Degradable cyclic amino alcohol ionizable lipids as vectors for potent influenza mRNA vaccines

CRS 2025

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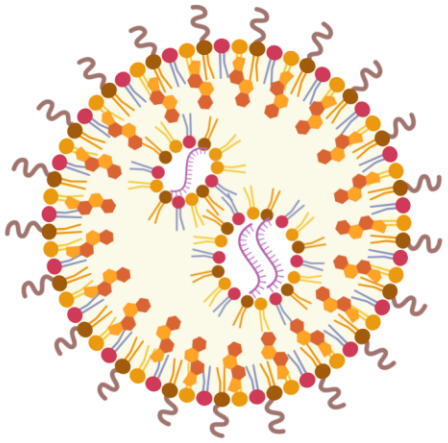
Components of a vaccine

mRNA vaccines

Delivery Component



LNPs

mRNA

Immunostimulatory pathways

cytokine release

cellular reprogramming

Viral protein/ neoantigen

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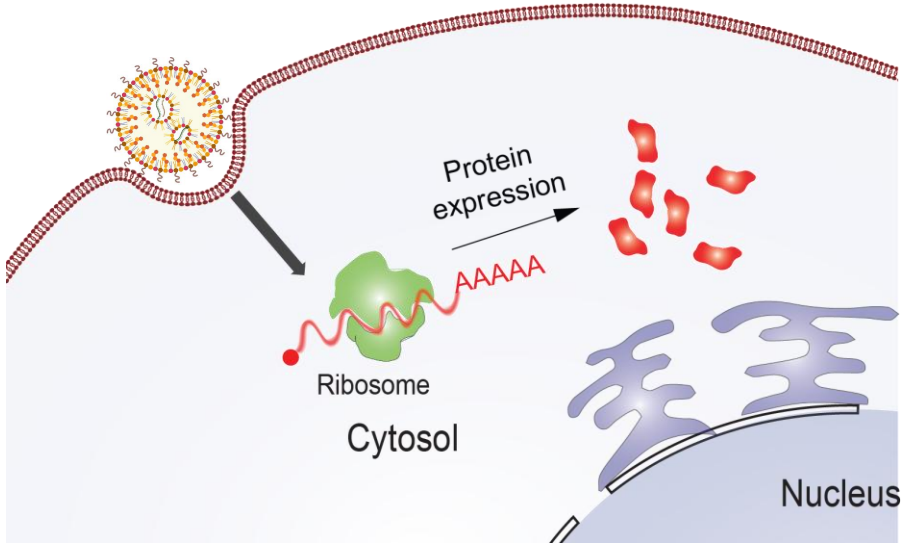
Gupta, A. et al. Nat Rev Drug Discov 23, 914–938 (2024).

Delivery of mRNA vaccines

mRNA

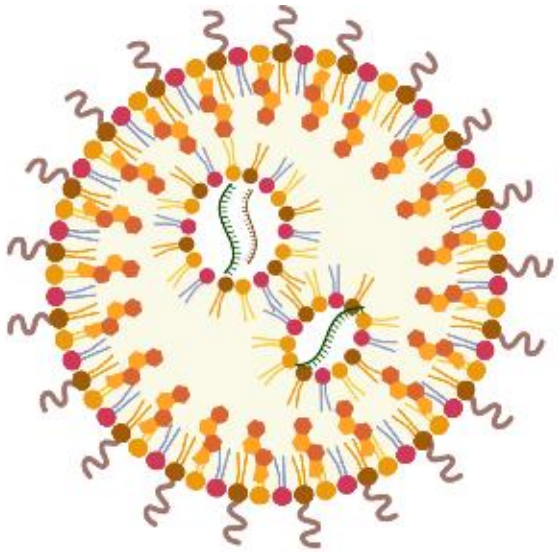
- Large molecule (~200-1500 KDa), highly charged, and does not cross cellular membranes
- prone to nuclease degradation
- induce immune response (TLR pathways)



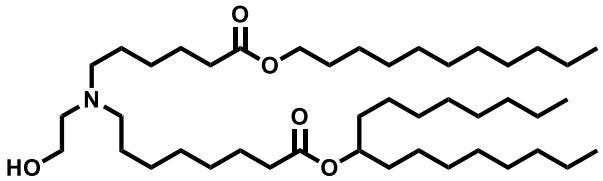
Lipid Nanoparticle

Lipid Components -:

- Ionizable lipids
- Helper Lipids
- Cholesterol
- PEGylated lipids



Ionizable lipid



- Nucleic acid complexation, endosomal escape
- Regulate transfection of cell types and immune stimulation
- Produced by combinatorial chemistry/rational design

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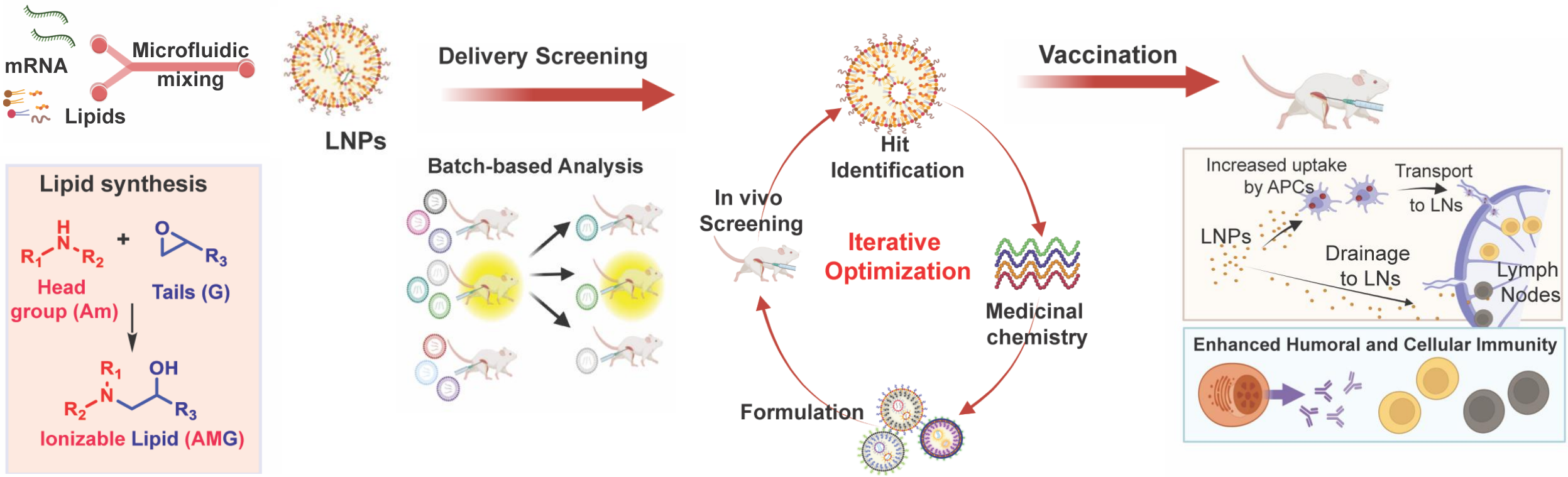
Gupta, A. *et al. Nat Rev Drug Discov 23, 914–938 (2024).*

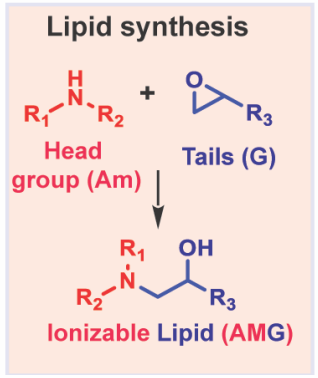
Hypothesis : *Investigating chemical design space can generate novel ionizable lipids for developing potent mRNA vaccines*

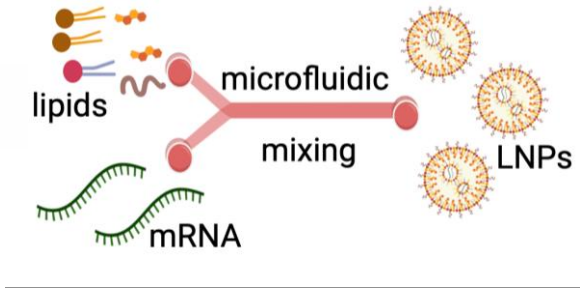
Strategy :



in vivo batch-analysis of lipid library

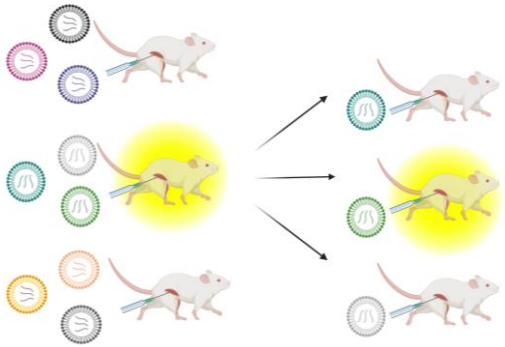
- Developed a library of **100 lipids**



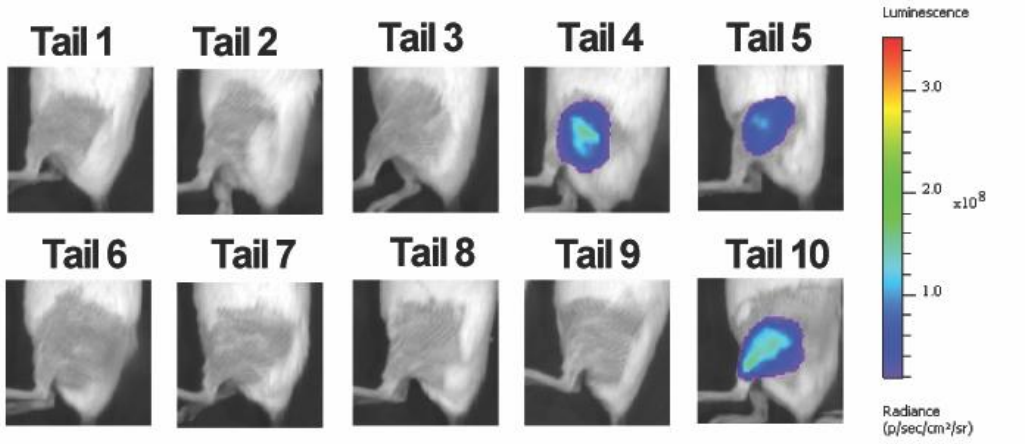


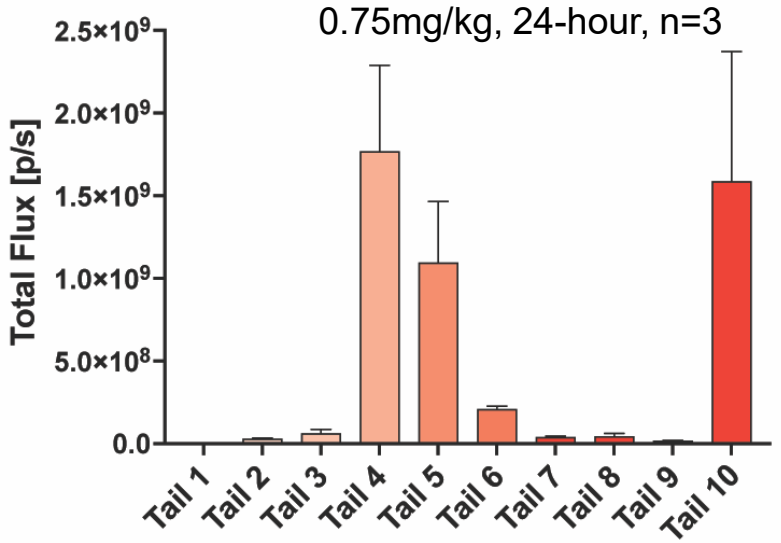
In vivo screening

Batch-Analysis



Screen 1- Pooled into groups based on tails





- Tail 4,5 & 10 LNPs showed highest mRNA transfection

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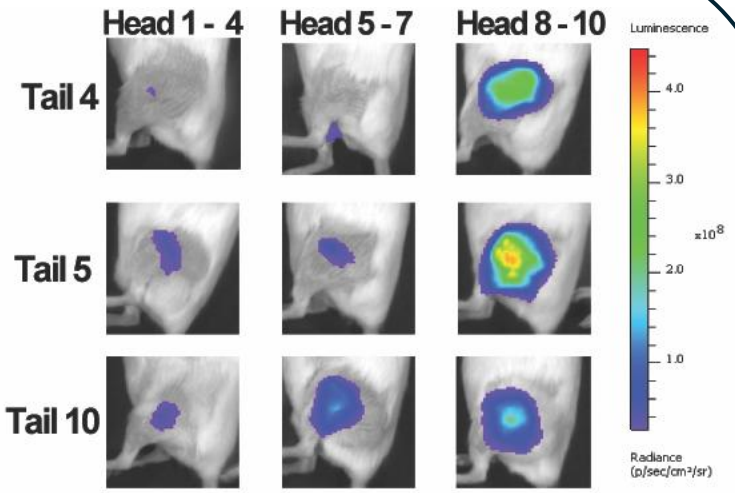
A. Gupta†, *et al. (accepted, Nature Nanotechnology)*

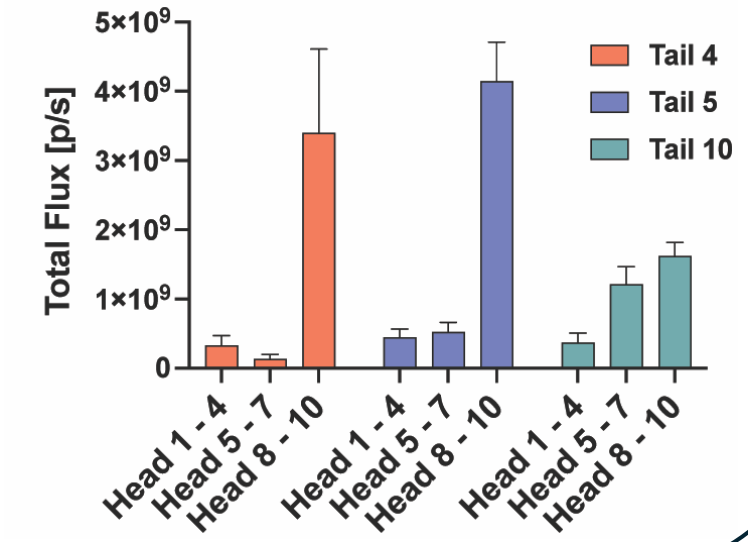
in vivo batch-analysis of lipid library

Screen 2

Smaller batches of hit tail groups

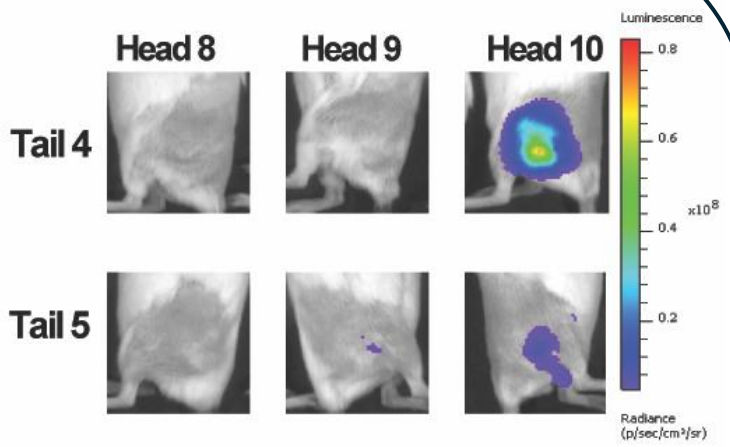


0.25mg/kg, 24-hour, n=3

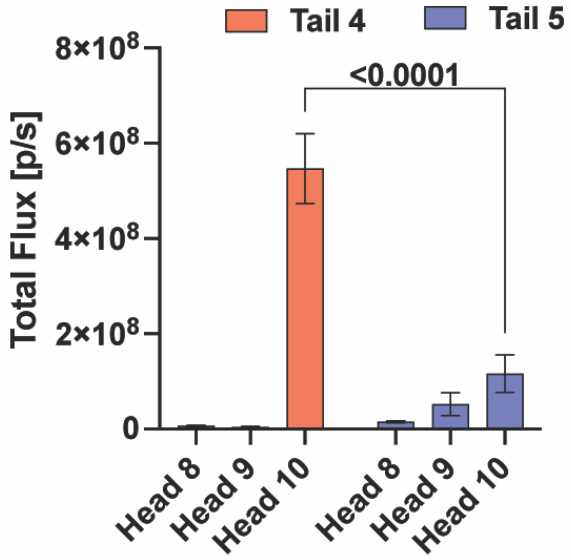


Screen 3

Individual lipid screening



0.05mg/kg, 24-hour, n=3



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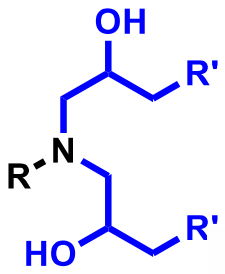
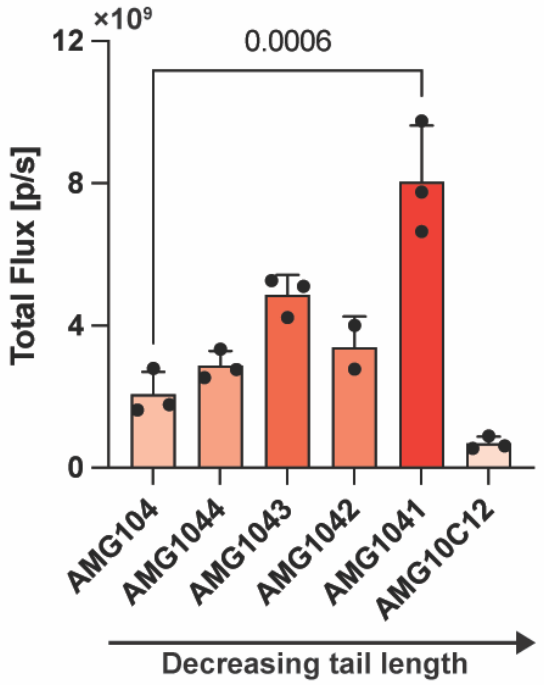
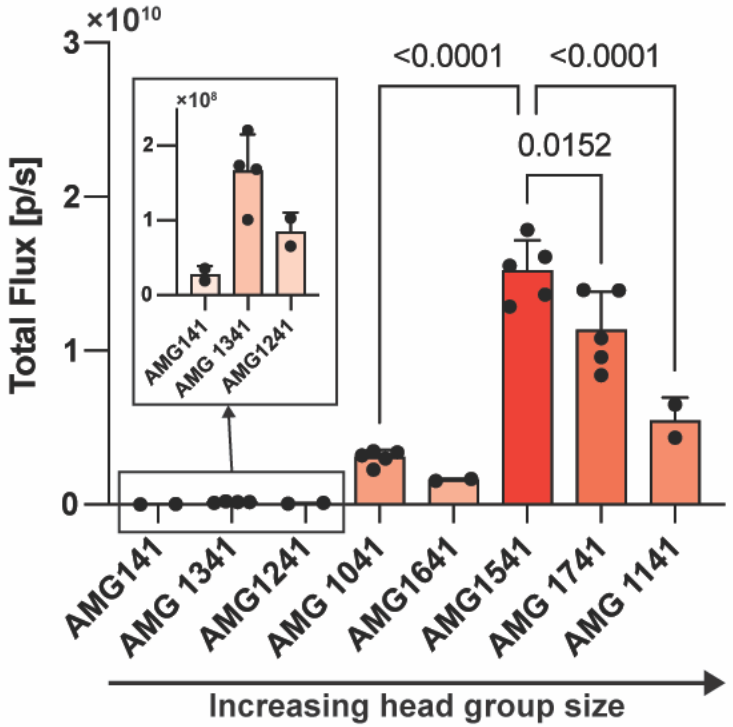
Head (Am) 10 + Tail (G) 4 → **AMG 104** was the most potent lipid

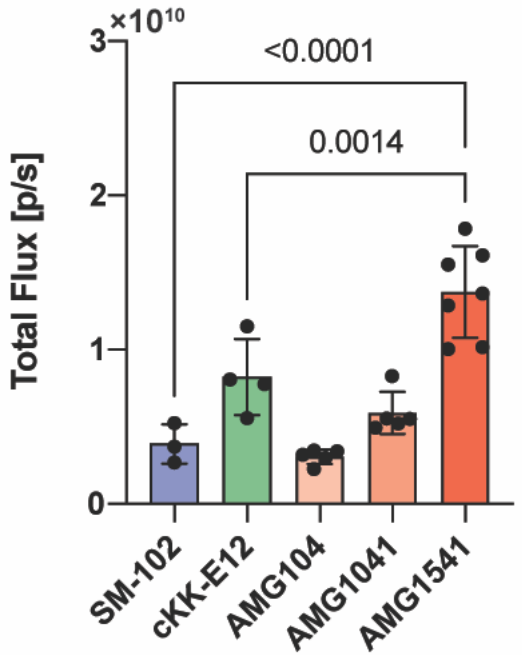
Medicinal chemistry to iteratively optimize ionizable lipid structure



R' – Tails
R-N - Headgroup



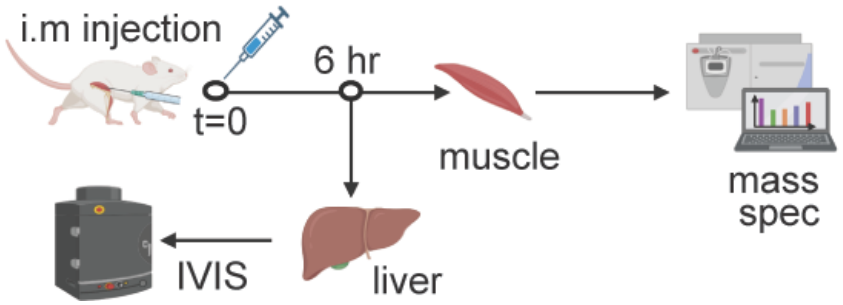




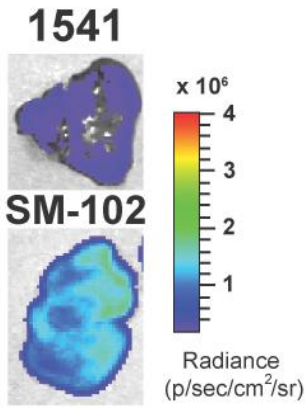
- Systematic optimization yielded **~4.5-fold** increase
- AMG 1541 exhibits **3-fold higher** mRNA transfection **than FDA approved SM-102** formulation

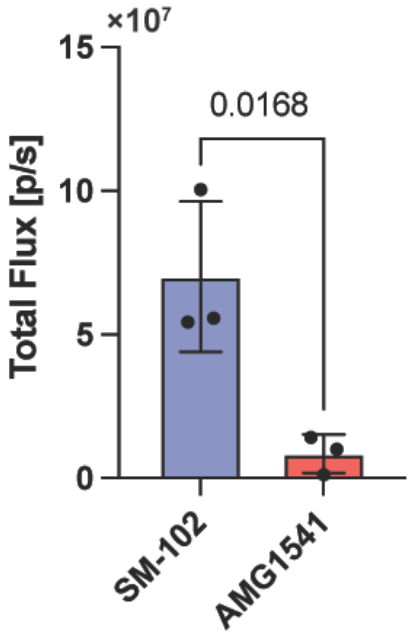
Novel Ionizable Lipids are Rapidly Cleared

- Rapid clearance may reduce injection site inflammation and associated side effects

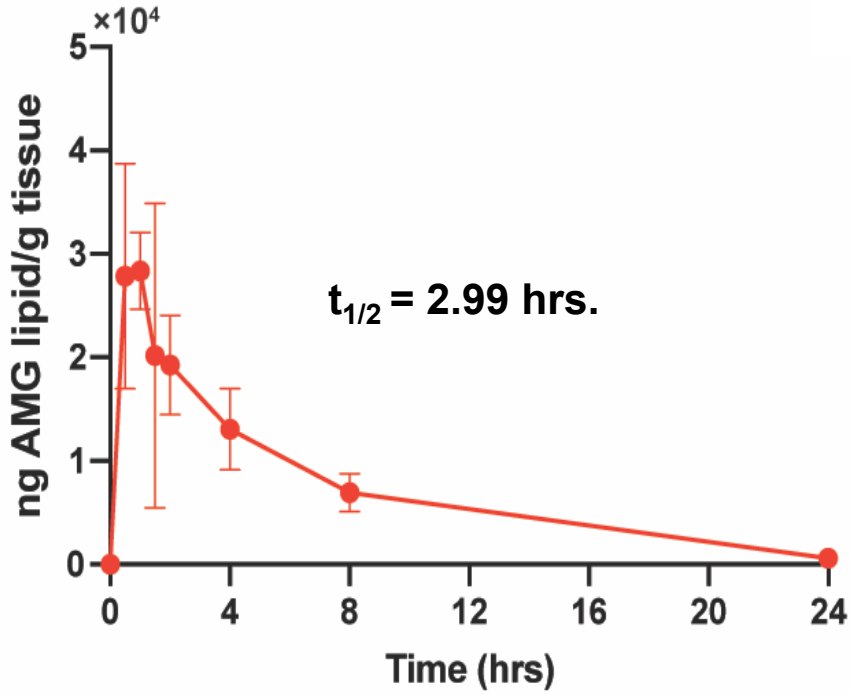


Minimal LNP accumulation in liver



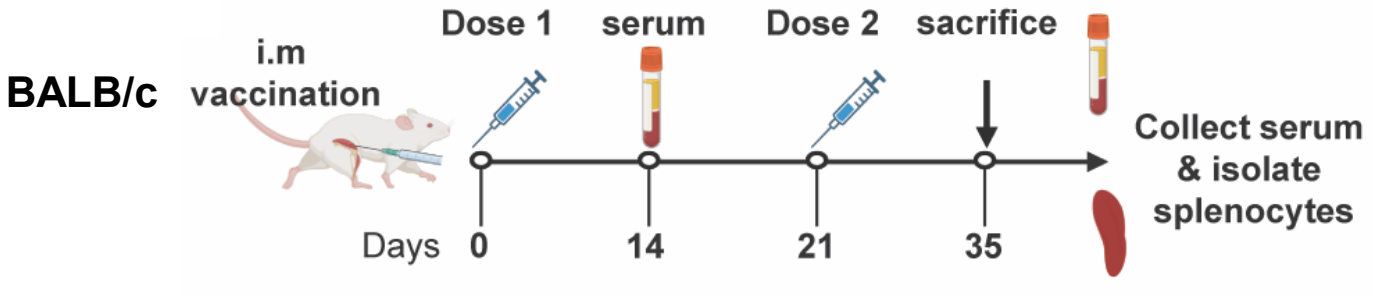


Degradation in muscle

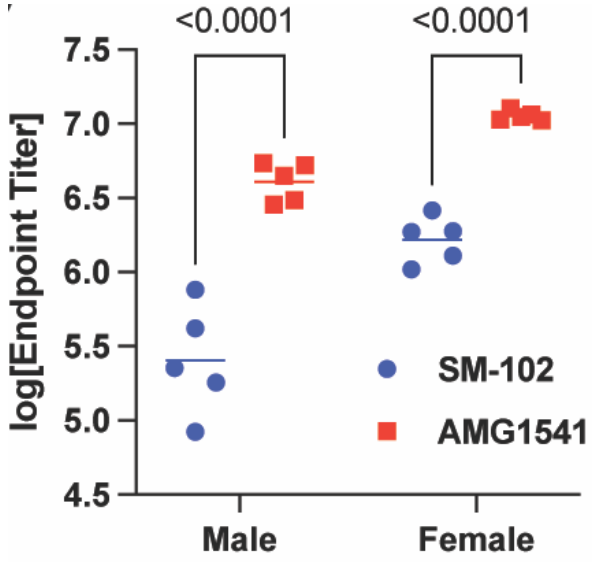


Does it translate to immunogenicity?

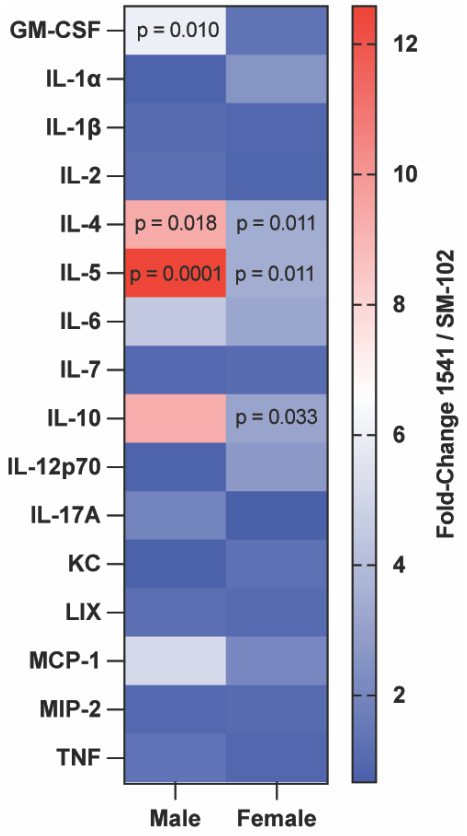
AMG1541 Induced Robust Immune Response in Male and Female Mice



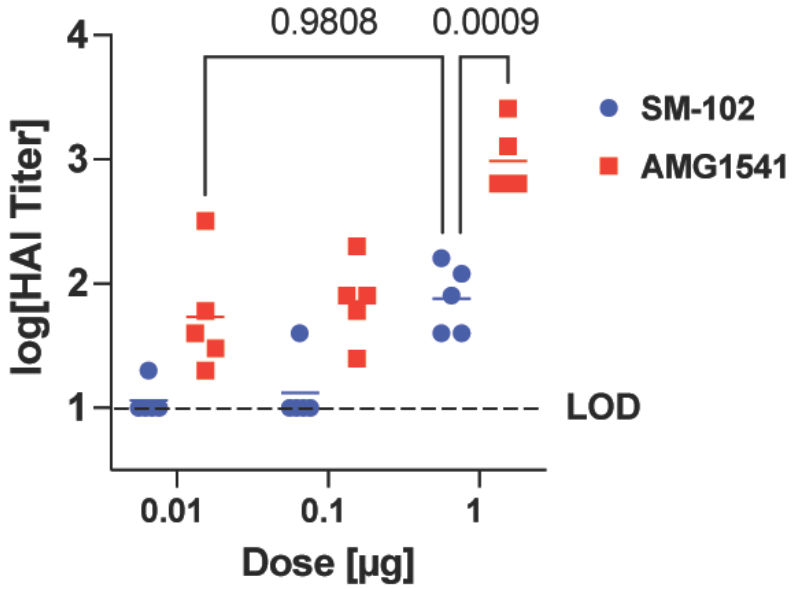
Humoral



Cellular



Neutralization Ab titers

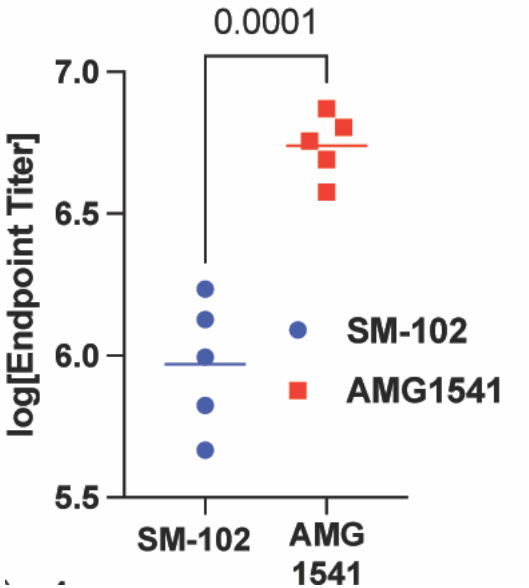


- More robust humoral and cellular response induced by AMG 1541.
- **Equivalent** neutralizing Ab titers at **100-fold** lower dose (0.01 µg vs. 1 µg)

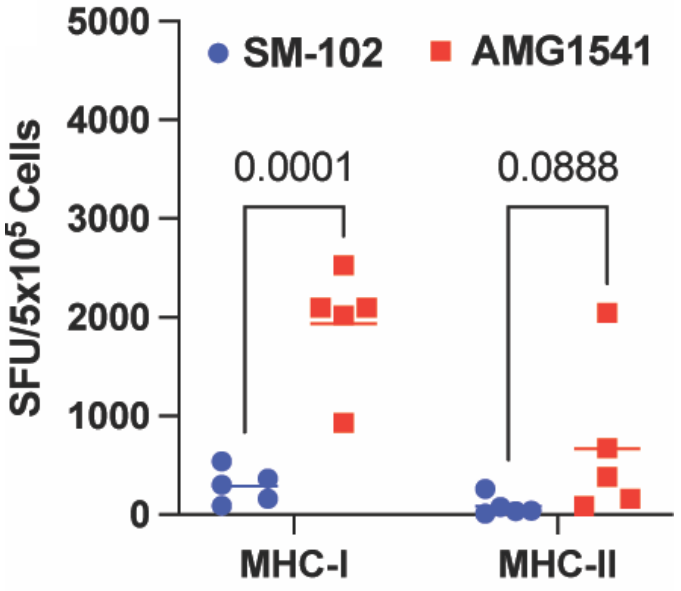
A. Gupta†, *et al. (accepted, Nature Nanotechnology)*

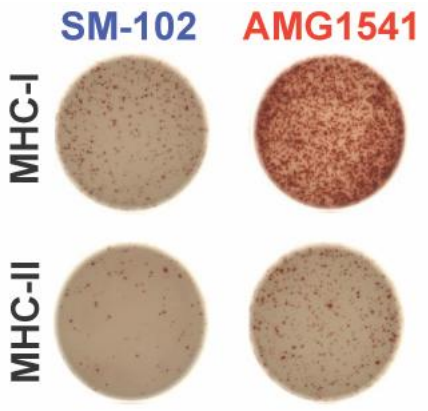
AMG1541 Induces Superior Immune Response in BL6 mice

Humoral



Cellular





- AMG 1541 induced 6.5 -fold higher humoral response
- 6.7-fold higher CD8+ T cell response in C57BL/6 mice

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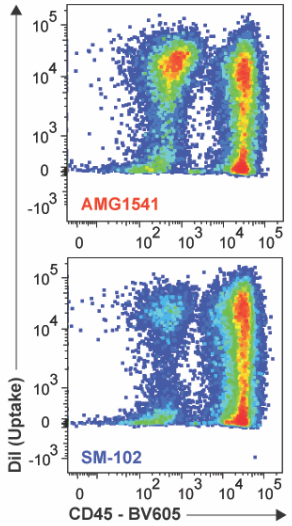
A. Gupta†, *et al. (accepted, Nature Nanotechnology)*

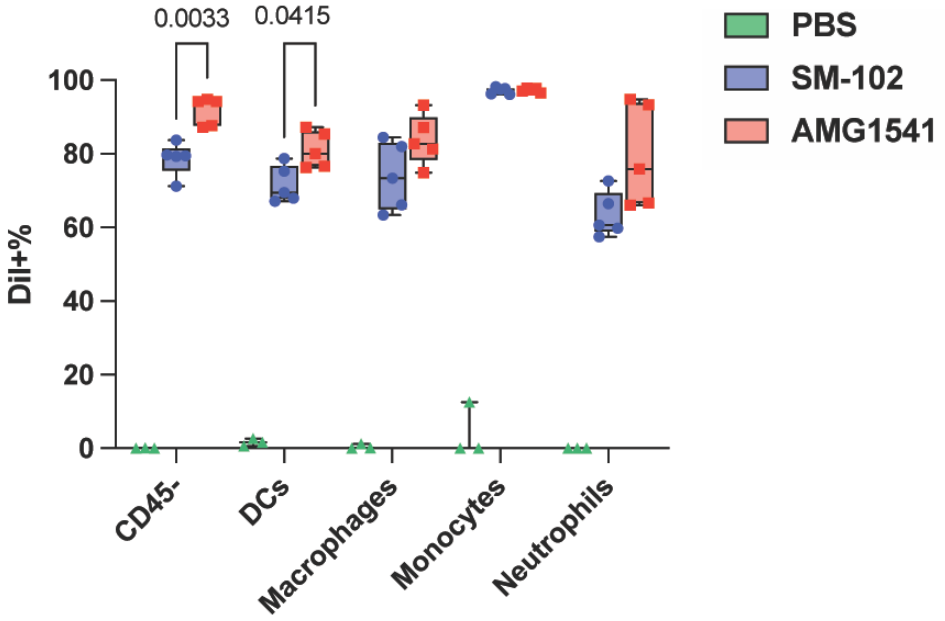
AMG 1541 demonstrated enhanced lymph node drainage

- DiI loaded LNPs to assess uptake

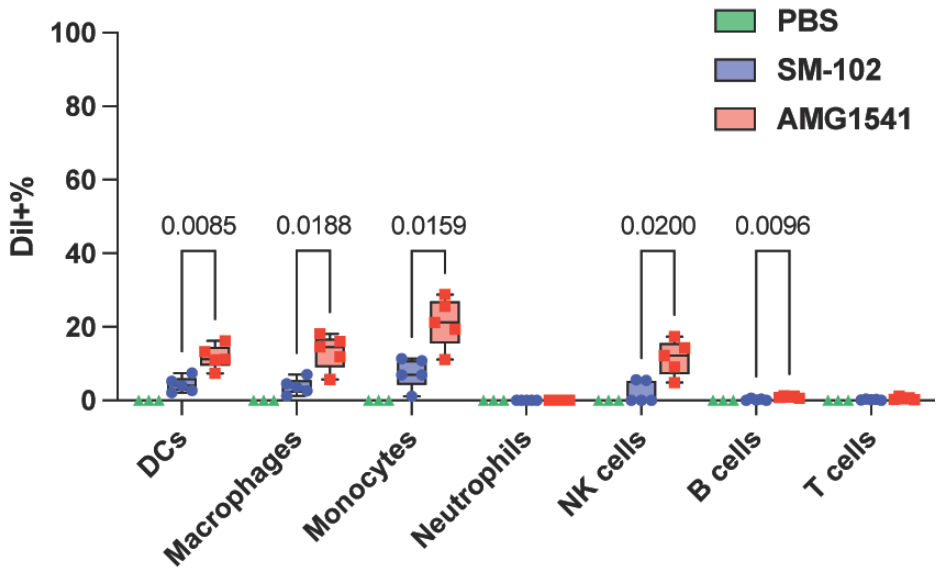
LNP injection → **24 hour** → Harvest Muscles Draining LNs

Muscle Uptake





Lymph Node Uptake



- Most immune cells isolated from muscles have LNPs
- AMG 1541 shows improved trafficking into lymph nodes

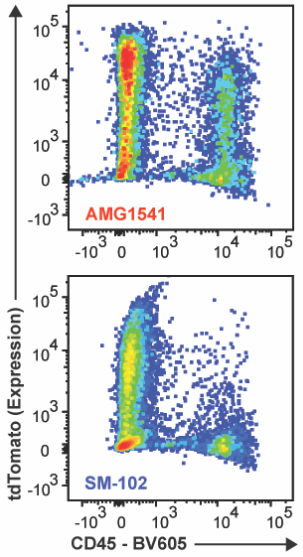
AMG 1541 demonstrated enhanced lymph node drainage

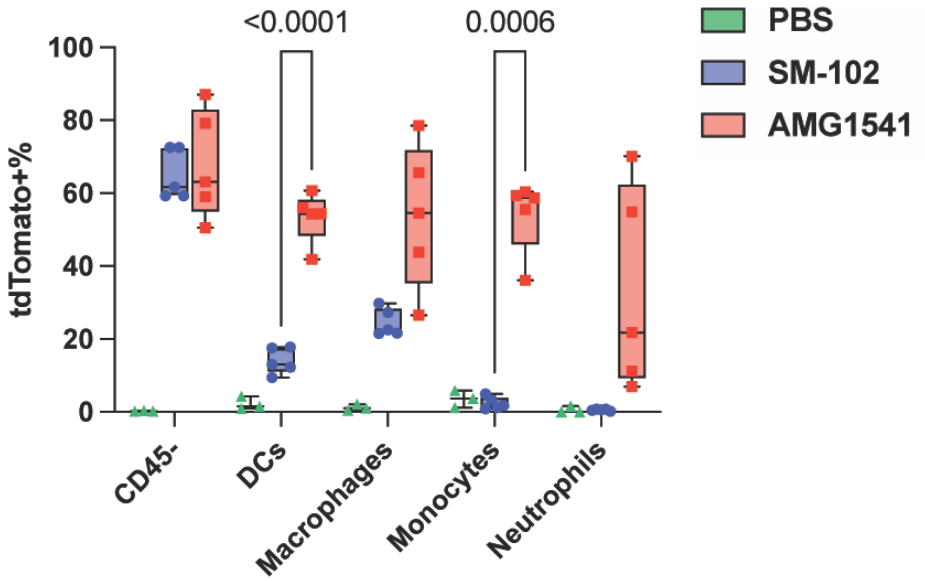
- LNPs encapsulating tdTomato mRNA

LNP injection → **24 hour**

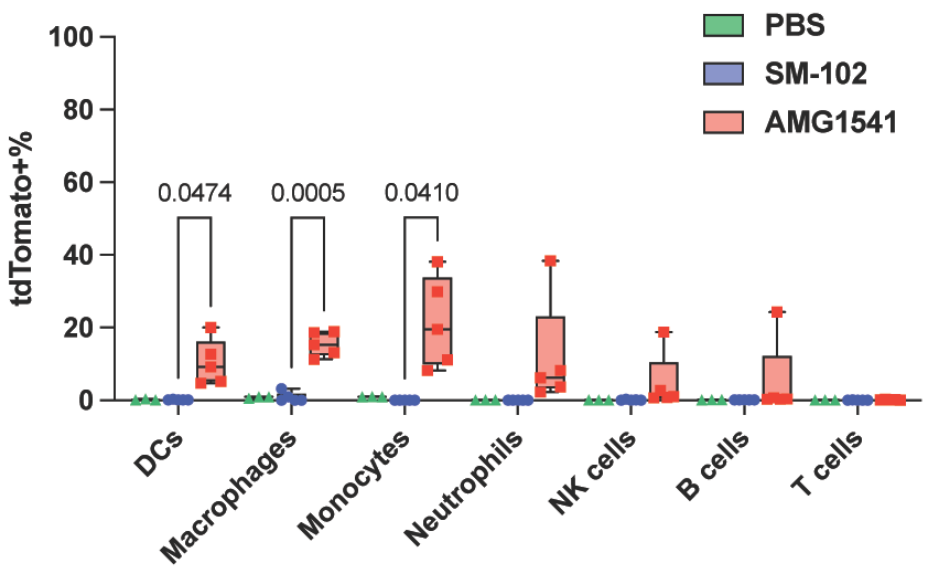
Harvest
Muscles
Draining LNs

Muscle Delivery





LN delivery



- In muscles, most of the expression comes from non-immune cells
- AMG 1541 exhibits higher expression in APCs and other immune cell subsets

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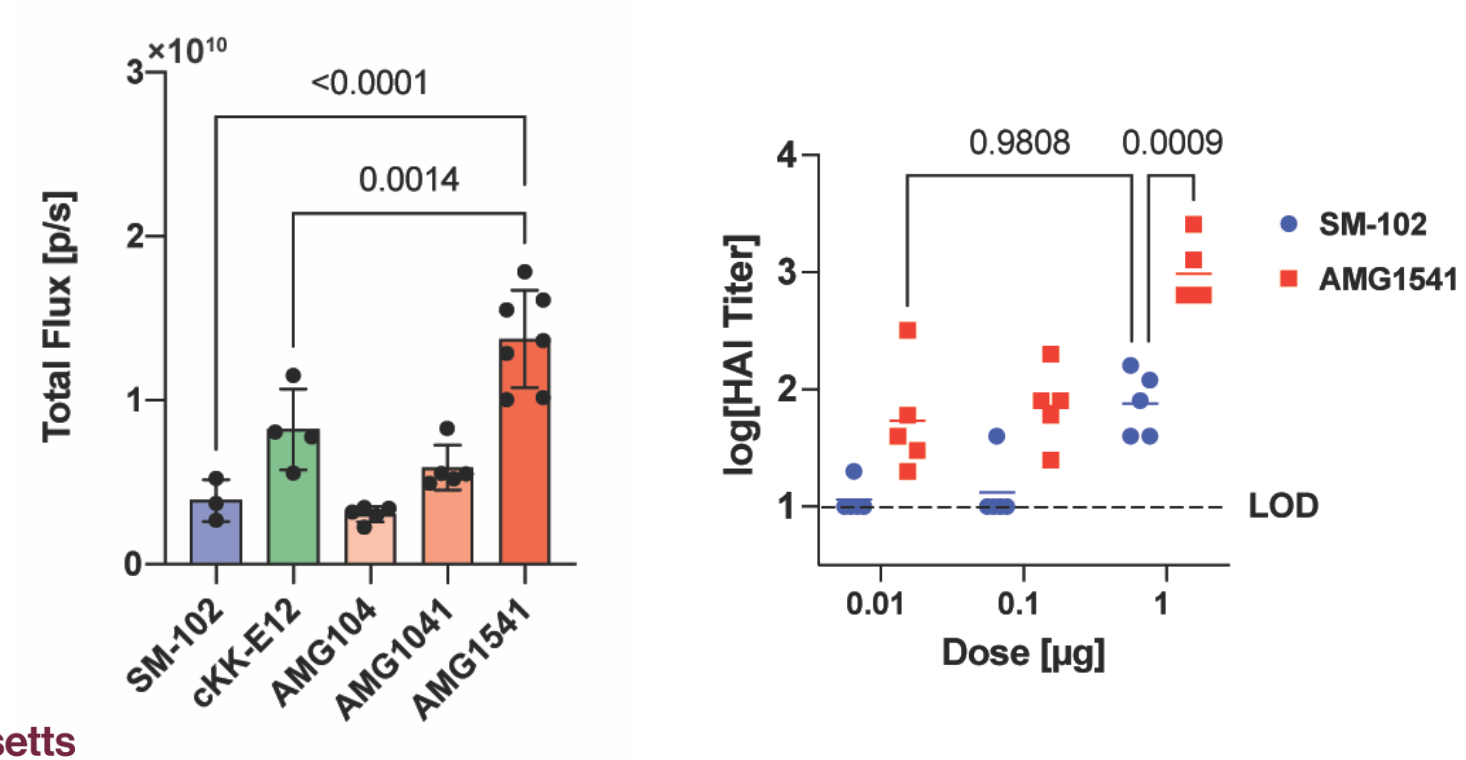
Summary

Potent mRNA vaccines can be generated by improving delivery vehicle design

- ✓ Ionizable lipid chemistry strongly enhances delivery efficacy of LNPs
- ✓ Increased antigen transfection strongly correlates with vaccine potency
- ✓ 100-fold dose reduction to generate similar immune response



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- Austin Danko
- Patrick McMullen
- Yizong Hu

KOCH INSTITUTE for Integrative Cancer Research at MIT

CRS 2025 ANNUAL MEETING & EXPOSITION

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