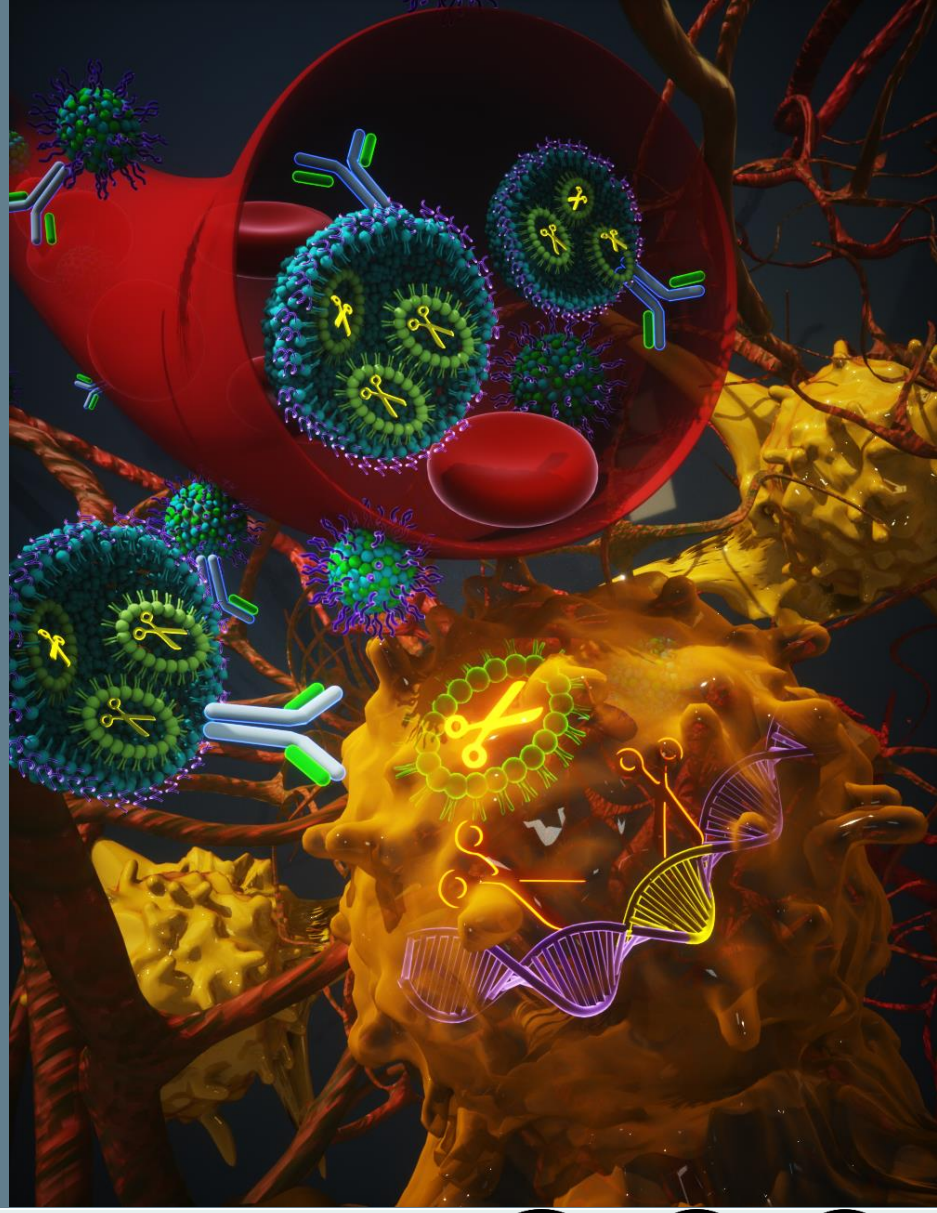
Targeted lipid nanoparticles are going beyond the liver: from vaccines to genome editing



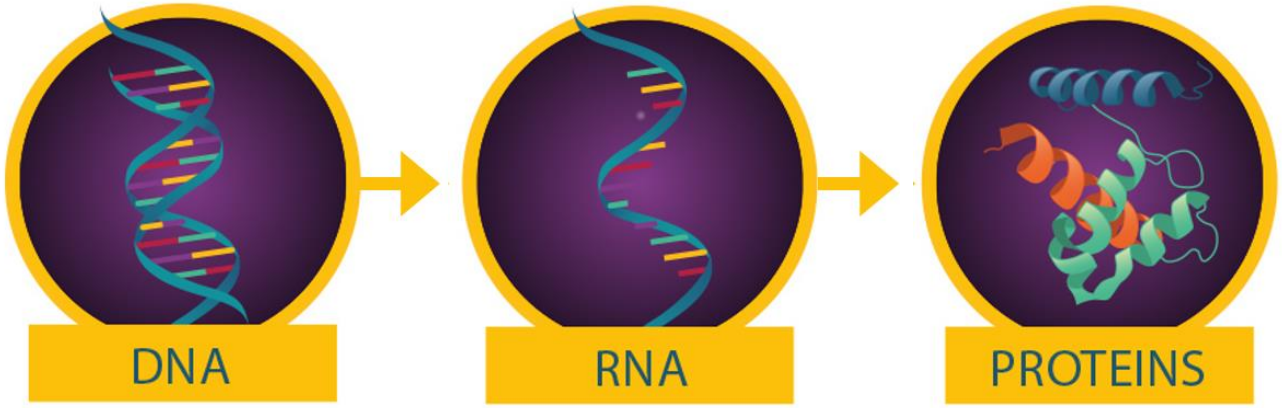
Tech Forum: Accelerating the development of mRNA vaccines and therapeutic

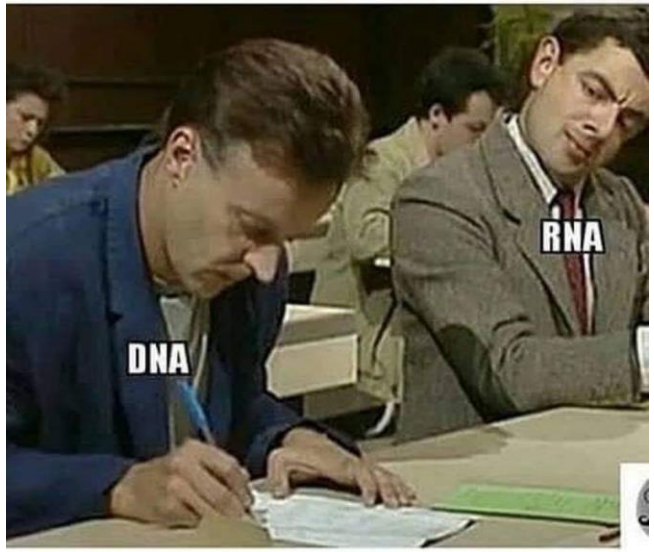
Dan Peer

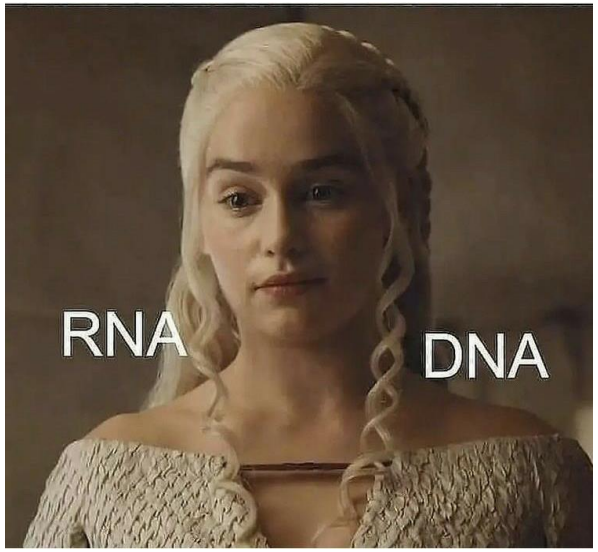
Peer Lab
Precision Nanomedicine

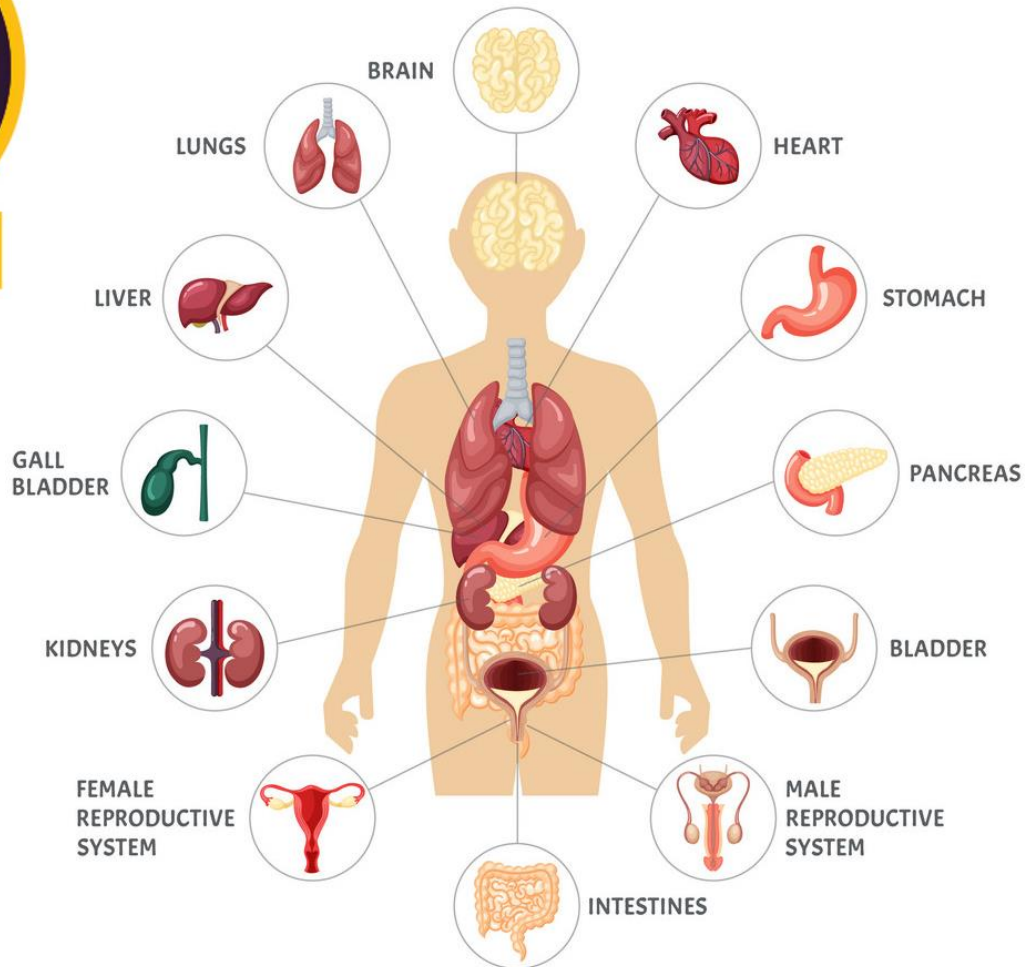
TEL AVIV UNIVERSITY
אוניברסיטת תל אביב

Central Dogma of Biology

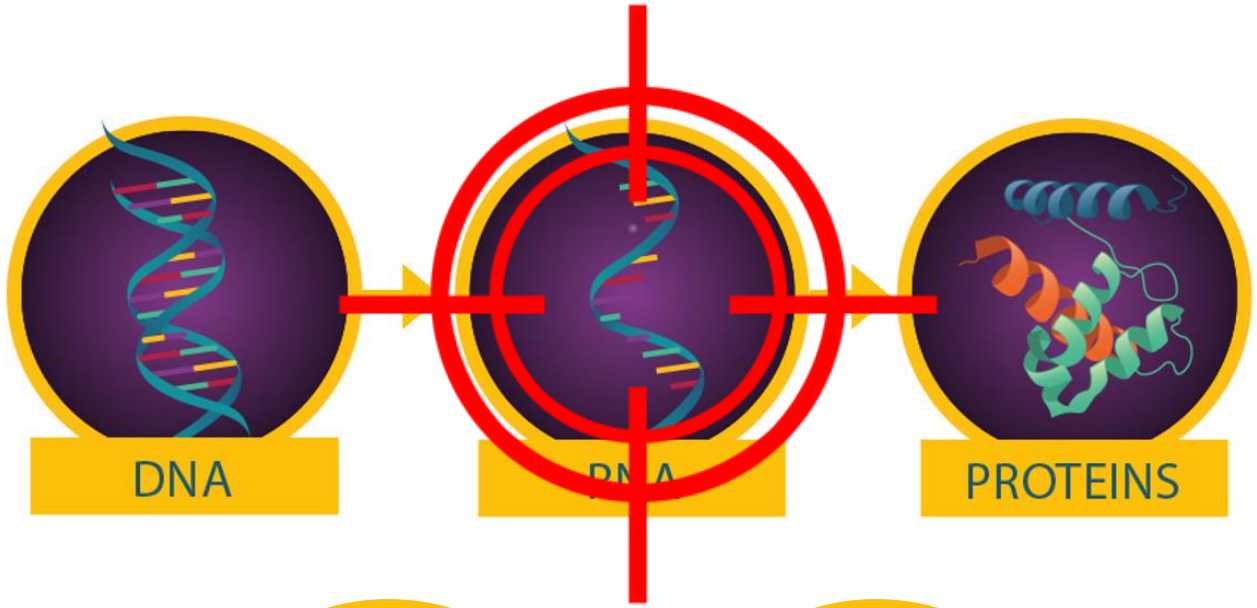








Therapeutic approaches



Silence

Activate

Edit

Replace

RNA based therapeutics

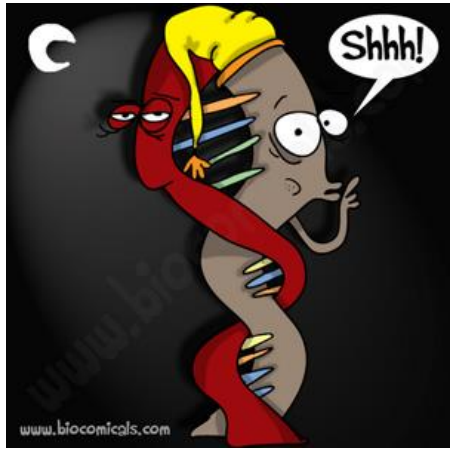
Silence

Activate

Edit

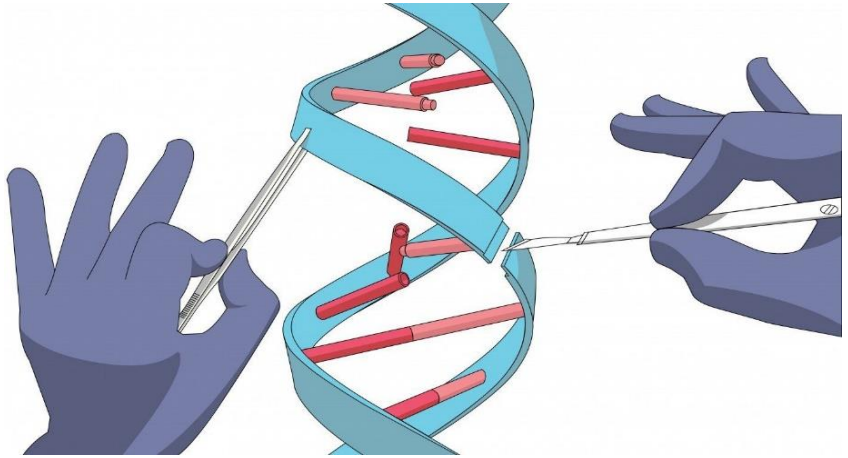
Replace

siRNA



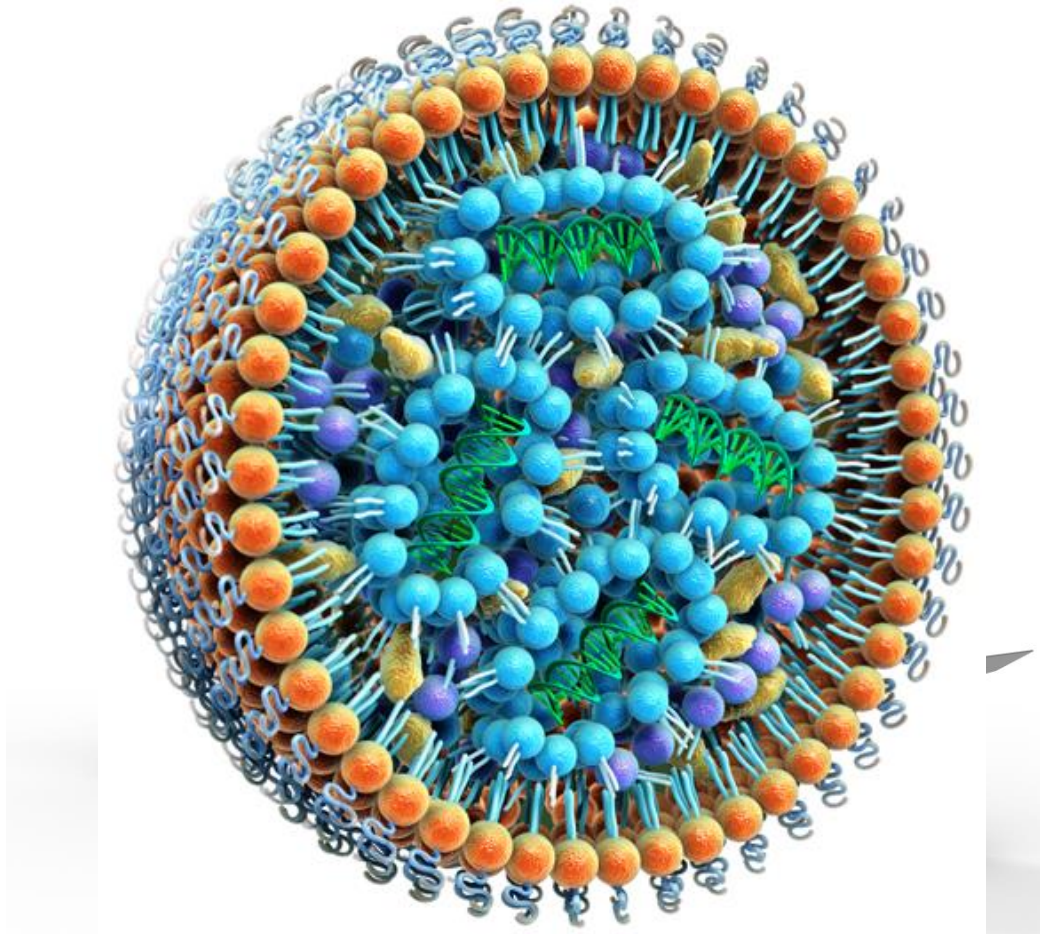
mRNA

Genome editing (e.g. CRISPR/Cas9)



The challenge in RNA therapeutics

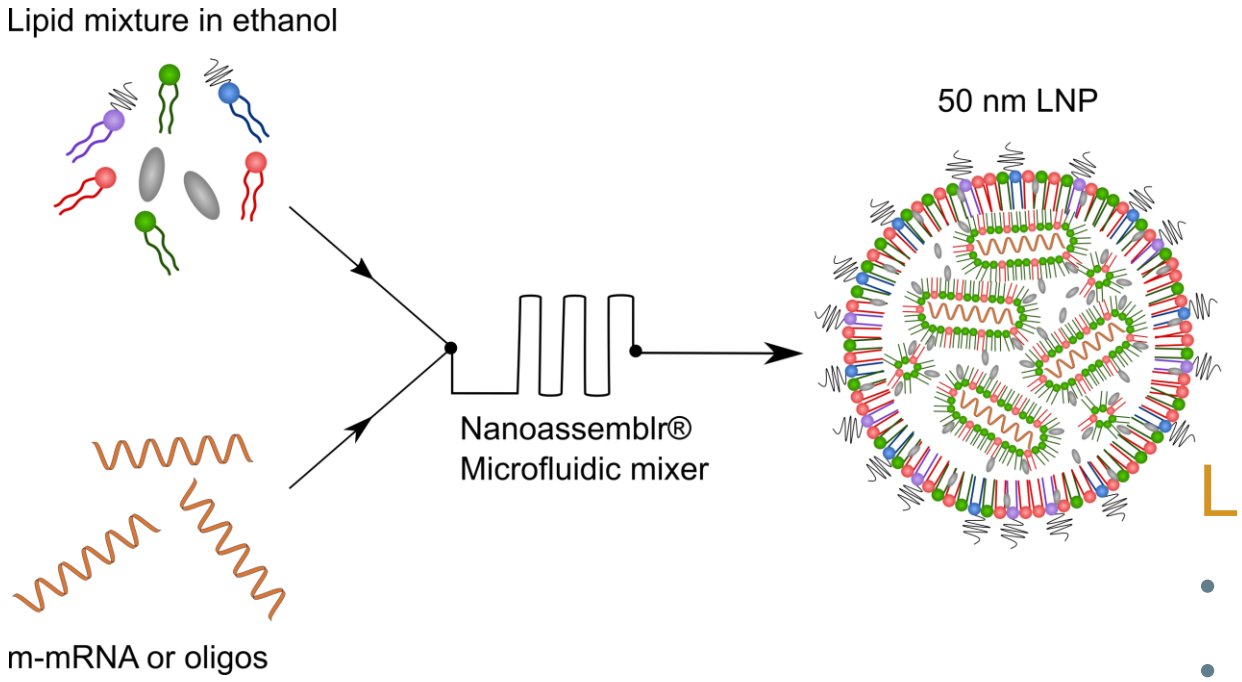
- An appropriate delivery system.
- Requirements:
  - Efficient RNA encapsulation
  - Evading clearance mechanism
  - Avoid toxicity and immune activation
  - Carrier internalization
  - RNA release
  - Specificity



Lipid nanoparticles (LNPs)

Components:

- Cholesterol
- DSPC
- PEG-DMG
- Ionizable lipid

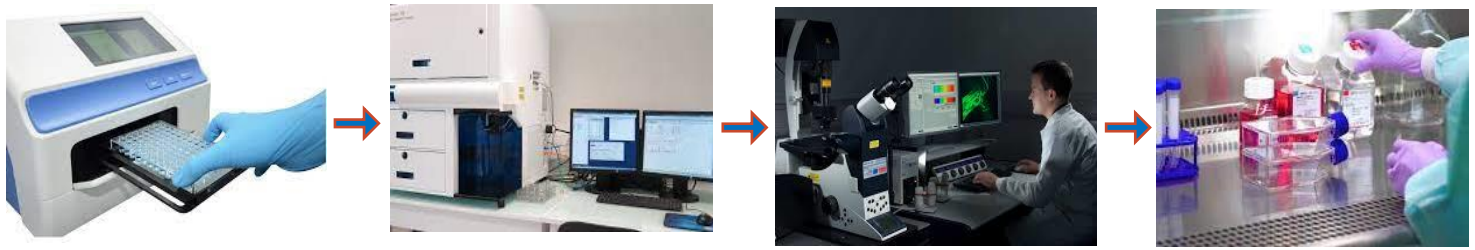


LNPs advantages:

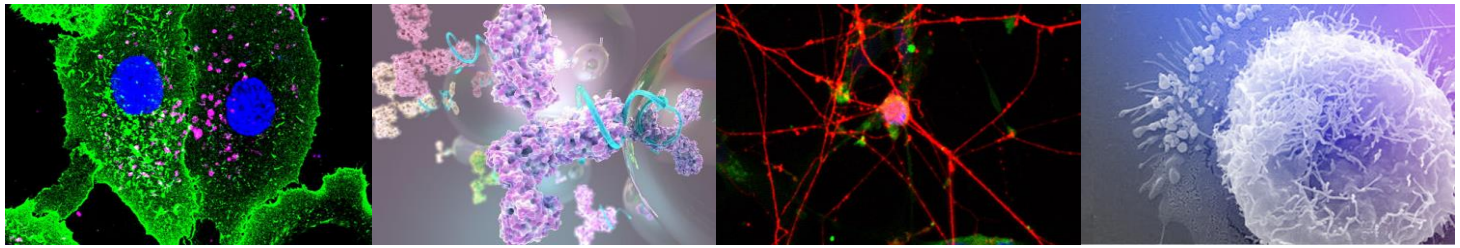
- Biocompatible
- Biodegradable
- Nucleic acid protection
- Increase stability in circulation
- Increase therapeutic effect
- Clinically approved

Laboratory of Precision NanoMedicine

Exome / Transcriptome analysis
Target Discovery & Validation

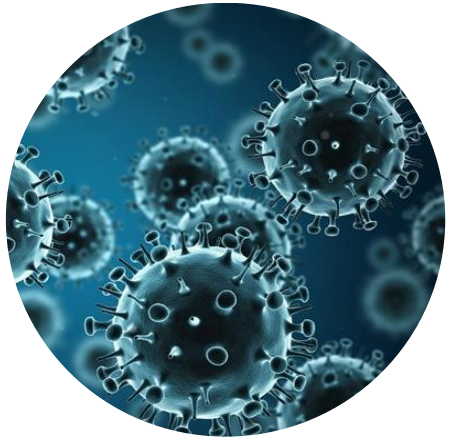


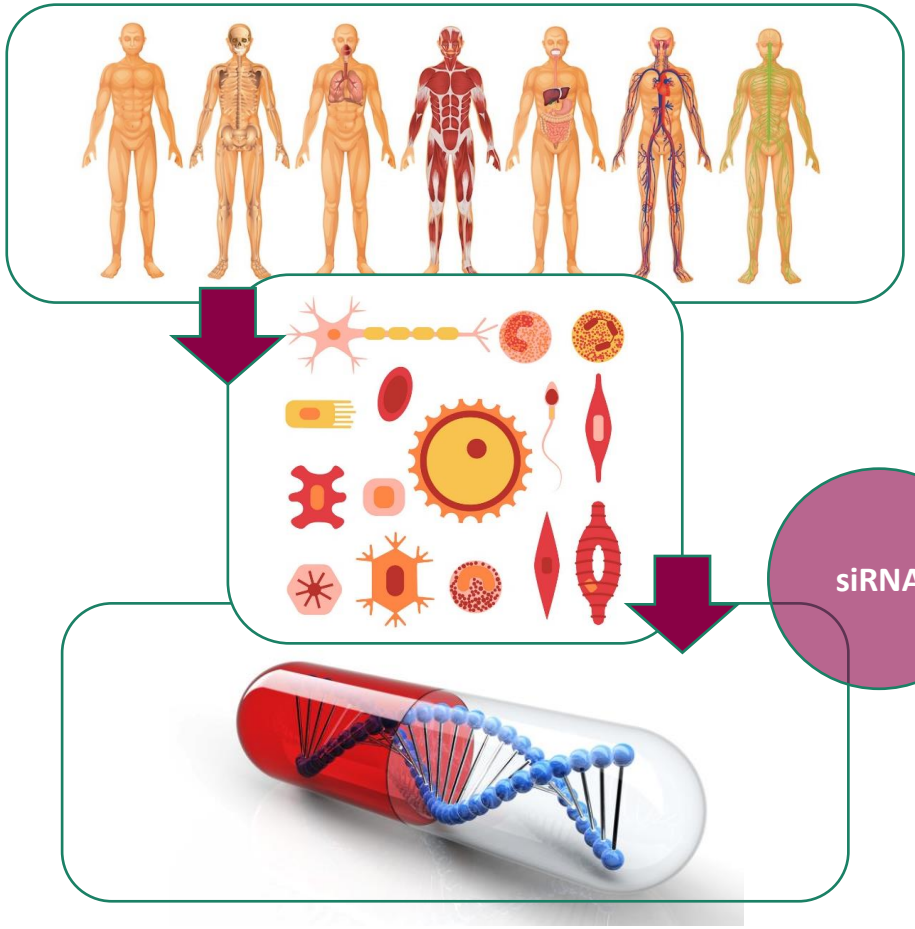
RNA Delivery systems (from lipid synthesis) to
Generation of mAbs & Protein Engineering



Tailor-made the appropriate nano-vehicle, selective targeting agent and specific therapeutic payload

The Complexity of Human Disorders





siRNA

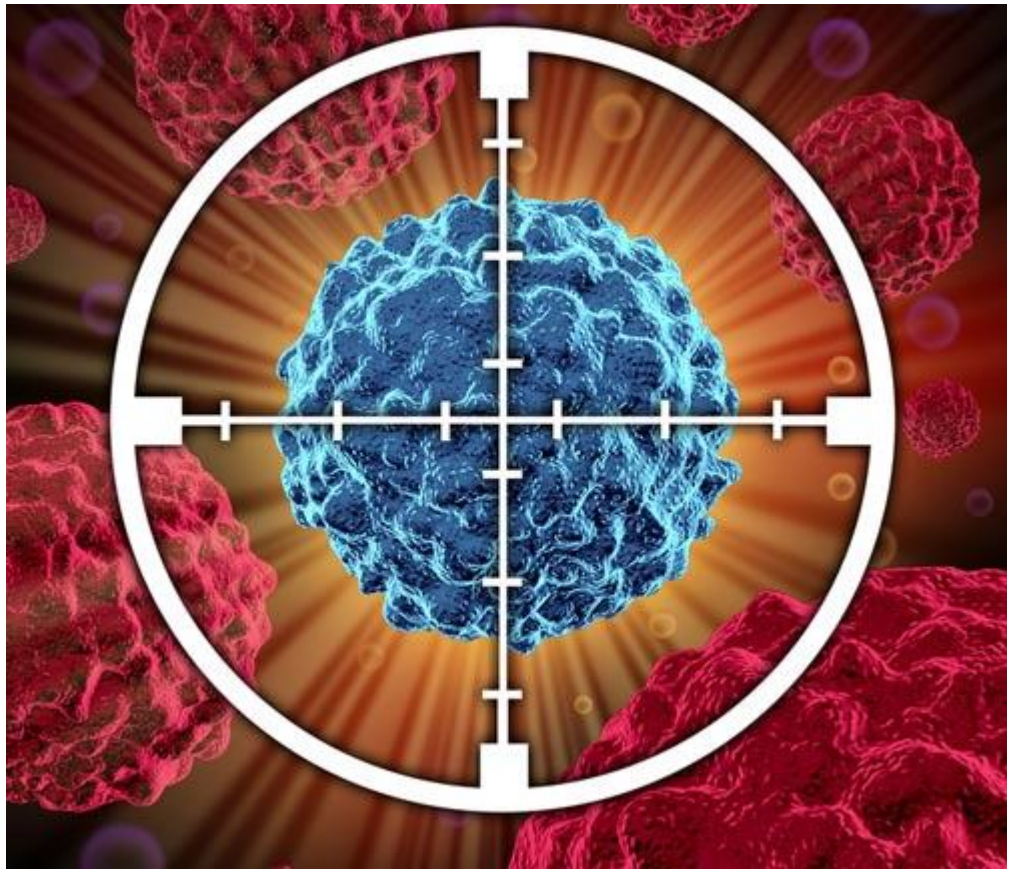
circRNA

saRNA

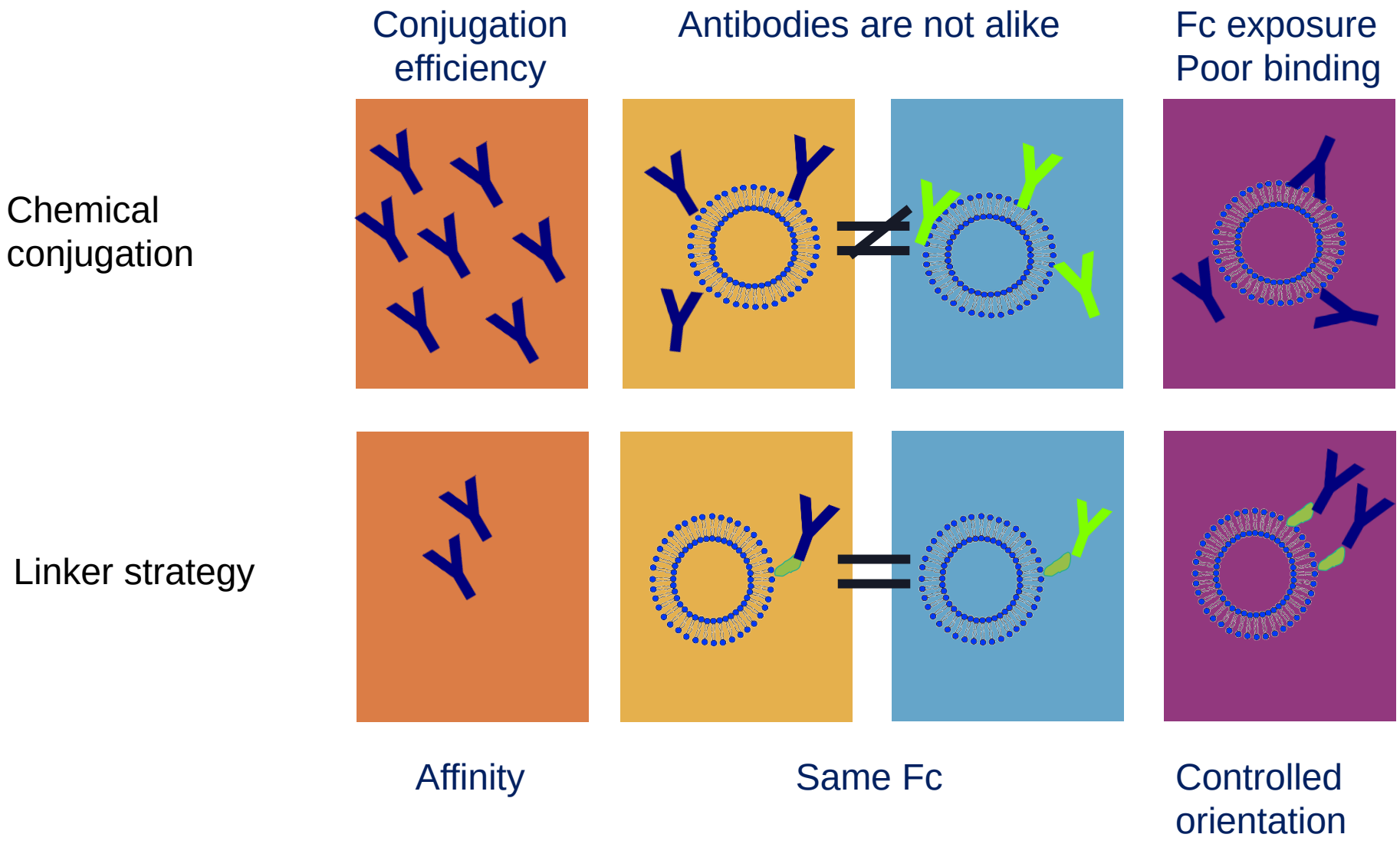
mRNA

Targeted delivery platform

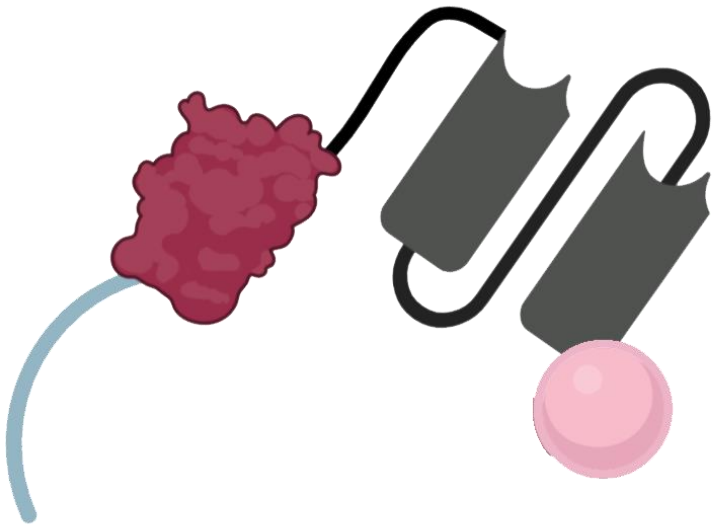
- Targeting moiety-
  - Antibodies
  - Peptides
  - Ligands
  - Aptamers



Antibody targeted LNPs construction



Anchored Secondary scFv Enabling Targeting – ASSET



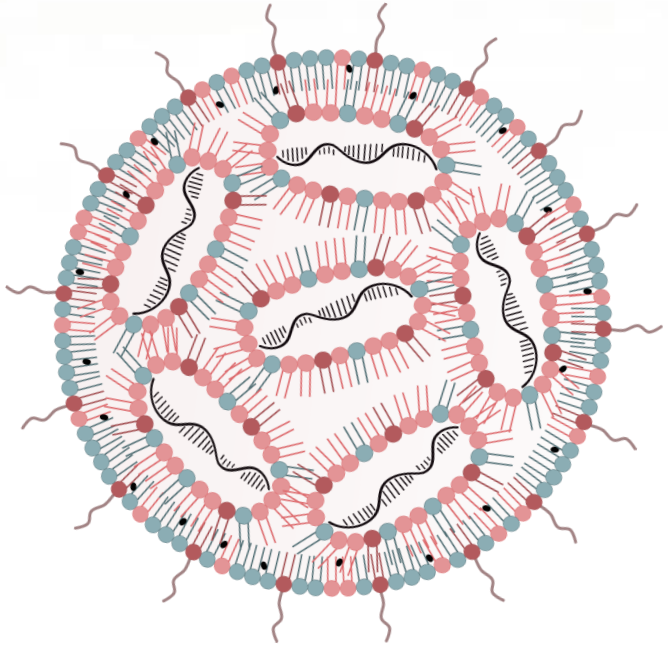
Dr. Ranit Kedmi

Dr. Nuphar Veiga

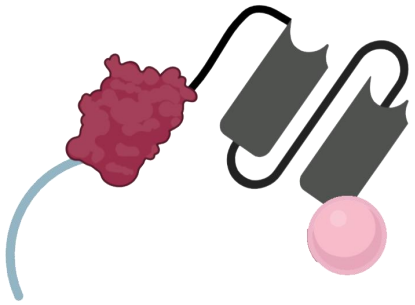
Kedmi, R. *et al. Nat. Nanotechnol.* **13**, 214–219 (2018).

ASSET as a Targeting Platform

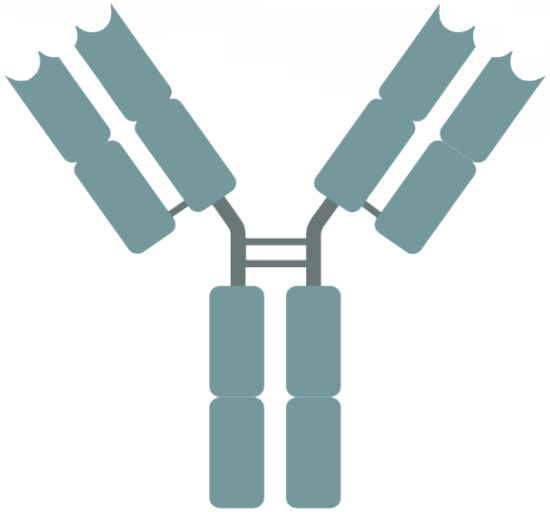
LNPs



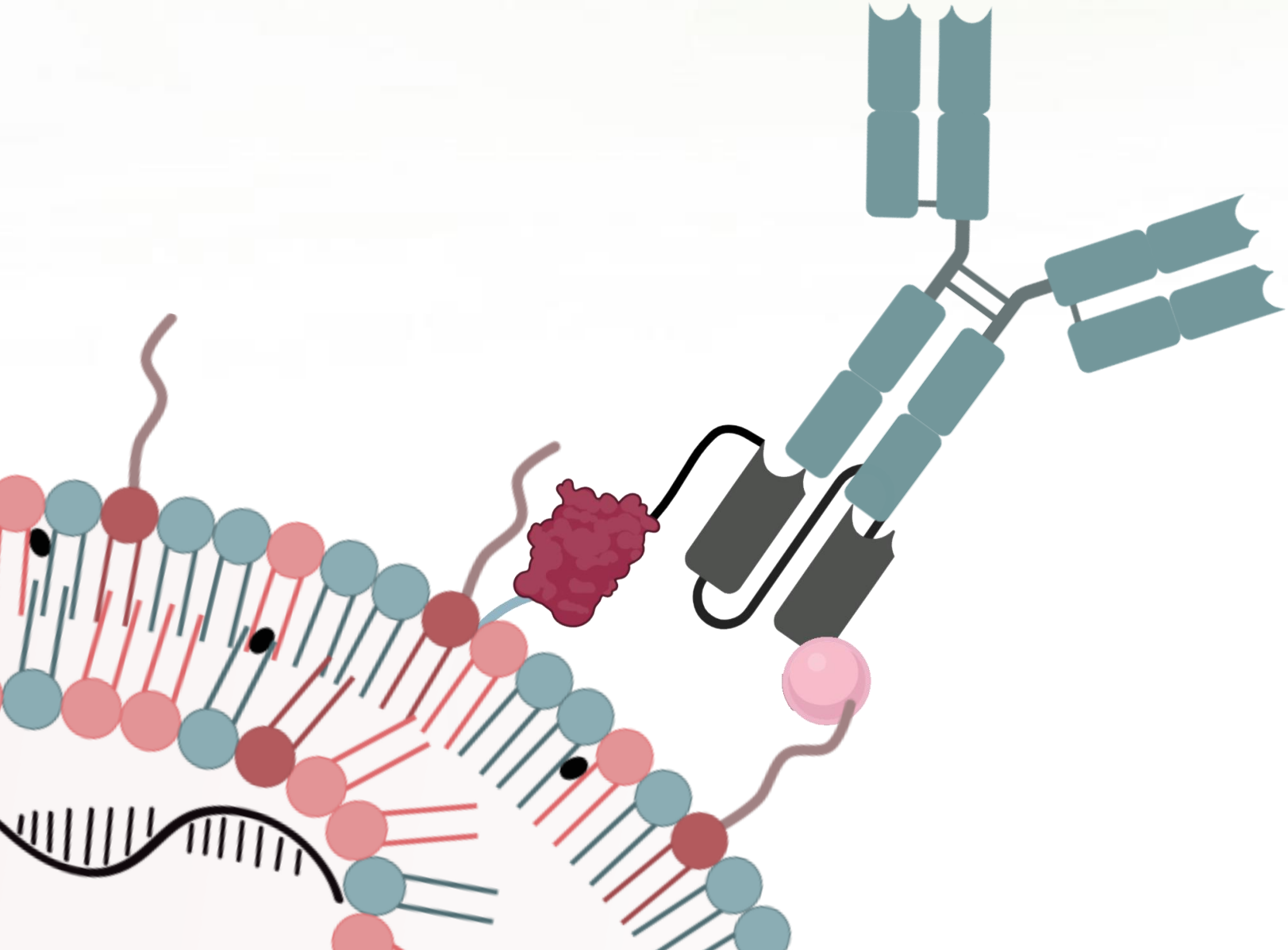
ASSET



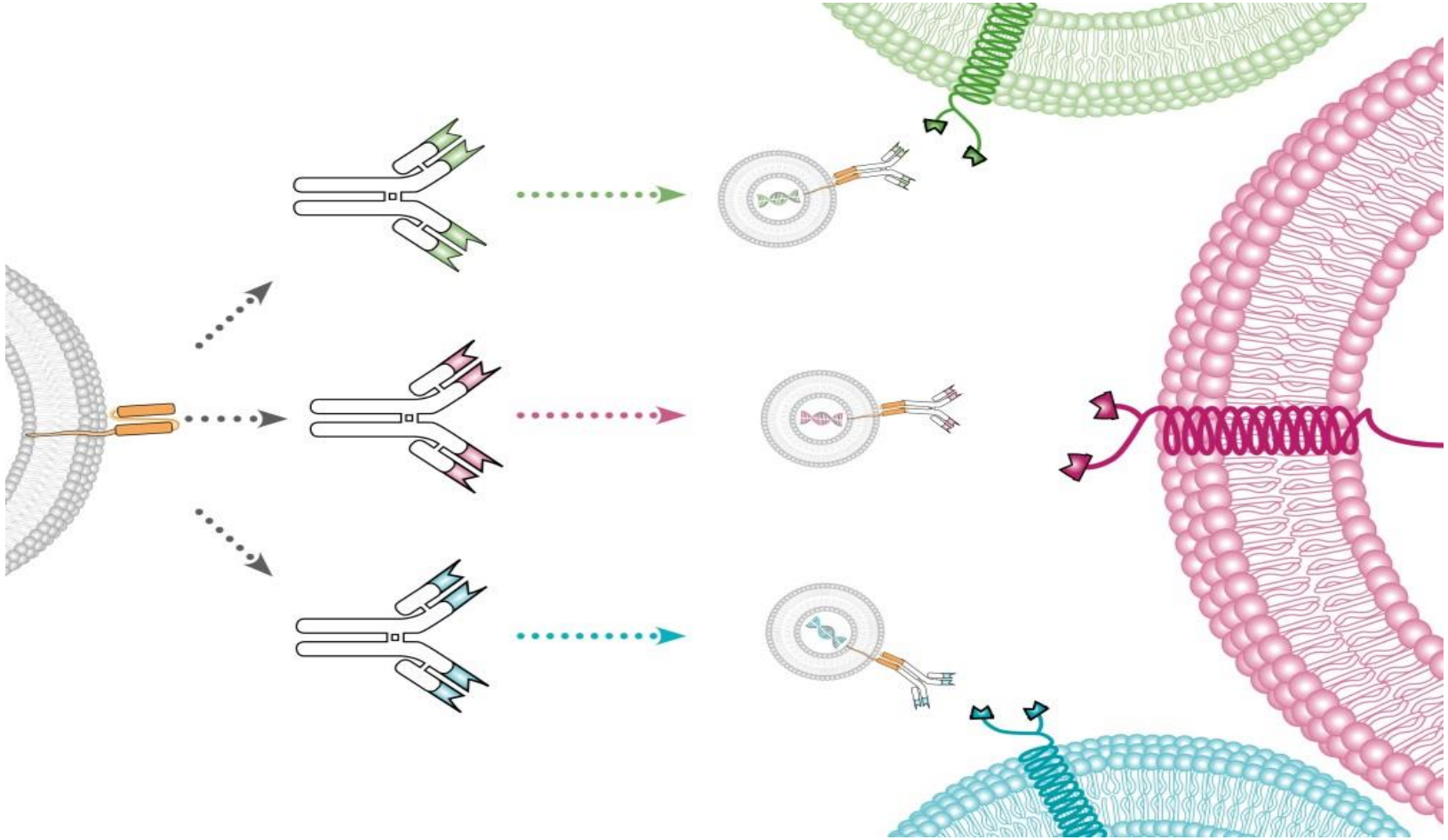
Rat IgG2a antibody



ASSET as a Targeting Platform

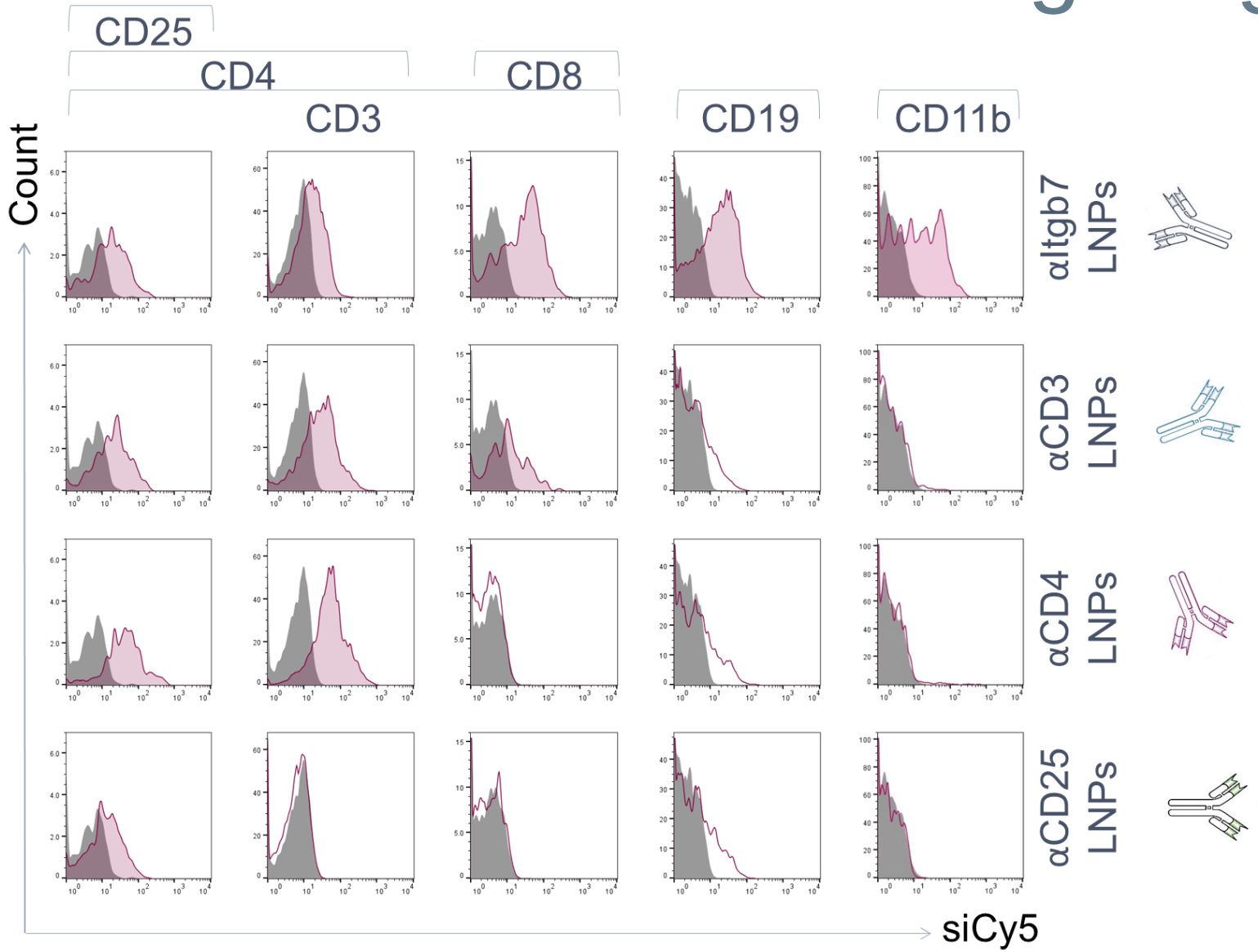


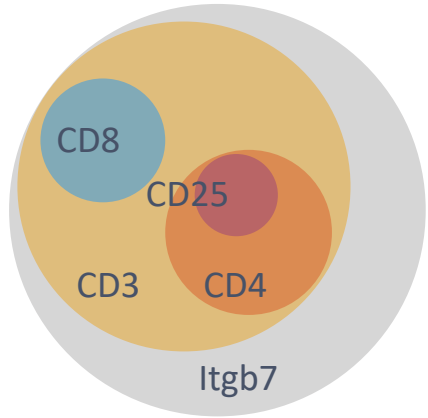
Modular platform



Kedmi R, Veiga N et al. Nature Nanotechnology 2018

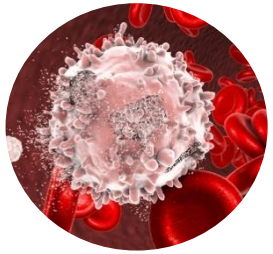
Versatile *in vivo* targeting





Kedmi R, Veiga N et al. Nature Nanotechnology 2018

Therapeutic applications



Hematological malignancies

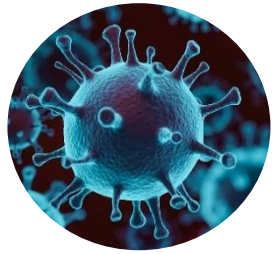
- Myeloma
- Leukemia
  - CML
  - AML
  - CLL
  - ALL
- Lymphoma
  - Hodgkin's
  - NHL: MCL

Solid Tumors

- Ovarian cancer
- GBM
- Head and neck

Inflammation

- Autoimmune diseases
  - Colitis
  - Crohn's
  - RA
  - Psoriasis
  - Lupus/SLE
  - MS
  - Allergy/Asthma
  - GVHD
  - IR injury



Viral infection

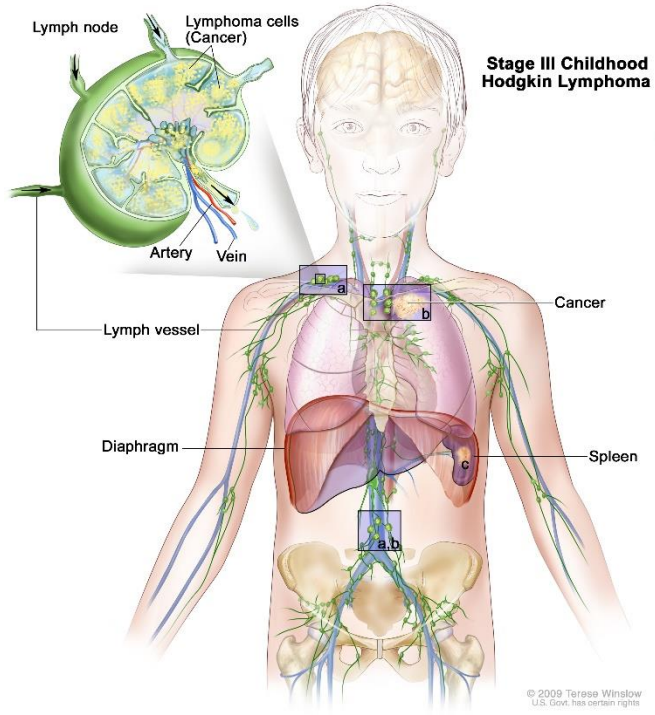
- HIV
- Ebola
- COVID - 19

Genetic disorders

- Duchenne Muscular Dystrophy
- CF
- XBP-1

Mantle cell lymphoma (MCL)

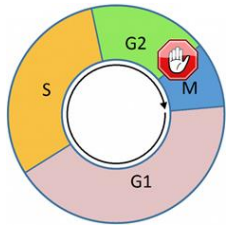
- Aggressive form of B cell non-Hodgkin lymphoma
- Relatively rare (~6% of all NHL cases)
- Considered “incurable” with traditional chemotherapy
- Median overall survival: 5-7 years



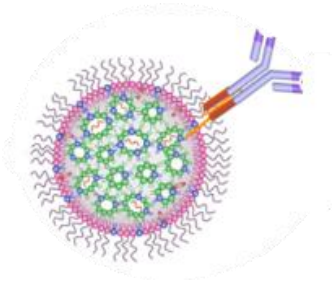
Mantle cell lymphoma (MCL)

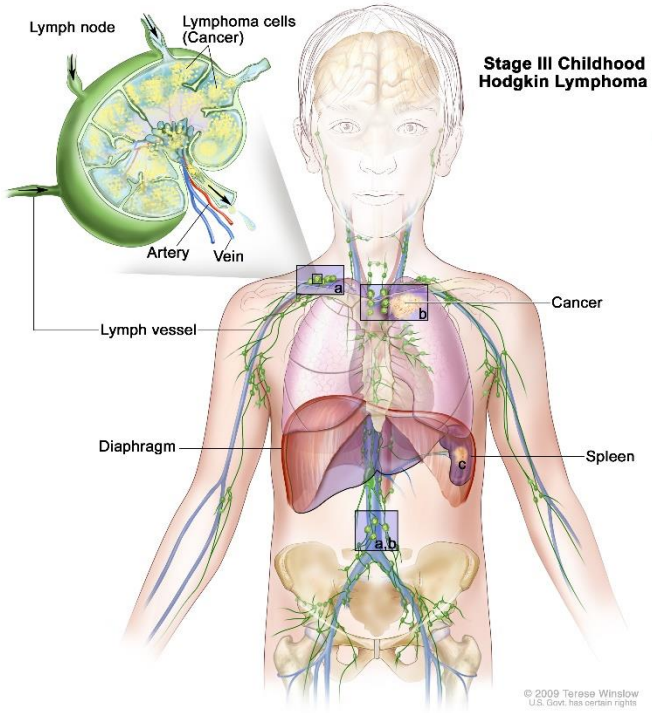
- Therapeutic approach: Silencing

- Target gene: PLK1

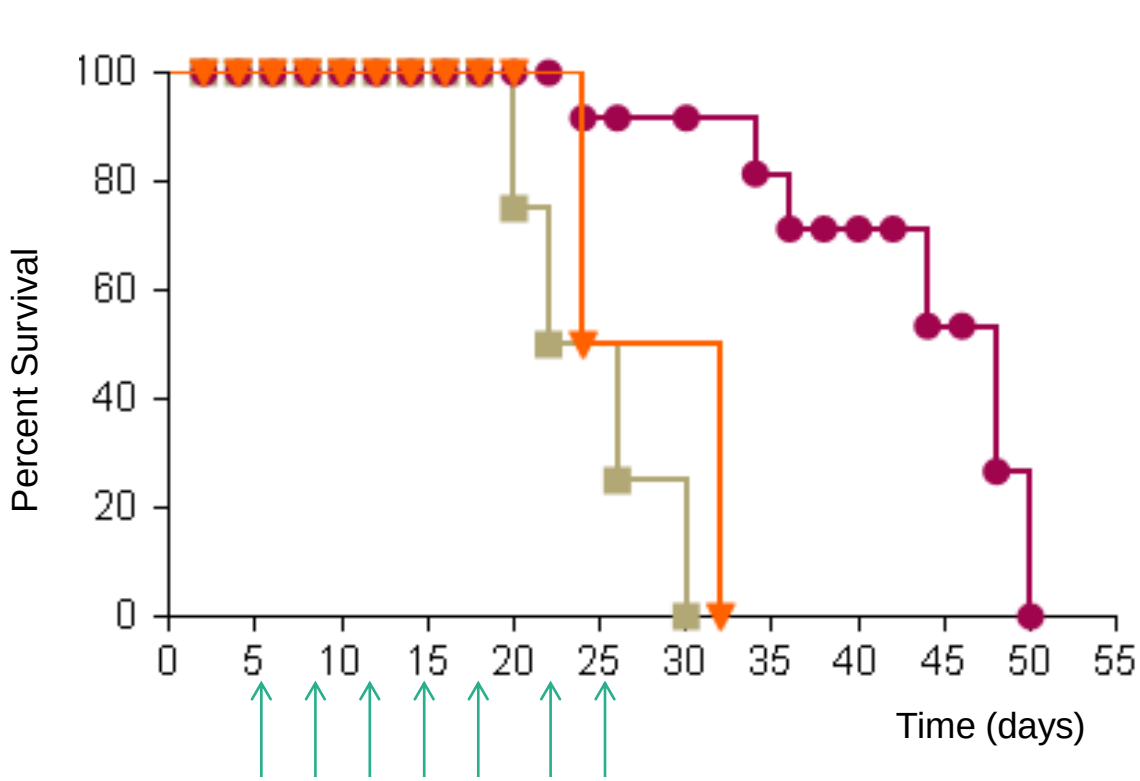


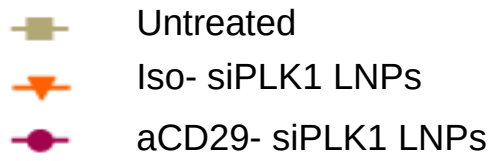
- Target cells: CD29

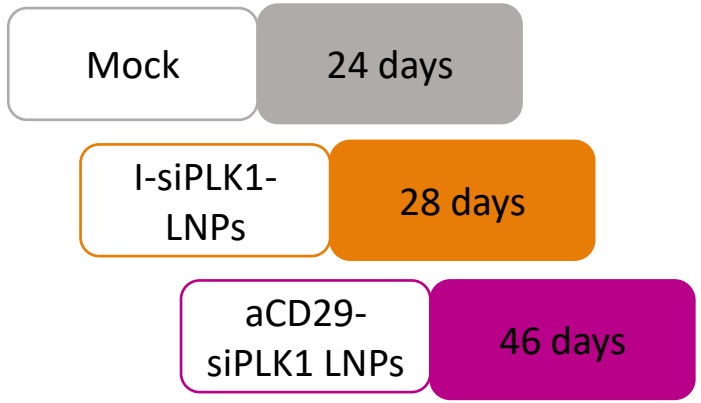




Prolonged Survival in MCL Model using CD29 targeted siPLK1-LNPs





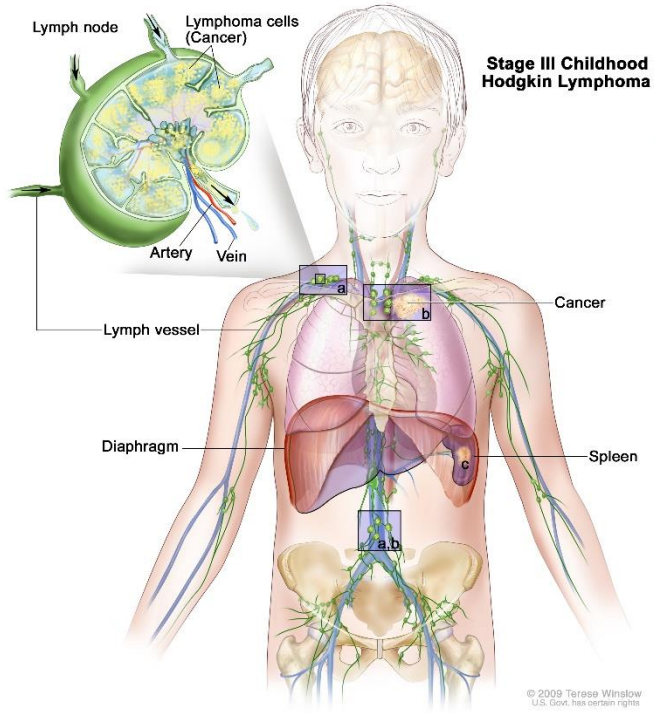


Kedmi R, Veiga N et al. Nature Nanotechnology 2018

n=10 mice/group

Therapeutic approach for MCL treatment

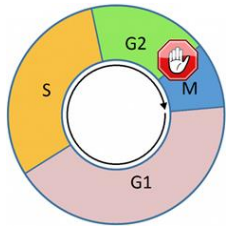
- Therapeutic approach: Silencing
- Target gene: PLK1
- Target cells: CD29



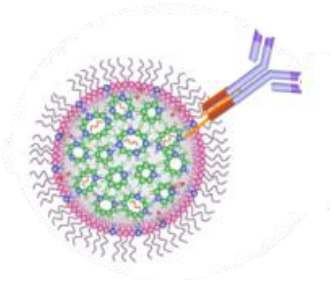
Cancer

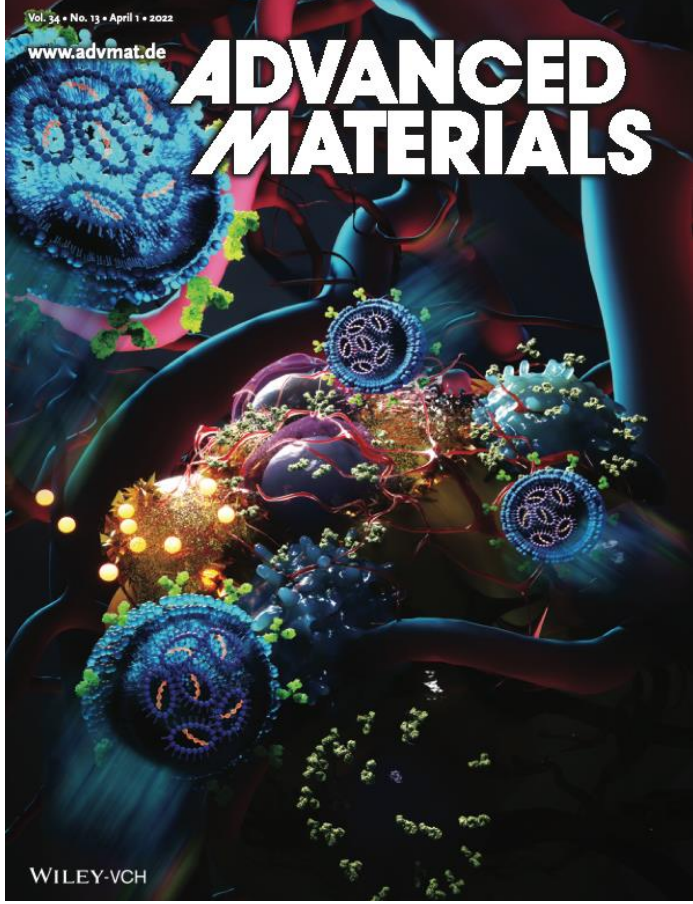
- Therapeutic approach: Silencing

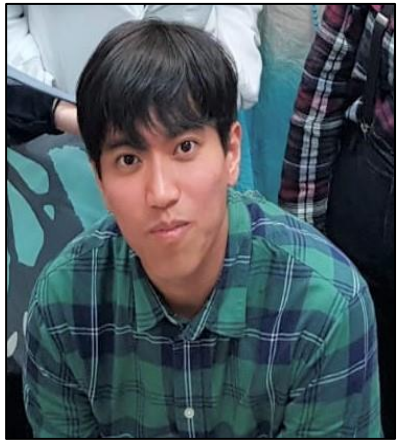
- Target gene: HO1



- Target Receptor: PDL-1







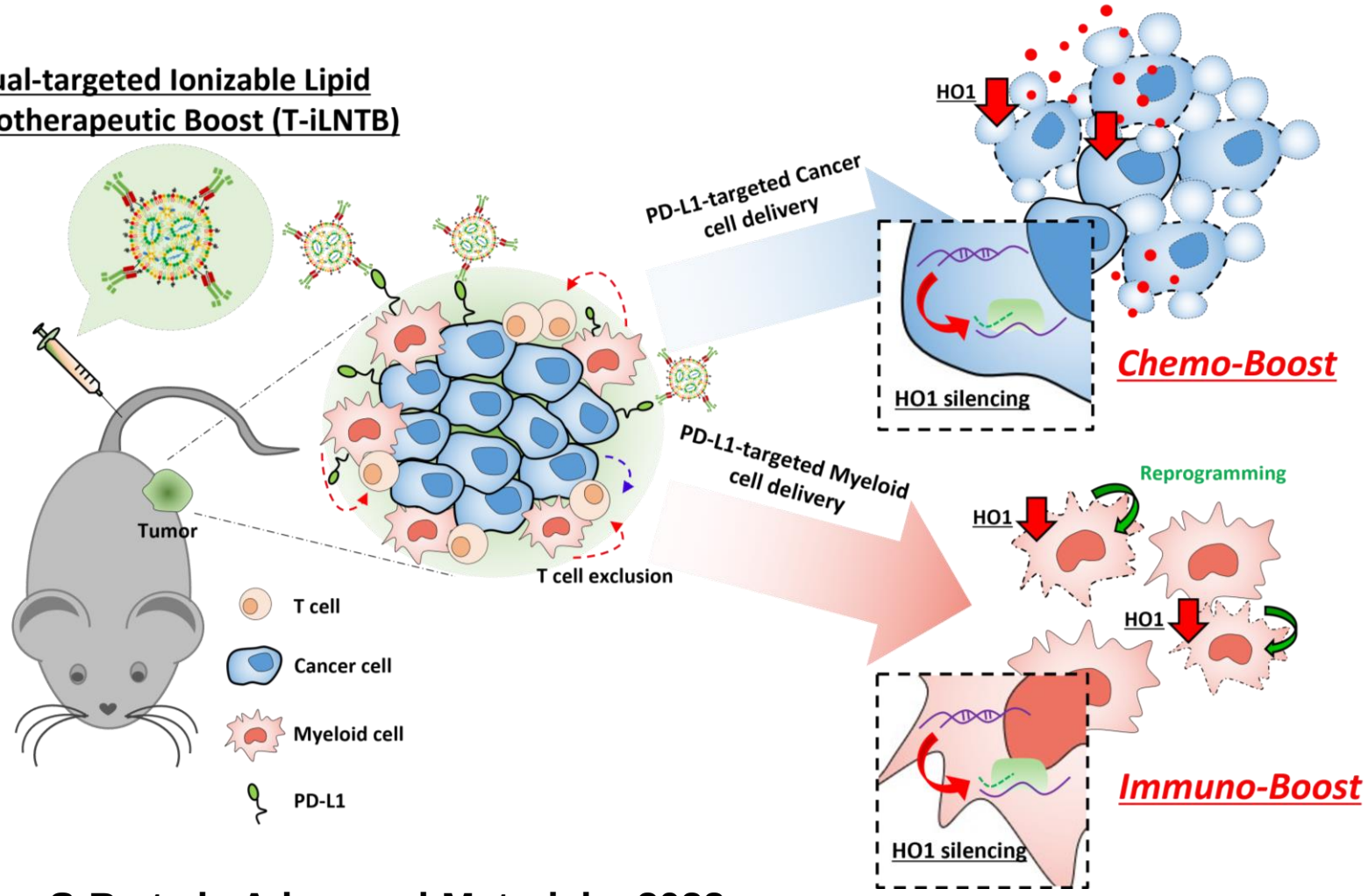
Seok-Beom Yong, Ph.D

Single gene (HO1)-targeted lipid nanoparticle for chemo-immunotherapeutic boost in cancer

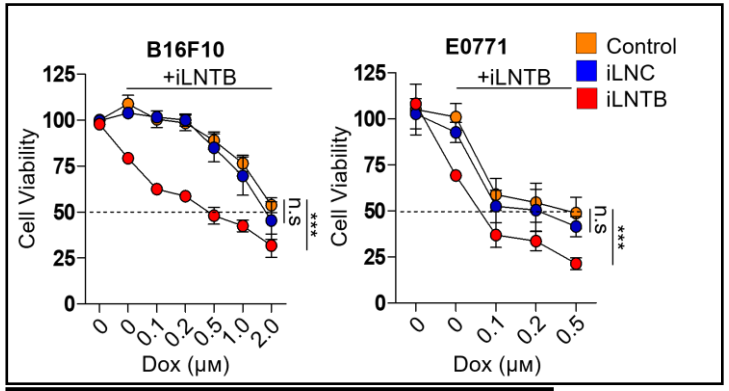
***Dual function of HO1 gene in tumor**

1. Chemo-resistance function in **Cancer cell**
2. Immune-modulatory function in **Tumor myeloid cell**

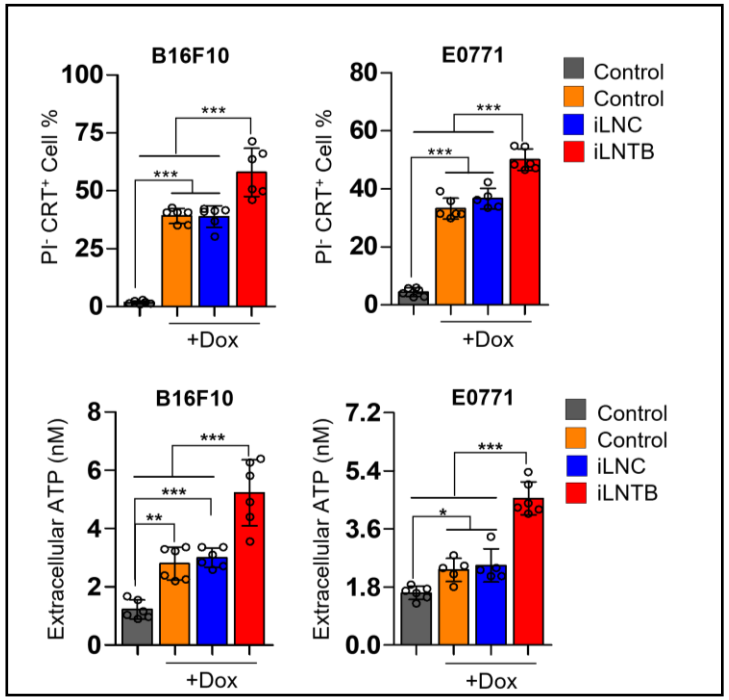
Dual-targeted Ionizable Lipid Nanotherapeutic Boost (T-iLNTB)



Chemo-sensitization effect



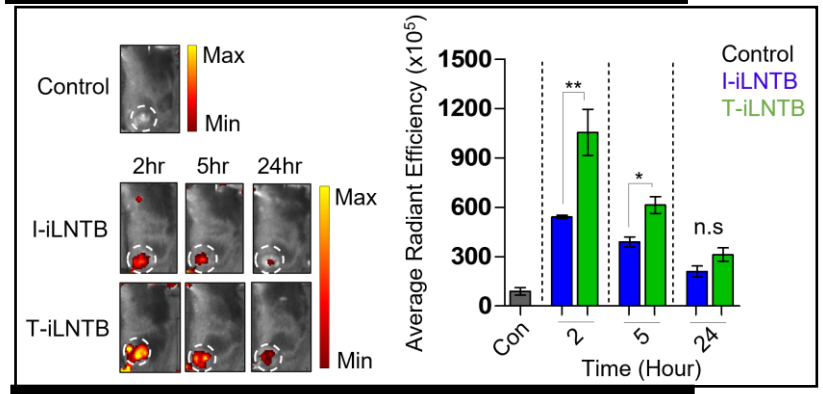
Increased Immunogenic cell death



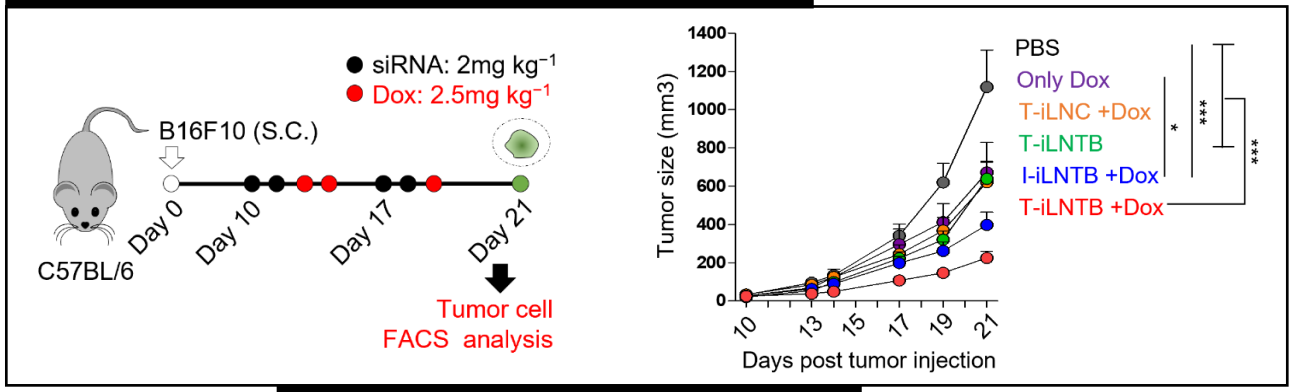
Yong S-B et al., Advanced Materials, 2022

In vivo anti-tumor effect in combination with chemotherapy (Dox) & immune reprogramming effect

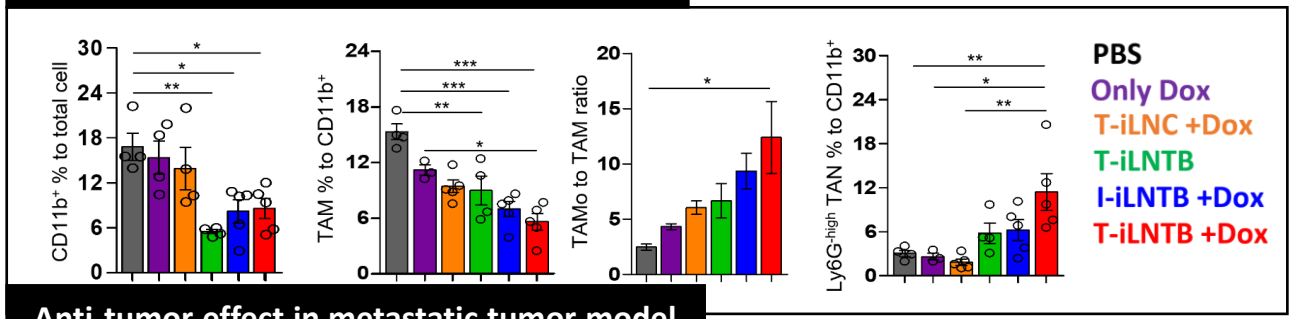
Increased tumor delivery by PD-L1-targeting



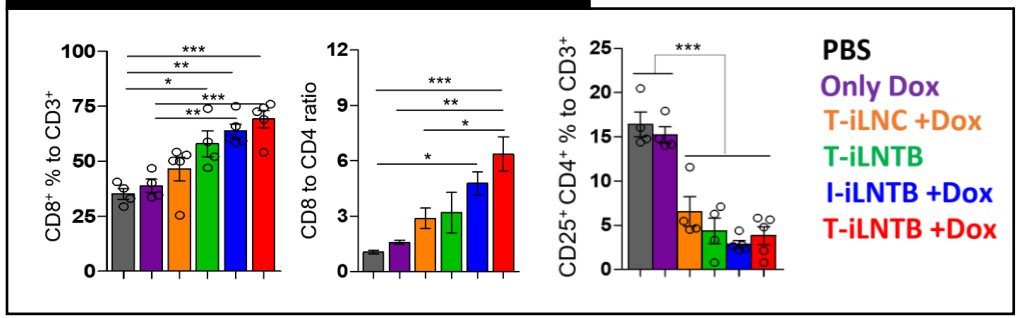
Anti-tumor effect in combination with chemotherapy



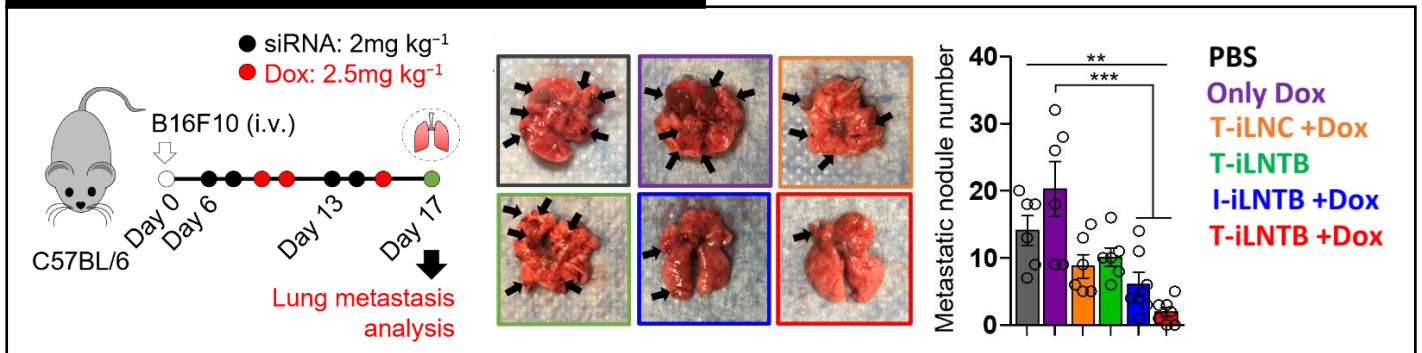
Tumor myeloid cell reprogramming effect



Tumor T cell reprogramming effect

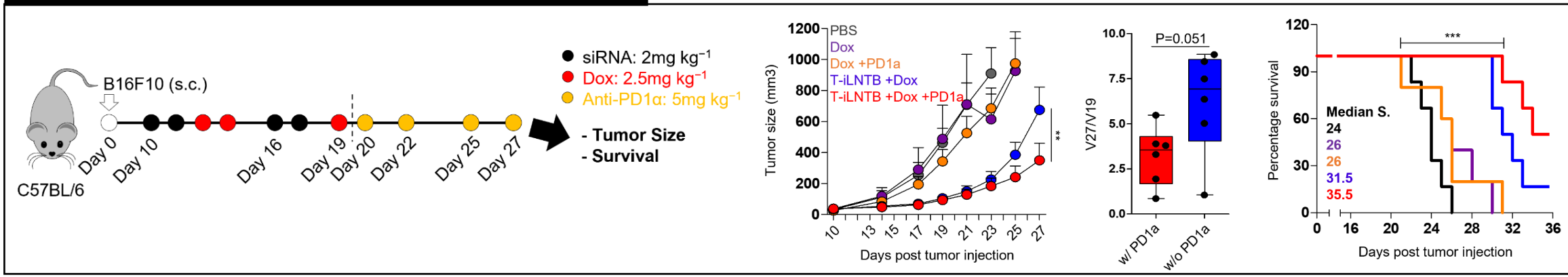


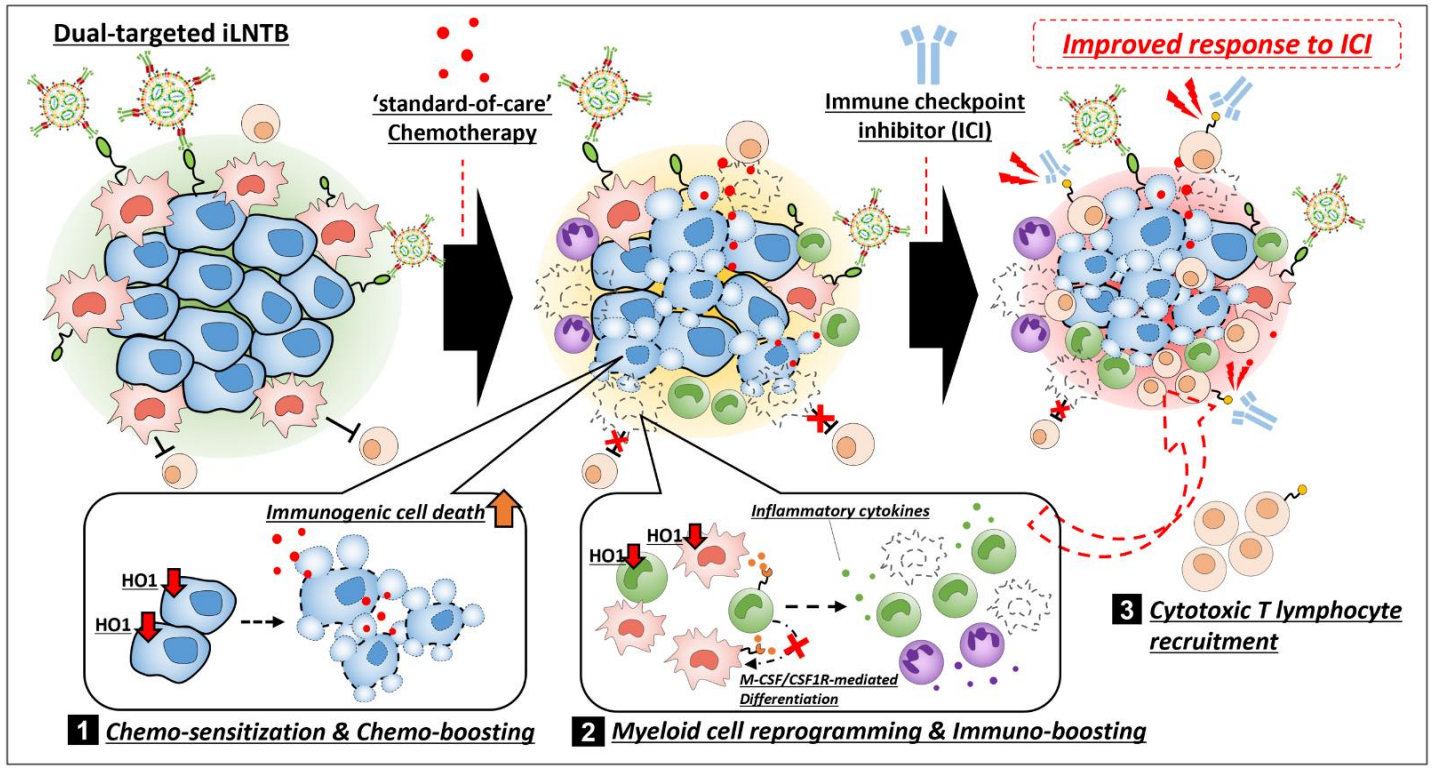
Anti-tumor effect in metastatic tumor model



Yong S-B et al., Advanced Materials, 2022

Anti-tumor effect in combination with chemo-immunotherapy

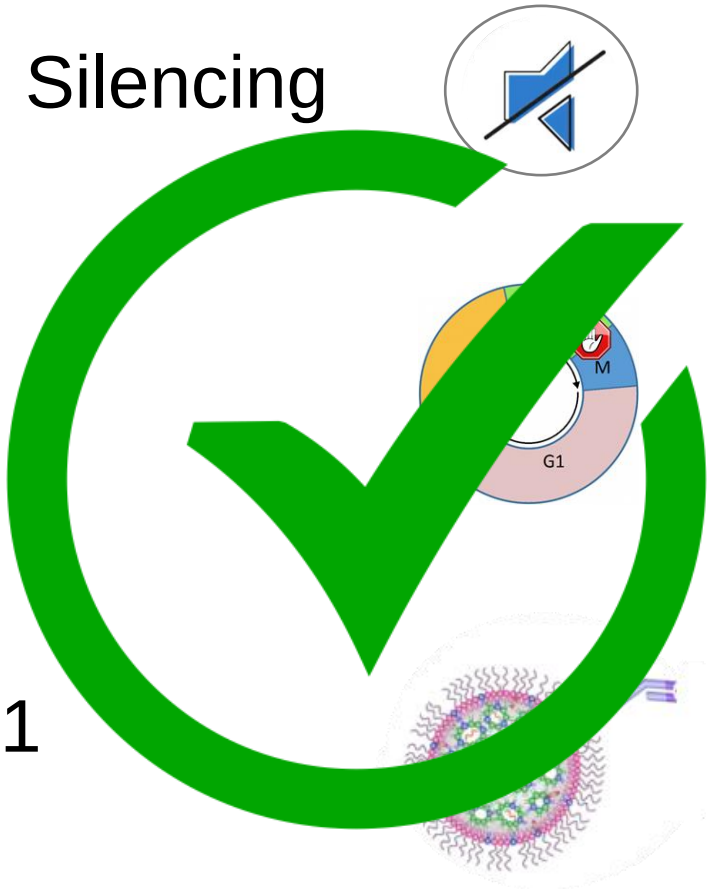


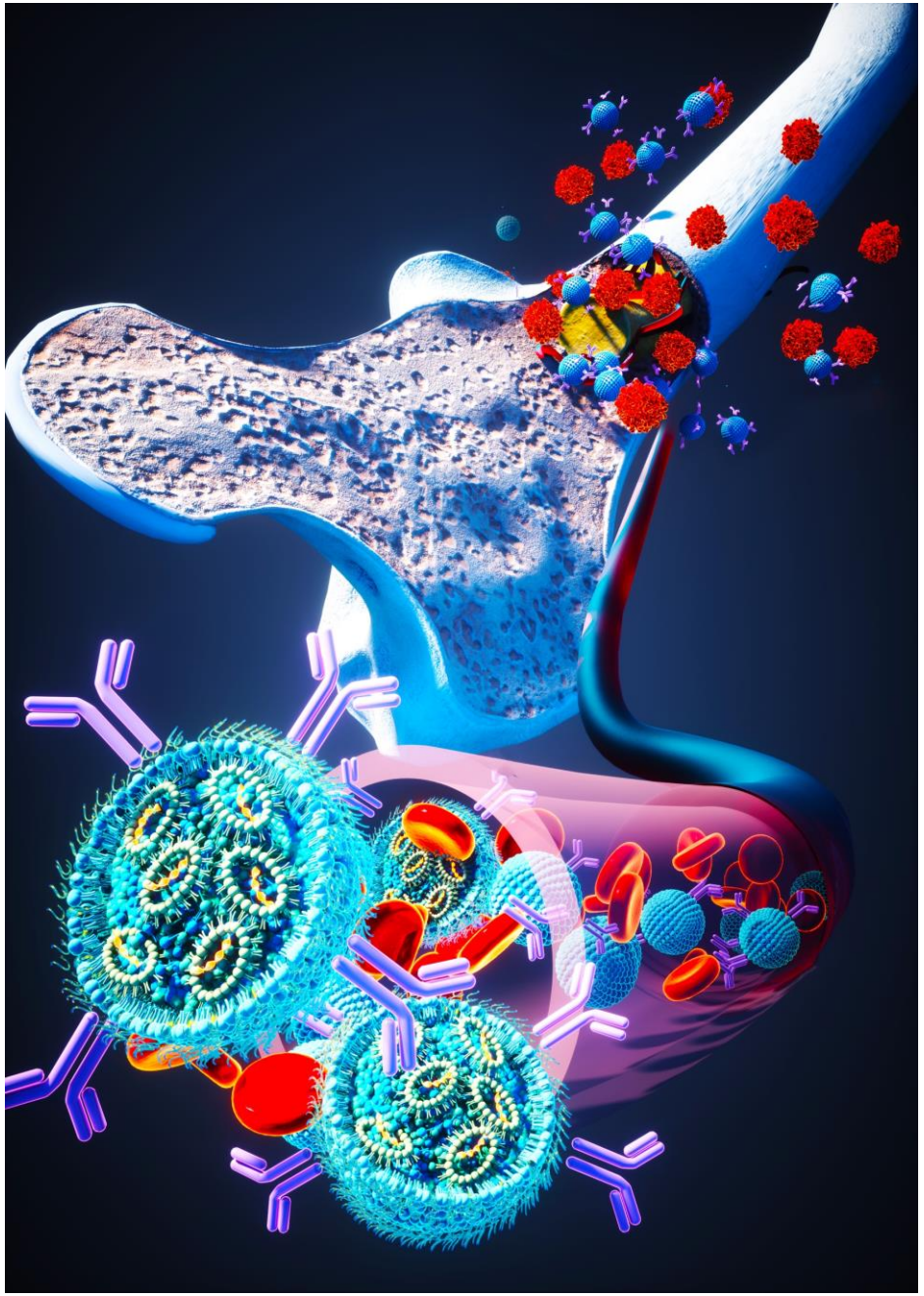


Yong S-B et al., Advanced Materials, 2022

Cancer

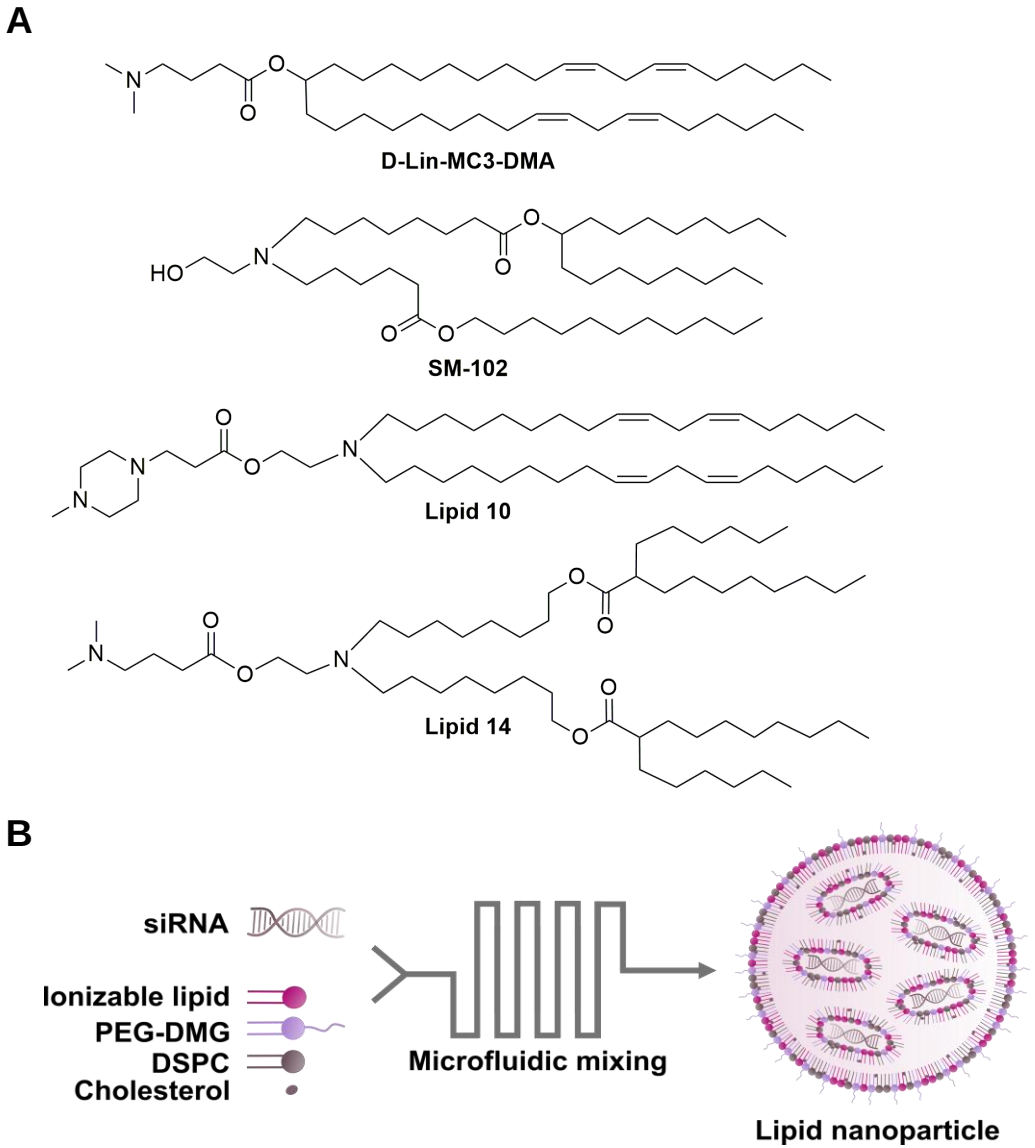
- Therapeutic approach: Silencing
- Target gene: HO1
- Target Receptor: PDL-1

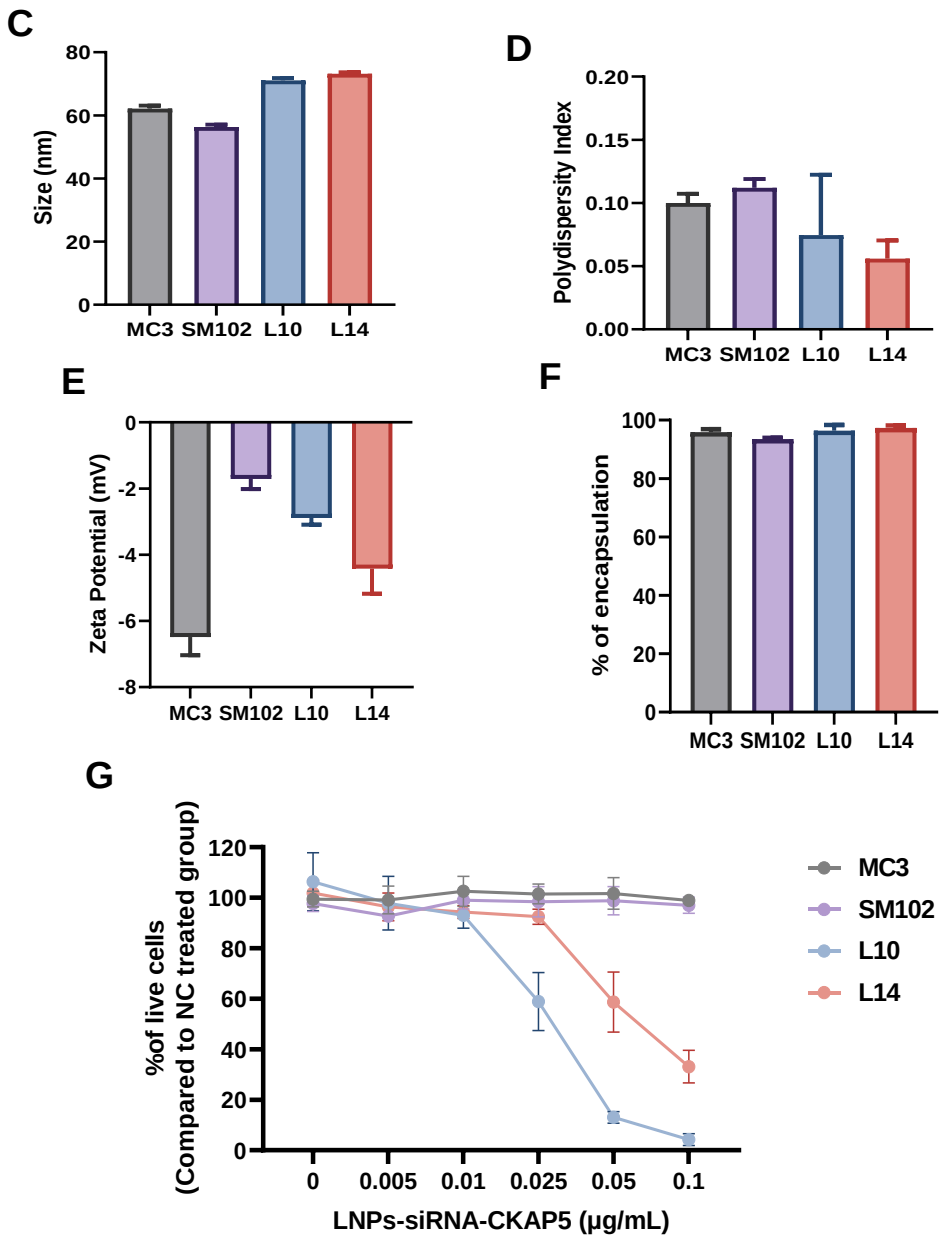




Delivery of Therapeutic RNA to the Bone Marrow in Multiple Myeloma Using CD38-Targeted Lipid Nanoparticles

Screen of ionizable cationic lipids for the transfection of human MM cells





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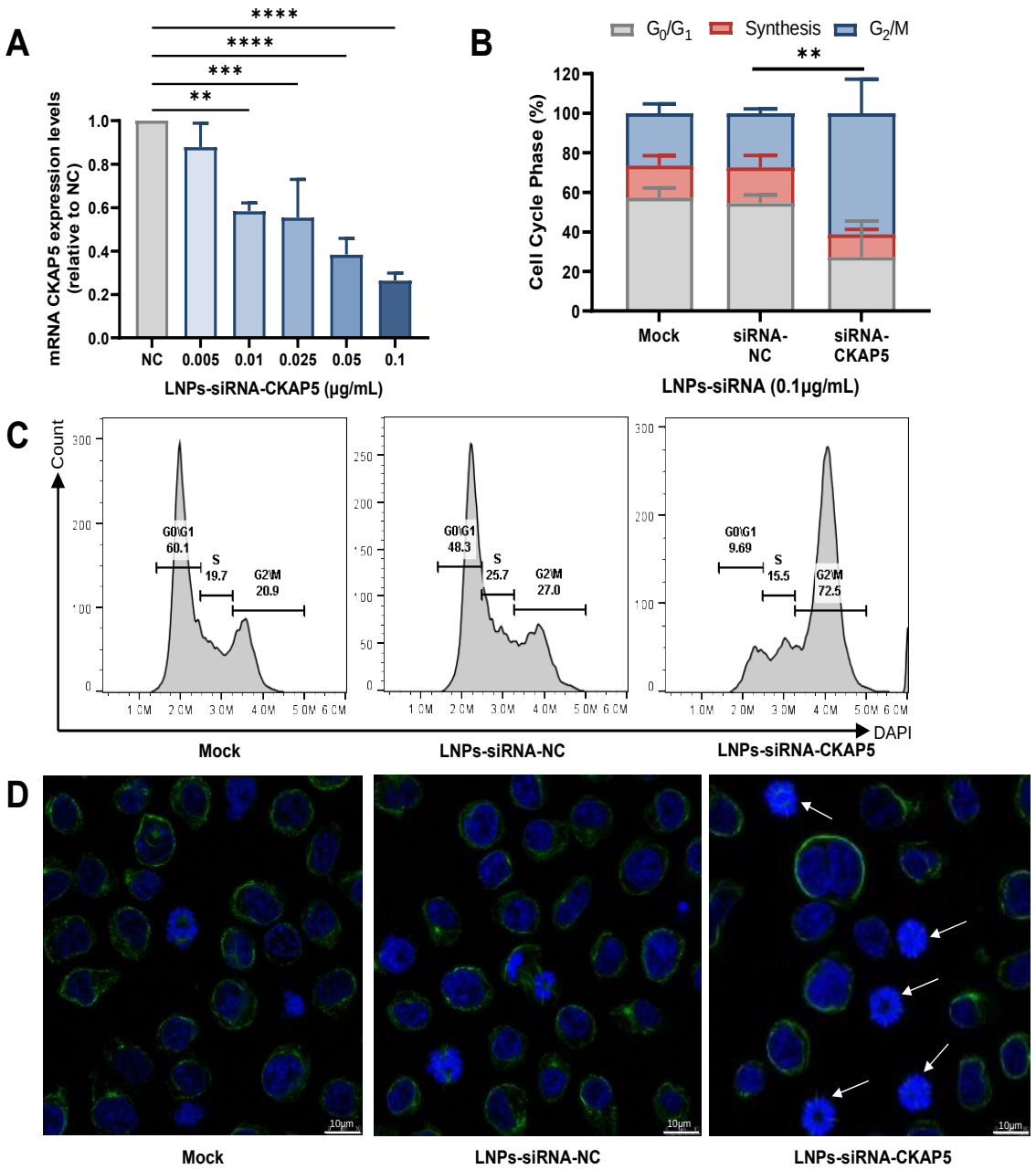
Therapeutic effects of LNPs-siRNA-CKAP5 on CAG cells in vitro



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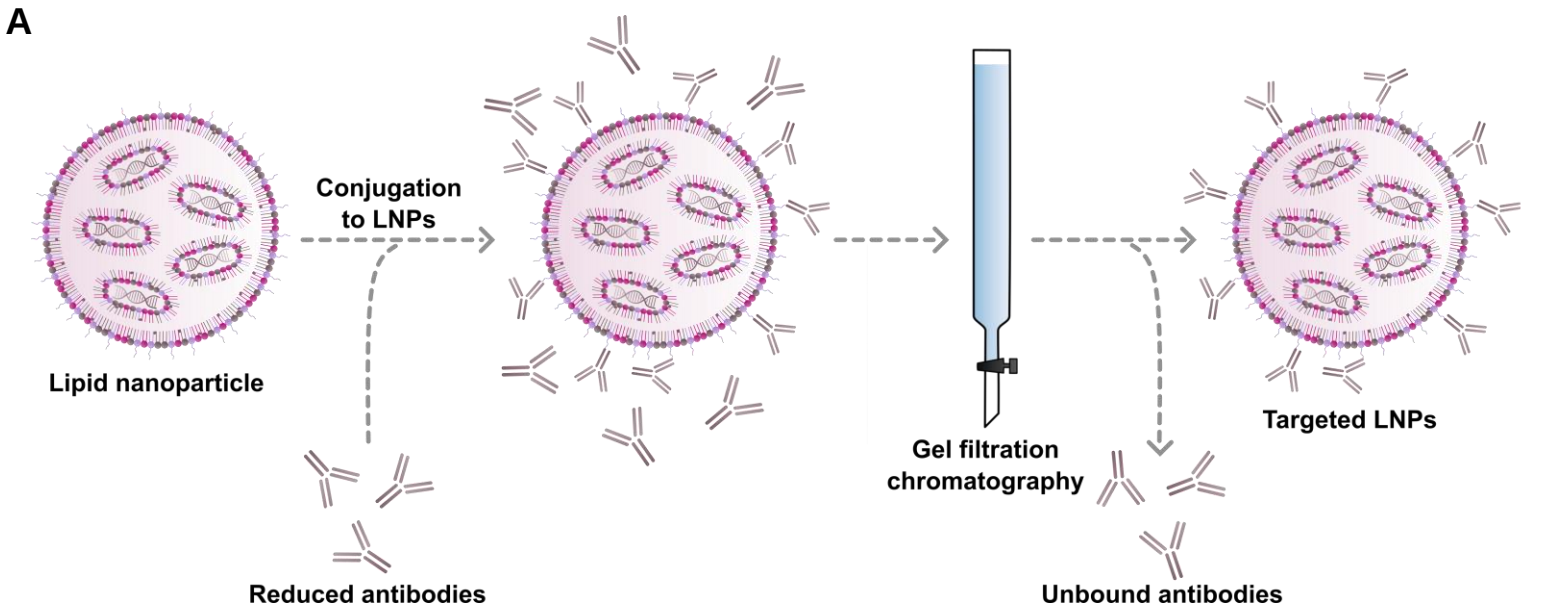
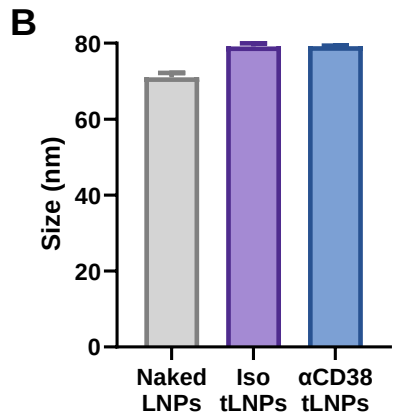
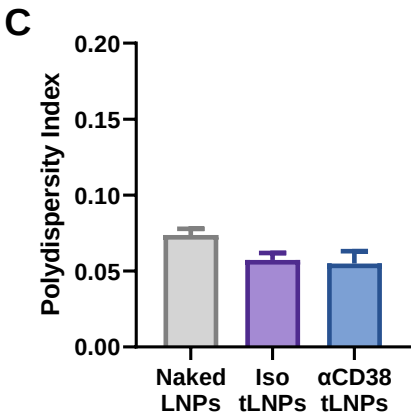
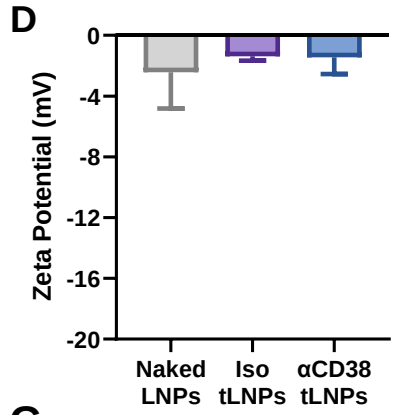
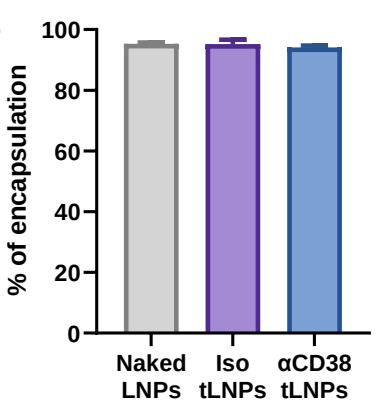
Characterization and evaluation of anti-CD38 targeted LNPs

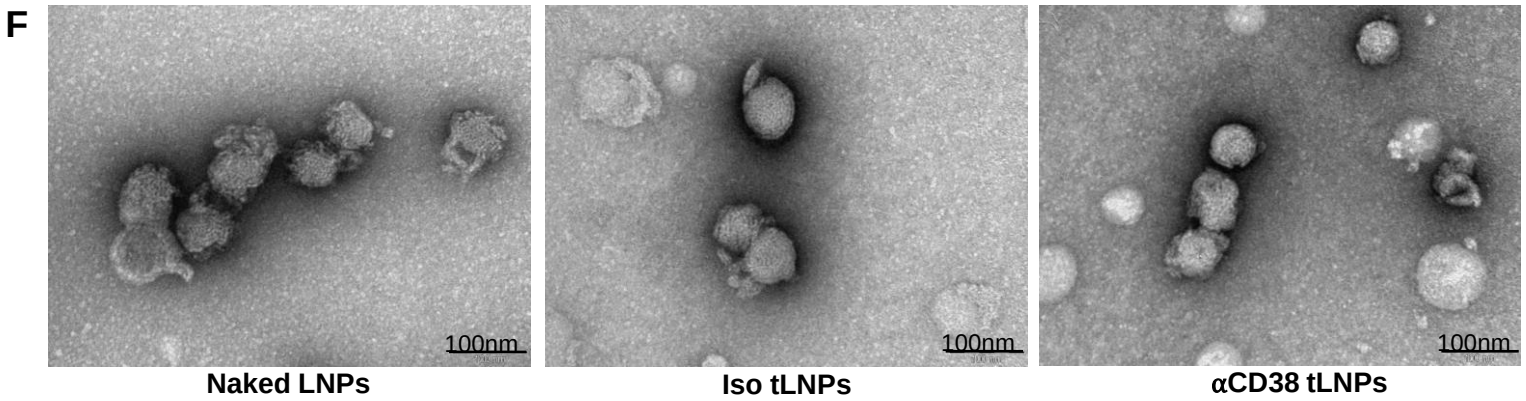


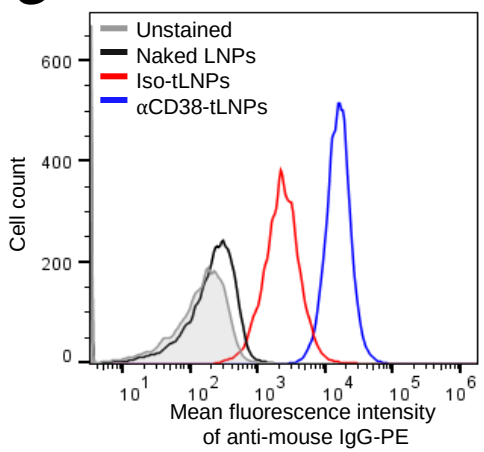












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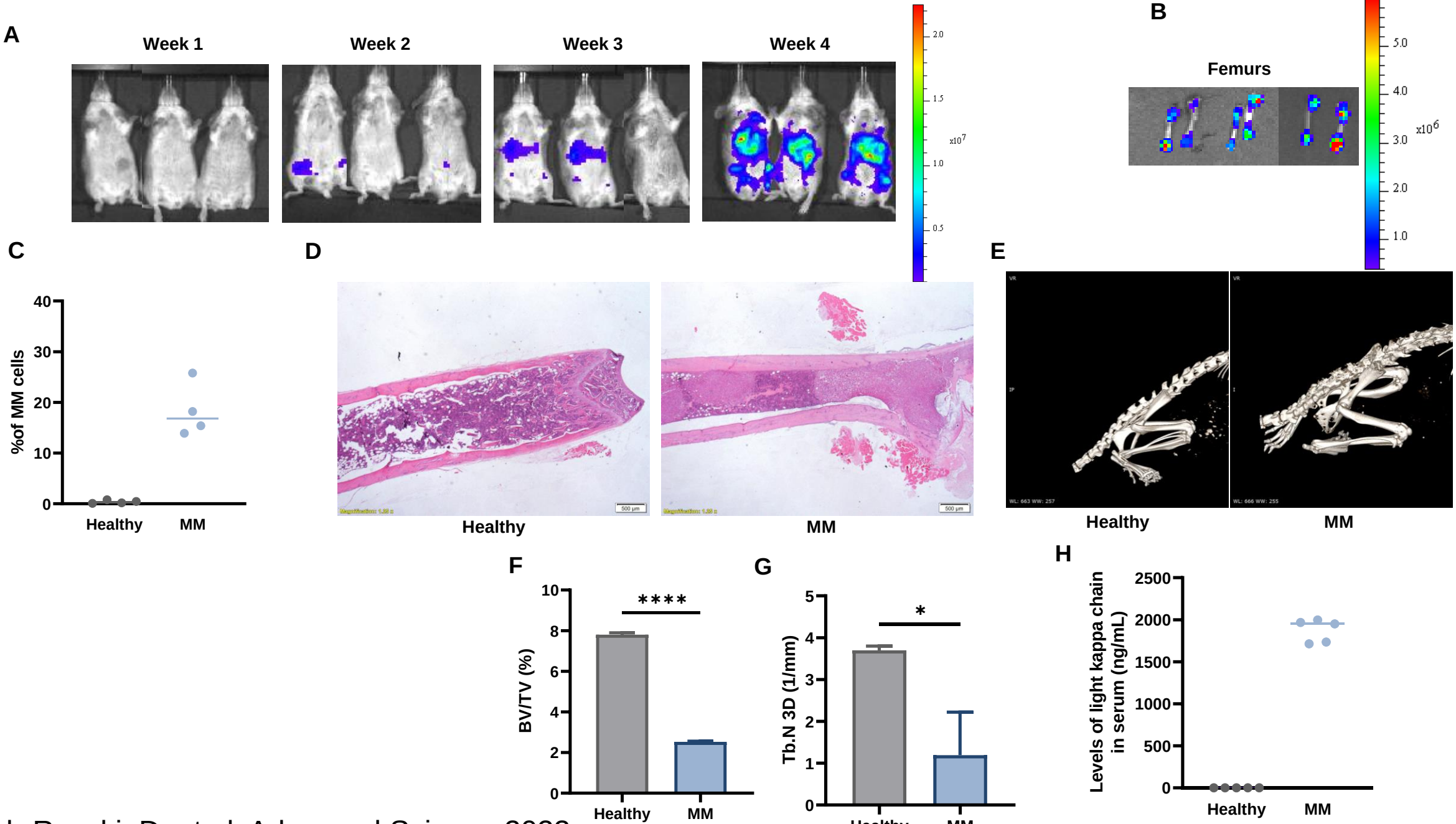
Establishment of novel xenograft MM mouse model



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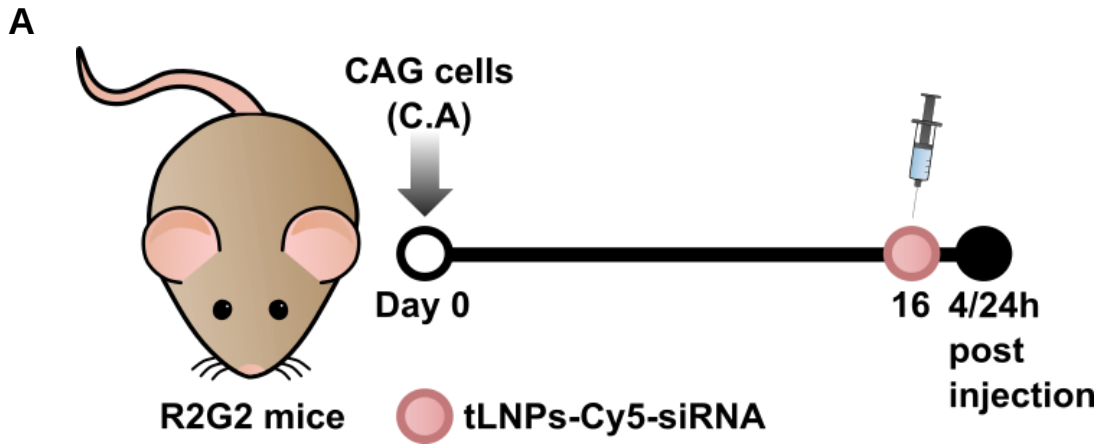
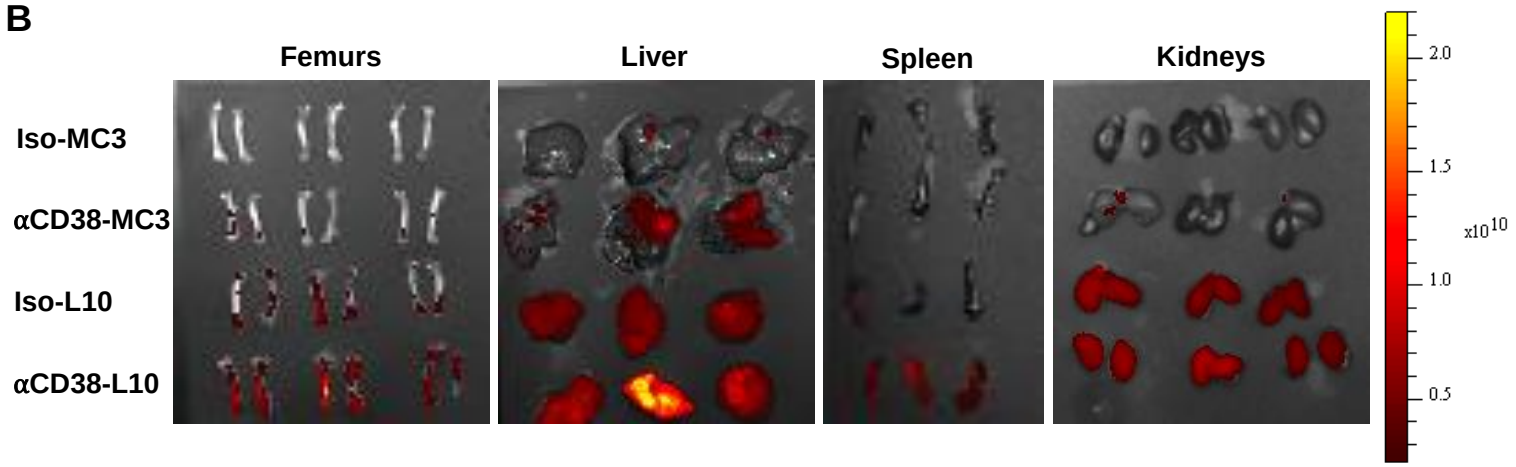
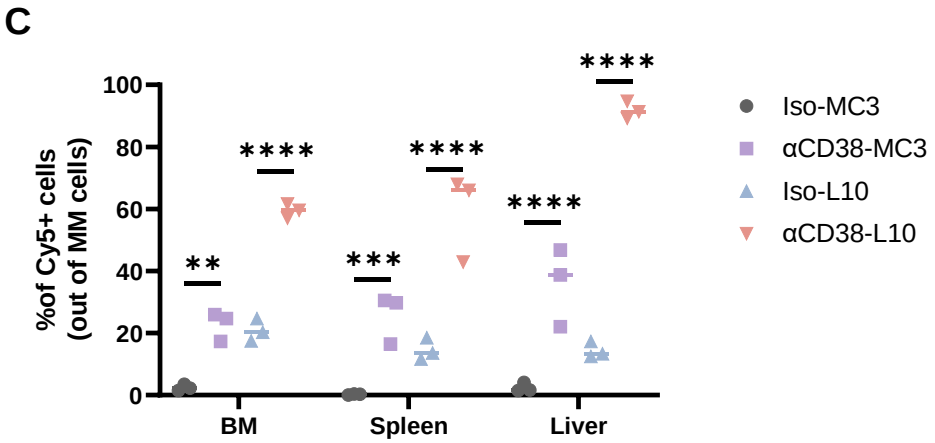
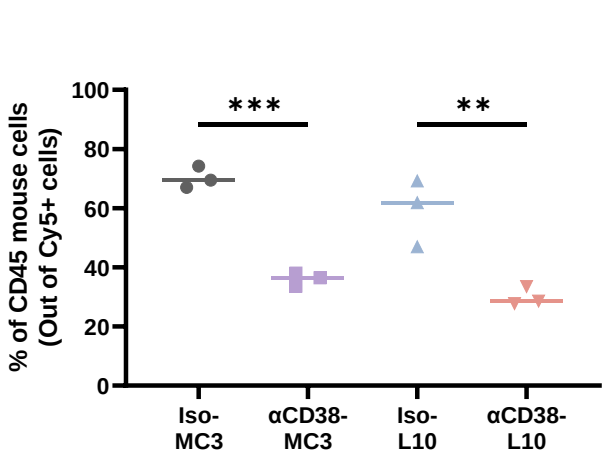
In vivo biodistribution of targeted LNPs in MM-bearing mice









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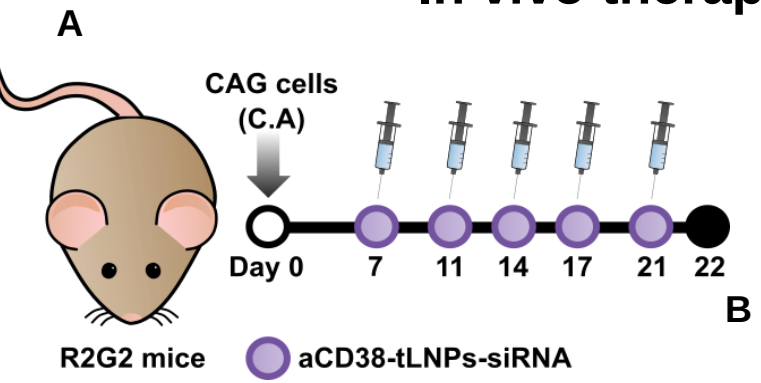
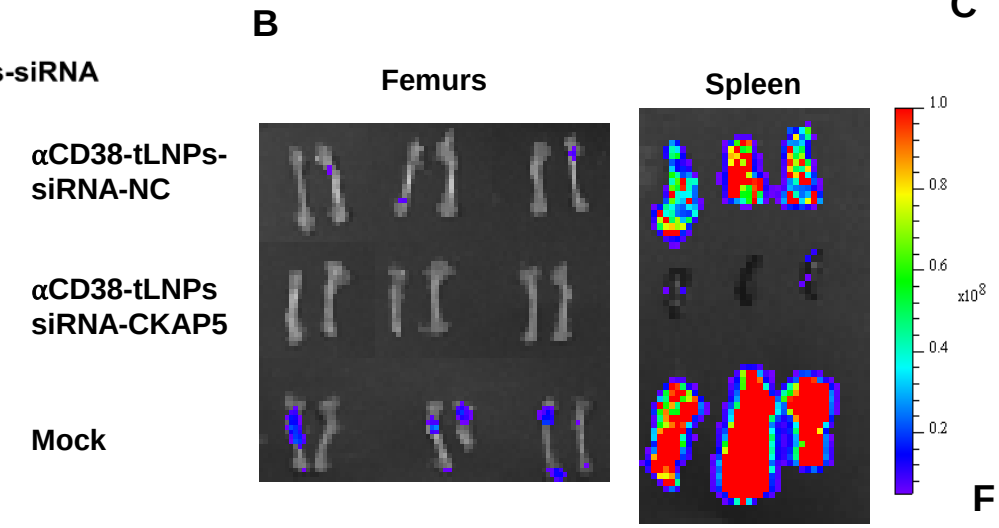
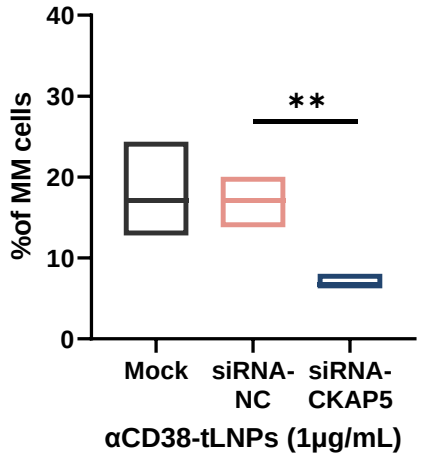
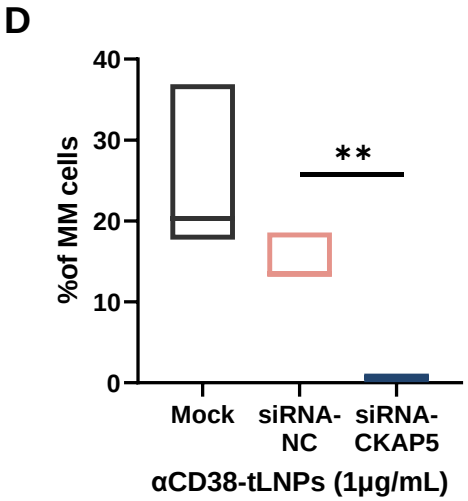
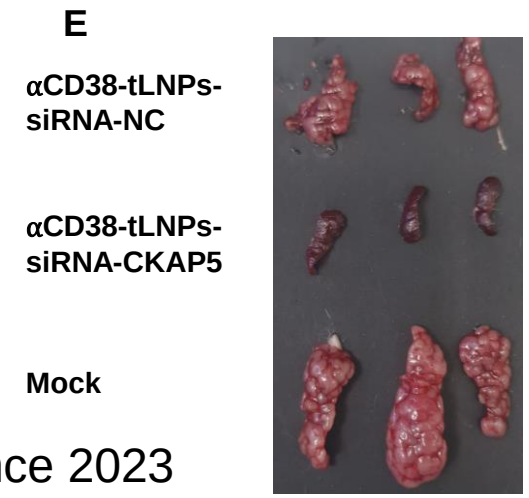
In vivo therapeutic effect of αCD38-tLNPs-siRNA-CKAP5 in MM-bearing mice

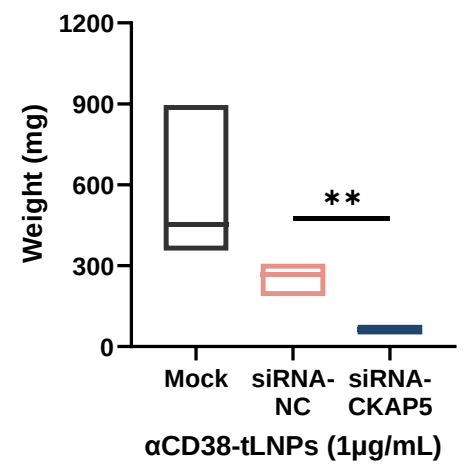


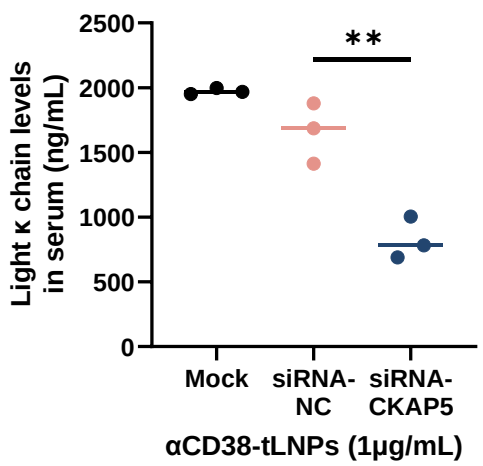












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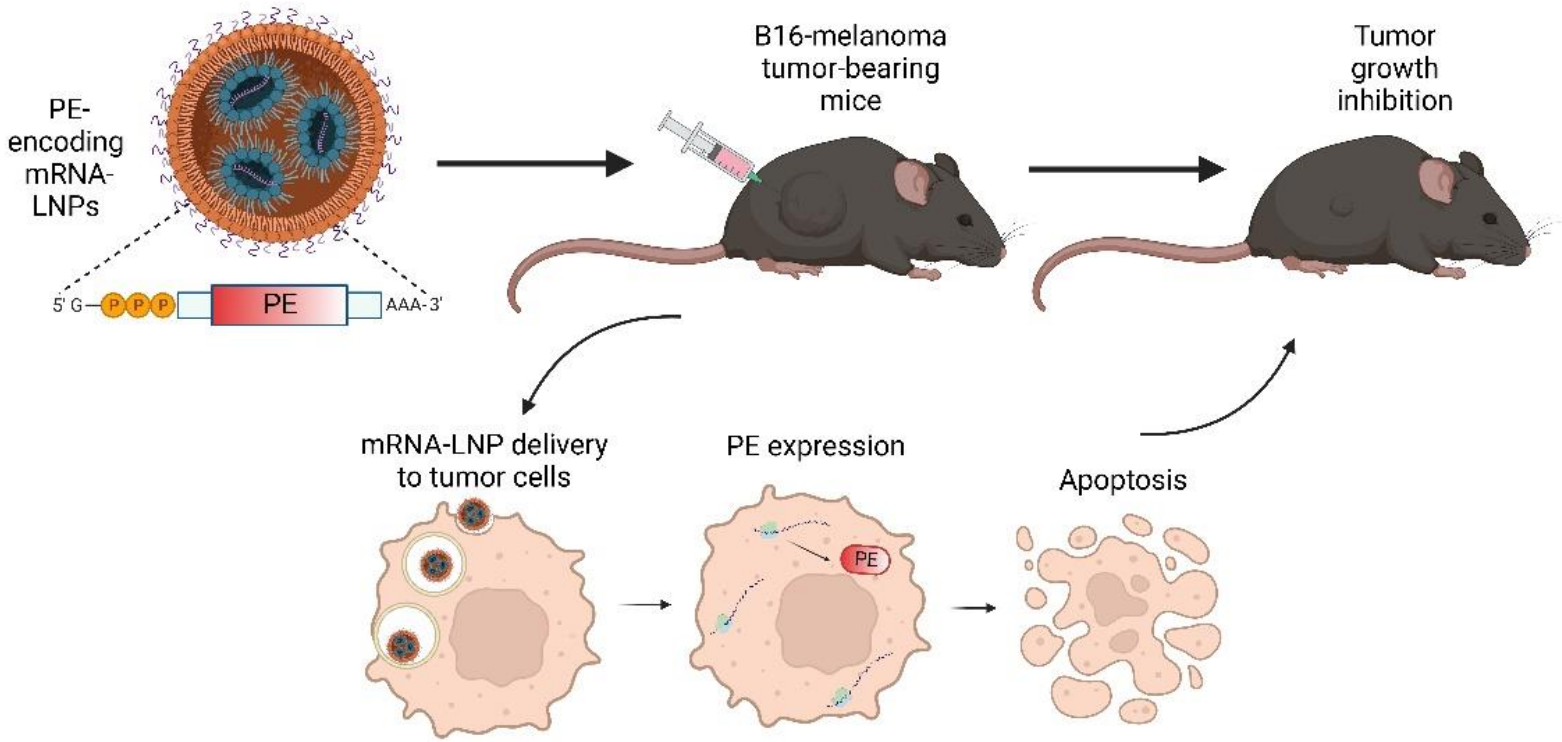
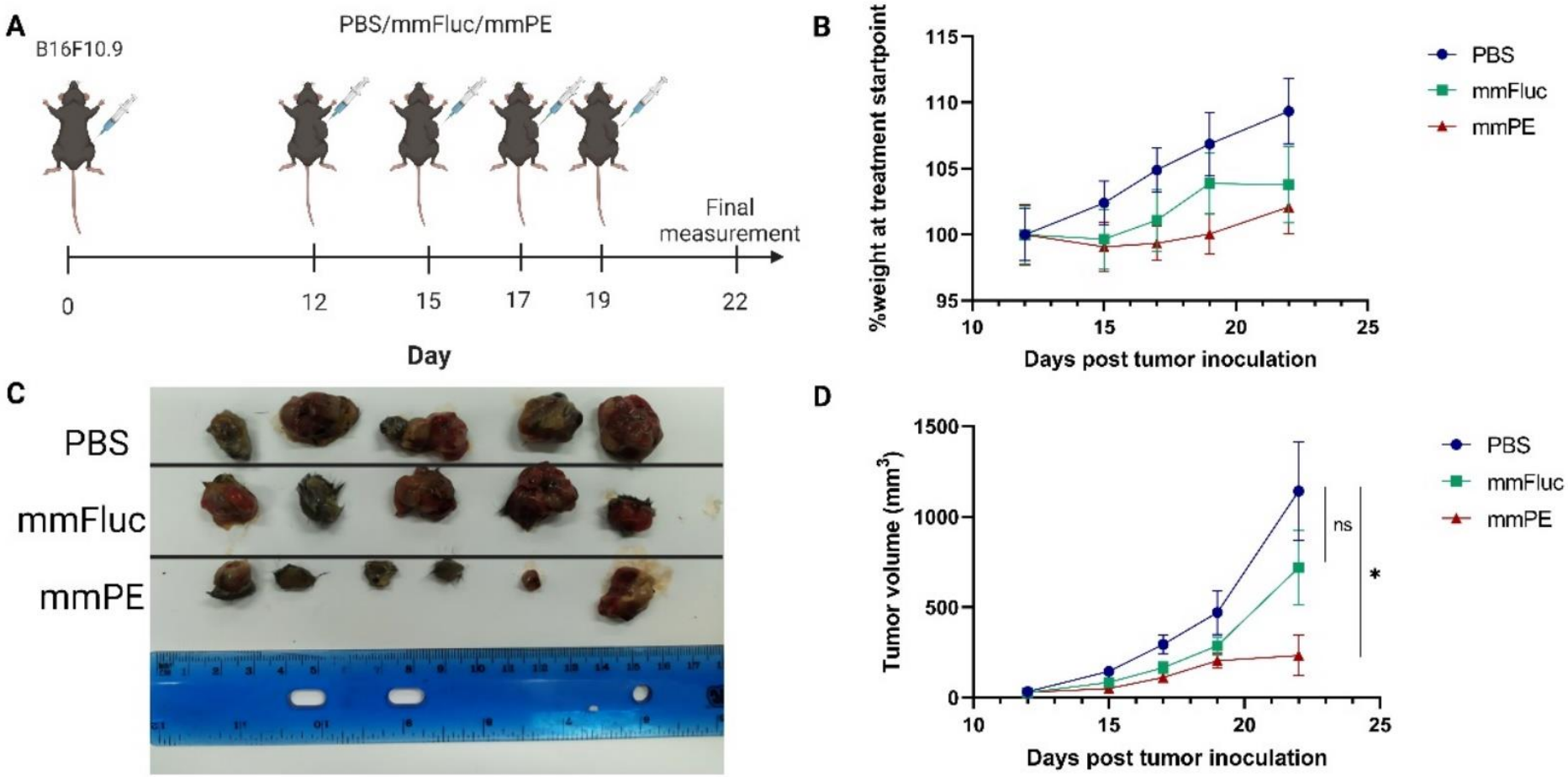
Using bacterial toxins as novel local therapy for solid tumors

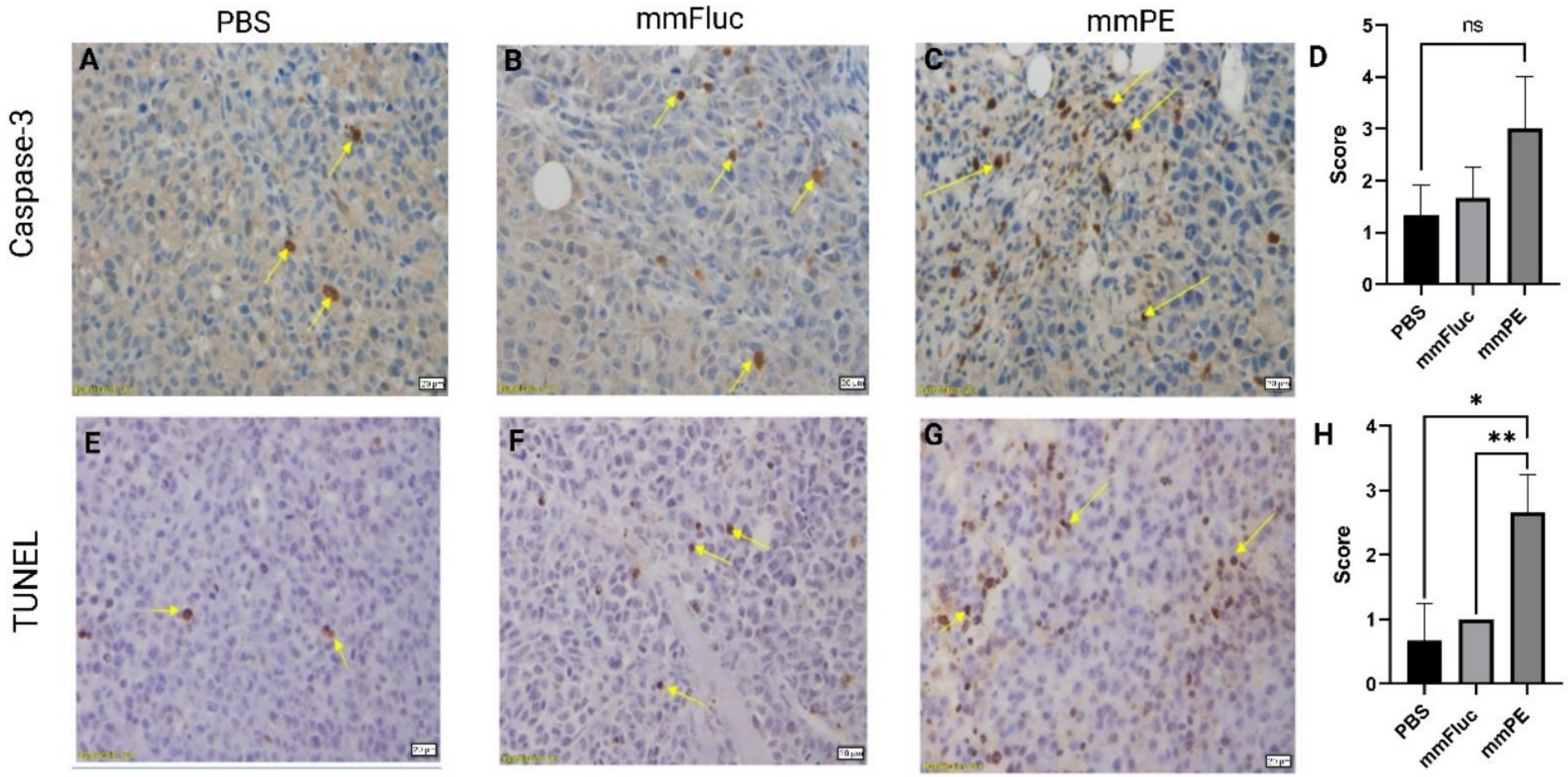


Granot-Matok Y. et al. Theranostics 2023



Granot-Matok Y. et al. Theranostics 2023

Intratumorally-injected, repeated doses of mmPE-LNPs caused *in-vivo* apoptosis in tumor cells



Granot-Matok Y. et al. Theranostics 2023

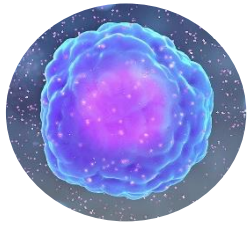
Inflammatory Bowel Disease (IBD)

- Chronic inflammation of the gastrointestinal (GI) tract.
- Types of IBD include:
  - Ulcerative colitis.
  - Crohn's disease.
- Current treatment:
  - Anti-inflammatory drugs
  - Immune system suppressors
  - Surgery

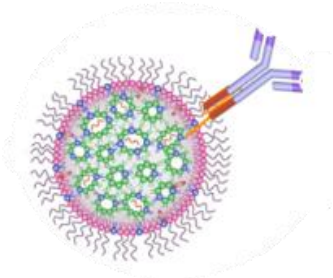
Therapeutic approach for IBD treatment

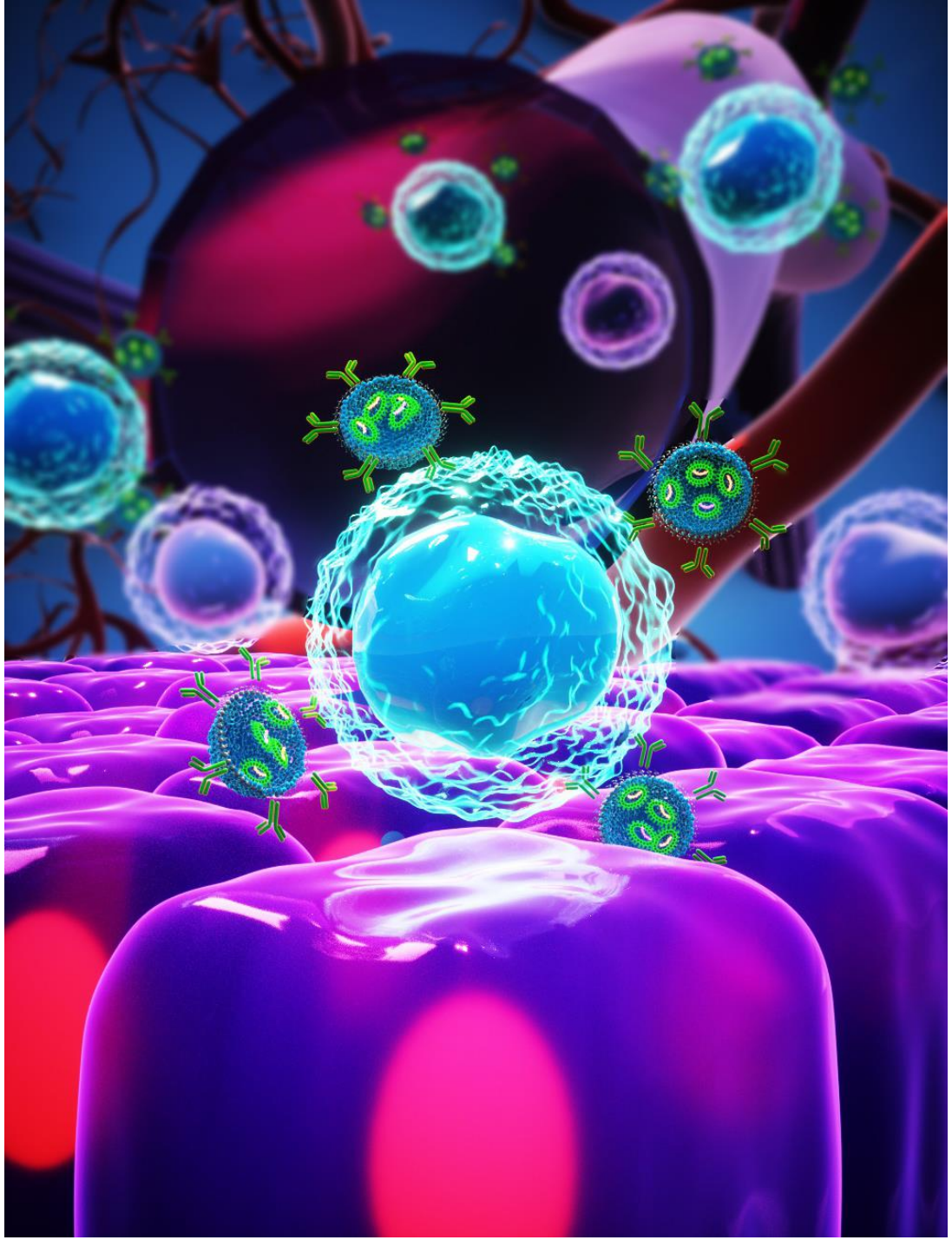
- Therapeutic approach: Silencing

- Target gene: IFN γ



- Target Receptor: Integrin $\alpha_4\beta_7$





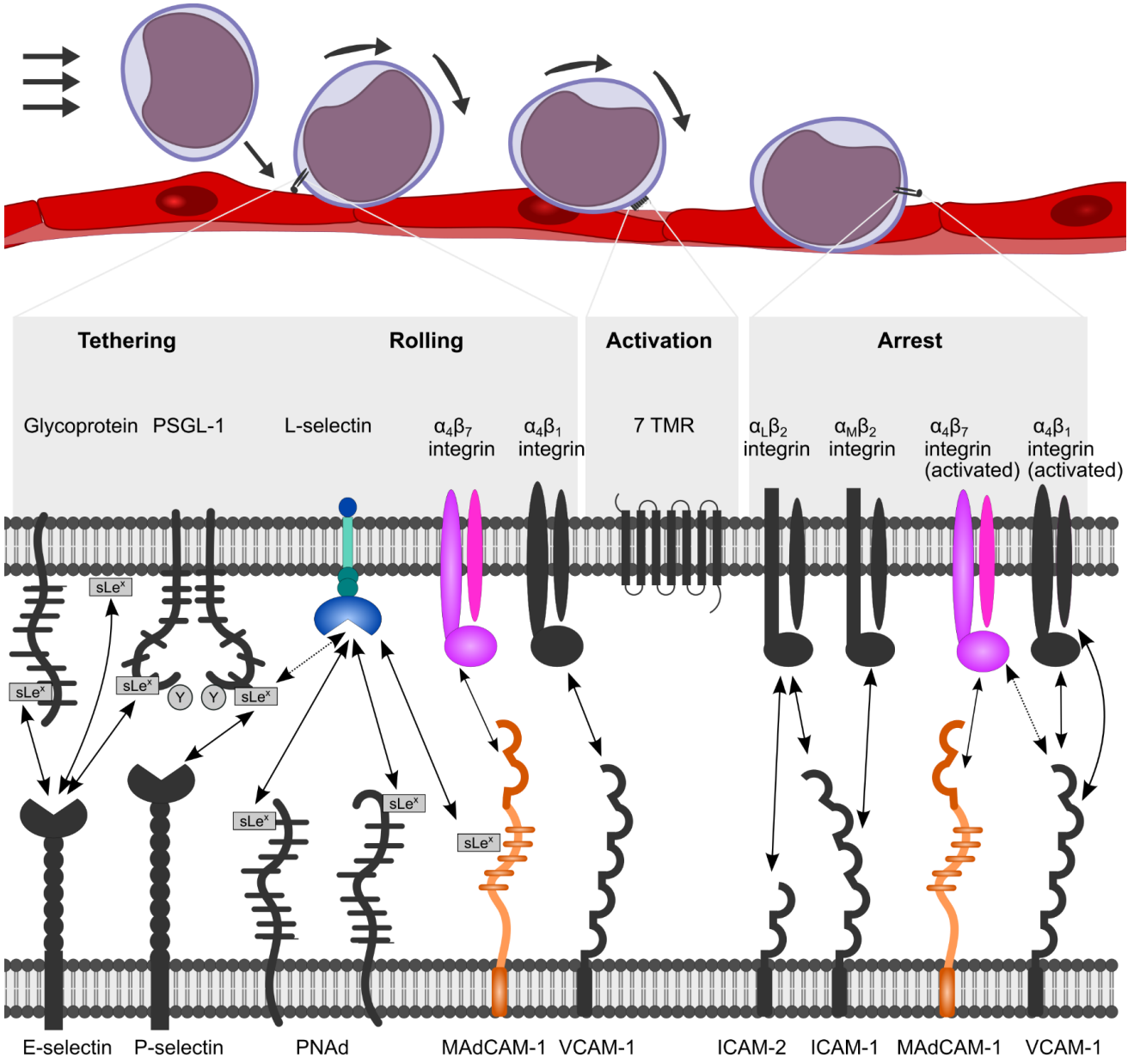
The next level of Targeting – selective targeting against receptor conformation

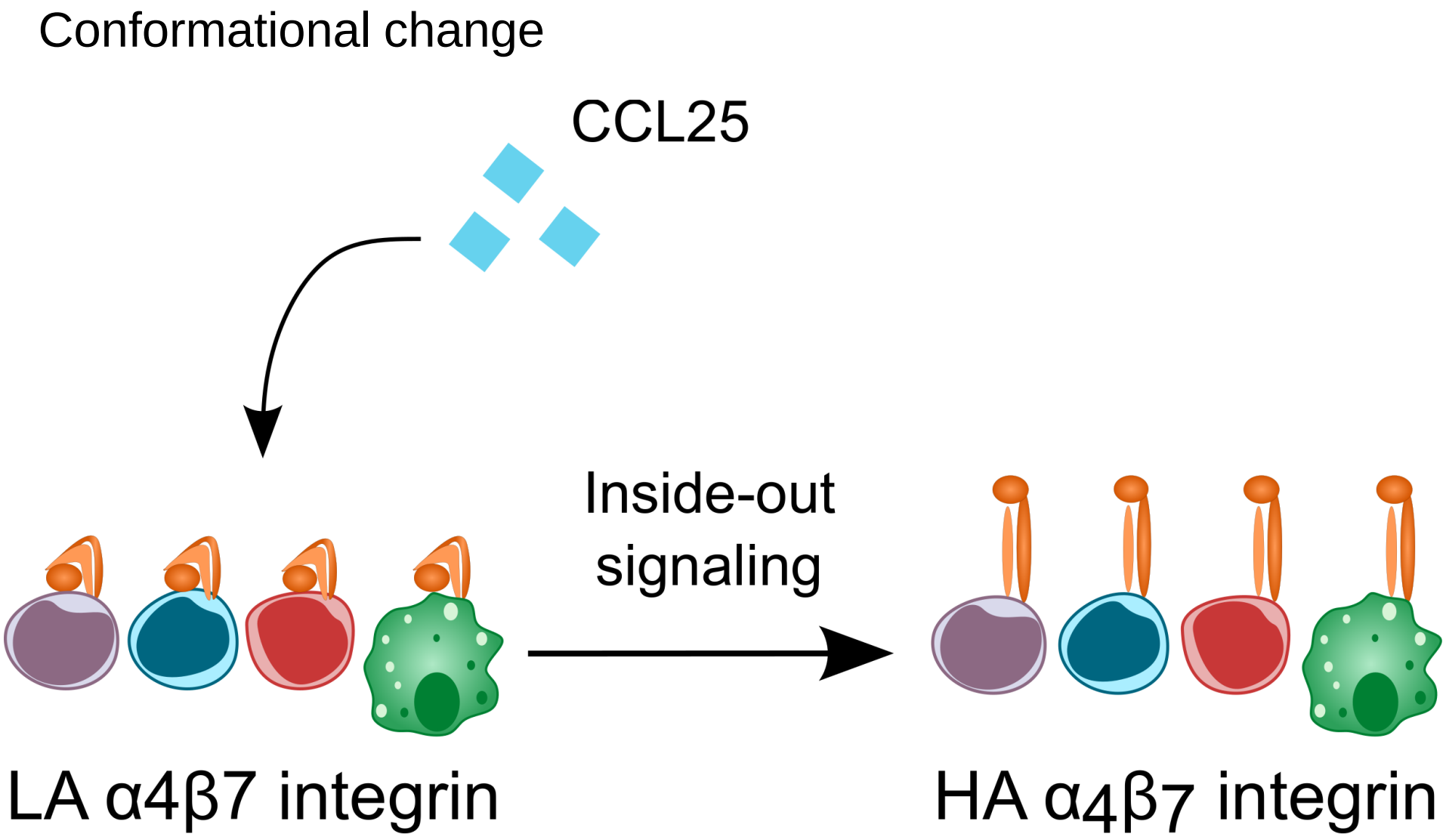
Dr. Niels Dammes

Inspired by leukocyte homing process

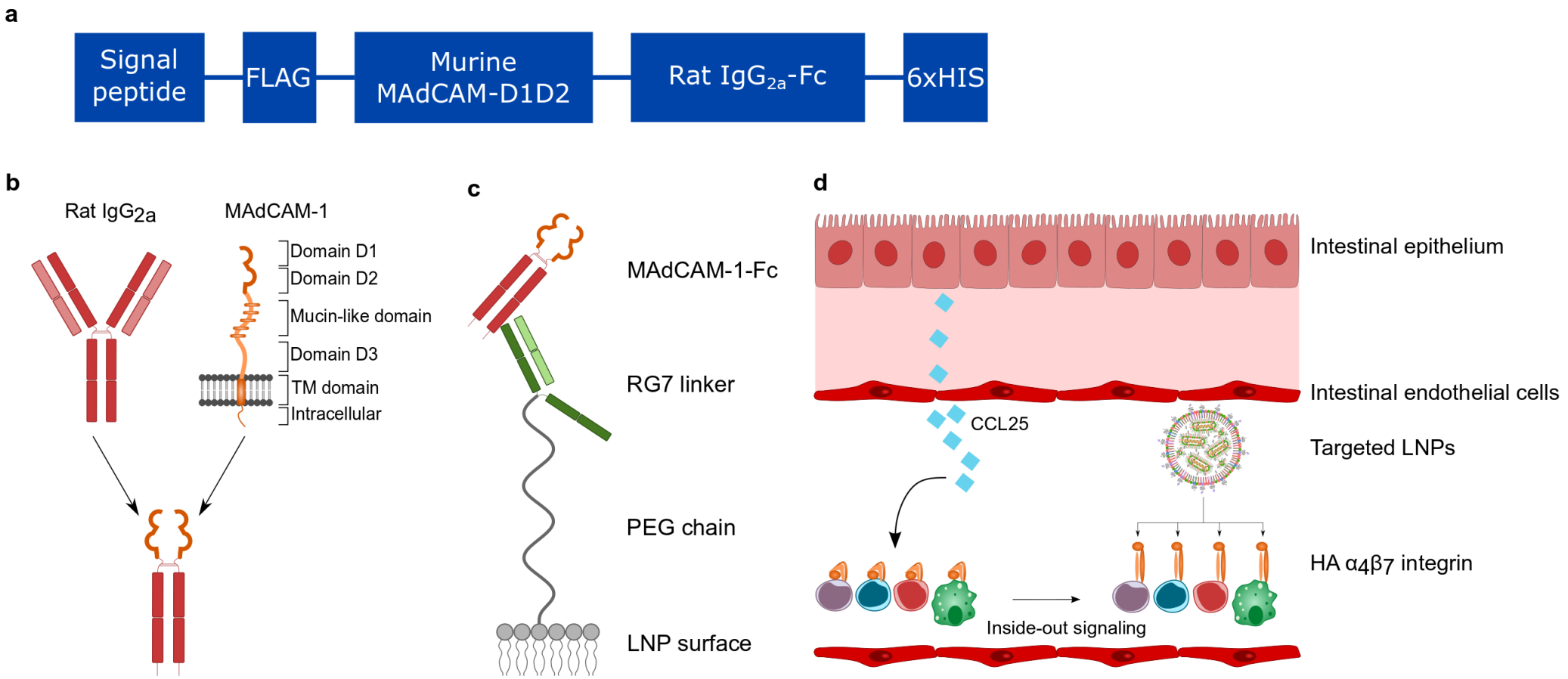
Modified from:
UH von Andrian & CR Mackay
New Engl. J. Med.
2000

Proteins related to MAdCAM are colored




Conformational change
CCL25
Inside-out
signaling
LA α4β7 integrin
HA α4β7 integrin

Targeting concept



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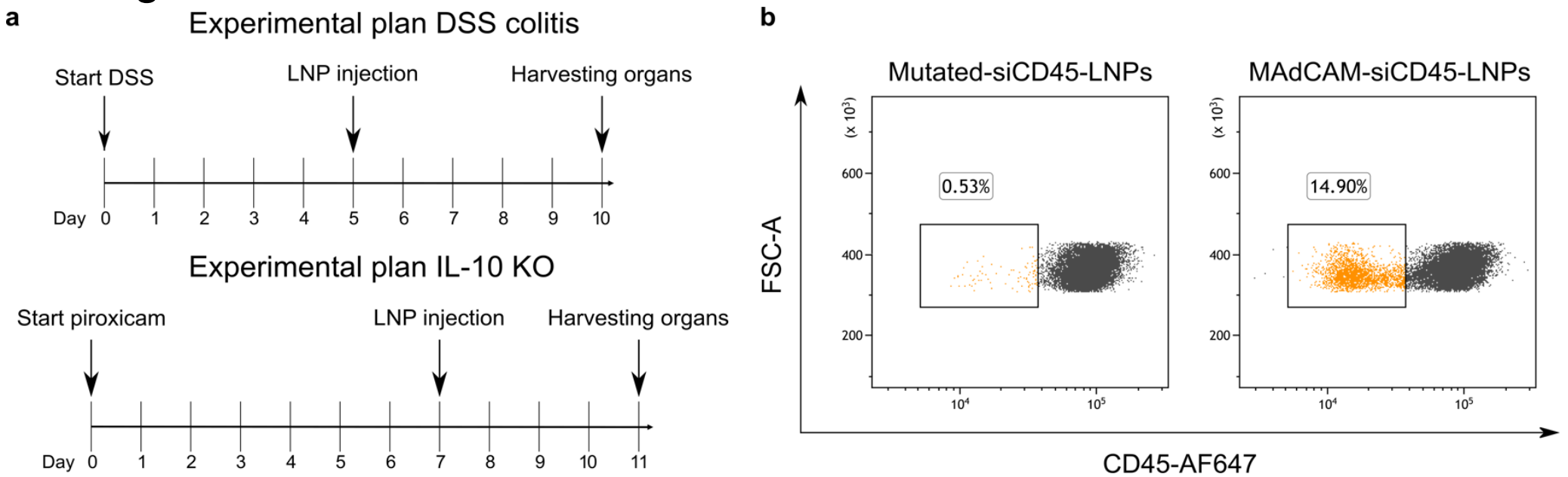
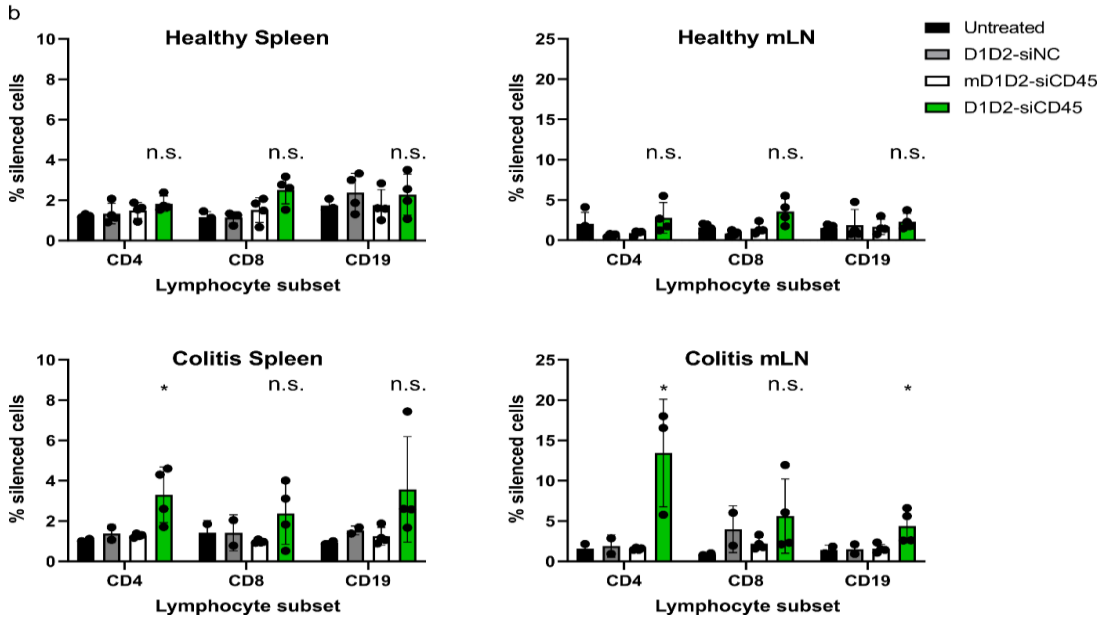
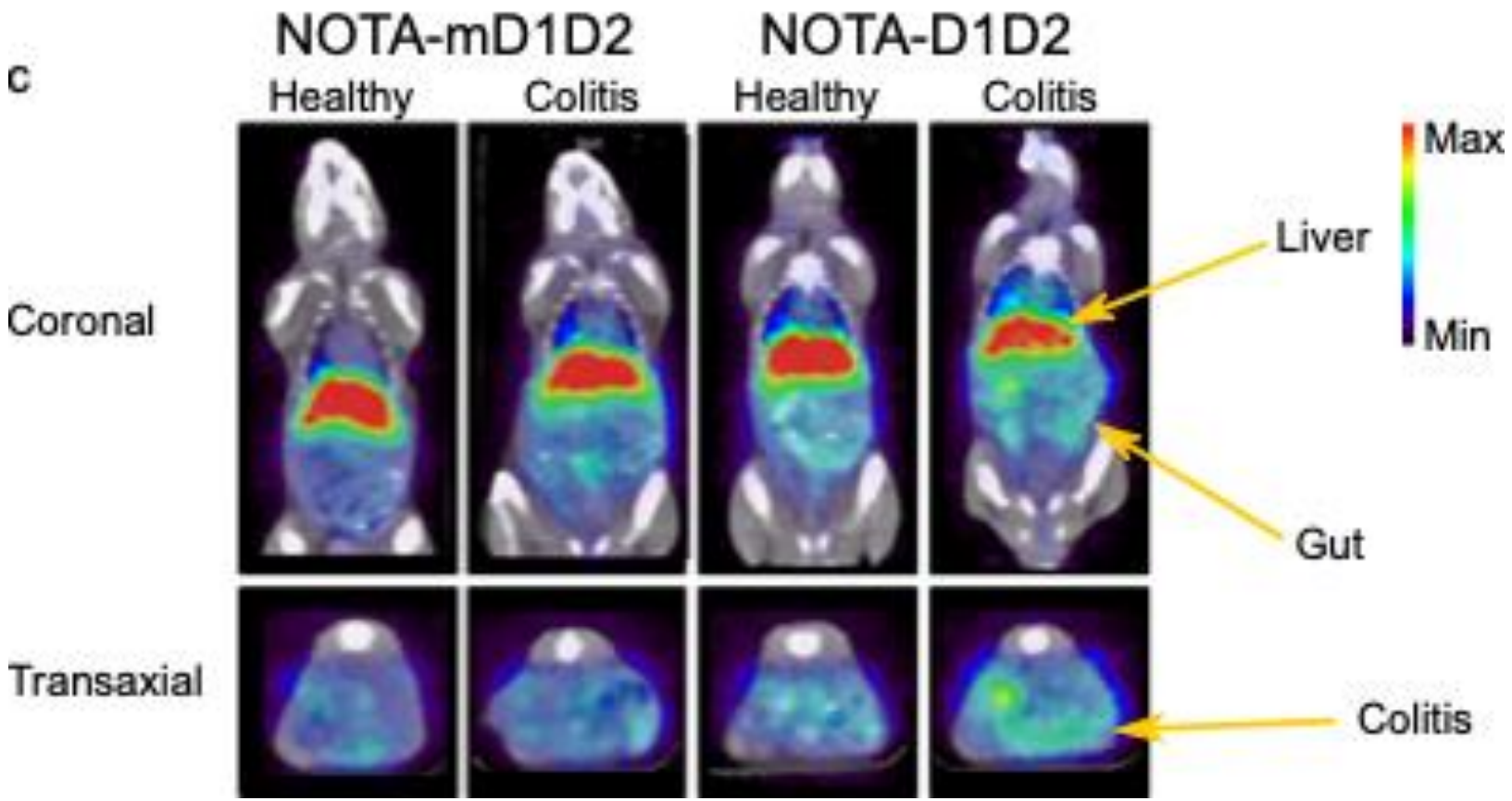
CD45 silencing





Dammes N. et al. Nature Nanotechnology 2021

Molecular imaging of inflammatory leukocytes in experimental colitis using PET/CT and D1D2-NOTA-^{64}Cu



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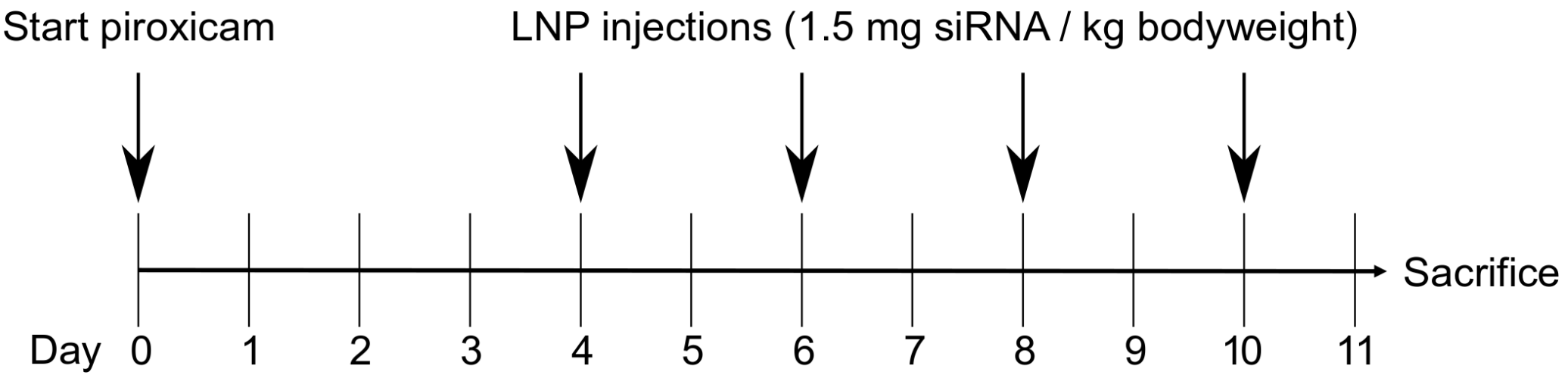
Injection scheme efficacy experiment IL-10KO



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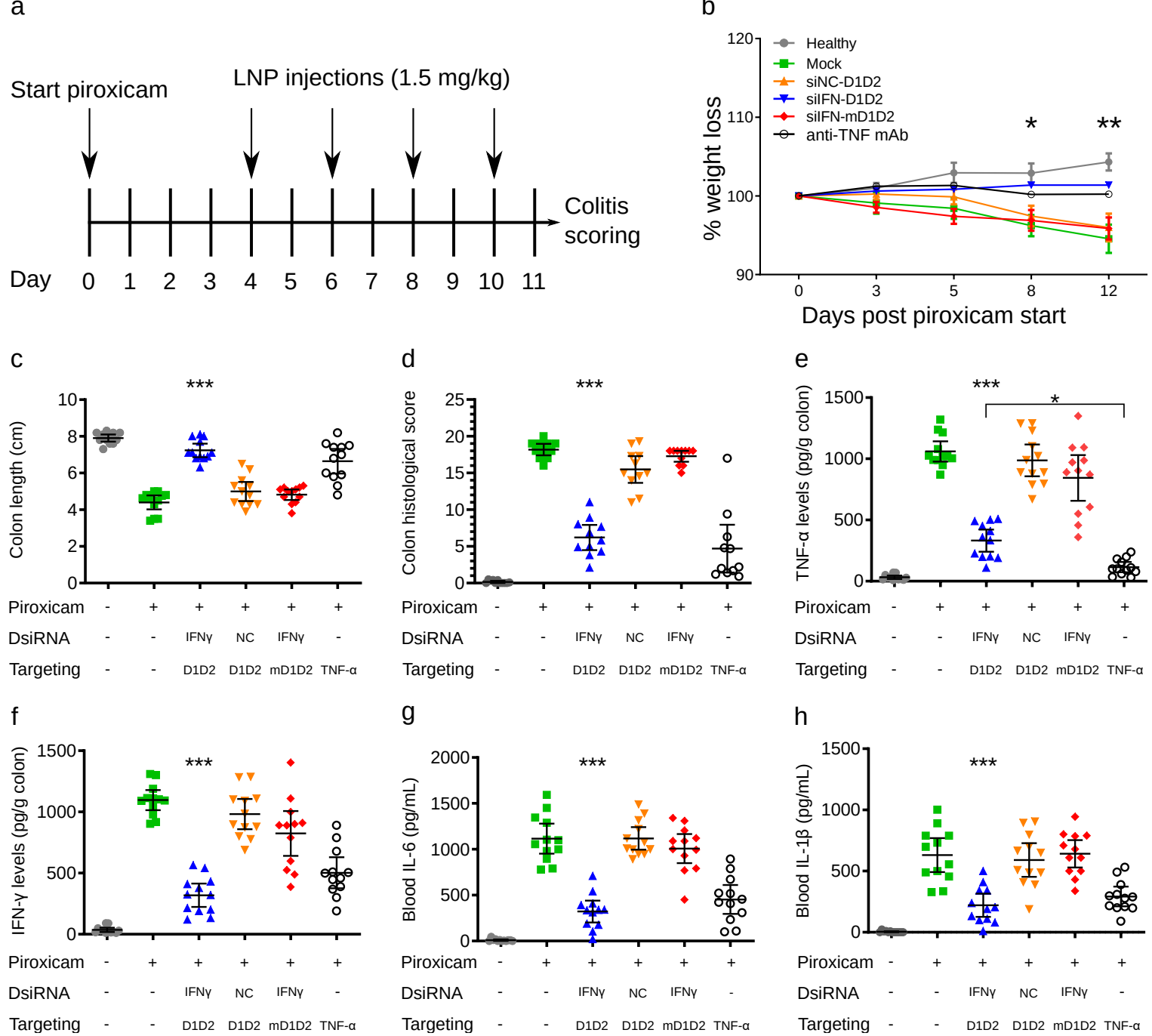

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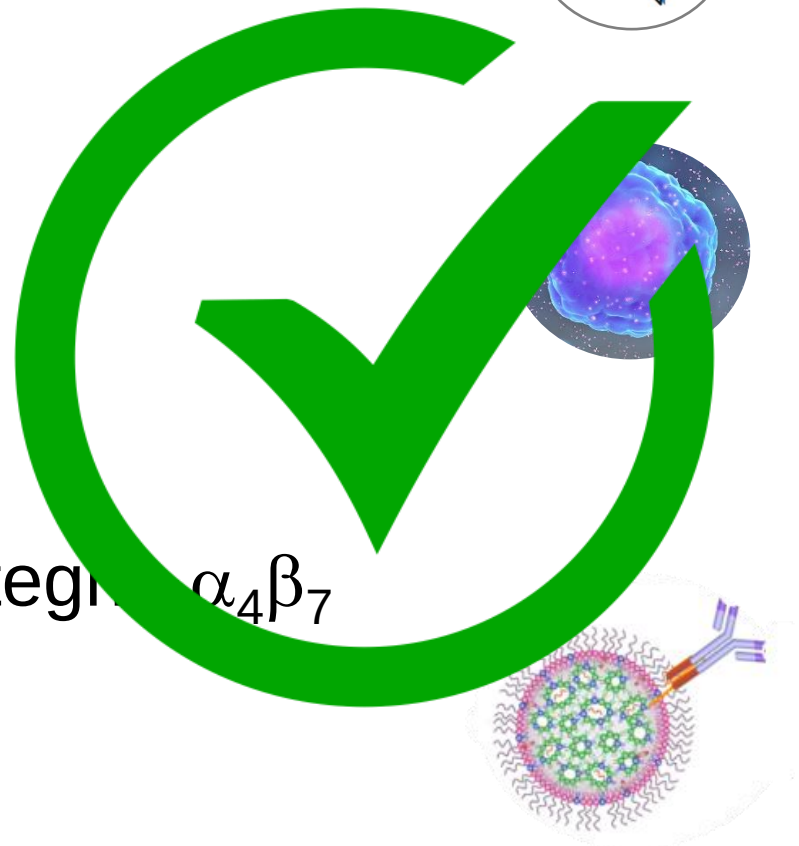
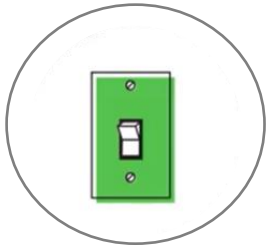
Therapeutic approach for IBD treatment

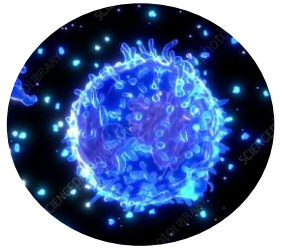
- Therapeutic approach: Silencing
- Target gene: IFN γ
- Target Receptor: Integrin $\alpha_4\beta_7$

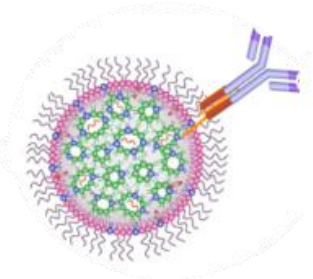


Alternative therapeutic approach for IBD treatment

- Therapeutic approach: Activate
- Target gene: IL-10
- Target cells: Ly6C^{Hi}



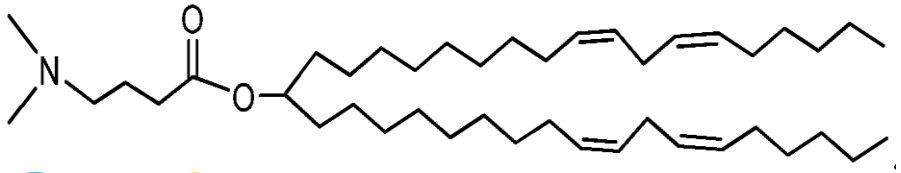


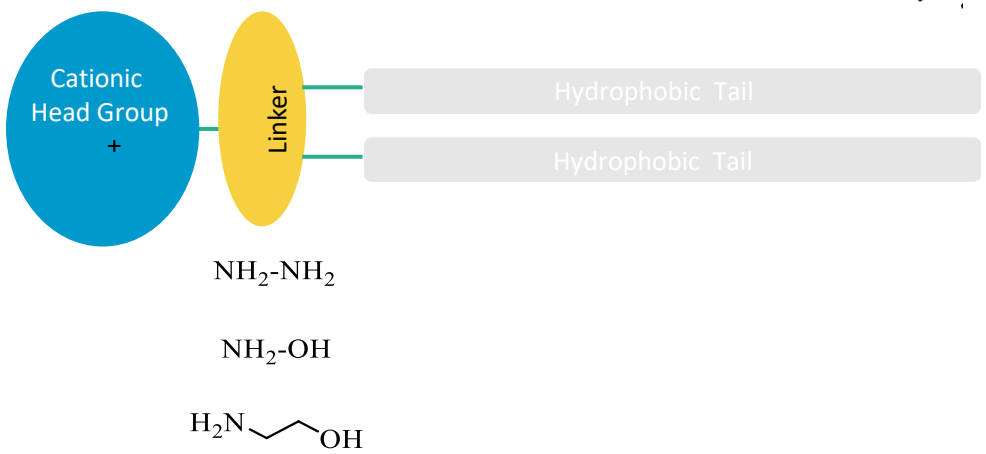


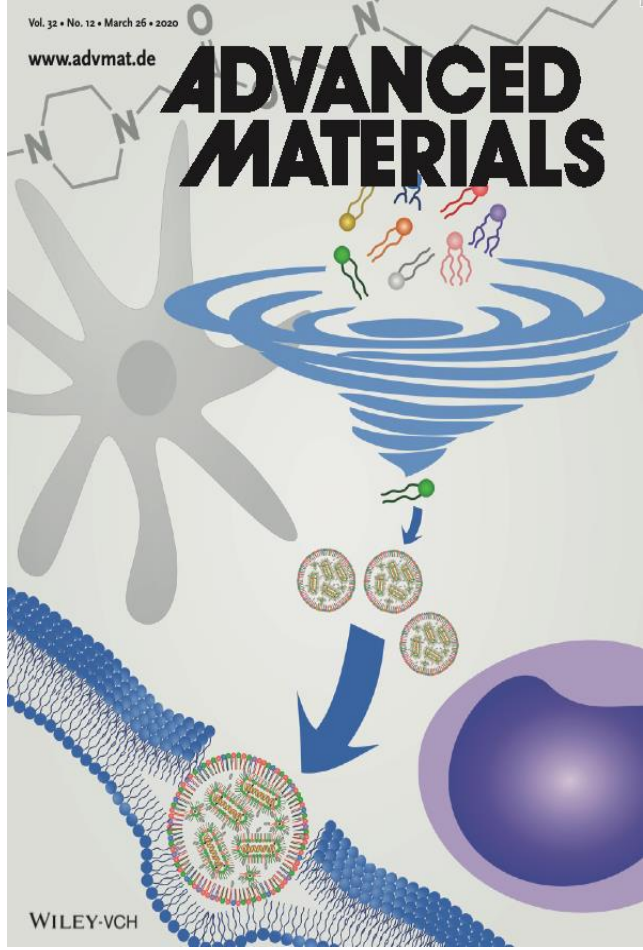
Structural Design of Ionizable Lipids

Dr. Srinivas Ramishetti

Dlin-MC3-DMA







Based on the gold standard we have synthesized 60 lipid families

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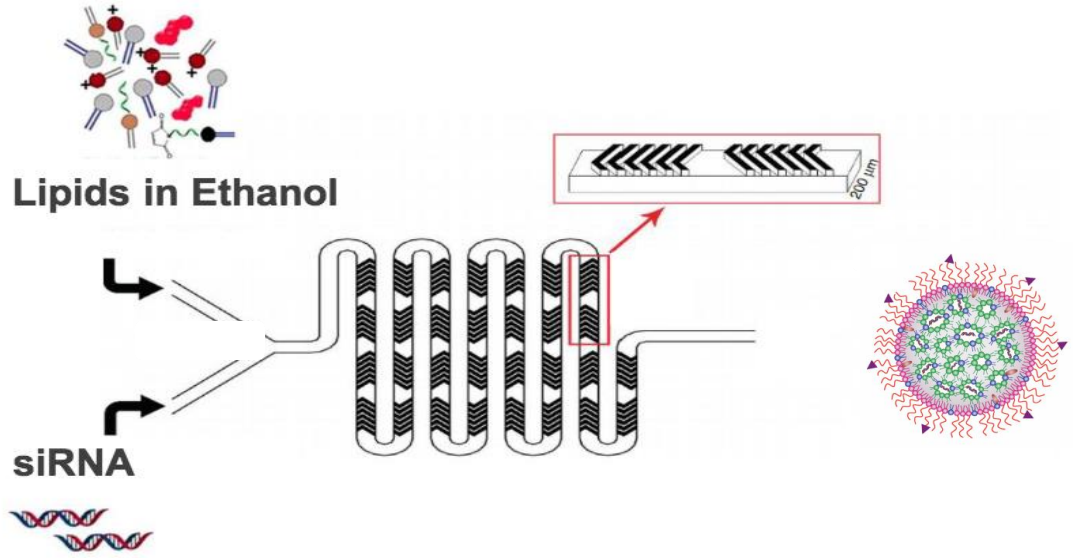
Using Microfluidic Mixing for Generating LNPs



Acetate buffer at pH-4.5

The starting point for formulation studies

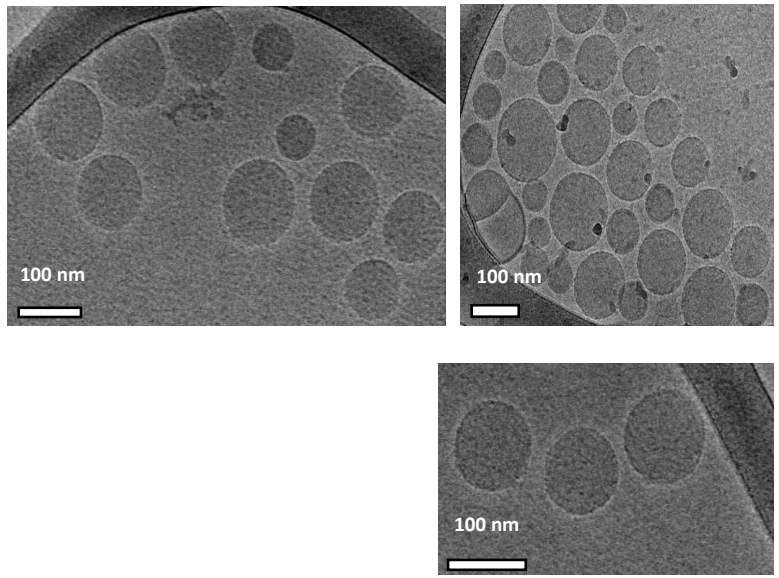
Lipids	mol (%)
Ionizable lipid	50%
DSPC	10%
Cholesterol	38.5%
DMG-PEG	1.5%

DSPC- 1,2-Distearoyl-Sn-glycero-3-PhosphoCholine
DMG-PEG- 1,2-Dimyristoyl-sn-glycerol, Methoxypolyethylene Glycol

LNPs Characterization

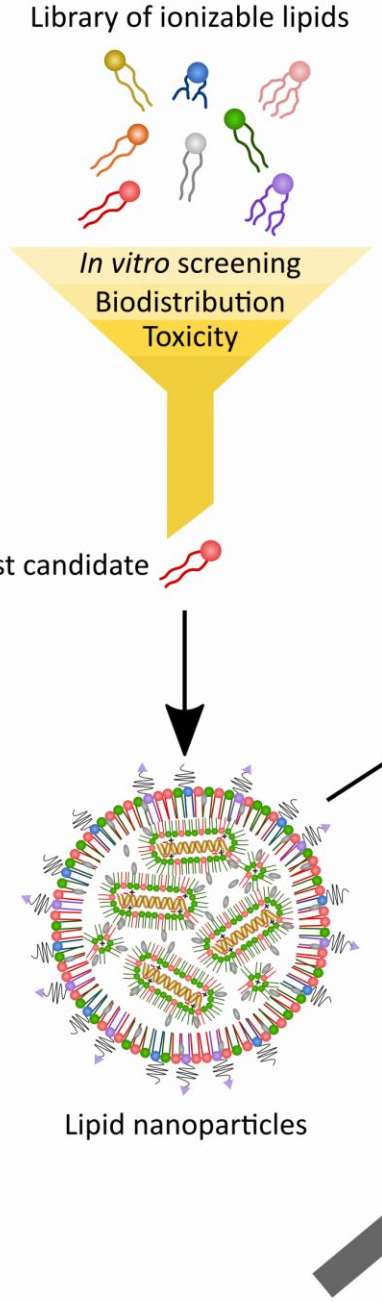
Formulation	Size (d.nm)	PDI	Zeta-potential (mV)
Dlin-MC3-DMA	44.5	0.18	-1
HZ-35	45.1	0.14	18.5
HZ-25	56.3	0.06	13.9
HZM-45	50.5	0.08	18.5
HZM-50	108.5	0.10	20.4
HZM-55	52.5	0.11	10.1
HZM-Im	46.0	0.15	-10.8
HZM-Pip	67.7	0.16	-2.8
HZM-Gly	52.8	0.07	-1.5
EA2	59.0	0.08	6.7
EA2-Pip	97.0	0.17	-1.28
EA-406	138.6	0.06	-1.82
HyAm-Gly	75.1	0.04	-13.6
HyAm-2	44.3	0.09	-3.6

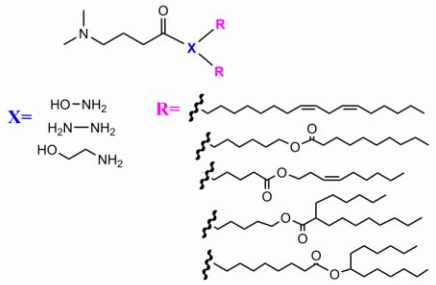
Structural characterization of siRNA-EA2-LNPs by cryoTEM

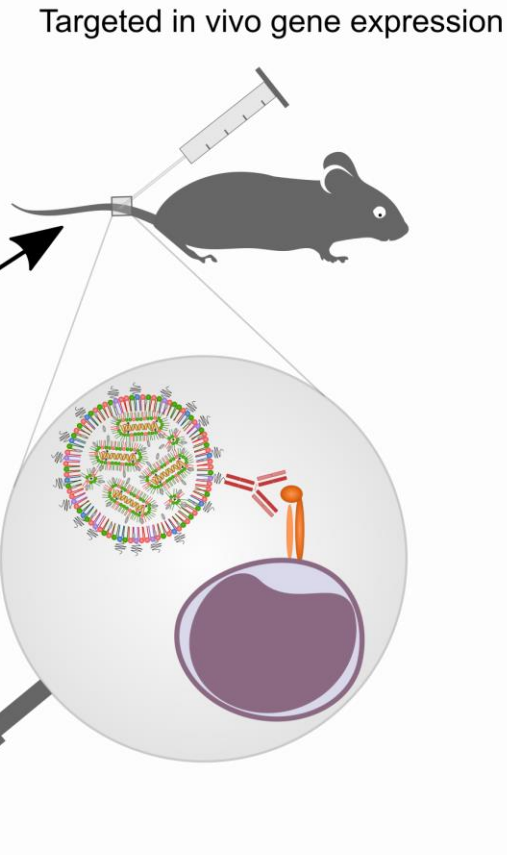


Ramishetti S. et al. Advanced Materials 2020

Dr. Inbal Hazan-Halevi

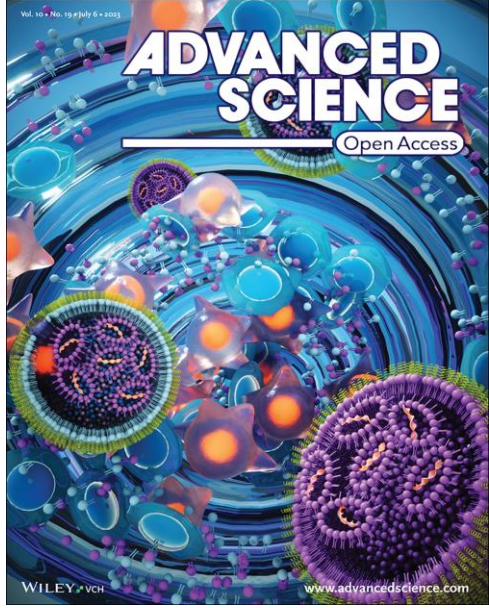


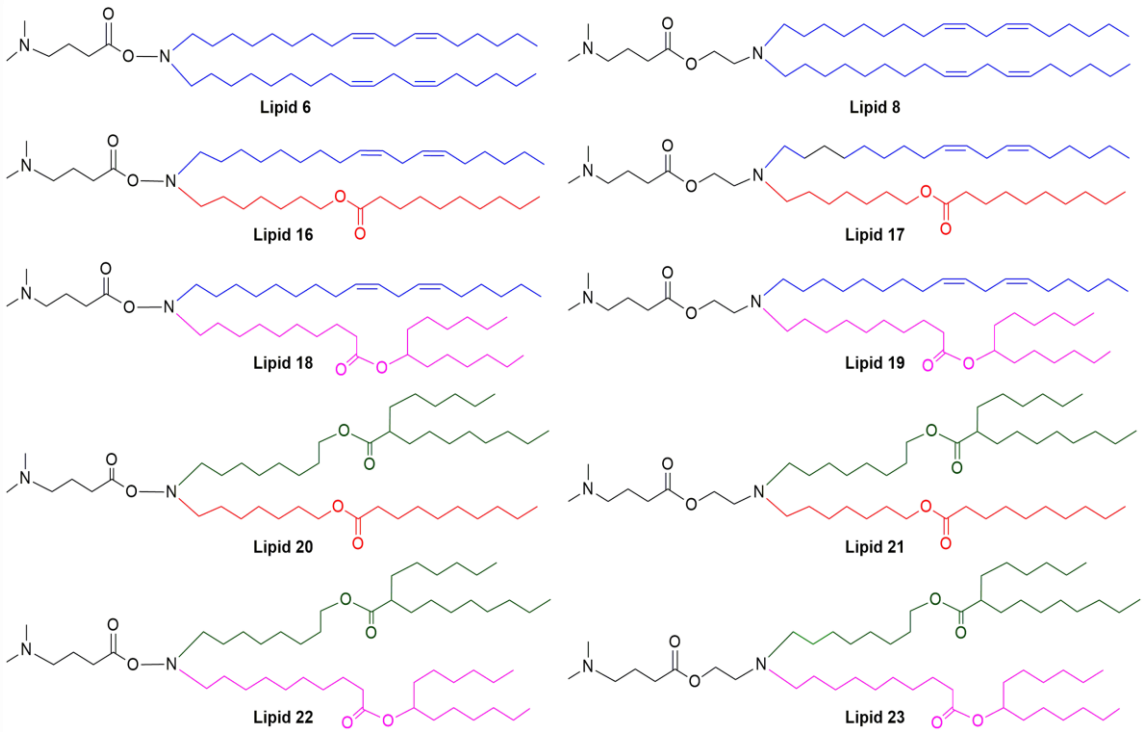




Dr. Srinivas Ramishetti

Dr. Somu Gonna Naidu





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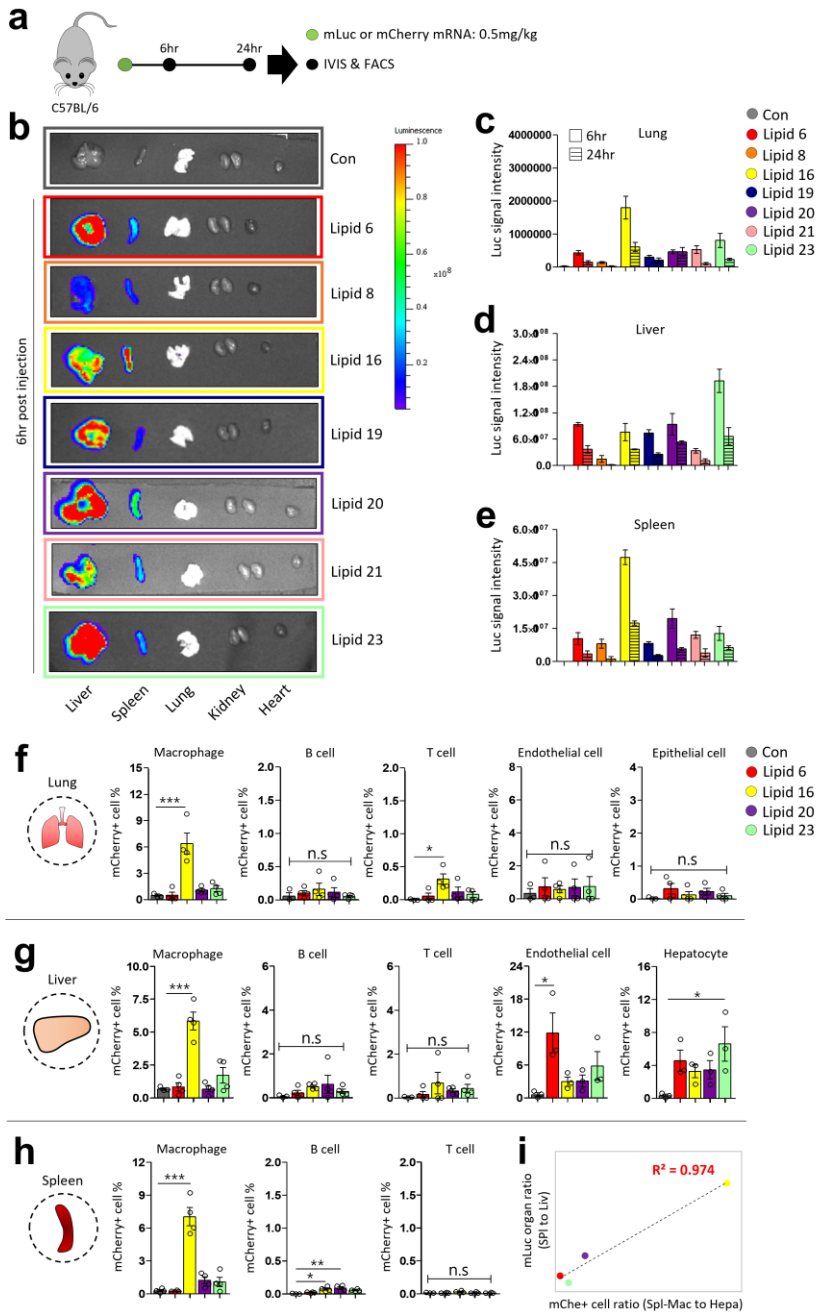
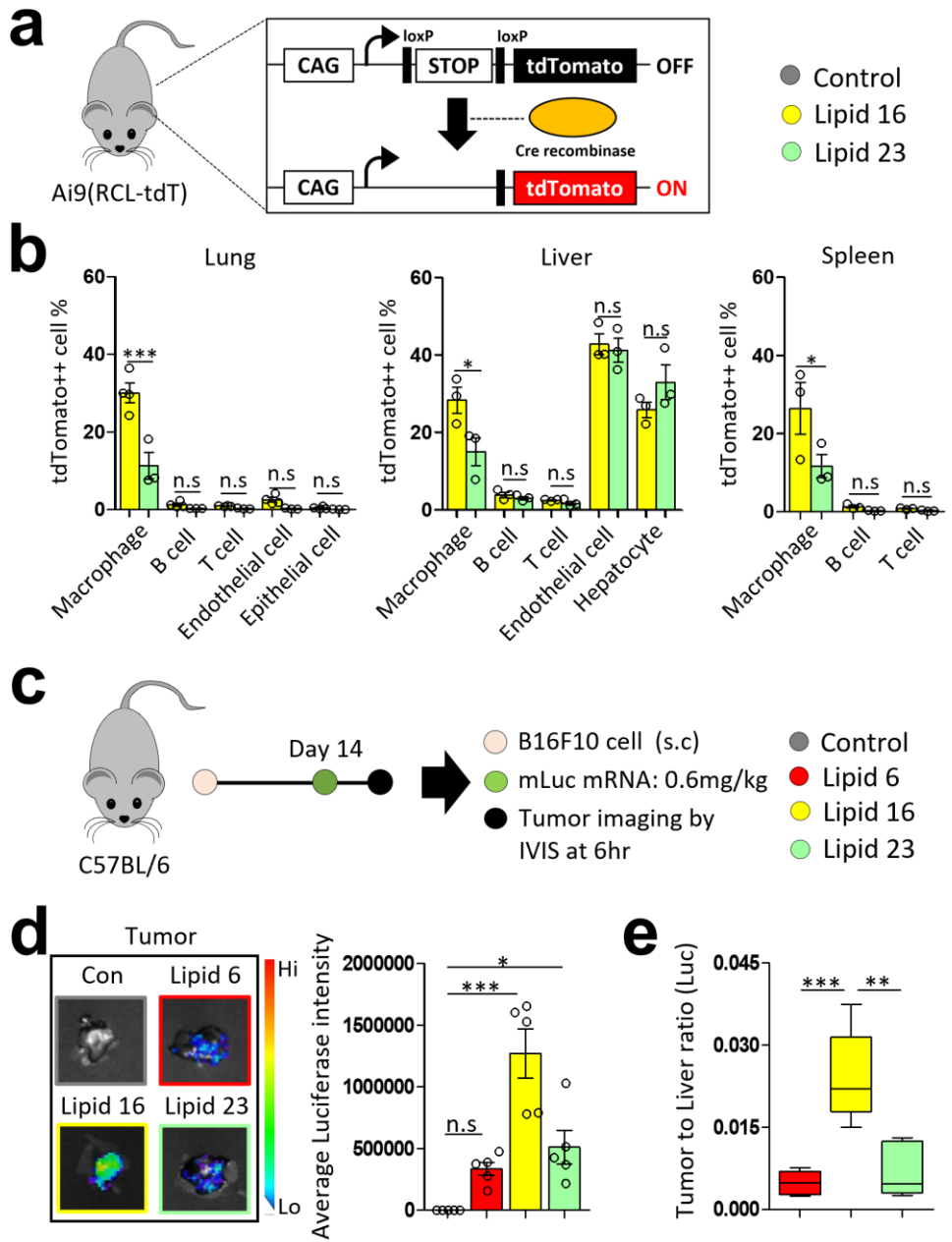
Organ specific delivery or cell specific ?

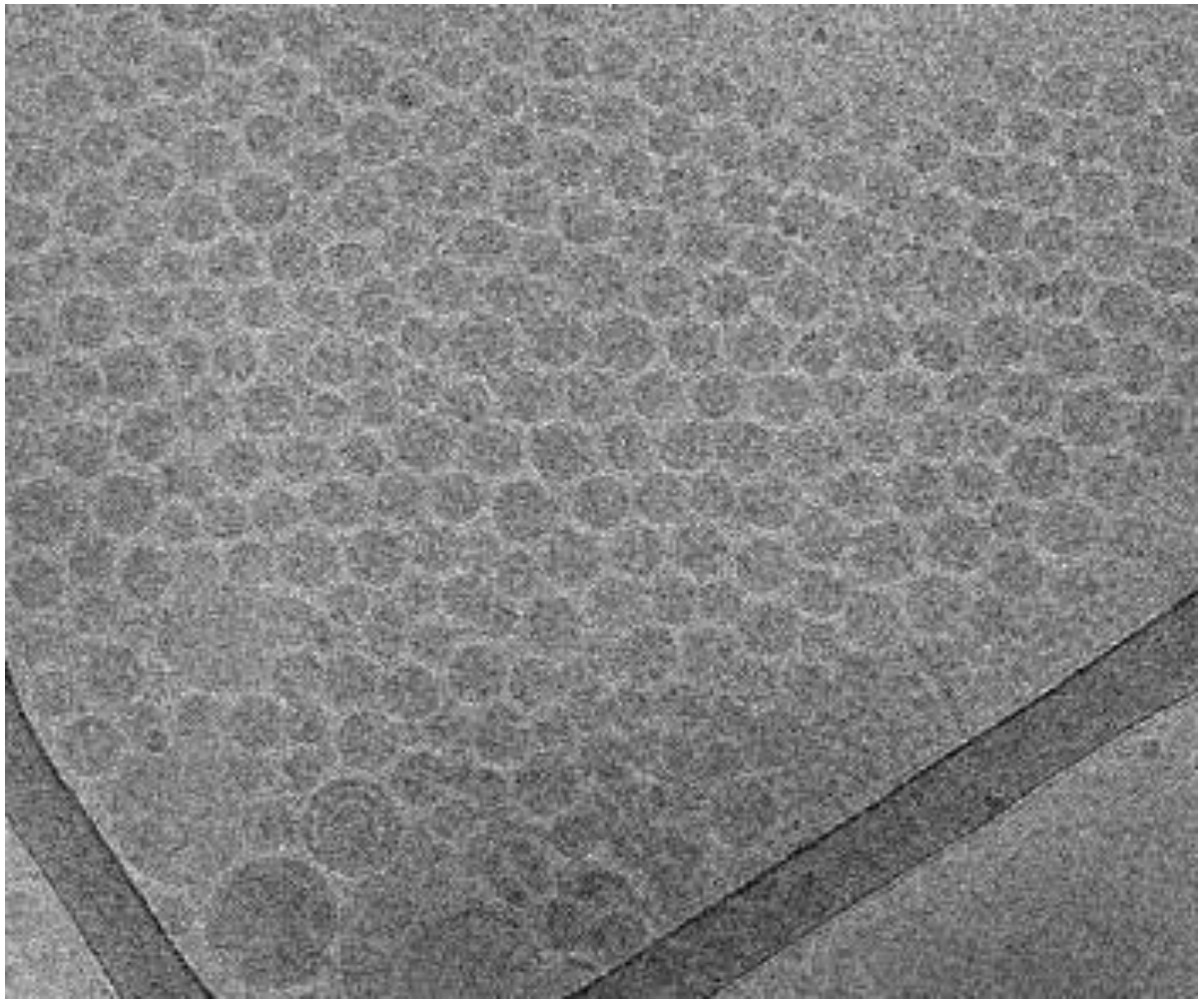




Naidu GS*, Yong S-B* et al. Advanced Science 2023

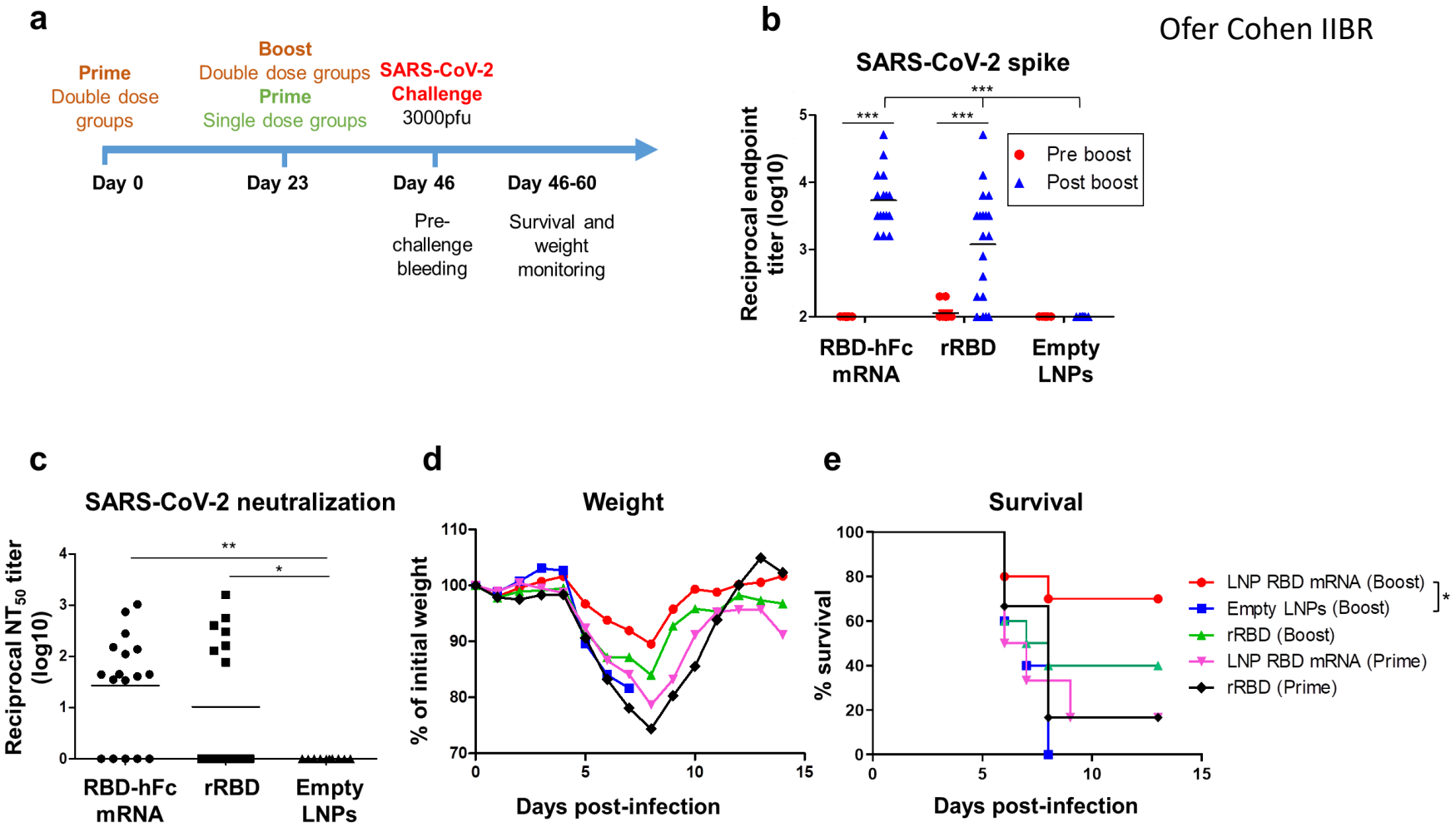
mRNA Vaccines

LNP RBD-hFc mRNA vaccine protects hACE2 trangenic mice against a lethal SARS-CoV-2 challenge .



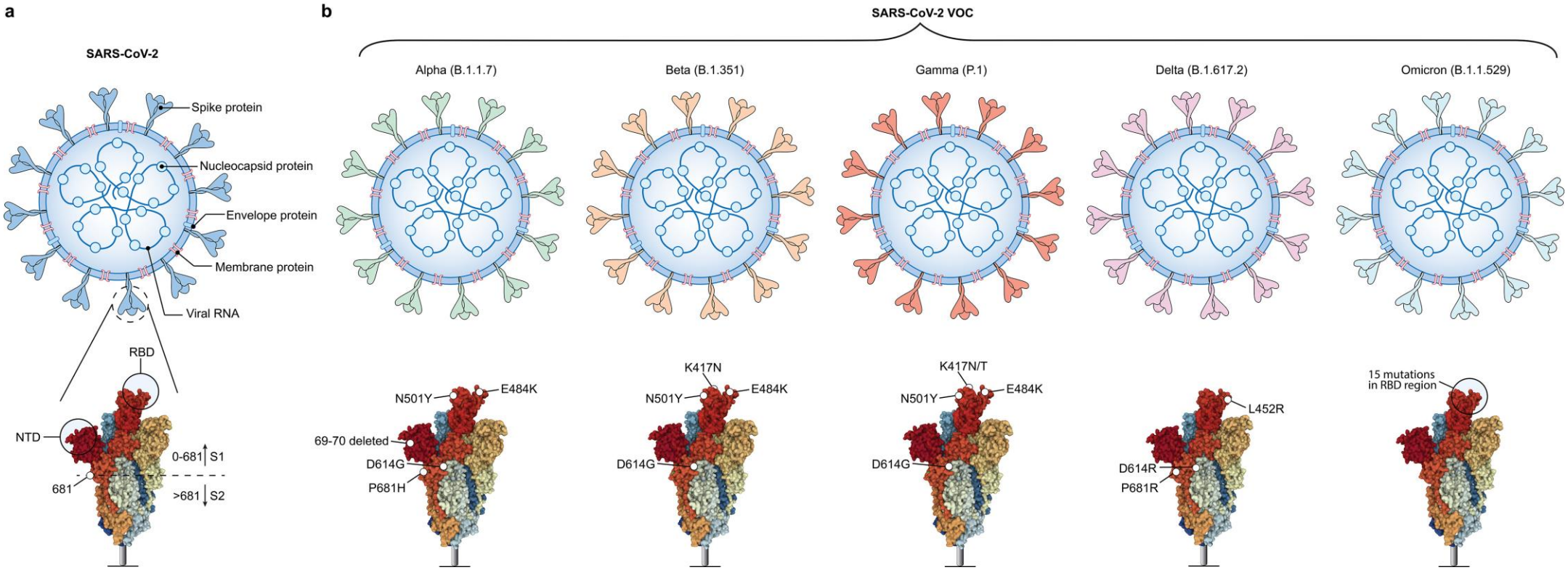
LNP RBD-hFc mRNA vaccine protects hACE2 trangenic mice against a lethal SARS-CoV-2 challenge

Collaboration – Dr. Ofer Cohen IIBR

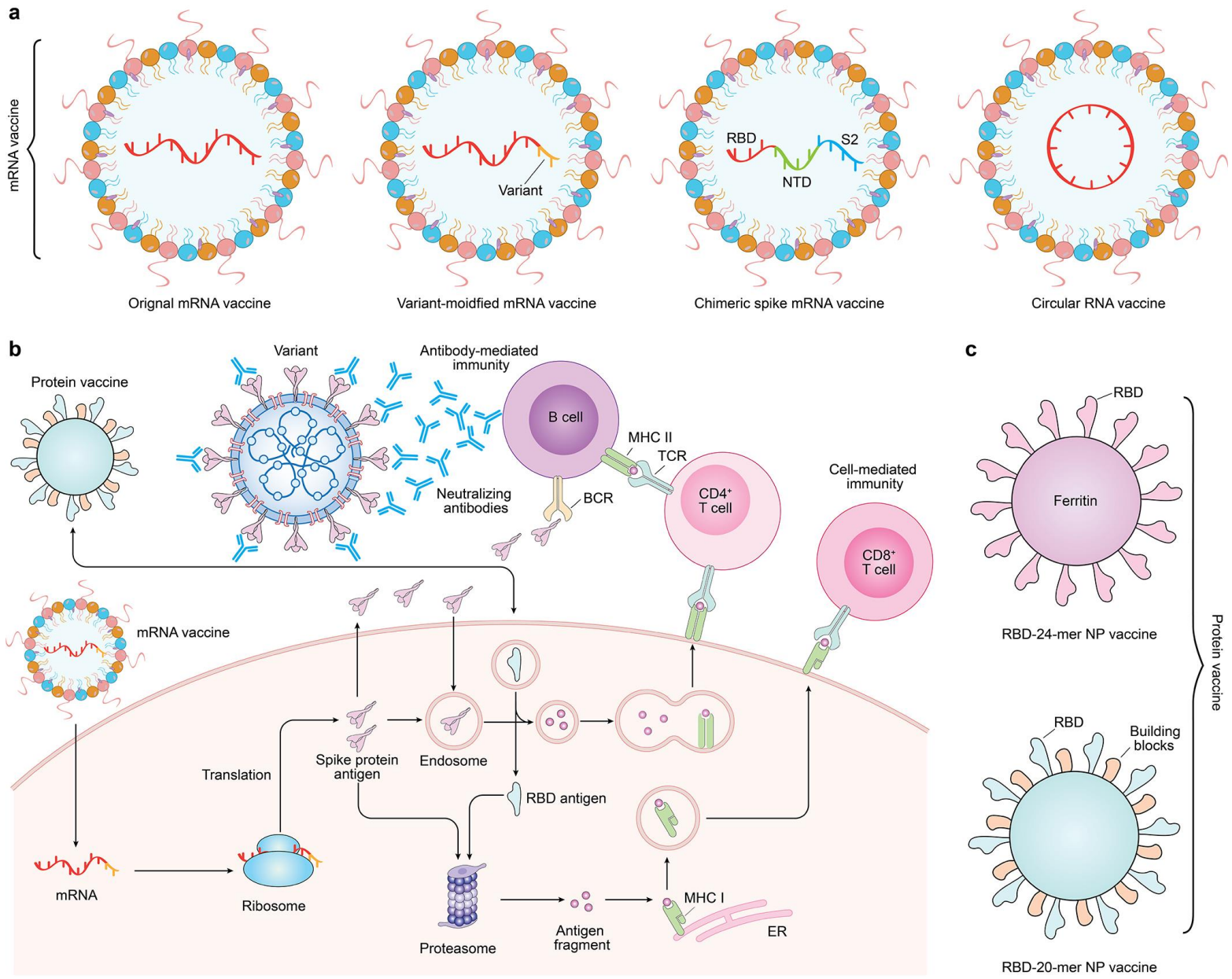


Elia U et al. ACS Nano 2021
Elia U. et al. Nano Letters 2021

Fast response for a highly mutated pathogen



Huang X., Kon E. et al. Nature Nanotechnology 2022



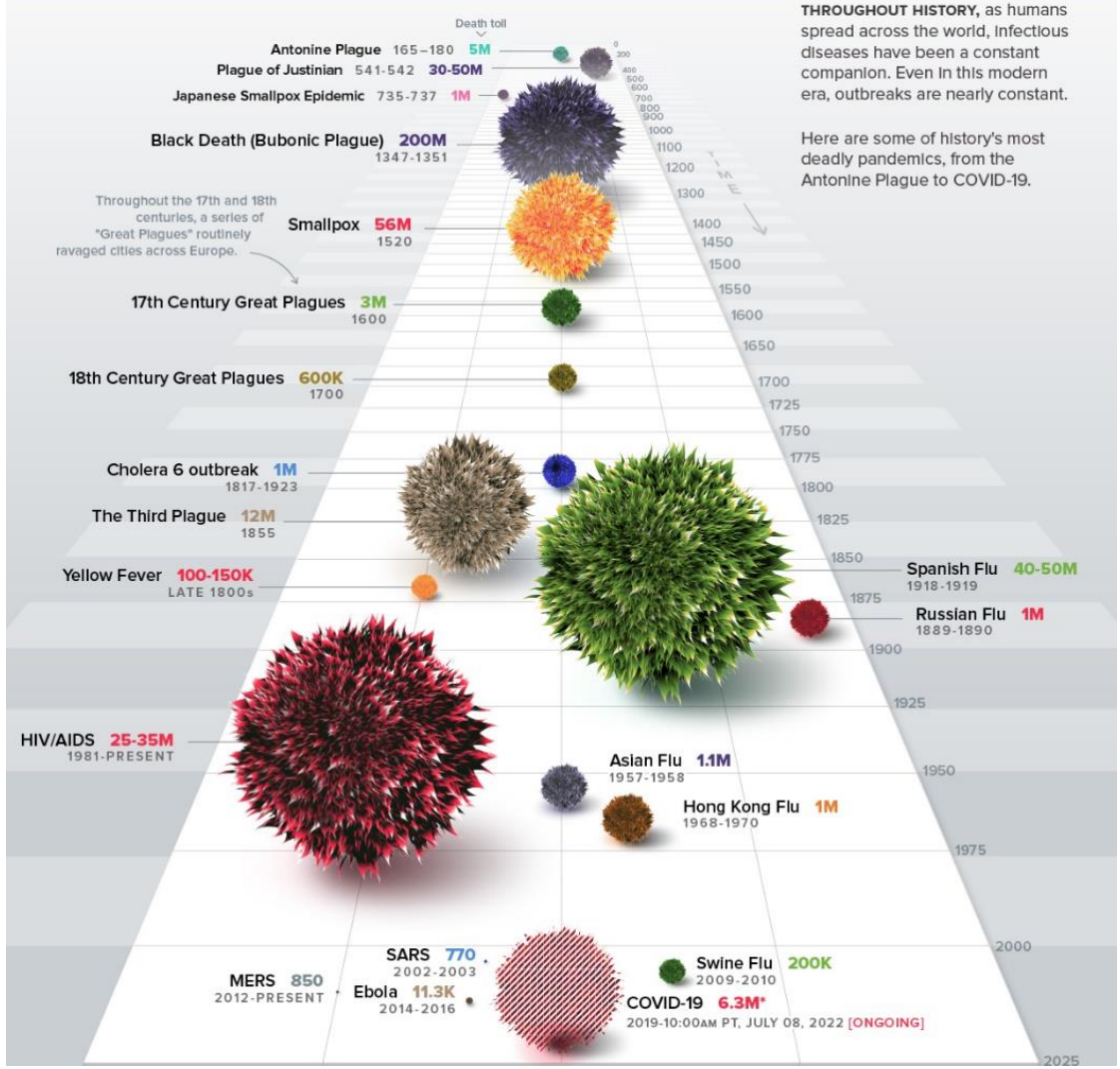
Huang X., Kon E. et al. Nature Nanotechnology 2022

HISTORY OF **PANDEMICS**

PAN·DEM·IC (of a disease) prevalent over a whole country or the world.

THROUGHOUT HISTORY, as humans spread across the world, infectious diseases have been a constant companion. Even in this modern era, outbreaks are nearly constant.

Here are some of history's most deadly pandemics, from the Antonine Plague to COVID-19.



WHO officially declared COVID-19 a pandemic on Mar 11, 2020.

It is hard to calculate and forecast the impact of COVID-19 because the disease is new to medicine, and data is still coming in.

*Johns Hopkins University estimates

DEATH TOLL
[HIGHEST TO LOWEST]

200M
Black Death (Bubonic Plague)
1347-1351

56M
Smallpox
1520

40-50M
Spanish Flu
1918-1919

30-50M
Plague of Justinian
541-542

The plague originated in rats and spread to humans via infected fleas.

The outbreak wiped out 30-50% of Europe's population. It took more than 200 years for the continent's population to recover.

Smallpox killed an estimated 90% of Native Americans. In Europe during the 1800s, an estimated 400,000 people were being killed by smallpox annually. The first ever vaccine was created to ward off smallpox.

The death toll of this plague is still under debate as new evidence is uncovered, but many think it may have helped hasten the fall of the Roman Empire.

25-35M HIV/AIDS 1981-PRESENT

12M The Third Plague 1855

6.3M* COVID-19 2019-10:00AM PT, JULY 08, 2022 [ONGOING]

5M Antonine Plague 165-180

3M 17th Century Great Plagues 1600

1.1M Asian Flu 1957-1958

1M Russian Flu 1889-1890

1M Hong Kong Flu 1968-1970

1M Cholera 6 outbreak 1817-1923

1M Japanese Smallpox Epidemic 735-737

600K 18th Century Great Plagues 1700

200K Swine Flu 2009-2010

100-150K Yellow Fever LATE 1800s

11.3K Ebola 2014-2016

850 MERS 2012-PRESENT

770 SARS 2002-2003

*Johns Hopkins University estimates

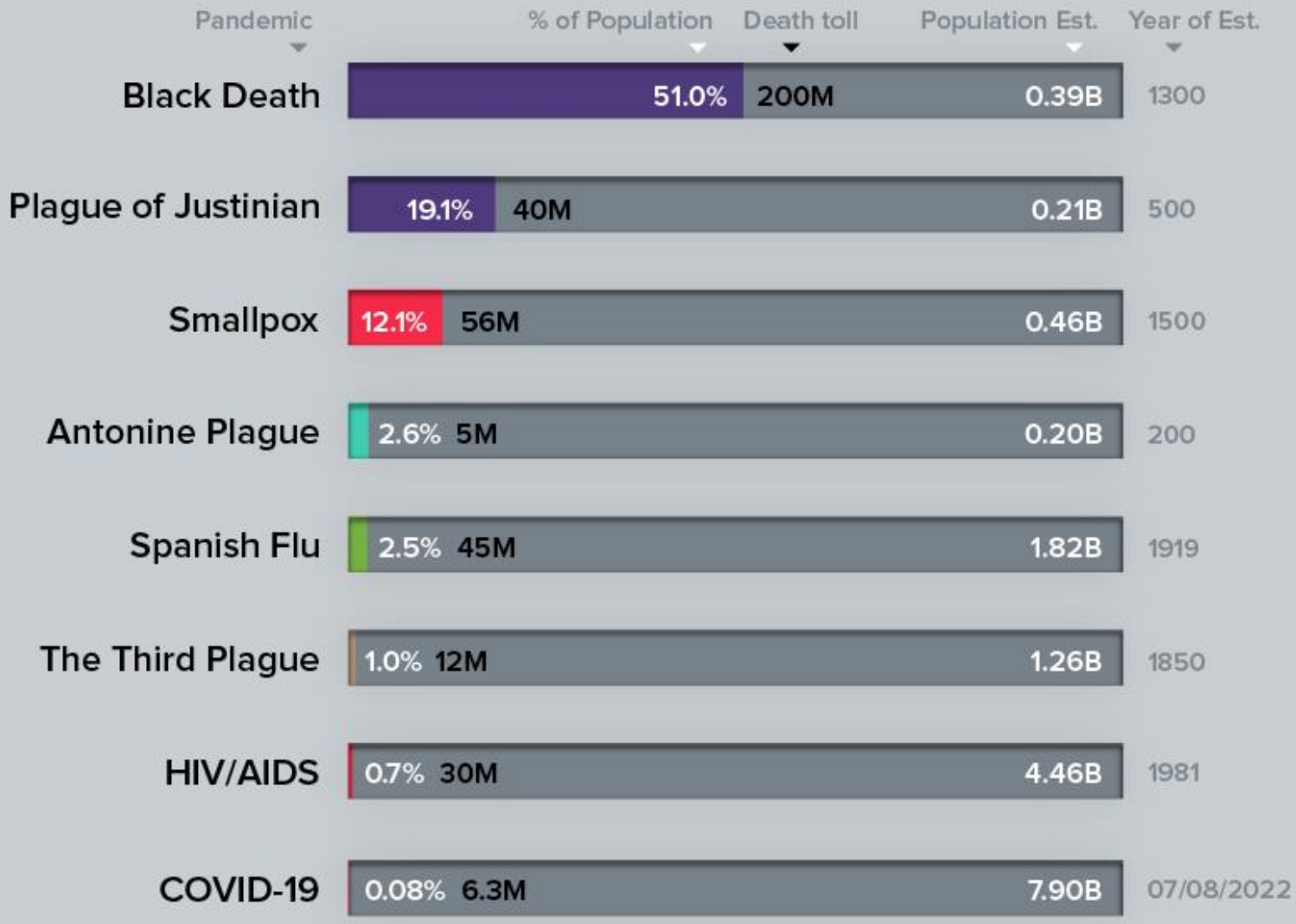
Sources:
CDC, WHO, BBC, Wikipedia, Historical records, Encyclopedia Britannica Johns Hopkins University

VISUAL CAPITALIST

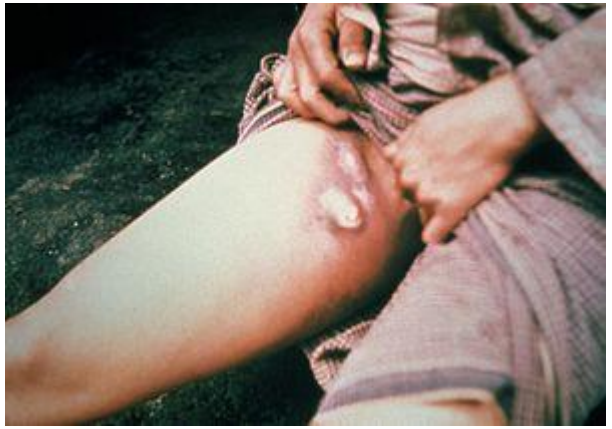
/visualcapitalist @visualcap visualcapitalist.com

COLLABORATORS RESEARCH + WRITING Nick LePan, Nick Routley | DESIGN Harrison Schell | ART DIRECTION Harrison Schell

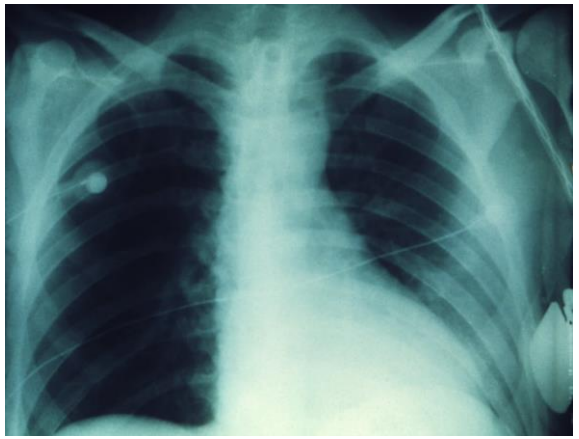
[DEATH TOLL AS A PERCENT OF THE POPULATION]



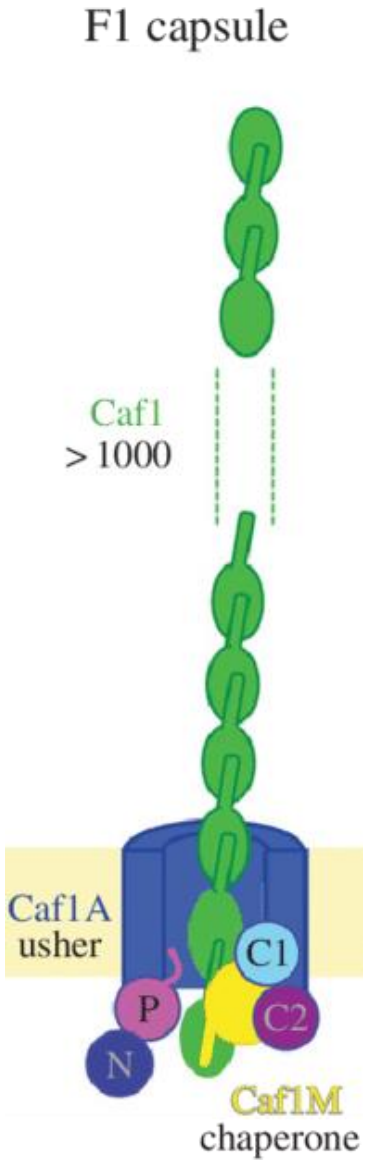
An mRNA-LNP Vaccine Against a Highly Lethal Bacterial pathogen – *Y. pestis*



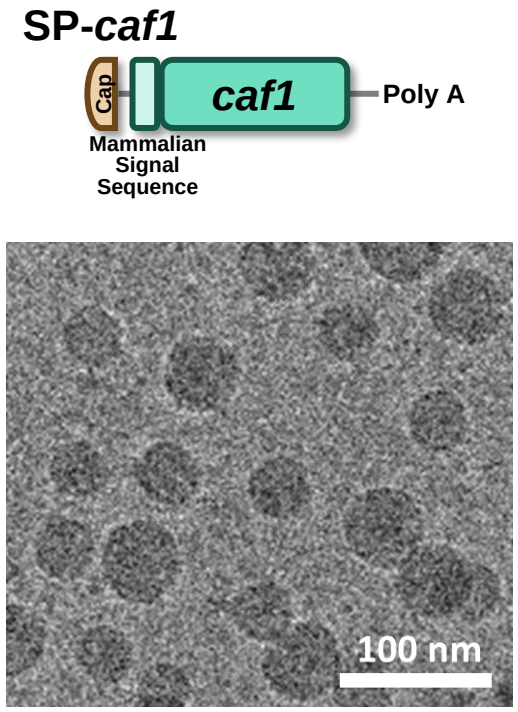
Bubonic
Lymph nodes affected
50-60% Mortality Rate

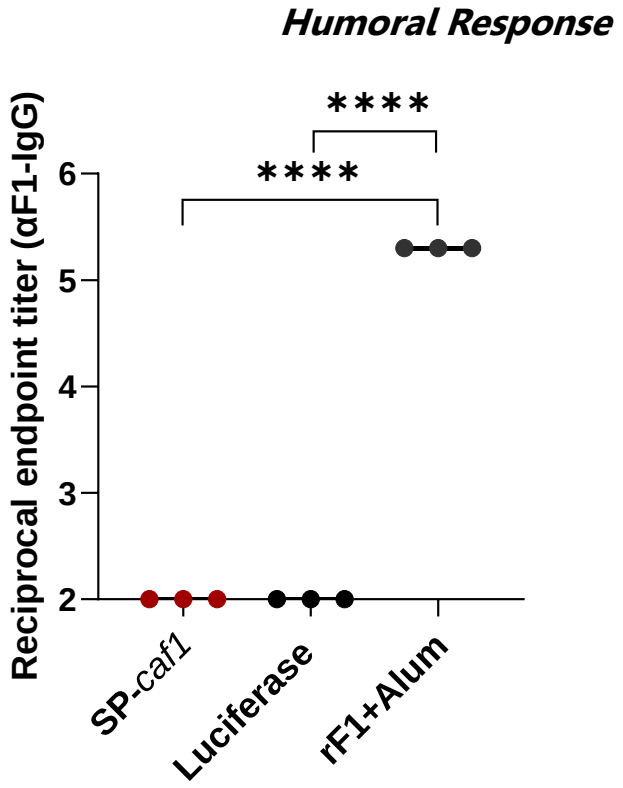


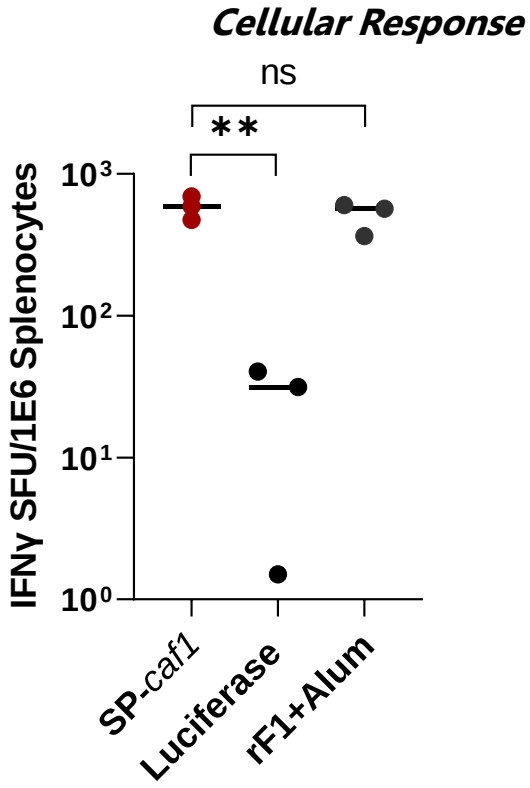
Pneumonic
Human to human transmission (droplets)
Short incubation period
Rapid disease progression
~100% mortality within days if untreated.



Vaccination with a signal-peptide *Caf1* encoding mRNA-LNP results in cellular responses without humoral responses

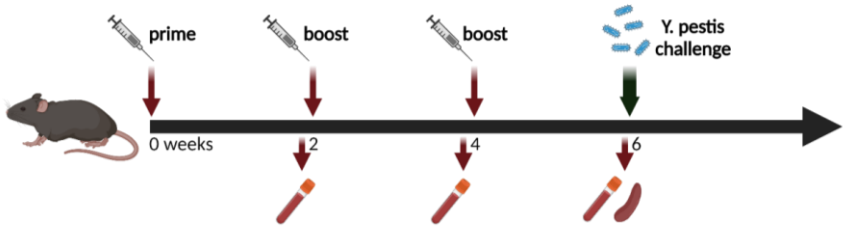




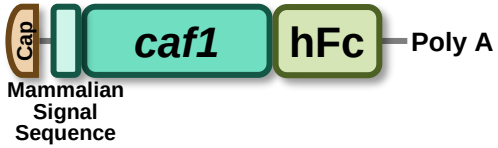


Kon, E et al. SCIENCE Adv. 2023

Vaccination with ΔSP-*caf1 or* SP-*caf1*-hFc encoding mRNA-LNPs results in strong cellular and humoral responses

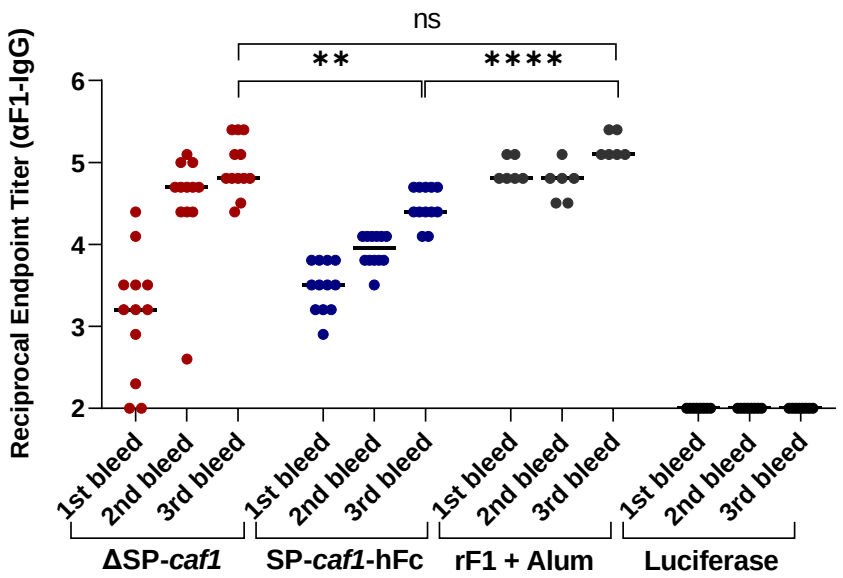


SP-*caf1*-hFc

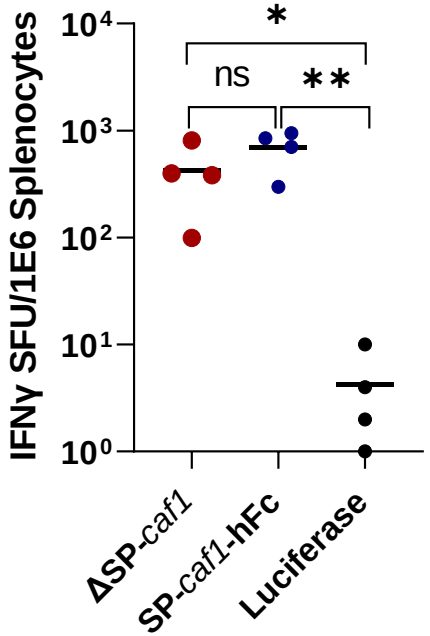


ΔSP-*caf1*

Humoral Response

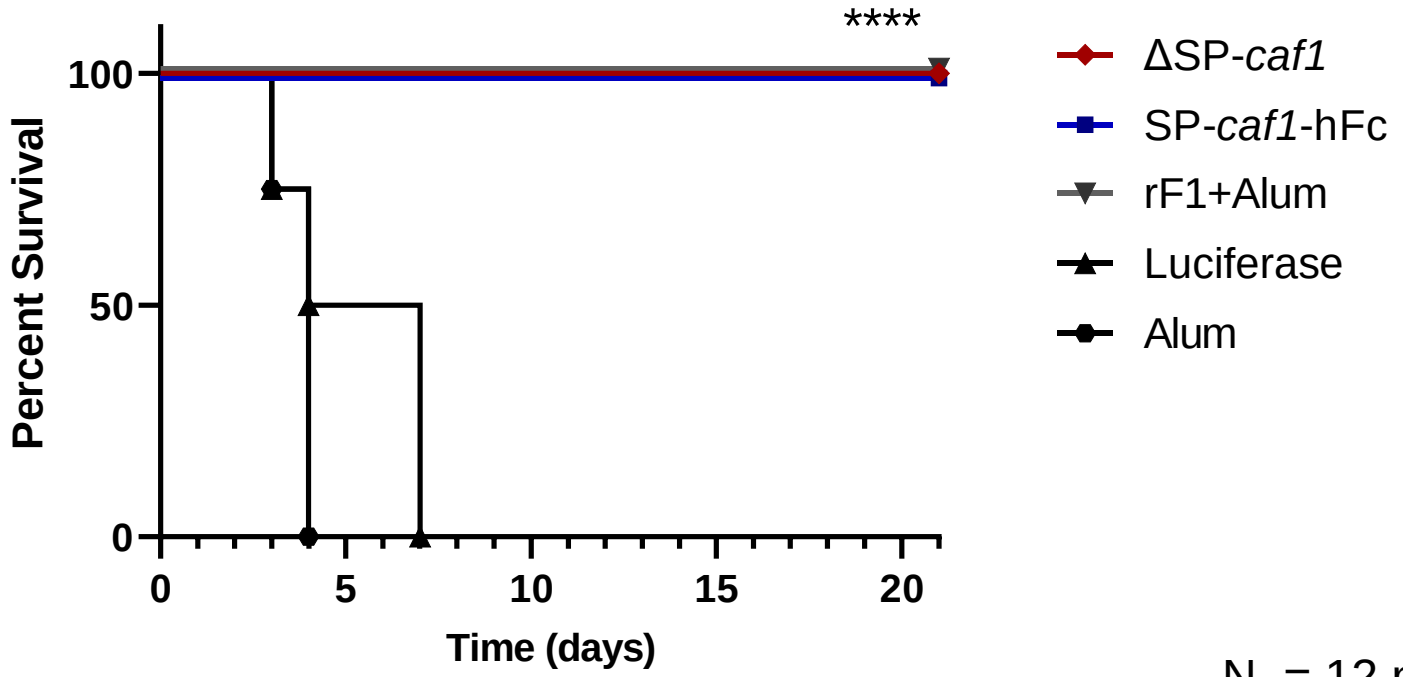


Cellular Response



Kon, E et al. SCIENCE Adv. 2023

Vaccination with ΔSP-*caf1 or* SP-*caf1*-hFc encoding mRNA-LNPs confer full protection against a lethal *Y. pestis* challenge



N = 12 mice / group.
Experiment repeated 3 times independently.

Kon, E et al. SCIENCE Adv. 2023

RNA based therapeutics

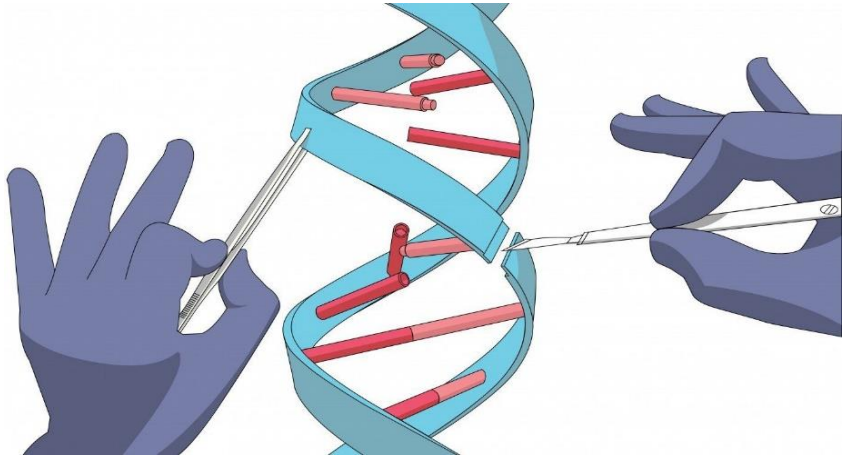
Silence

Activate

Edit

Replace

CRISPR/Cas9

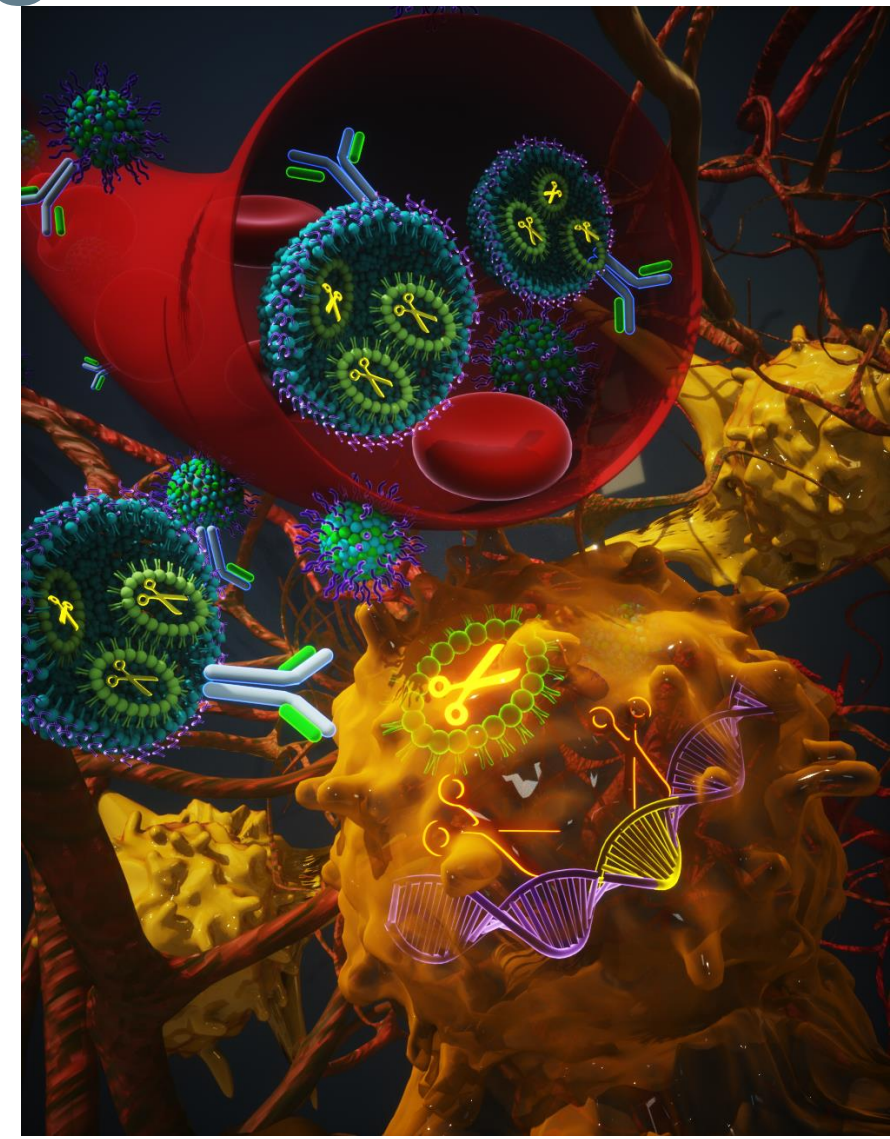


Targeted CRISPR/Cas9 nanomedicine for therapeutic genome editing in cancer

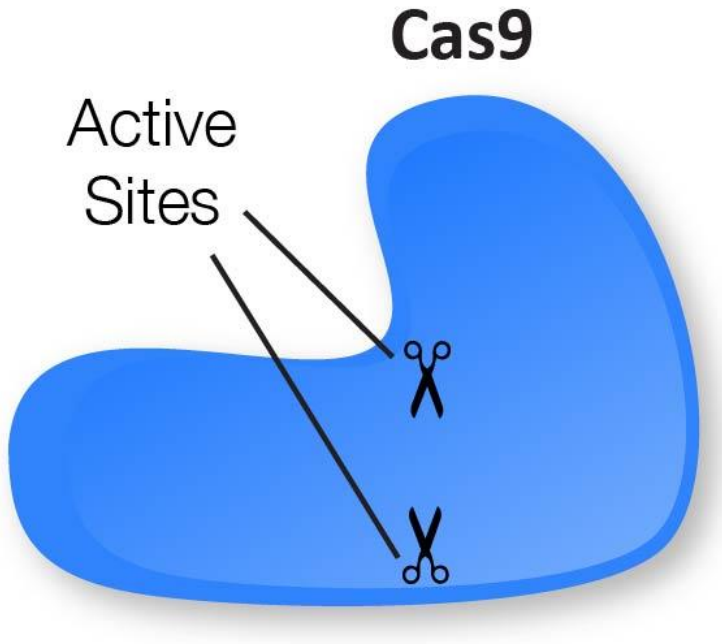
Dr. Daniel Rosenblum

Dr. Anna Gutkin

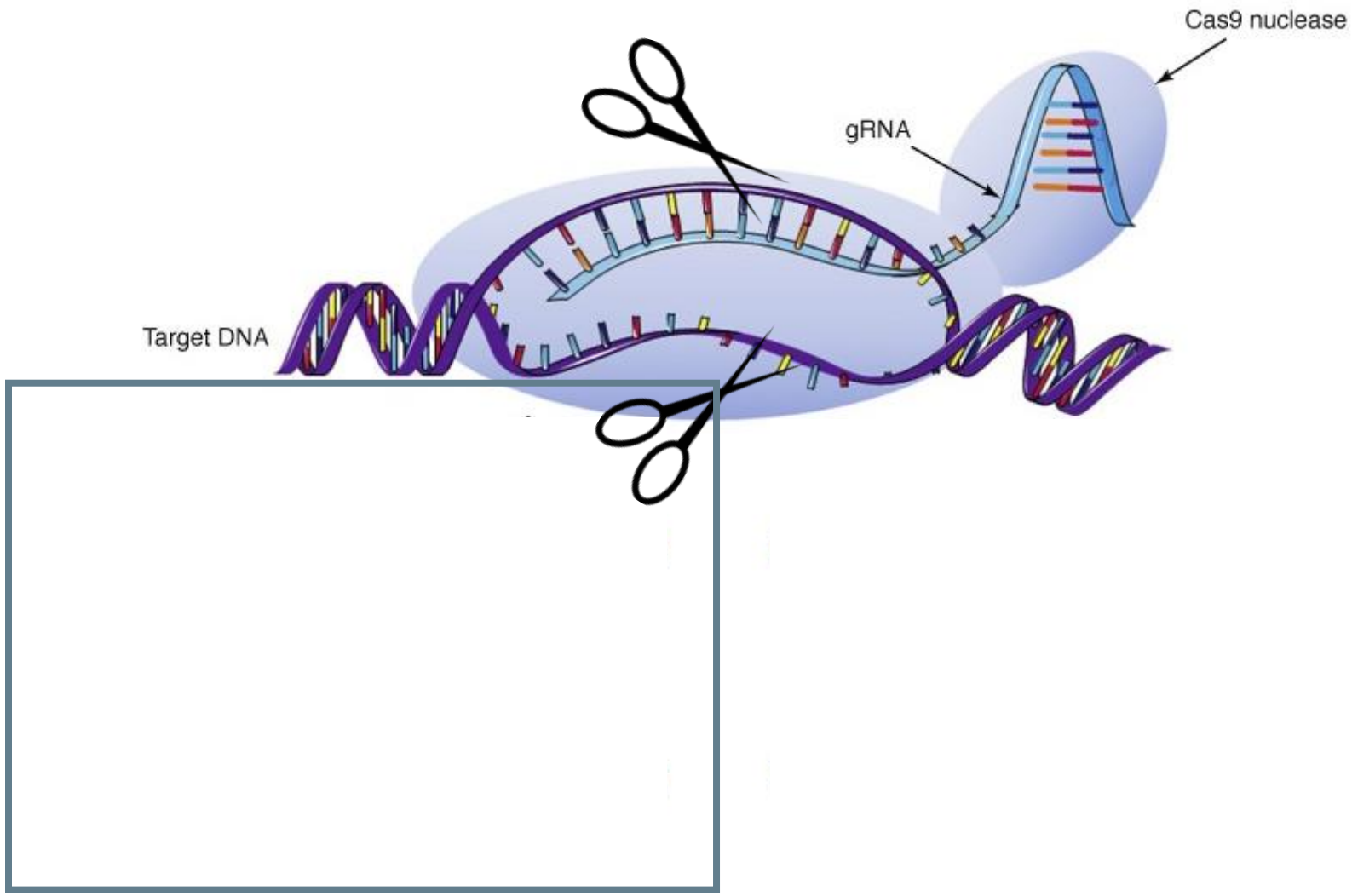
Rosenblum D.*, Gutkin A* et. al. Science Advances 2020
Gutkin A**,** Rosenblum D.*, Peer D. Nature Biotechnology 2021
Elia U*, Kon E*, Peer D. Nature Biotechnology 2023



CRISPR/Cas9 mediated genome editing



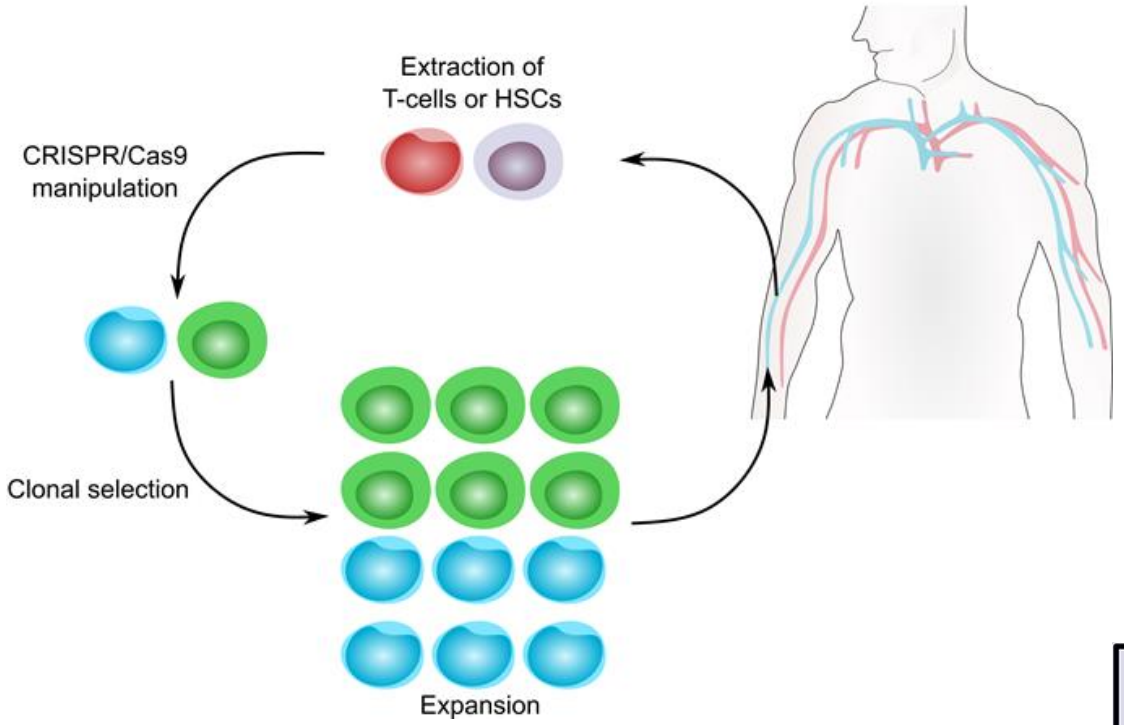
CRISPR/Cas9 mediated genome editing



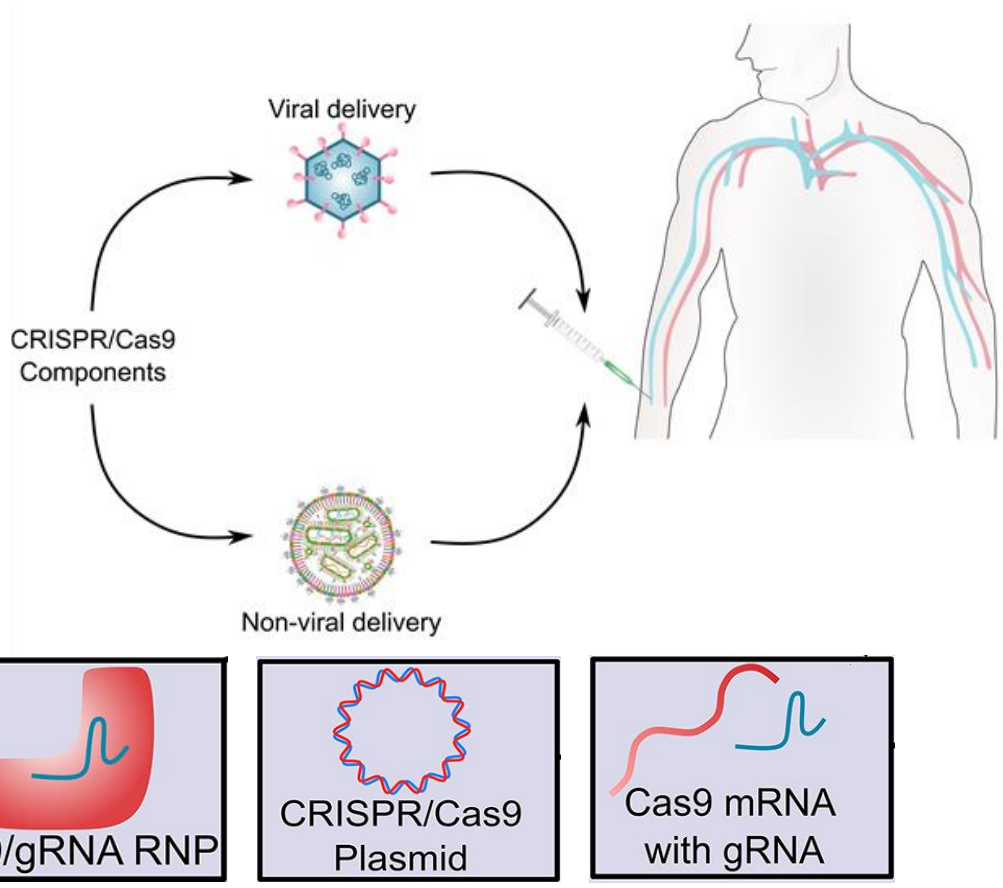
Bin L. et.al, Drug Discovery Today (2019)

Clinical applications of CRISPR/Cas9

Ex vivo editing



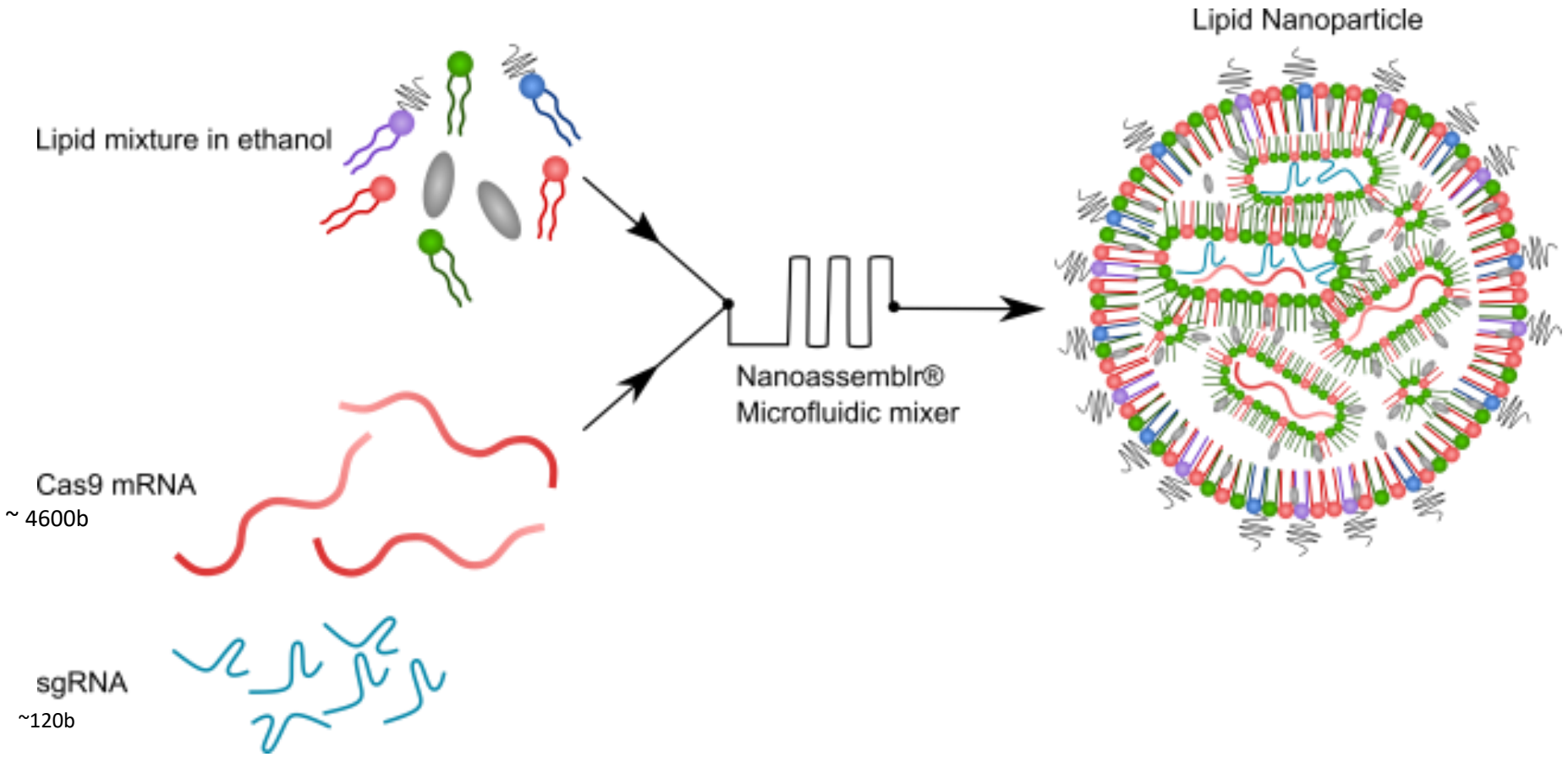
In vivo editing



Rosenblum D., Gutkin A. et. al. ADDR (2020)

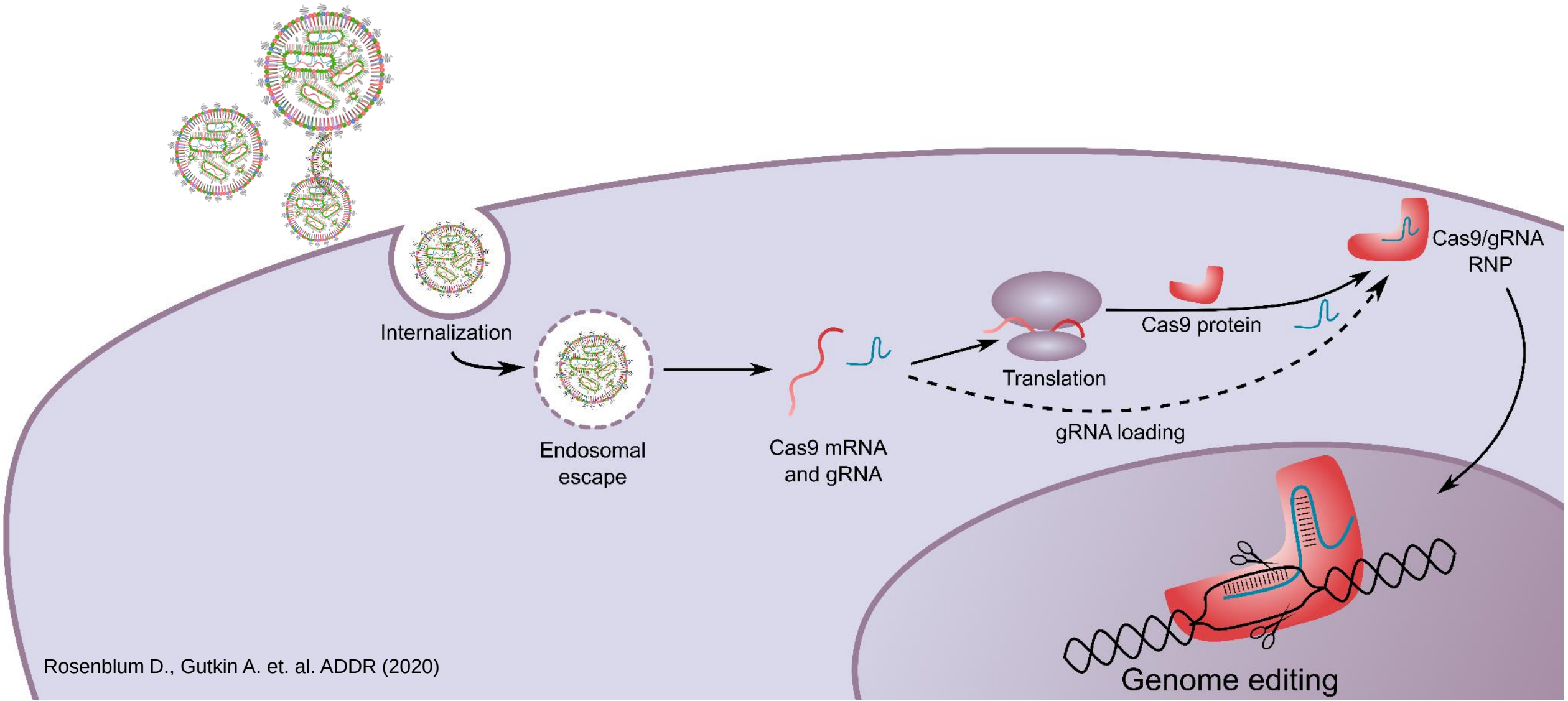
Cas9 nuclease: 160Kda, ~4600b

CRISPR/Cas9 Lipid nanoparticles (cLNPs)



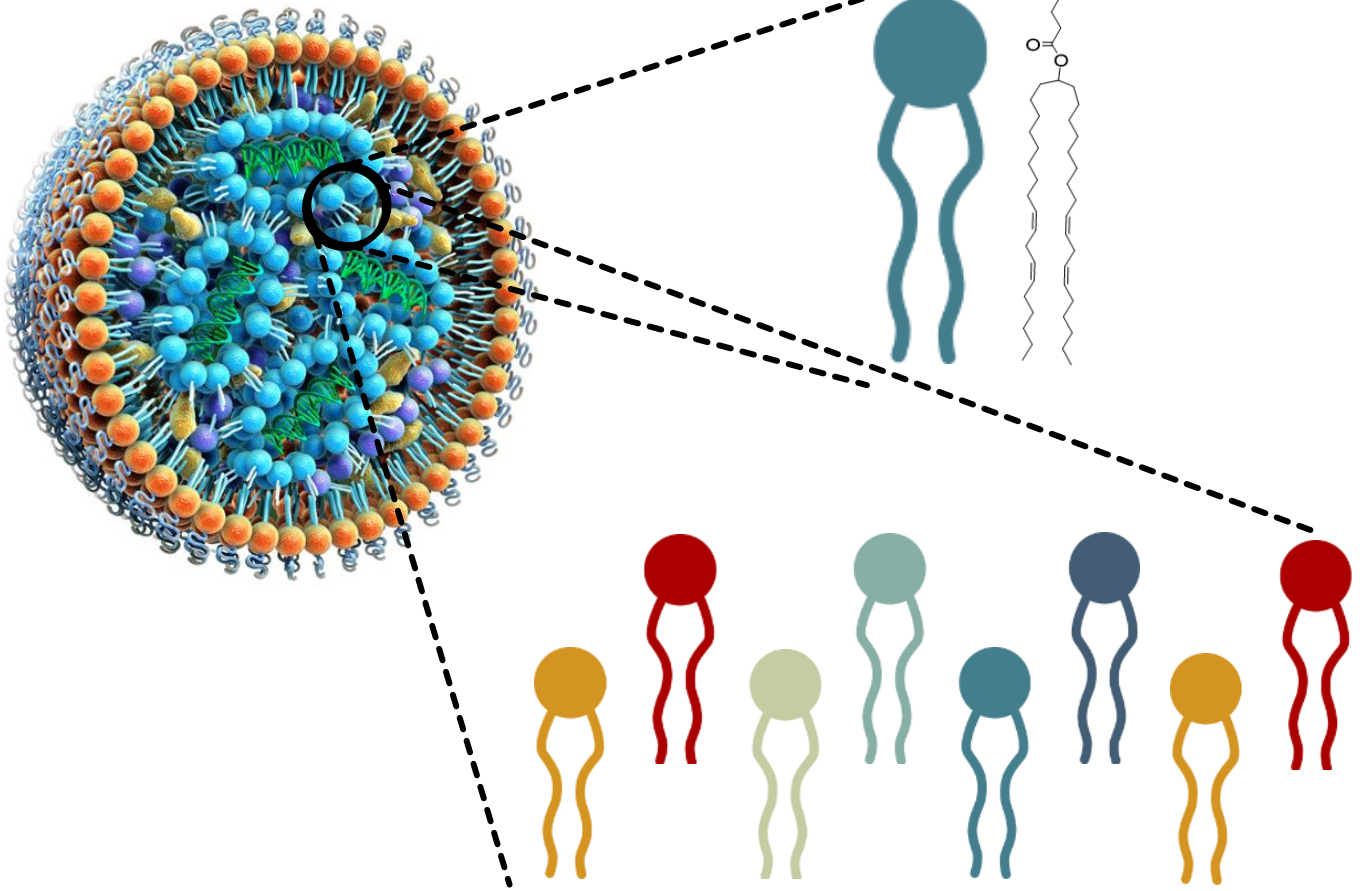
Rosenblum D., Gutkin A et. al. Science Advances (2020)

cLNPs mechanism of action



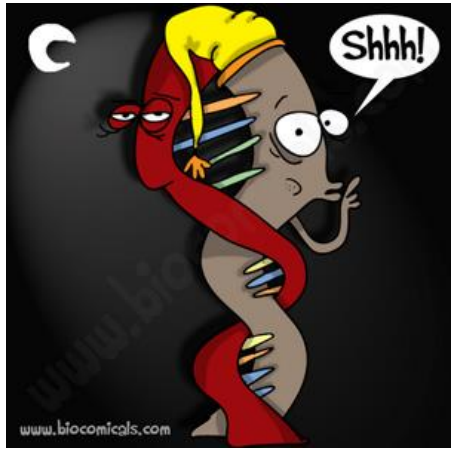
Rosenblum D., Gutkin A. et. al. ADDR (2020)

Design and construction of cLNPs



Dlin-MC3-DMA

siRNA

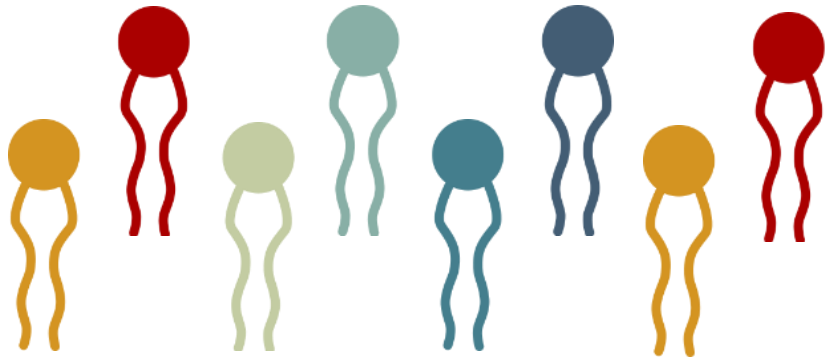


~20-24b

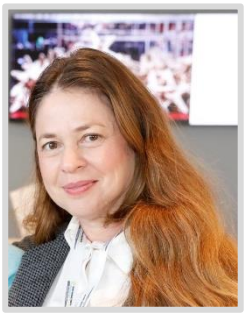
mRNA

~4600b

Design and construction of cLNPs

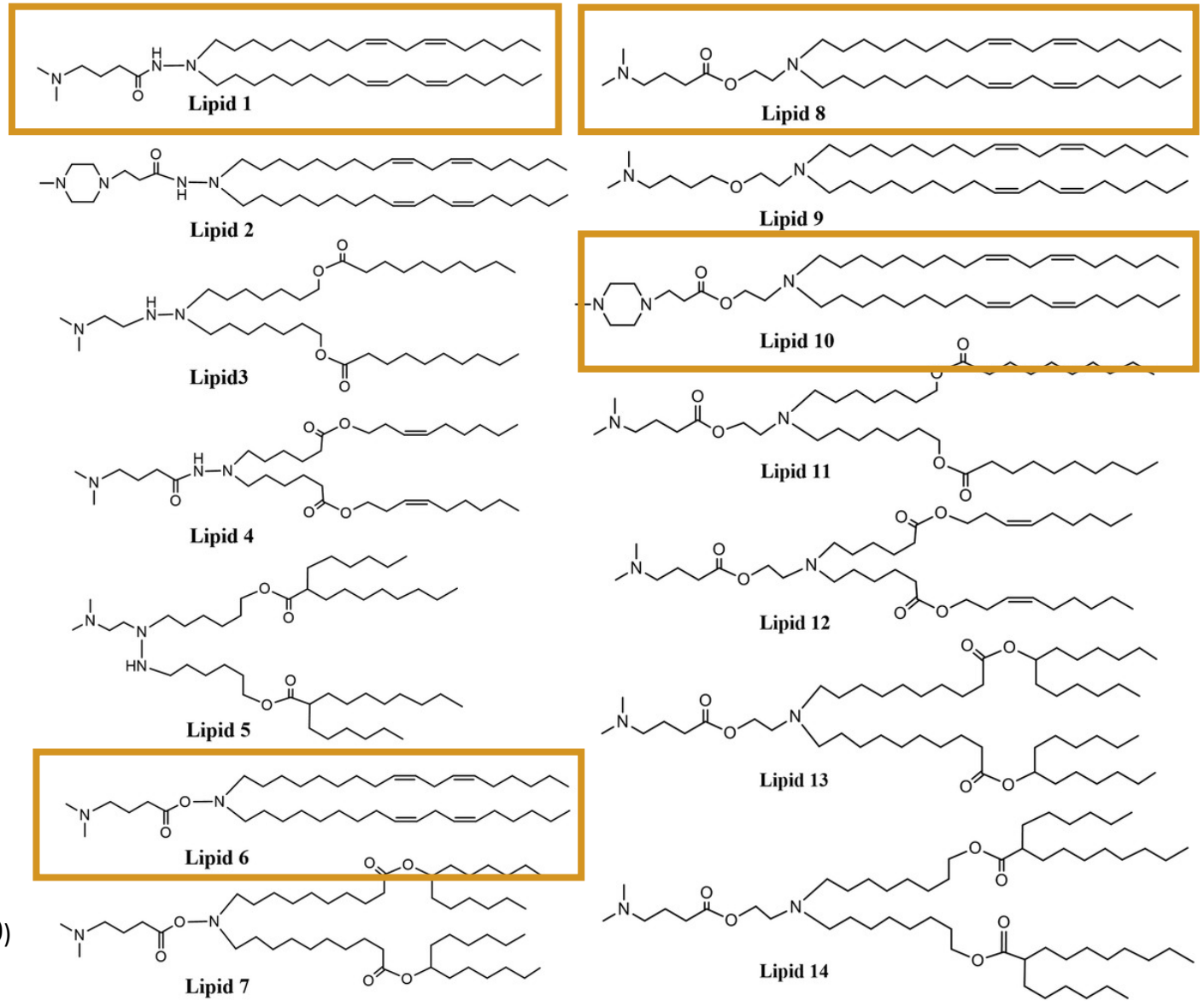


Dr. Srinivas Ramishetti



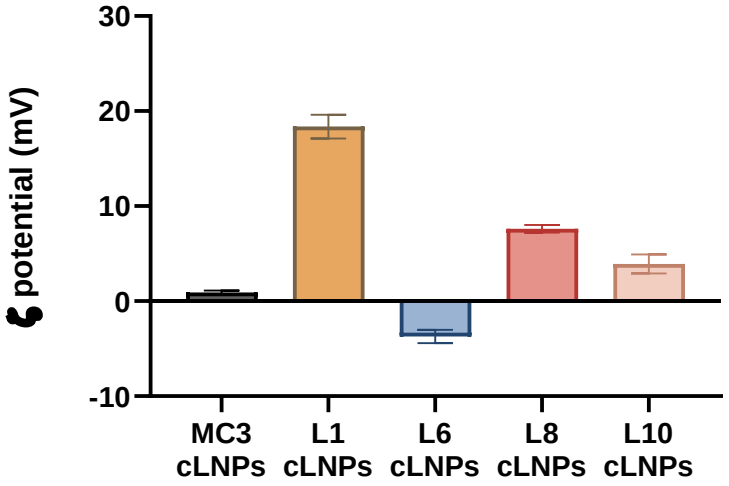
Dr. Inbal Hazan-Halevi

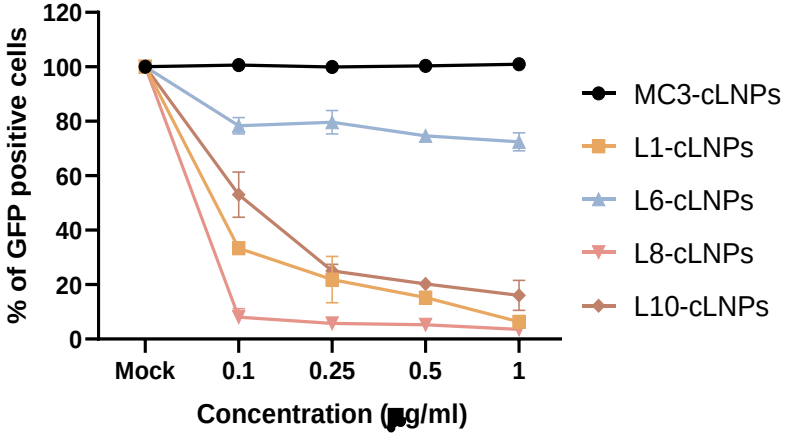
Dr. Ramesh Palakuri

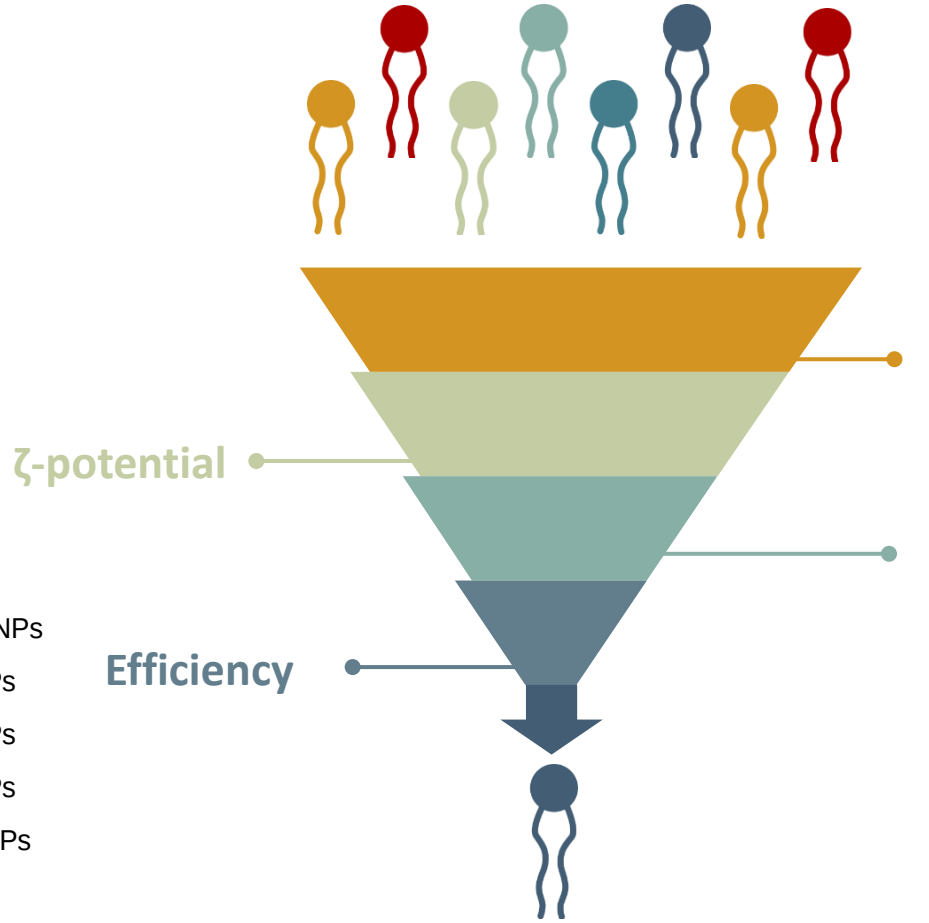


Ramishetti S, Hazan-Halevy I, Palakuri R et. al. Adv Mater (2020)

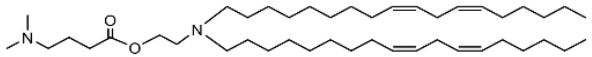
Design and construction of cLNPs

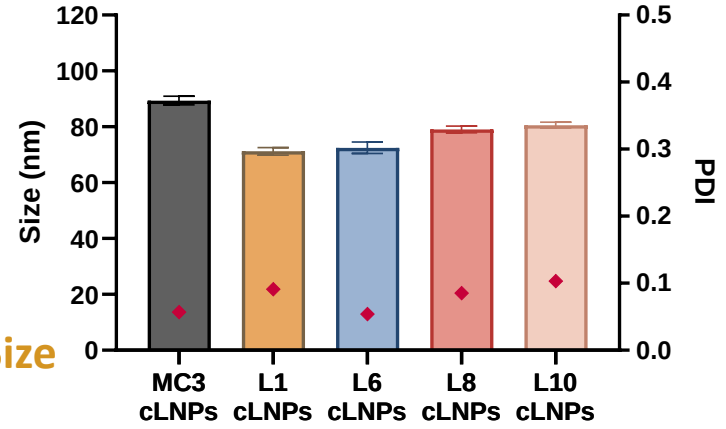


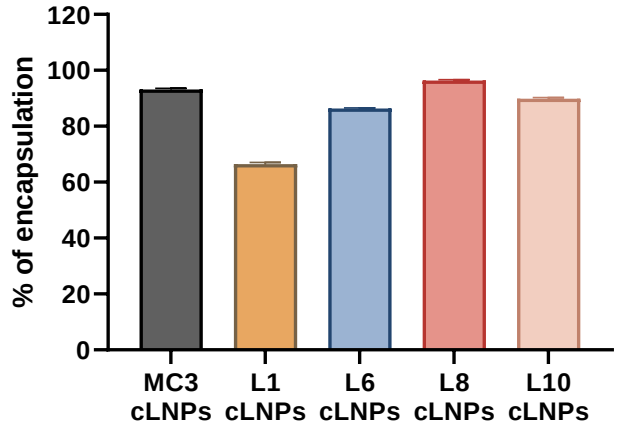




Lipid 8

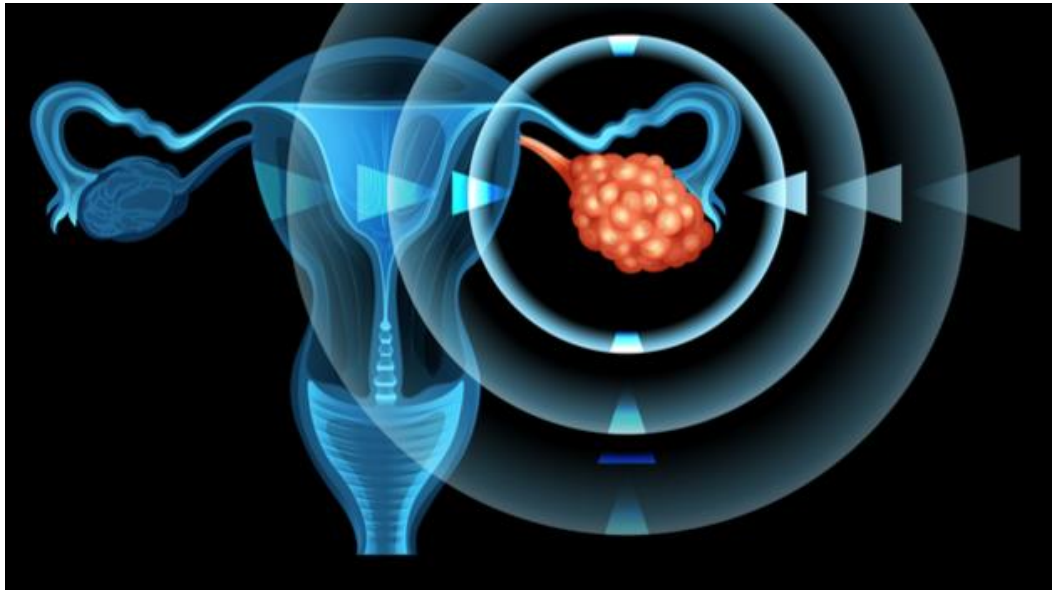






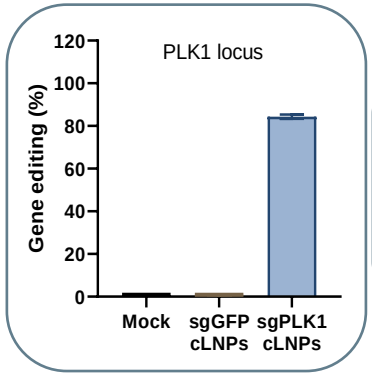
Tumor models

- Murine orthotopic glioblastoma multiforme (GBM)
- Human metastatic ovarian adenocarcinoma



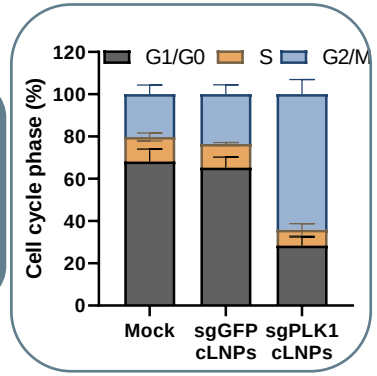
PLK1 gene disruption in two tumor models *in vitro*

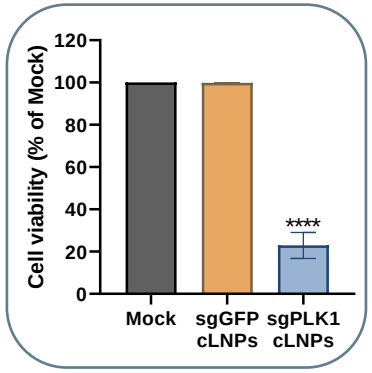
GBM



Gene editing- **85%**

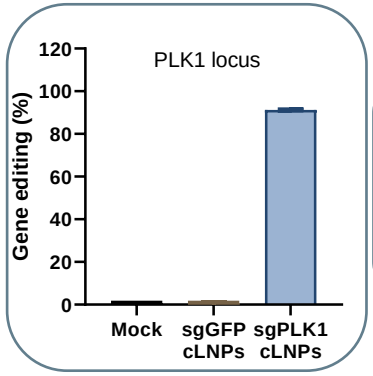
Cell cycle arrest- 3 fold





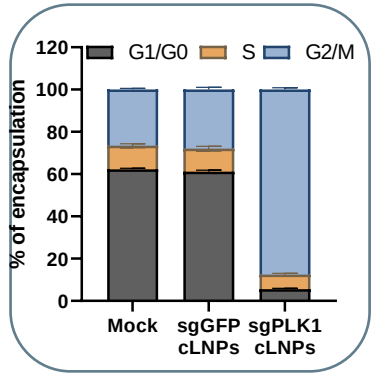
Cell death- 5 fold

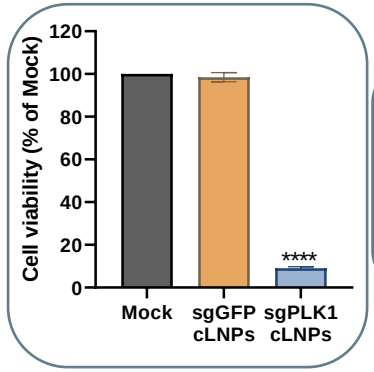
Ovarian cancer



Gene editing- **91%**

Cell cycle arrest- 4 fold

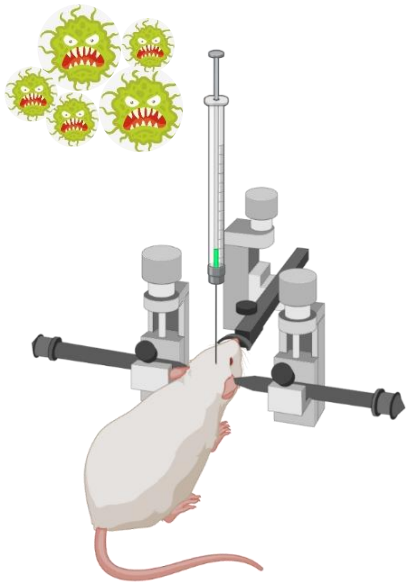


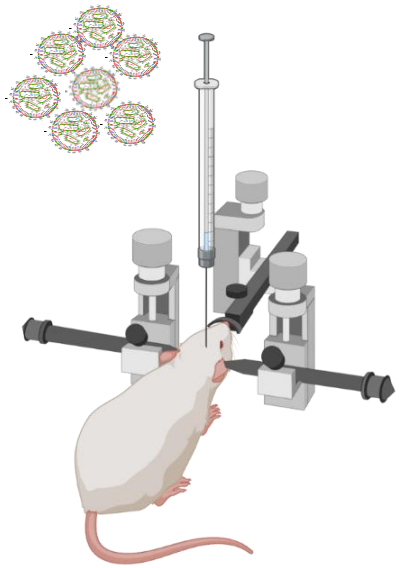


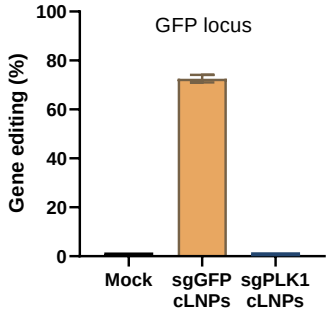
Cell death- 10 fold

Local delivery of cLNPs to GBM

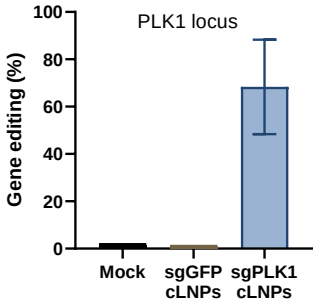
GBM cells injection → 10d → cLNPs injection → 2d → Gene disruption analysis → 1d → Apoptosis induction analysis



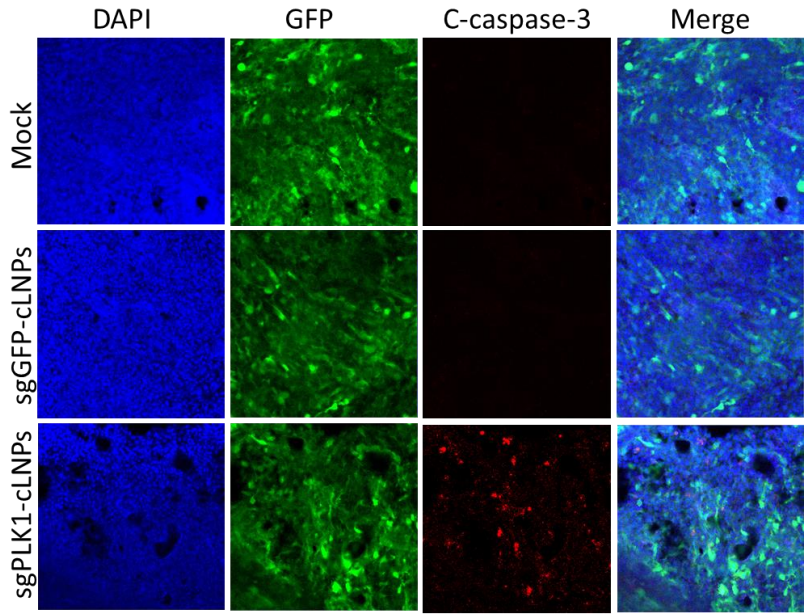




Gene editing- **75%**



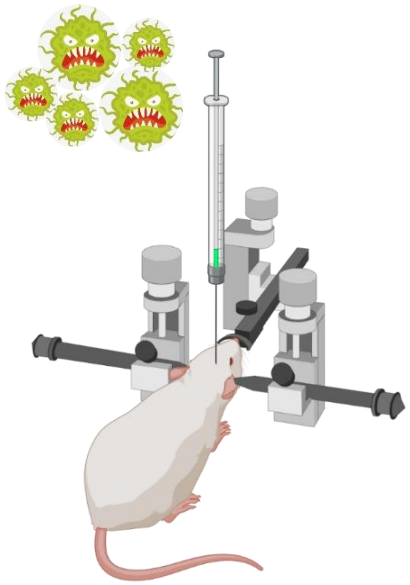
Gene editing- **70%**

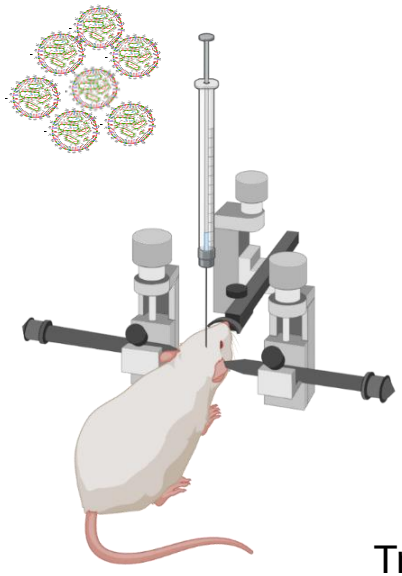


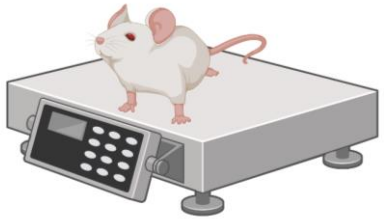
Rosenblum D., Gutkin A. et. al. Science Advances (2020)

Schematic experimental design: Local delivery of cLNPs to GBM bearing mice

GBM cells injection → 10d → cLNPs injection → 60d follow up



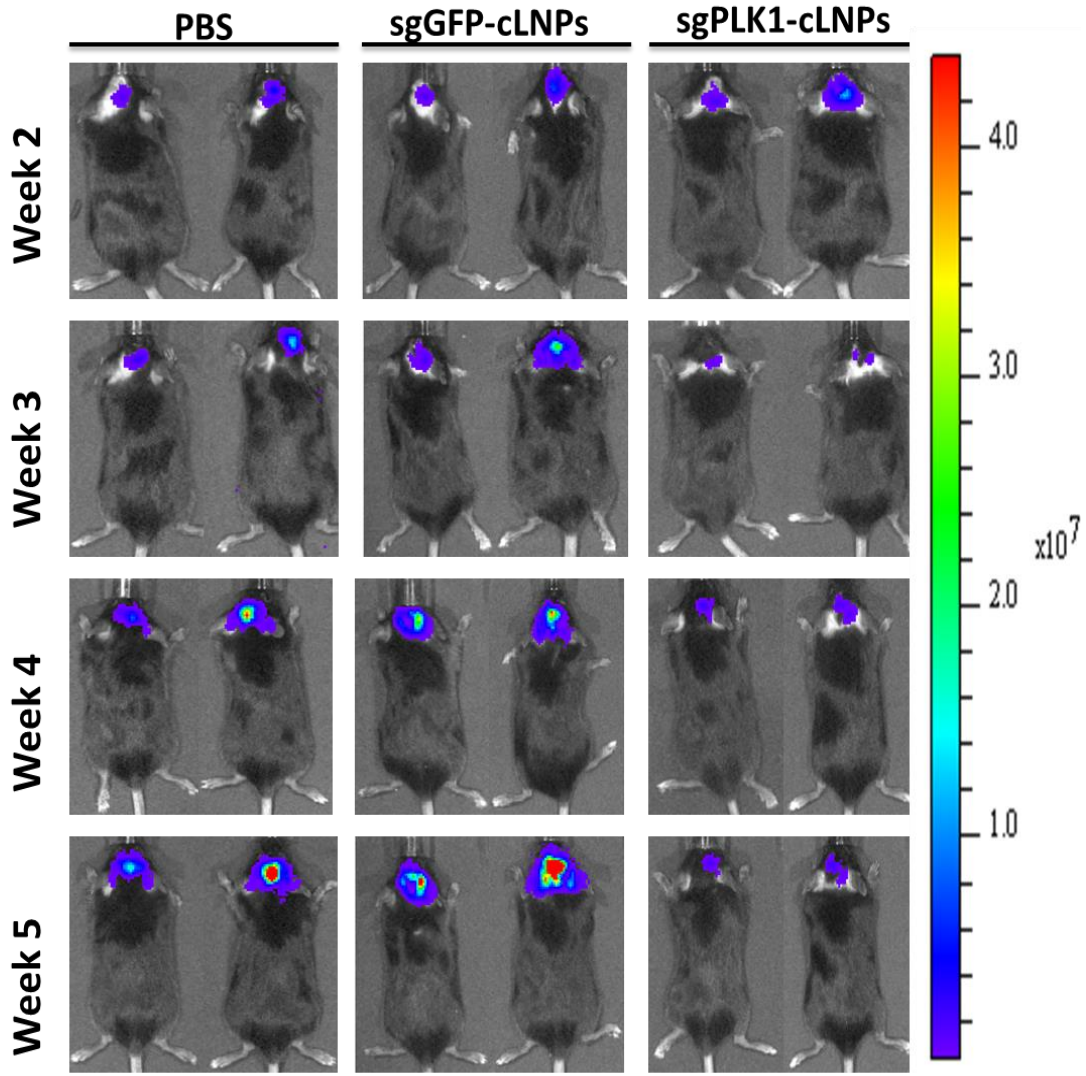


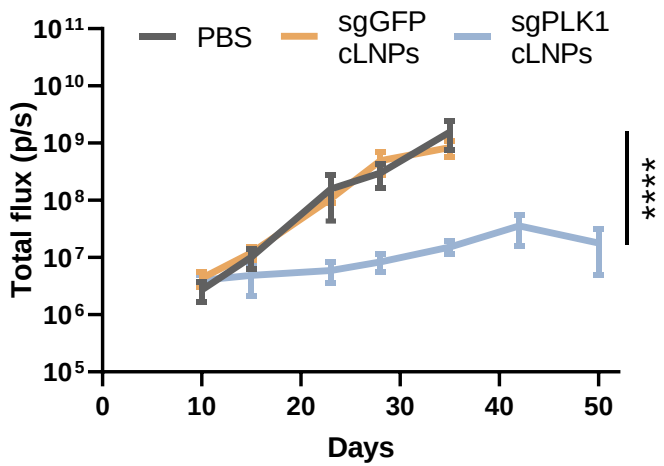


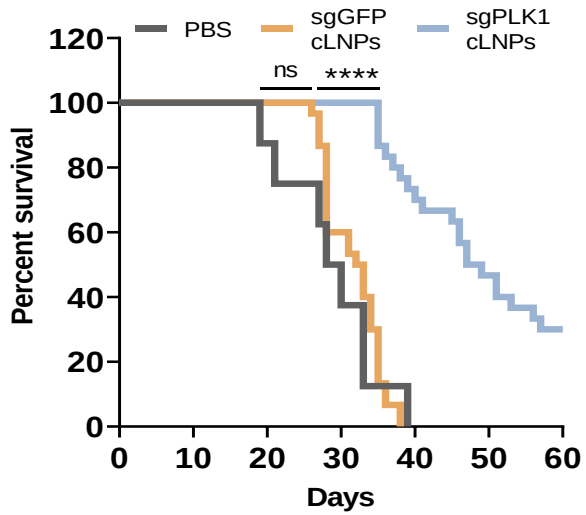
Treatments:

1. PBS
2. sgGFP- cLNPS
3. sgPLK1- cLNPS

Single administration of sgPLK1-cLNPs results in potent tumor growth inhibition and prolong survival of GBM bearing mice



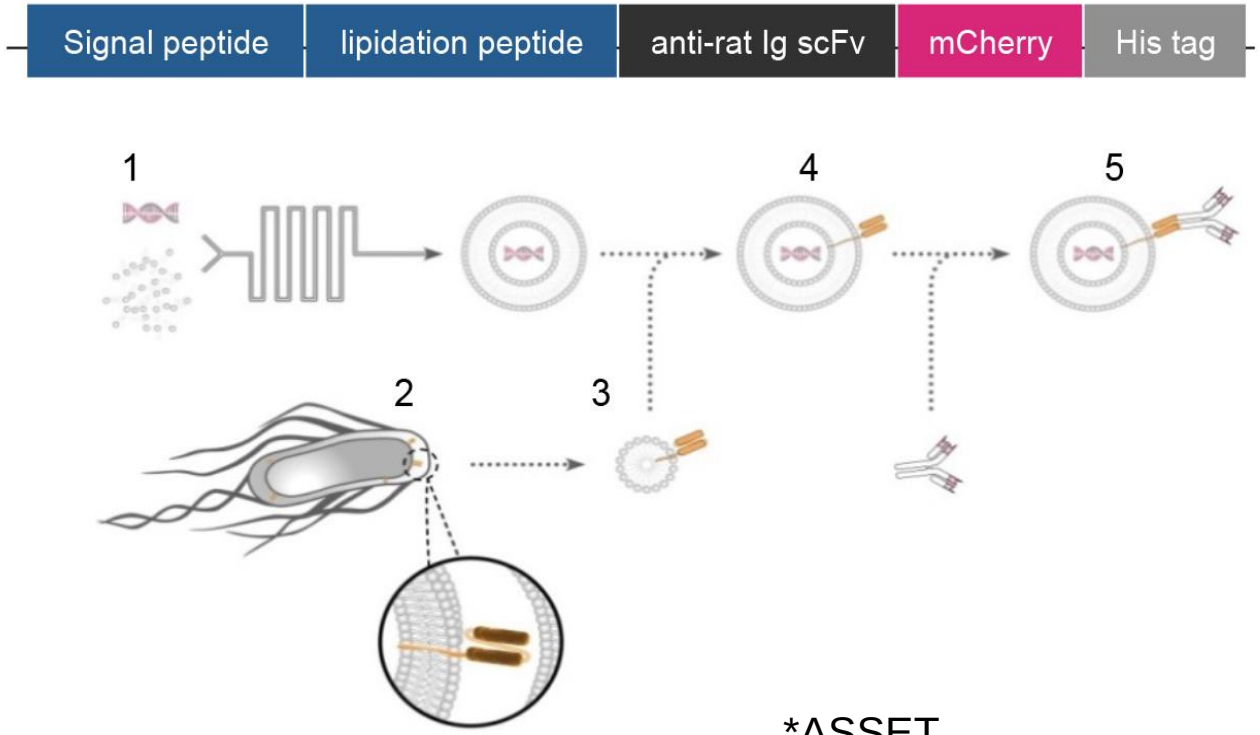




Increase:
Median survival 50%
Overall survival 30%

Systemic delivery to metastatic ovarian adenocarcinoma

- ASSET* incorporation to LNPs.
- Targeting the overexpressed epidermal growth factor receptor (EGFR).




Dr. Ranit Kedmi

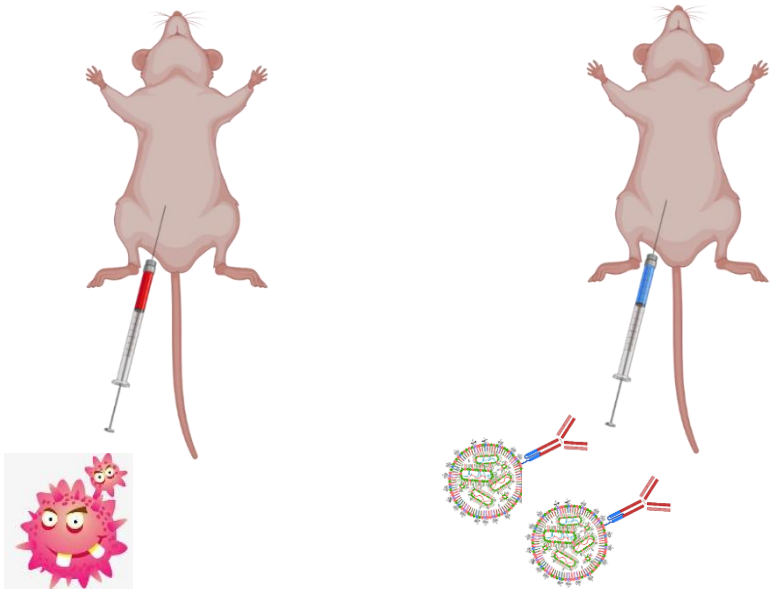

Dr. Nuphar Veiga

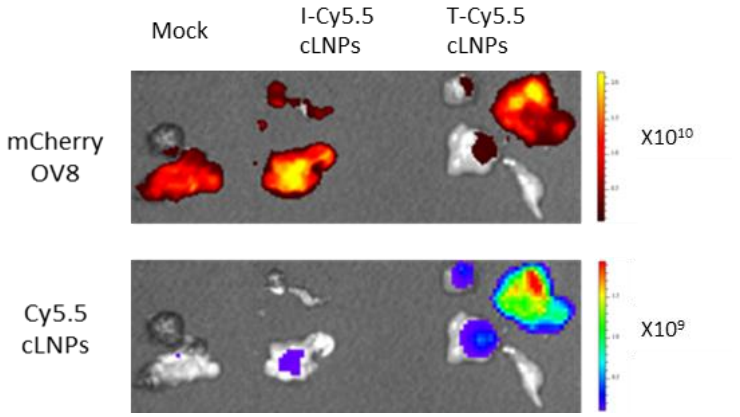
*ASSET
Anchored Secondary ScFv Enabling Targeting

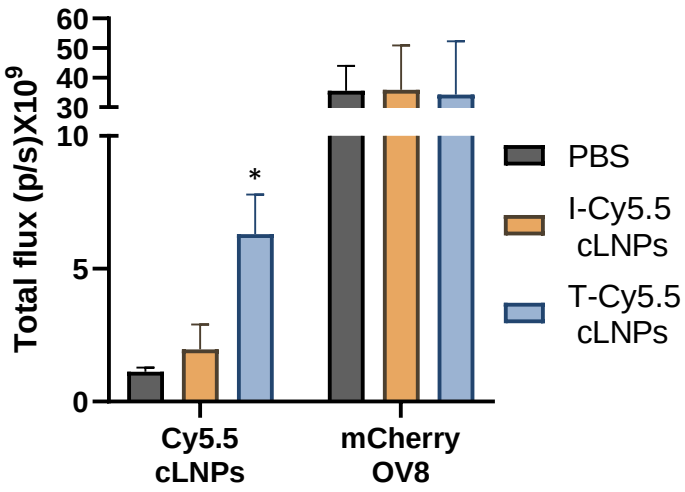
Kedmi R., Veiga N. et al. Nature Nanotechnology 2018

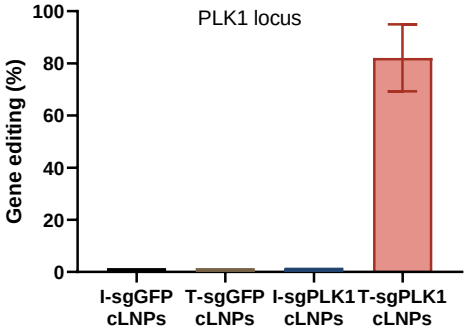
Systemic delivery of targeted cLNPs to metastatic ovarian adenocarcinoma

OV8 cells injection → 10d → cLNPs injection → 4h → Tumor targeting analysis → 3d → Gene disruption analysis







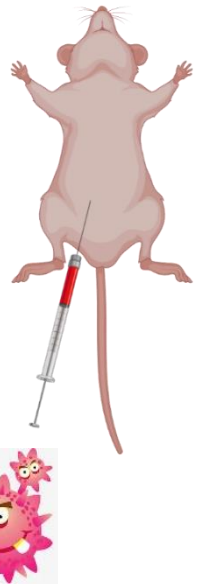


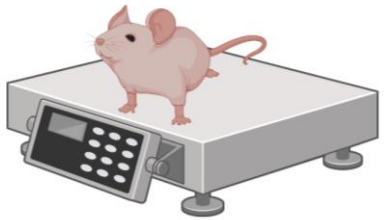
Gene editing at the PLK1 locus- 80%

Rosenblum D., Gutkin A. et. al. Science Advances (2020)

Schematic experimental design: Systemic delivery of targeted cLNPs to metastatic ovarian adenocarcinoma

OV8 cells injection → 10d → cLNPs injection → 60d follow up

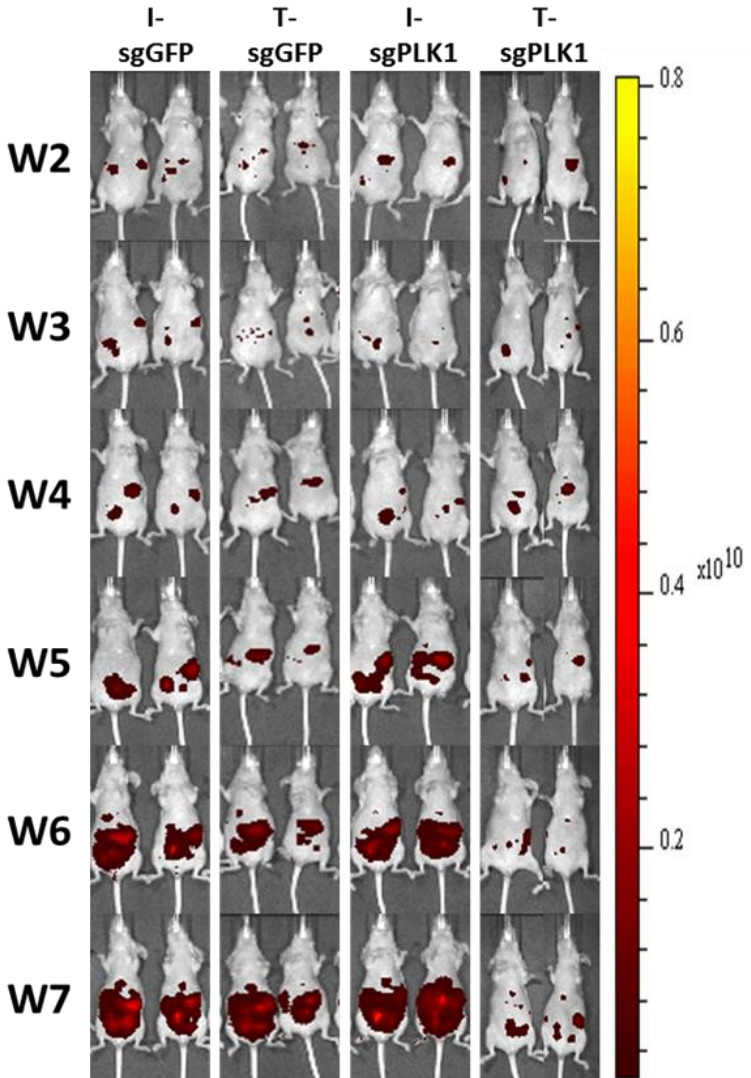


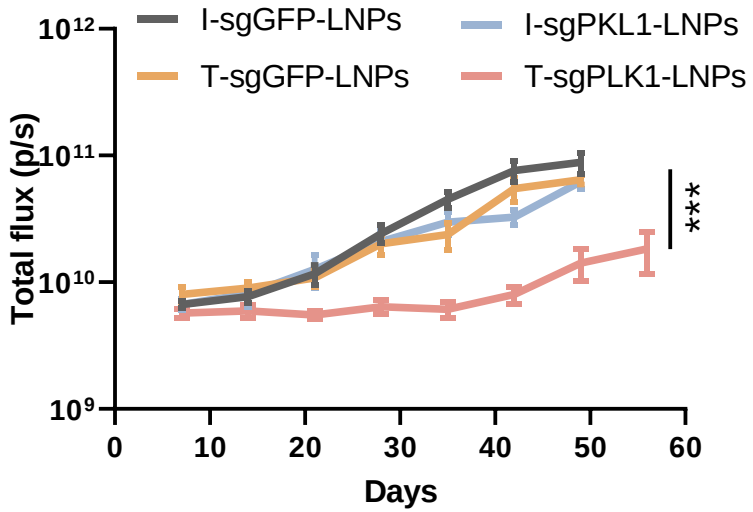


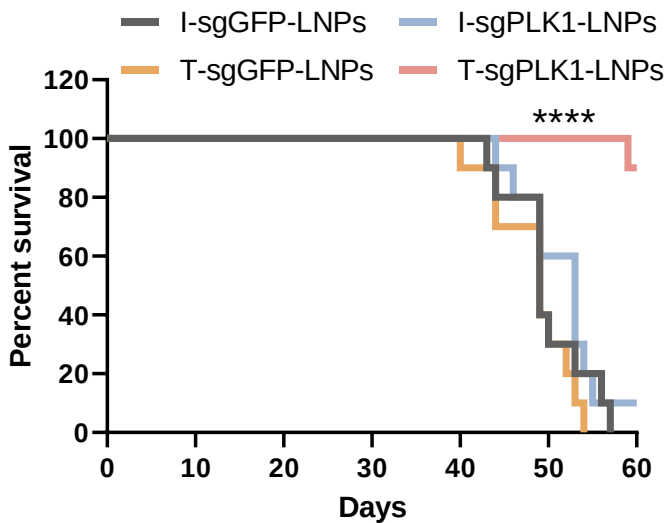
Treatments:

1. I-sgGFP-cLNPs
2. T-sgGFP- cLNPS
3. I-sgPLK1- cLNPS
4. T-sgPLK1-cLNPS

EGFR targeted sgPLK1-cLNPs potently inhibit tumor growth and increased overall survival in metastatic OV8 bearing mice







Increase:
Overall survival 80%

n=10 mice/group

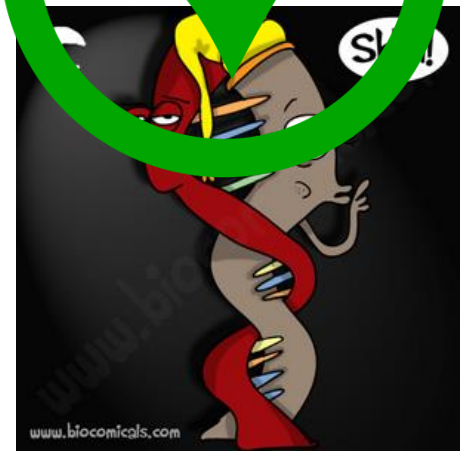
RNA based therapeutics

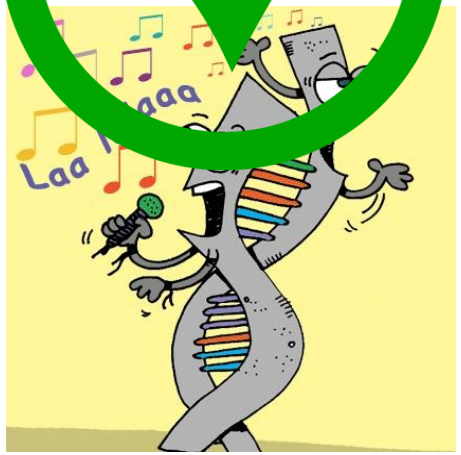
Silence

Activate

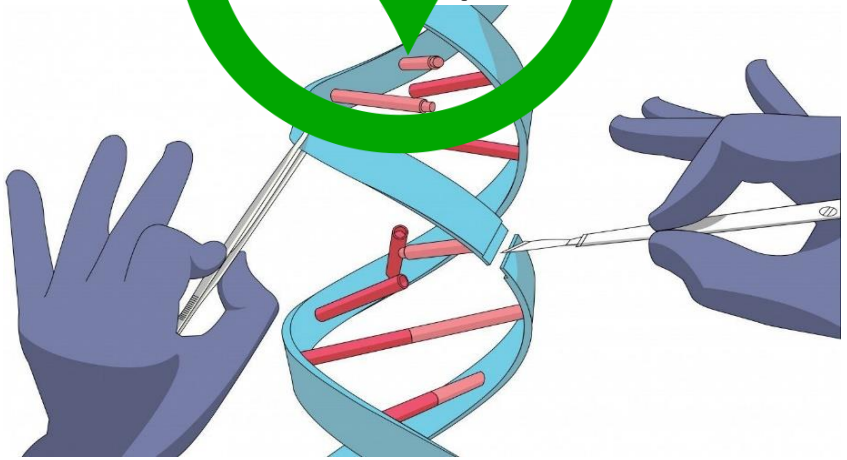
Edit

Replace





CR R/Cas9



Vision

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המרכז לחקר המטאונקולוגי
על שם ורדה ובעז דותן

National Institute of Allergy and Infectious Diseases

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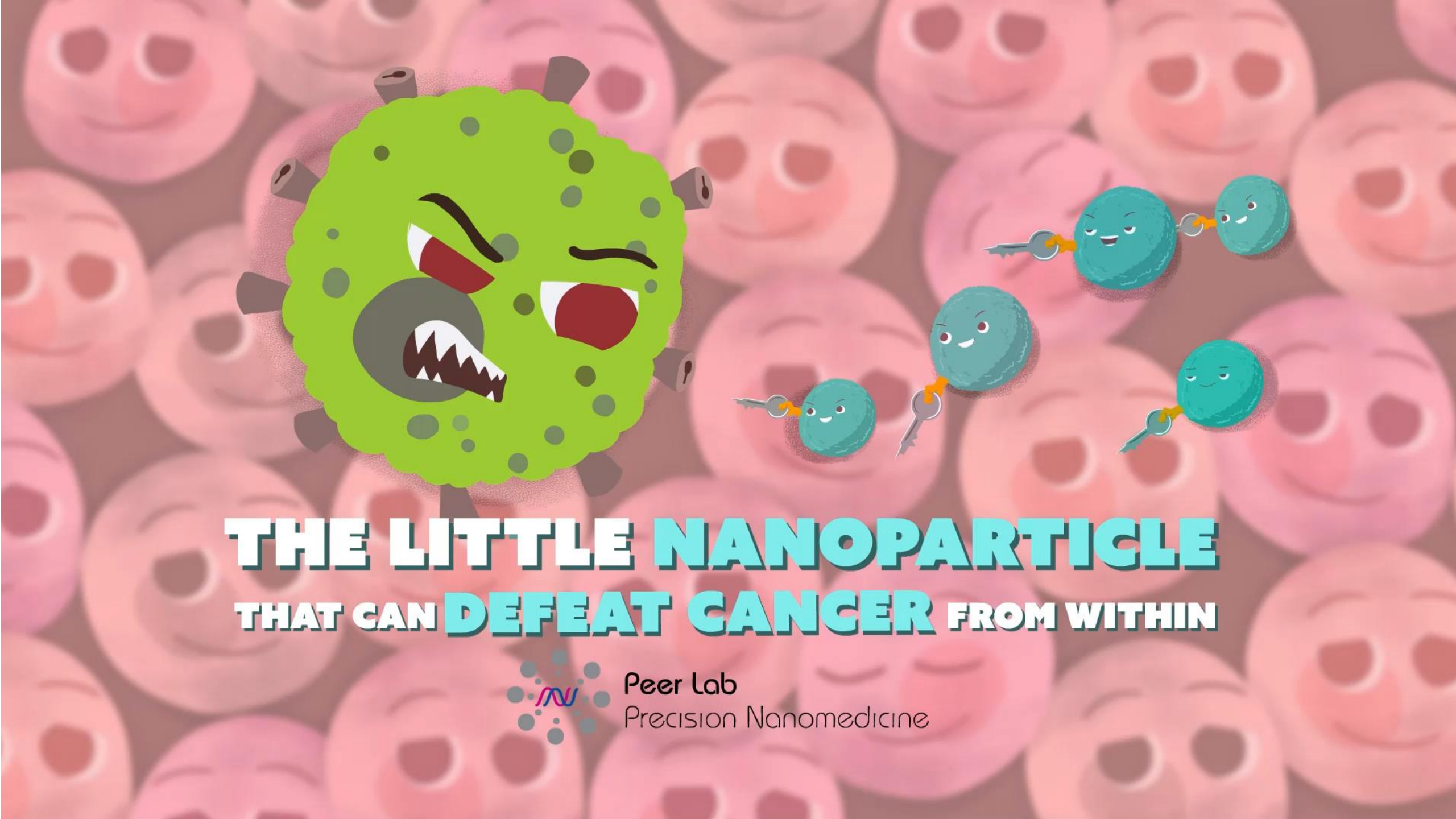
ART BioScience
BRIDGING THE THERAPEUTIC GAP

Alnylam EXPERT


THE LITTLE NANOPARTICLE
THAT CAN DEFEAT CANCER FROM WITHIN
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