

Multi-epitope- and siRNA- based dendritic cell-targeted nano-vaccine against SARS-CoV-2

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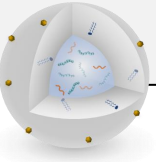
CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Advanced Delivery Science



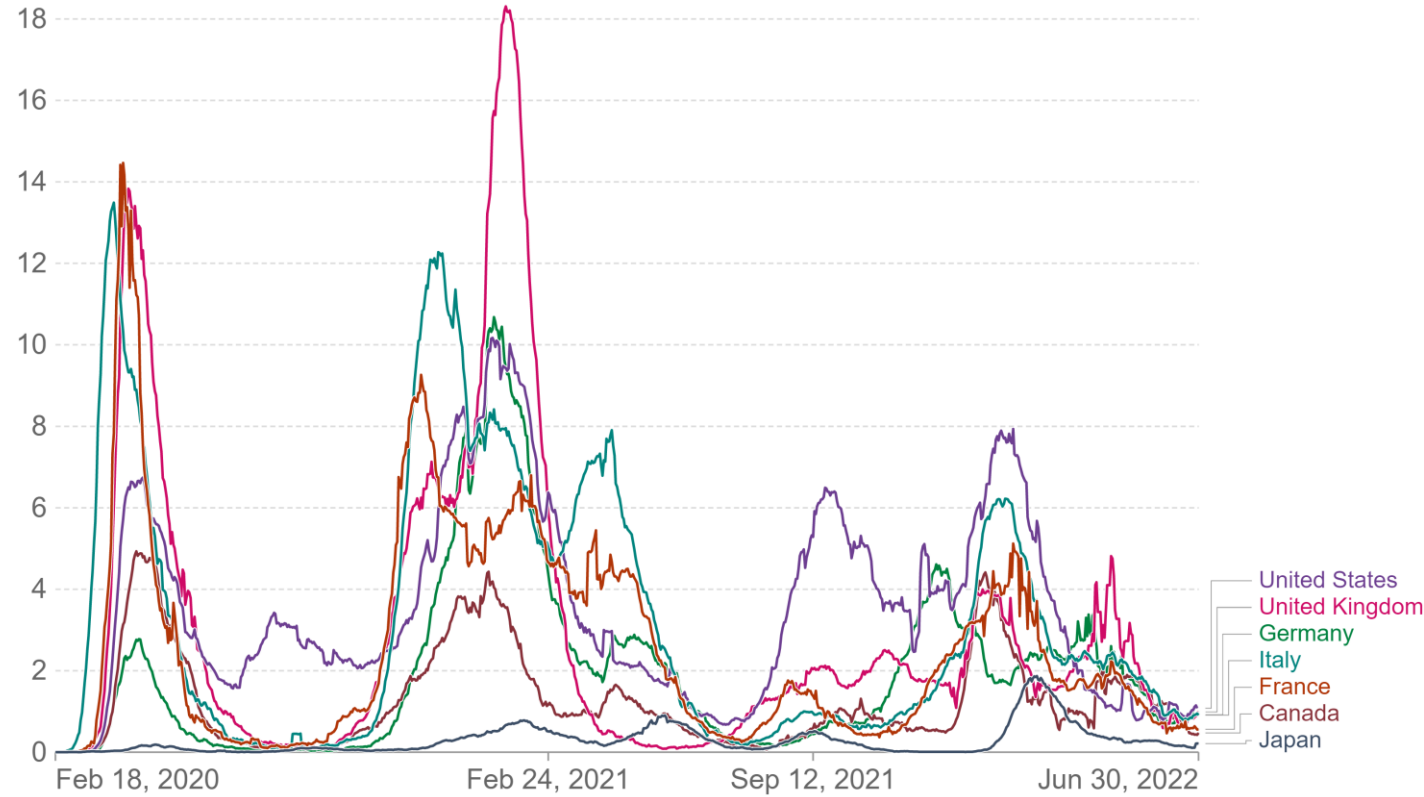
COVID-19: Morbidity and vaccination status



Daily new confirmed COVID-19 deaths per million people

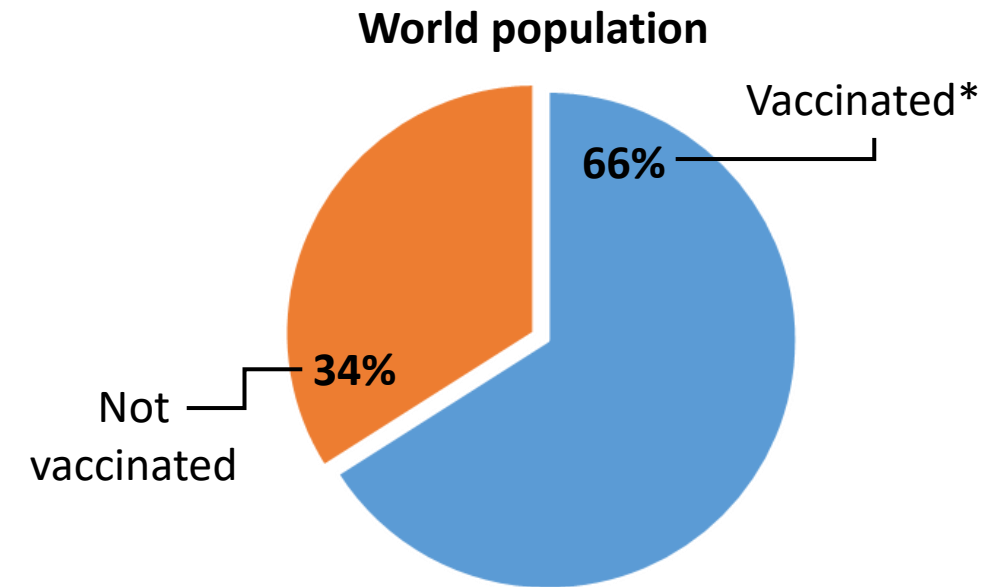
7-day rolling average. Due to varying protocols and challenges in the attribution of the cause of death, the number of confirmed deaths may not accurately represent the true number of deaths caused by COVID-19.

Our World
in Data



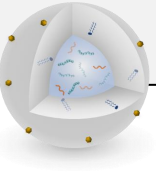
Source: Johns Hopkins University CSSE COVID-19 Data

CC BY



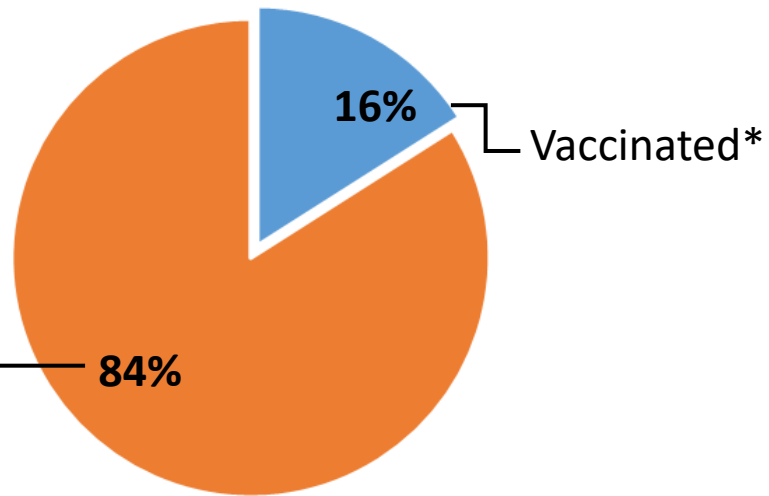
*Received at least one dose of COVID-19 vaccine

COVID-19: Morbidity and vaccination status



Our World
in Data

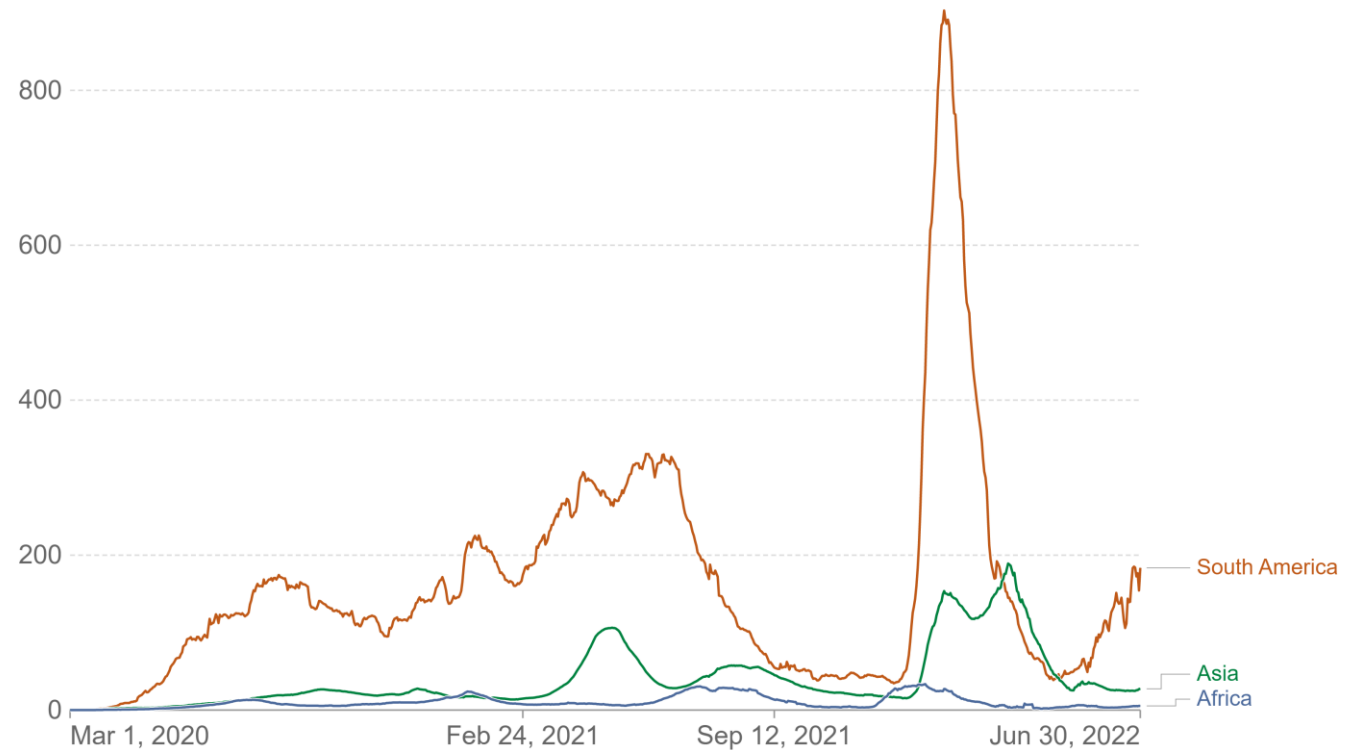
Low-income countries



*Received at least one dose of COVID-19 vaccine

Daily new confirmed COVID-19 cases per million people

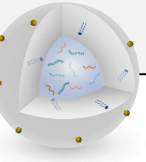
7-day rolling average. Due to limited testing, the number of confirmed cases is lower than the true number of infections.



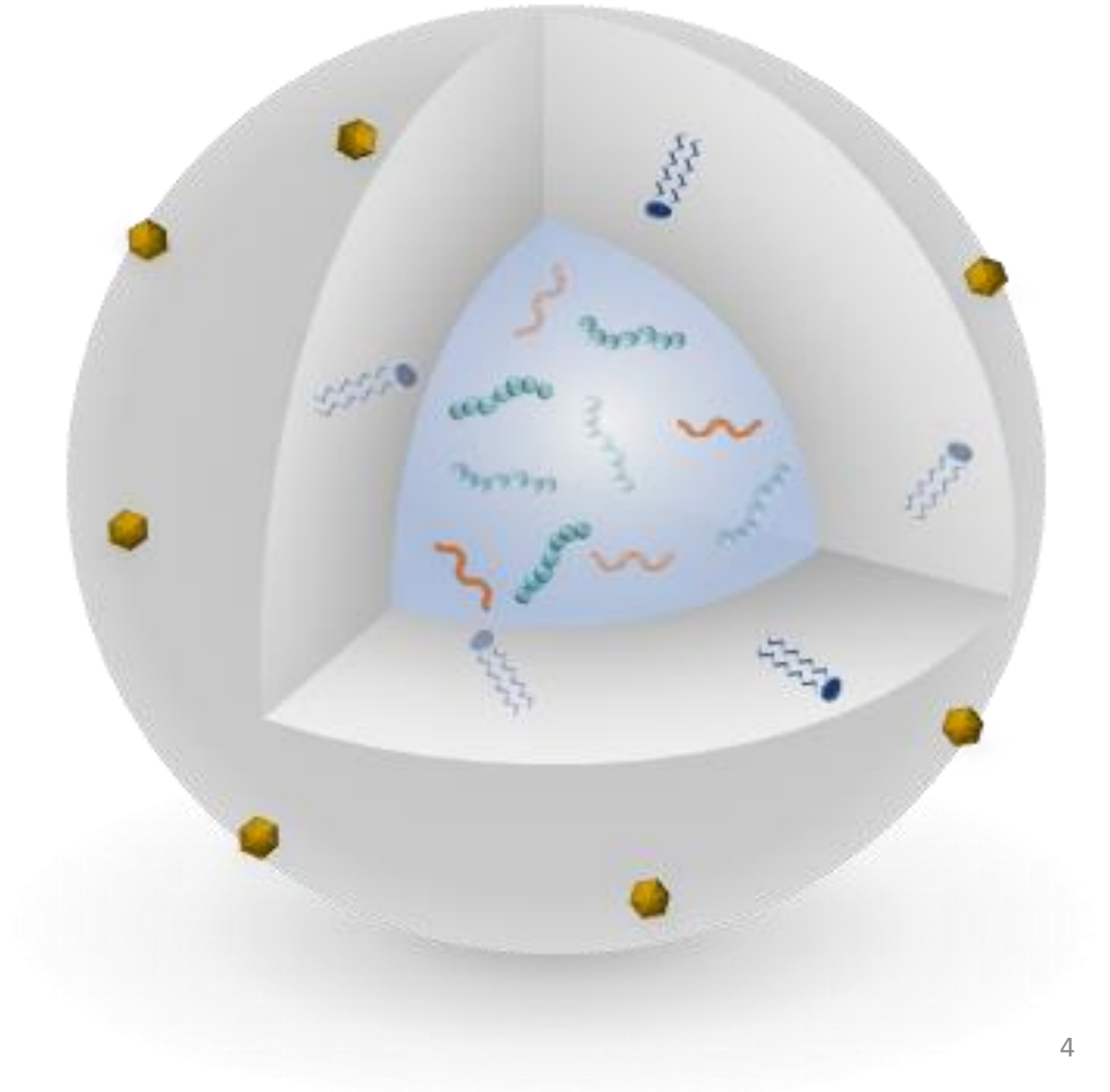
Source: Johns Hopkins University CSSE COVID-19 Data

CC BY

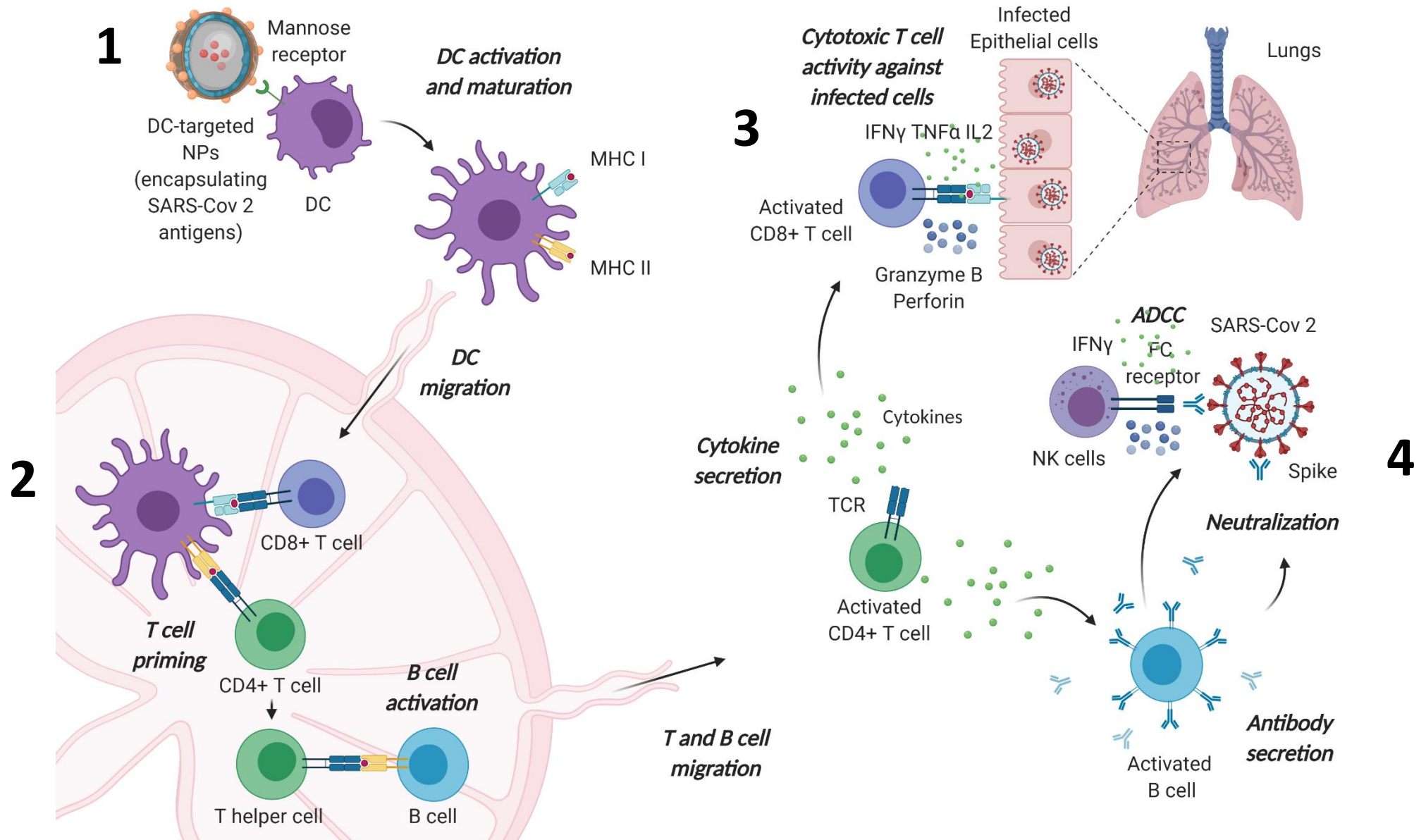
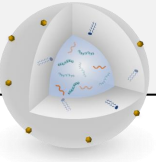
Multi-epitope- and siRNA-based Nano-Vaccine



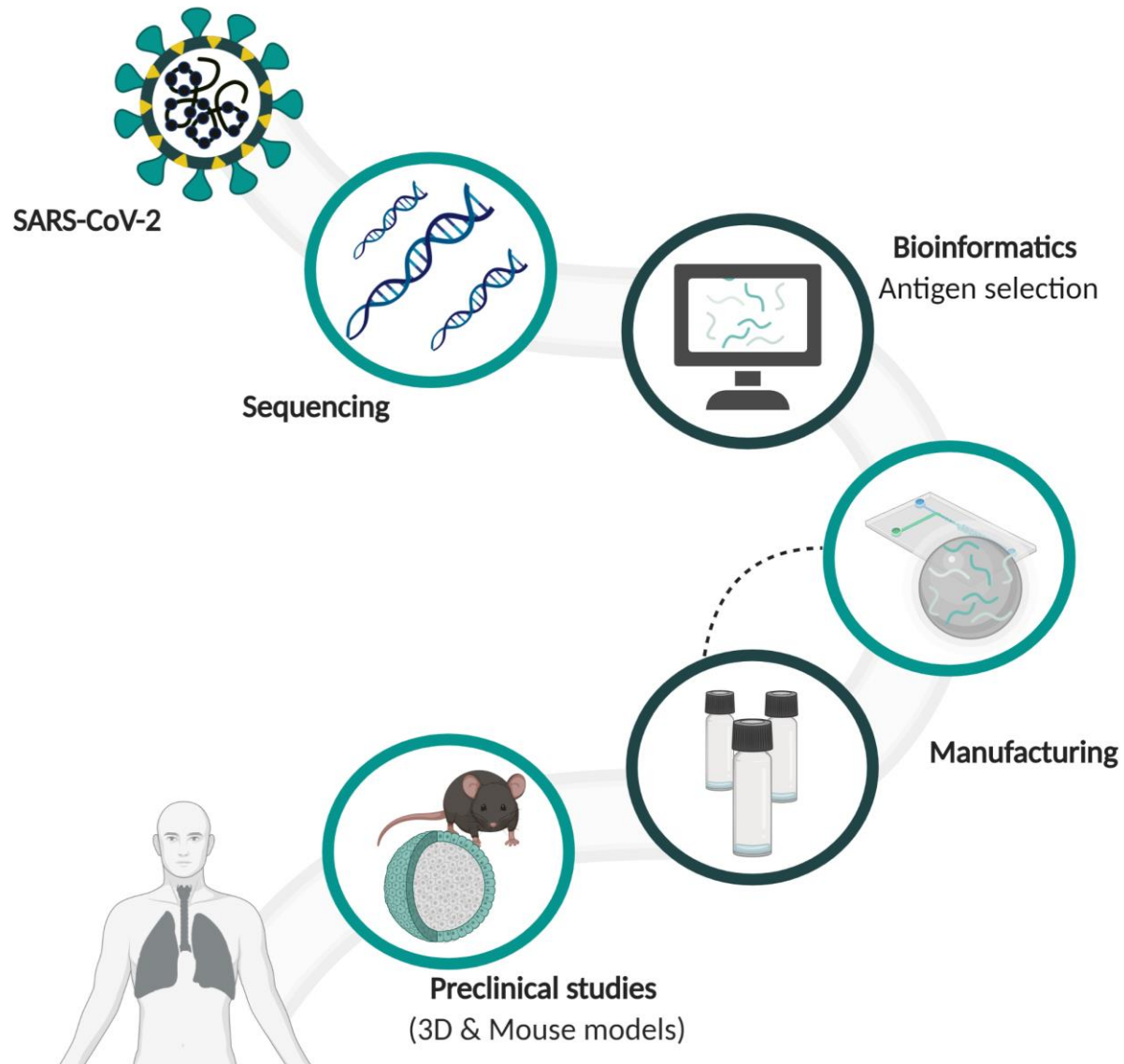
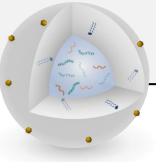
- **Safe**
- **Specific**
- **Immunogenic**



DC-Targeted Nano-Vaccine against SARS-CoV-2



SARS-CoV-2 Nano-Vaccine: Strategy



Rita Acúrcio

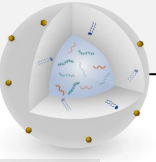


Daniella Vaskovich



- **Bioinformatic-assisted selection of SARS-CoV-2 immunogenic MHC class I and MHC class II peptides:**
 - Surface accessibility
 - Absence of reported mutations
 - High population coverage
- **Antigen epitopes validated for T cell and B cell activation**

COVID-19 Nano-Vaccine: Characterization



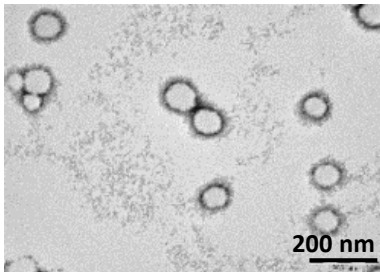
SARS-CoV-2-associated
Spike (S) Antigens
(MHC class I and II)

Modulators of
DC function
(CpG and Poly(I:C))

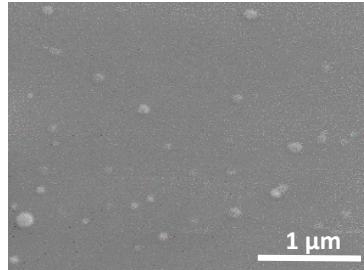
Polymeric matrix
(PLA/PLGA)

Targeting ligands
(Mannose)

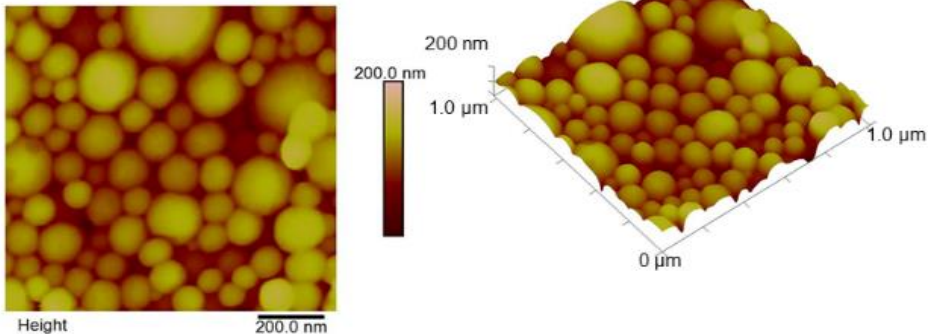
TEM



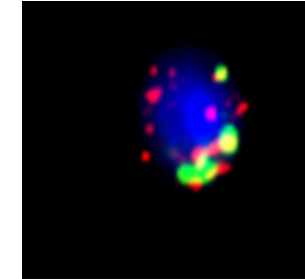
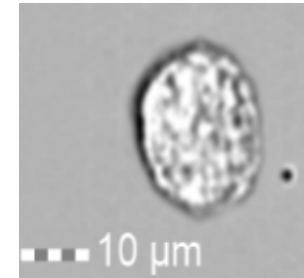
SEM



AFM



Bone marrow DC (BMDC) internalizing Cy5-labeled NP

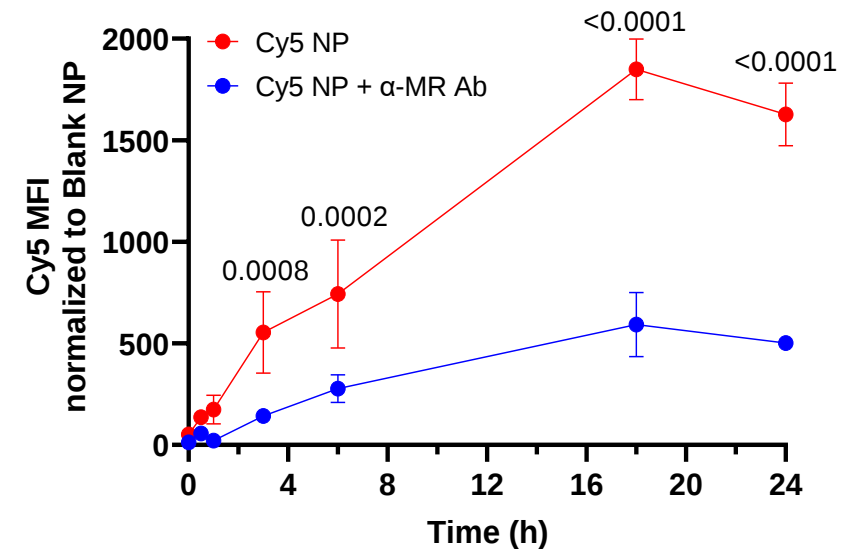


Nanovaccine

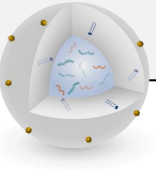
Lysotracker

Nucleus

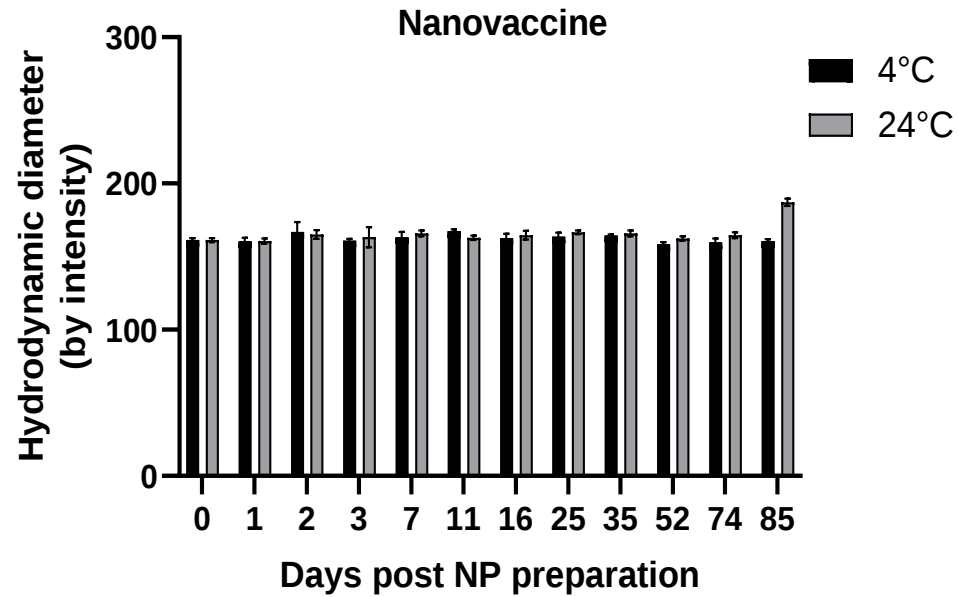
Cy5-labeled NP internalization profile by BMDC



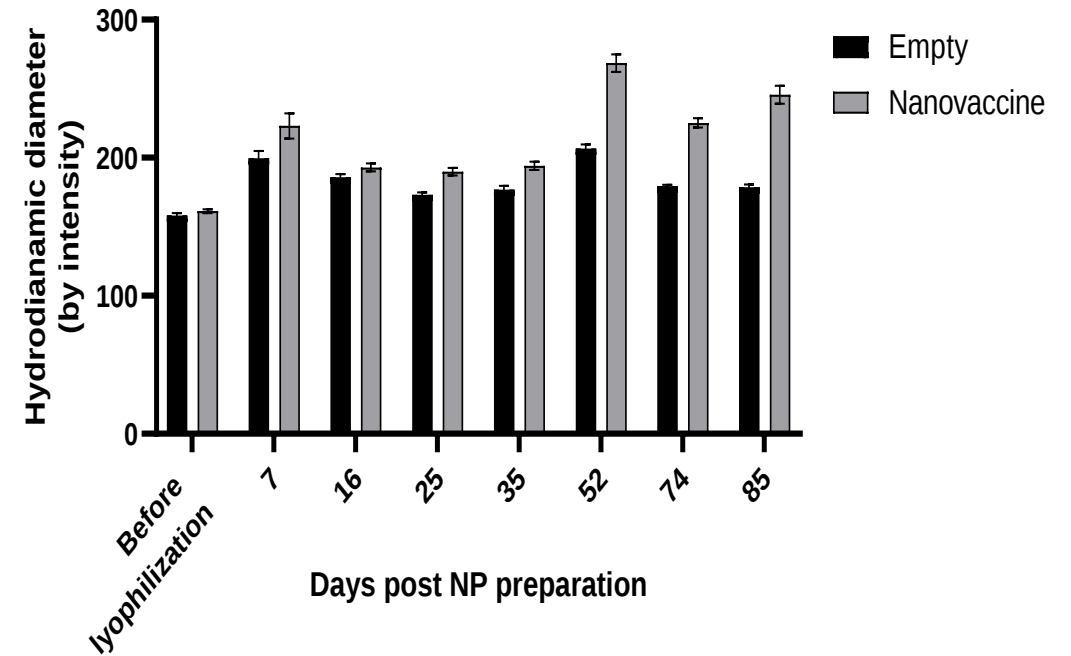
SARS-CoV-2 Nano-Vaccine: Stability at RT



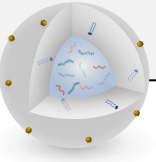
Stable in suspension



Stable as a lyophilized powder

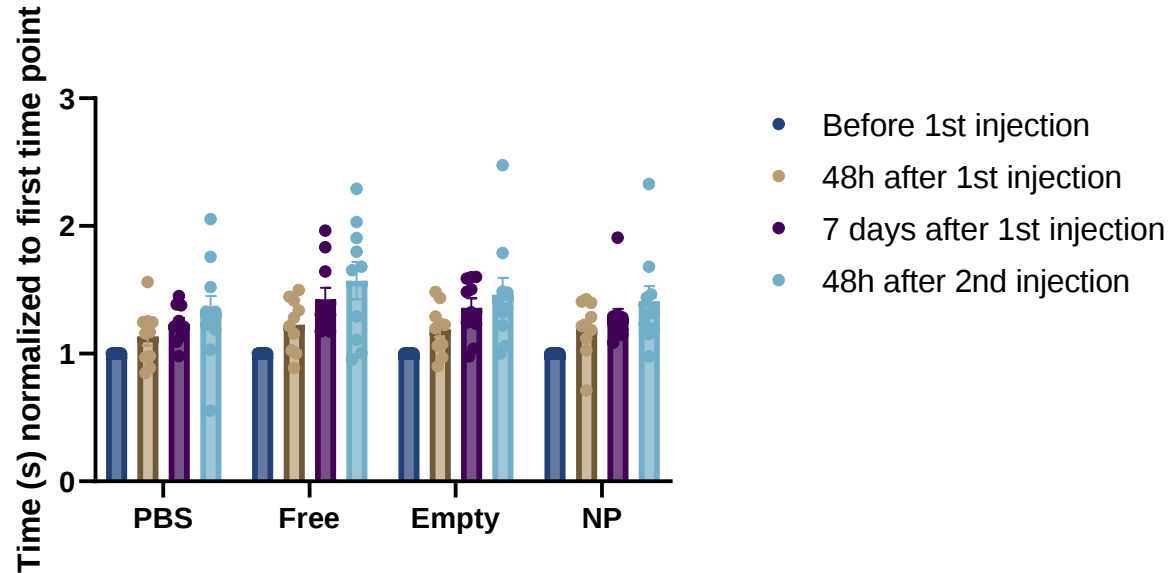


SARS-CoV-2 Nano-Vaccine: Preclinical Safety

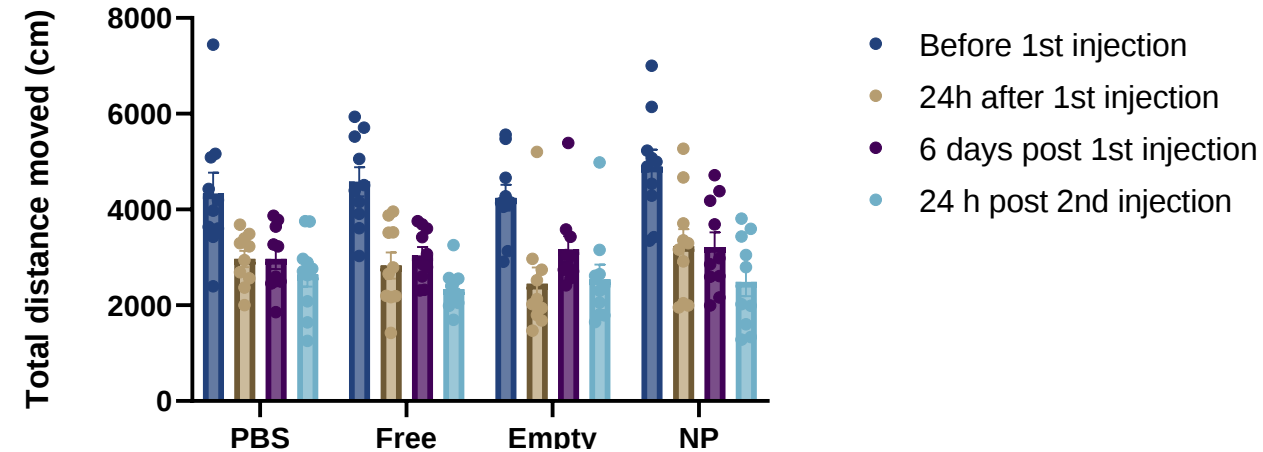
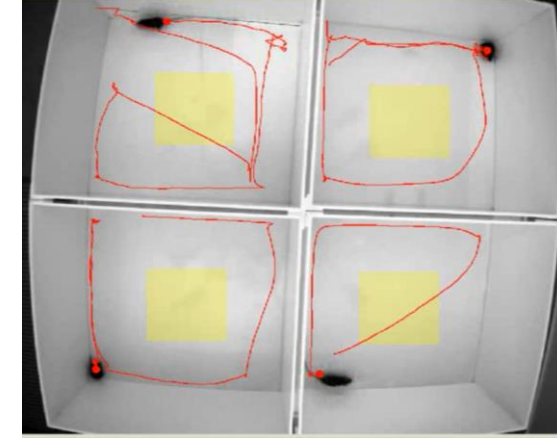


S.C. administration of *Nano-Vaccine* did not induced neurotoxicity or affect mice motor coordination

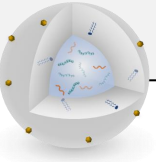
Rotarod



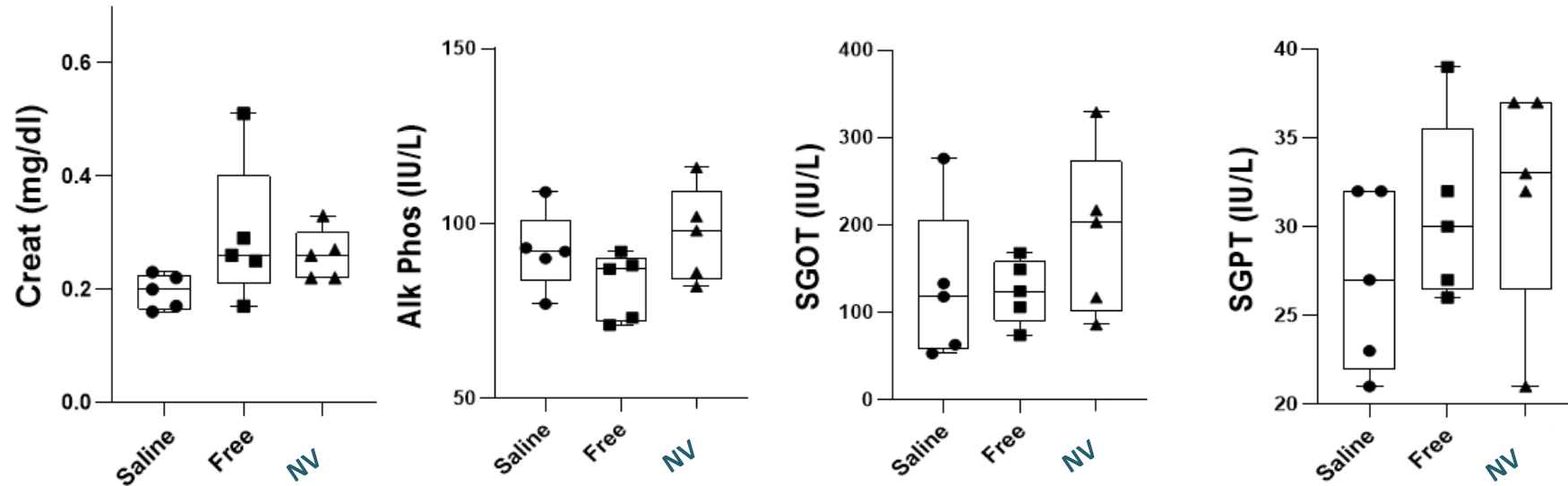
Open Field



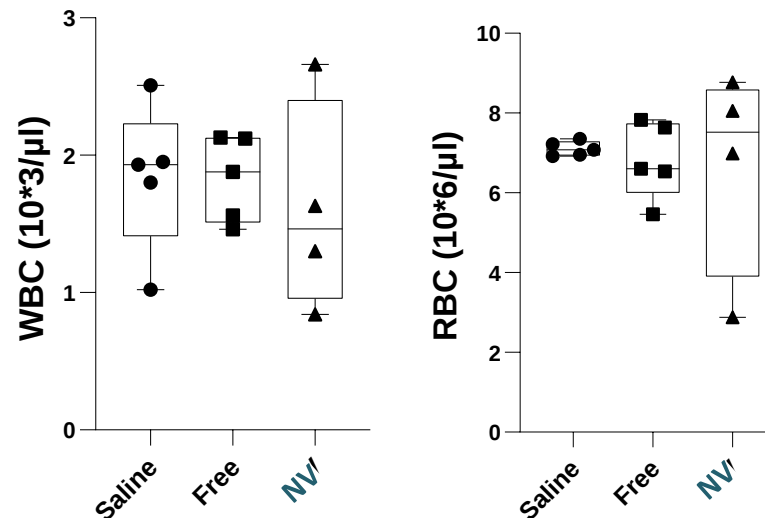
SARS-CoV-2 Nano-Vaccine: Preclinical Safety



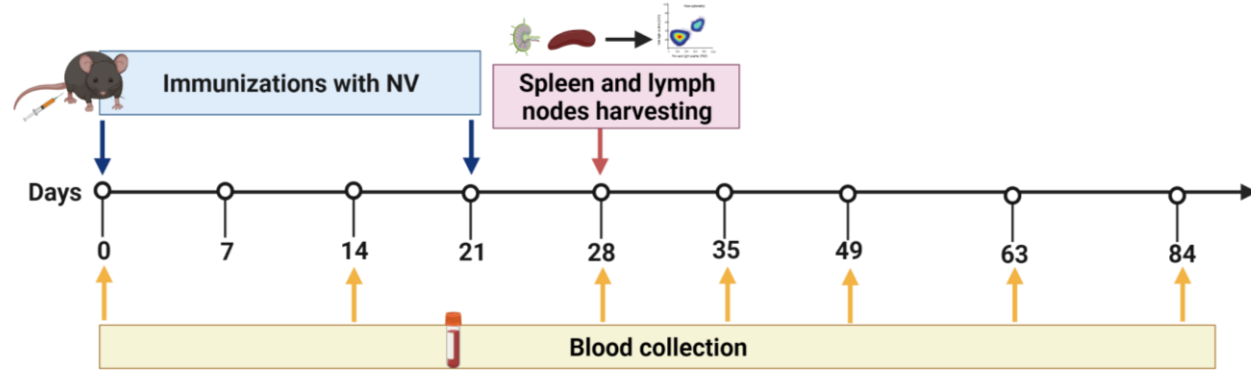
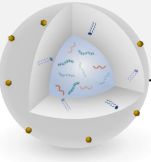
S.C. administration *Nano-Vaccine* did not show any abnormal toxicity in blood chemistry



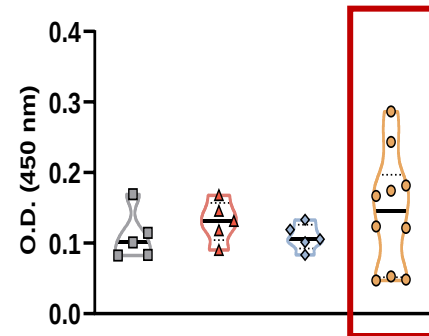
S.C. administration of *Nano-Vaccine* did not show any abnormal toxicity in blood hematology



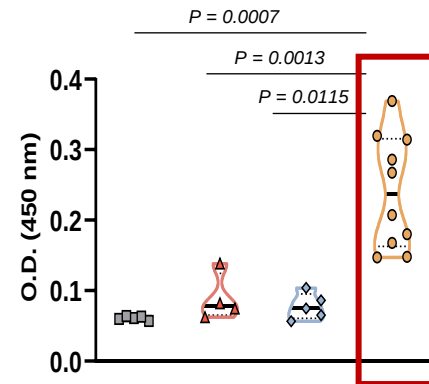
SARS-CoV-2 Nano-Vaccine: Elicits robust humoral immune response



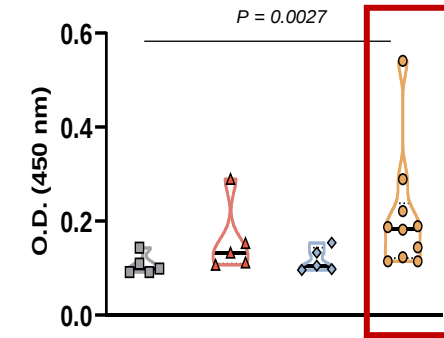
IgG MHC-I coating



IgG MHC-II coating

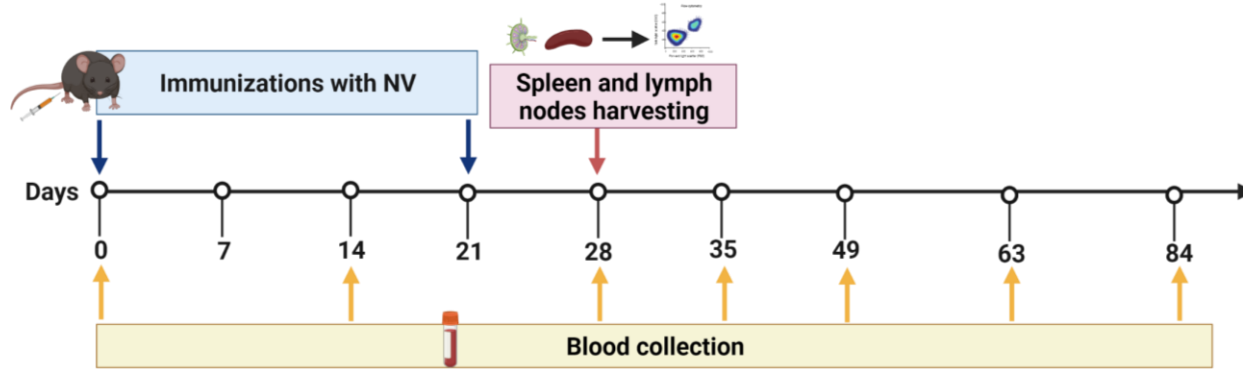
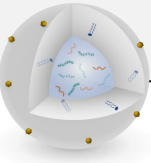


IgG RBD WT coating

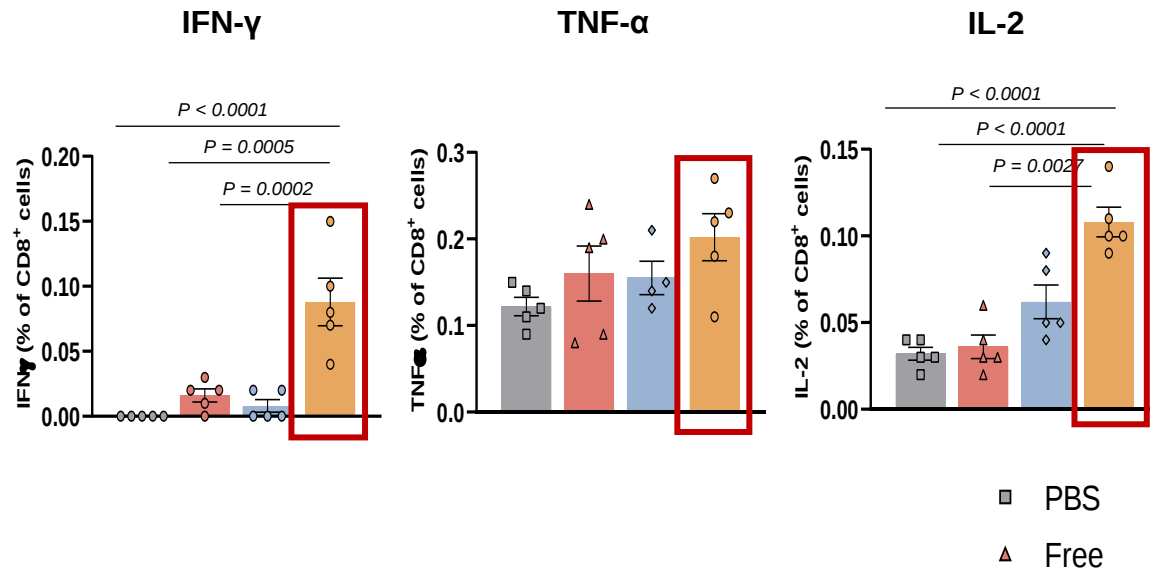


- PBS
- ▲ Free
- ◆ Empty NP
- NV

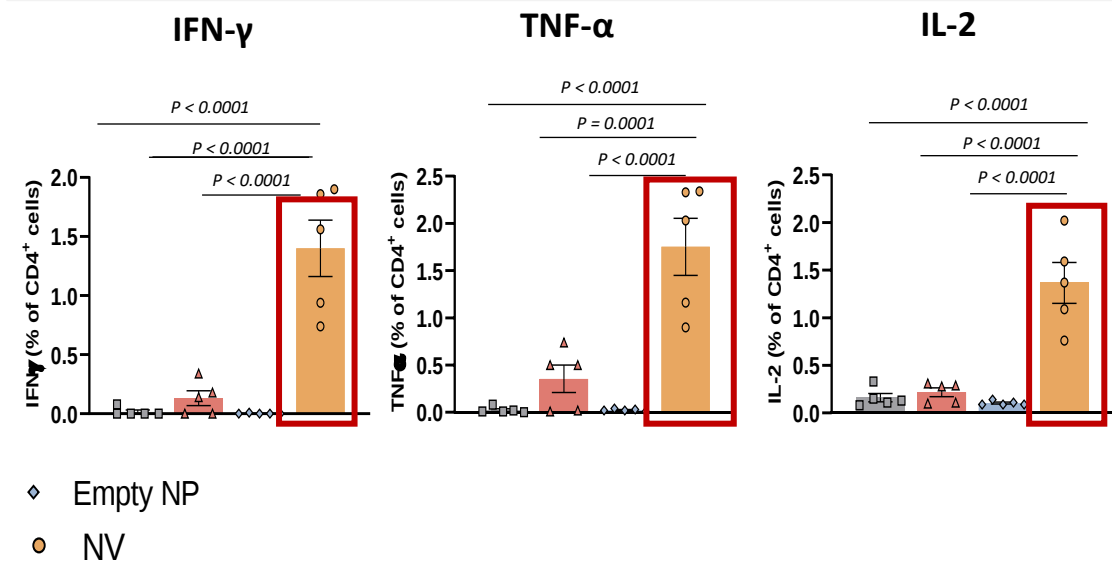
SARS-CoV-2 Nano-Vaccine: Elicits robust cellular immune response



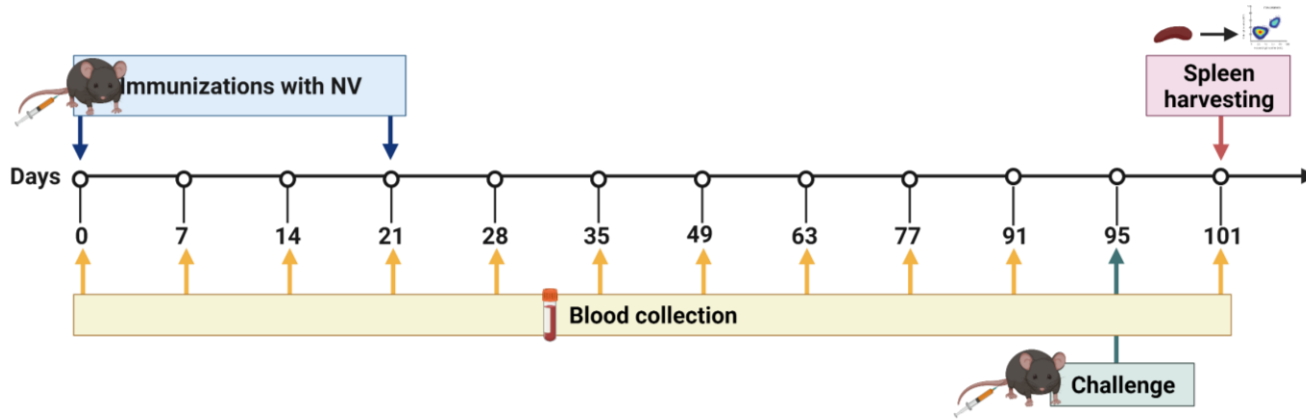
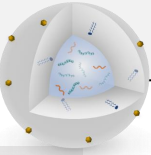
CD8⁺ T cells



CD4⁺ Th1 cells

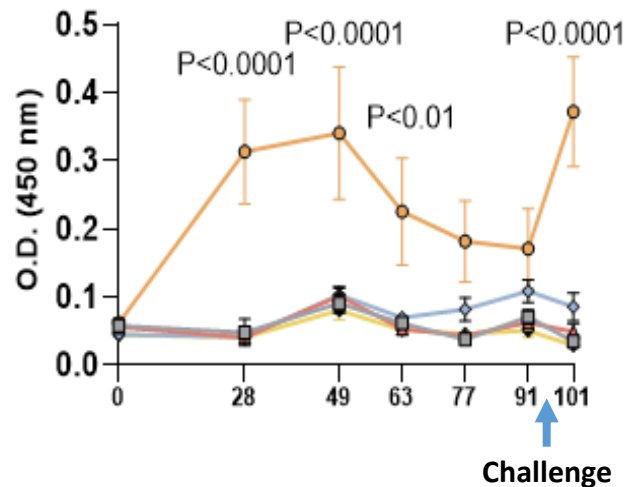


SARS-CoV-2 Nano-Vaccine: induced memory immunity

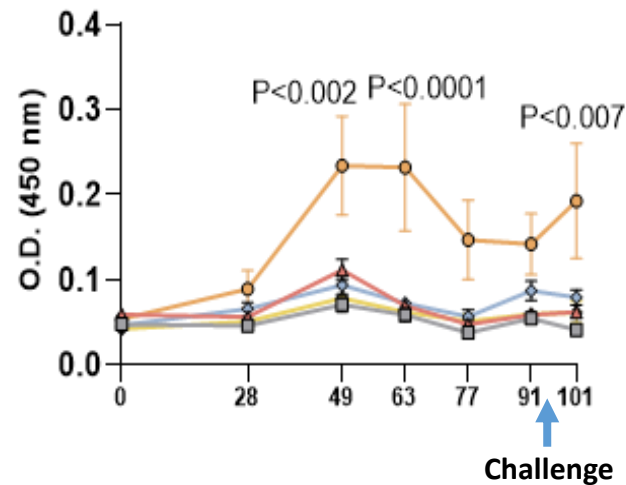


Humoral response

IgG MHC-I coating

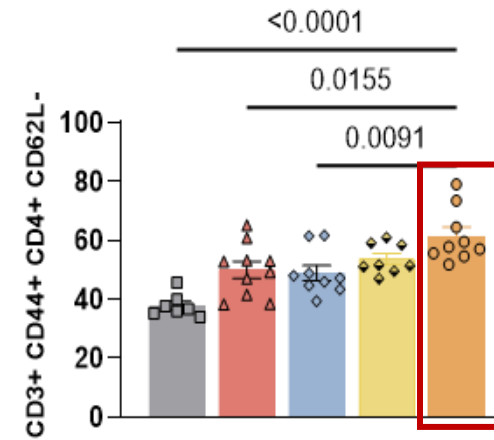


IgG MHC-II coating

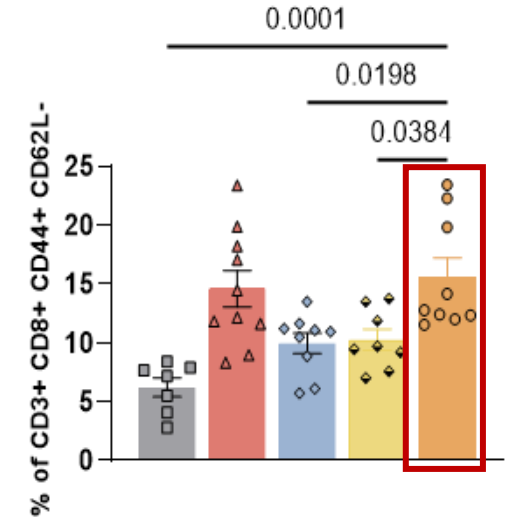


Cellular response

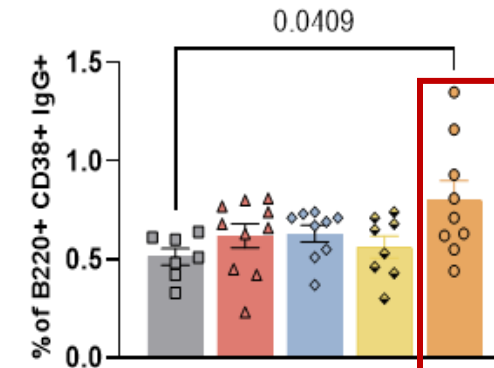
CD4+ effector memory



CD8+ effector memory

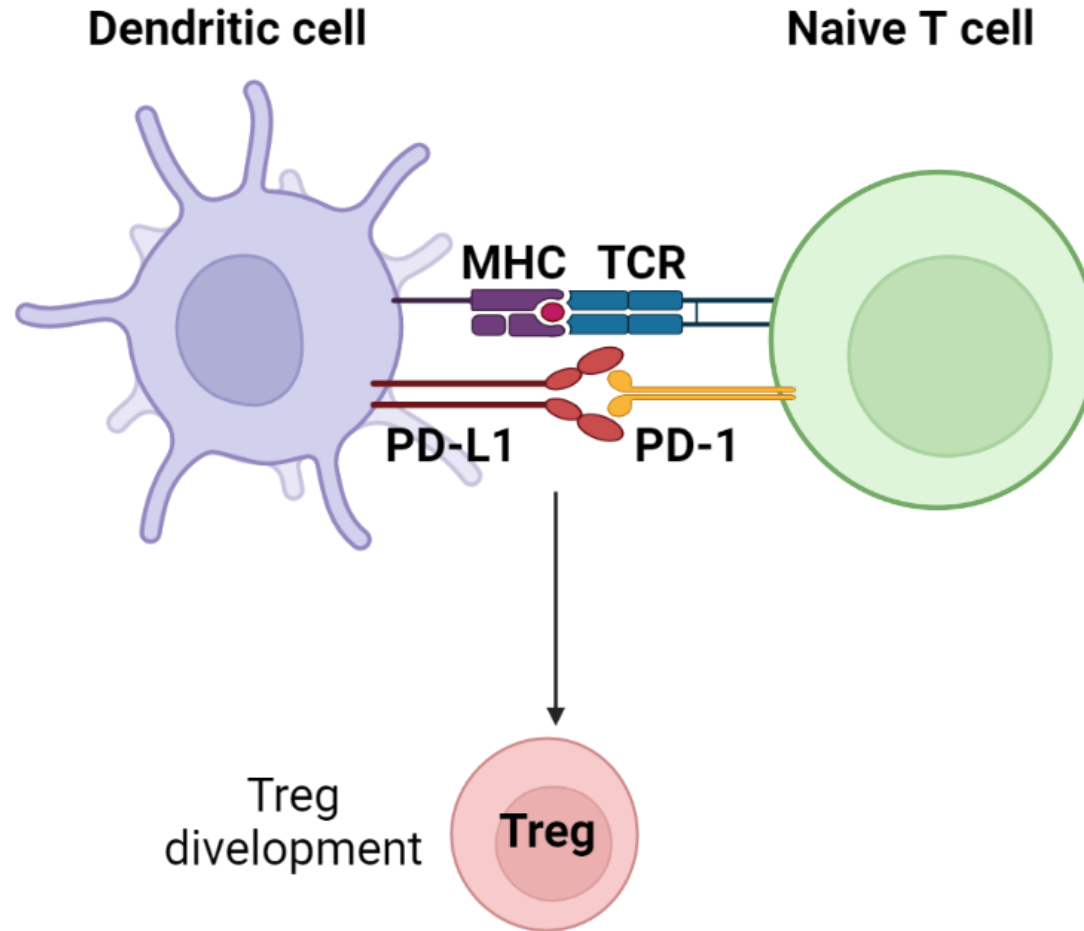
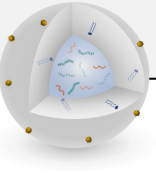


Memory B cells

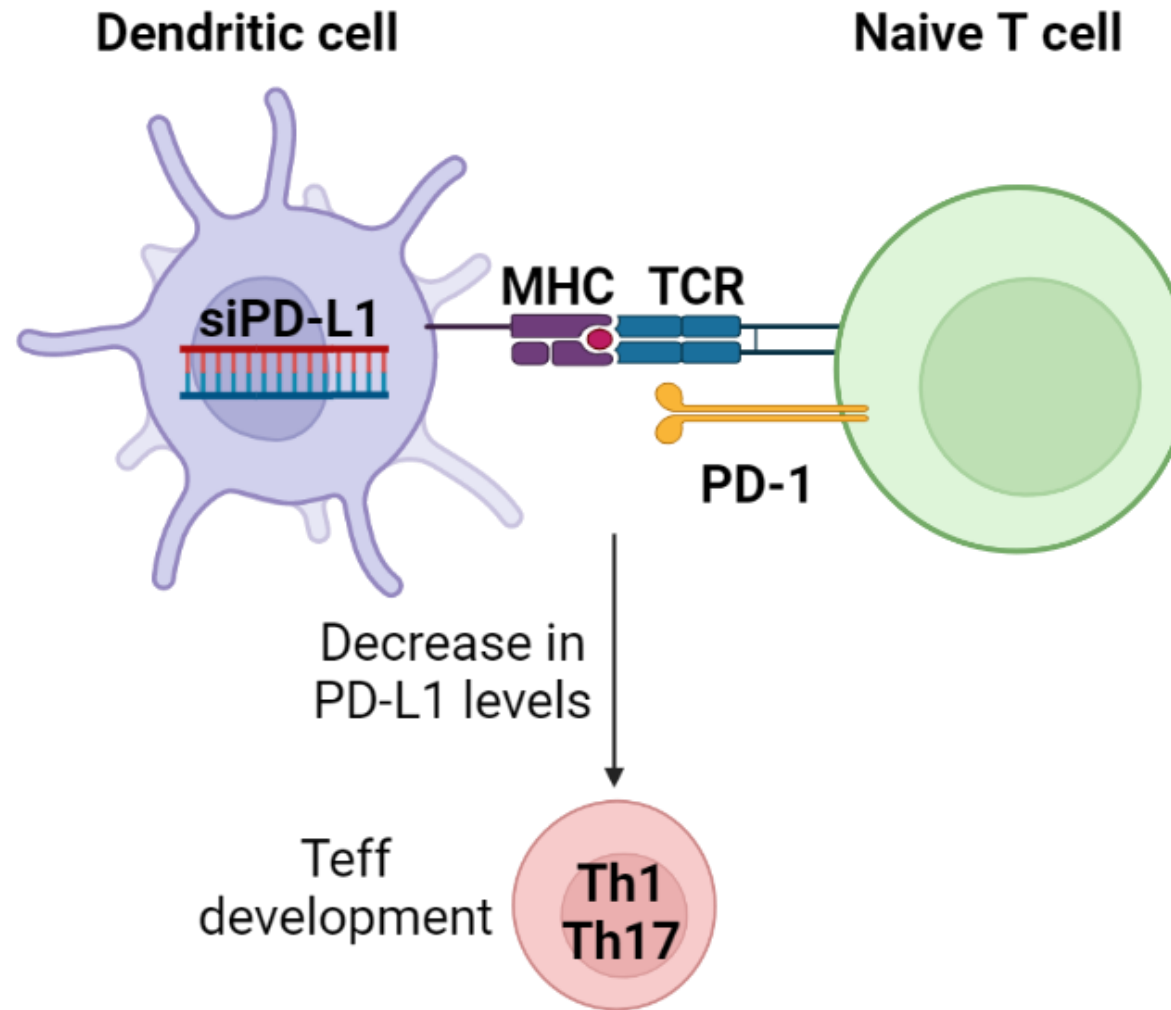
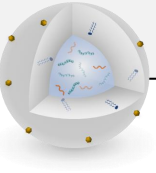


- PBS
- ▲ Free
- ◆ Empty NP
- ◆ Empty NP+Adj
- NV

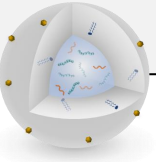
SARS-CoV-2 Nano-Vaccine: siPD-L1



SARS-CoV-2 Nano-Vaccine: siPD-L1

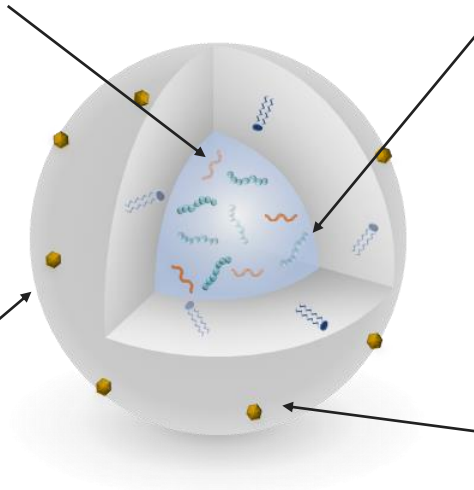


Multi-epitope and siRNA Nano-Vaccine



**SARS-CoV-2-associated
Spike (S) Antigens**
(MHC class I and II)

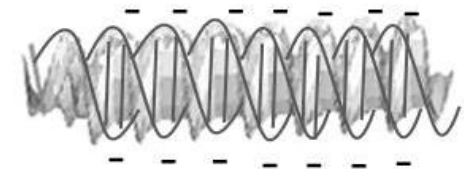
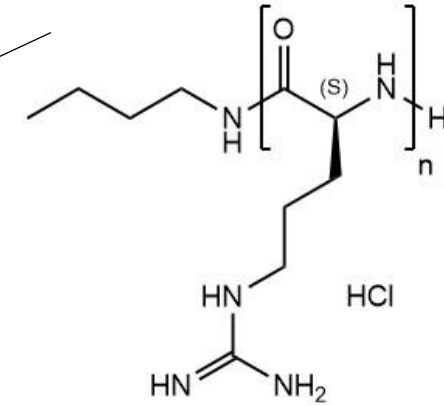
**Polymeric
matrix**



**Modulators
of
DC function**
(CpG, Poly(I:C)
and **siPD-L1**)

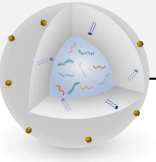
Targeting ligand
(Mannose)

Polyarginine

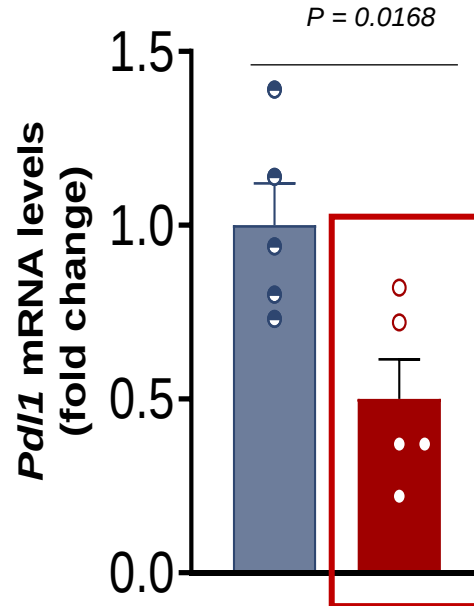


siRNA

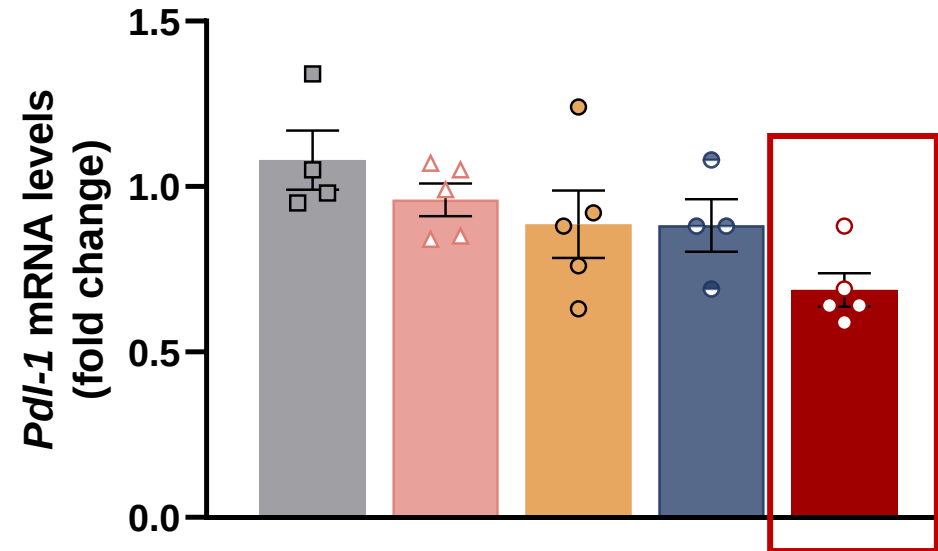
siPD-L1 cause to a decrease in PD-L1 levels in the spleen



55 h post Nano-Vaccine injection



28 days post Nano-Vaccine injection



■ PBS

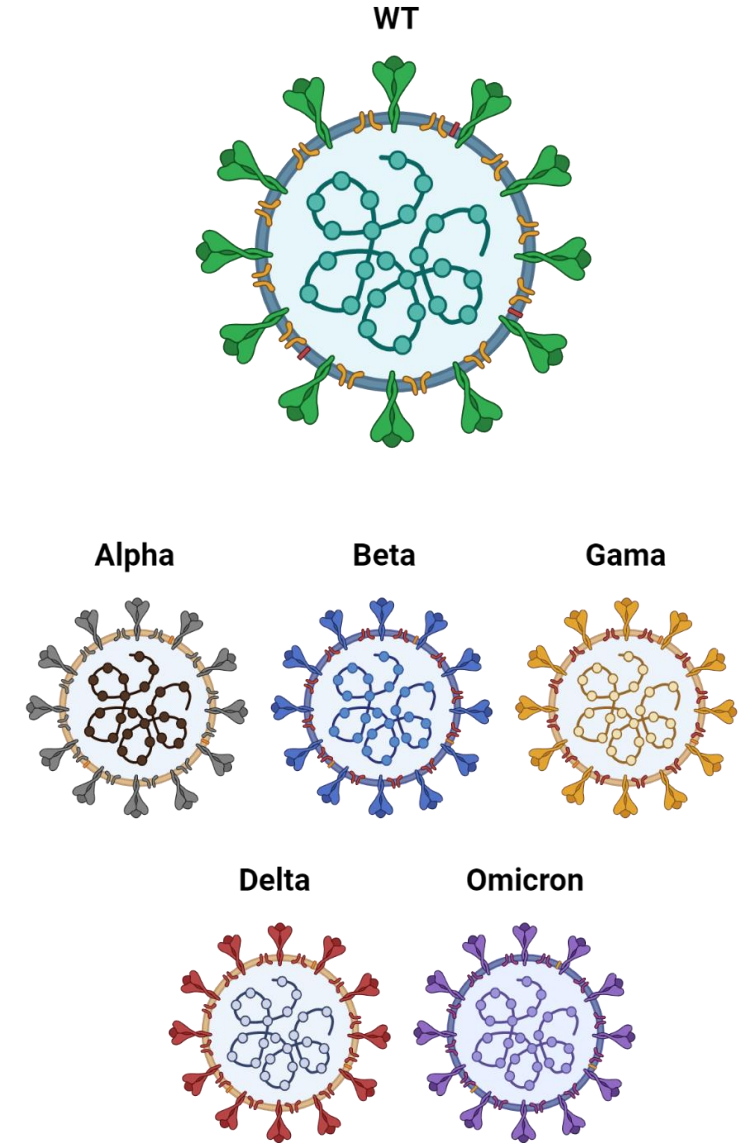
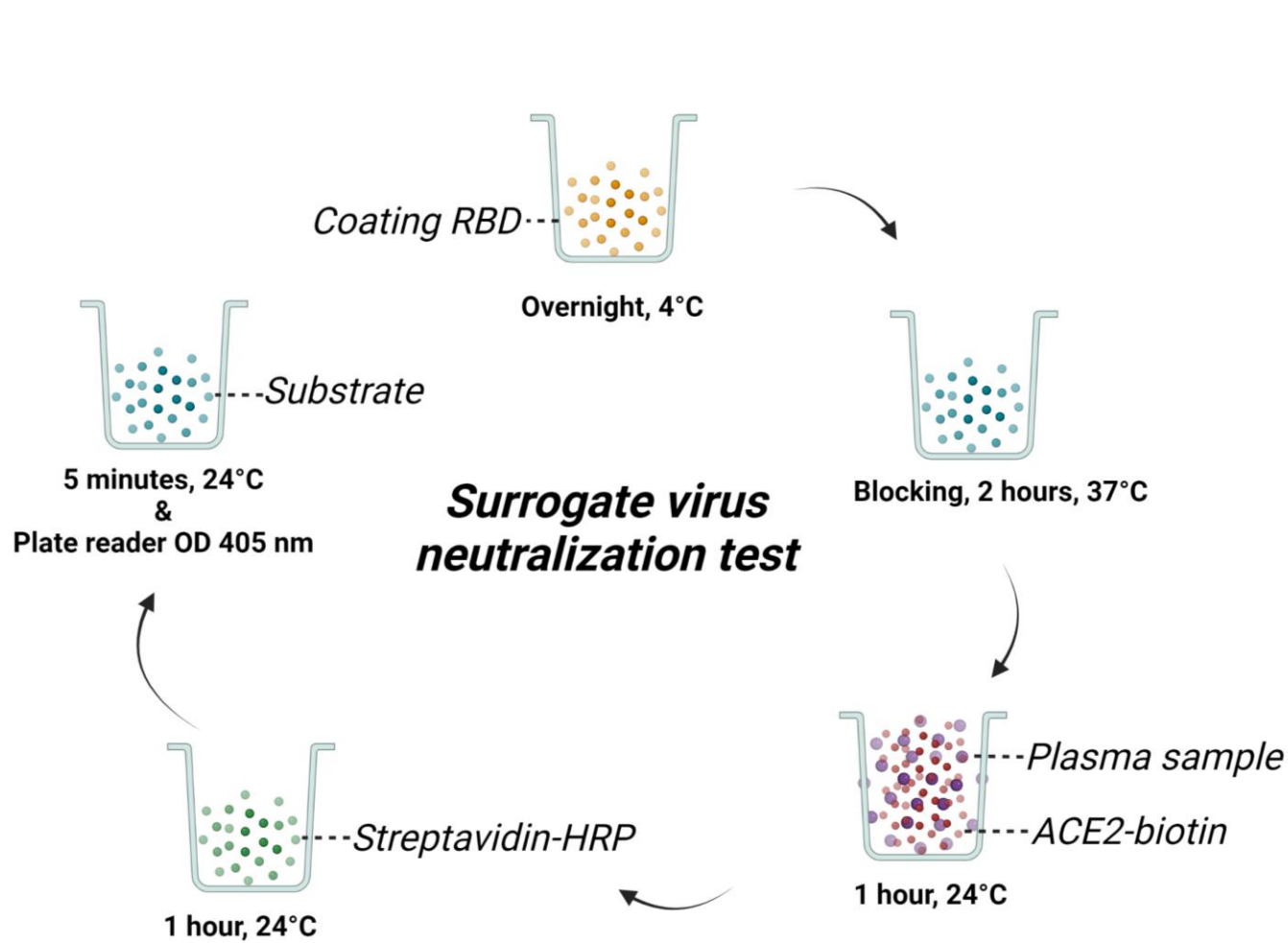
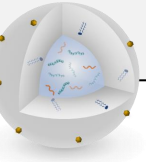
△ Free MHC-I and MHC-II + CpG + Poly (I:C) + siRNA PD-L1

● NP MHC-I / NP MHC-II + CpG + Poly (I:C)

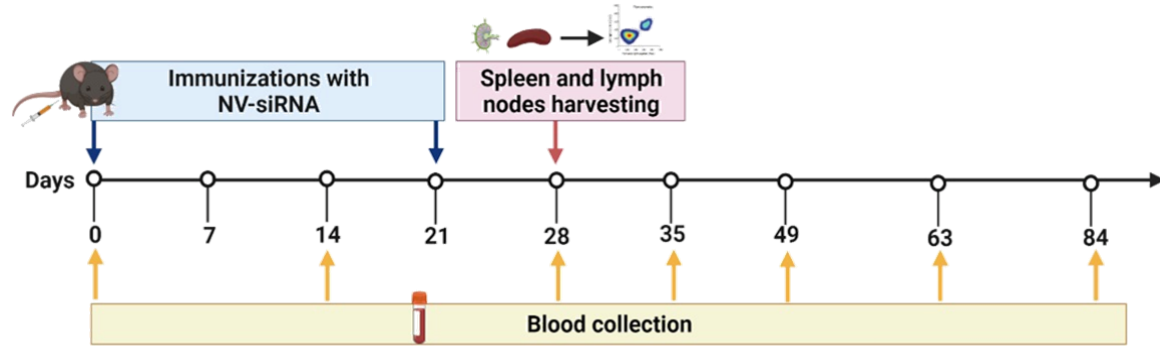
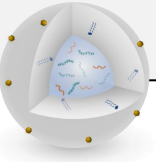
● NP MHC-I / NP MHC-II + CpG + Poly (I:C) + siRNA NC

○ NP MHC-I / NP MHC-II + CpG + Poly (I:C) + siRNA PD-L1

SARS-CoV-2 Nano-Vaccine: Neutralization test



SARS-CoV-2 Nano-Vaccine: Neutralization test



■ PBS

△ Free + siRNA PD-L1

● NV

● NV + siRNA NC

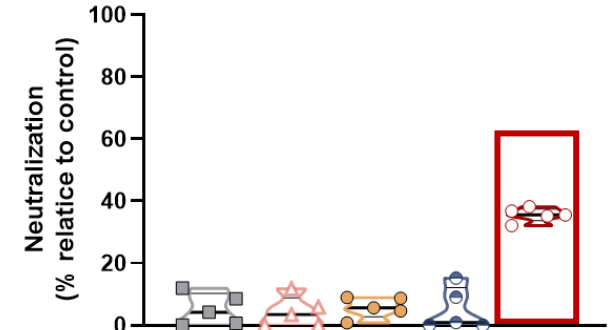
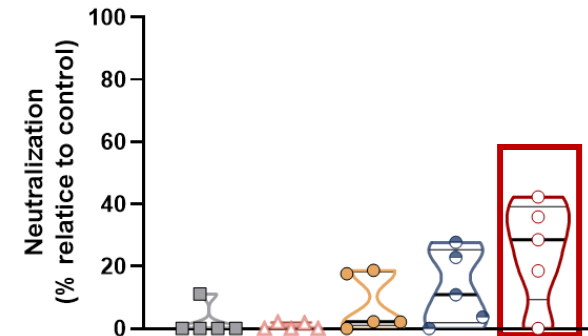
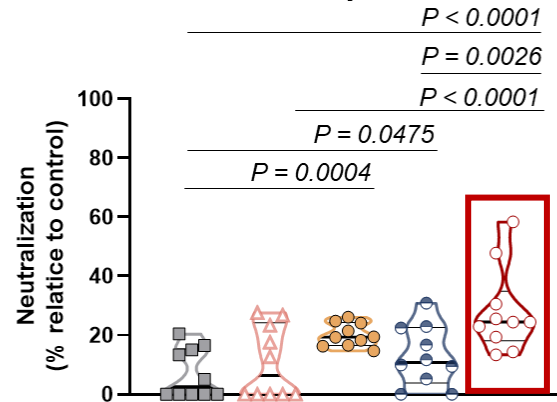
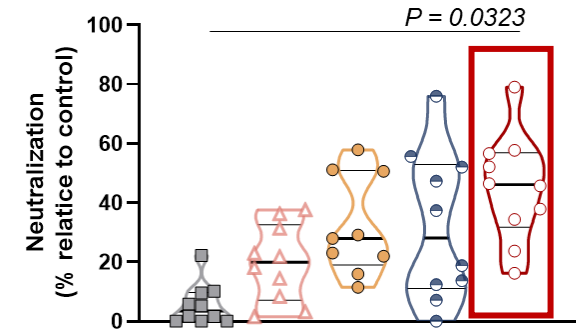
○ NV+ siRNA PD-L1

WT

Alpha

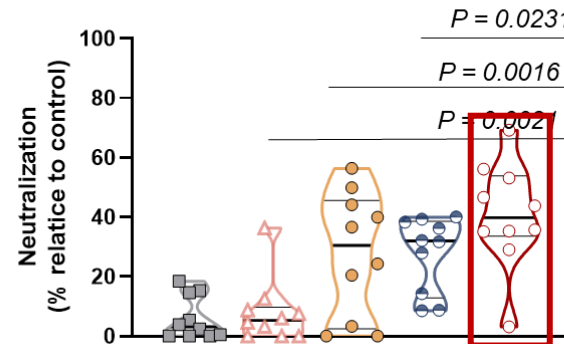
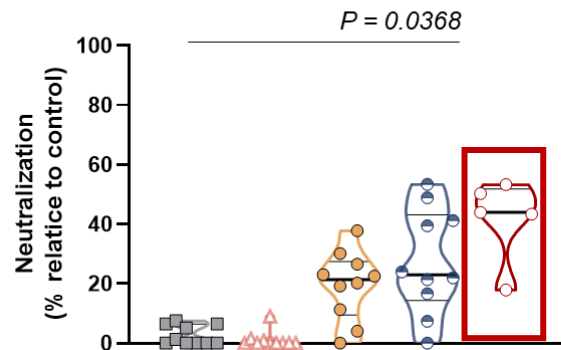
Beta

Gamma

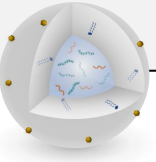


Delta

Omicron



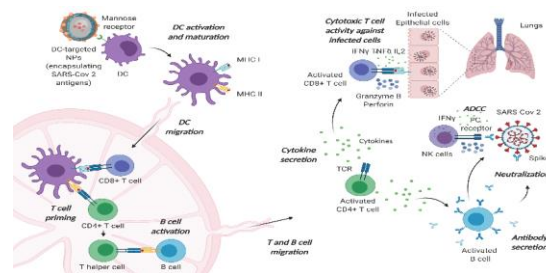
Conclusions



- Next-generation **multi peptide-, TLR agonists-, siRNA- based Nano-Vaccine**
- **Safety – Nano-Vaccine** did not reveal any abnormal toxicity.
- **Specificity - Nano-Vaccine** successfully induced antigen-specific secretion of IgM, IgG and IgA antibodies with high binding affinity to SARS-CoV-2 RBD.
- **Neutralization** - Specific neutralization of SARS-CoV-2 RBD, WT and all relevant mutations by sera of immunized mice with our **Nano-Vaccine**.
- **Efficacy - Nano-Vaccine** successfully increased the cellular activity of effector T cells and B cells.
- **Shipping advantage** - our **Nano-Vaccine** does not require cold-chain management and is stable in RT.
- **Administration routes** - the **Nano-Vaccine** can be administered intranasally (“needle-free”).



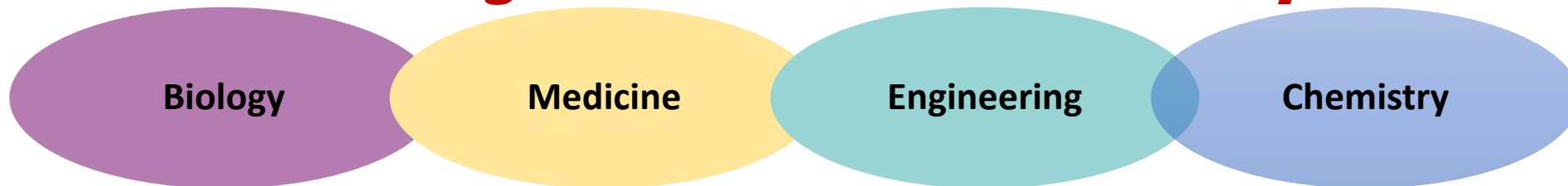
Safe to Use



**Specific, safe and immunogenic
next-generation Nano-Vaccine**



Easy to Distribute



Establishment and molecular characterization of pairs of dormant vs fast-growing, primary vs metastatic and resistant vs drug-sensitive orthotopic tumor models

Anat Eldar-Boock, PhD
Eilam Yeini
Sabina Pozzi

Sahar Dangoor-Israeli
Yael Shtilerman
Christian Burgos
Opal Avramoff
Yulia Liubomirski, PhD
Paula Ofek, PhD
Galia Tiram, PhD
Shiran Ferber, PhD
Hadas Gibori, PhD
Dikla Ben-Shushan, PhD
Noa Reisman
Sapir Golan
Roni Blatt
Keren Miller, PhD
Liron Stern
Nitzan Albeck

Patient-derived tumor models

Daniella Vaskovich
Lidar Fridrich
Rachely Grossman, MD
Miki Goldenfeld
Shelly Sofer, MD
Roni Shreiberk-Atidim, MD
Ilanit Shetzigovski-Meller, MD
Gal Bachar, MD

3D tumor models

Lena Neufeld
Ron Kleiner
Gal Shenbach
Tal Zur
Zohar Shatsberg
Ehud Segal, PhD
Liora Omer

Design, synthesis and characterization of 20 novel nanomedicines

Yana Epshtein
Shani Koshrovski
Daniel Rodriguez
Pradip Dey, PhD
Alexis Wolfel, PhD
Anna Scomparin, PhD
Shay Eliyahu, PhD
Hemda Baabur-Cohen, PhD
Ela Markovsky, PhD
Dina Polyak, PhD
Adva Krivitsky, PhD
Racheli Blau, PhD
Evgeny Pisarevsky, PhD
Joao Coniot, PhD
Alessio Malfanti, PhD
Ruri Ruperti
Marina Buzhor

Administration
Ayelet Hashdi

