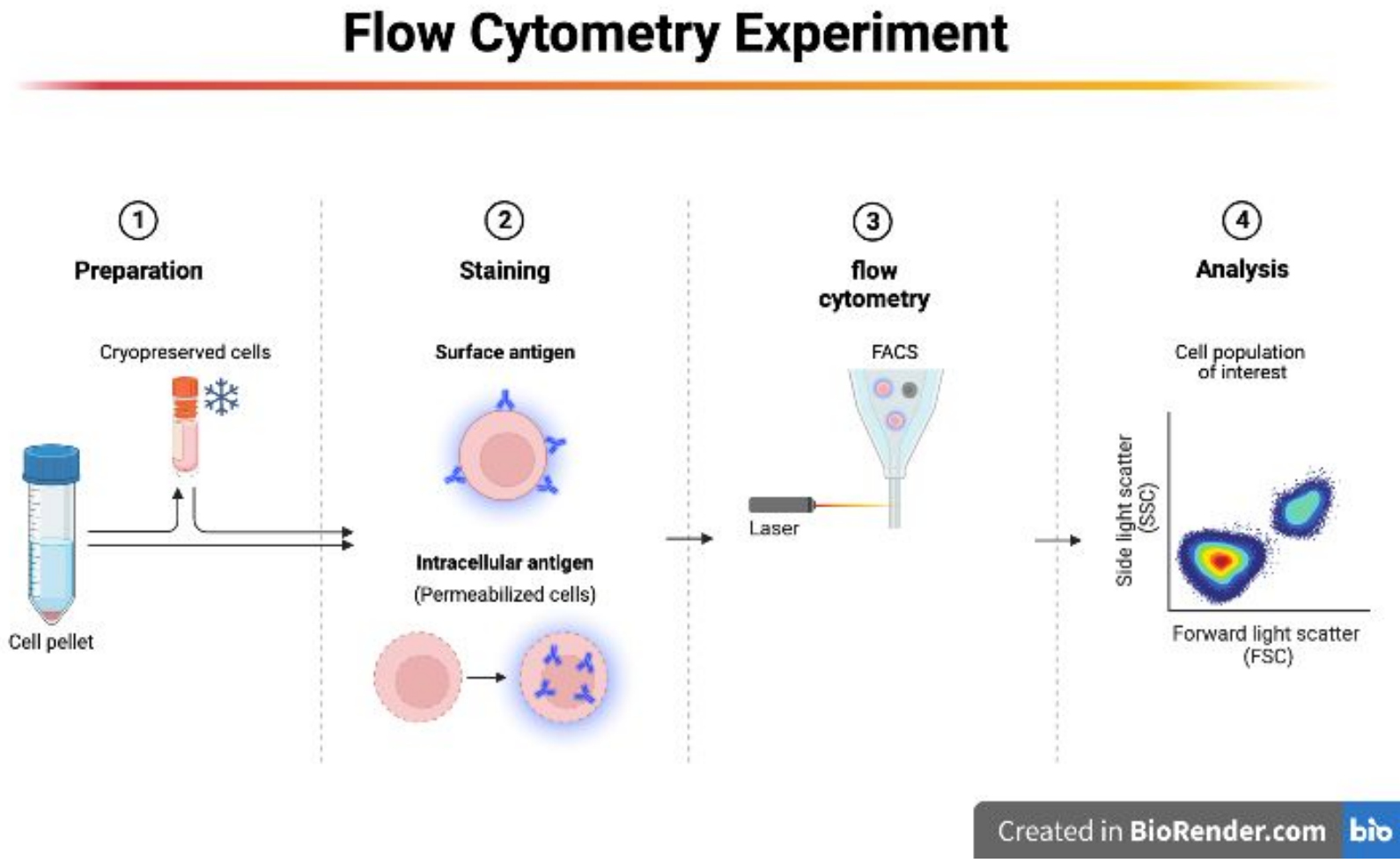


Procedures

DETERMINE THE TOTAL EXPRESSION OF S309-CAR-NK CELLS USING WESTERN BLOT

Procedures

- Determine the surface expression of S309-CAR-NK cells using flow cytometry
- Investigate whether S309-CAR-NK cells bind to Omicron subvariant XBB.1.5 Spike protein



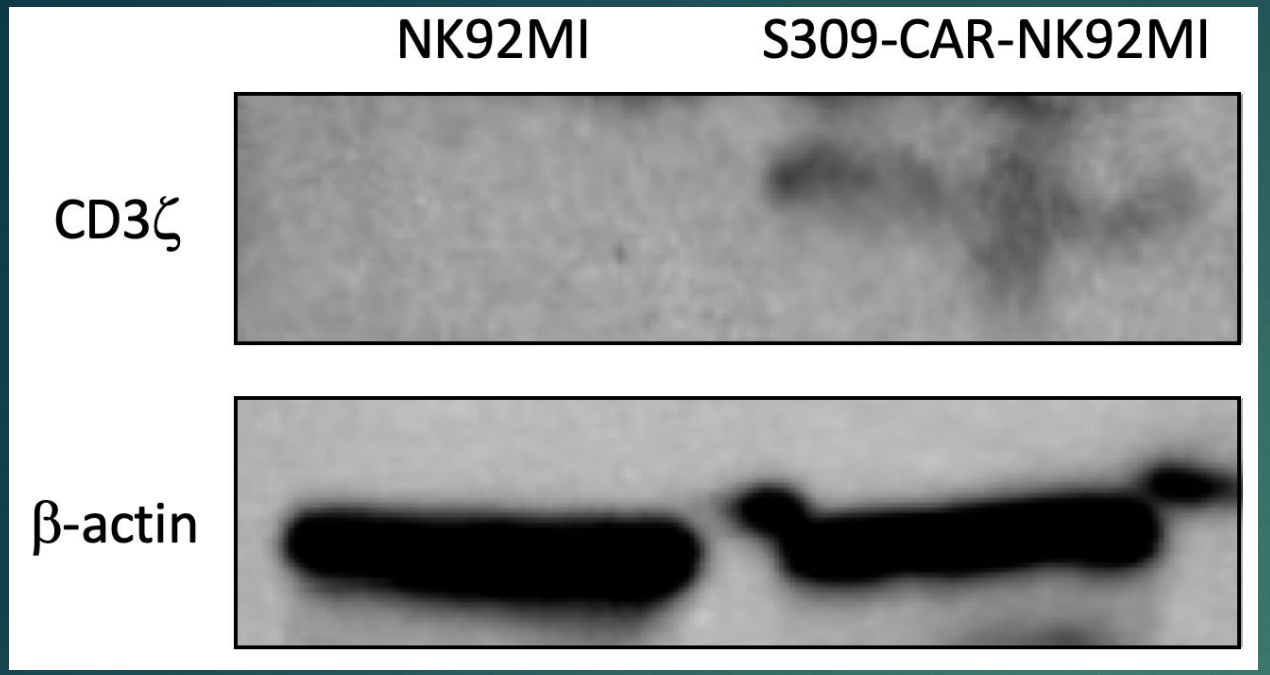


Figure 1: Snapshot of the intensity of S309-CAR-NK92MI and NK92MI expression bands

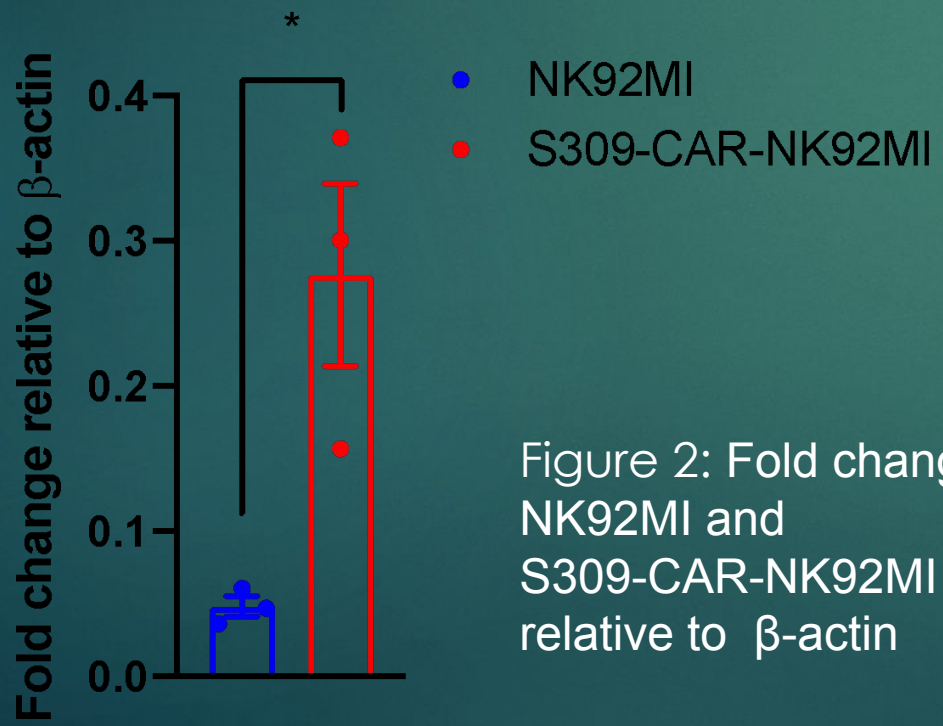


Figure 2: Fold change of NK92MI and S309-CAR-NK92MI relative to β-actin

Results

INTRACELLULAR EXPRESSION OF S309-CAR-NK CELLS DETERMINED BY WESTERN BLOT

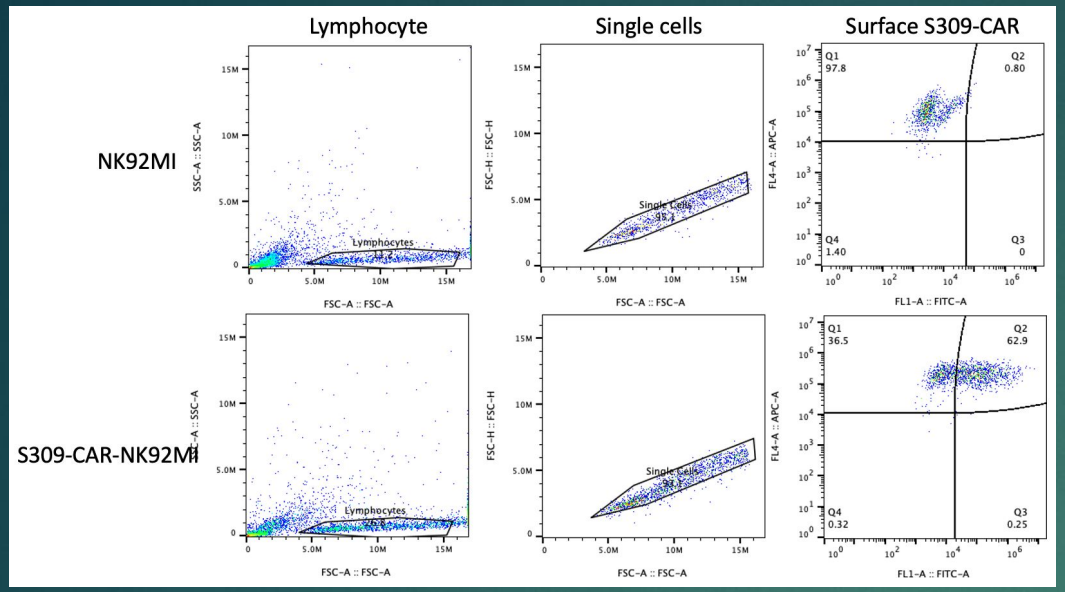


Figure 3: Technical replicate of the surface expression of S309-CAR-NK cells

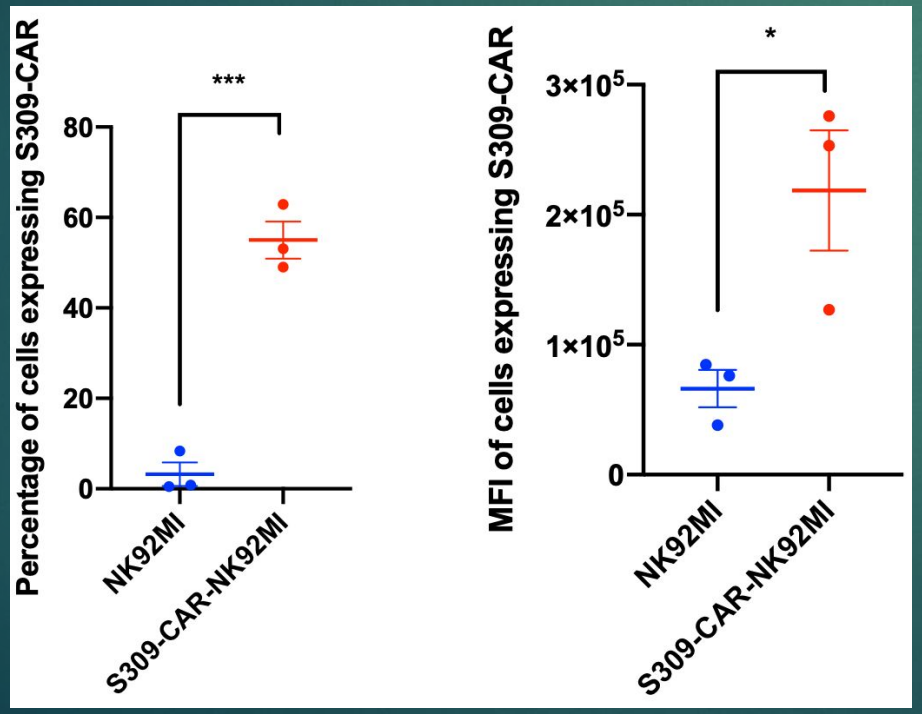


Figure 4: Statistical significance of the surface expression of S309-CAR-NK cells

Results

SURFACE EXPRESSION OF S309-CAR-NK CELLS DETERMINED BY FLOW CYTOMETRY

Results

BINDING OF S309-CAR-NK CELLS TO S OMICRON PROTEIN DETERMINED BY FLOW CYTOMETRY

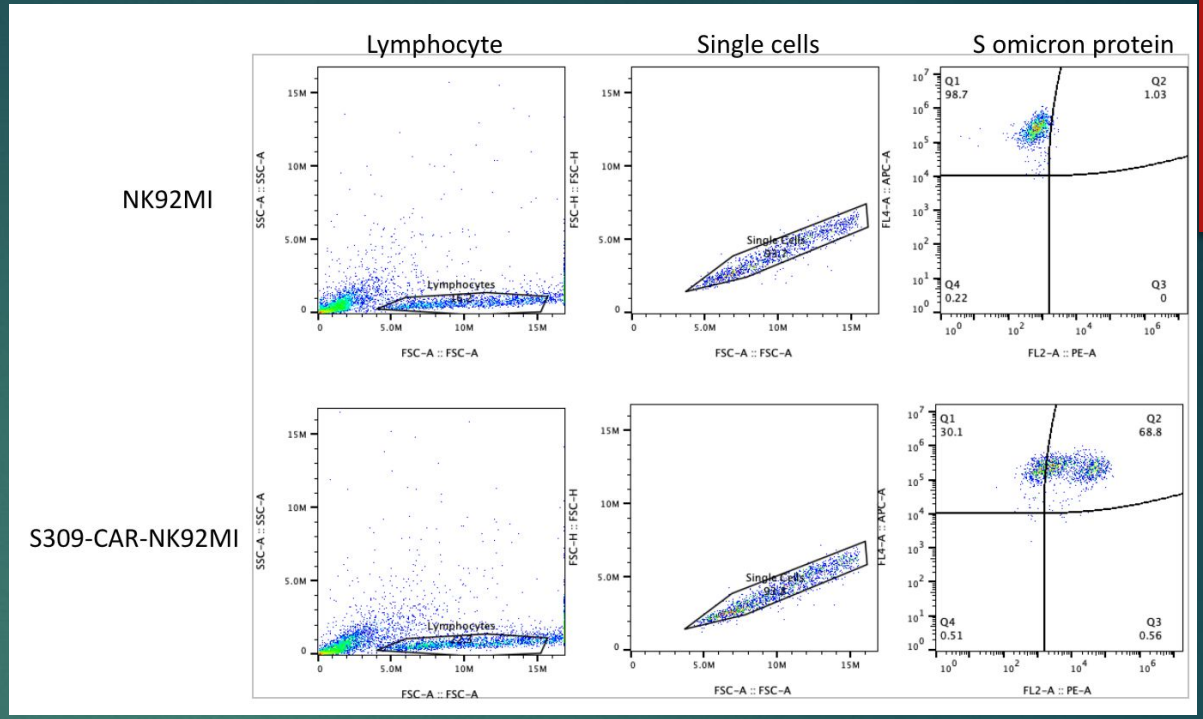


FIGURE 5: Technical replicate of the binding of S309-CAR-NK cells to Spike Omicron protein

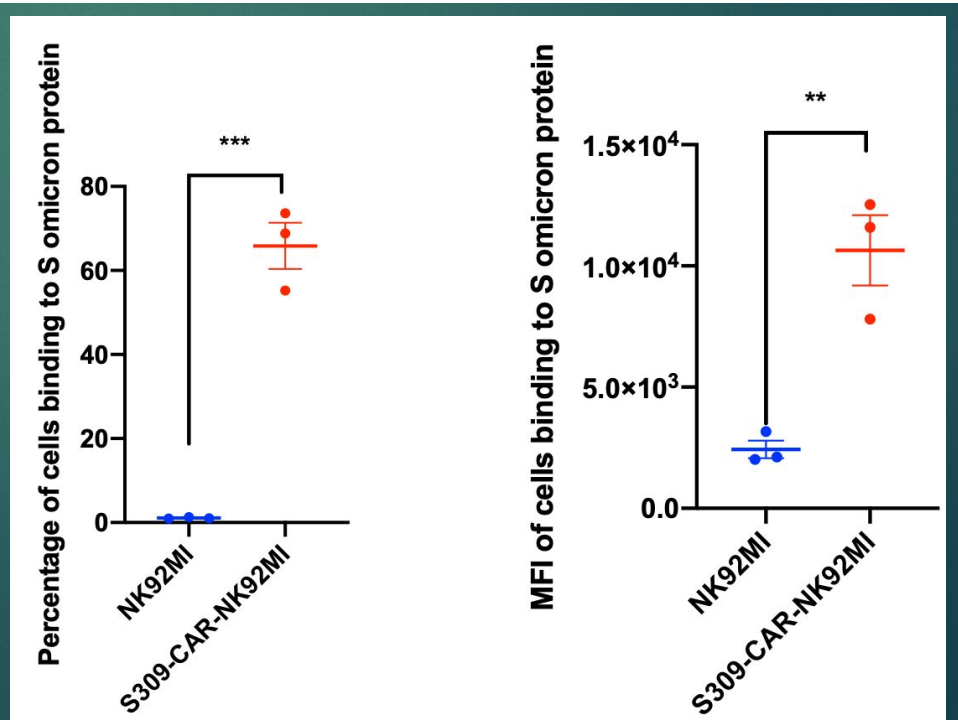


FIGURE 6: Statistical significance of the binding of S309-CAR-NK cells of Spike omicron protein

Analysis

- The values by the BCA assays were obtained from Biotek Synergy HT Microplate Reader. The values were plotted in Excel as shown in Tables 1 and 2 for the generation of the standard curve and protein concentrations determination.
- The Western blots images were captured by ChemiDoc Imaging System by Bio-Rad and converted into TIFF (Figure 7). The intensities of the bands were quantified using Fiji or ImageJ by first subtracting any background noise followed by creating rectangles (with the same area) around the bands. The raw intensity values are shown in Table 3 with appropriate normalization to β-actin, which the is graphed in Figure 8.
- Flow cytometry data were obtained by Dr. Liu's lab member using an Accuri C6 Plus Flow Cytometer by BD Biosciences. The flow data analyses were assisted by Dr. Liu's lab member. The mean fluorescent intensities (MFI) and percentage were graphed in Prism version 8 to calculate for the statistics using unpaired t-tests.
- Analysis Software includes Excel, FlowJo version 8 by BD Biosciences, Fiji, and Prism version 8.

Summary

- RATIONALE: Properties of SARS-CoV-2 influence the effectiveness of public health interventions: increase transmissibility, change antigenicity, avoid immunity caused by previous infection/vaccination, and evade immune response → increase in the virus's severity.
- Natural Killer Cells (immune white blood cell) has granules that can destroy tumors and other virus-infected cells. Are host's immune defense against pathogens preventing the establishment of infection and the viral spreading through the body.
- SARS-CoV2 interrupts equations of immune responses, disrupting cytolytic antiviral effects of NK cells and induce "cytokine storm" by activating infected immune cells.
- CAR-NK cells are a safer alternative to CAR-T cell therapy → Advantages: less antigen loss relapse, minimal on-target, off-tumor toxicity, and antibody-dependent cellular cytotoxicity (ADCC).

- RESULTS AND ANALYSIS: All data have P less than 0.05 (significant)
- 60% of cells express S309-CAR-NK
- 60% cells binding to protein → binding efficiency almost 100%