Optimized lipid nanoparticles for CRISPR/Cas9 delivery: a breakthrough in targeting KRAS mutations in lung cancer

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PLRC

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Disclosure Statement

Olivia Merkel is a Co-Founder of RNhale, SAB member of Corden Pharma, AMW, and Coriolis Pharma, as well as a Consultant for AbbVie, PARI Pharma, and Boehringer-Ingelheim.

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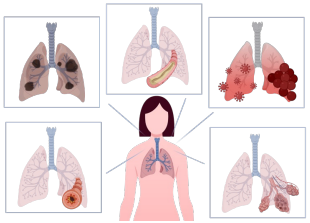
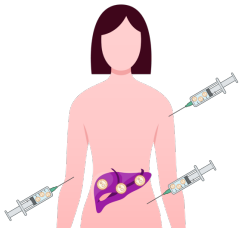
Unmet medical need



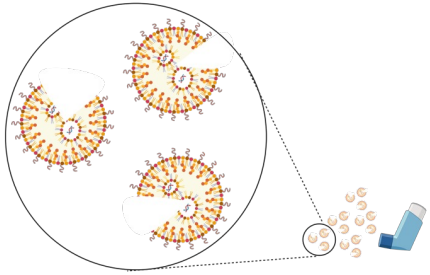
1) Lung Diseases Are Amongst the Leading Causes of Death Globally (WHO)

Solution: RNA Medicines to "Drug the Undruggable"



2) Approved RNA-Nano-Formulations Accumulate in the Liver

Solution: Local Administration Routes



3) Approved RNA-Nano-Formulations Are Not Suited for Inhalation Delivery

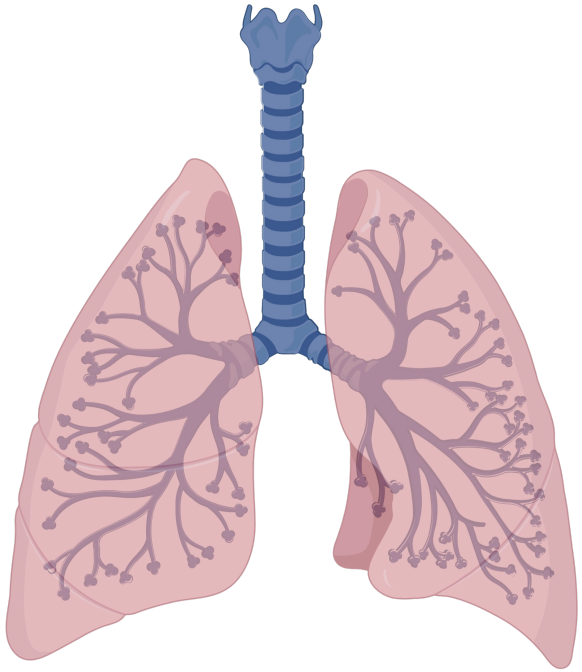
Solution: Biodegradable Polymer Nanoparticles

4) Nanocarrier Materials Are Commonly Optimized by Trial-And-Error Methodology

Solution: Rational and Computational Predictions

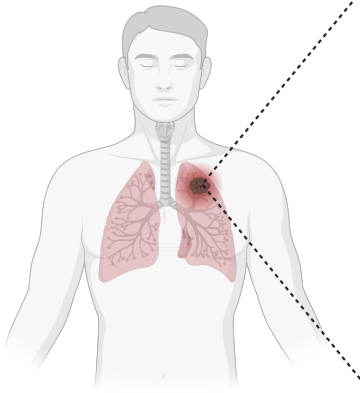
Pulmonary drug delivery

- ✓ Non-invasive
- ✓ <u>Local delivery</u>
  FPE ⇩
  Systemic side effects ⇩
- ✓ Lung retention ⇧
- ✓ <u>Physiology</u>
  Large surface area
  High vascularity
  No serum proteins
  Thin epithelium

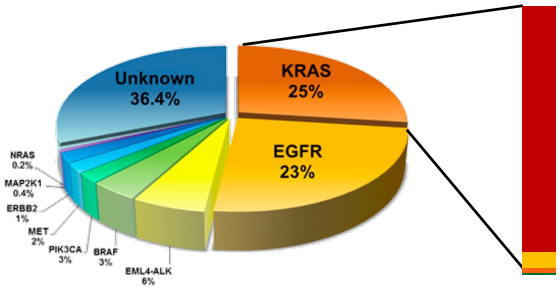


- ➢ Mucus / Surfactant
- ➢ Size requirements

Lung cancer



- A leading cause of cancer-related deaths worldwide
- Diagnosis at a later stage ⟶ hard to implement treatment ⟶ low survival rate



Codon 12 (91%) Codon 61 (2%)
Codon 13 (6%) Others (0.5%)

KRAS mutations:

- 15–30% of lung cancer cases
- Limited effectiveness and resistance to standard therapies (tyrosine kinase inhibitors) ⟶ poor prognosis and reduced survival
- Undruggable target ⟶ Sotorasib and Adagrasib

(Siegel et al., 2018; Dogan et al., 2012; Ferrer et al., 2018)

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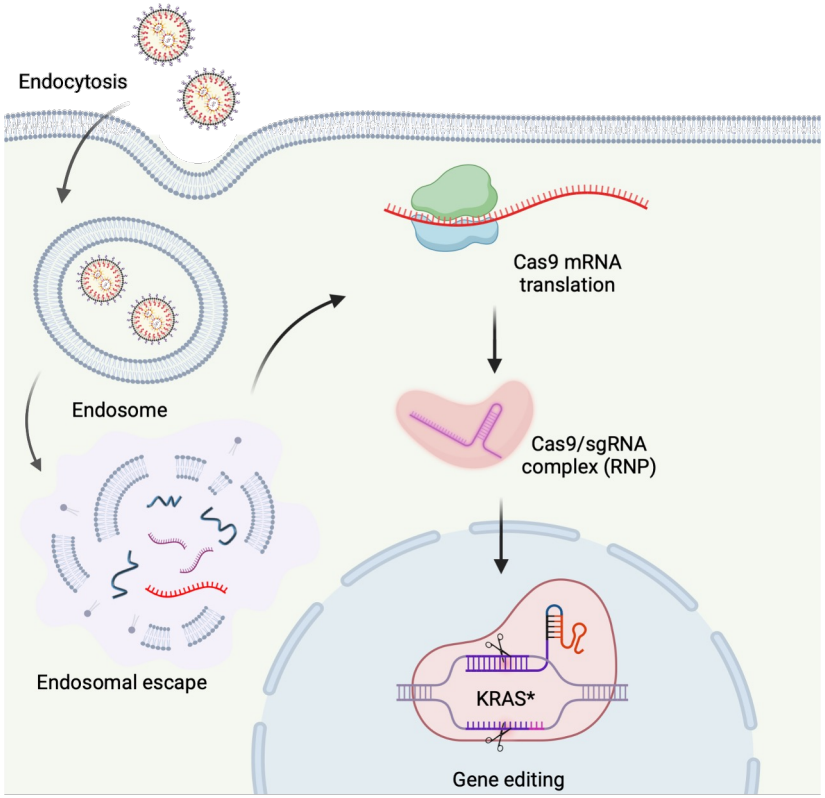
KRAS and CRISPR/Cas9

CRISPR/Cas9-NPs for the precise editing of KRAS mutations



(Lenova et al., 2020. Chen et al., 2025)

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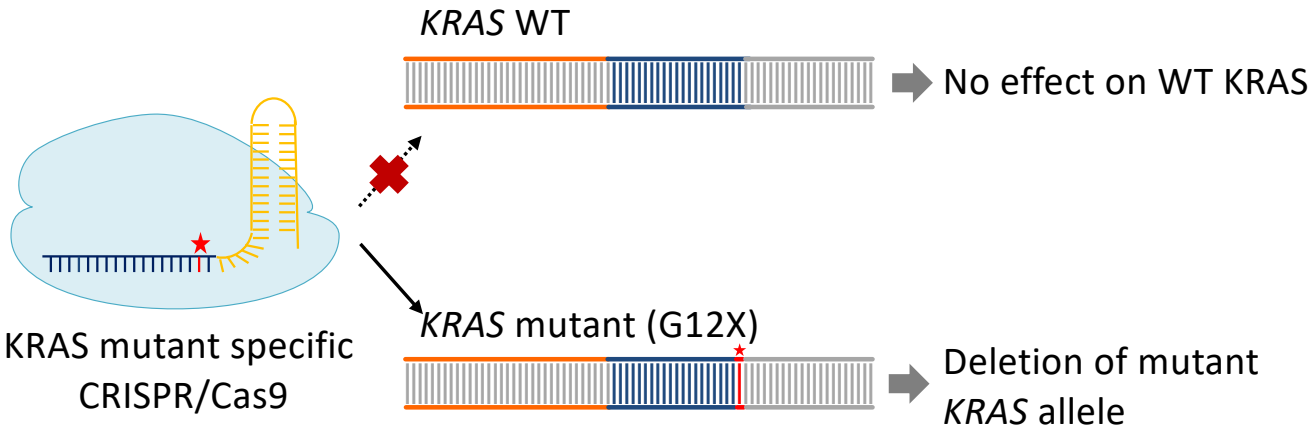
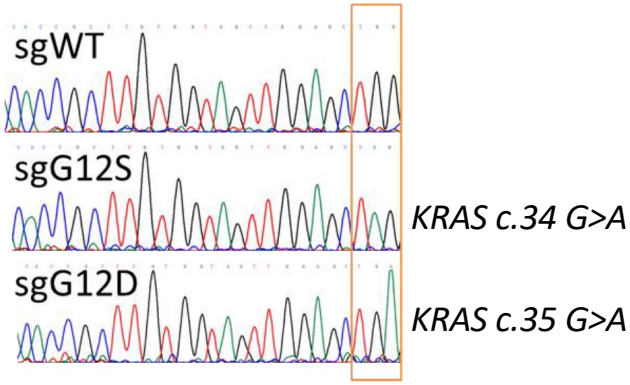
Allele specific deletion of KRAS mutants





(Mehta et al., unpublished)

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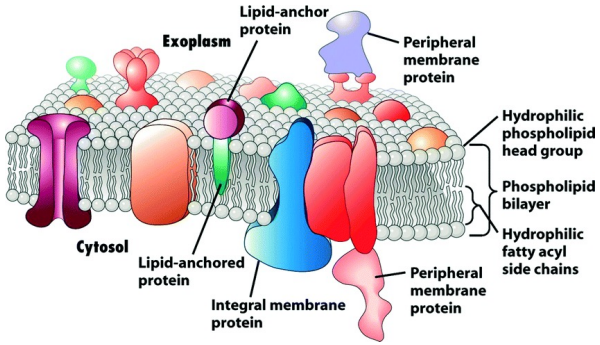
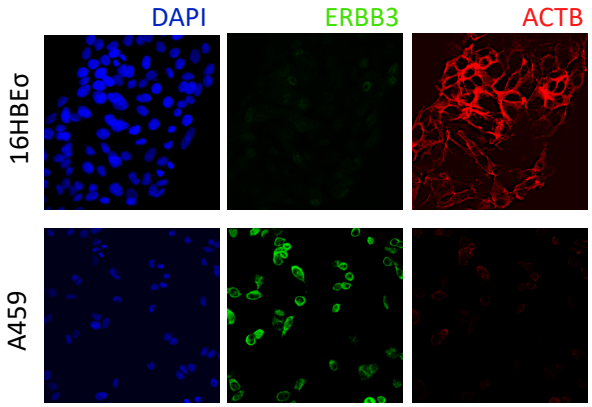
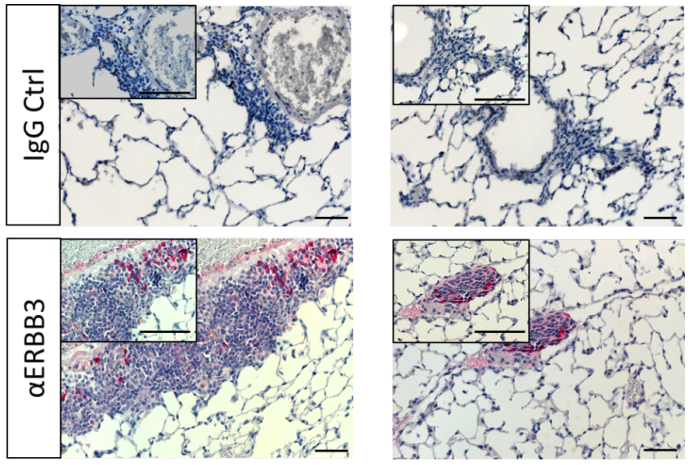
Membrane protein signature





Intratracheal Adenoviral CRE → 4m → Histology

KRAS LSL G12D



Scale 50µm

(Mehta et al., unpublished)

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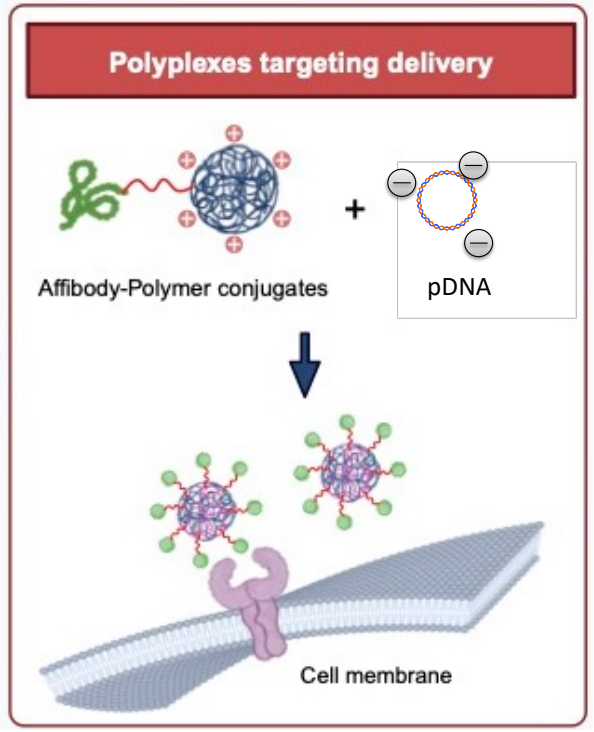
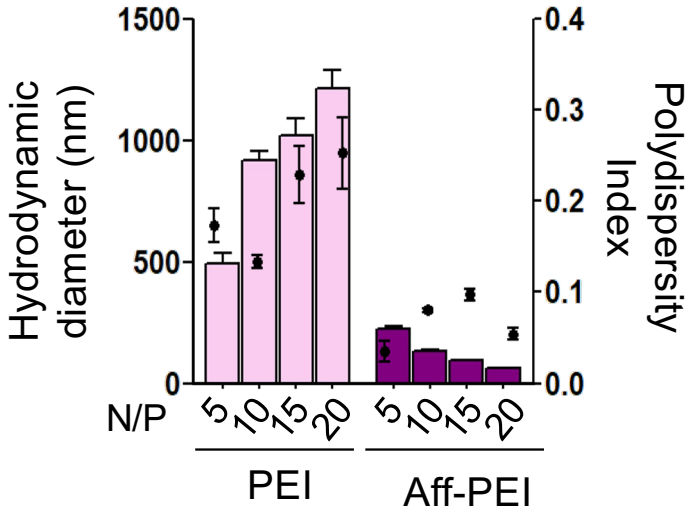
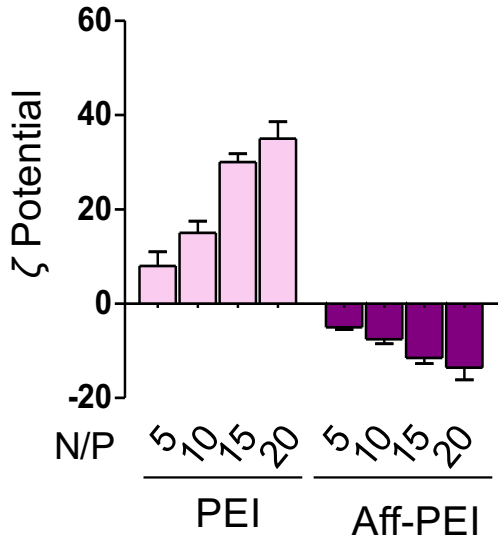
Polyplex characterization







(Mehta et al., unpublished)

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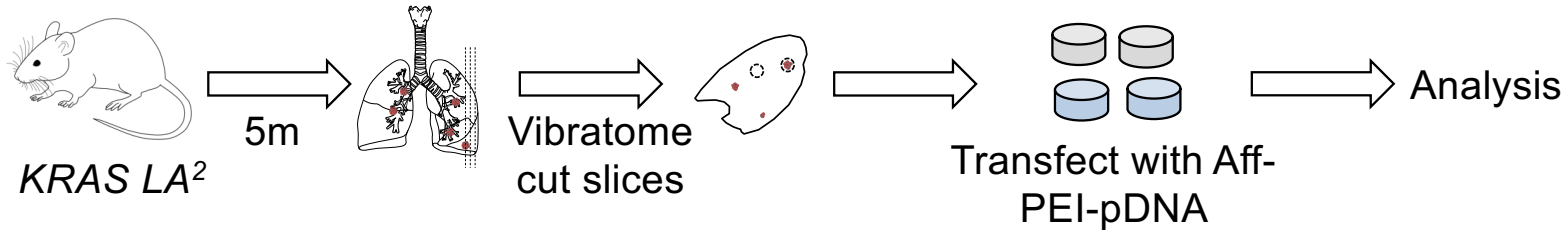
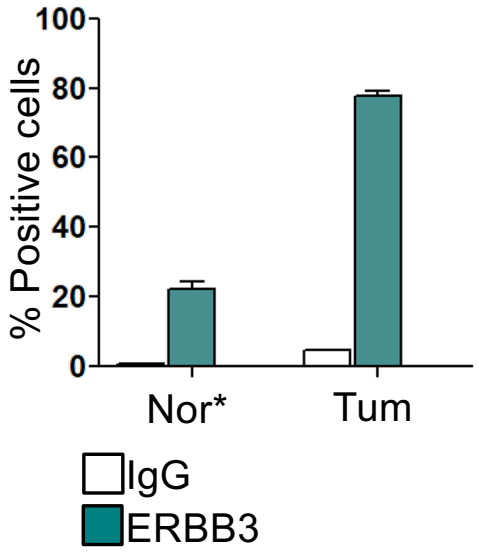
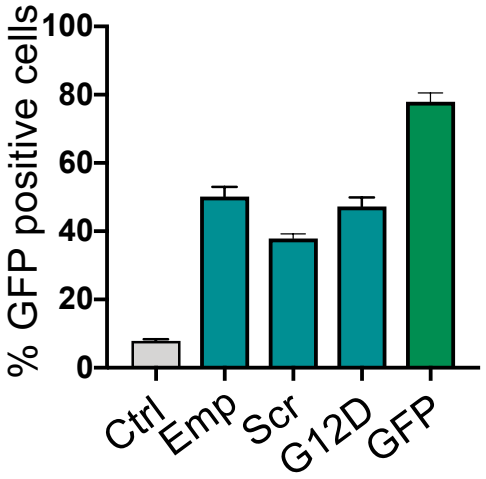
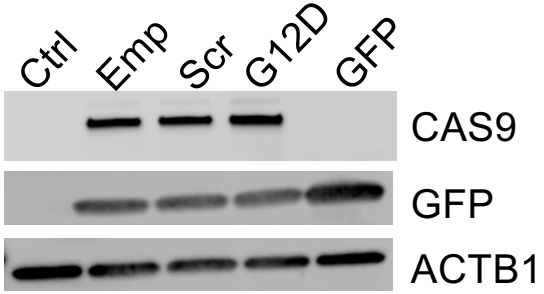
Tissue targeting









(Mehta et al., unpublished)

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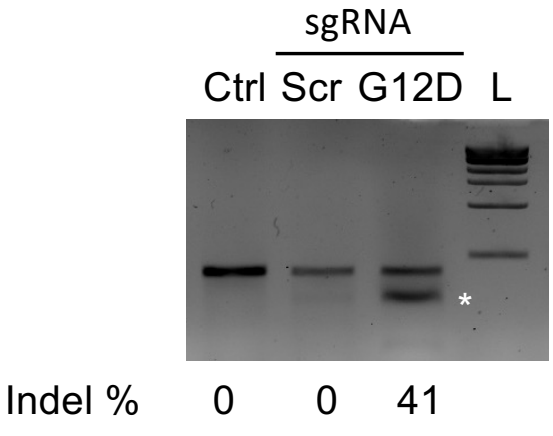
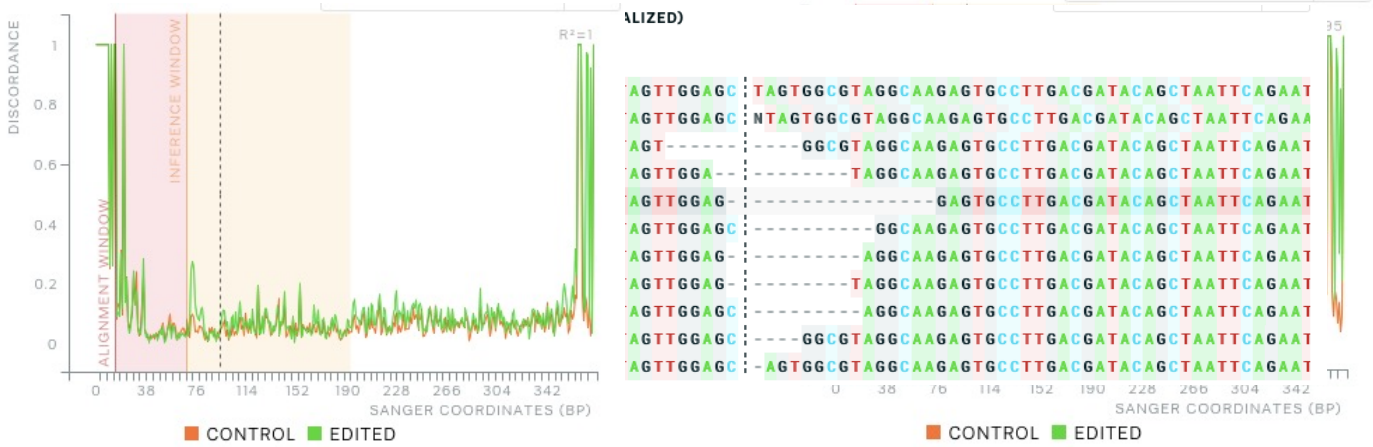
Tissue CRISPR activity





(Mehta et al., unpublished)

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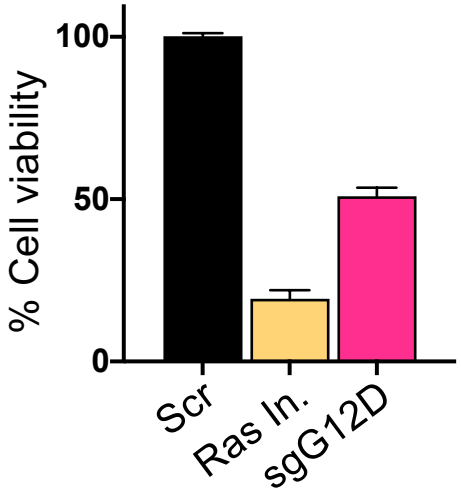
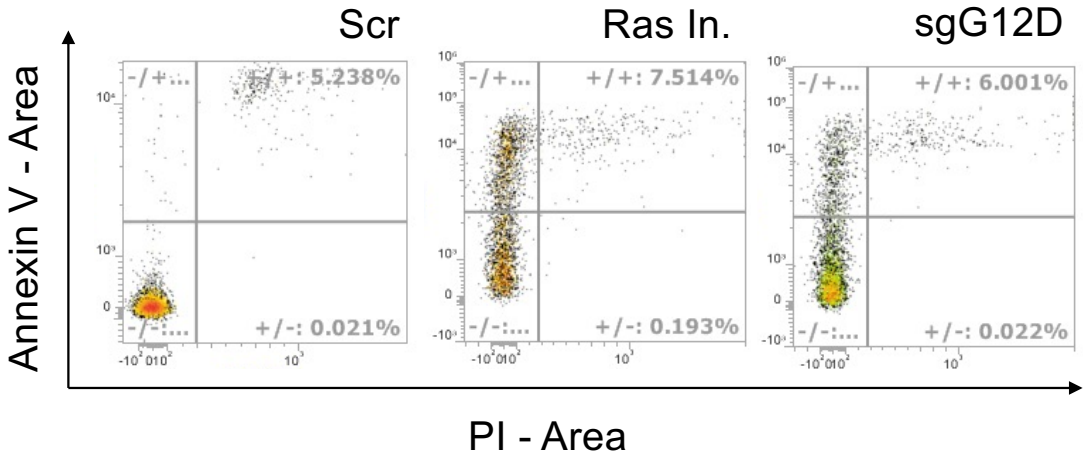
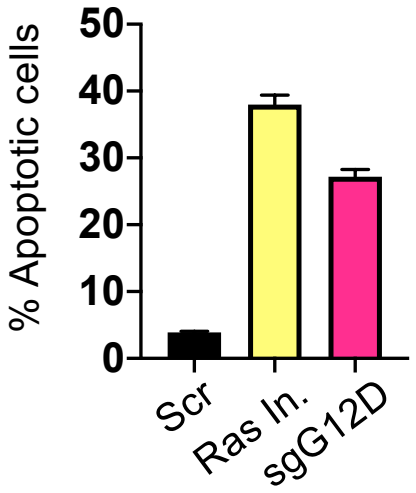
Tissue CRISPR activity







(Mehta et al., unpublished)

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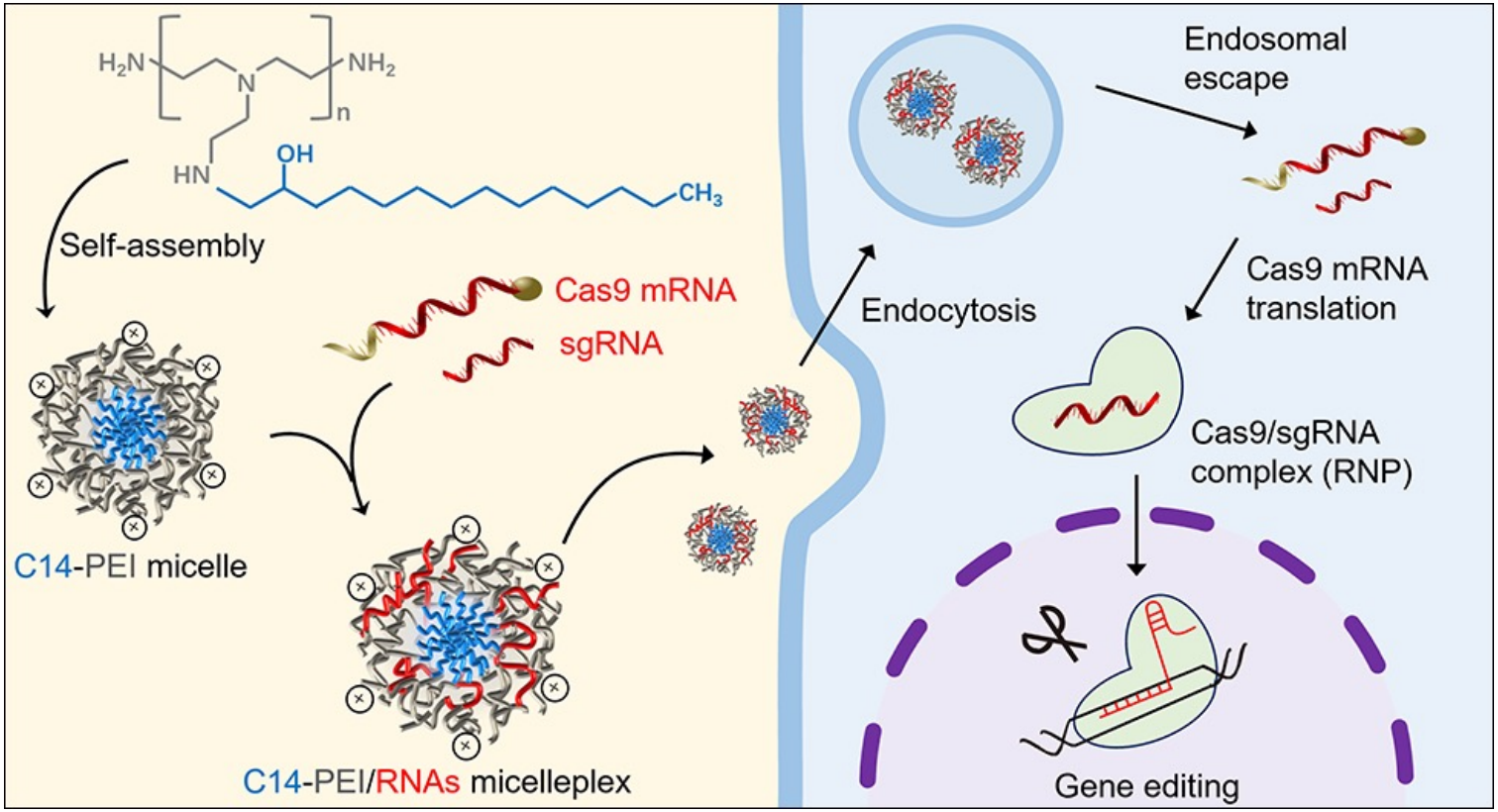
CRISPR/Cas9 mRNA/sgRNA Polyplexes



(Chen et al., Nanoscale 2025)

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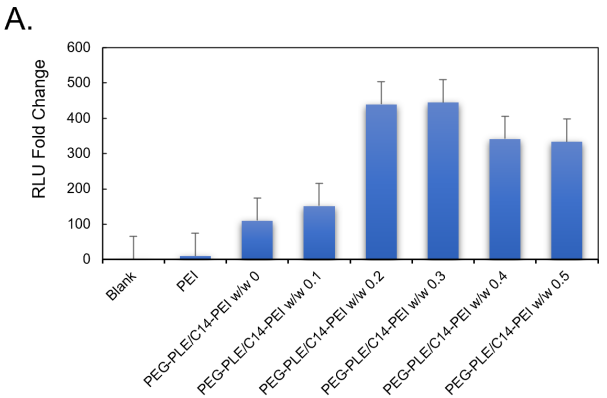
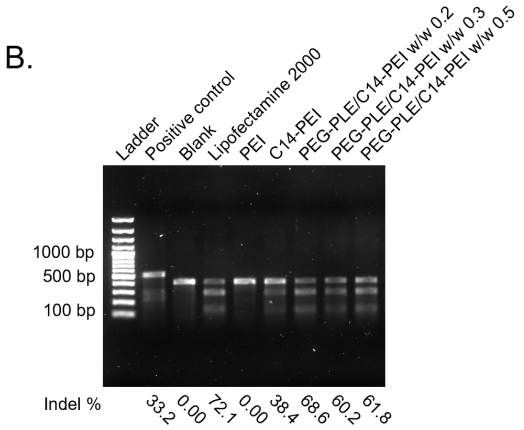
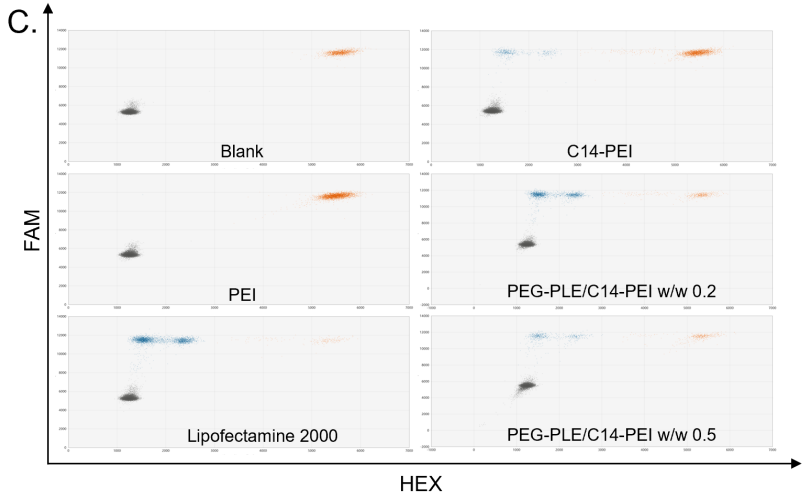
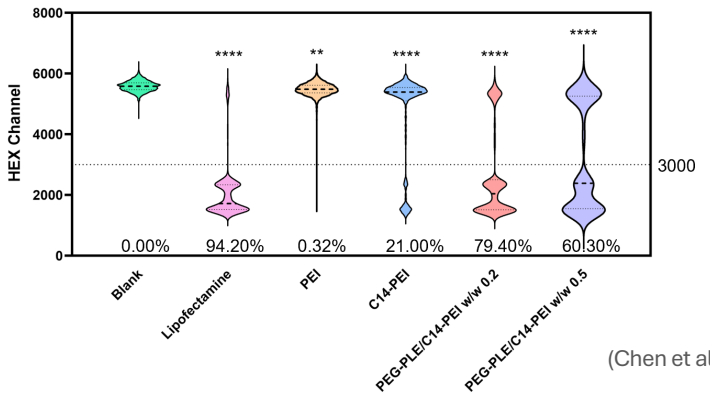
In vitro CRISPR activity









(Chen et al., Biomaterials Science 2025)

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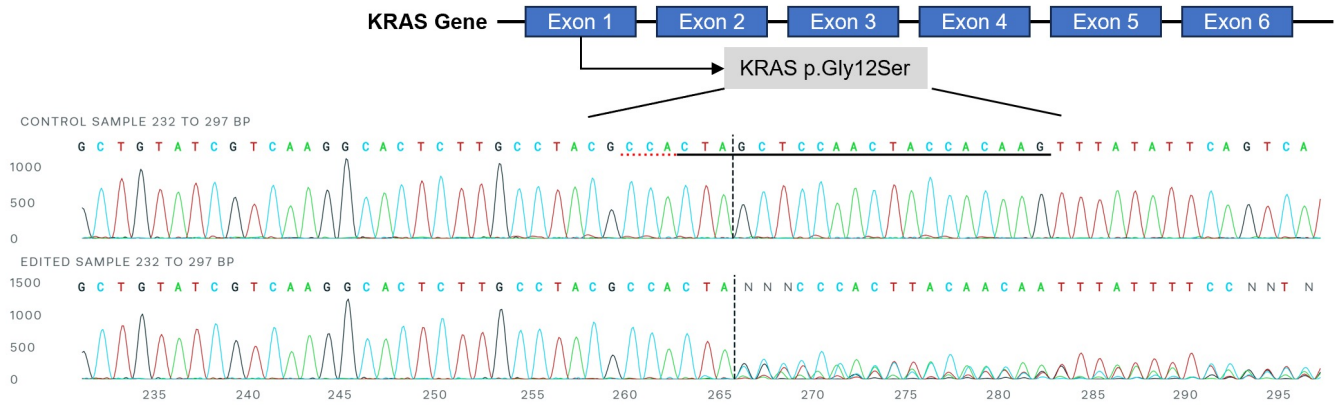
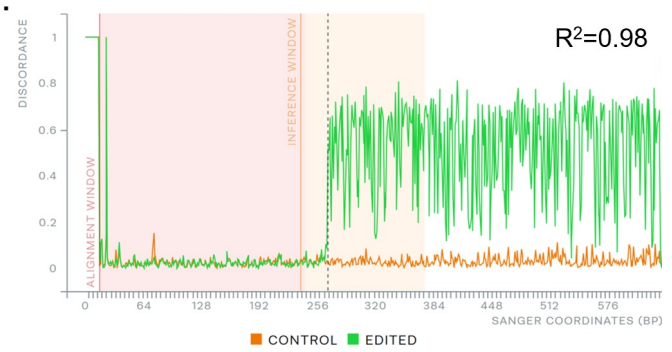
In vitro CRISPR activity

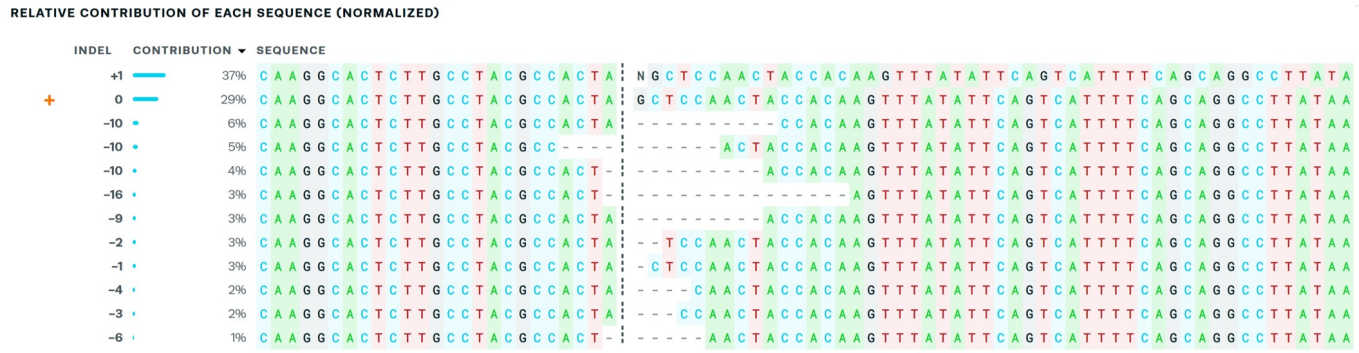
A.



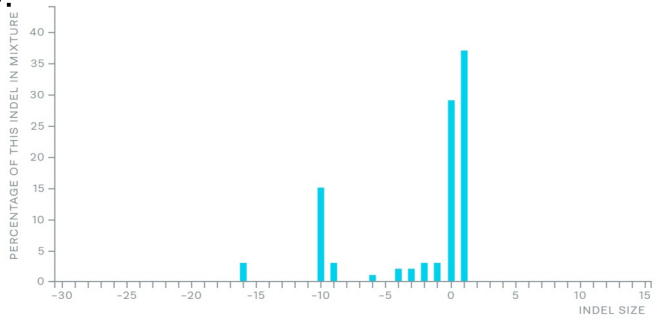
B.



D.



C.



(Chen et al., Biomaterials Science 2025)

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In vitro CRISPR activity

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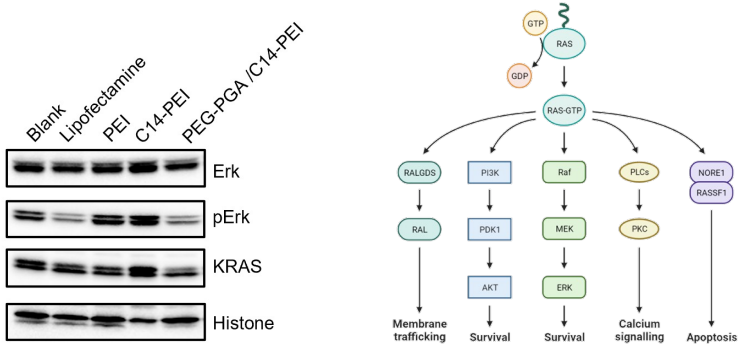

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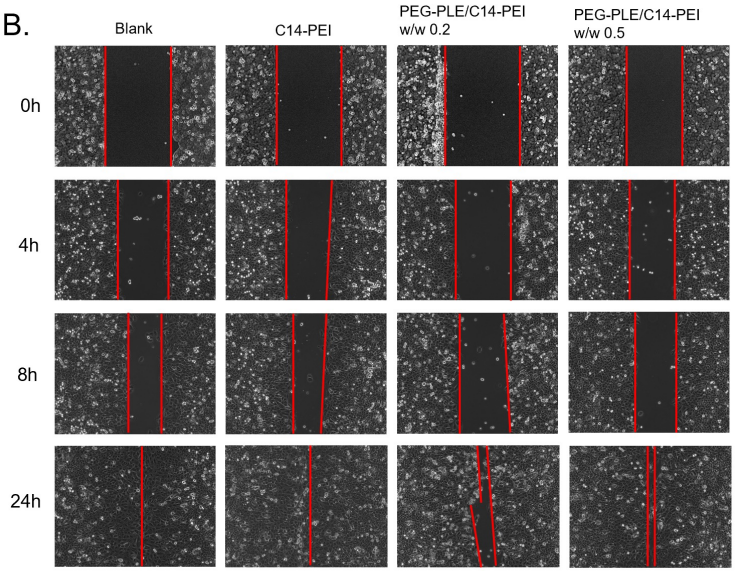

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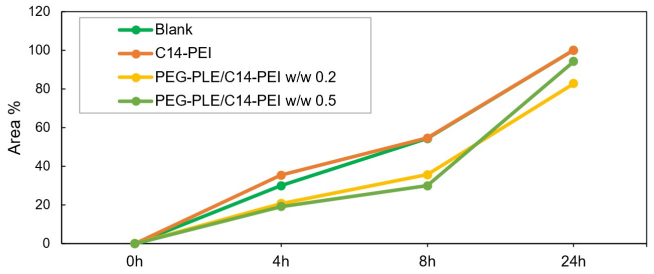
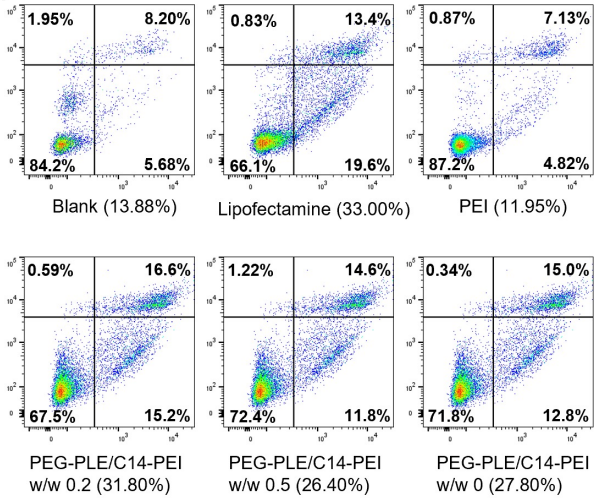


D.



Chen et al., Biomaterials Science 2025)

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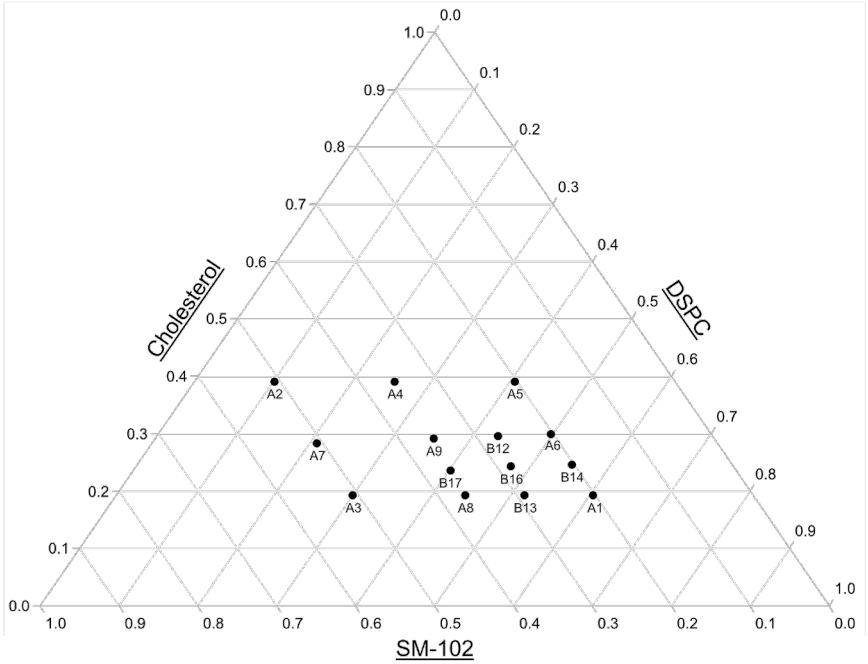
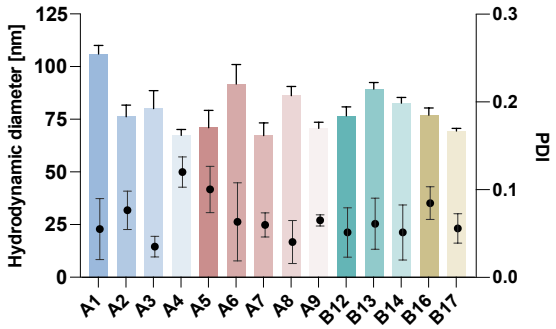
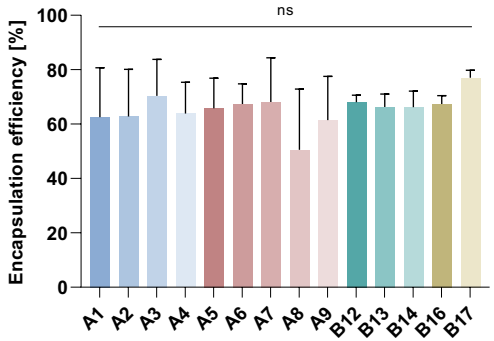
Optimization of mRNA-LNPs

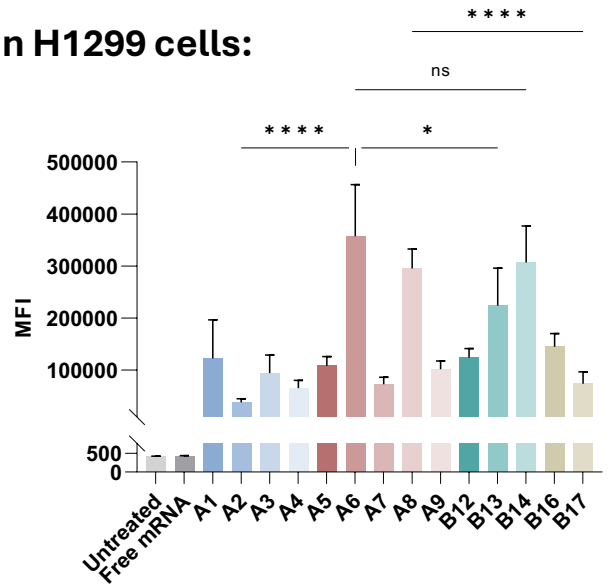
Screening lipid compositions:







eGFP expression in H1299 cells:



(Carneiro et al., subm.)

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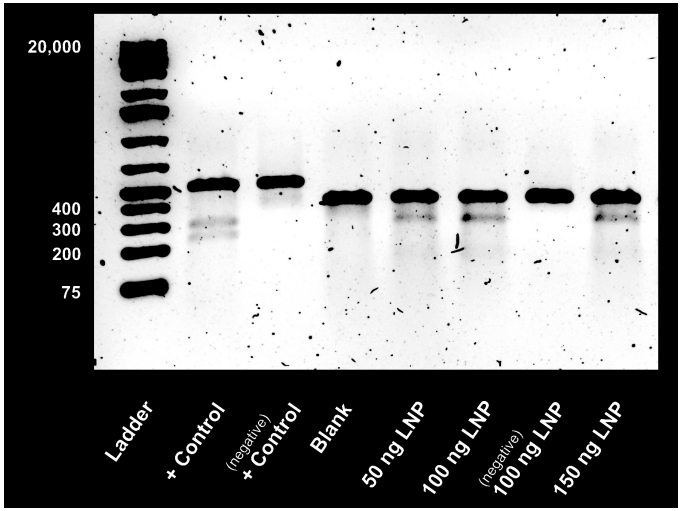
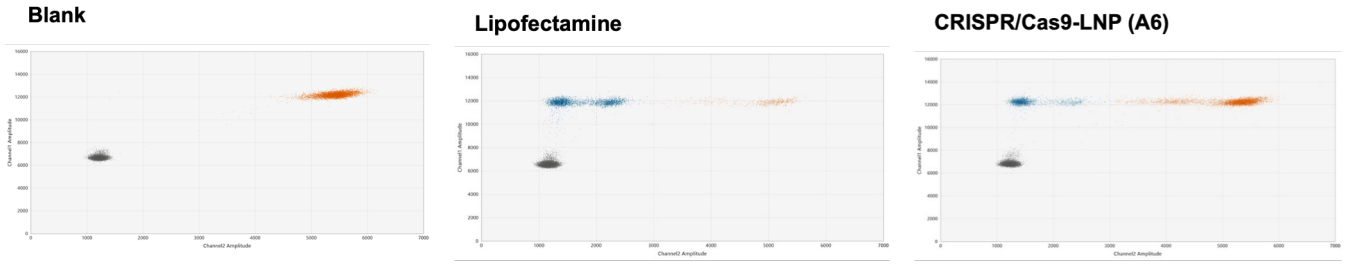
CRISPR/Cas9-LNPs

Gene editing efficacy of A6 in A549 cells

T7EI indel detection





Sample	Gene editing efficacy (%)
Blank	0.04
Lipofectamine	87.20
CRISPR/Cas9-LNP (A6)	35.50

(Carneiro et al., subm.)

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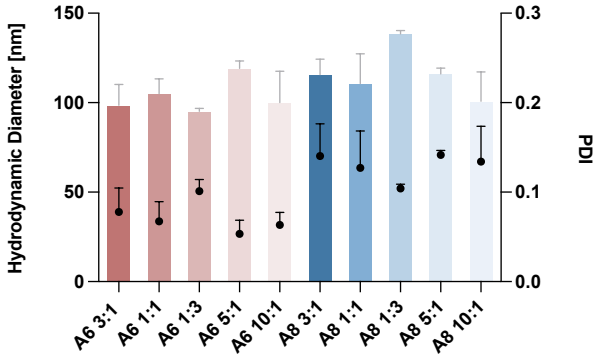
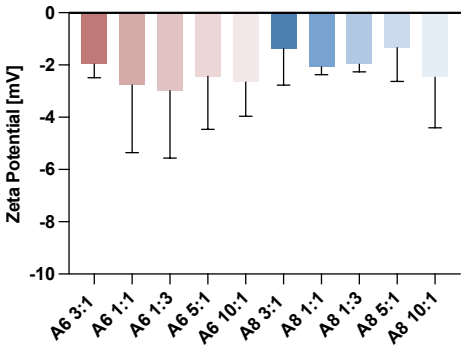
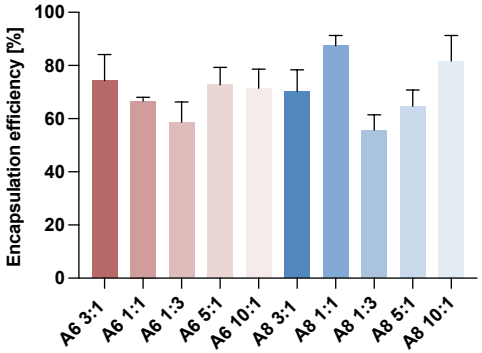
CRISPR/Cas9-LNPs

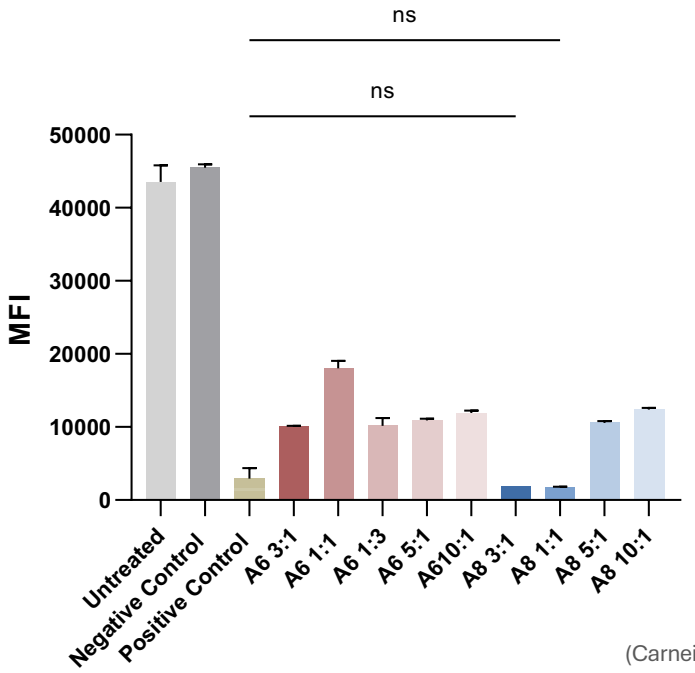
Optimization of mCas9:sgRNA w/w ratio:







eGFP knockout in eGFP-HeLa cells



(Carneiro et al., subm.)

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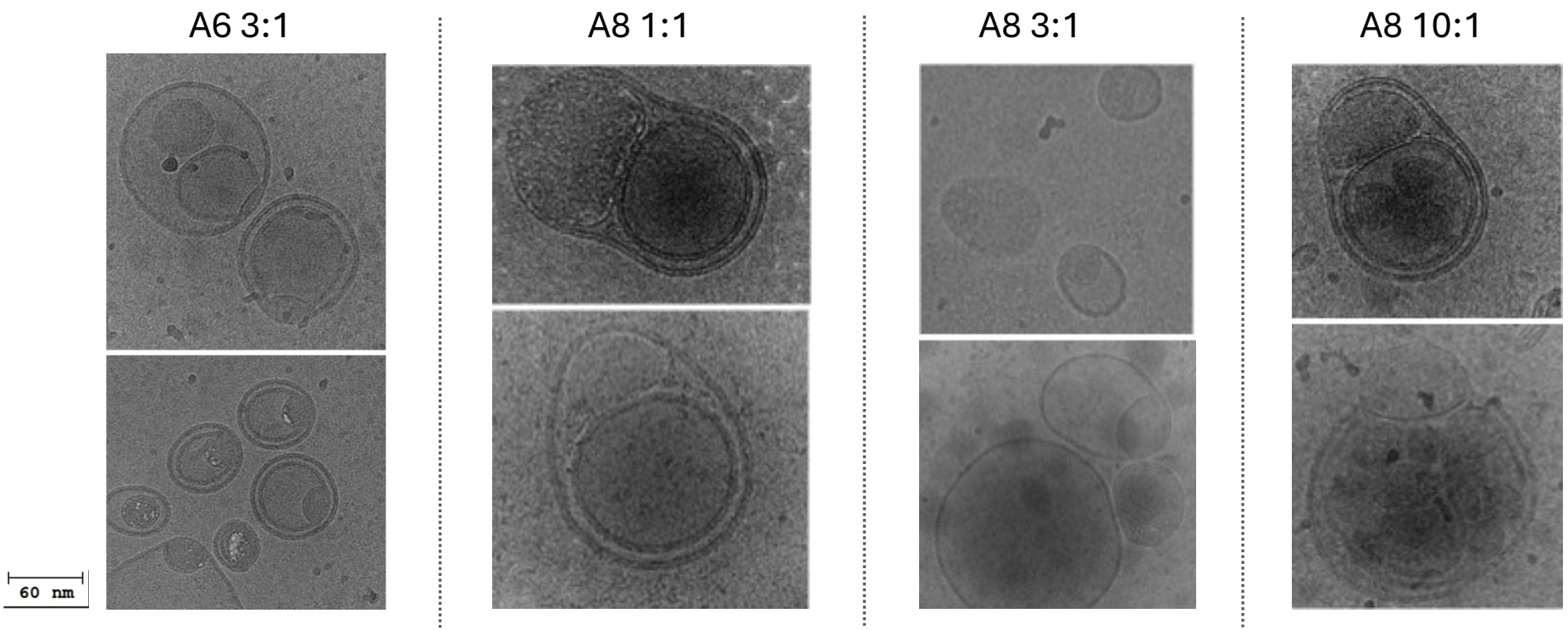
CRISPR/Cas9-LNPs



(Carneiro et al., subm.)

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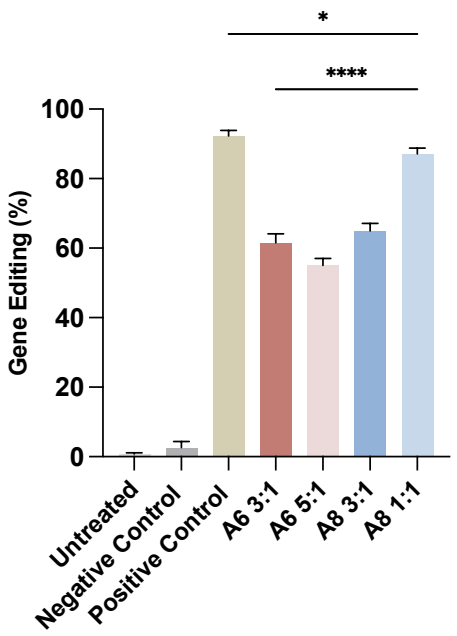
Optimization of mCas9:sgRNA w/w ratio

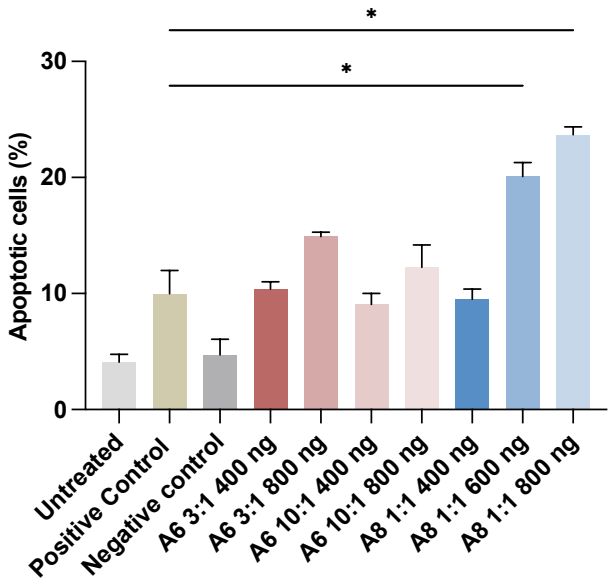
Gene editing efficiency in A549 cells



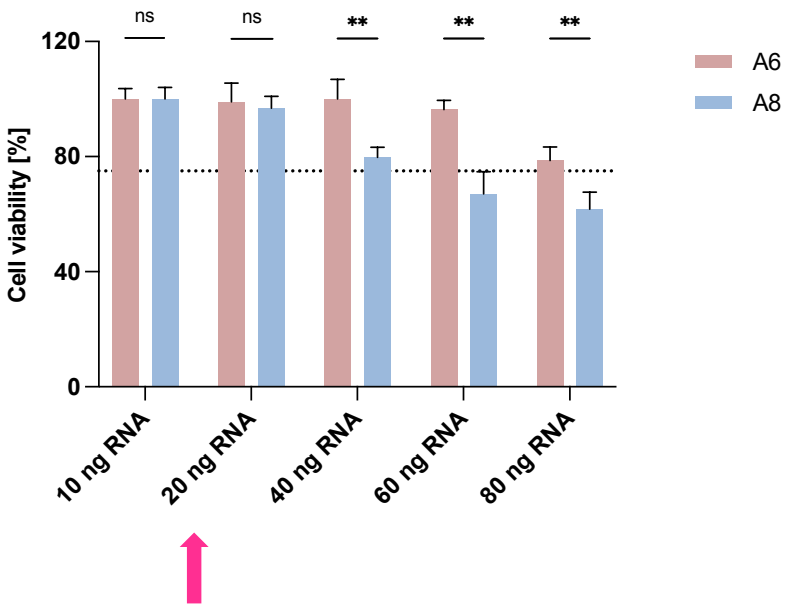
(Carneiro et al., subm.)

Optimization of mCas9:sgRNA w/w ratio

Cell apoptosis

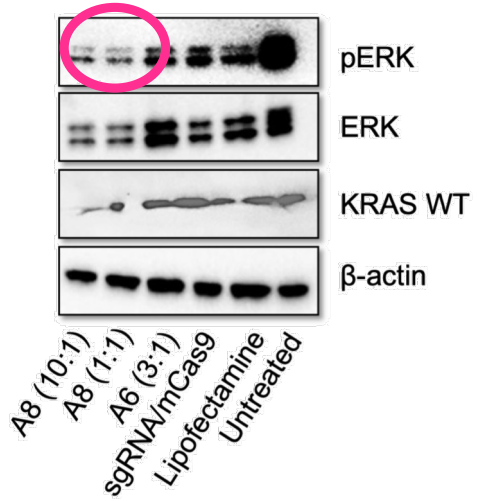


Cytotoxicity



Working concentration

Efficacy



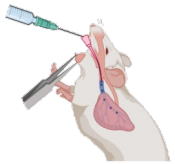
(Carneiro et al., subm.)

Toxicity evaluation in an animal model

- Goal: to obtain the maximum tolerated dose after a single administration (intratracheal)

Day 1 — **Day 21**

Start of the experiment



Intratracheal administration

Dose:
1 mg/kg, twice a week

Healthy Swiss mice

N = 12 animals
Group 1: Untreated (PBS)
Group 2: NC-LNPs
Group 3: LNP A8 (1:1)
Group 4: LNP A6 (3:1)

Days 1 – 21

Clinical observation
Body weight recorded twice a week, fur condition, respiratory rate, behavior

Endpoint

Surviving animals underwent hematological and clinical biochemistry analysis

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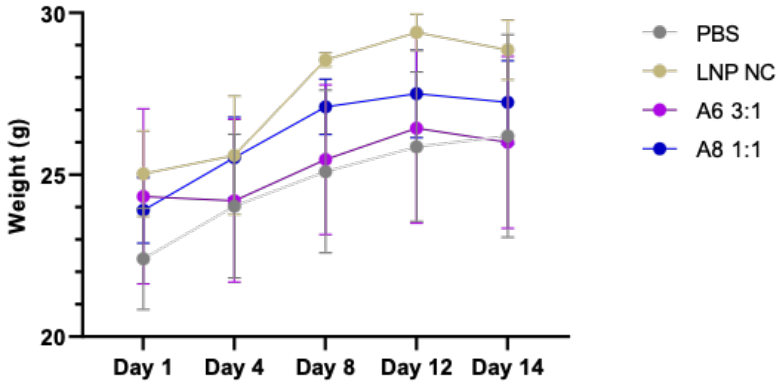
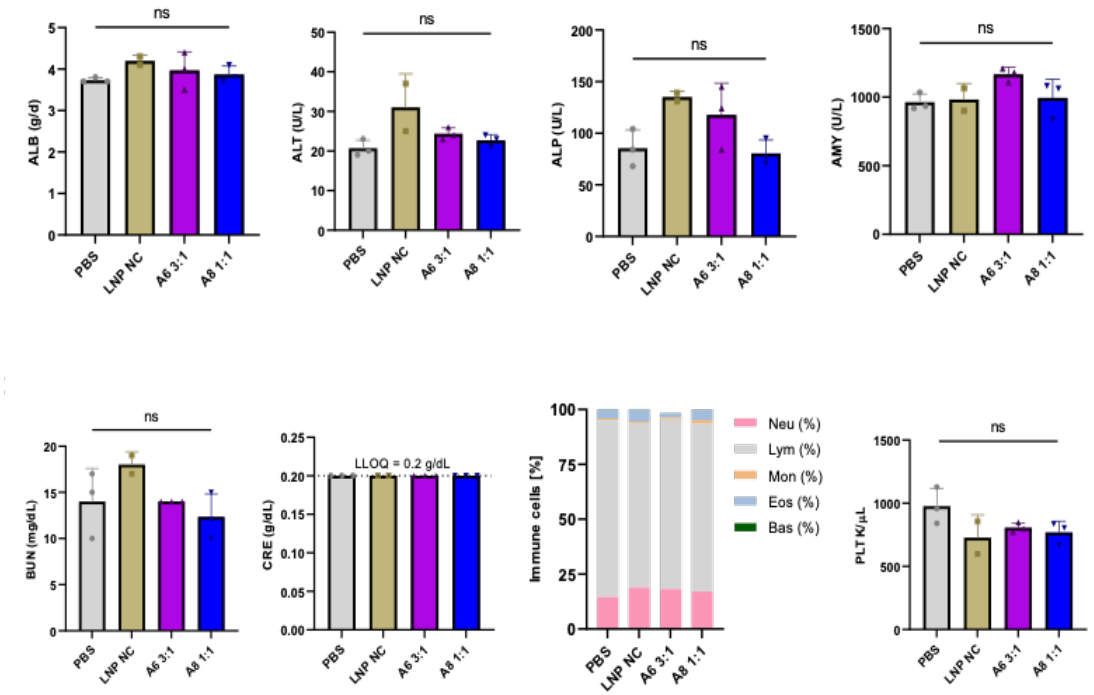
Toxicity evaluation in an animal model

- No signs of toxicity were reported after repeated administration of the best-performing formulations (A6 3:1 and A8 1:1) in healthy animals (N = 12)





(Carneiro et al., subm.)

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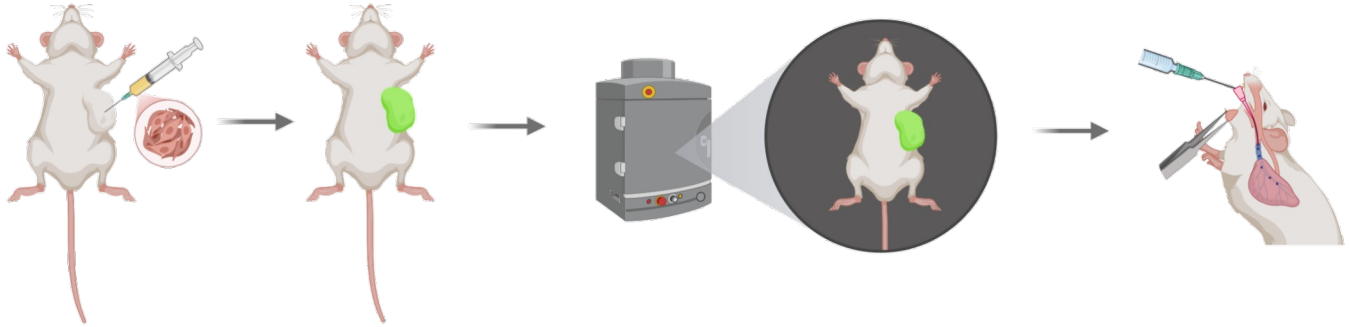
Gene editing evaluation in an animal model

- Efficacy study (ongoing):
  - Goal: to evaluate the anti-tumor effect of experimental treatments using an orthotopic mouse model of lung cancer



Tumor injection
(Injection of 10^6 A549-Luc cells into the intercostal space of the left lung in immunocompromised mice)

(Carneiro et al., subm.)

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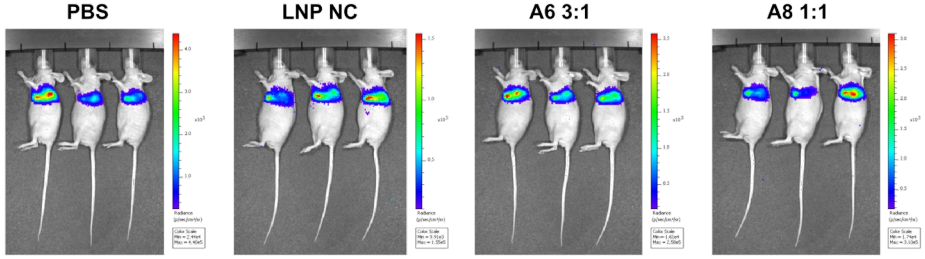
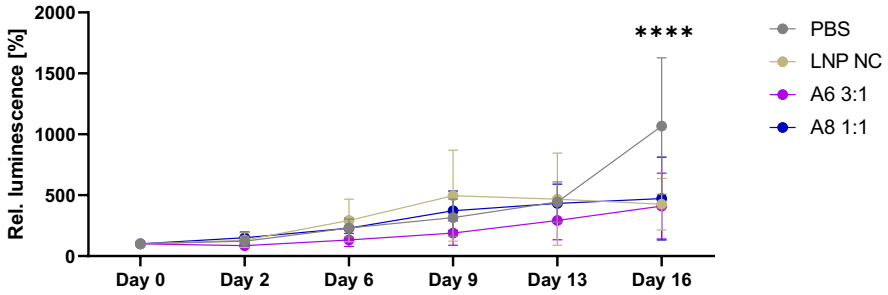
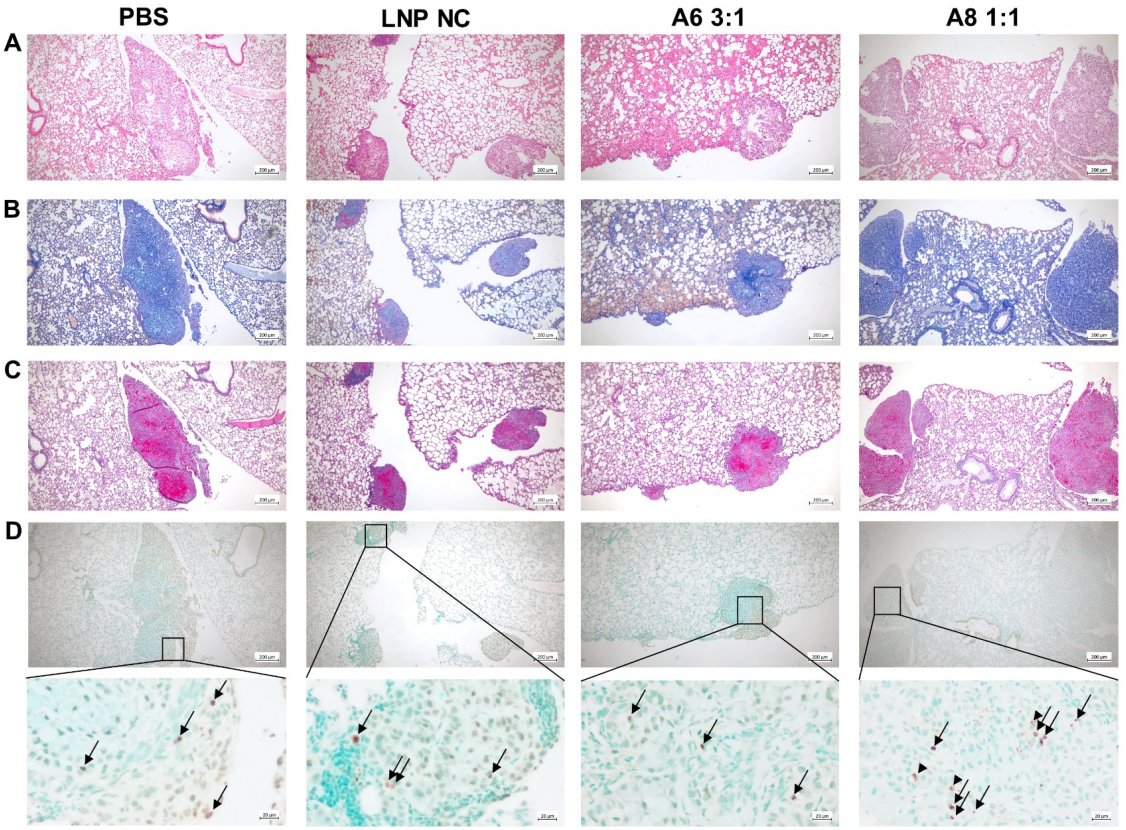
Efficacy evaluation in an animal model







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Summary and outlook

- Summary:
  - The potential of CRISPR-Cas9 delivery in KRAS mutated lung cancer
  - Efficacy testing in *in vitro*, *ex vivo*, and *in vivo* models

- Outlook for pulmonary RNA delivery
  - Ongoing assessment in *in vivo* disease model
  - Nebulization and spray drying of the formulation

- Aim: Development of nanoparticle based nucleic acid therapies

Thank you!

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