Pioneering intranasal siRNA-based Nanovaccine

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imed Research Institute for Medicines
TEL AVIV UNIVERSITY אוניברסיטת תל אביב
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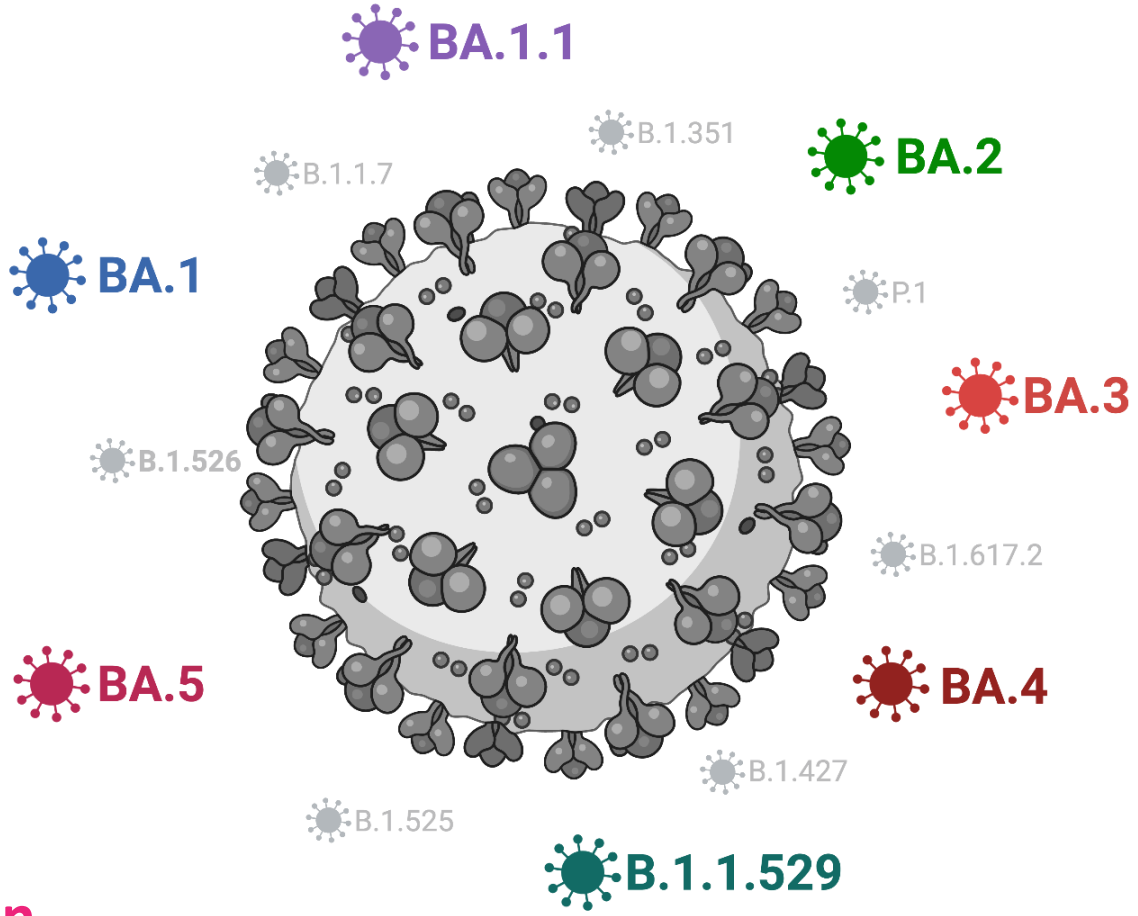
COVID-19 Challenges & Opportunities

Multiple vaccines are authorized or received approval for emergency use against SARS-CoV-2

67% of the world population has received at least one dose of a COVID-19 vaccine

SARS-CoV-2 variants of concern are a threat to currently approved COVID-19 vaccines



Alternative and more effective immunization strategies to block the transmission of SARS-CoV-2

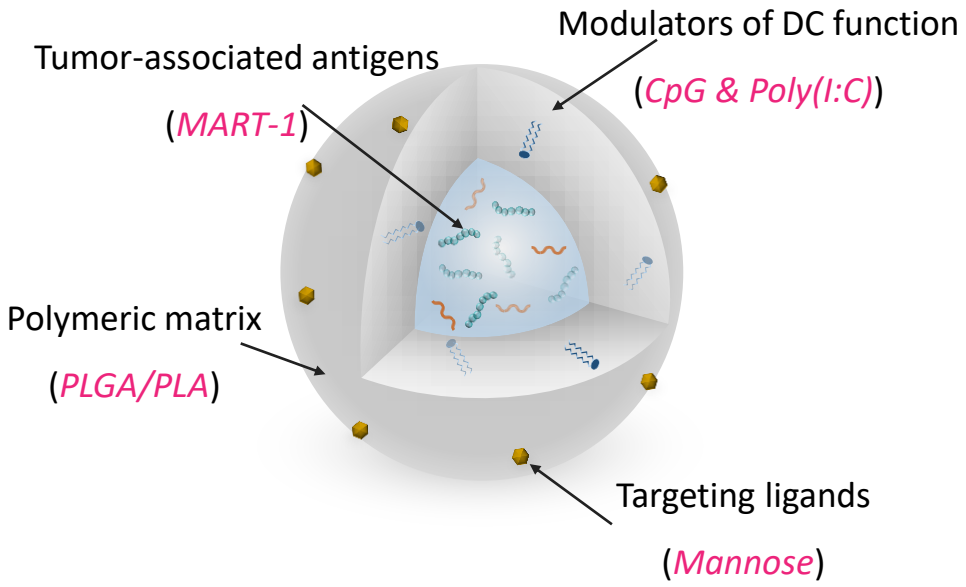
The Multifunctional Nanoplatform

nature nanotechnology

ARTICLES

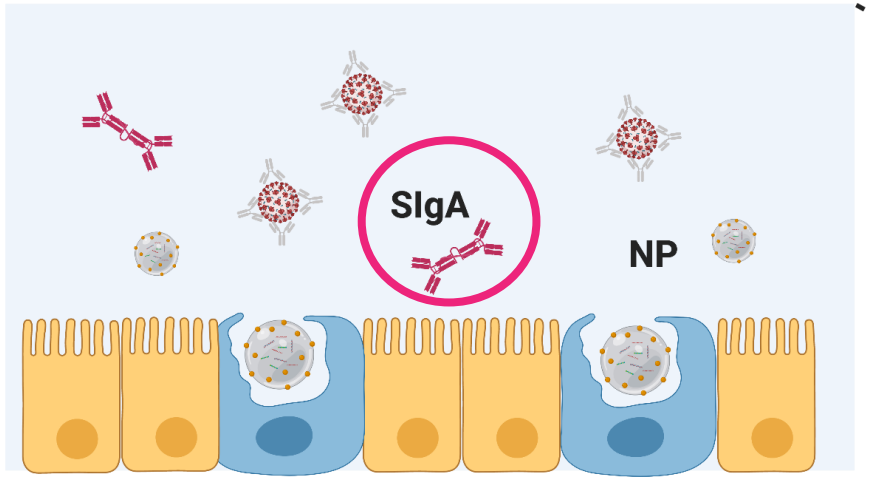
https://doi.org/10.1038/s41565-019-0512-0

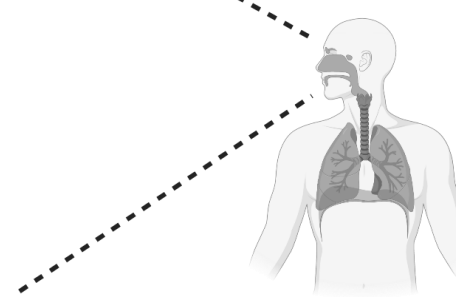
Immunization with mannosylated nanovaccines and inhibition of the immune-suppressing microenvironment sensitizes melanoma to immune checkpoint modulators



Conniot, Scomparin *et al. Nature Nanotechnology*, 2019 Sep;14(9):891-901.

The Multifunctional Nanoplatform



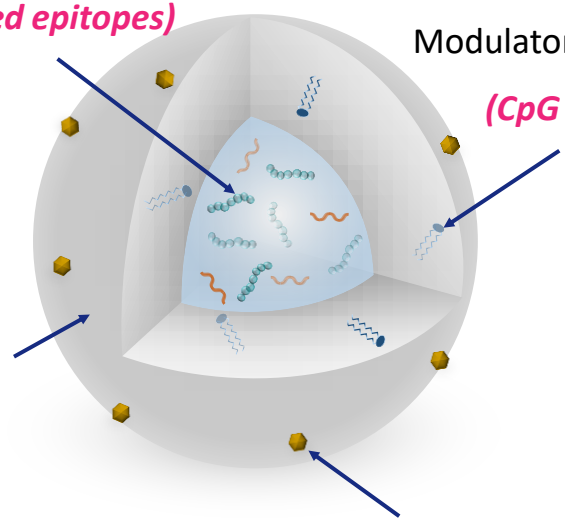


SARS-CoV-2-associated Antigens

(Spike-associated epitopes)

Modulators of DC function

(CpG & Poly(I:C))



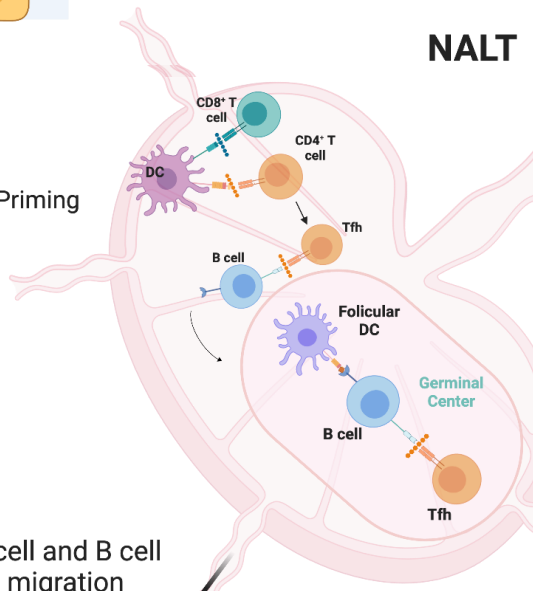
Polymeric matrix

(PLGA/PLA)

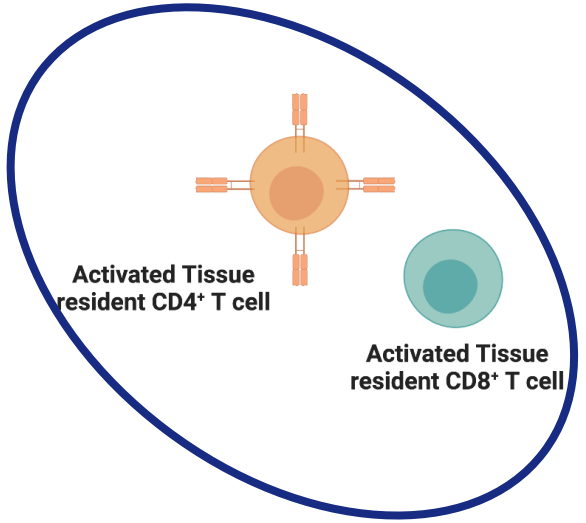
Targeting ligands

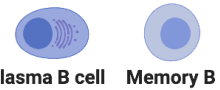
(Mannose)

NALT



T cell and B cell migration





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Intranasal DC-Targeted COVID-19 Nanovaccine

1

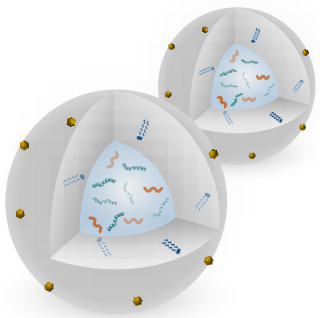
UniProt

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

Antigen Selection

Bioinformatic & Immuno-assay

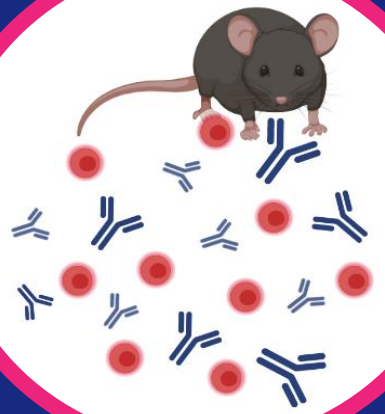
2



Nanovaccine Preparation & Characterization

Polymeric Nanoparticles

3



Nanovaccine Efficacy

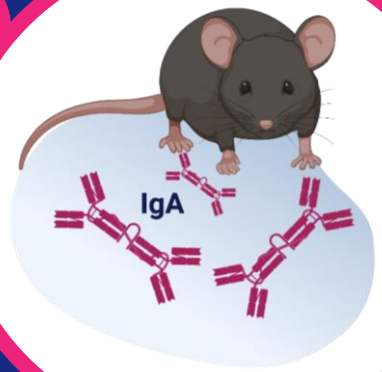
Cellular & Humoral response

5

Nano-vaccine Efficacy

SARS-CoV-2 Neutralization

4



Intranasal nanovaccine Efficacy

Mucosal immunity

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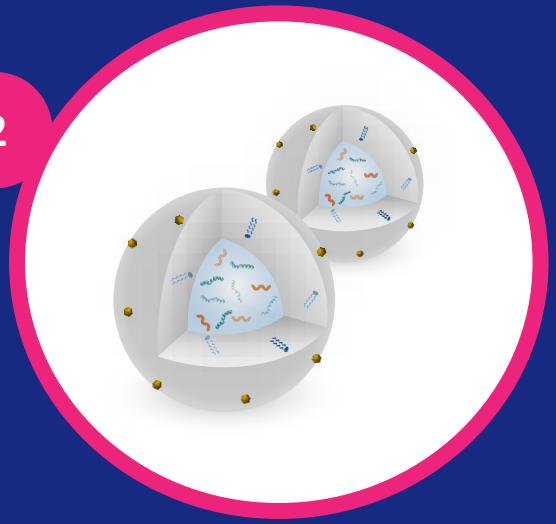
Intranasal DC-Targeted COVID-19 Nanovaccine

1

2



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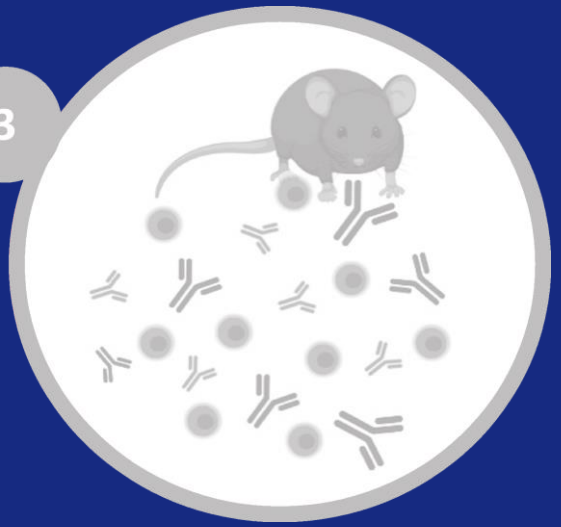
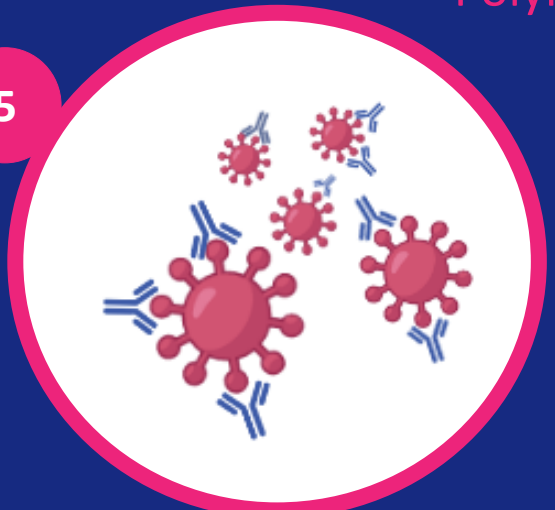


Nanovaccine Preparation & Characterization

Polymeric Nanoparticles

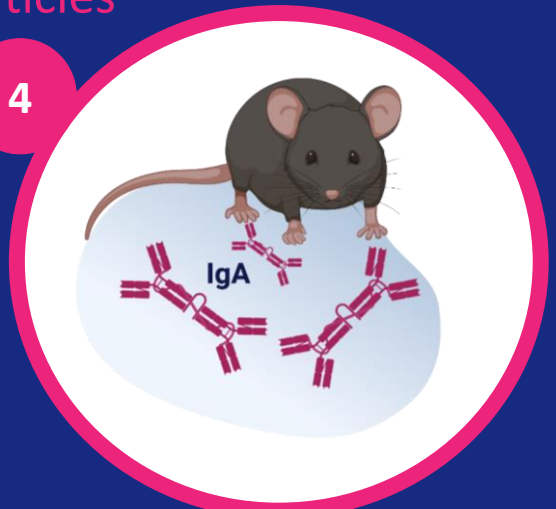
5



Nano-vaccine Efficacy

SARS-CoV-2 Neutralization

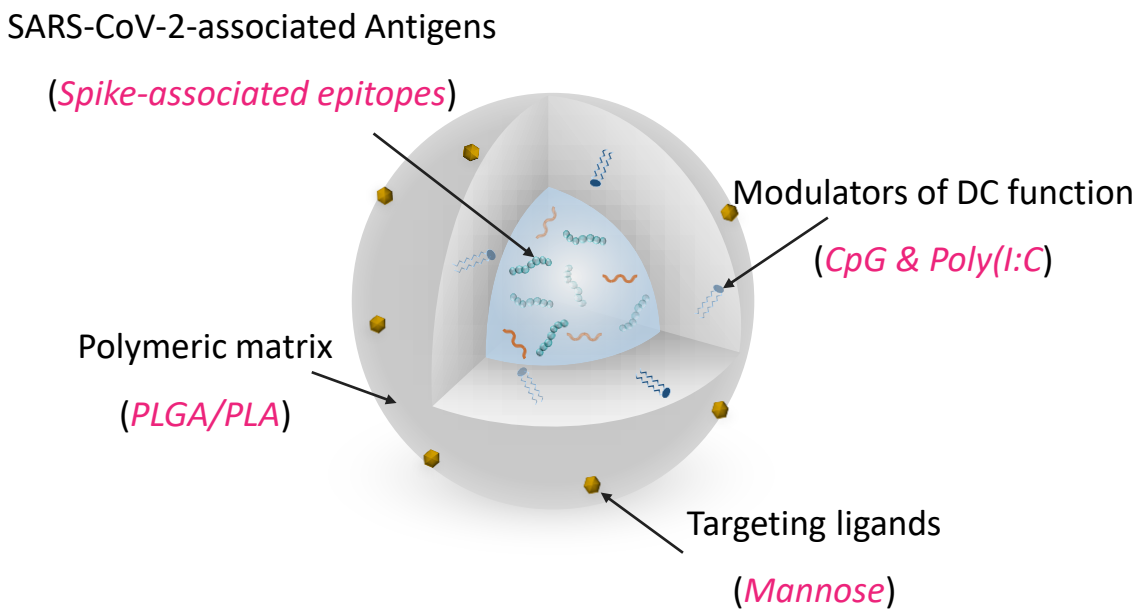
4

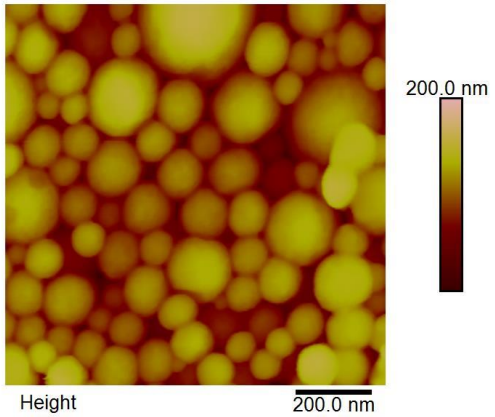


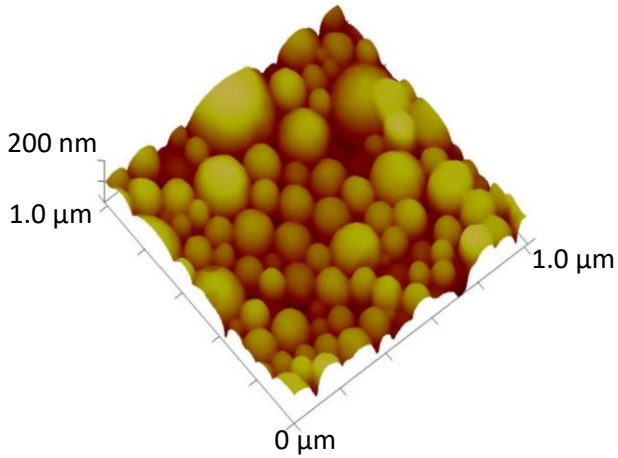
Intranasal nanovaccine Efficacy

Mucosal immunity

Intranasal DC-Targeted COVID-19 Nano-vaccine – Nanoplatform Characterization







Target product specifications for optimal DC activation

Size: < 200 nm

PDI: < 0.2

Peptides: Moderate entrapment efficiency

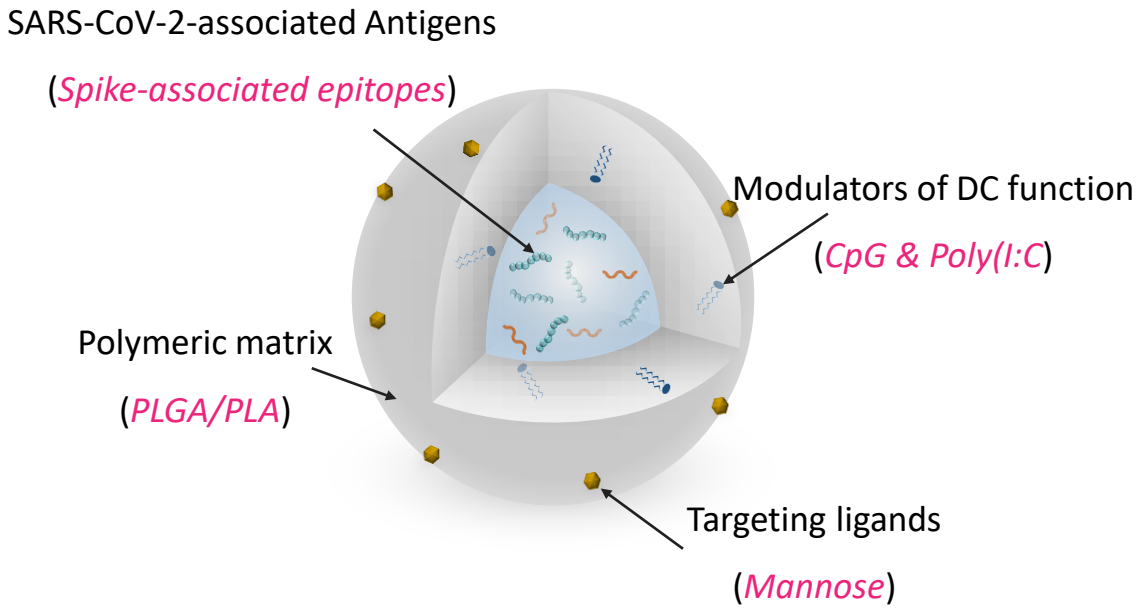
Adjuvants: High entrapment efficiency

Table 1. Nanoparticle (NP) size, polydispersity index (Đ), entrapment efficiency (EE) and loading capacity (LC) of antigens into NP.

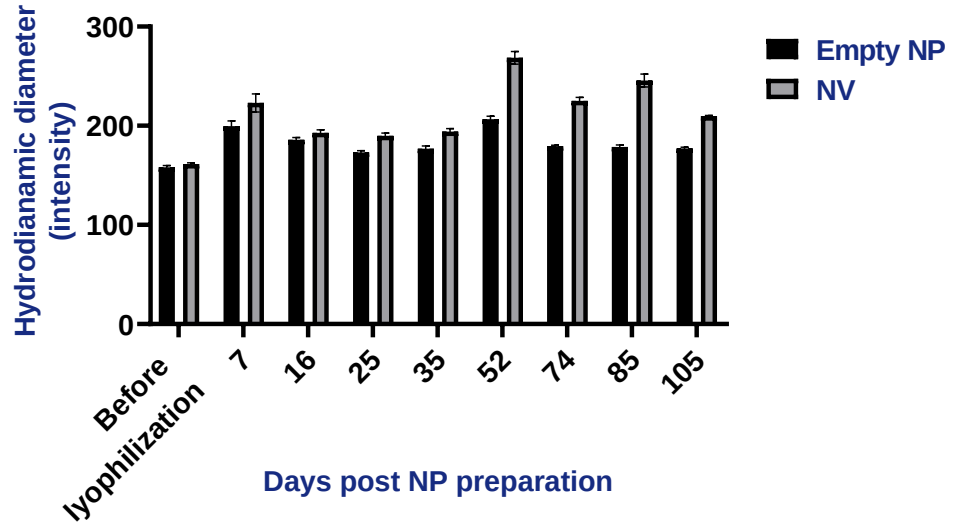
NP	Size[1] (nm ± SD[2])	PdI ± SD[2]	Z-Potential (mV ± SD[2])	Peptide EE (% ± SD[2])	Peptide LC (µg/mg ± SD[2])	CpG EE (% ± SD[2])	CpG LC (µg/mg ± SD[2])	Poly (I:C) EE (% ± SD[2])	Poly (I:C) LC (µg/mg ± SD[2])
P14-loaded NP	203 ± 1	0.147 ± 0.01	-34.2 ± 1.7	74.0 ± 0.5	74.0 ± 0.5	94.6 ± 1.8	3.3 ± 0.08	99.0 ± 0.3	6.9 ± 0.02
P19-loaded NP	188 ± 8	0.154 ± 0.06	-34.4 ± 1.6	58 ± 6.0	58 ± 6.0	95.2 ± 9.00	3.3 ± 0.2	98.7 ± 0.2	7.9 ± 0.01

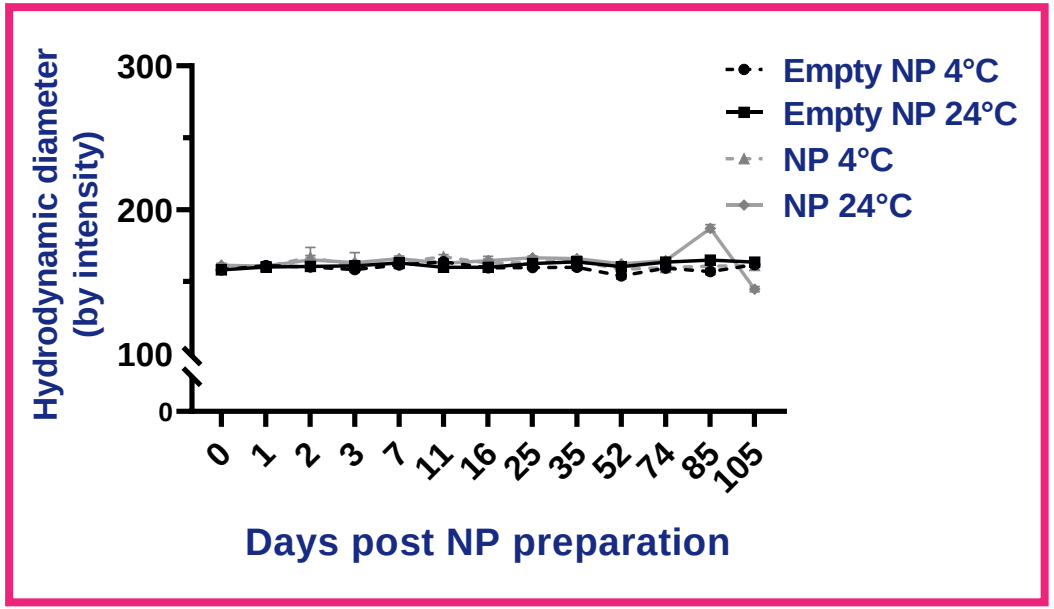
***Adv. Sci.* 2024**, 11, 2404159.

Intranasal DC-Targeted COVID-19 Nano-vaccine – Nanoplatform Characterization



Nanoplatform stable as a powder.
NP mean diameters and Đ
at different time-points after lyophilization.





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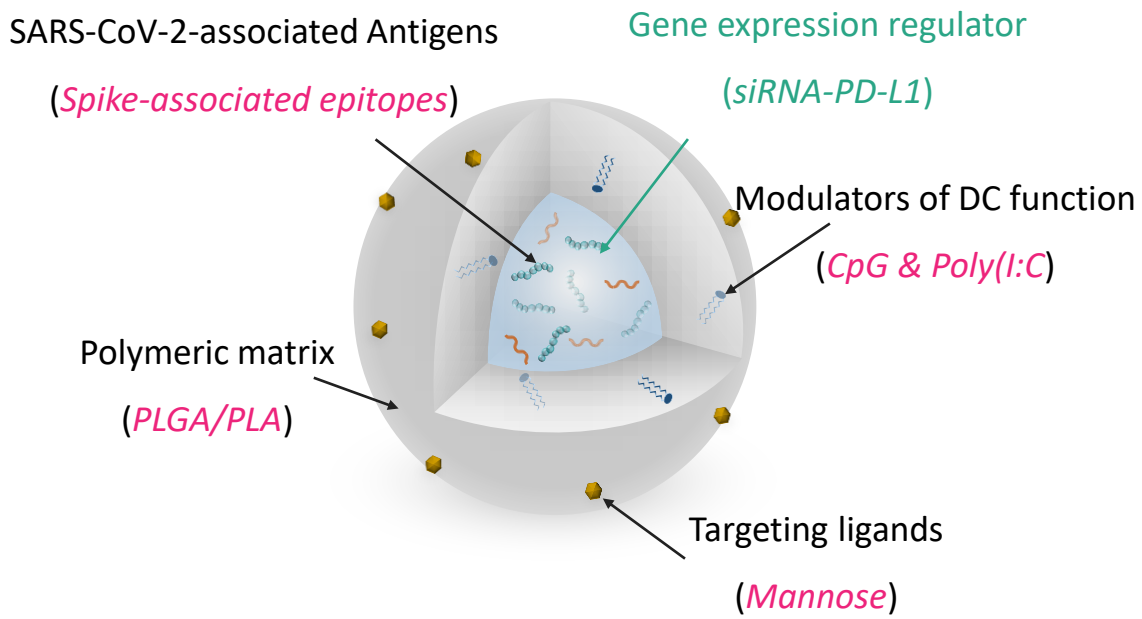
DC-Targeted COVID-19 Nano-vaccine – Efficacy studies

Explore additional immune-reactive effects by incorporating siRNA in our NV to further test the boundaries of our nanoplatform.

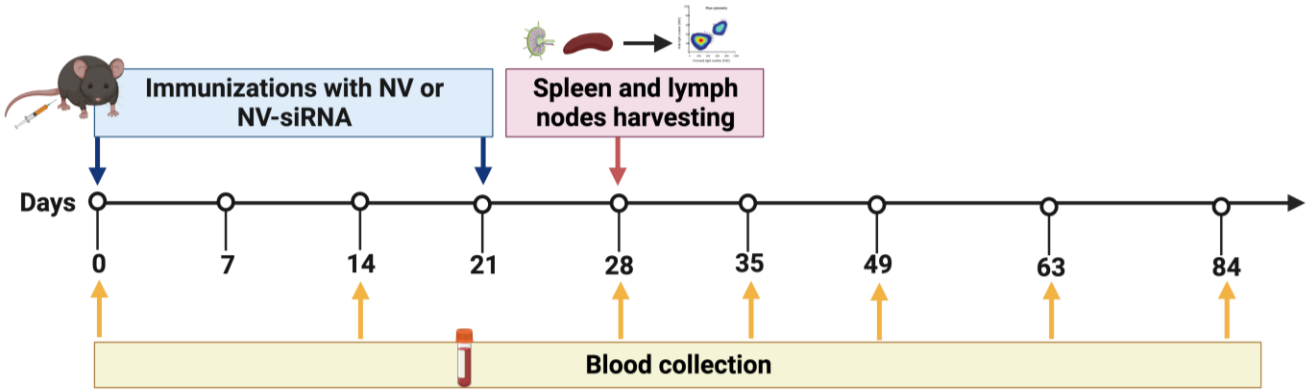


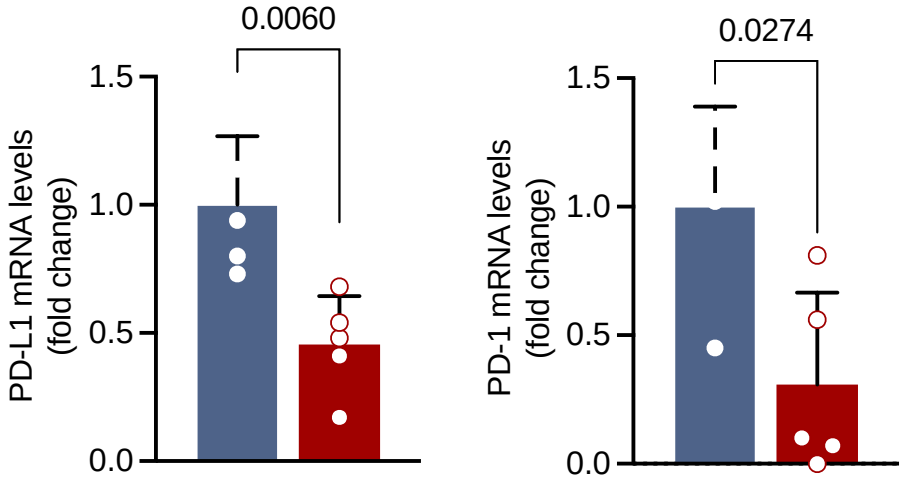
↑ PD-L1 expression on the DC surface

↑ Tfr cells, which inhibit GC response

Downregulating PD-L1 in DC would improve DC-T-B cell interactions during antigen presentation.

DC-Targeted COVID-19 Nano-vaccine – Efficacy studies

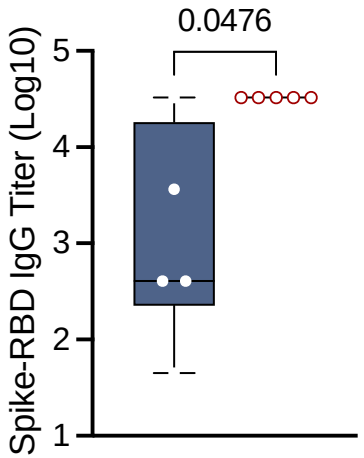




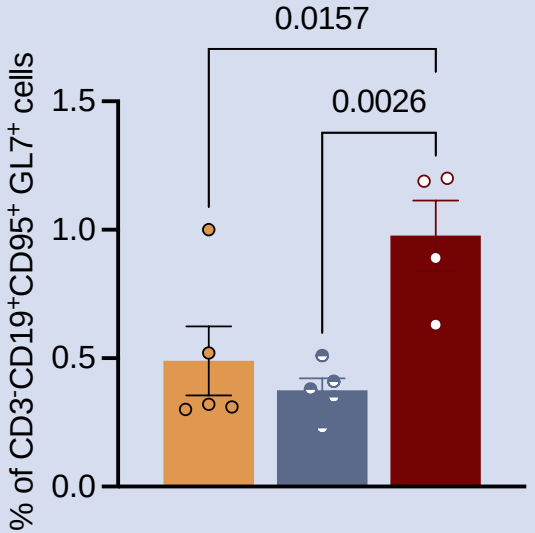
- NP MHCI- / NP MHCII-binding peptides + CpG + Poly (I:C)
- NP MHCI- / NP MHCII-binding peptides + CpG + Poly (I:C) + **siRNA NC**
- NP MHCI- / NP MHCII-binding peptides + CpG + Poly (I:C) + **siRNA PD-L1**

Animals immunized with **siPD-L1 NV** showed **significant GC B cell proliferation** essential for antibody diversification, affinity and maturation, supporting robust antibody production.

Spike-RBD IgG



Germinal Center B cells



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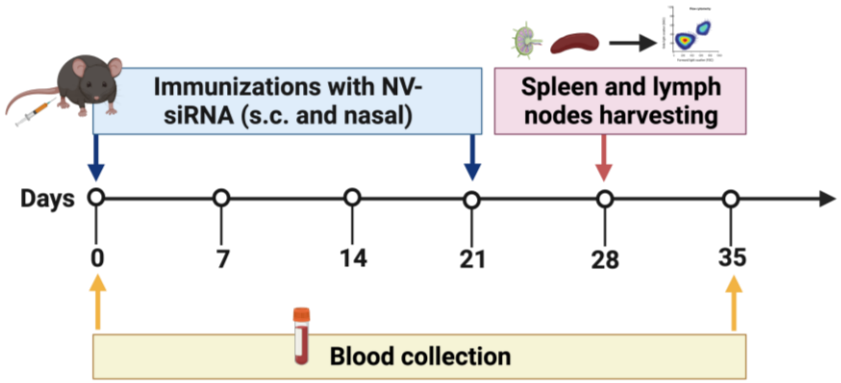
DC-Targeted COVID-19 Nano-vaccine – Efficacy studies

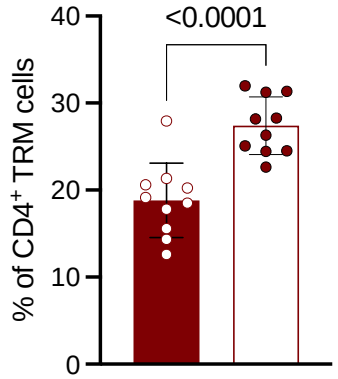


○ NP MHCI- / NP MHCII-binding peptides + CpG + Poly (I:C)+ siRNA PD-L1 ***SC booster***

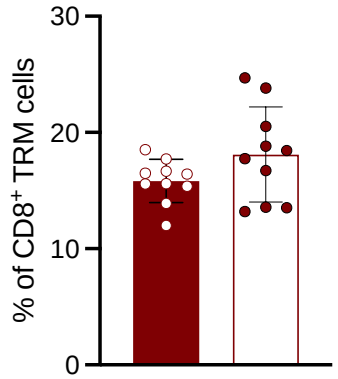
● NP MHCI- / NP MHCII-binding peptides + CpG + Poly (I:C)+ siRNA PD-L1 ***IN booster***

Animals immunized with siPD-L1 NV induced robust mucosal cellular & humoral immunity

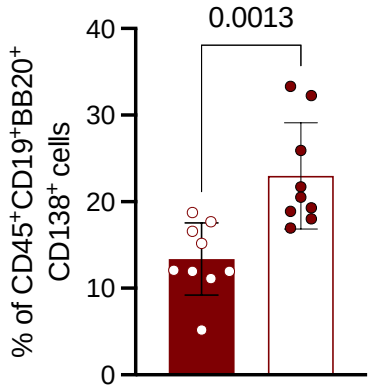
Tissue-Resident CD4⁺ T cells



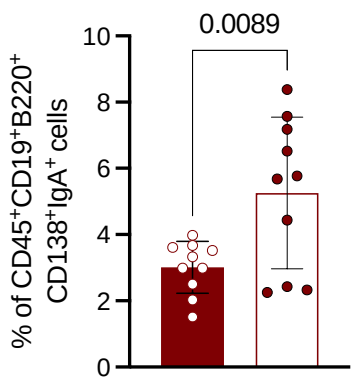
Tissue-Resident CD8⁺ T cells



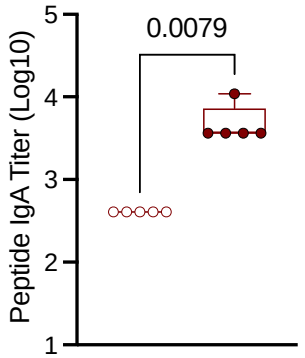
Antibody-Secreting B cells



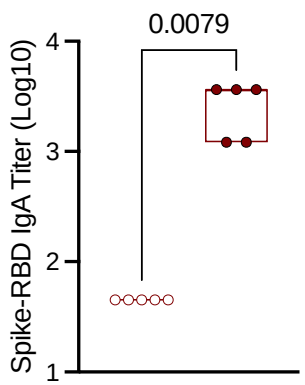
Nasal mucosa IgA

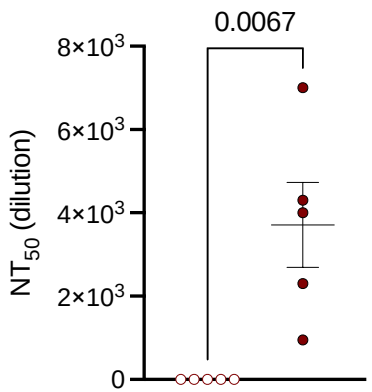


BALF Peptide SIgA



BALF Spike-RBD SIgA





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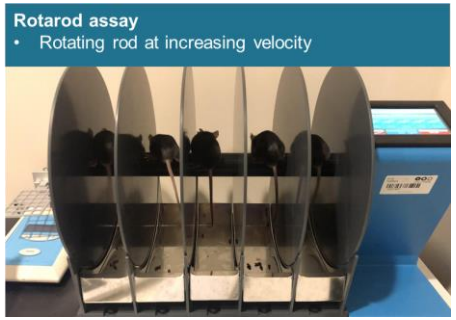
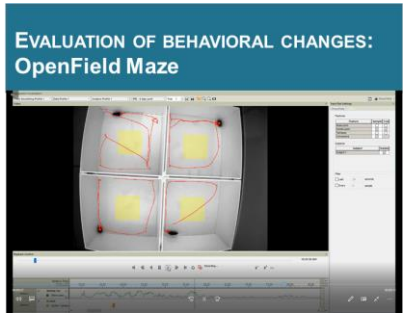
DC-Targeted COVID-19 Nano-vaccine – Efficacy studies



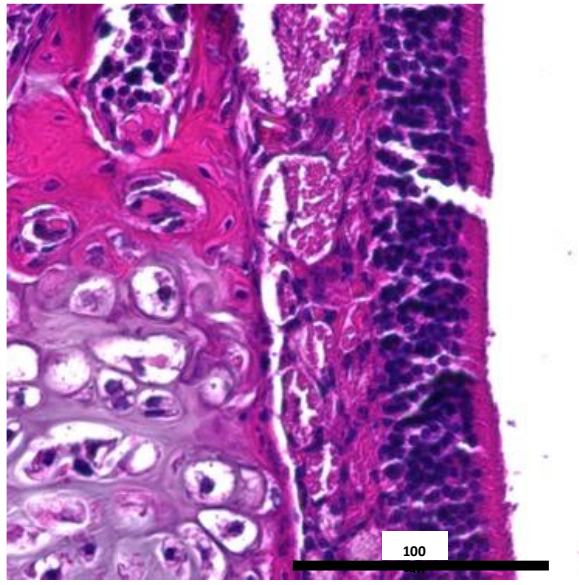
No effect on:

- **body weight, survival;**
- **motor coordination and imbalance;**
- **locomotor and anxiety-like behavior**

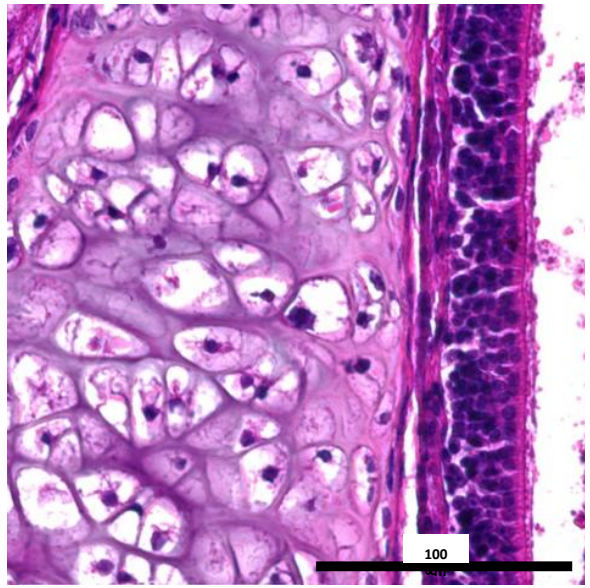


siPD-L1 NV did not cause any damage or extensive inflammation to the nasal mucosa tissue when administered IN

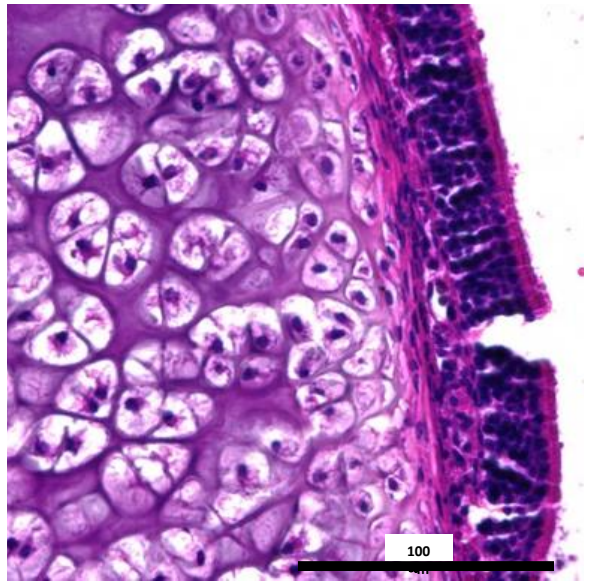
PBS



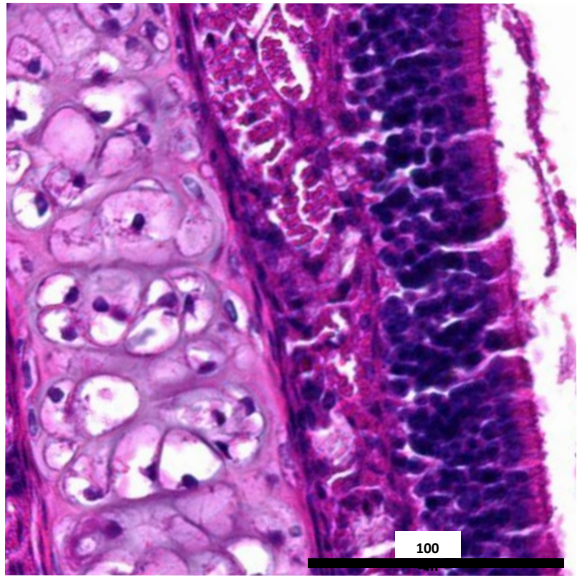
Free MHCI/II-binding peptides + CpG + Poly (I:C) + siPD-L1



Empty NP



NP MHCI- /NP MHCII-binding peptides + CpG + Poly (I:C) + siPD-L1



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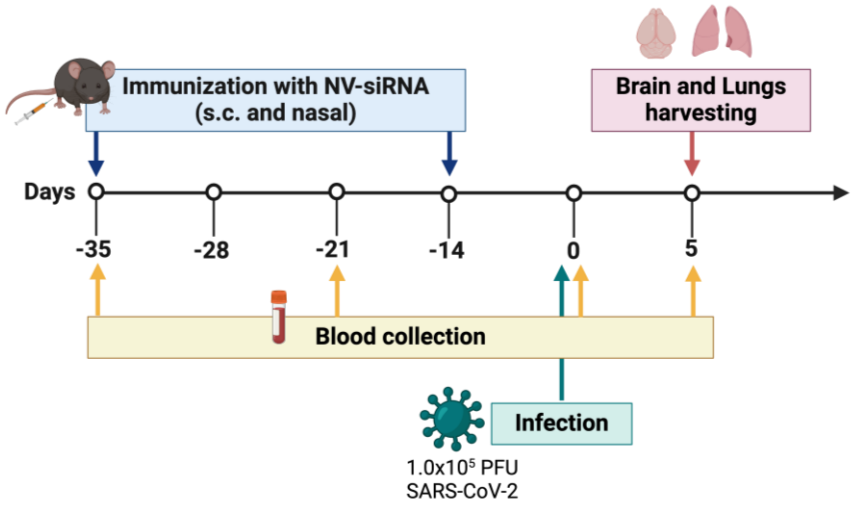
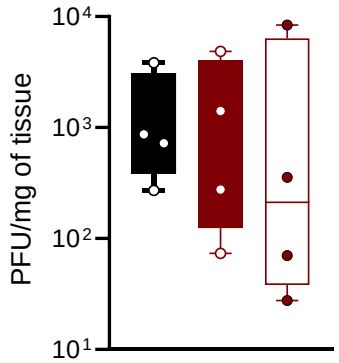
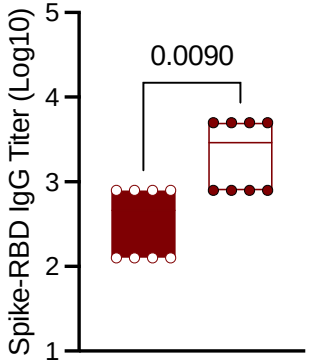
DC-Targeted COVID-19 Nano-vaccine – Efficacy studies



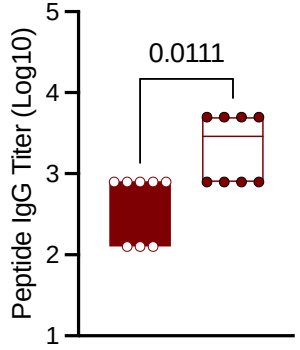
TCID$_{50}$ Lungs



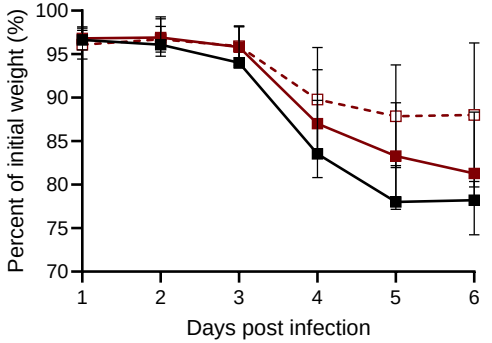
Serum Spike-RBD IgG



Serum Peptides IgG



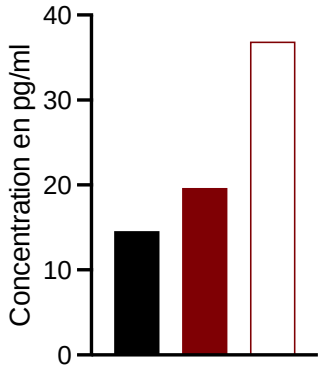
Prof Ana Zarubica



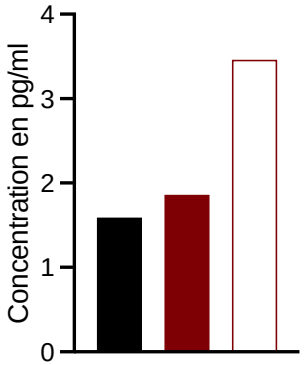
- Positive Control
- NP MHCI- / NP MHCII-binding peptides + CpG + Poly (I:C)+ siRNA PD-L1 ***SC booster***
- NP MHCI- / NP MHCII-binding peptides + CpG + Poly (I:C)+ siRNA PD-L1 ***IN booster***

Assessment of weight loss and clinical scoring suggested delayed disease progression

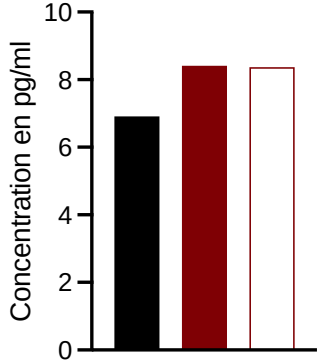
Lungs IFN-γ



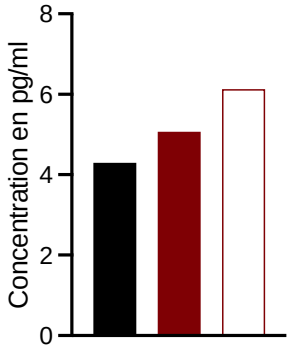
Lungs IFN-β



Brain IFN-γ



Brain IFN-β



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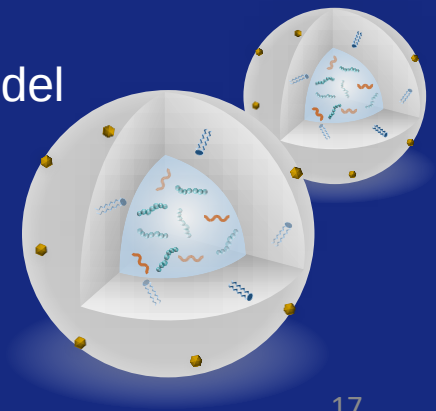
Multifunctional nanoplatform can entrap multiple immunomodulators.

Nanovaccine successfully **increased** the cellular activity of effector T cells.

Nanovaccine successfully **induced the secretion of antigen-specific IgG** and **sIgA** antibodies.

Neutralization of SARS-CoV-2 WT and variants (α, Δ, O) by sera of mice immunized with our nano-vaccine.

SARS-CoV-2 challenge study in humanized transgenic SARS-CoV-2 infection mouse model **suggested delayed disease progression.**



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No breakthrough is too small

Thank you!

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