Gene Delivery for Glioblastoma: At the Interface of Immunotherapy and Precision Medicine

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CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
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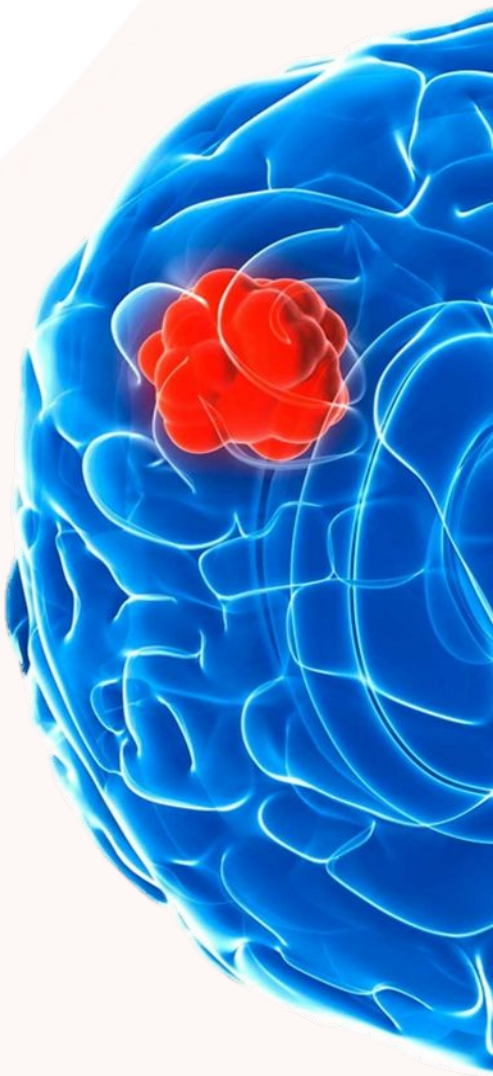
NEXT-GENERATION DELIVERY INNOVATIONS

The need

Glioblastoma remains incurable

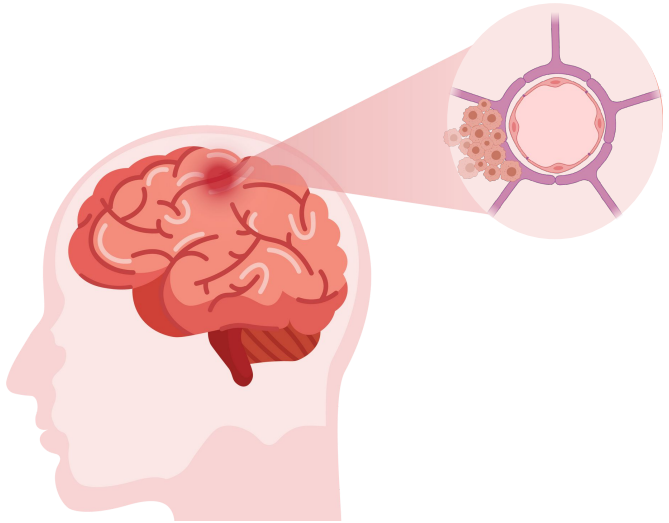


5-year survival – 5.7%

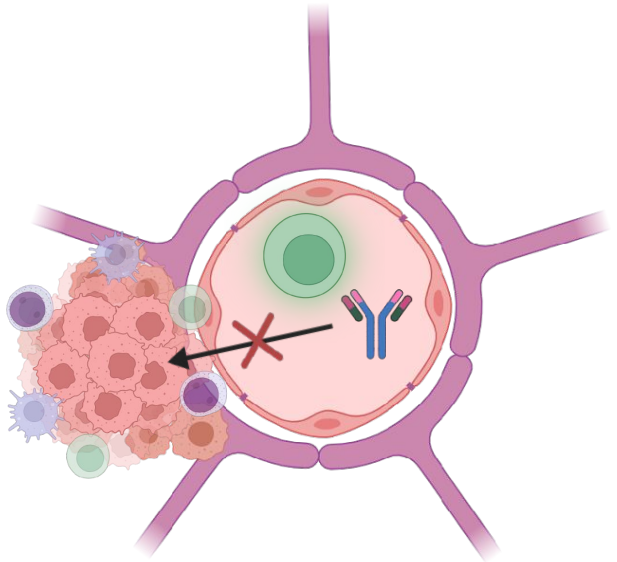
F. Hameedat,..., **F. Sousa*,** Nat Rev Mat, 2024

F.Sousa*, et al, Drug Deliv Transl Res, 2023

The challenges



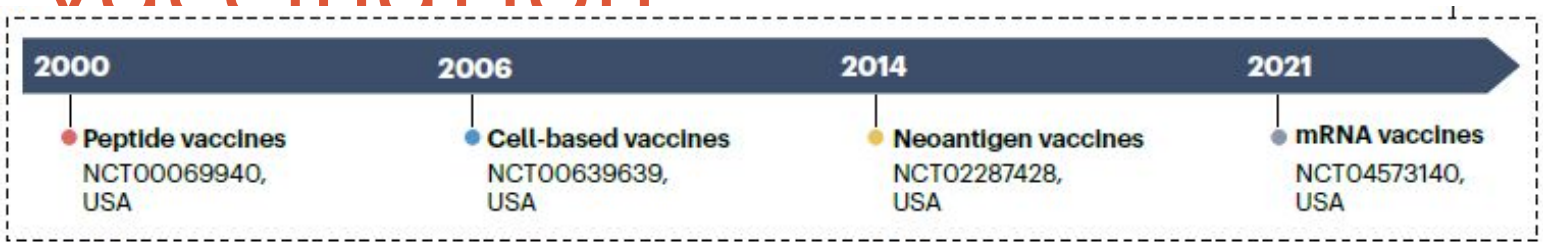
Presence of the blood-brain barrier

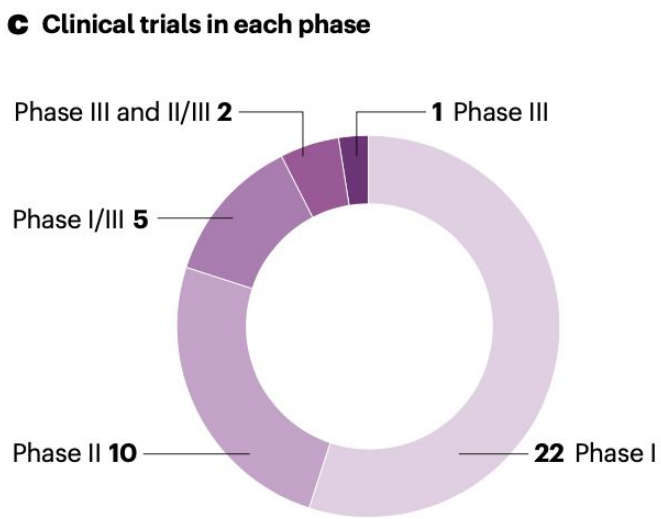


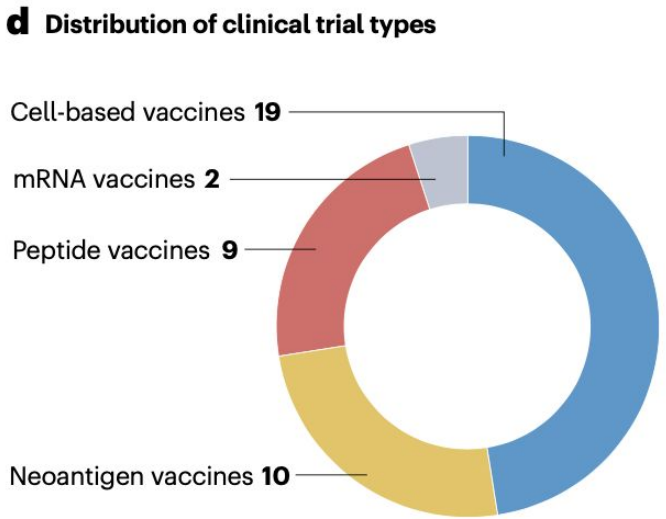
Immunosuppressive tumour microenvironment (TME)

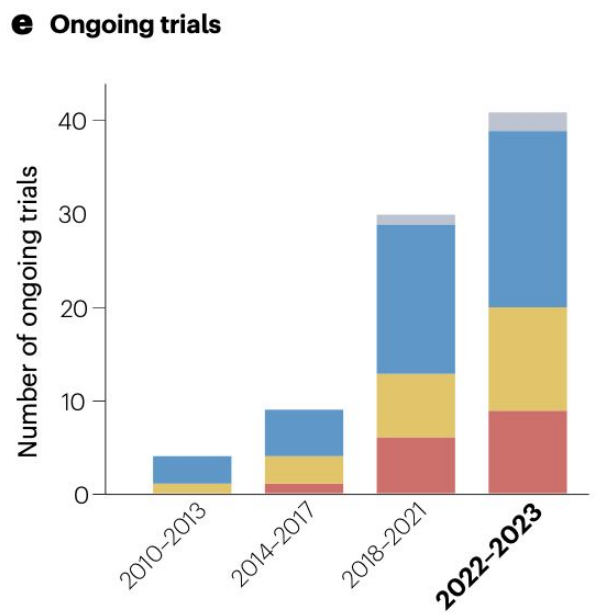
F. Sousa et al., J Control. Release, 2021, 339:381-390
F. Sousa et al, J. Control Release, 2019, 22;309:37-47

Glioblastoma vaccination





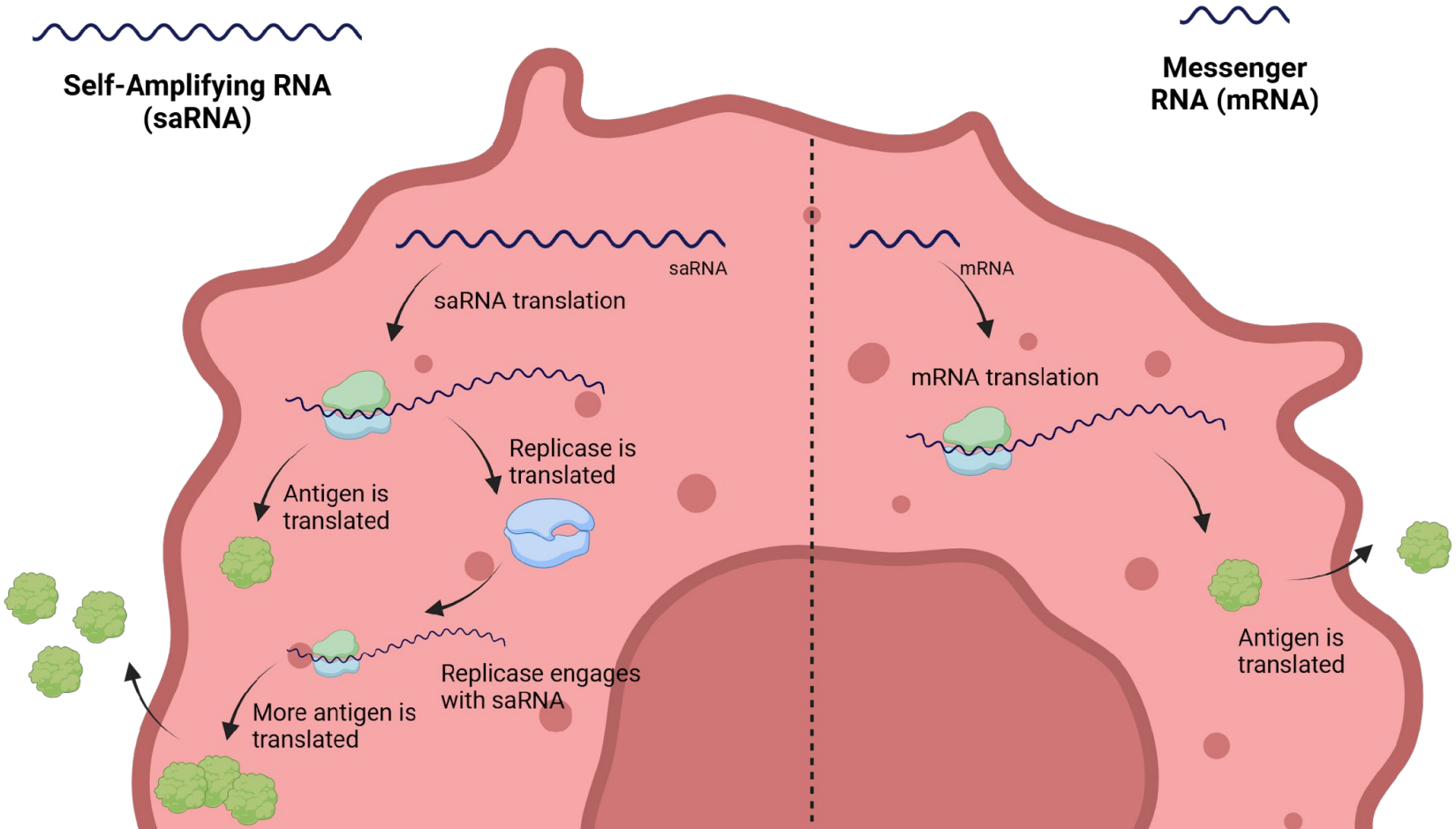




F. Hameedat,..., **F. Sousa,** Nat Rev Mat, 2024

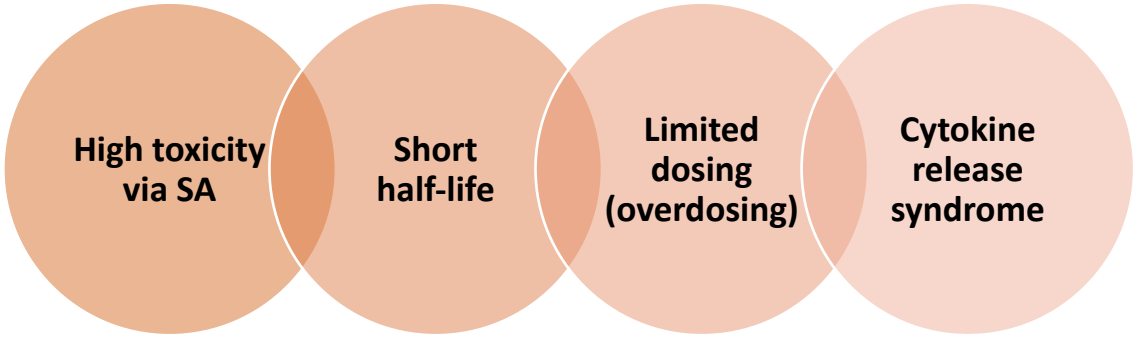
New generation of nanovaccines for brain cancer immunotherapy

saRNA nanovaccines



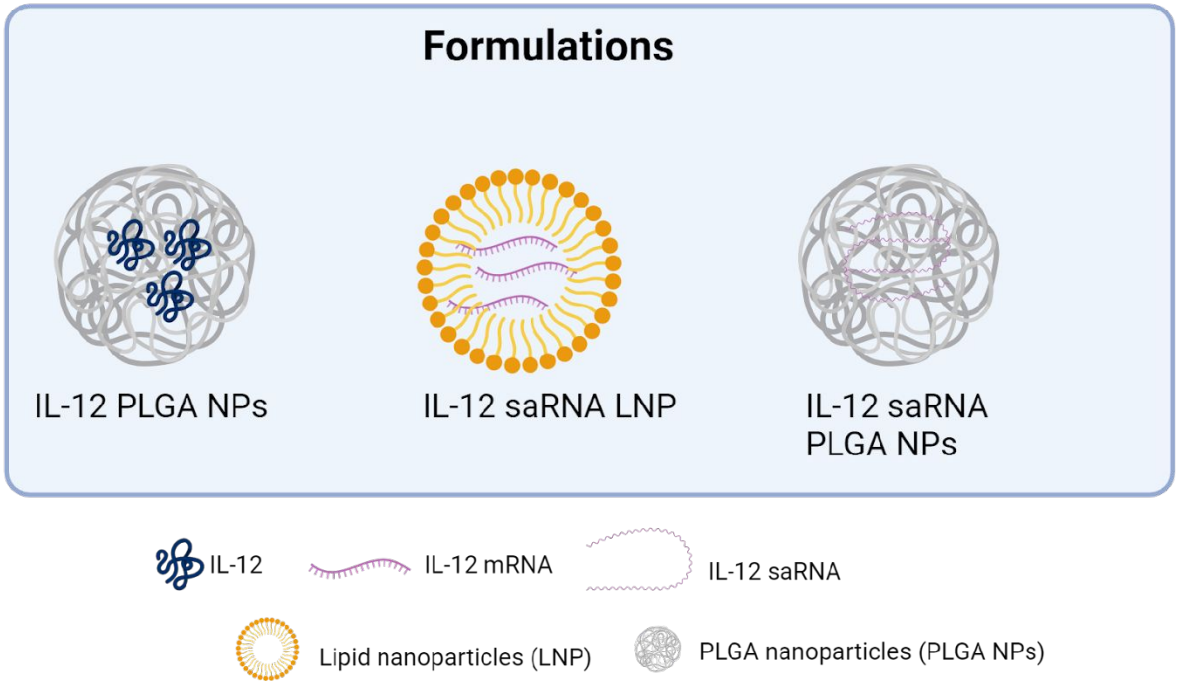
Power of IL-12

- Convert immunologically **“cold” tumor microenvironment to inflamed “hot” tumor.**
- Stimulates production **of interferon-γ (IFN-γ)**
- Changes the tumor microenvironment from one that contains M2-type phenotype macrophages to one that has more inflammatory M1-type phenotype



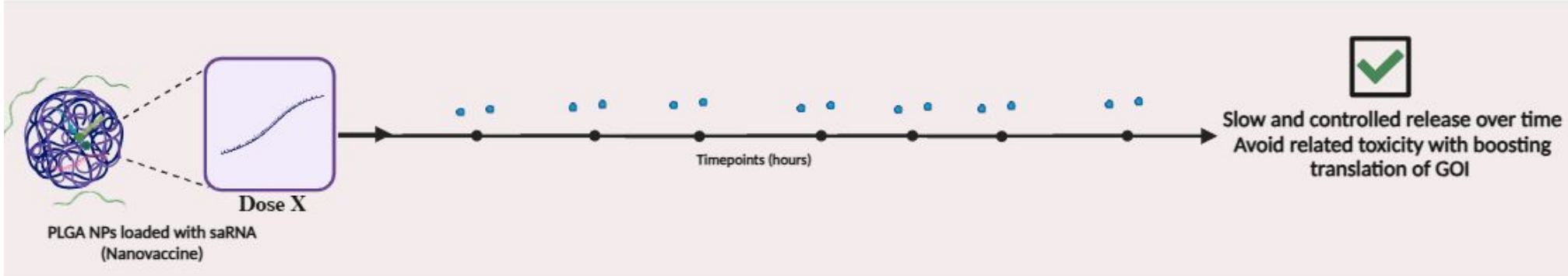
F.Sousa et al, Drug Deliv Transl Res, 2023

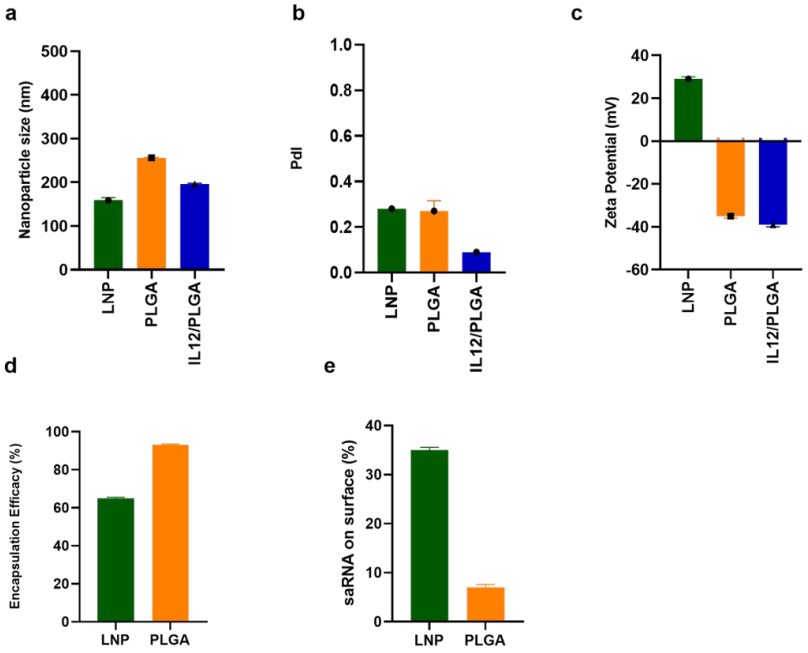
Non-viral vectors for gene therapy

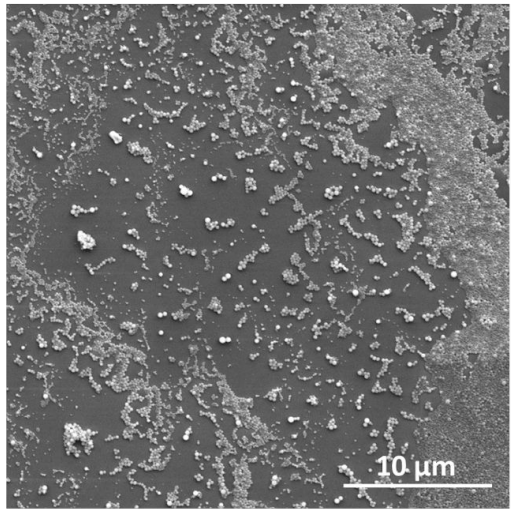


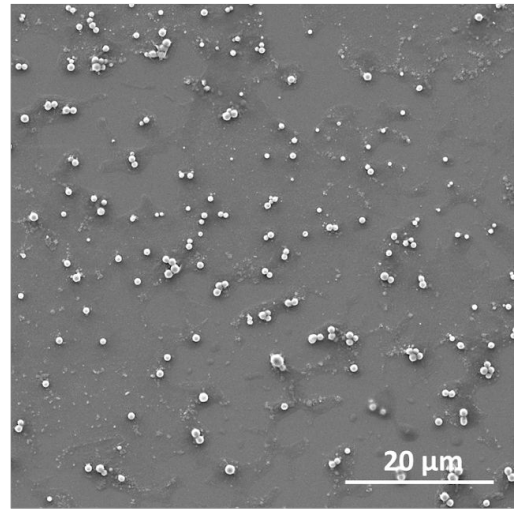
- Established a reproducible & automated methodology to produce PLGA NPs loaded with saRNA
- Optimized the **PLGA NPs** formulation accordingly for gene delivery

PLGA as a non-viral vector



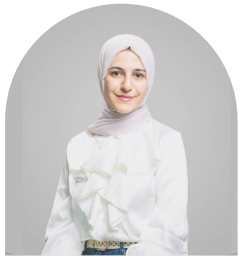


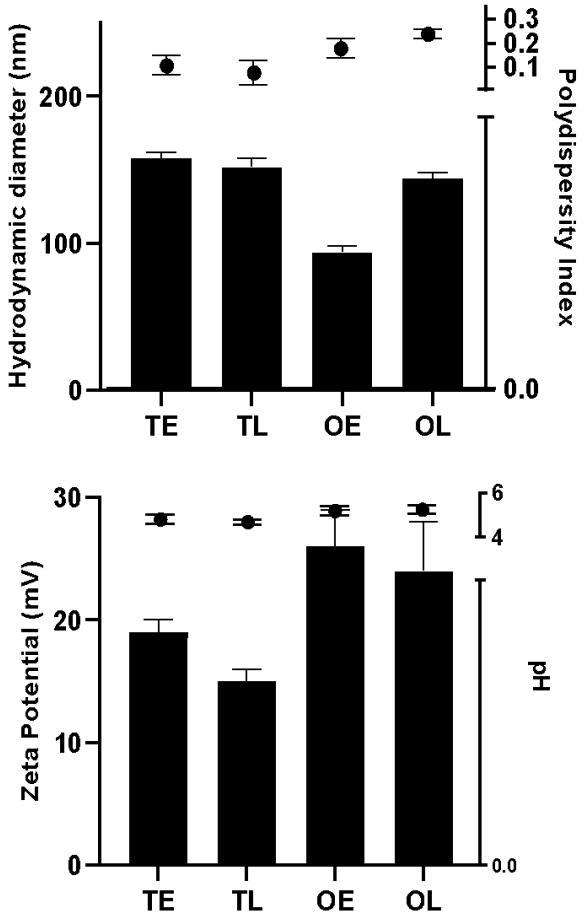


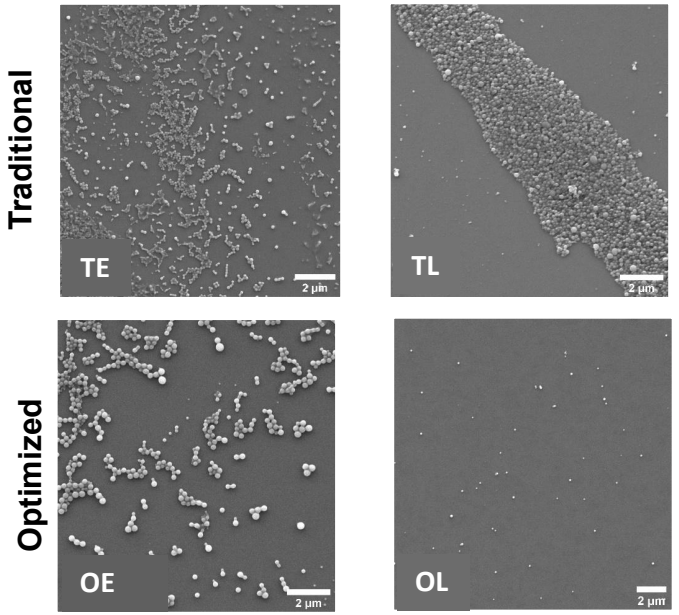


F. Hameedat et al, *manuscript in preparation*

Optimized methodology



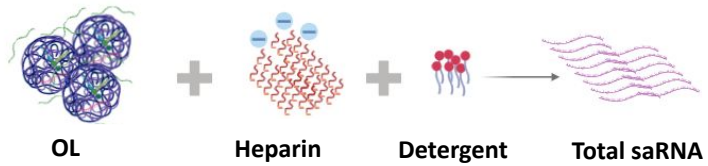


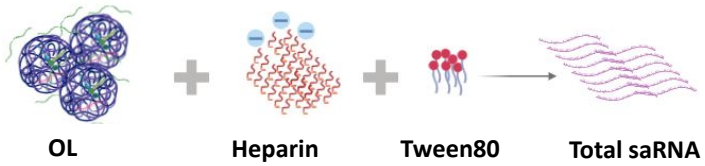


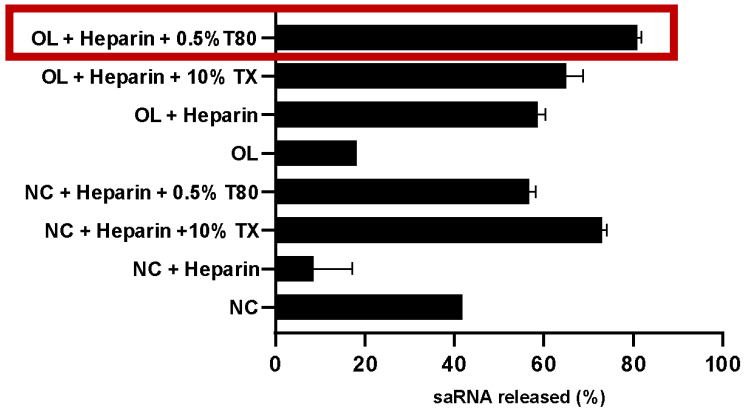
	Diameter number (nm)	Total concentration (particles/mL)
TE	145.9	5.49E+11
TL	156.3	5.18E+11
OE	93.3	3.2E+11
OL	109.5	3.36E+11

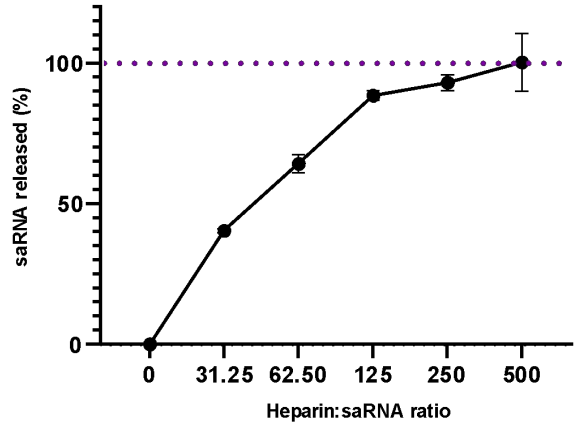
F. Hameedat et al, *manuscript in preparation*

Quantification of loaded saRNA









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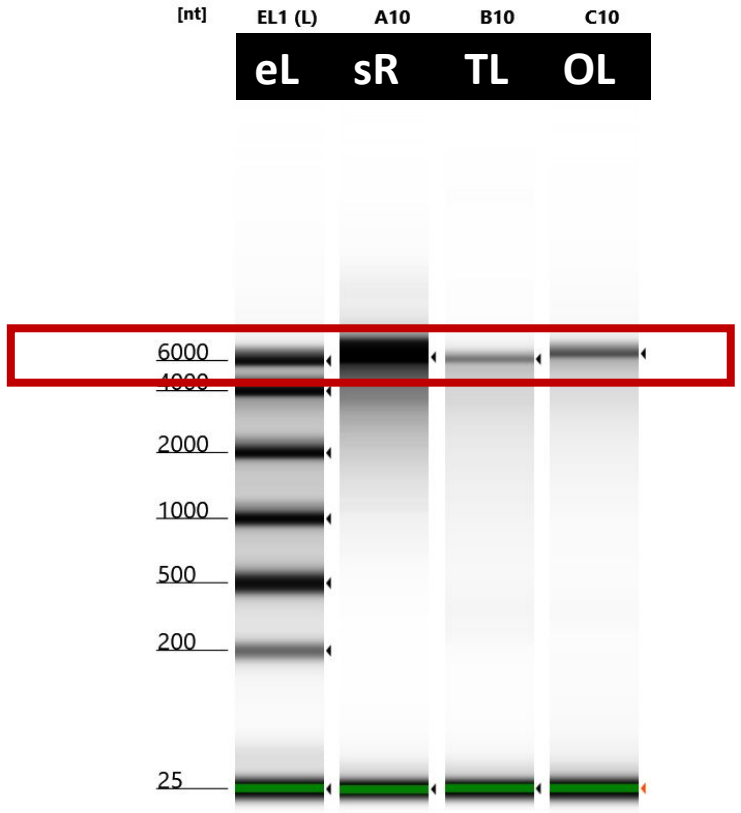
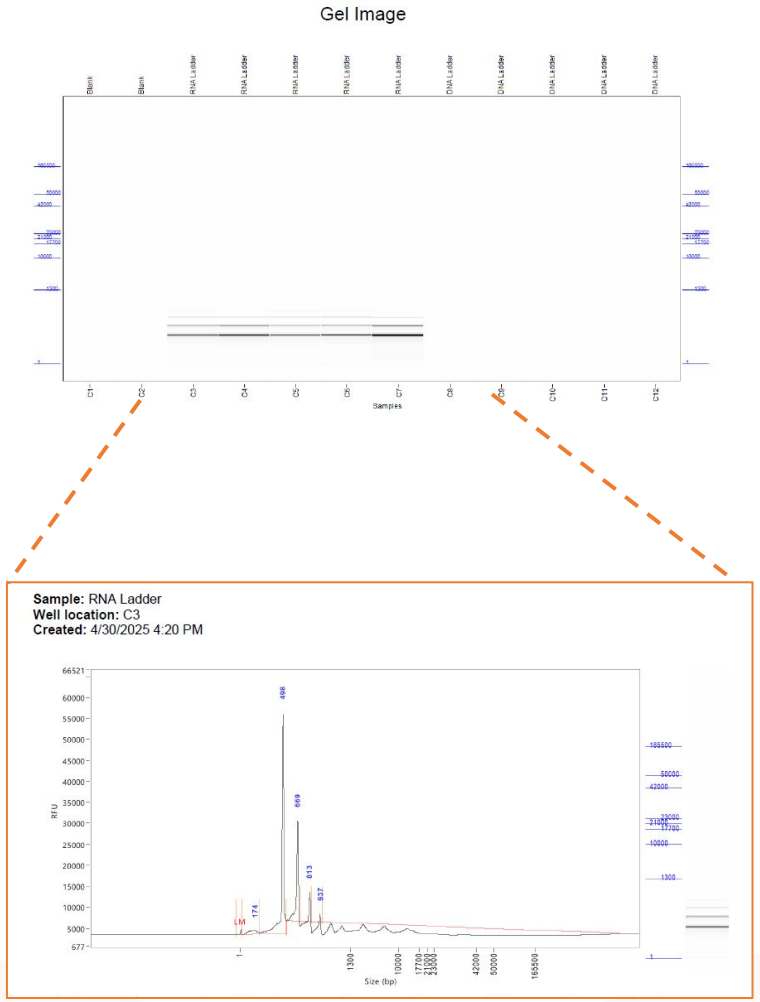
Integrity of saRNA

TapeStation



Fragment Analyzer



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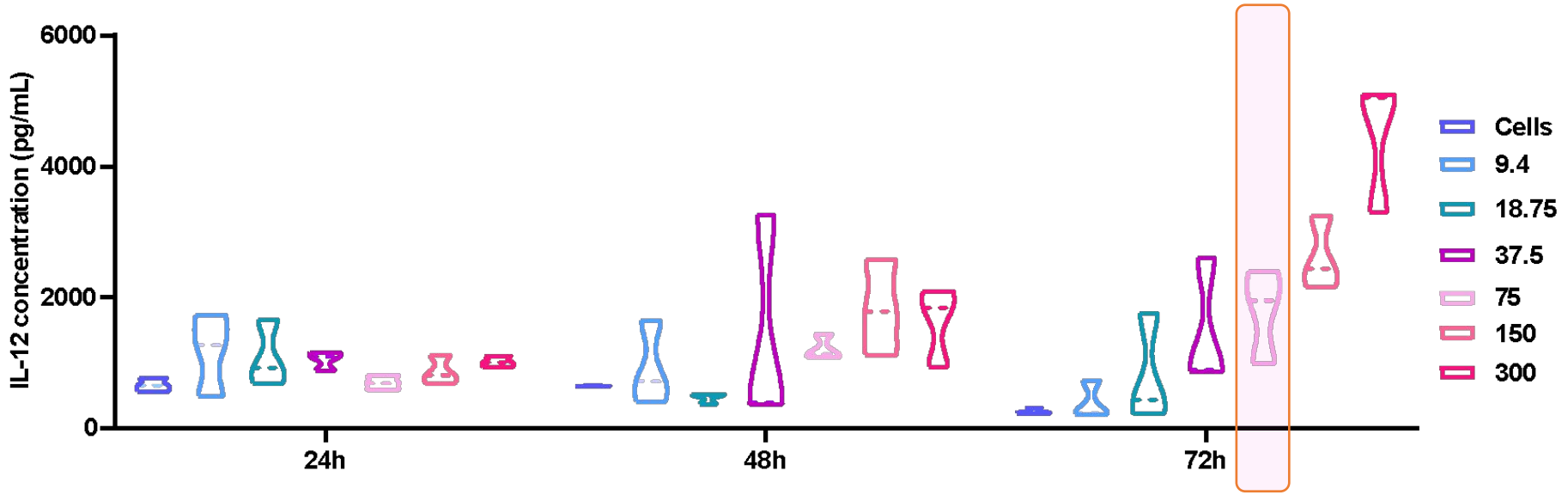
Transfection efficacy of saRNA



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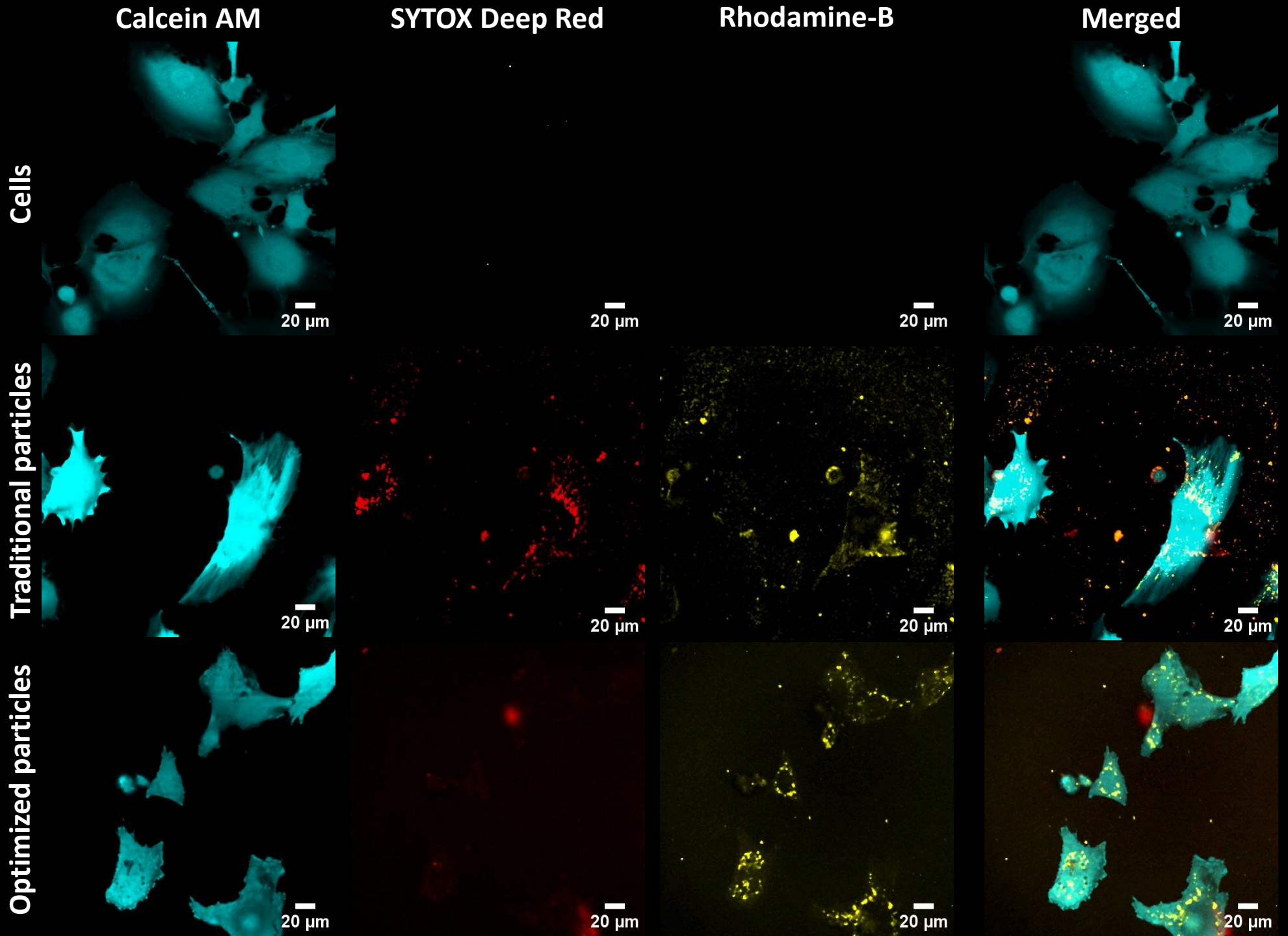
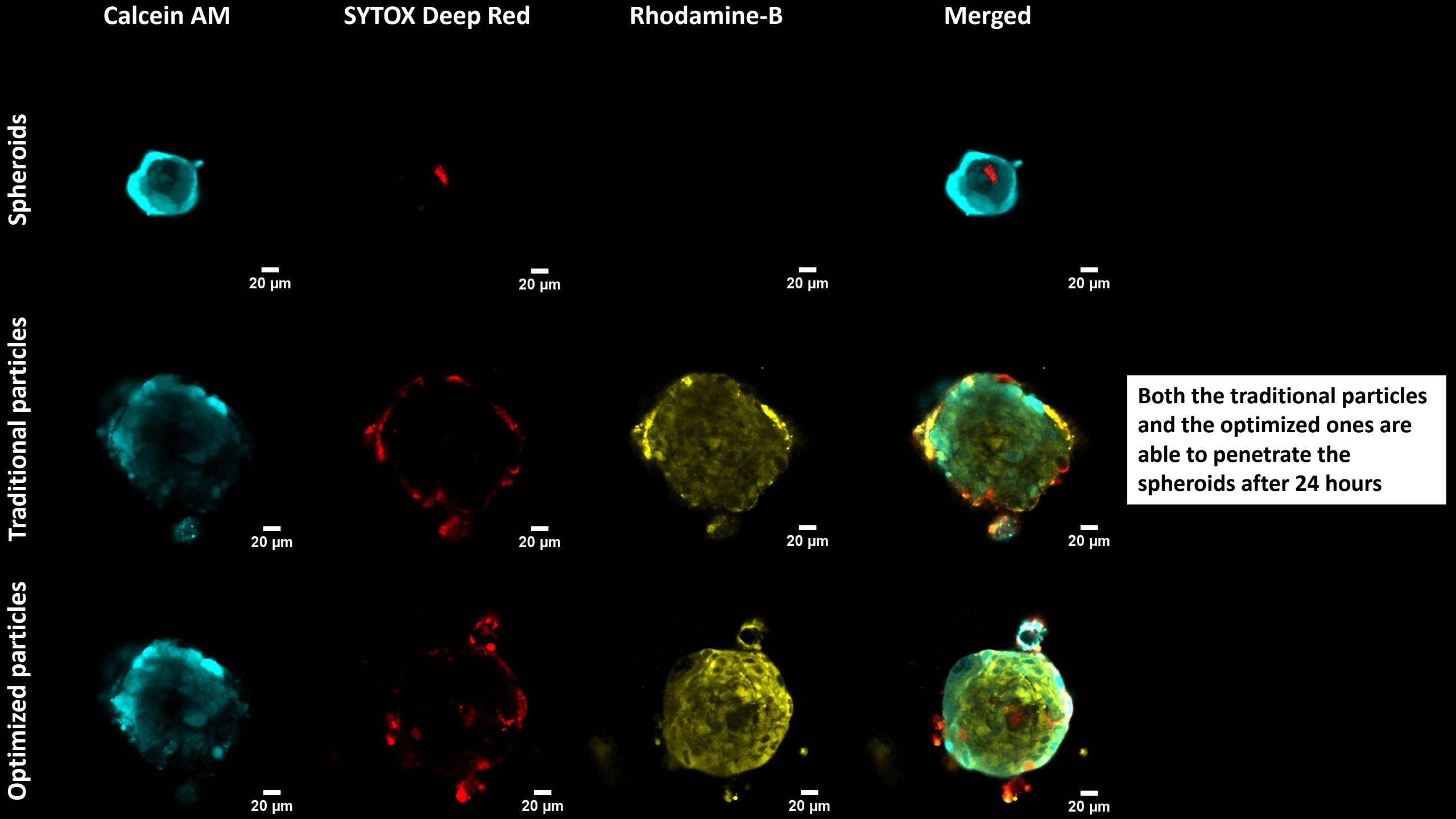
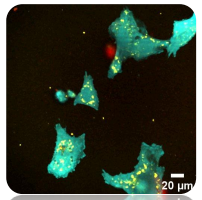


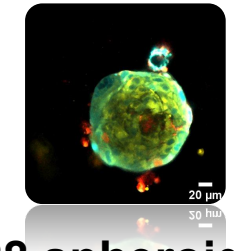
Both the traditional particles and the optimized ones are able to penetrate the SB28 cells after 24 hours


Calcein AM
SYTOX Deep Red
Rhodamine-B
Merged
Spheroids
Traditional particles
Optimized particles
20 µm
Both the traditional particles and the optimized ones are able to penetrate the spheroids after 24 hours

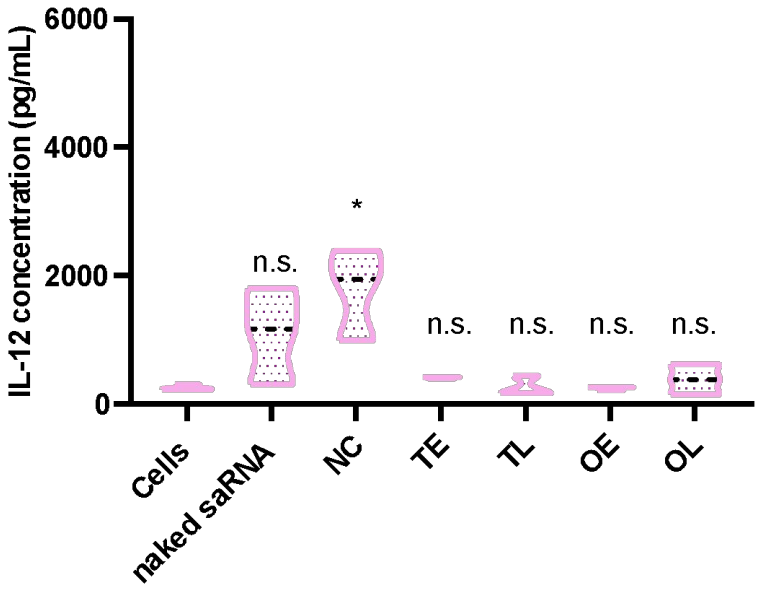
Transfection efficacy of nanovaccines

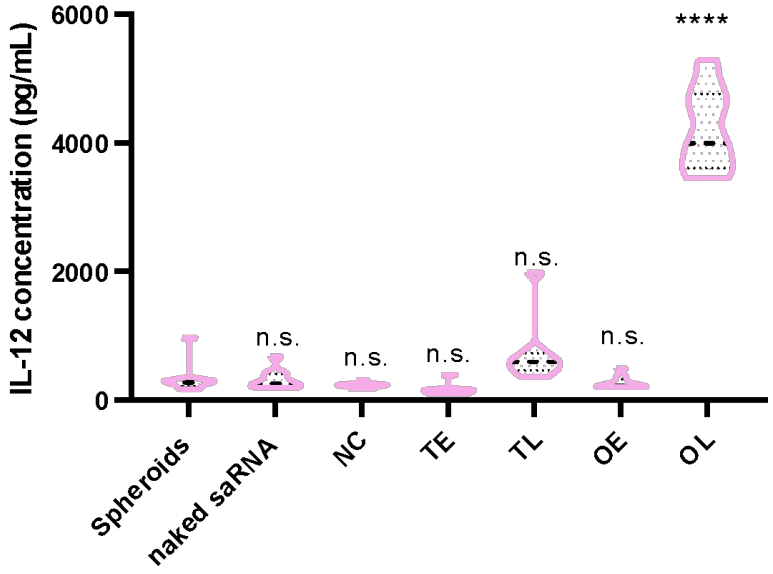


SB28 cells



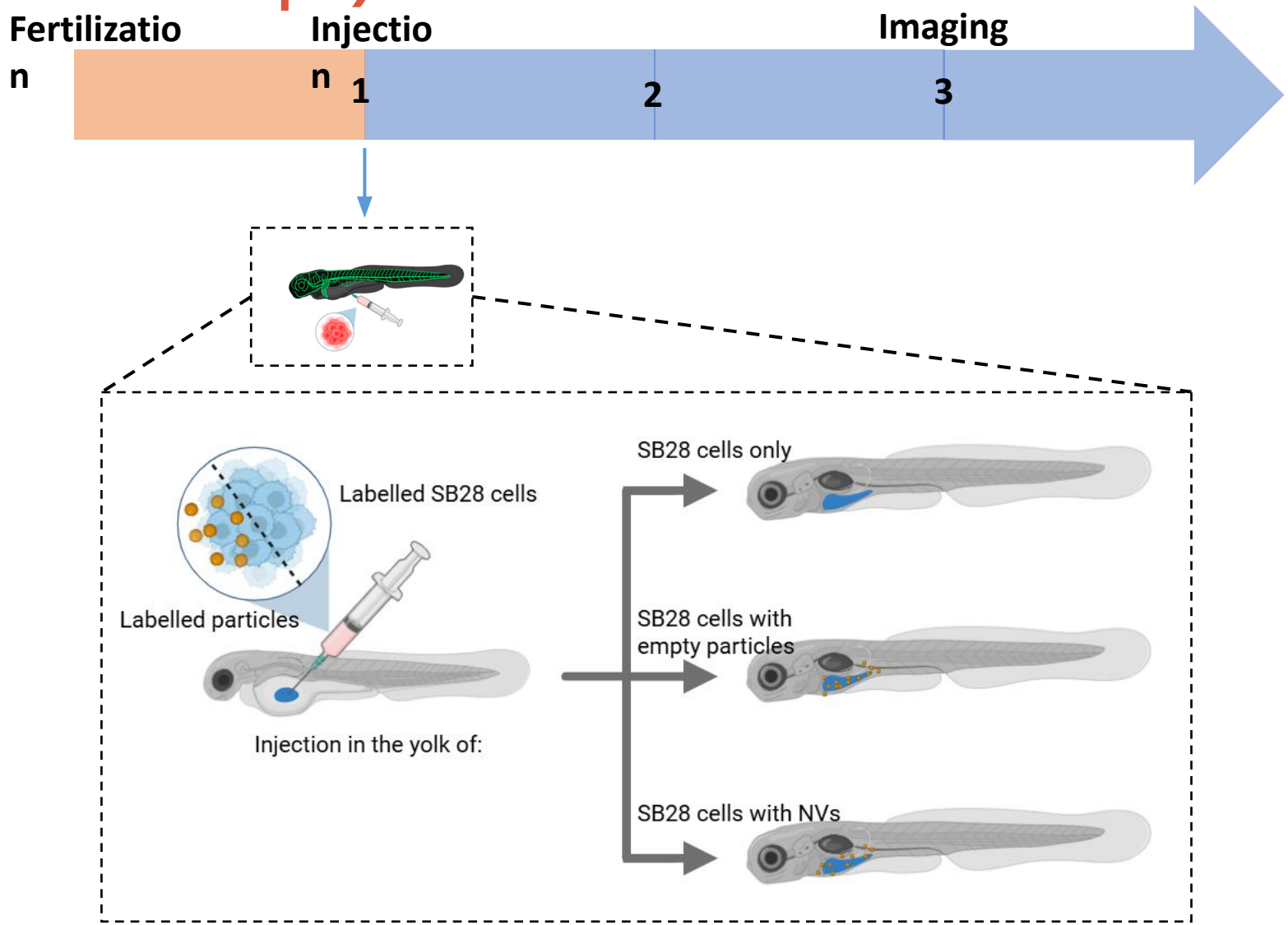
SB28 spheroids

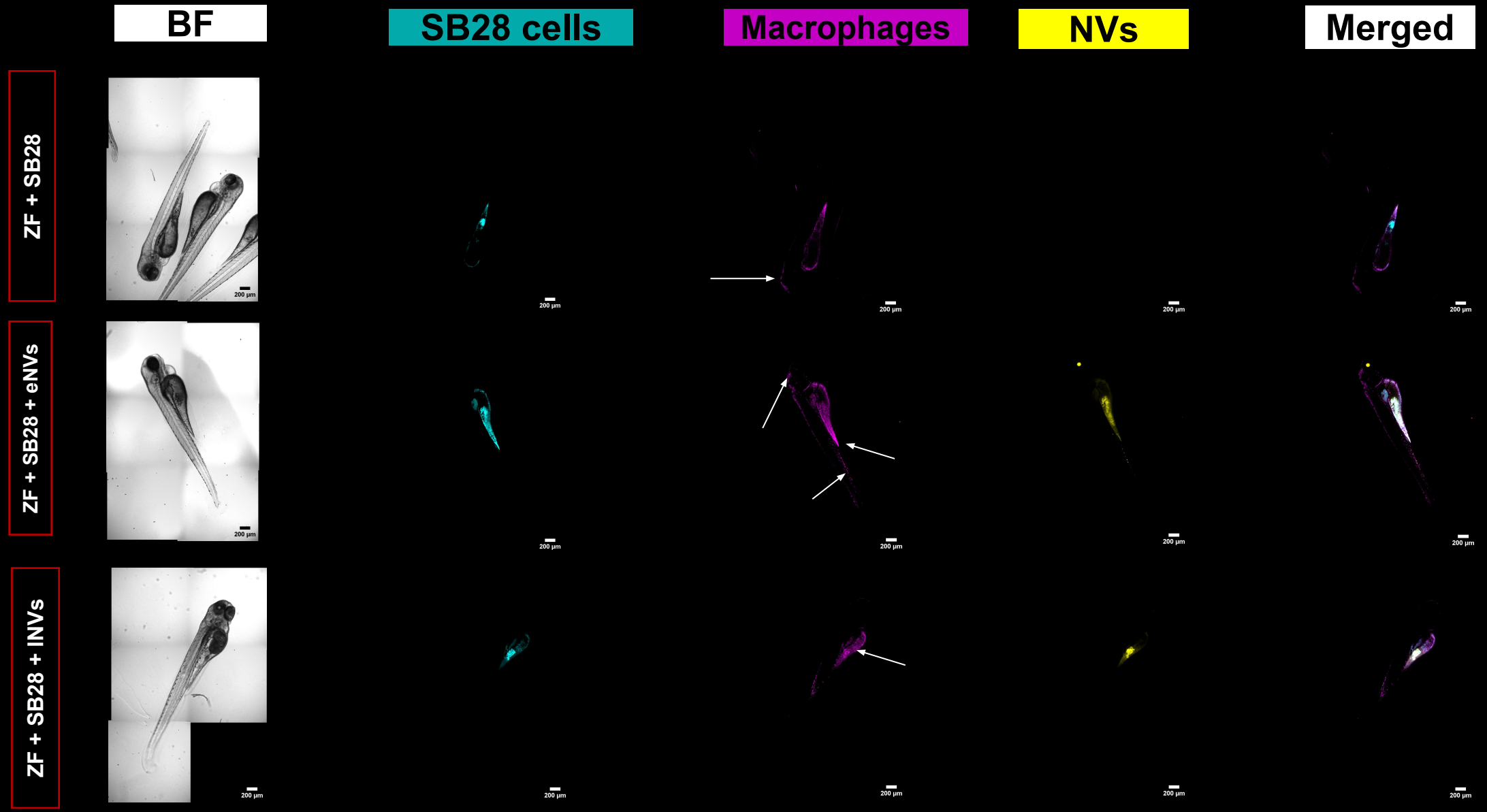




The optimized polymeric nanovaccine platform is able to transfect SB28 cells due to NVs penetration

Zebrafish xenograft model – Tg (mfap4:mTurq2)

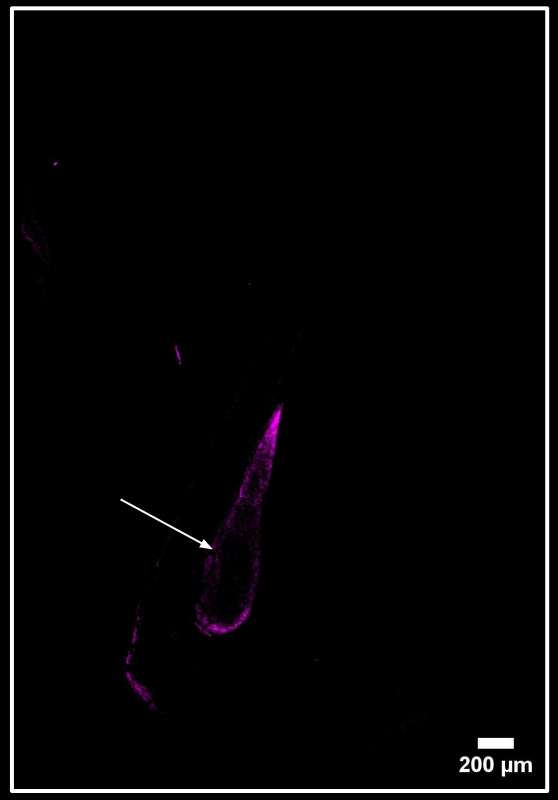




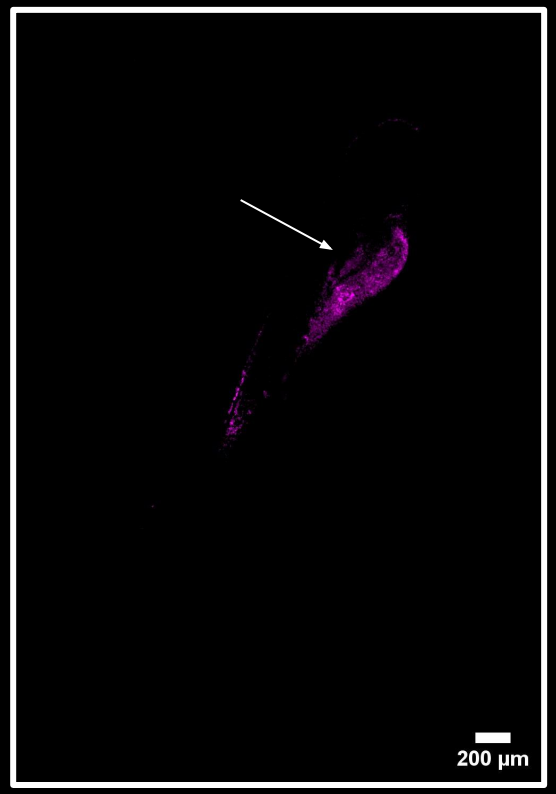
The macrophages showed a migration towards the yolk after 48 hours of injection with NVs.

Macrophage migration

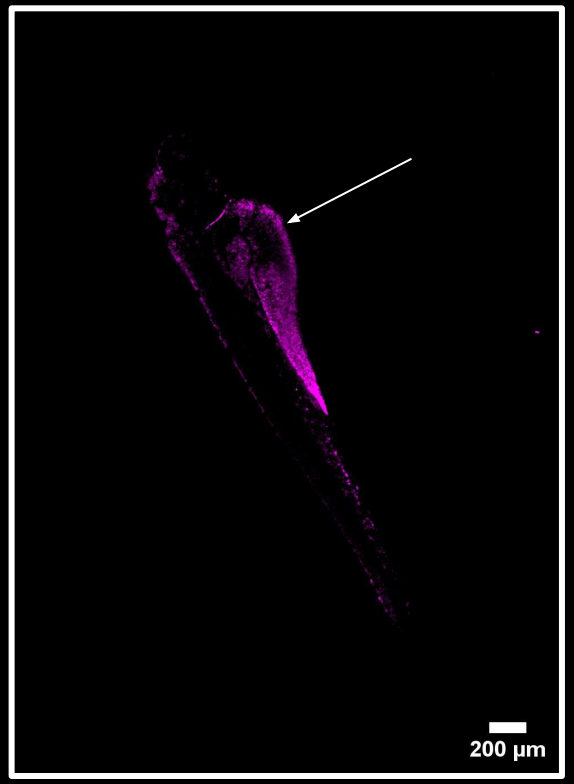
SB28 cells



Empty NVs



Loaded NVs



SB28 cells

Macrophages

Is nanovaccine platform just allowing macrophage migration? Or also changing the phenotype?

IL-12 reprograms CAR-T cells


nature cancer

Article

https://doi.org/10.1038/s43018-023-00668-y

Cytokine-armed dendritic cell progenitors for antigen-agnostic cancer immunotherapy


nature communications

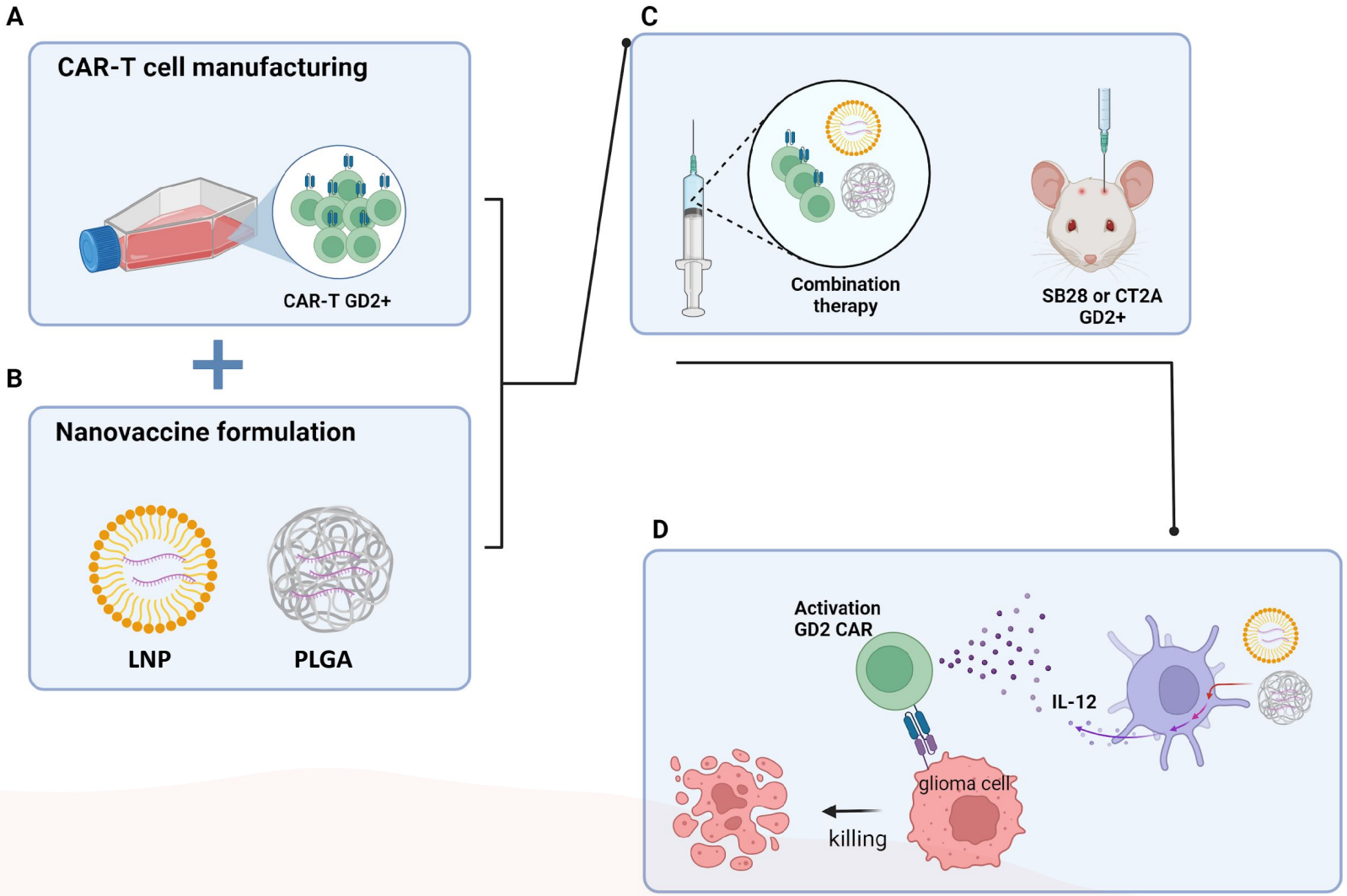
Article

https://doi.org/10.1038/s41467-023-44310-y

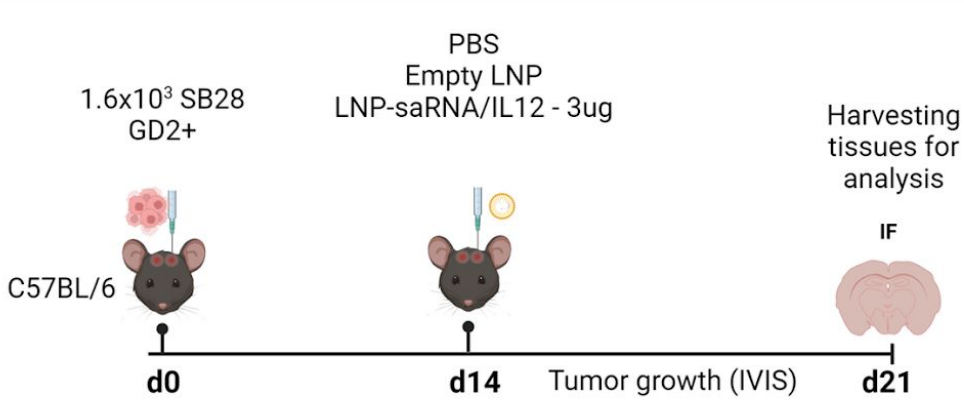
IL-12 reprograms CAR-expressing natural killer T cells to long-lived Th1-polarized cells with potent antitumor activity

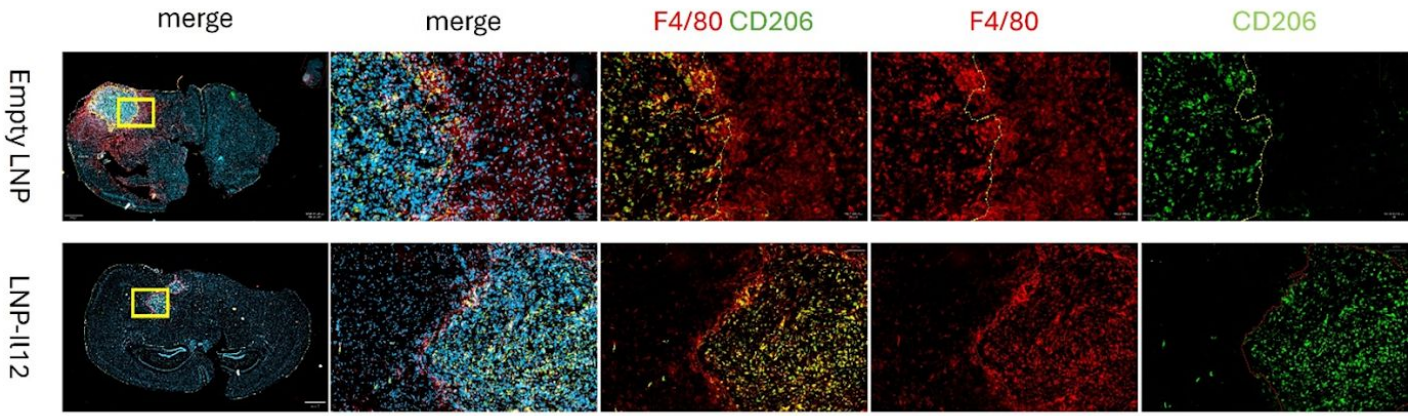
Can nanovaccines enhance PK/PD of **CAR-T cells into the brain** and kill target cells?

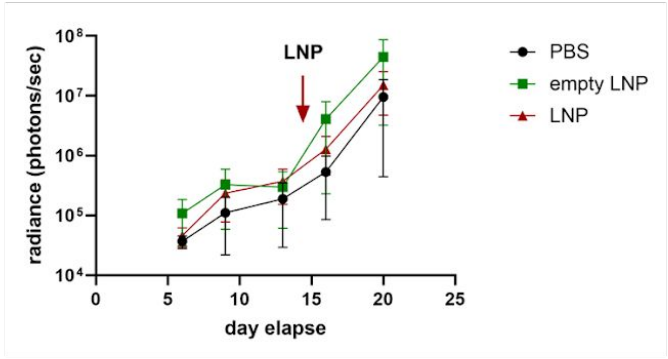
GD-2 CAR-T cells

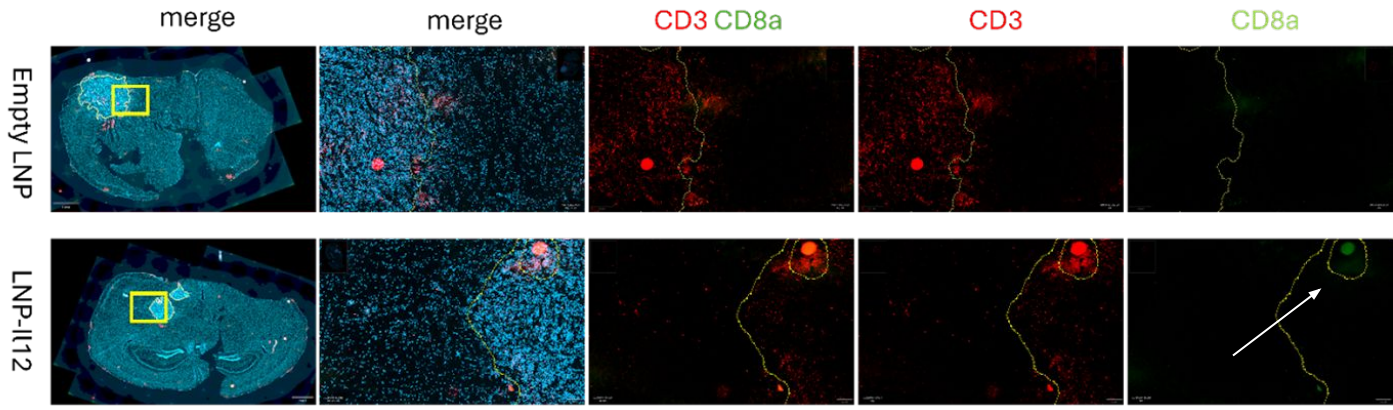


Migration of CD8 T cells



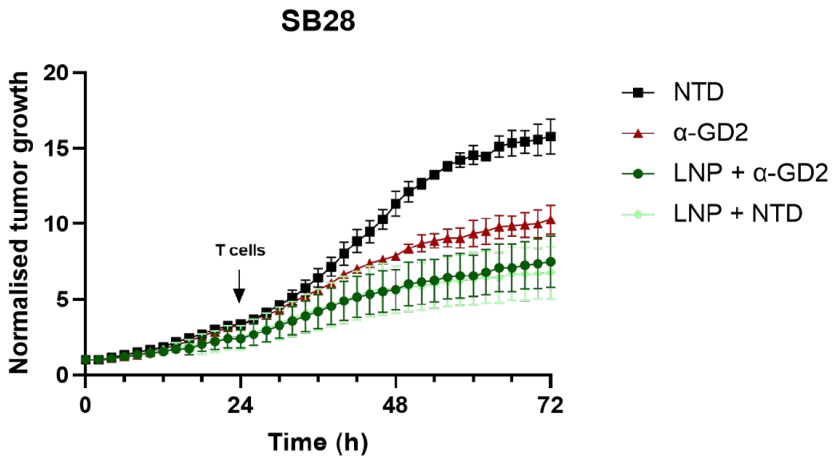


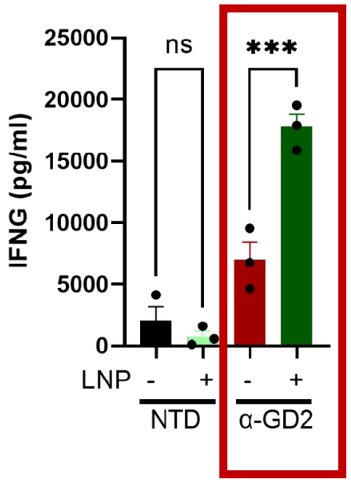


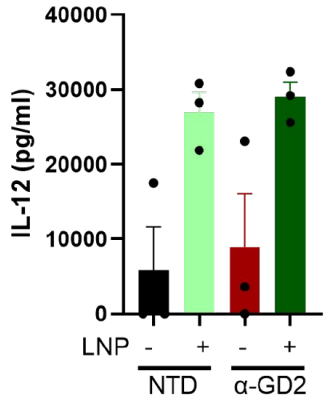


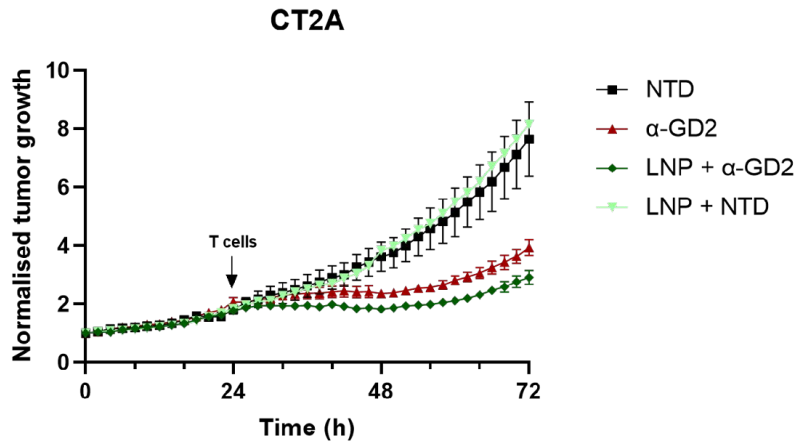
F. Sousa et al, *manuscript in preparation*

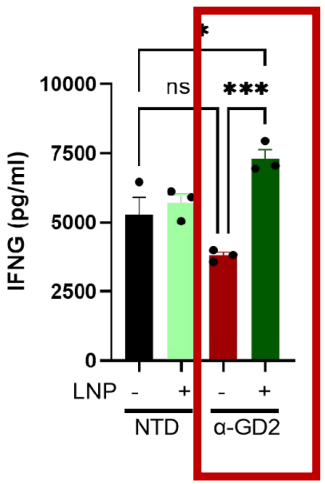
CAR-T killing assay, LNPs

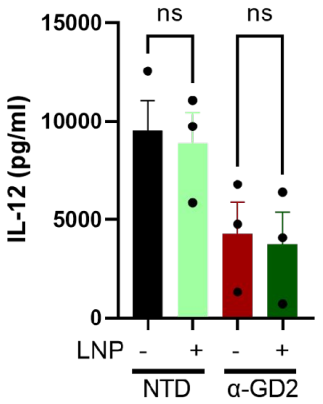












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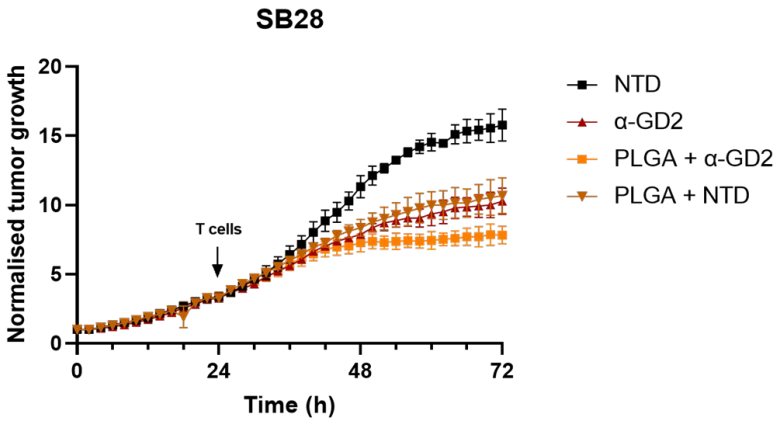
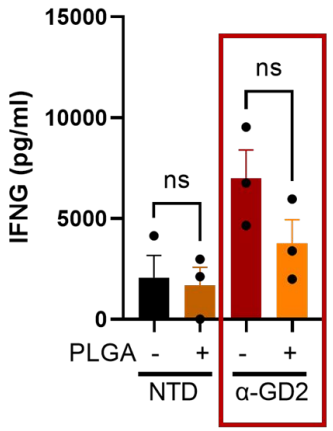
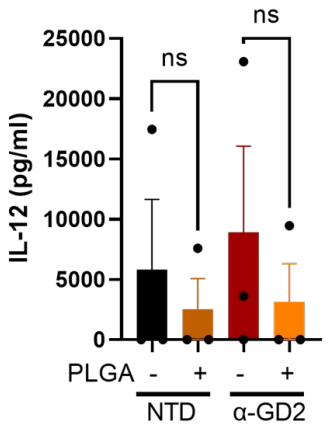
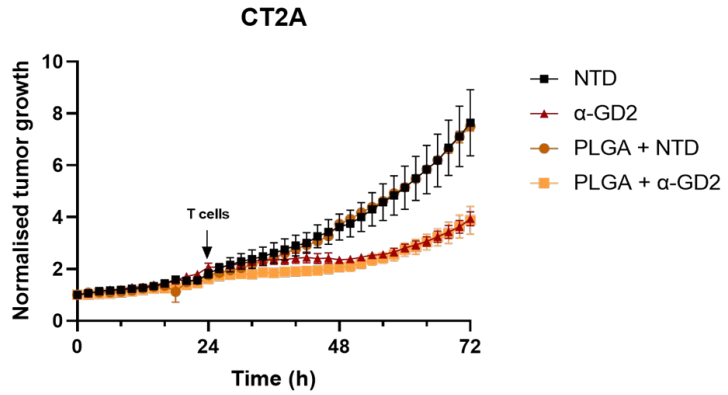
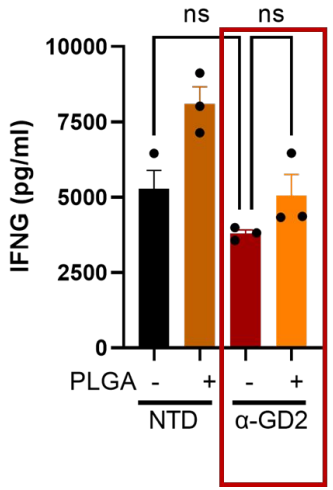
CAR-T killing assay, PLGA NPs

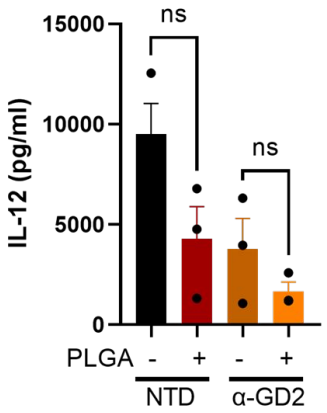








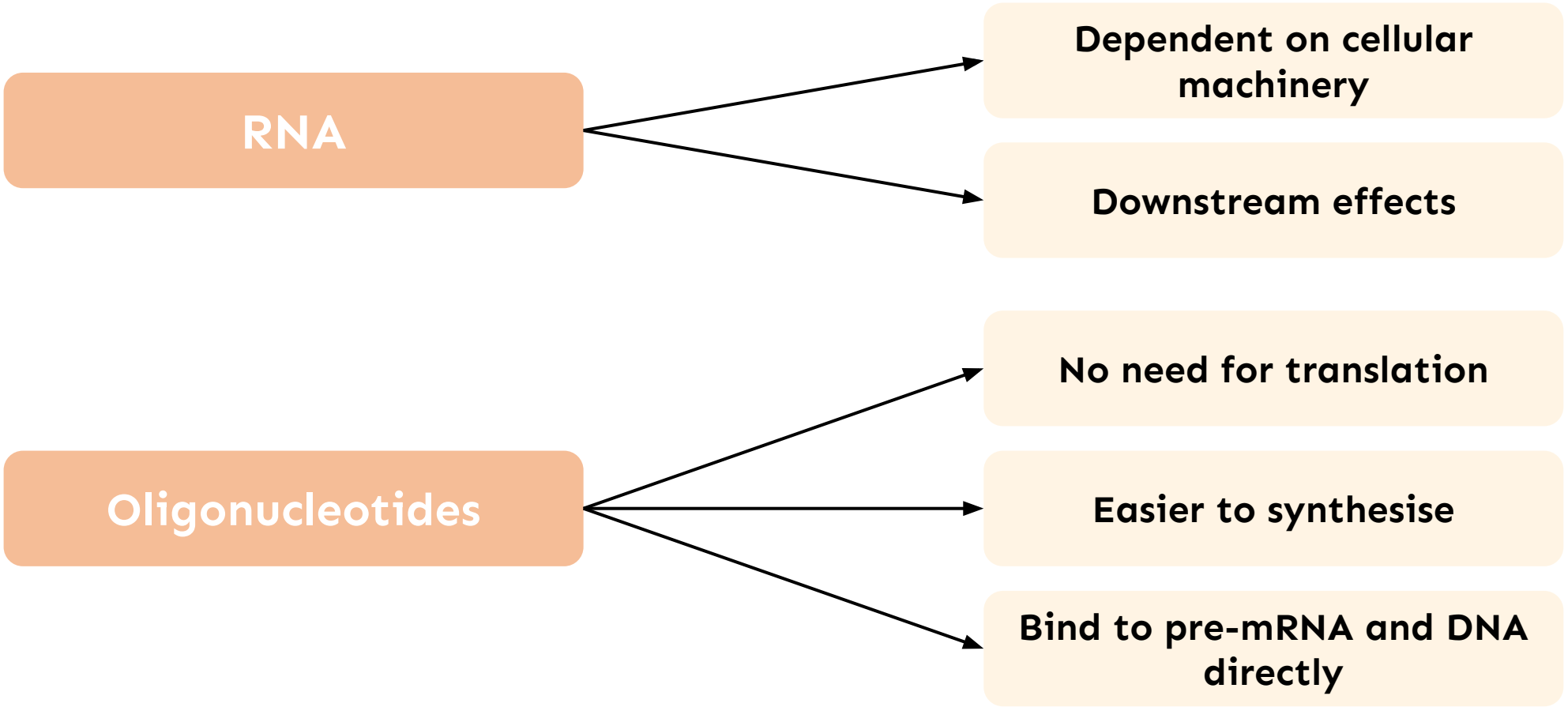




saRNA encoding IL-12 is able to transfect efficiently GBM cancer cells and macrophages

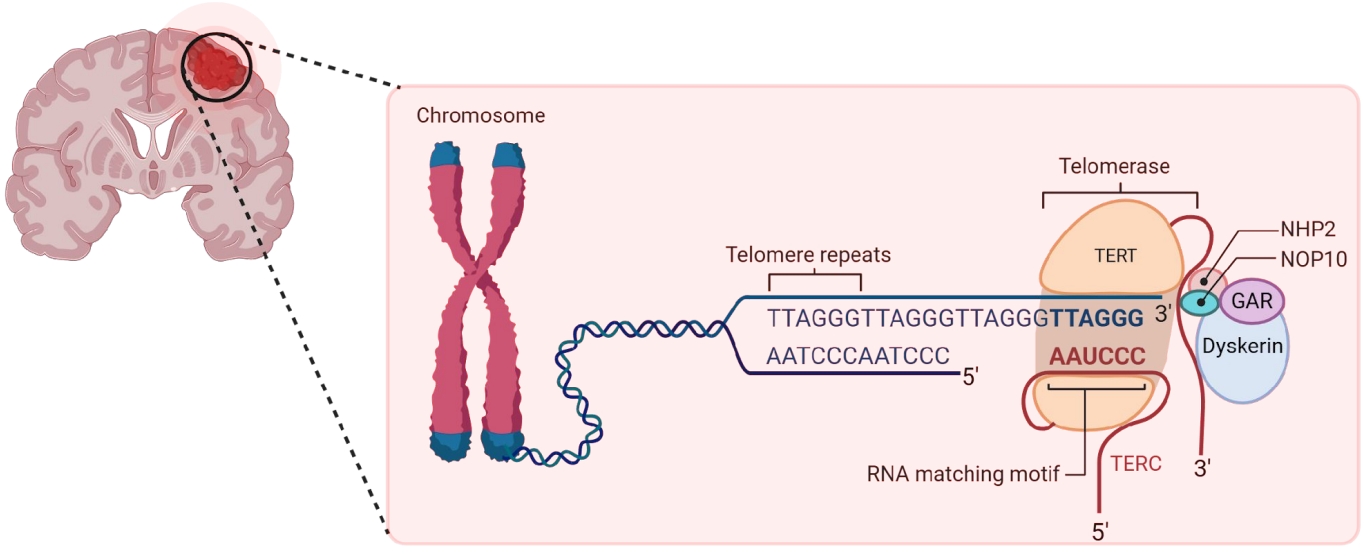
saRNA as a vaccine platform is able to boost CAR-T therapy

Gene therapy



Telomerase inhibition



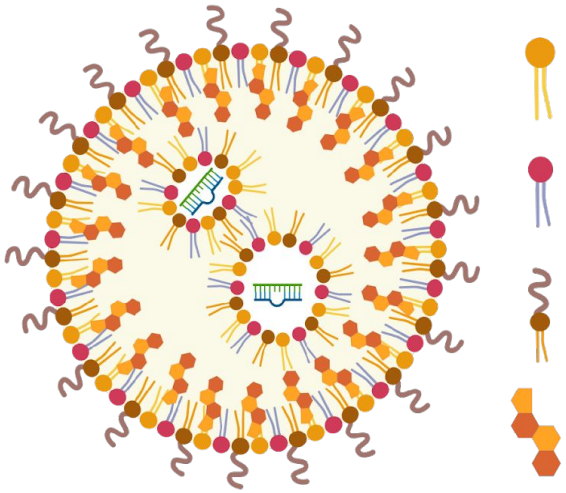


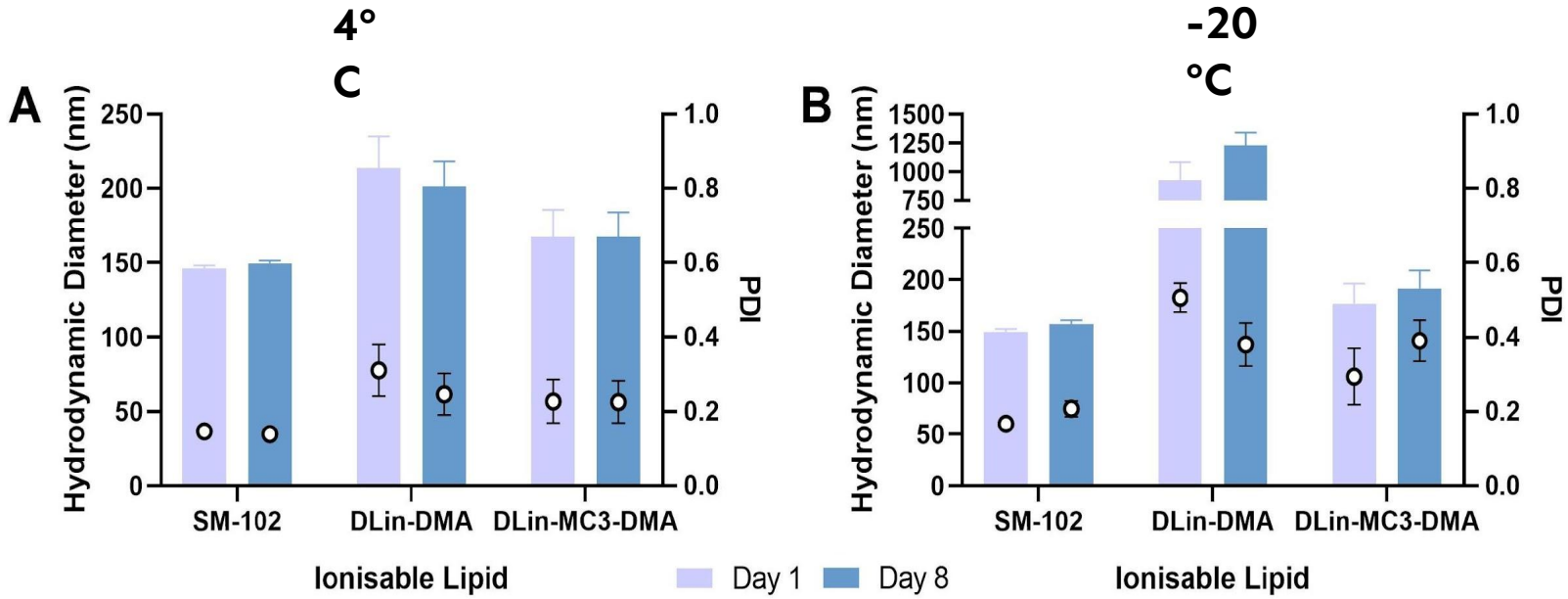
Imetelstat, a 13-mer oligonucleotide (ON), inhibits the TERC domain of telomerase

Clinical trial (2016) was ended prematurely, due to 2 fatalities

Imetelstat was administered through IV, leading to thrombocytopenia

LNPs as a vehicle for ON delivery





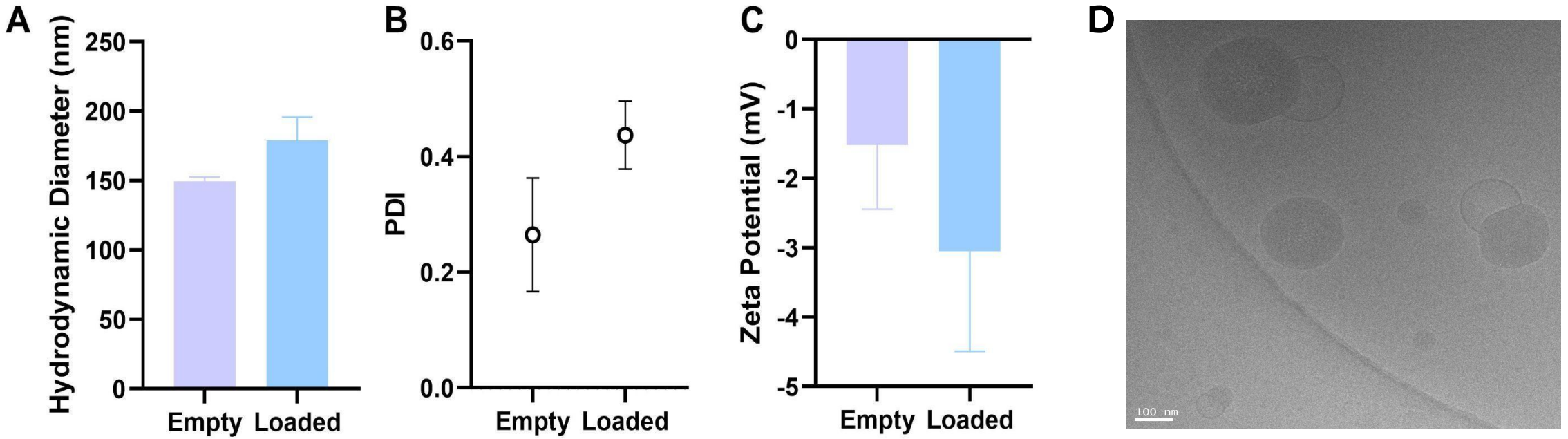
DLin-DMA is not stable at -20 °C.

PDI of SM-102 is significantly lower than DLin-MC3-DMA on day 8 at -20°C ($p=0.0133$).

SM-102 is the most desirable lipid for our LNPs.

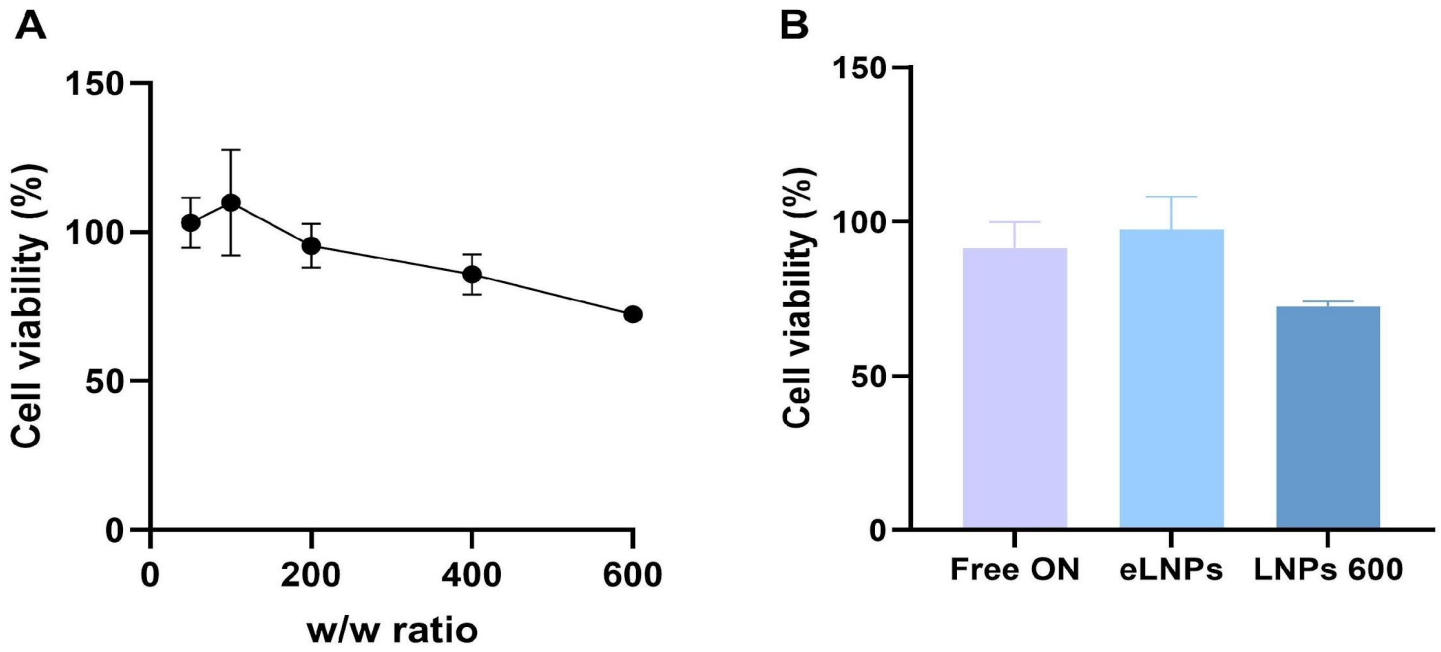
Preliminary data

LNPs as a vehicle for ON delivery



Preliminary data

LNPs as a vehicle for ON delivery



Preliminary data

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Funding Awards