Development of SORT lipid nanoparticles (LNPs) for genome correction of disease-causing mutations

Daniel J. Siegwart, Ph.D.
Professor
Department of Biomedical Engineering
University of Texas Southwestern Medical Center

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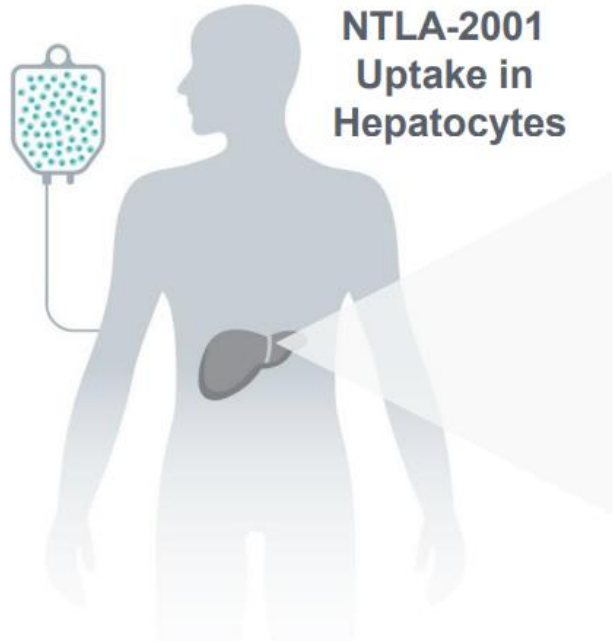
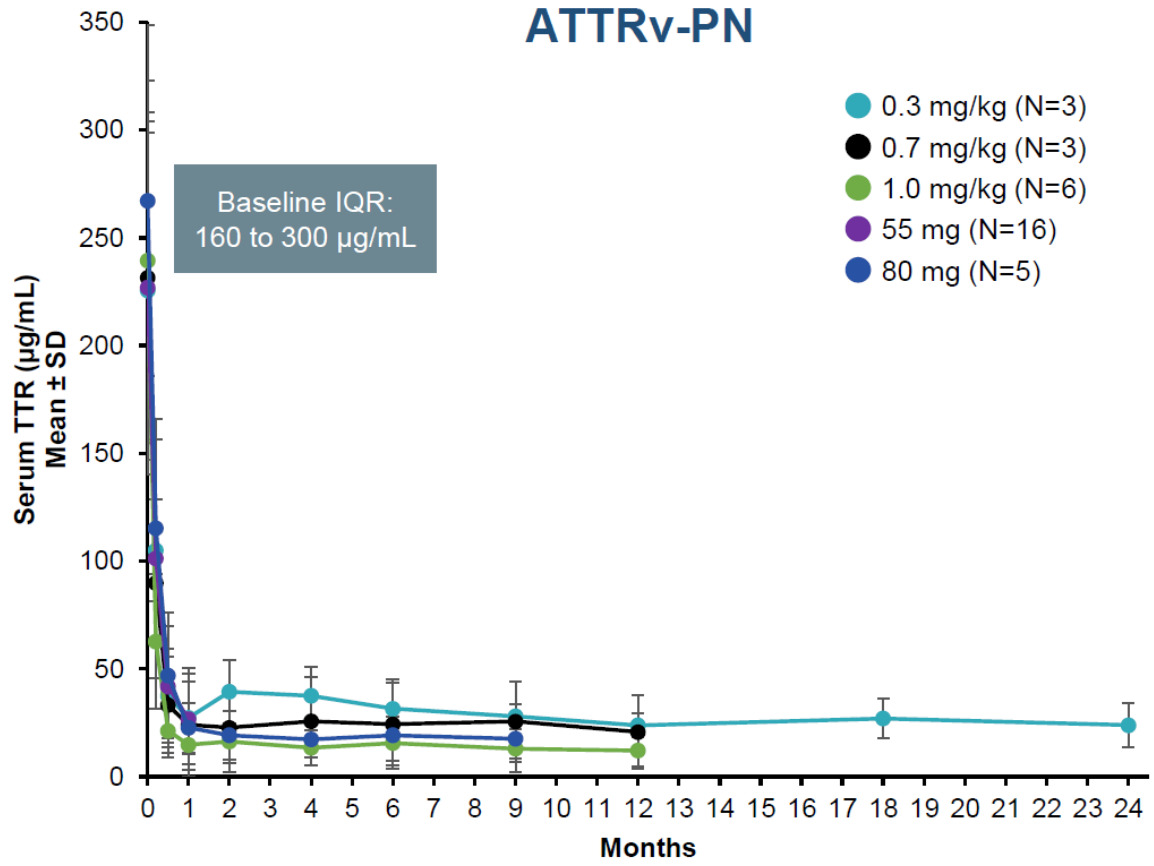
NEXT-GENERATION DELIVERY INNOVATIONS

Conflict of interest disclosures

	Co-founder	Scientific Advisory Board	Research collaboration
ReCode Therapeutics	●	●	●
Signify Bio	●		
Jumble Therapeutics	●		
Pegasus Bio	●		
Pfizer			●

Ongoing clinical trials suggest that LNP delivery of nucleic acid-encoded genome editors in the liver has the potential to create durable therapy for some diseases



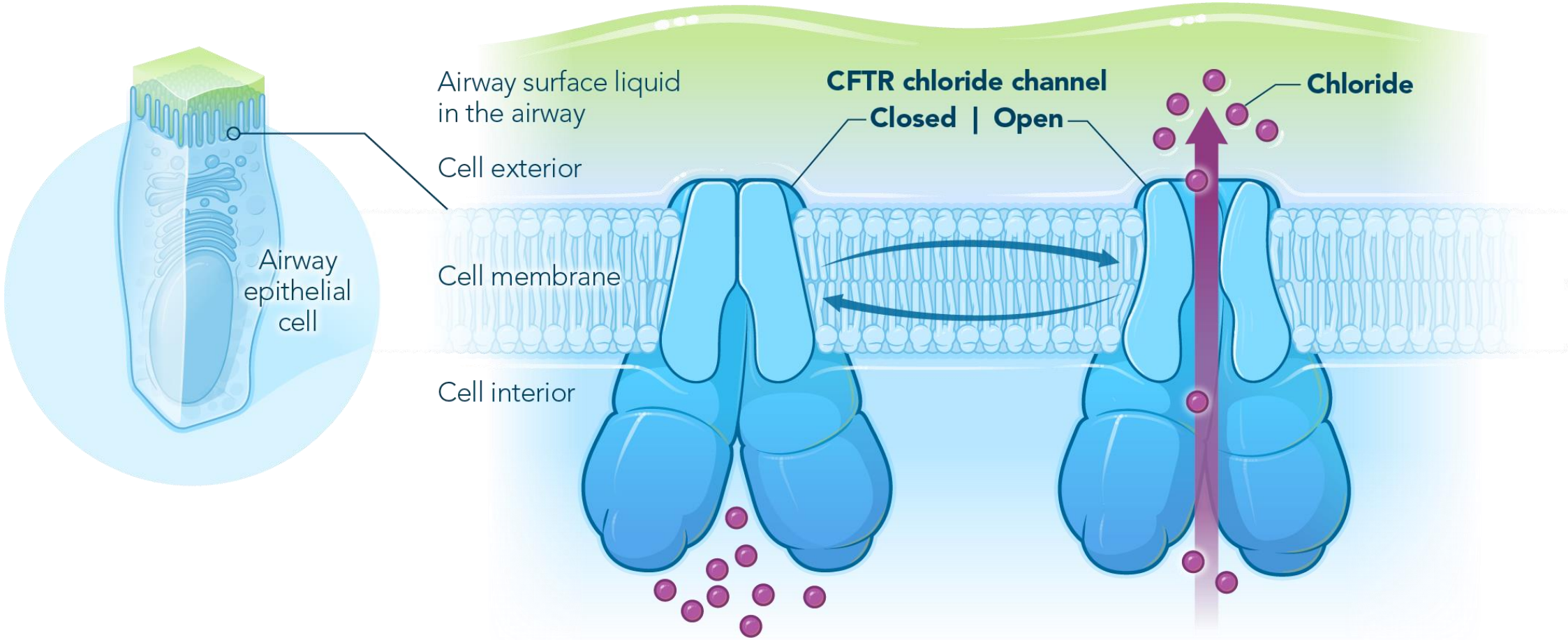


Editing the transthyretin (TTR) gene offers potential to provide permanently low serum [TTR] without the need for chronic therapy of TTR amyloidosis

Slide courtesy Intellia Therapeutics
https://www.intelliatx.com/wp-content/uploads/Intellia_Follow_On-Dosing_PNS-2024_06.25.24.pdf

Cargoes: Cas9 mRNA + sgRNA
LNP: LP001 / DSPC / Cholesterol / DMG-PEG2000

Cystic fibrosis occurs when the CFTR protein is either not made correctly or not made at all



"CFTR" = Cystic fibrosis transmembrane conductance regulator

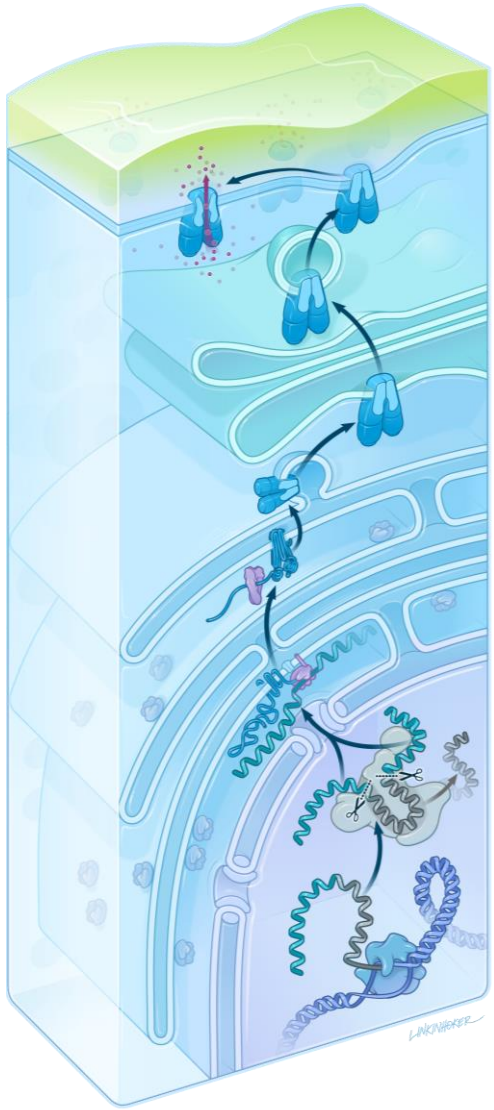
Source: The Cystic Fibrosis Foundation

For durable gene editing therapy, the ideal editing target are the self-renewing basal stem cells that can differentiate into other cells in the lungs, including epithelial cells

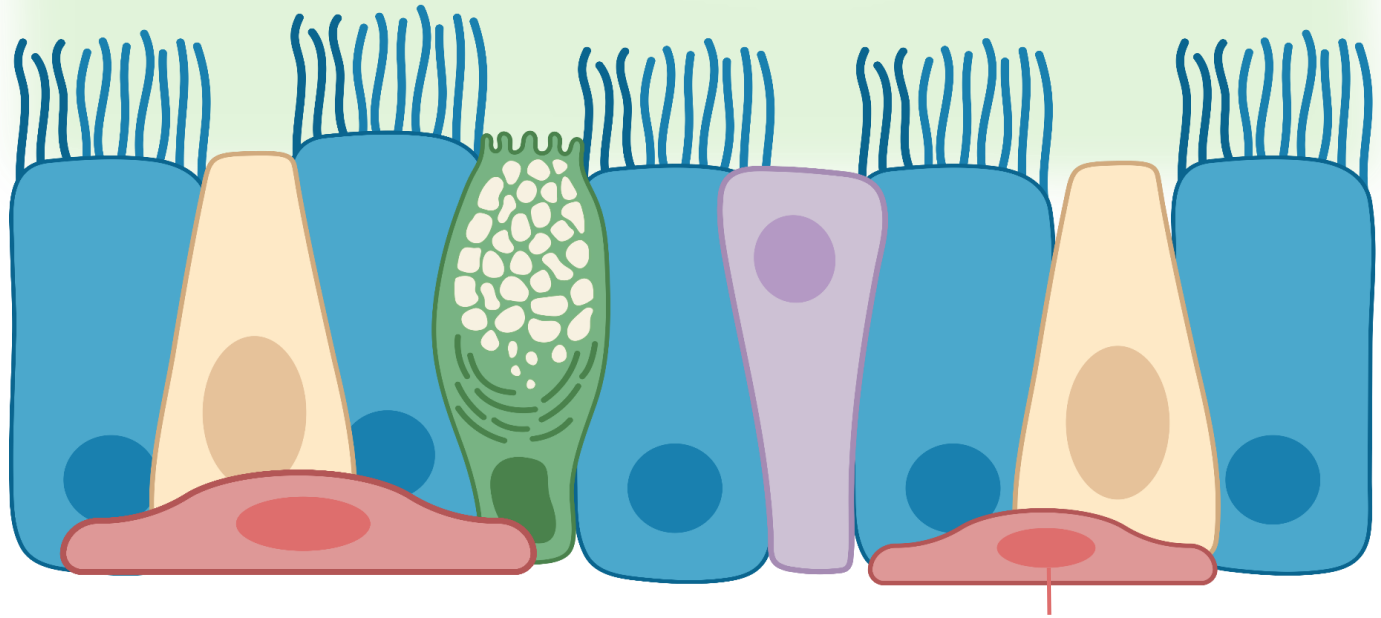
CFTR Protein

CFTR mRNA

CFTR DNA



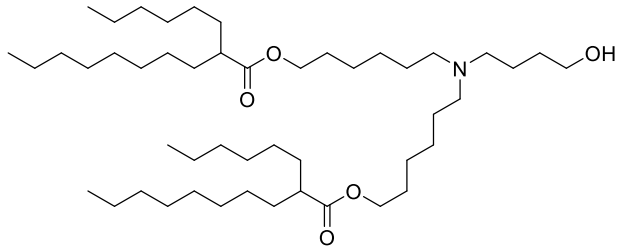
Permanent therapy: Airway stem cells persist



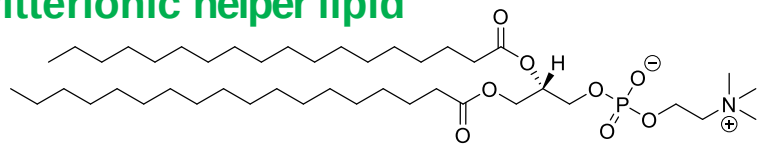
Basal stem cells

Genome editing medicines need help… that comes from self-assembled lipid nanoparticles (LNPs)

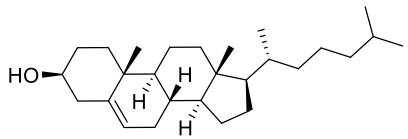
Ionizable amino lipid



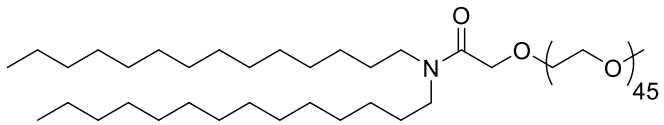
Zwitterionic helper lipid



Cholesterol

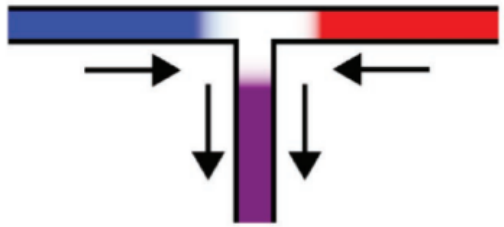


Poly(ethylene glycol) PEG-lipid

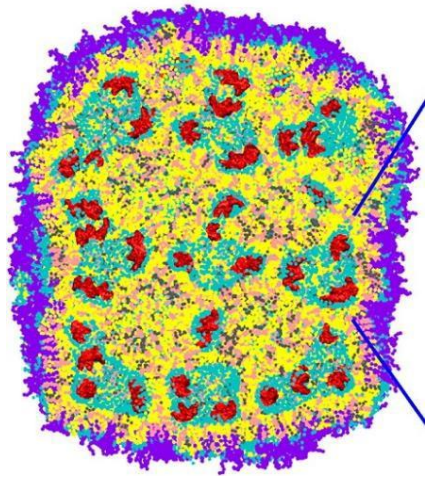


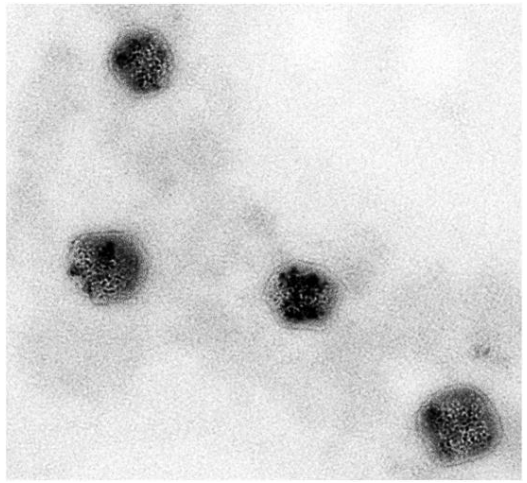
Lipid components in ethanol

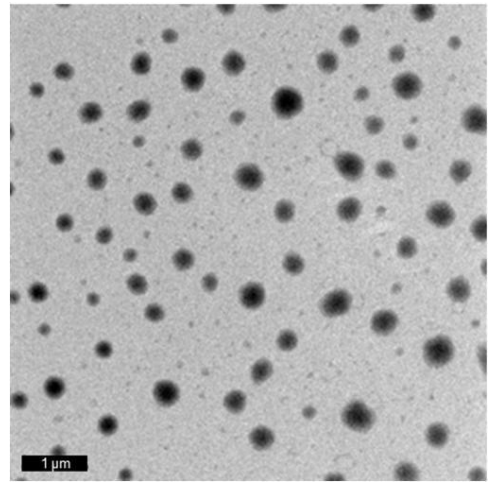
Rapid mixing



mRNA in acidic buffer (*aq.*)





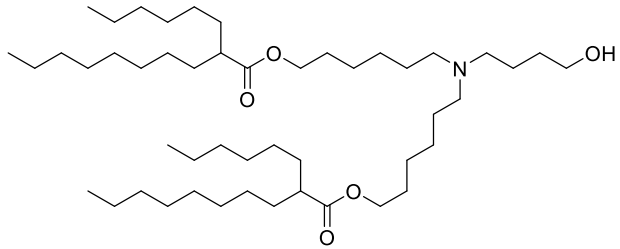


Kejin Zhou et al. **PNAS** *113*, 520 (2016).

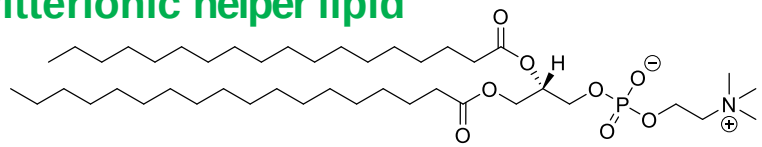
Xu Wang et al. **Nat. Protocols** *18*, 265 (2023).

Genome editing medicines need help… that comes from self-assembled lipid nanoparticles (LNPs)

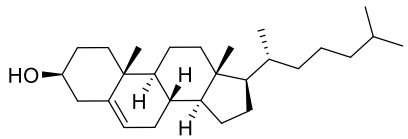
Ionizable amino lipid



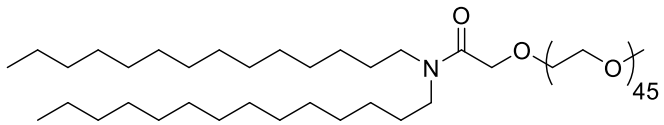
Zwitterionic helper lipid

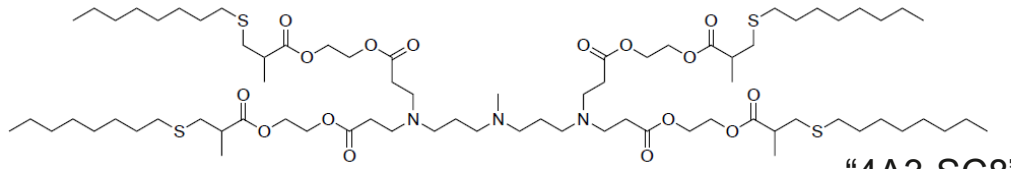


Cholesterol

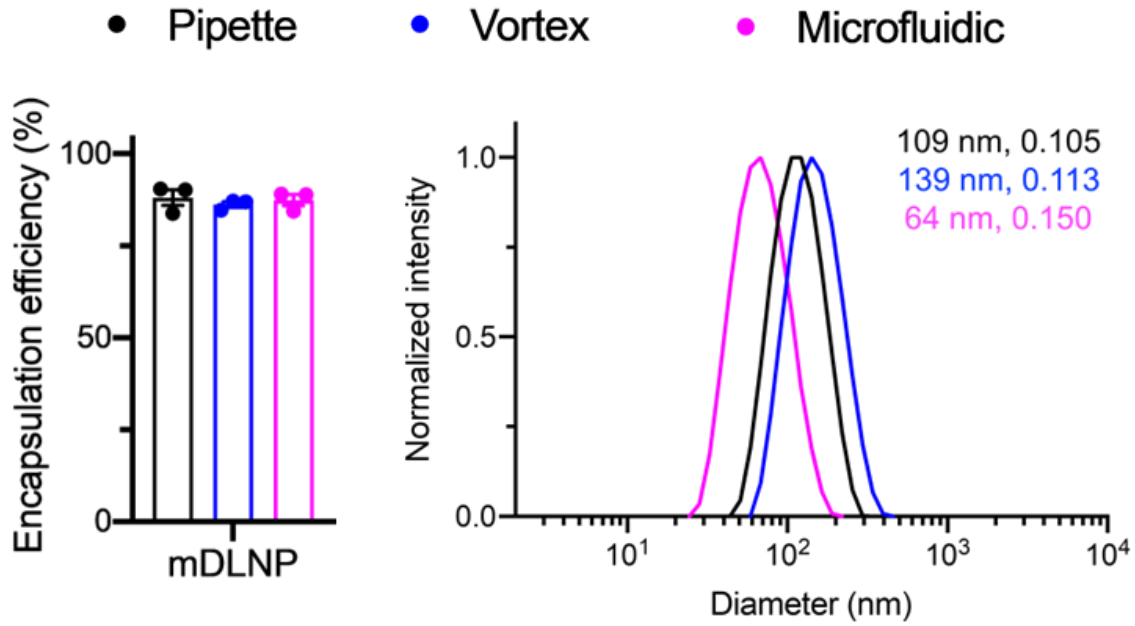


Poly(ethylene glycol) PEG-lipid





Molar ratios (%)				
Name	4A3-SC8	DOPE	Chol	DMG-PEG
4A3-SC8 LNPs	23.8	23.8	47.6	4.8



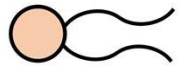
Kejin Zhou et al. **PNAS** *113*, 520 (2016).

Xu Wang et al. **Nat. Protocols** *18*, 265 (2023).

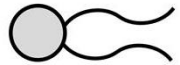
Alteration of lipid molar ratios significantly improves delivery of different nucleic acid cargoes but generally results in hepatic delivery

Traditional LNP

Ionizable cationic lipid

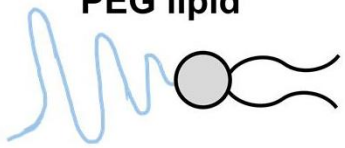


Zwitterionic phospholipid

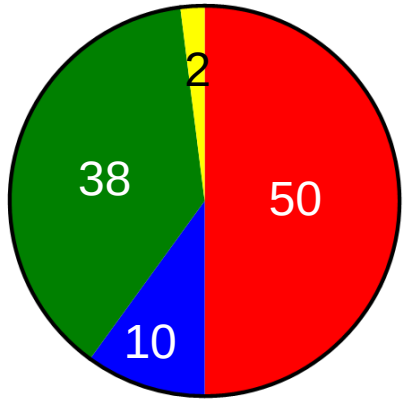


Cholesterol

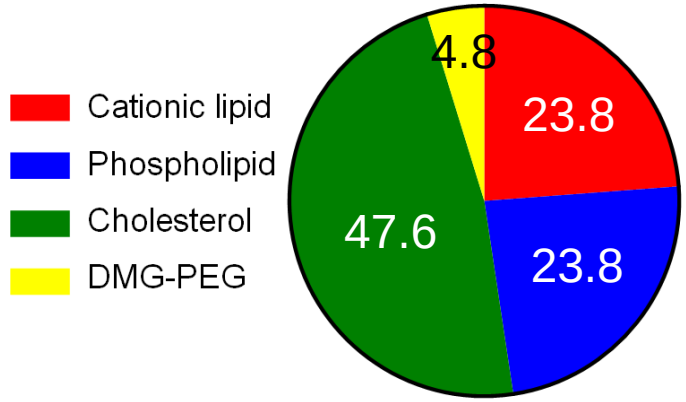
PEG lipid



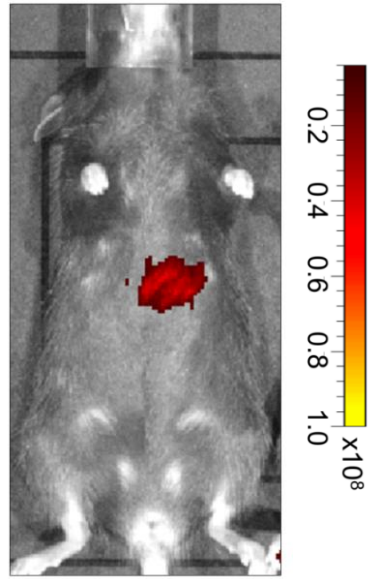
(fixed internal ratios)

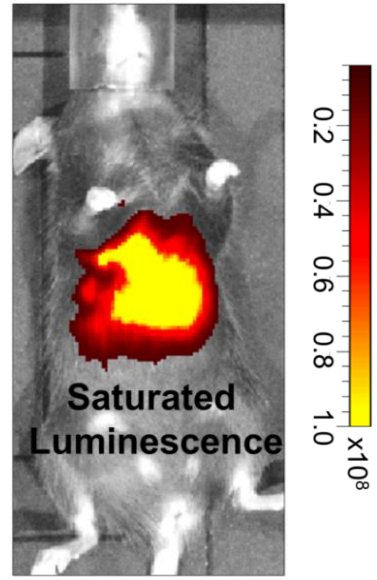


Starting siRNA formulation



Optimized mRNA formulation

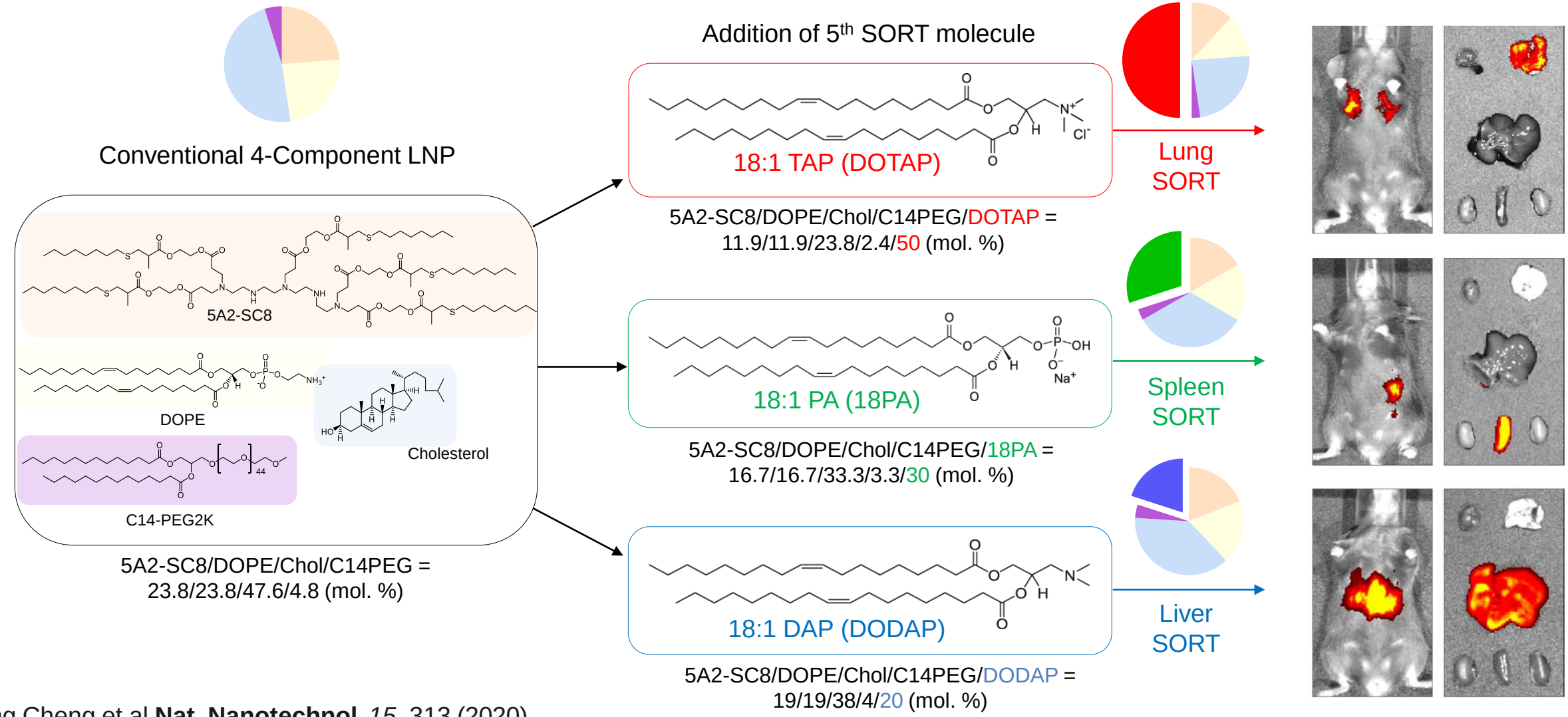




siRNA formulations: Kejin Zhou et al. **PNAS** *113*, 520 (2016).

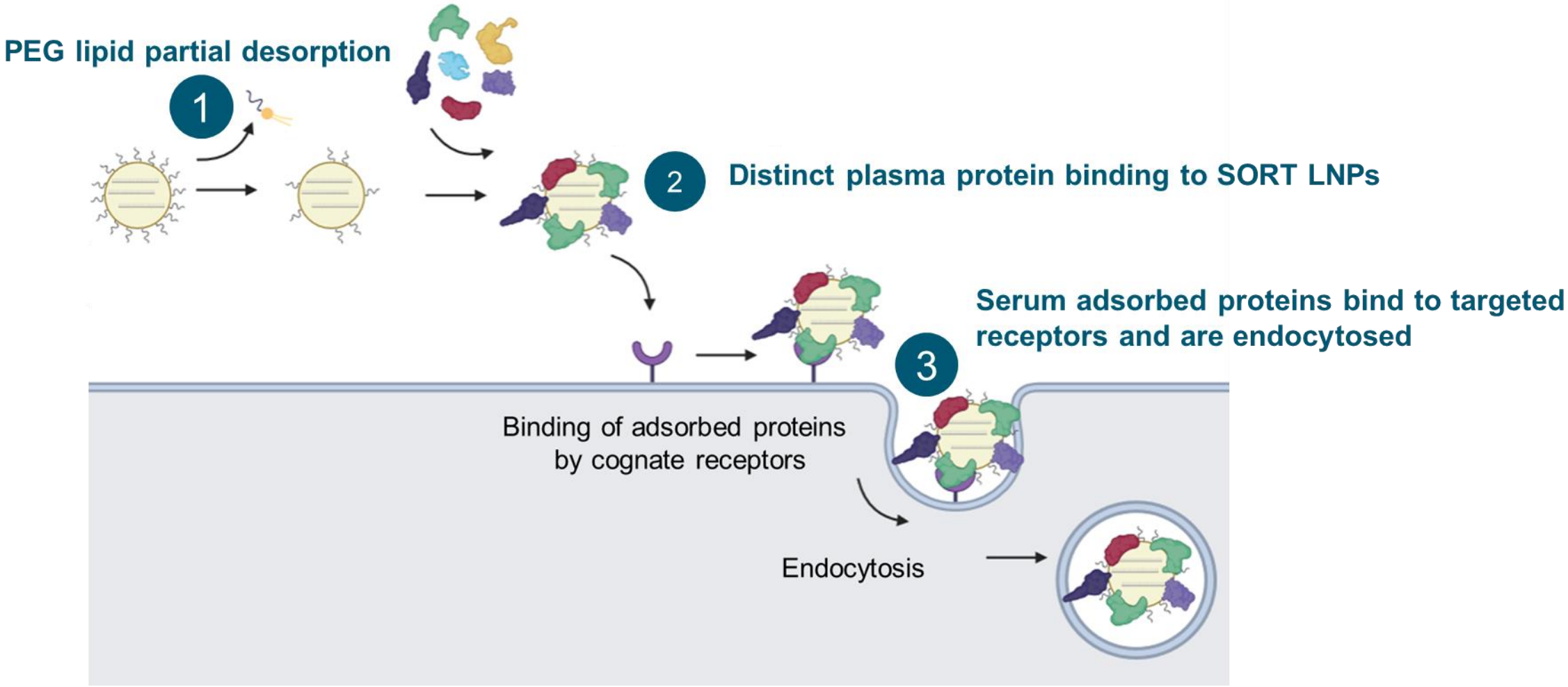
mRNA formulations: Qiang Cheng et al. **Adv. Mater.** *30*, 1805308 (2018).

Selective ORgan Targeting (SORT) allows LNPs to be systematically and predictably engineered to accurately edit cells in specific organs



Qiang Cheng et al **Nat. Nanotechnol.** *15*, 313 (2020).
Tuo Wei et al. **Nat. Commun.** *11*, 3232 (2020).
Shuai Liu et al. **Nat. Mater.** *20*, 701 (2021).
Xu Wang et al. **Nat. Protocols** *18*, 265 (2023).
Ester Álvarez-Benedicto et al. **Angew. Chem. Int. Ed.** *62*, e202310395 (2023).

SORT LNPs use an “endogenous targeting” mechanism of action via adsorption of distinct plasma proteins

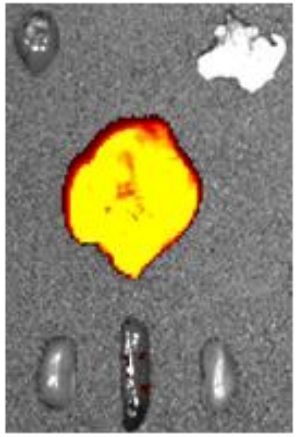


Sean Dilliard et al. **PNAS** *118*, e2109256118 (2021).

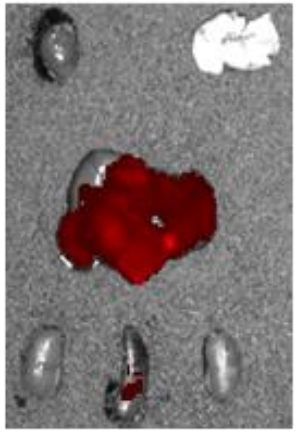
Extrahepatic delivery of SORT LNPs occurs via an ApoE-independent mechanism

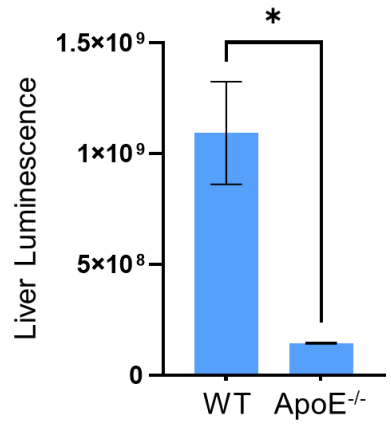
Liver SORT | Spleen SORT | Lung SORT

Wild-type



ApoE$^{-/-}$





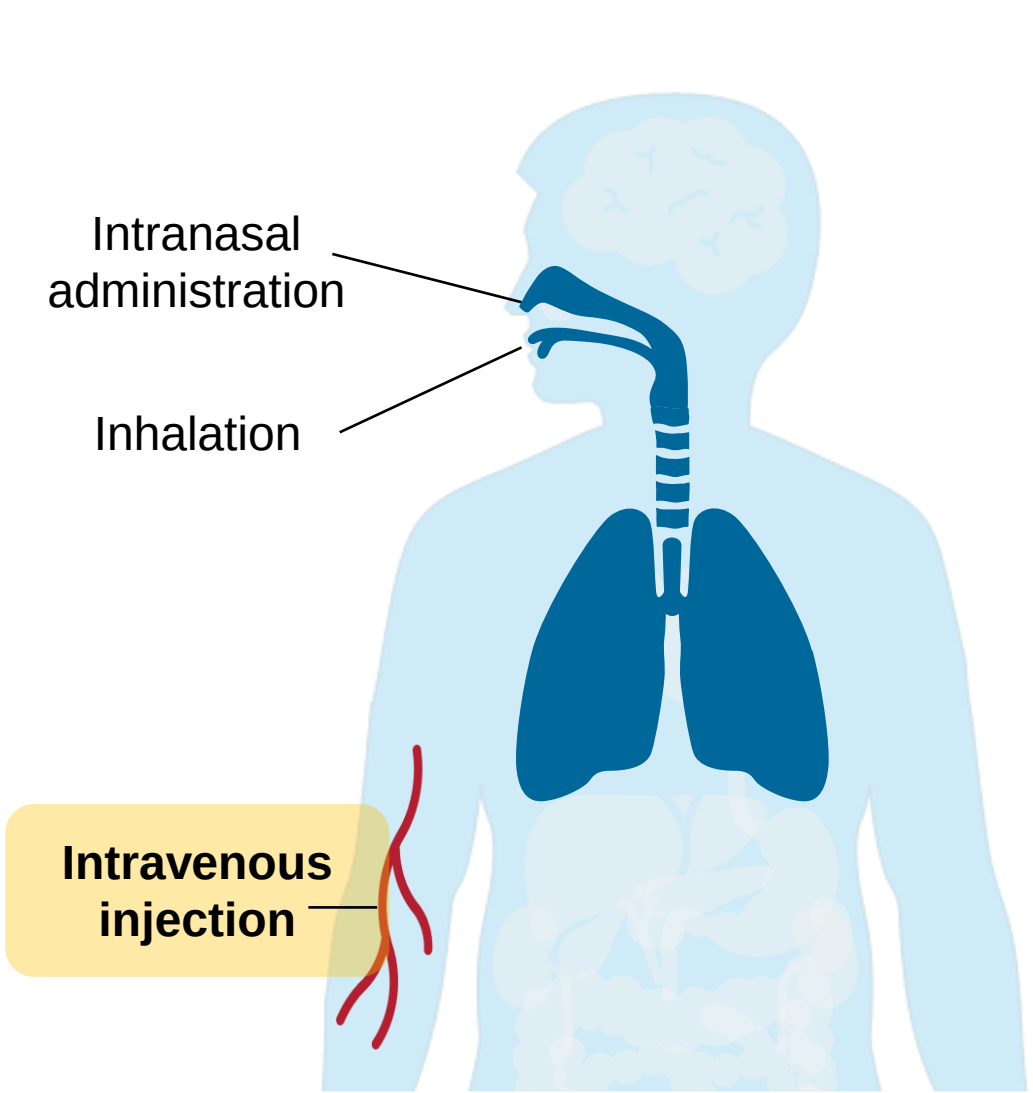
0.1 mg/kg Luciferase mRNA IV

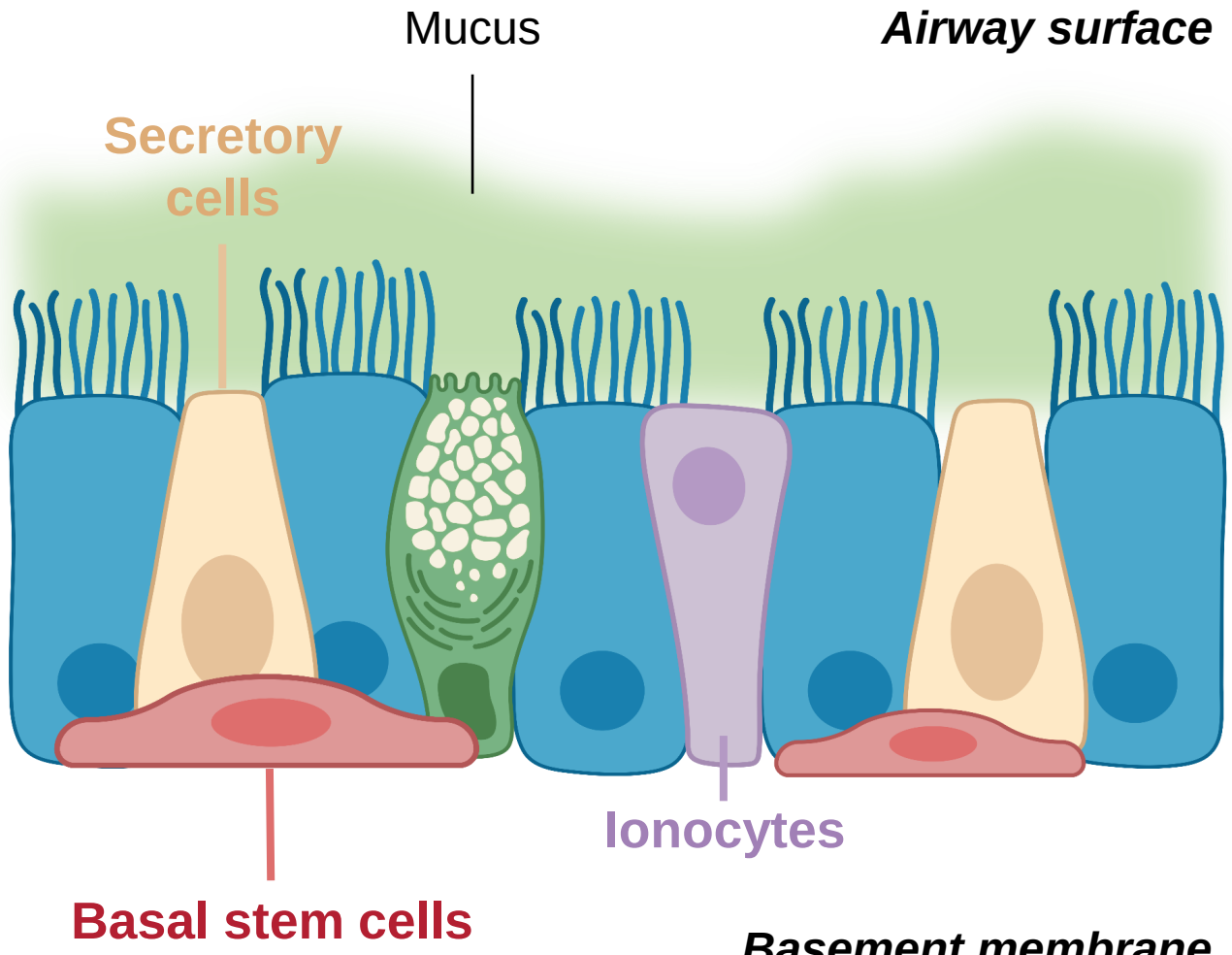
Sean Dilliard et al. **PNAS** *118*, e2109256118 (2021).

Sean Dilliard et al. **J. Controlled Release** *361*, 361 (2023).

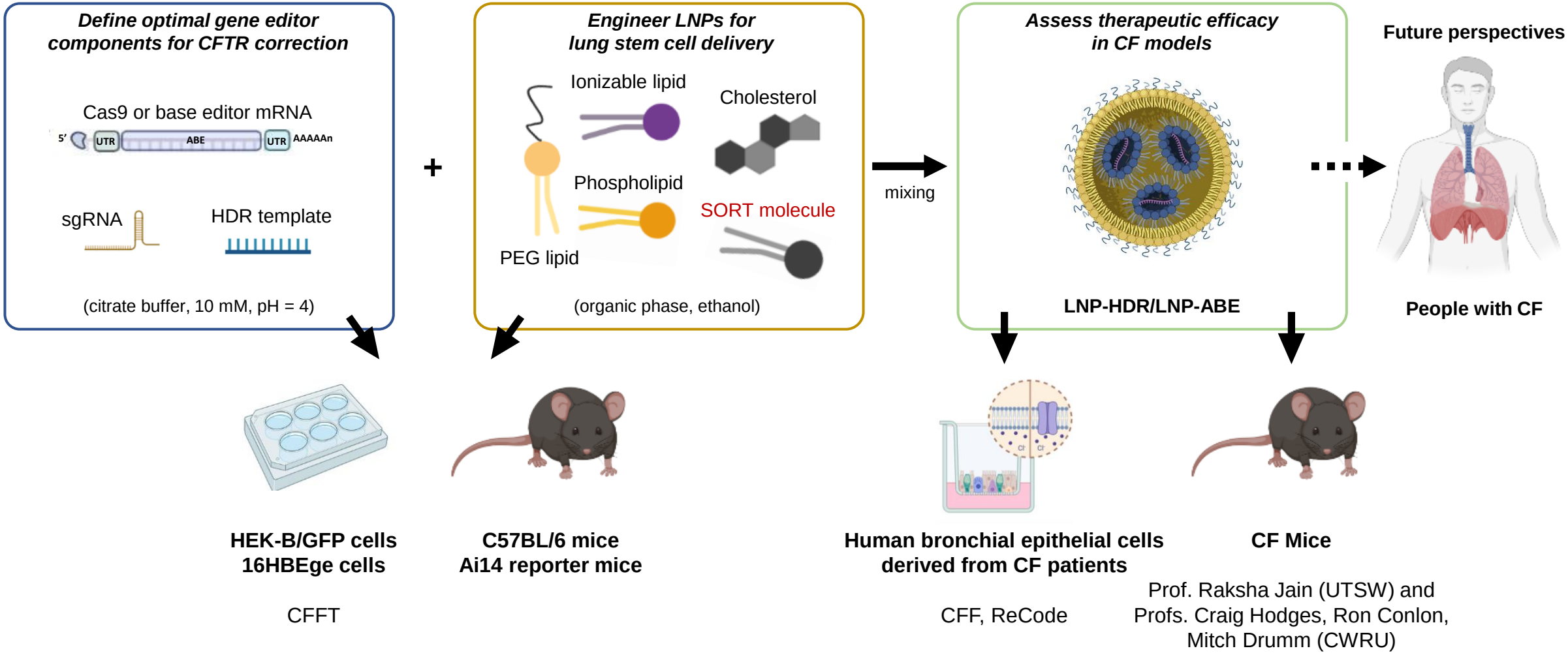
Minjeong Kim et al. **Nat. Biotechnol.** published online June 2 (2025).

Intravenously administered LNP therapies may permanently restore CFTR function via genome correction in stem and progenitor cells





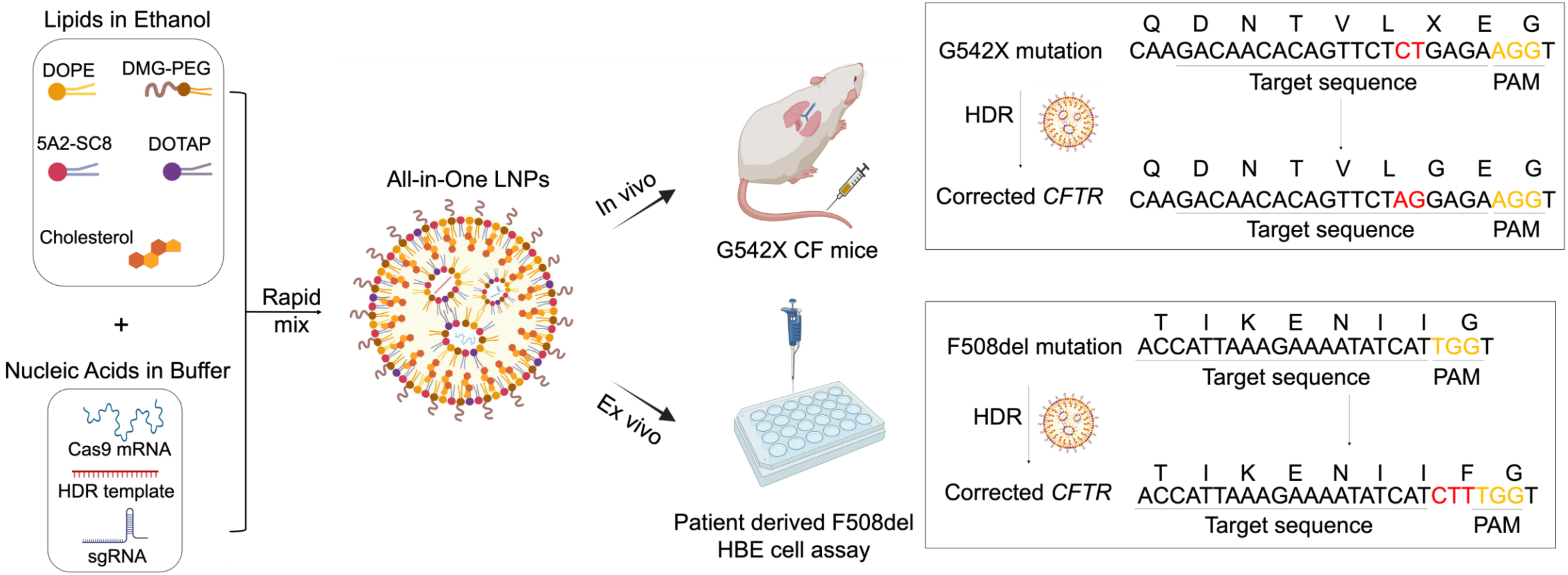
We employed a systematic workflow to engineer SORT LNPs for non-integrative *in vivo* gene correction of CFTR by CRISPR/Cas homology-directed repair and base editing



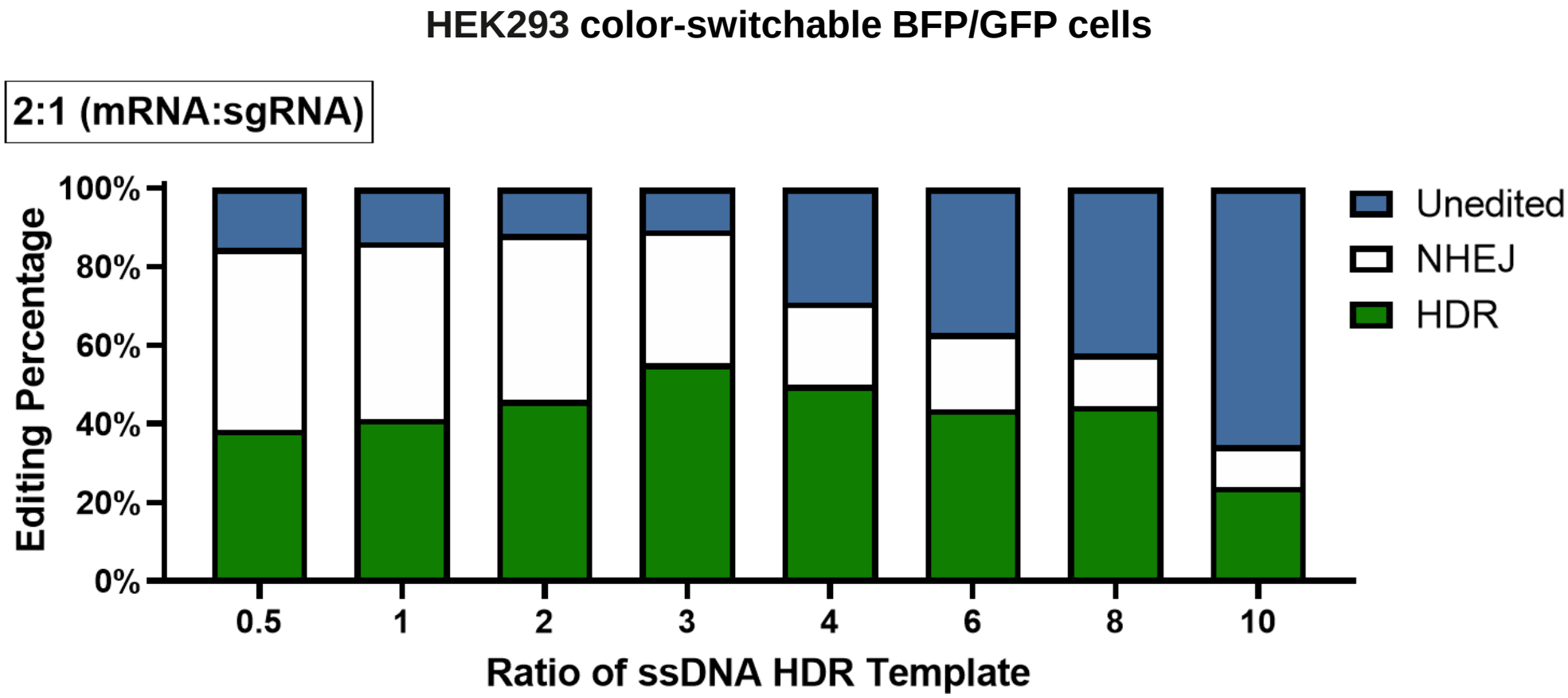
Tuo Wei,* Yehui Sun,* et al. **Nat. Commun.** *14*, 7322 (2023).

Yehui Sun,* Sumanta Chatterjee,* et al. **Science** *384*, 1196 (2024).

We initially evaluated Lung SORT LNPs for homology-directed repair mediated CRISPR/Cas correction of G542X mutations



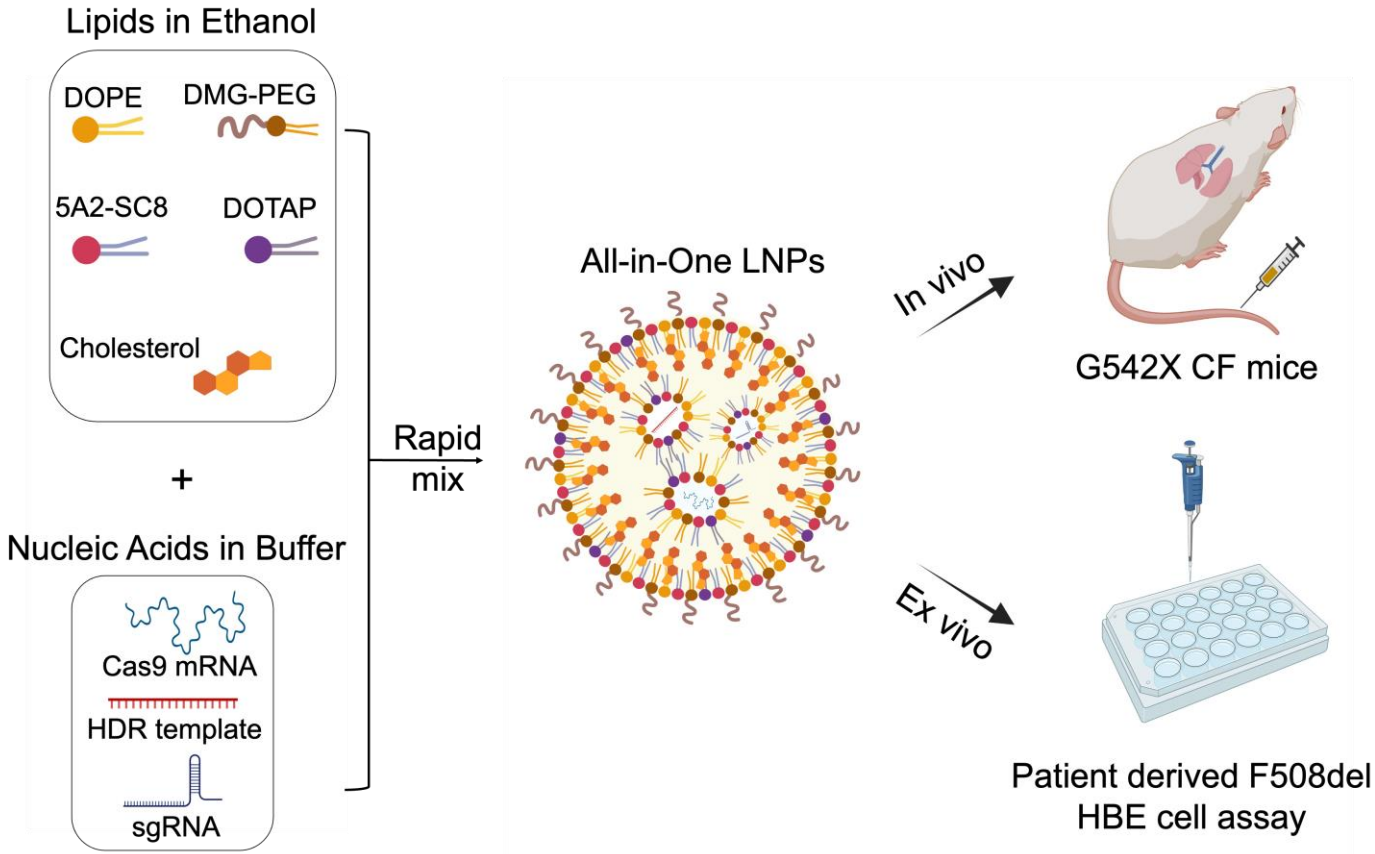
Optimization of encapsulated cargo ratios inside “all in one” LNPs improved CRISPR/Cas homology directed repair (HDR) correction

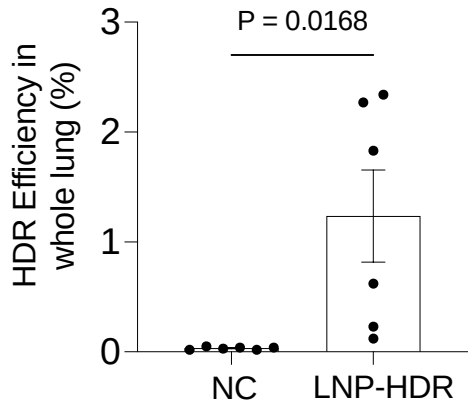


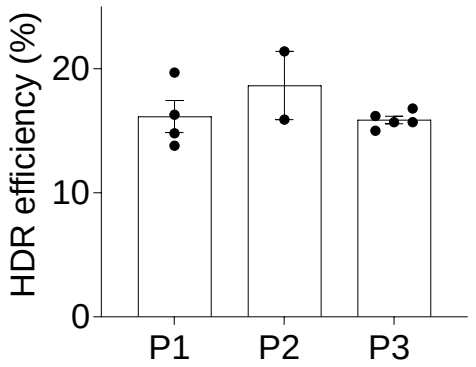
Cells shared by Jacob Corn lab

Lukas Farbiak et al. **Adv. Mater.** *33*, 2006619 (2021).

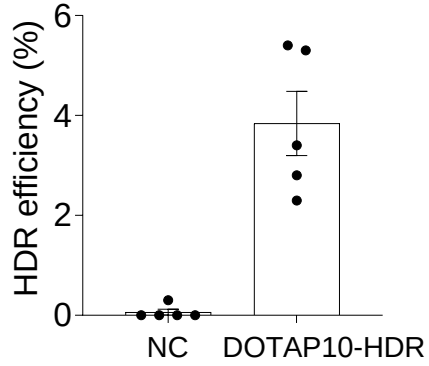
While genome correction could be achieved, the levels reached were not therapeutically meaningful







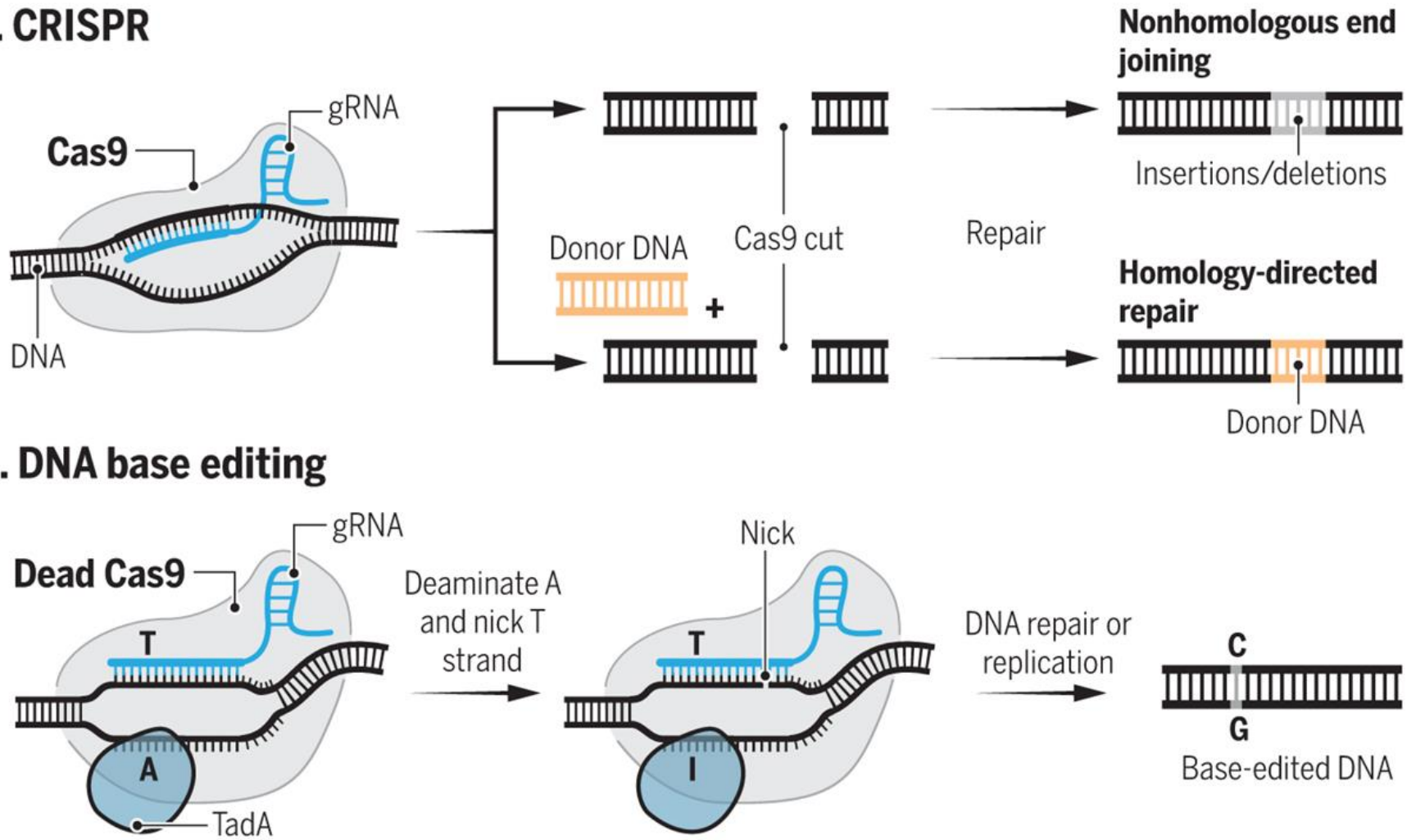
Undifferentiated cells



Fully differentiated cells

Tuo Wei,* Yehui Sun,* et al. **Nat. Commun.** *14*, 7322 (2023).

Base editing offers key advantages that could aid genome correction in stem cells



Potential advantages

- Works in non-dividing cells
- No DNA cleavage, no HDR
- No donor DNA template required
- Minimal *CFTR* gene disruption

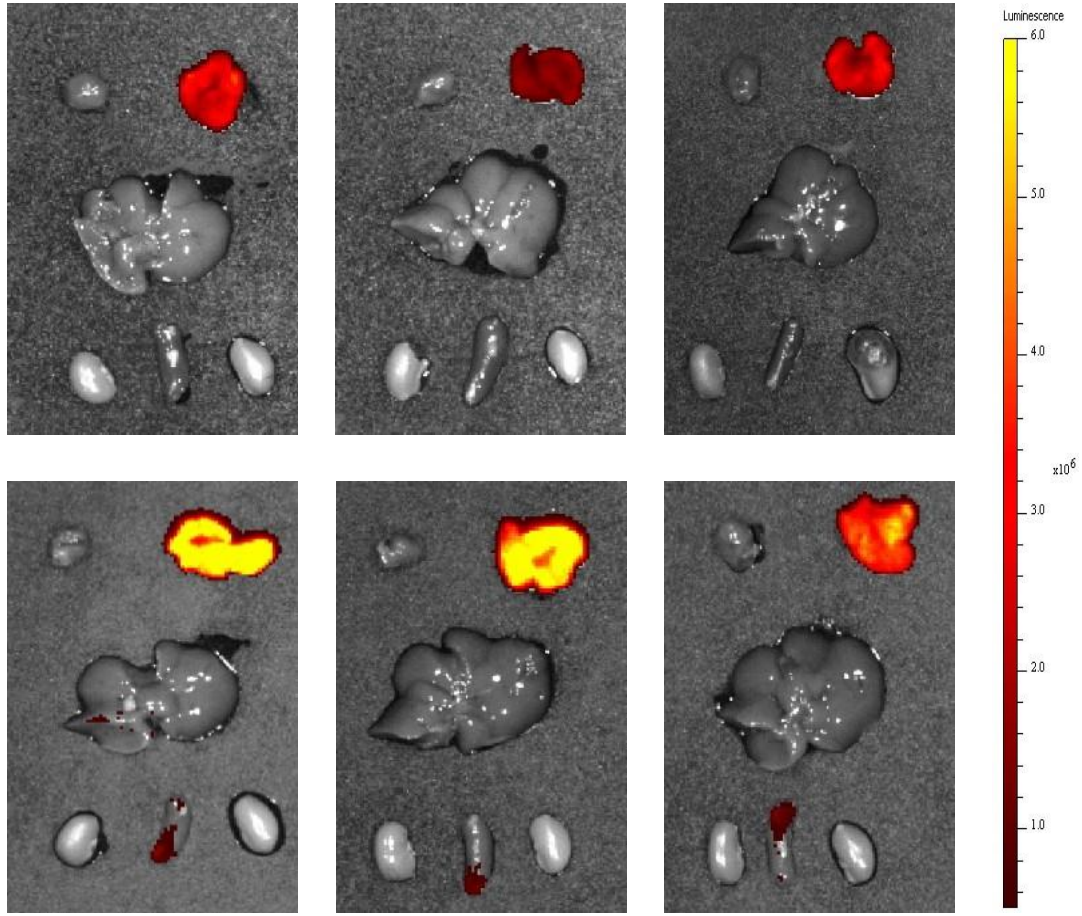
Cohen. **Science** *358*, 432 (2017).

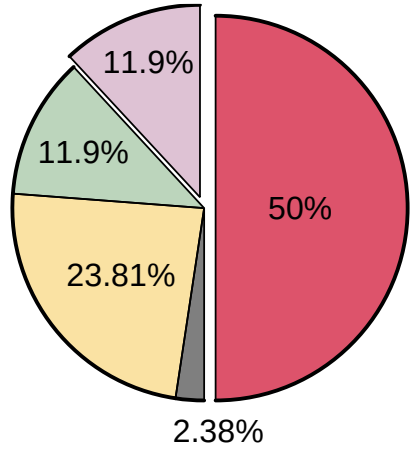
Xizhen Lian et al. **Nat. Nanotechnol.** *19*, 1409 (2024).
Minjeong Kim et al. **Nat. Biotechnol.** doi.org/10.1038/s41587-025-02675-z (2025).

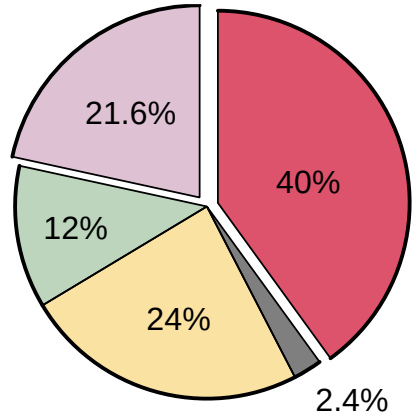
Improved SORT LNPs were optimized by adjusting the molar ratio of lipid components to increase potency and decrease toxicity

Lung SORT-01

Lung SORT-02



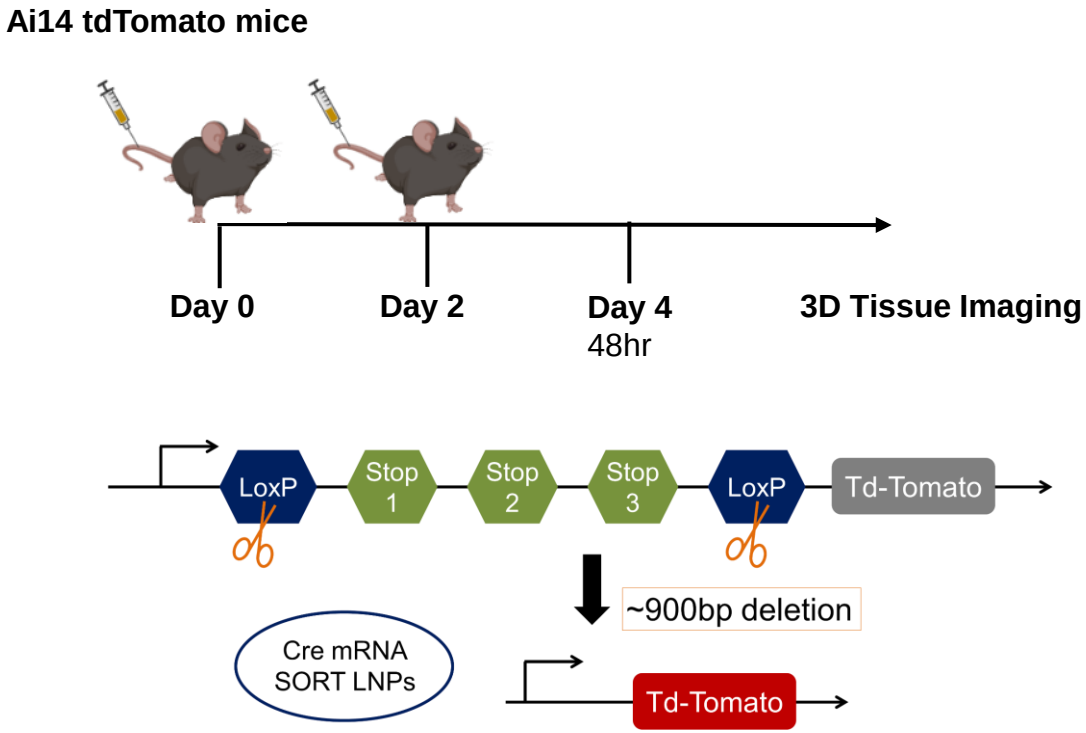


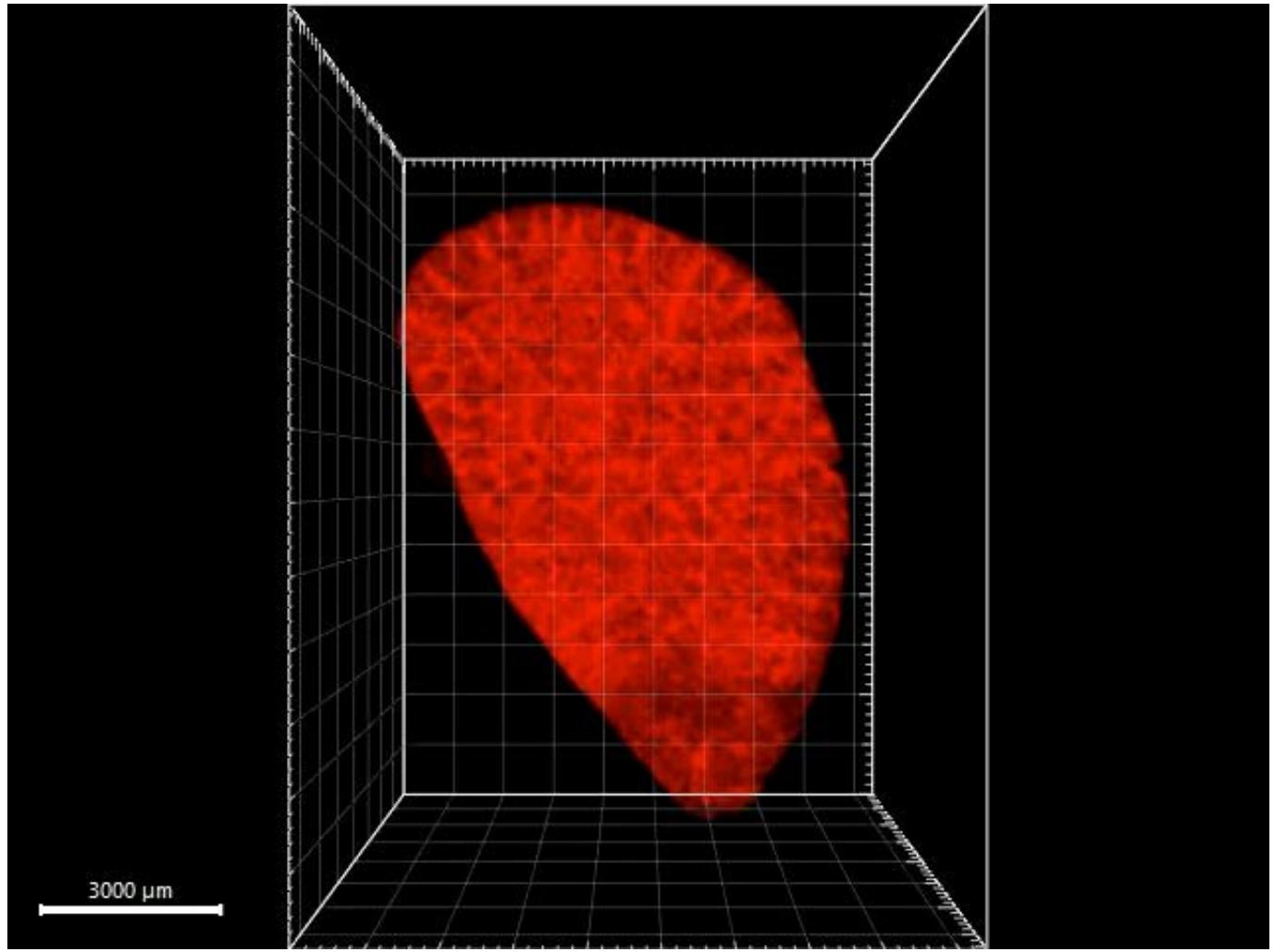


Ionizable lipid (5A2-SC8)
DOPE
Cholesterol
PEG-DMG
DOTAP

0.1 mg/kg Luc mRNA, IV
Analysis 6 hours after injection

Cre recombinase mRNA Lung SORT LNPs edit lung cells across the entire organ

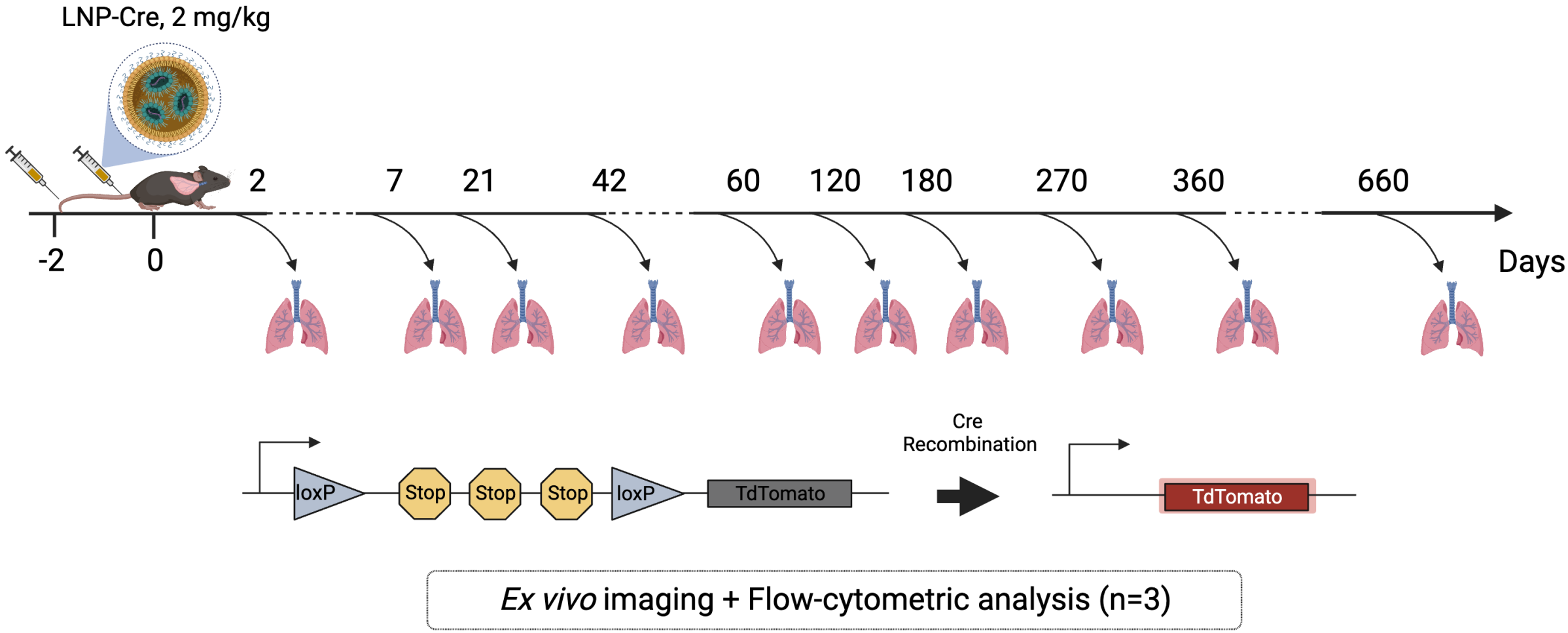




Jason Miller et al. **Angew. Chem. Int. Ed.** *129*, 1079 (2017). Published online December 2016.

Inflated left lung lobe imaged at the Whole Brain Microscopy Facility (Denise Ramirez)

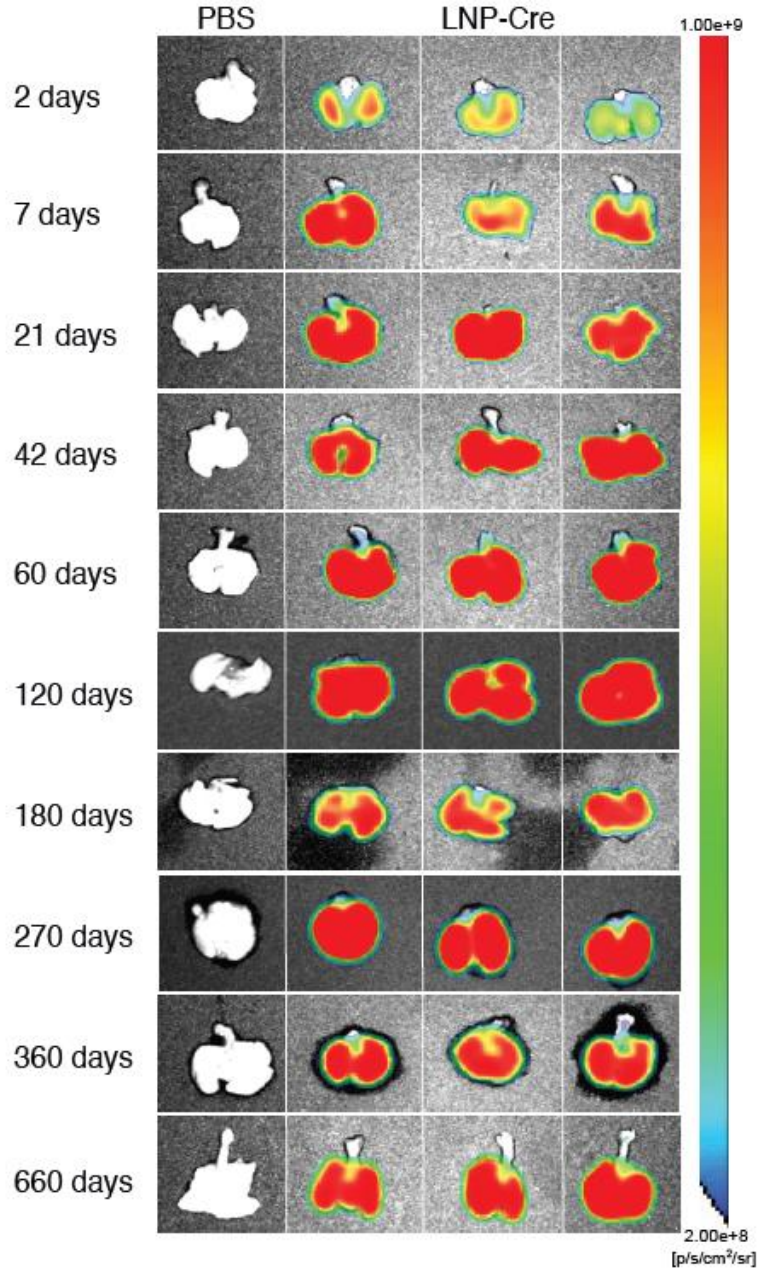
Cre mRNA Lung SORT LNPs were administered IV to Ai14 mice to evaluate the rates and long-term persistence of genetic changes in the lungs

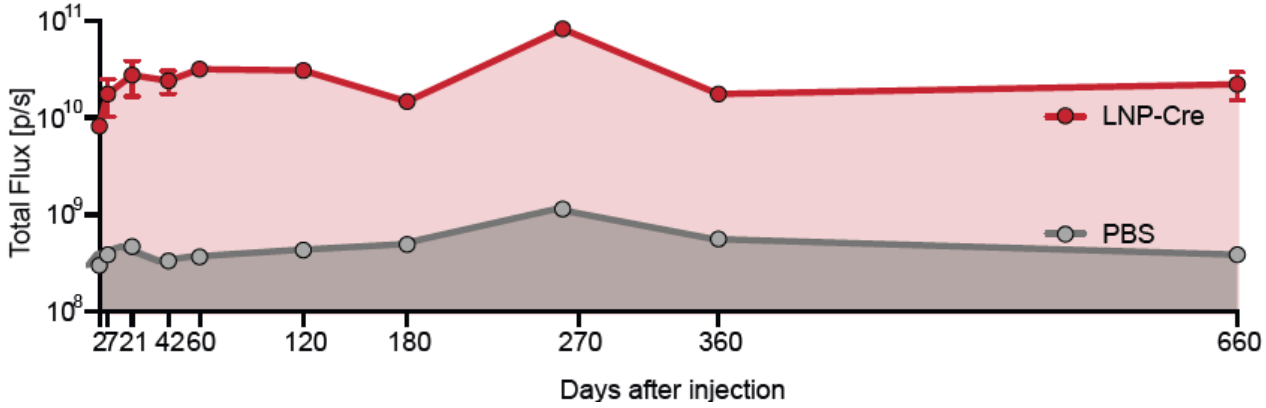


DOTAP40-02, 2 mg/kg Cre mRNA
IV, two injections in total

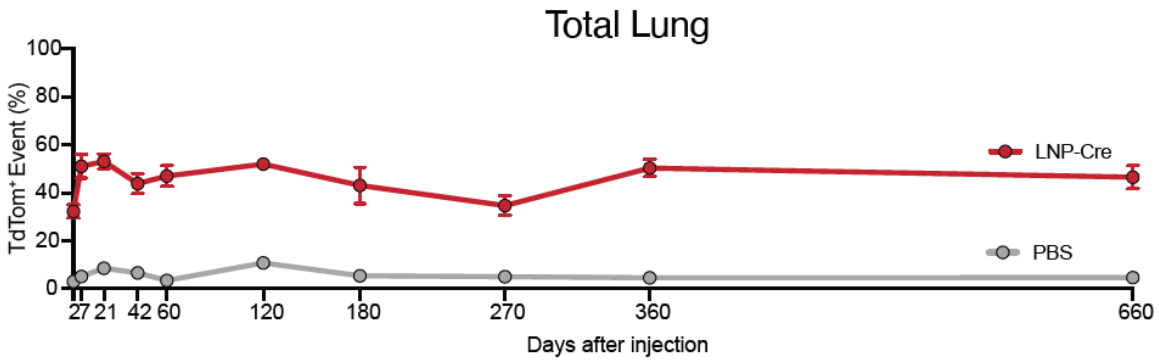
Yehui Sun,* Sumanta Chatterjee,* et al. **Science** *384*, 1196 (2024).

Persistent tdTomato fluorescence was quantified throughout the 22 months' long study

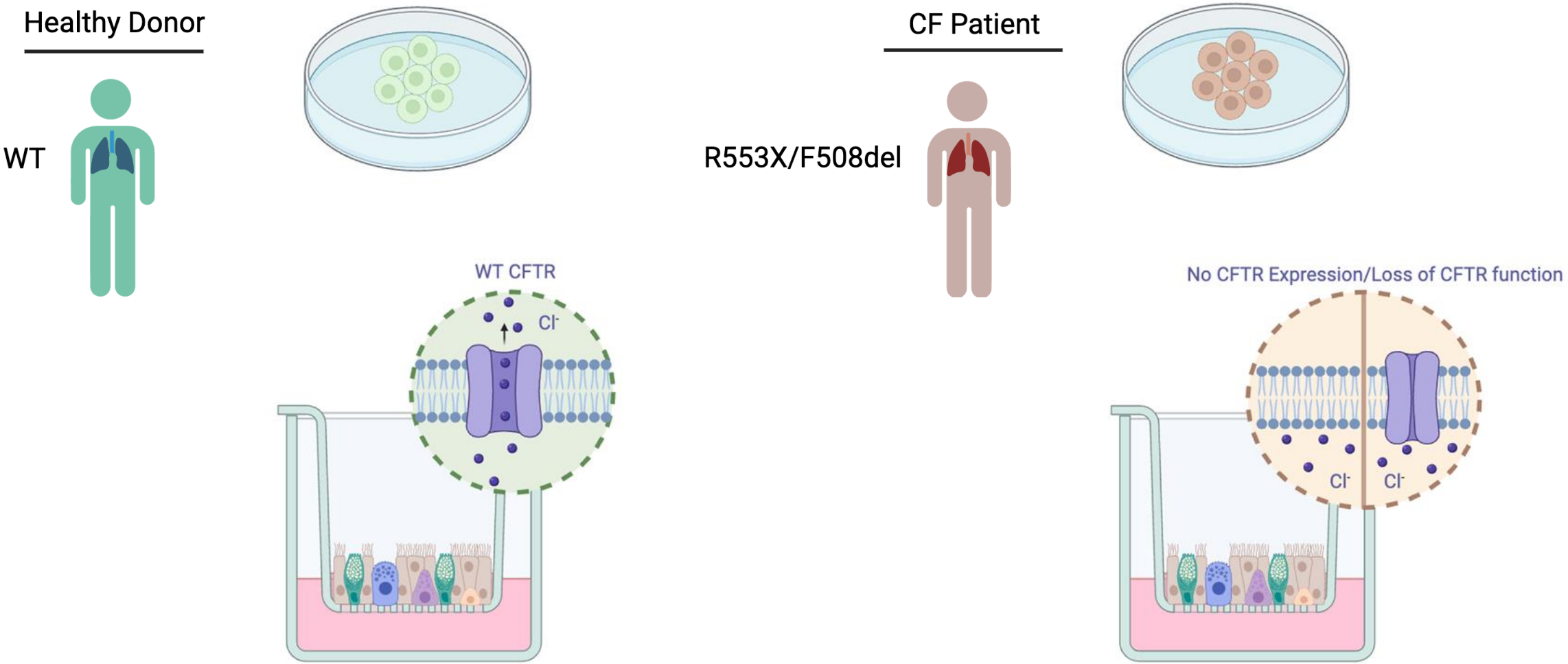




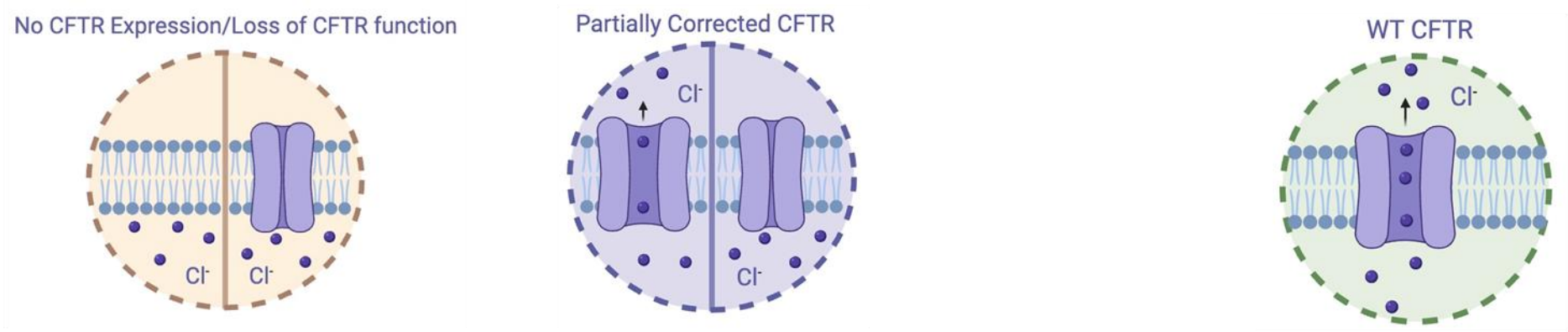
A diverse array of lung cell types encompassing endothelial, epithelial, immune, and stem cells were durably edited

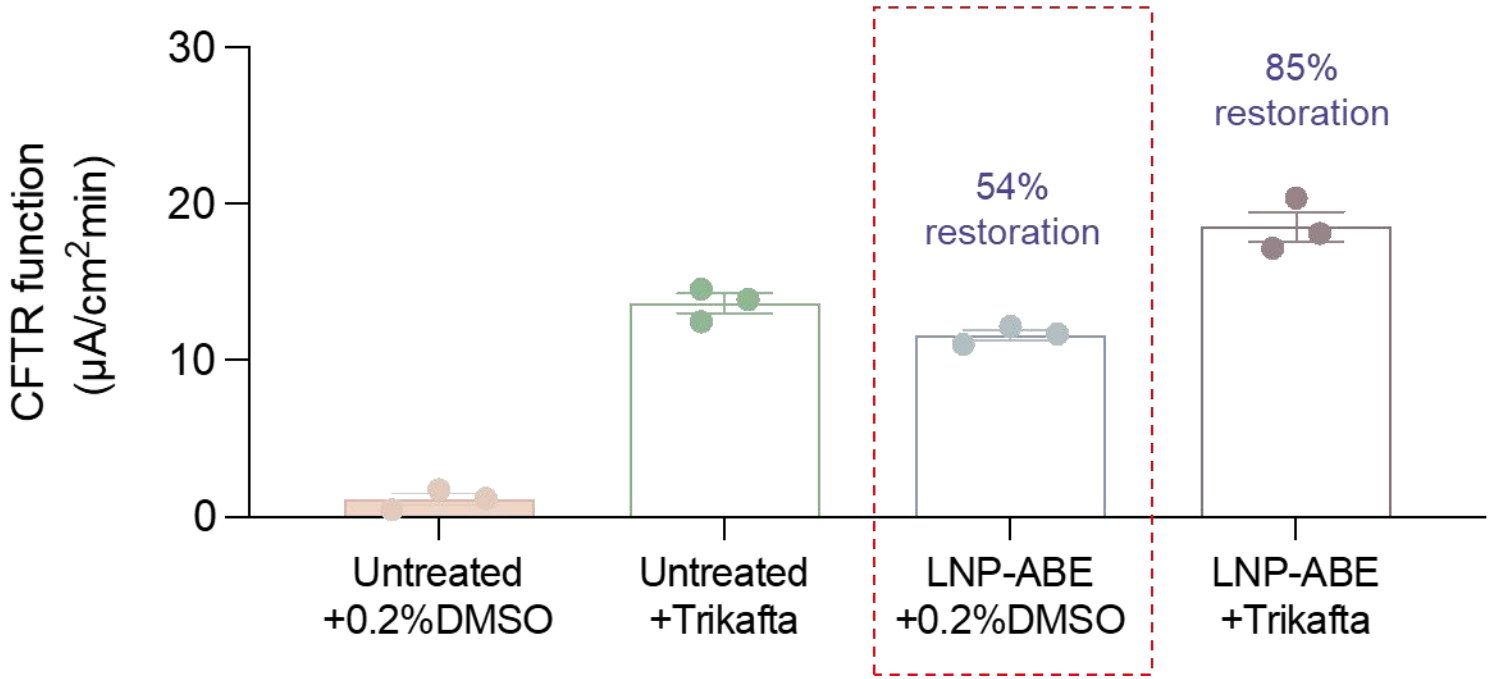


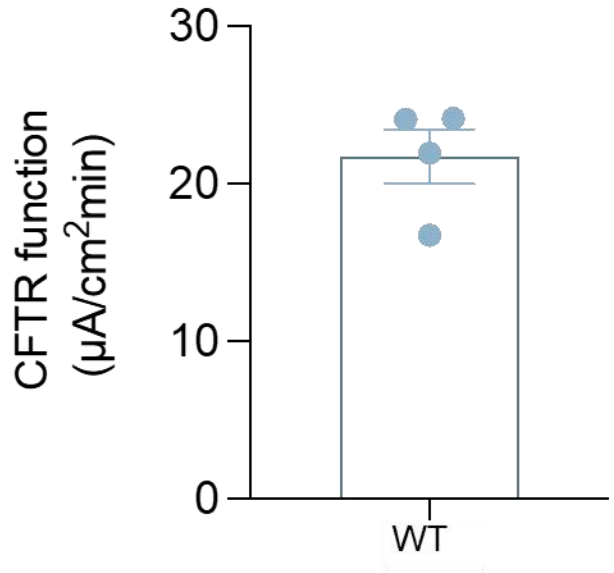
CFTR function was measured in differentiated human bronchial epithelial (HBE) cells cultured at an air-liquid interface



Lung SORT LNPs enabled 70% allelic gene correction via adenine base editing (ABE) and 54% restoration of CFTR function in R553X/F508del hBEs

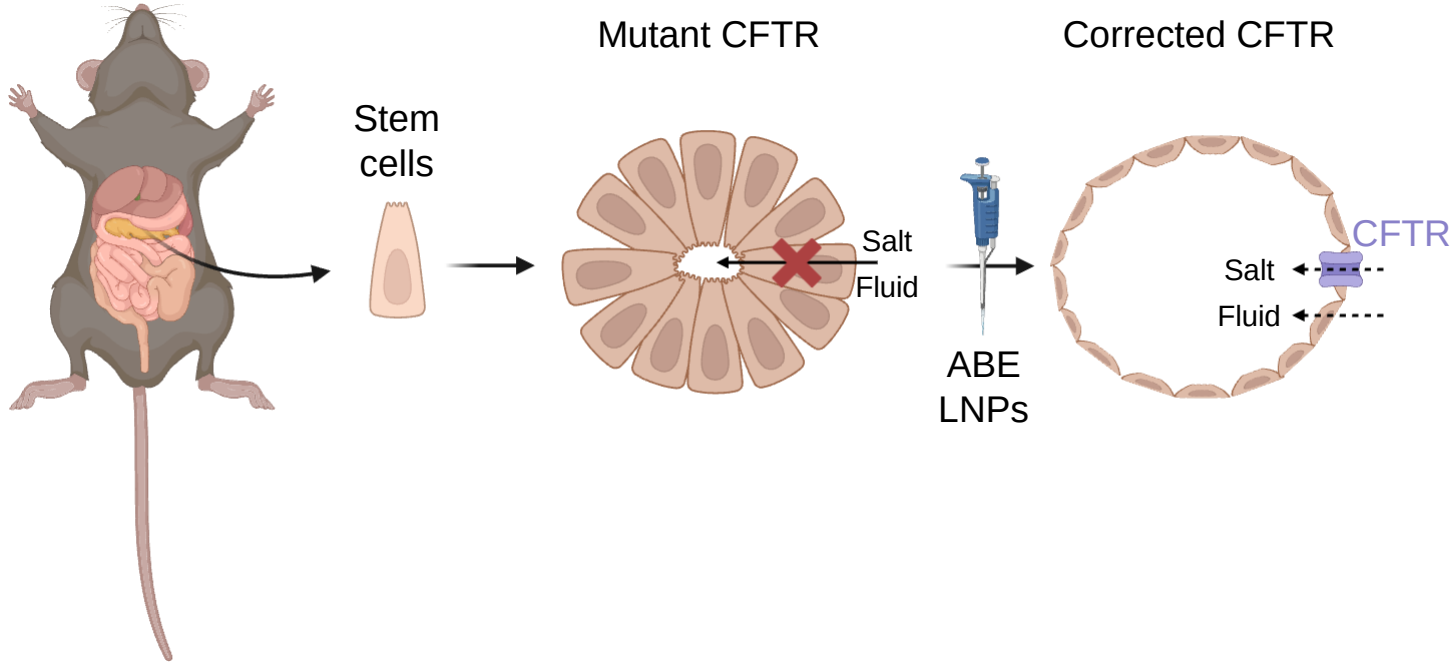


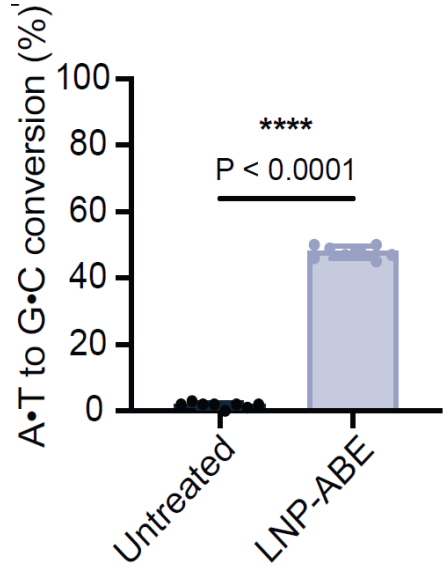




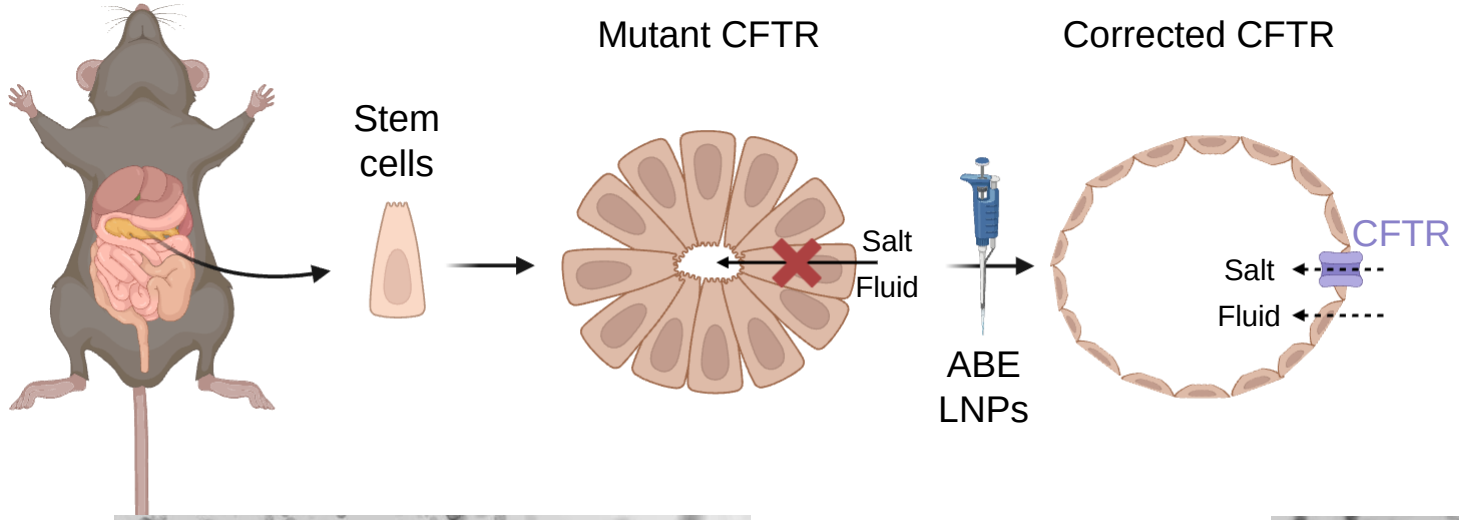
Collaboration with ReCode Therapeutics

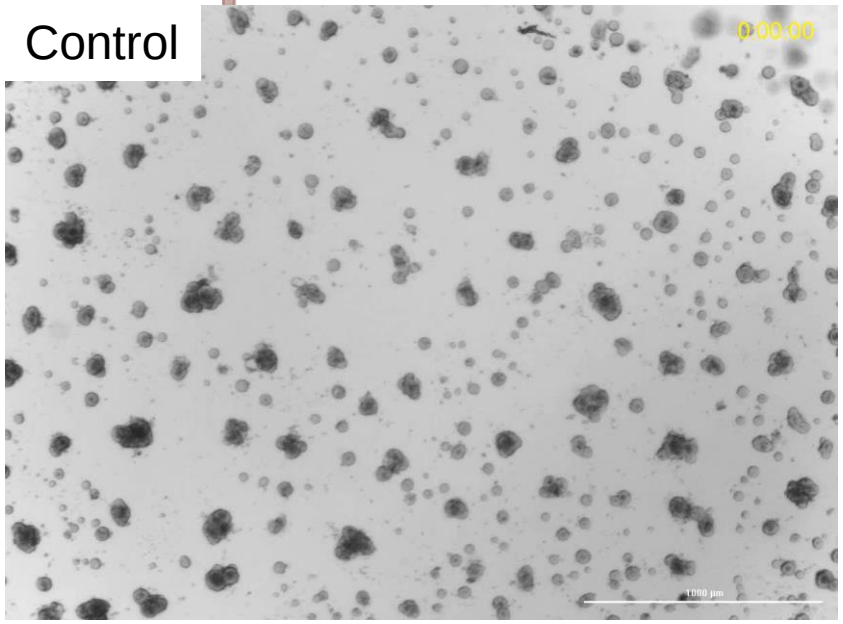
Gene correction via ABE Lung SORT LNPs resulted in functional CFTR-induced swelling in organoids derived from R553X CF mice

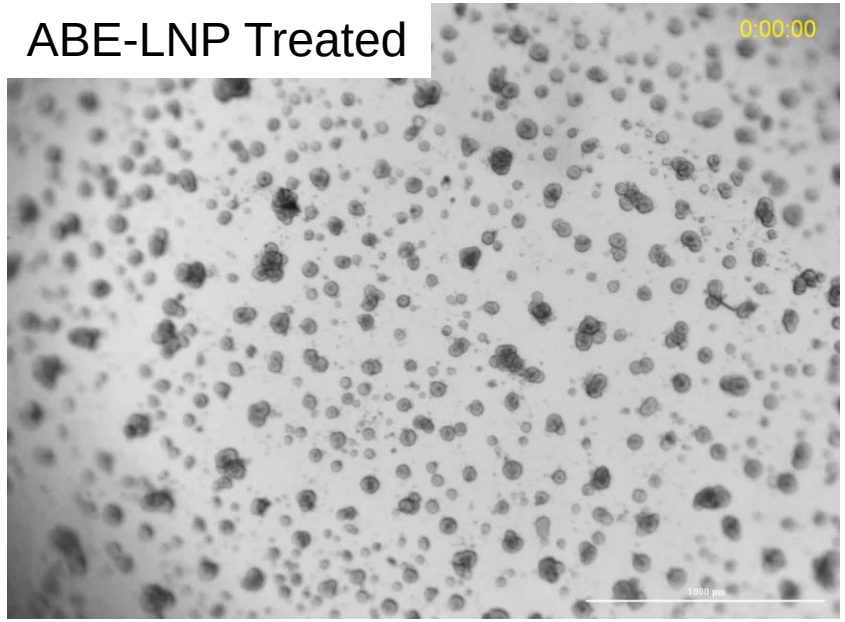


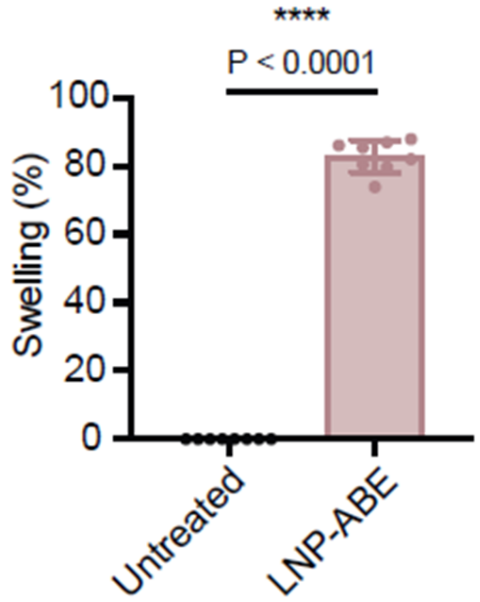


Gene correction via ABE Lung SORT LNPs resulted in functional CFTR-induced swelling in organoids derived from R553X CF mice

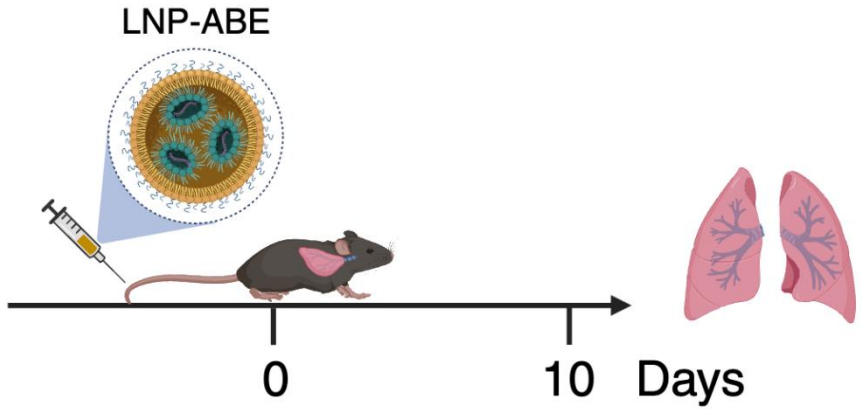


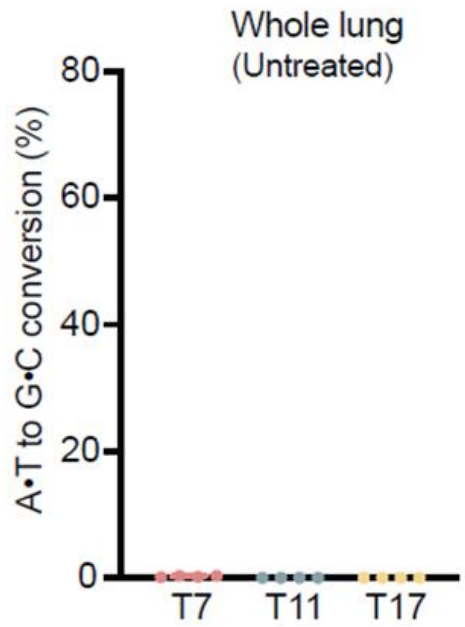


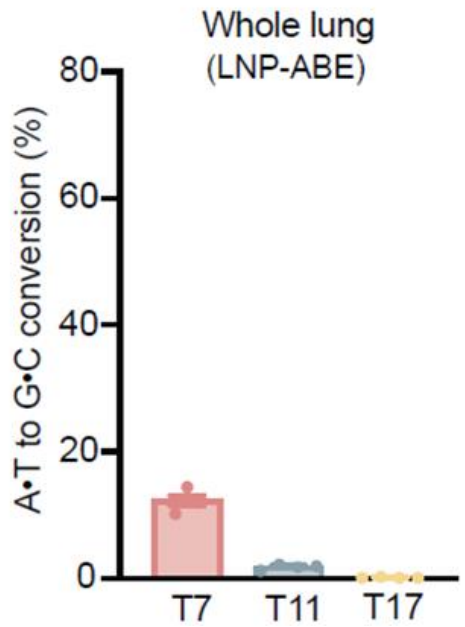


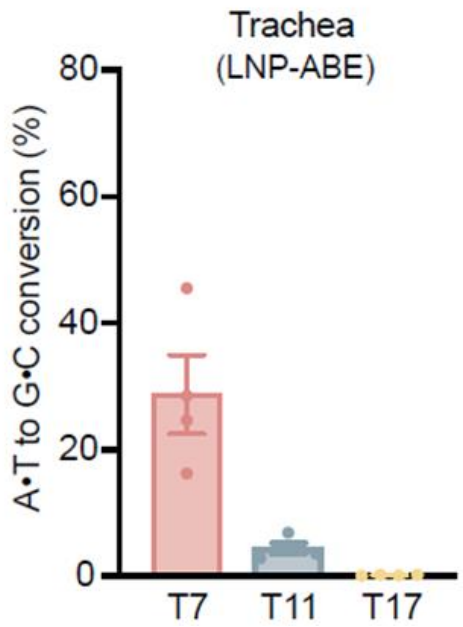


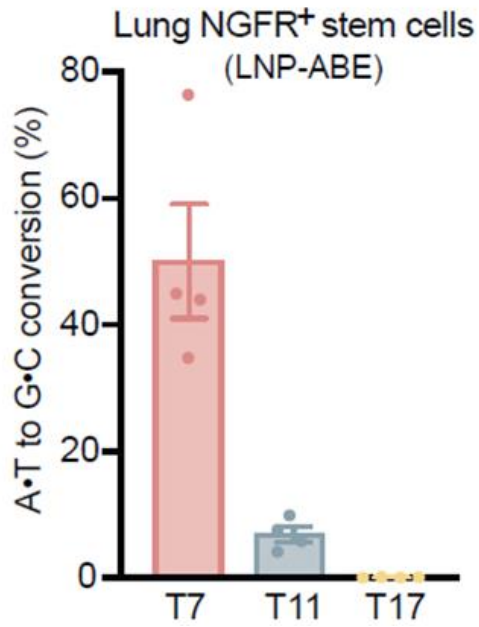
50% CFTR gene correction was quantified in lung stem cells of R553X CF mice following IV administration of ABE Lung SORT LNPs









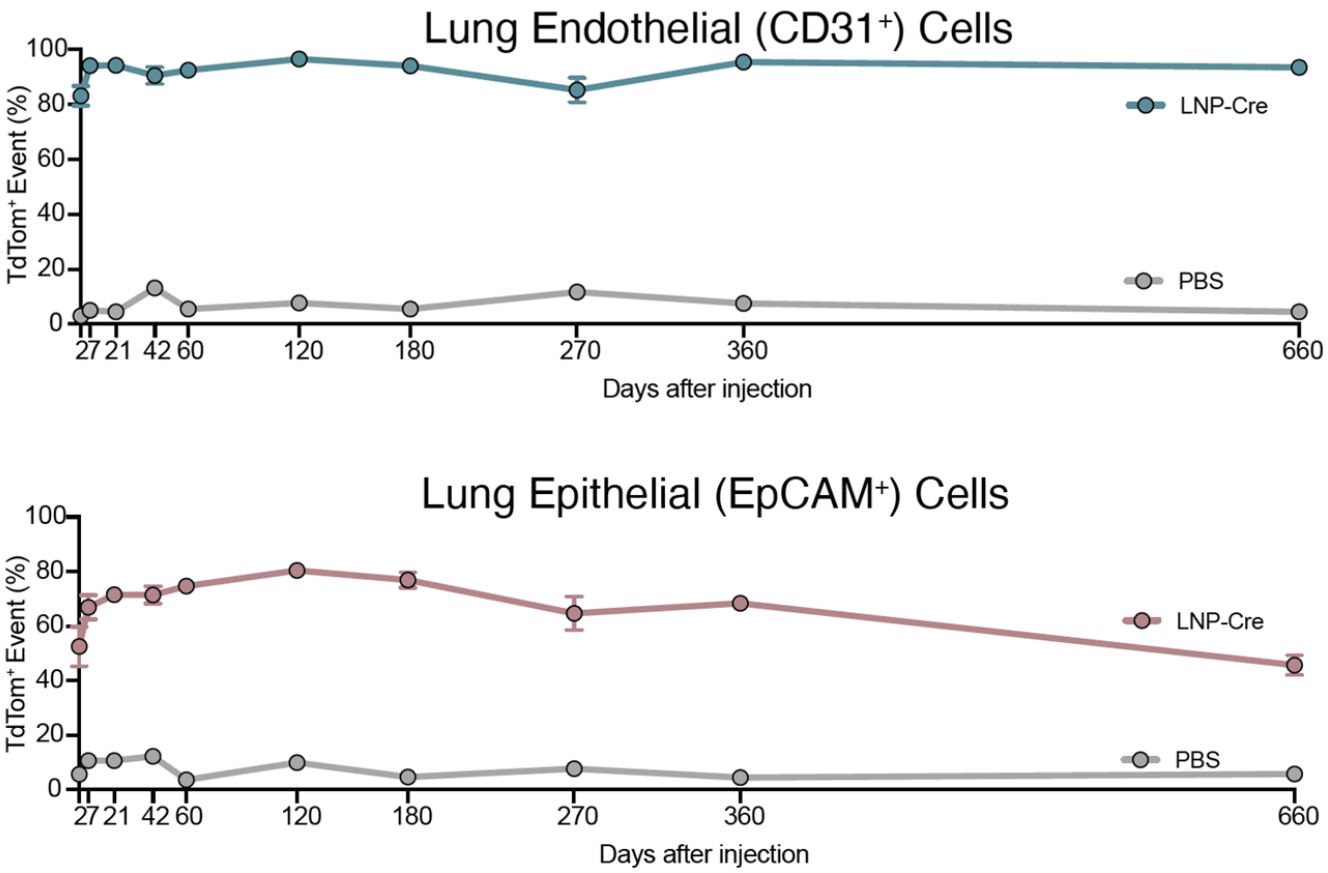


LNP-ABE (1.5 mg/kg total RNA, ABE mRNA:sgR553X = 2:1), IV.

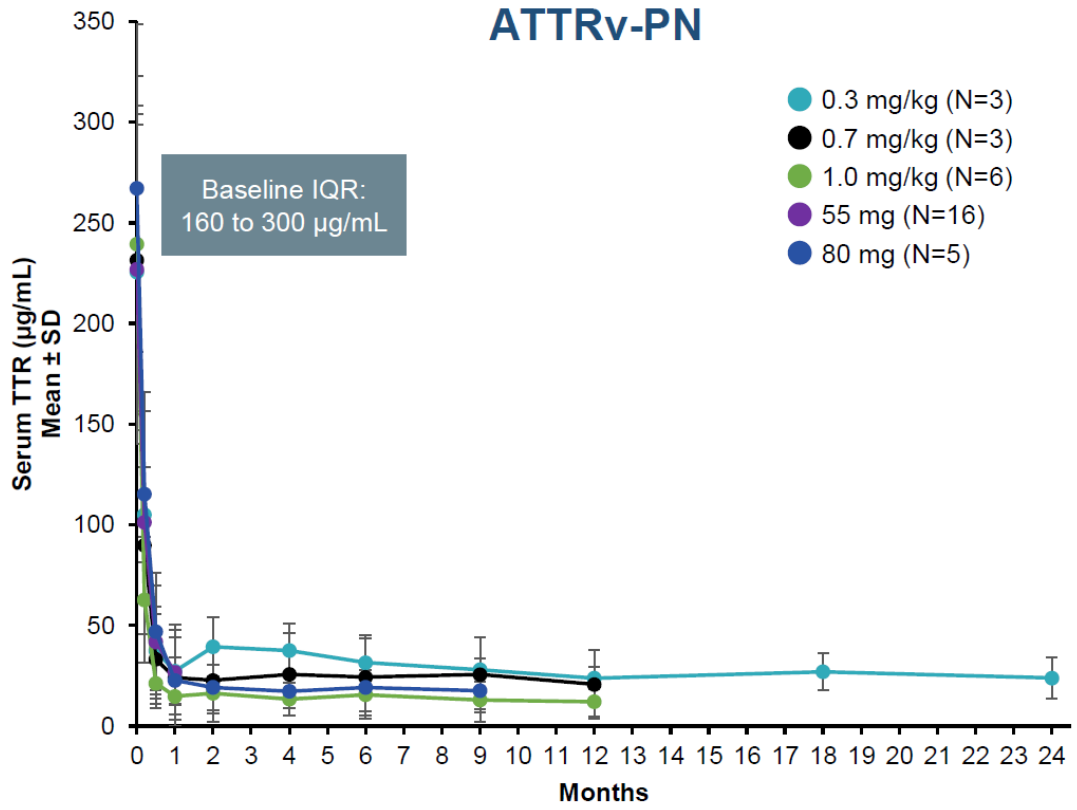
Collaboration with Craig Hodges (CWRU)

If this finding in mice can be translated to humans, it may indeed be possible to someday realize durable therapies for CF

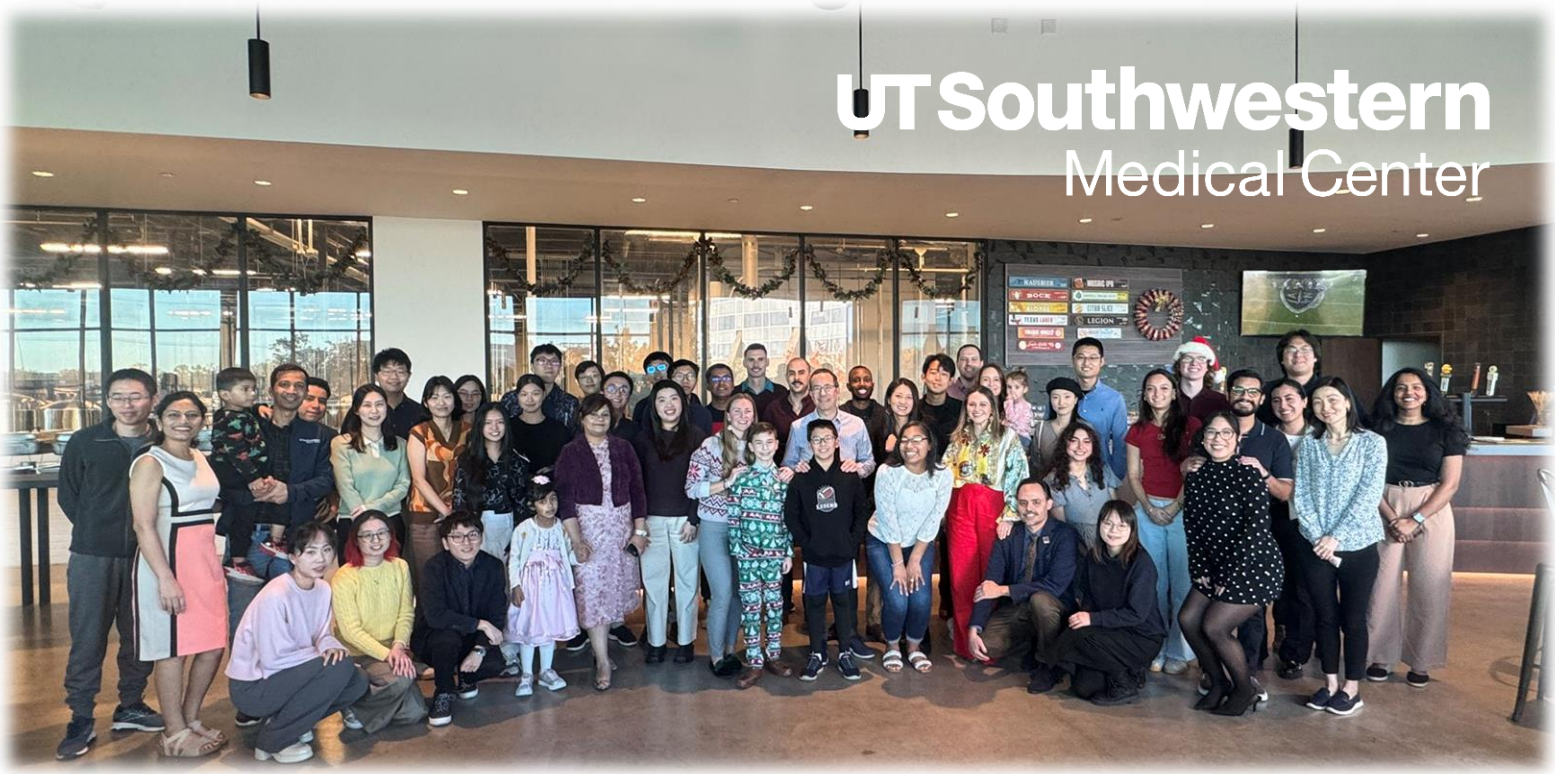
Mouse lungs



Human livers



The Program in Genetic Drug Engineering



Collaborators: Eric Olson, Bruce Beutler, Hao Zhu, Raksha Jain, Richard Wang, James Brugarolas, Uttam Tambar, Tian Qin, Craig Hodges, Mitch Drumm, Ron Conlon, Brian Davis, Sam Sternberg, David Liu, Mitch Weiss, Jonathan Yen, Charlie Emerson, Scot Wolf, Erik Sontheimer, Jonathan Watts, Hillary Valley, Jed Mahoney, Martin Mense, Phil Thomas

Past and current funding: Cystic Fibrosis Foundation, National Institutes of Health, Welch Foundation, CPRIT, DOD, American Cancer Society, Mary Kay Foundation, ReCode, Pfizer, NIH St. Jude Sickle Cell Consortium

Program Leadership

Lukas Farbiak, Ph.D.
Assistant Professor
Sumanta Chatterjee, Ph.D.
Instructor
Pratima Basak, Ph.D.
Jing Liu, Ph.D.
Laytonique Bacon

Program Scientists

Gabriela Ayala Pazzi
Alexis Pacheco Benitez
Xiaoyan Bian
Zexiang Chen, Ph.D.
Yaou Duan, Ph.D.
Lukas Farbiak, Ph.D.
Erick Guerrero
Haixia He
Lindsay Hill, M.D., Ph.D.
Hsi-Chien Huang, Ph.D.
Michaela Jeong, Ph.D.
Lisa Kim
Minjeong Kim, Ph.D.
Minwoo Kim
Sang Lee, Ph.D.
Tsung-Ying Lee
Xi Liu, Ph.D.
William Miller
Stephen Moore
Priyanka Patel
Vaidehi Patel
Clarissa Pham
Joshua Robinson
Julien Santelli, Ph.D.
Eunice Song
Yehui Sun
Yun-Chieh Sung, Ph.D.
Zeru Tian, Ph.D.
Amogh Vaidya
AP Wang
Xu Wang, Ph.D.
Jingwen Wei, Ph.D.
Shiying Wu
Yufen Xiao, Ph.D.
Victoria Yang
Kohki Yamada
Chuhan Zhang, Ph.D.
Fangyu Zhang, Ph.D.

Advertisement for another talk from our lab

Friday, July 18, 2025

8:54 AM - 9:05 AM EDT

Polymer lipids reduce anti-PEG antibody binding for repeated administration of mRNA therapeutics

Location: 119 B

Oral Abstract Speaker: Yufen Xiao, PhD – UT Southwestern Medical Center

Gene Delivery and Gene Editing

Yufen Xiao, Ph.D.

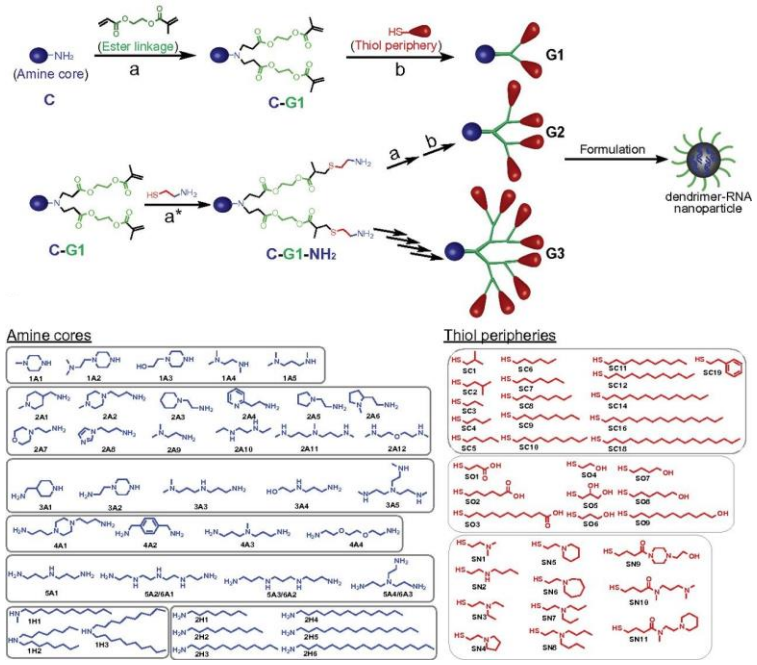
CRS 2025 ANNUAL MEETING & EXPOSITION

PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Our laboratory utilizes materials chemistry and engineering to enable organ and cell enriched nanoparticle delivery of genetic medicines

Lipid and Polymer Chemistry



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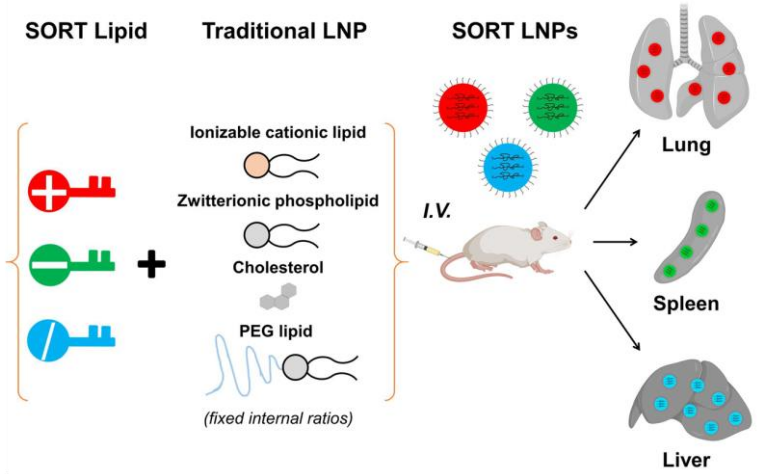
LNP Engineering



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Nature Nanotechnology *15*, 313 (2020).
Advanced Materials *33*, 2006619 (2021).
PNAS *118*, e2109256118 (2021).
Nature Protocols *18*, 265 (2023).
Nature Materials *in press* (2025).
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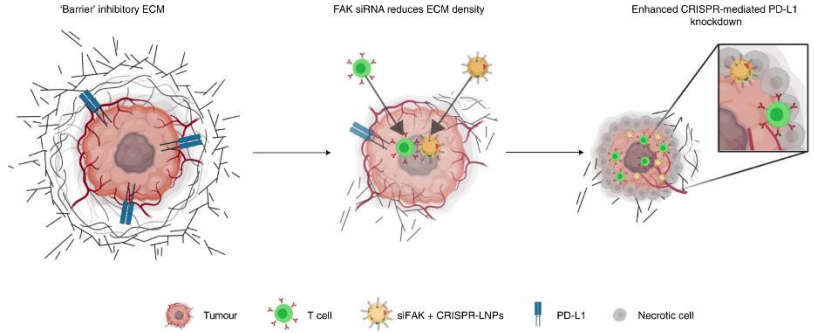
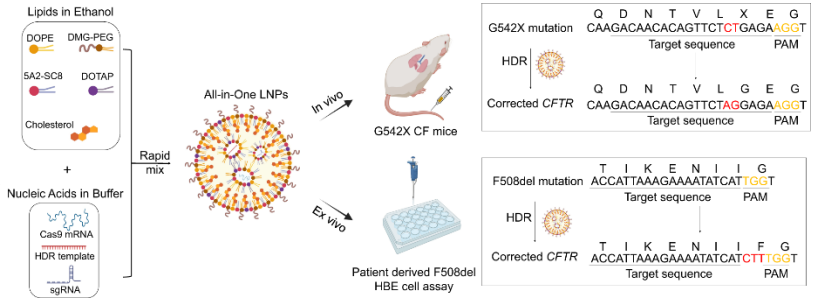
Therapeutic Applications

Cancer therapy



Cystic fibrosis treatment



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