Pulmonary delivery of circular mRNA (circRNA) therapeutics for cystic fibrosis (CF)

Ri Tang, MD, College of Pharmacy, University of Michigan
PI: Guizhi (Julian) Zhu, PhD, College of Pharmacy, University of Michigan

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CRS 2025 ANNUAL MEETING & EXPOSITION
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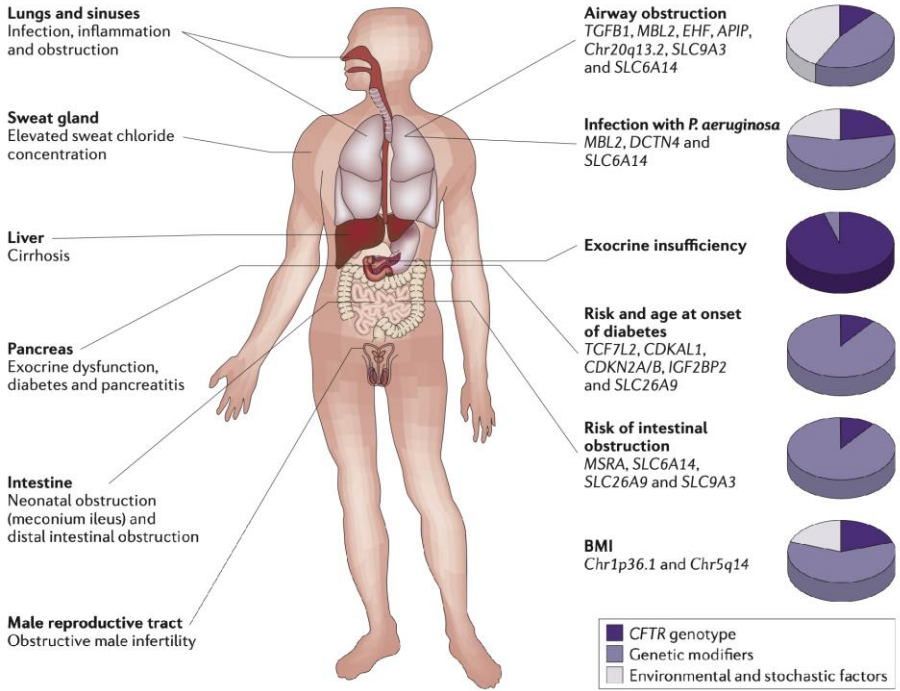
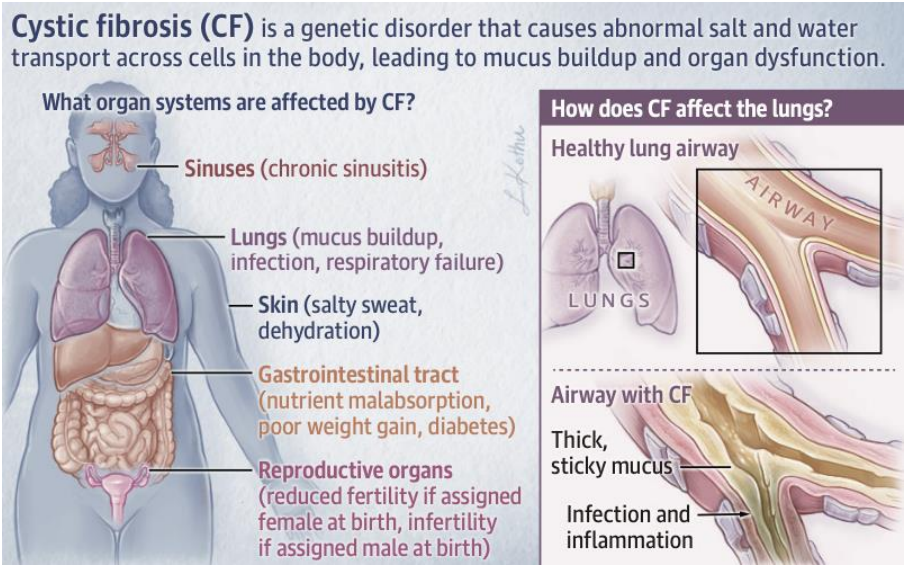
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Clinical background of Cystic fibrosis



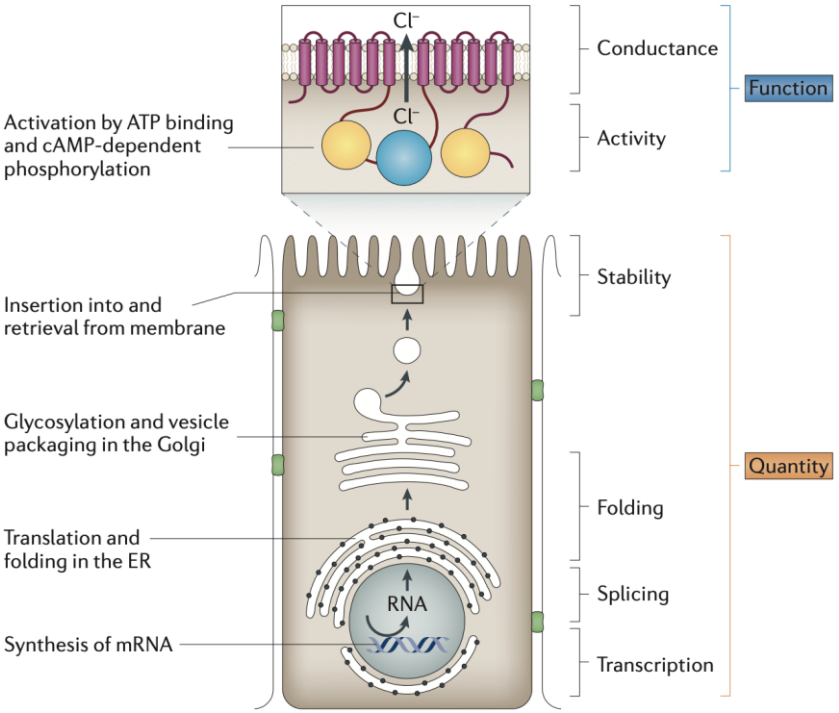
Nat Rev Genet. 2015 Jan



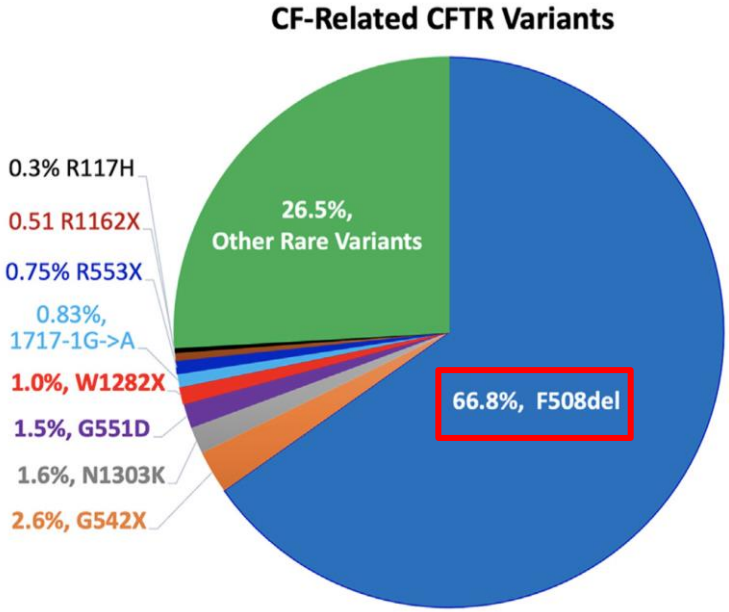
Cystic fibrosis affects more than 30,000 children and adults in the US and 70,000 people worldwide. It is most common in non-Hispanic White individuals.

JAMA. 2022 Jan

CF is caused by CF Transmembrane Conductance Regulator (CFTR) mutation



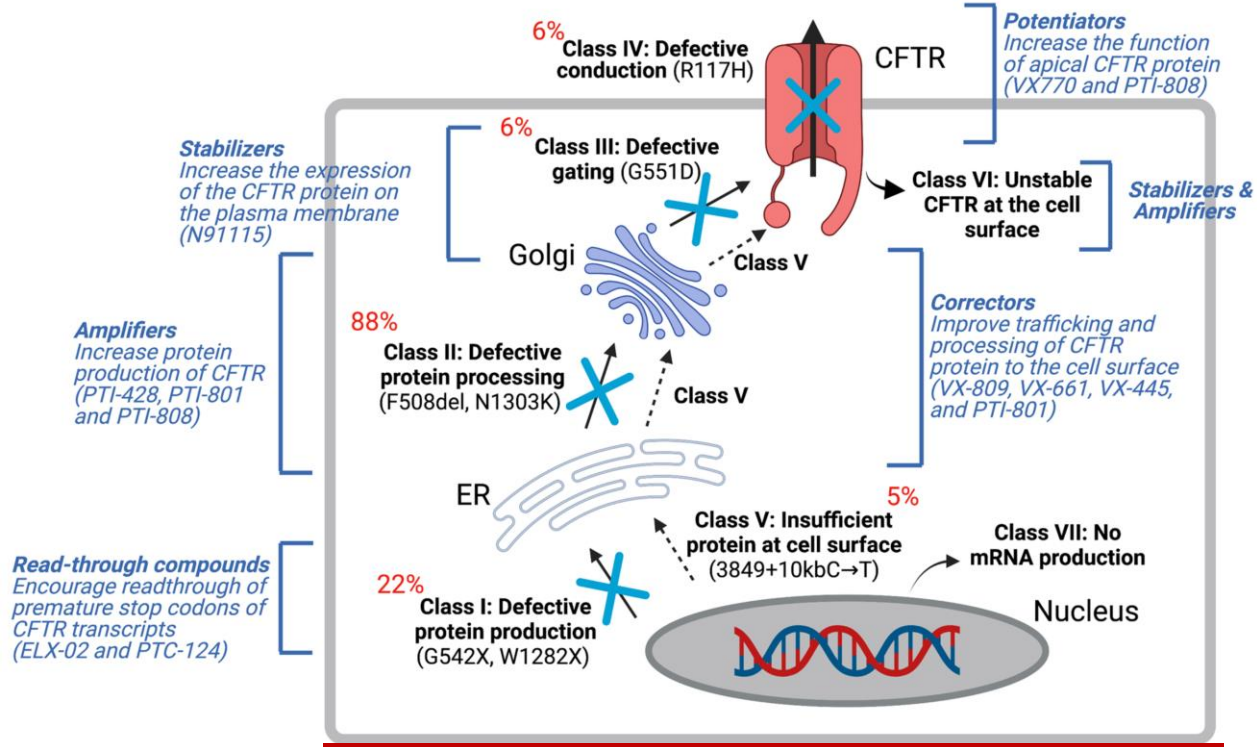
Nat Rev Genet. 2015 Jan



Restoring CFTR functions can be a promising therapeutic strategy for CF.

Front Pharmacol. 2022 Oct

CFTR modulator therapy



Rare CFTR variants remain uncured !

correctors | potentiators

stabilizers | amplifiers

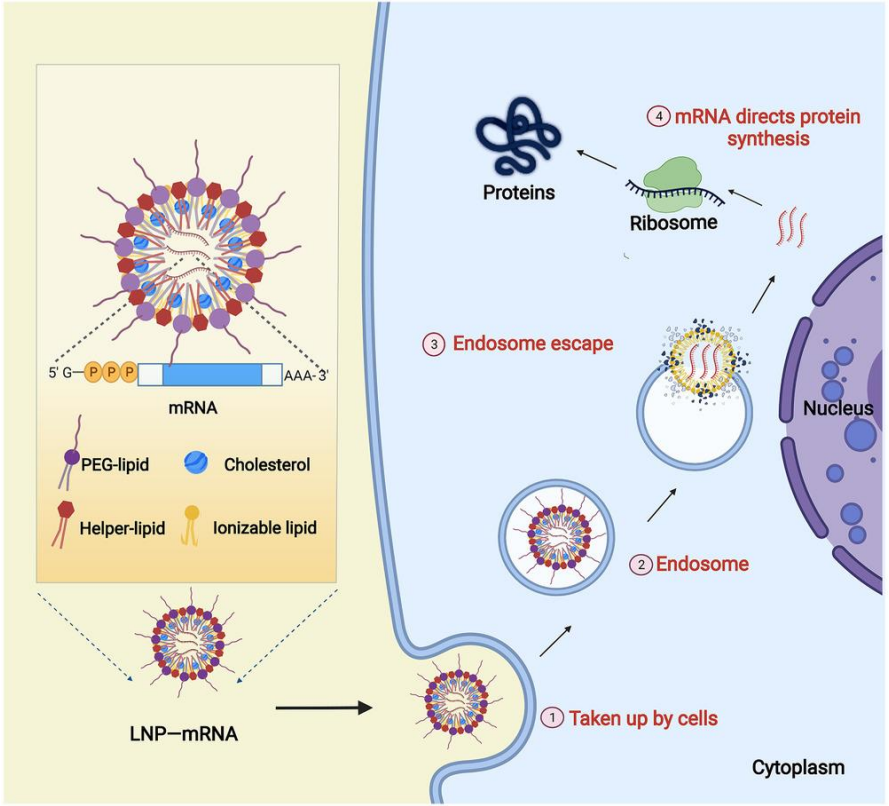
The modulator isn't universally effective and rather limited to specific classes of mutations.

J Transl Med. 2021 Oct

CFTR-encoding circRNA (circRNA-CFTR) for CF protein replacement therapy?

- Studies in humans suggested that **as little as 8%** of the normal CFTR transcript was sufficient to preserve normal lung function.

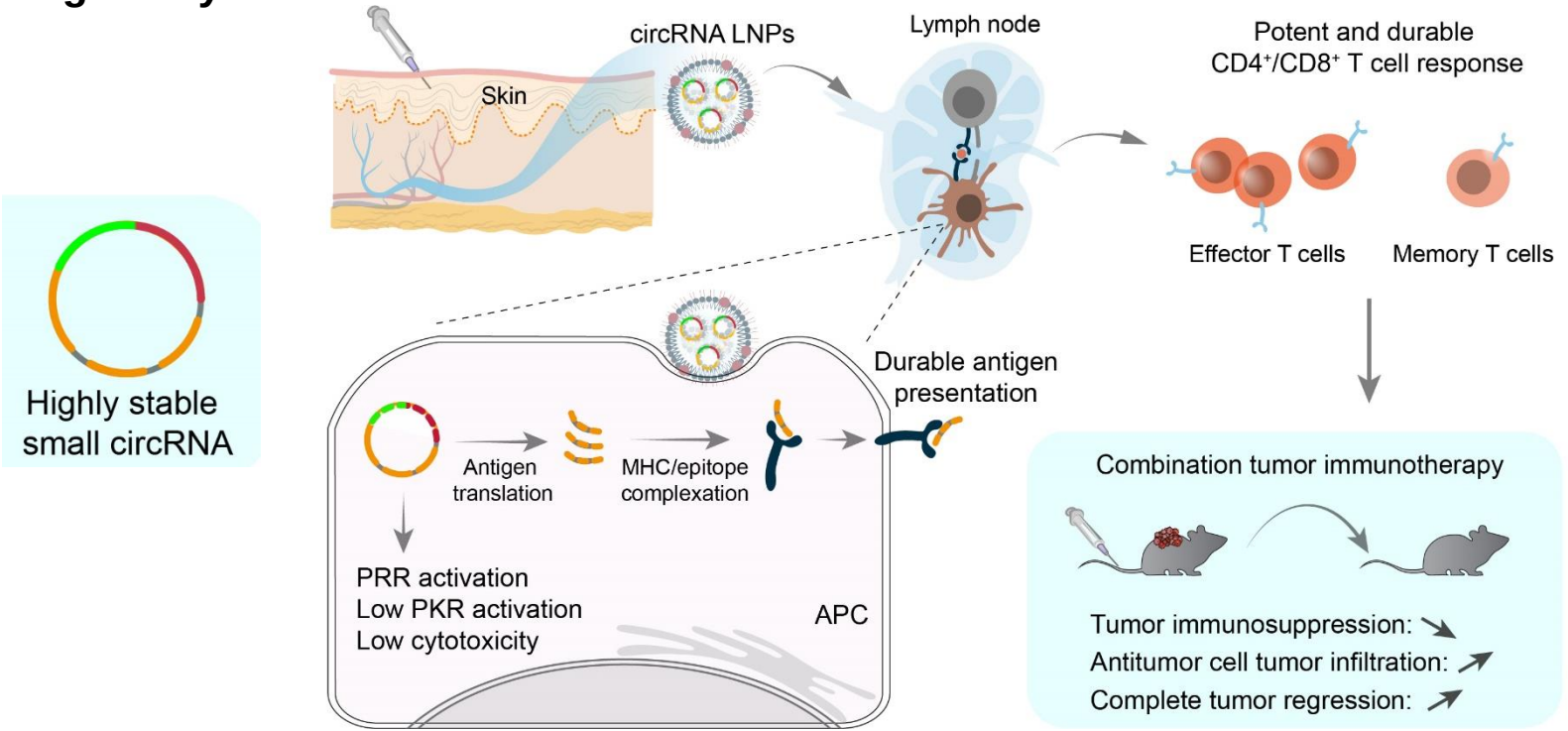
- Restoring **10% of CFTR mRNA** or **10%–35% of protein function** would be sufficient to ameliorate disease.



Am J Respir Cell Mol Biol. 2002 Nov

MedComm. 2022 Aug

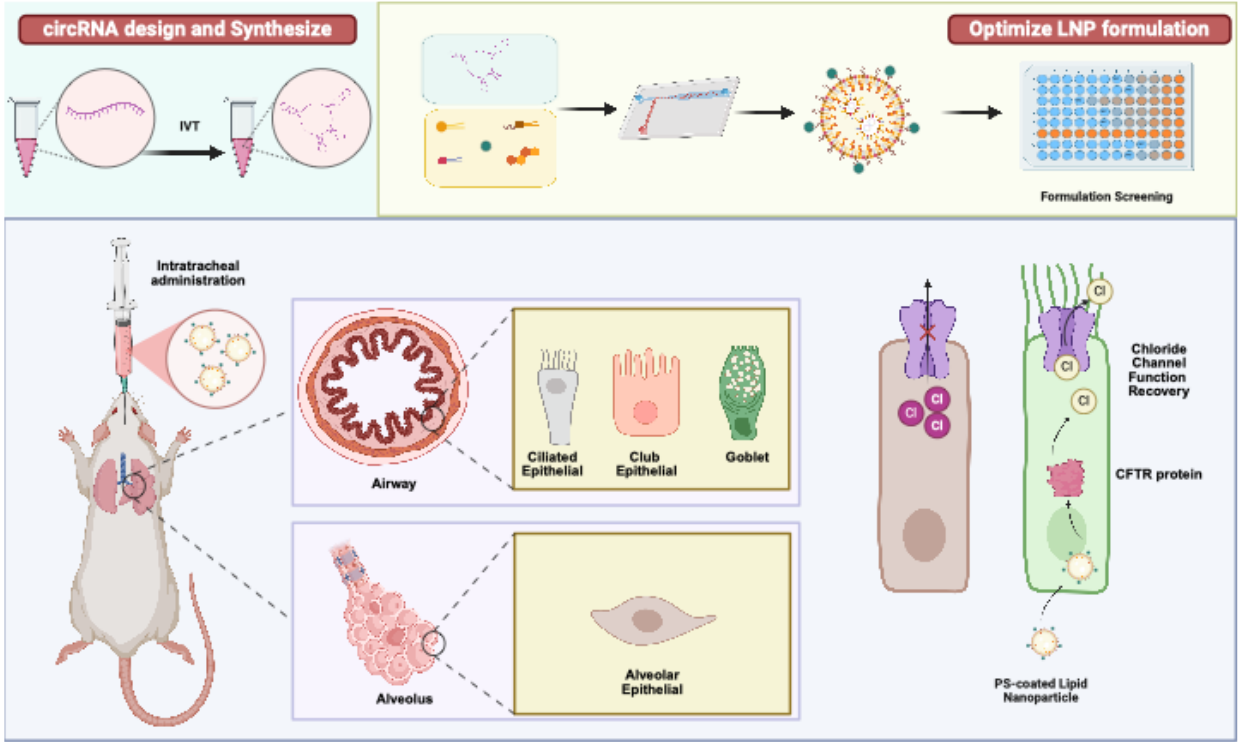
Protein-encoding circRNA: highly stable, long-lasting protein translation, low innate immunogenicity.



IRES: Internal Ribosome Entry Site.

Nat Biomed Eng. 2025 Feb

Scheme: pulmonary delivery of circRNA-CFTR using lipid nanoparticles (LNPs) for CF treatment



Experiment designment

1: circRNA-CFTR design and synthesis

2: Design of Experiment (DOE) screening of LNPs for pulmonary RNA delivery

3: Evaluate circRNA-CFTR LNPs in a human CF cell line

4: Evaluate circRNA-CFTR LNPs in a CF mouse model (ongoing)

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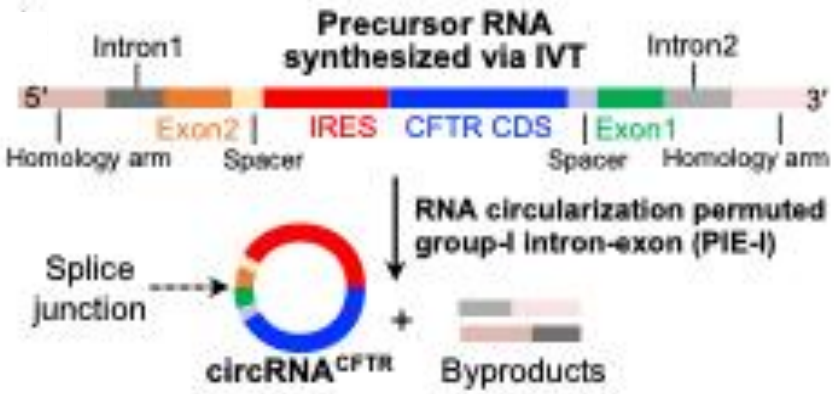
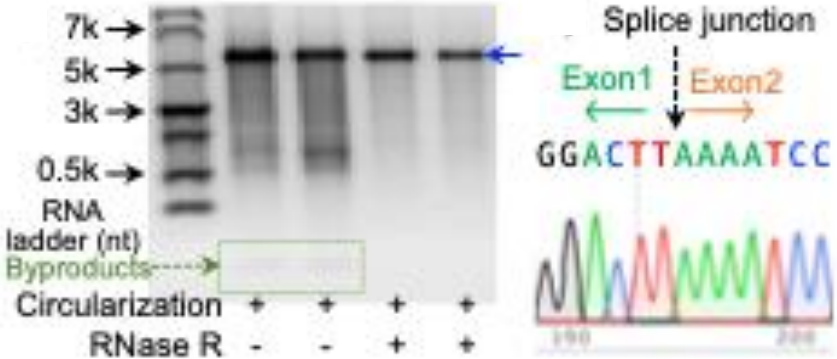
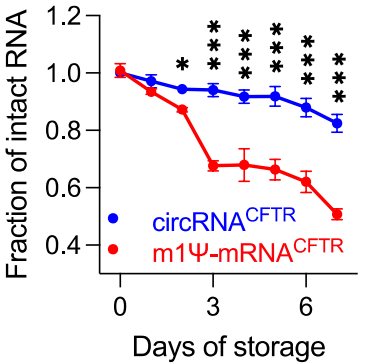
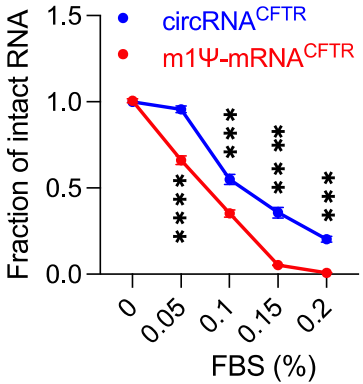
circRNA-CFTR design and synthesis









Experiment designment

1: circRNA-CFTR design and synthesis

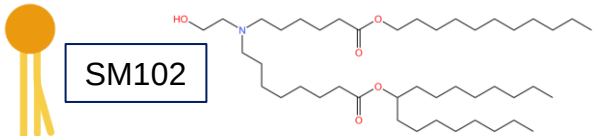
2: Design of Experiment (DOE) screening of LNPs for pulmonary RNA delivery

3: Evaluate circRNA-CFTR LNPs in human CF cells

4: Evaluate circRNA-CFTR LNPs in CF mouse model (ongoing)

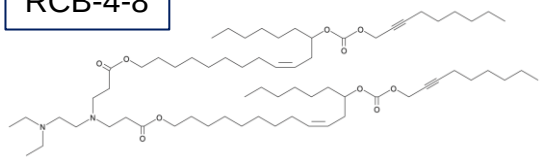
Compositions of LNPs studied for pulmonary delivery of RNA

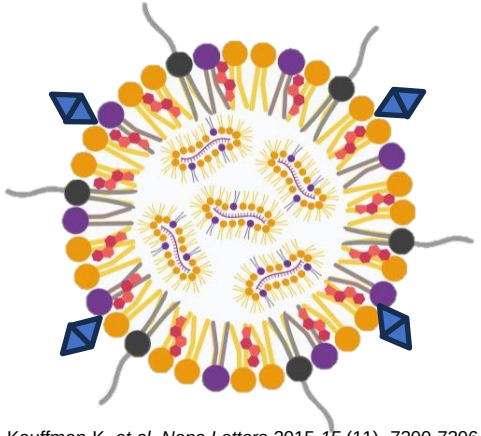
Ionizable lipid: complexed with RNA



SM102

RCB-4-8

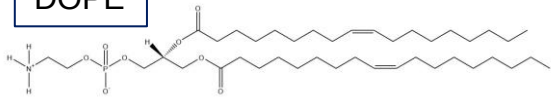




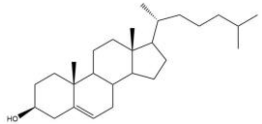
Kauffman K, *et al*. *Nano Letters* 2015 *15* (11), 7300-7306

Helper lipid: Improves packing

DOPE

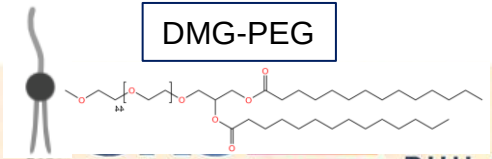


Cholesterol: Stabilizing LNPs



PEG-lipid: Prevents LNP aggregation

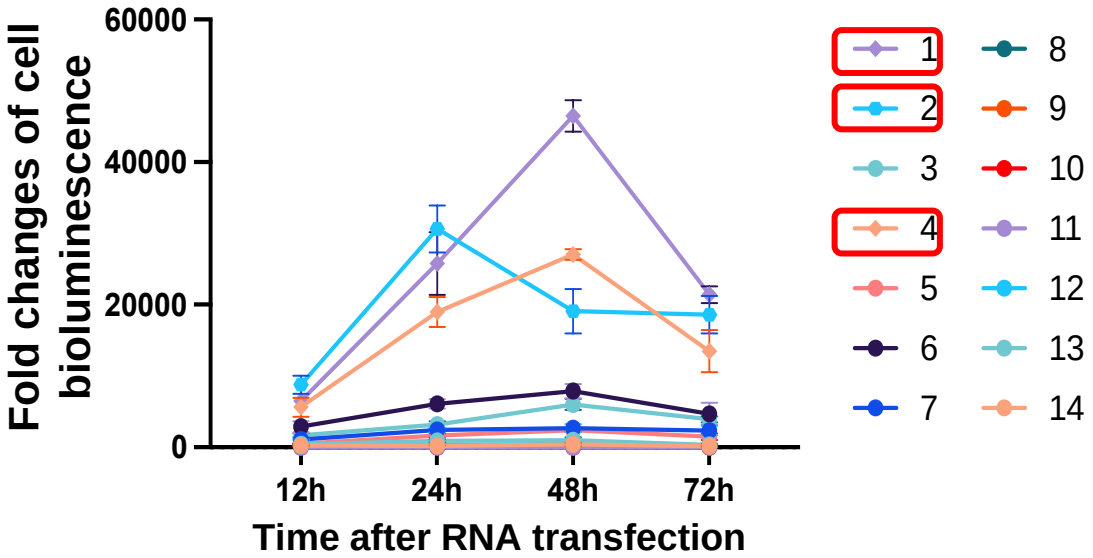
DMG-PEG



Surfactant: Increase pulmonary delivery efficiency, anti-inflammation

LNP delivery of circRNA-fLuc in CFBE41o- (F508del) human CF airway epithelial cells

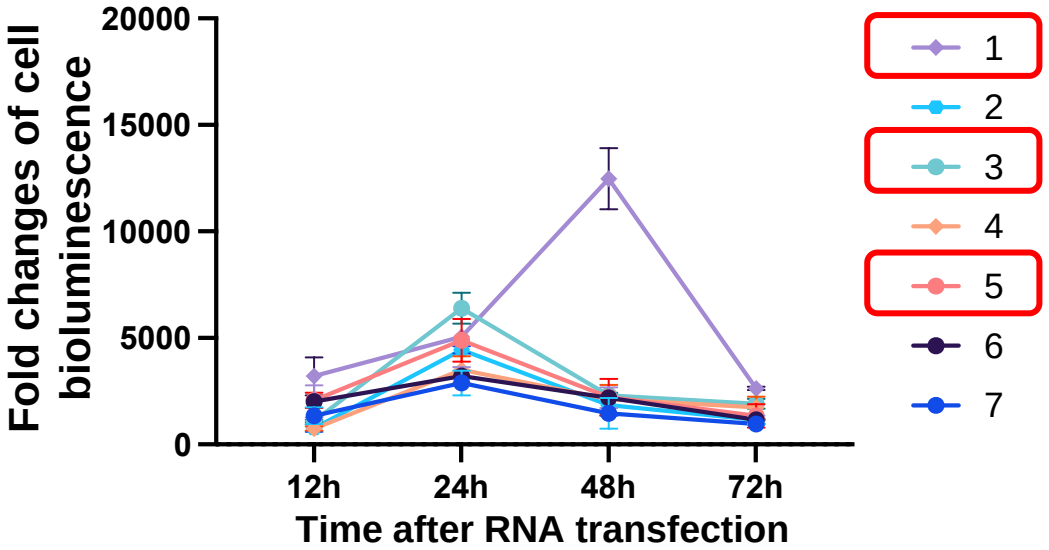
	Ionizable lipid	Surfactant	Surfactant ratio & formulation methods
1	SM102	-	-
2	SM102	Surfactant1	20% Coated
3	SM102	Surfactant2	10% Coated
4	SM102	Surfactant2	30% Coated
5	SM102	Surfactnant1	Mixed
6	SM102	Surfactnant3	10:1 Mixed
7	SM102	Surfactnant3	5:1 Mixed
8	RCB-4-8	-	-
9	RCB-4-8	Surfactnant1	20% Coated
10	RCB-4-8	Surfactnant2	10% Coated
11	RCB-4-8	Surfactnant2	30% Coated
12	RCB-4-8	Surfactnant1	Mixed
13	RCB-4-8	Surfactnant3	10:1 Mixed
14	RCB-4-8	Surfactnant3	5:1 Mixed



0.25ug circRNA-fLuc was used in each well (48 well plates) and treated for **12h to 72h**.

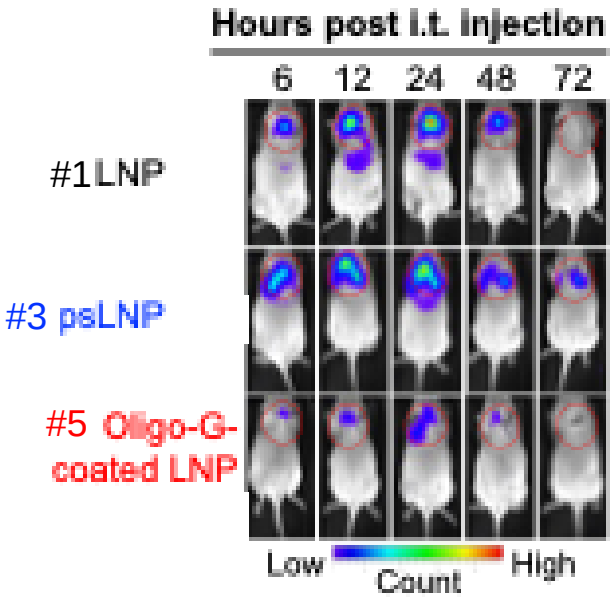
DOE study (Stage II): SM-102 LNPs and surfactant-coated SM-102 LNPs show high transfection efficiency in cells

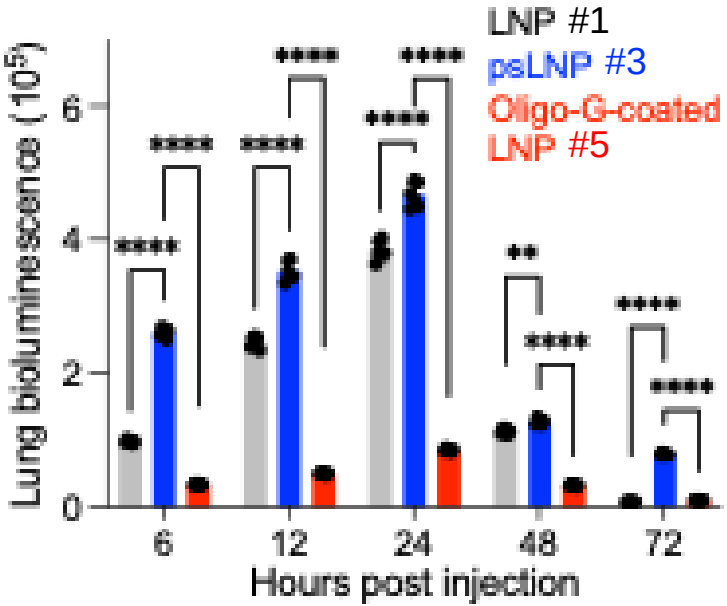
	Ionizable Lipid	Surfactant	
1	SM102	-	-
2	SM102	Surfactant1	10% Coated
3	SM102	Surfactant1	20% Coated
4	SM102	Surfactant1	30% Coated
5	SM102	Surfactant2	20% Coated
6	SM102	Surfactant2	30% Coated
7	SM102	Surfactant2	40% Coated



- **0.25ug** circRNA-fLuc was used in each well (48 well plates) and treated for **12h to 72h**.
- CFBE41o- (F508del): human CF airway epithelial cells.

DOE study (Stage III): Surfactant-coated SM-102 LNPs showed slightly better circRNA delivery than SM-102 LNPs in mice





- **10 ug** circRNA-fLuc;
- FVB mice.

Experiment designment

1: circRNA-CFTR design and synthesis

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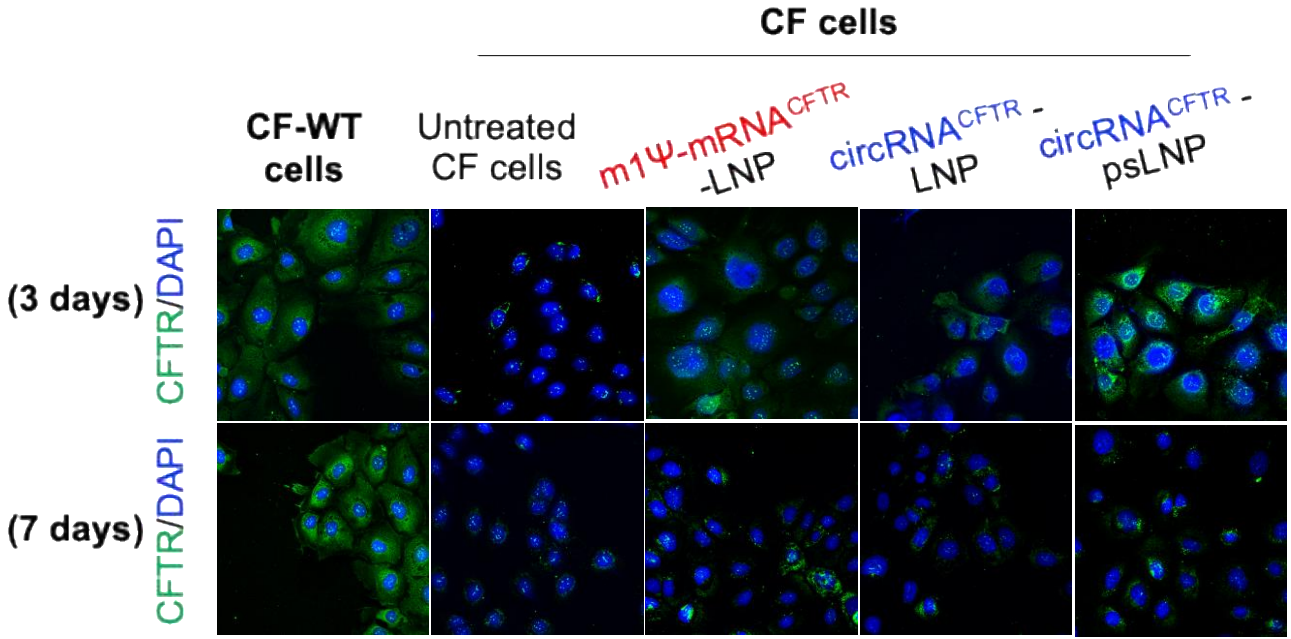
3: Evaluate circRNA-CFTR LNPs in human CF cells

4: Evaluate circRNA-CFTR LNPs in CF mouse model (ongoing)

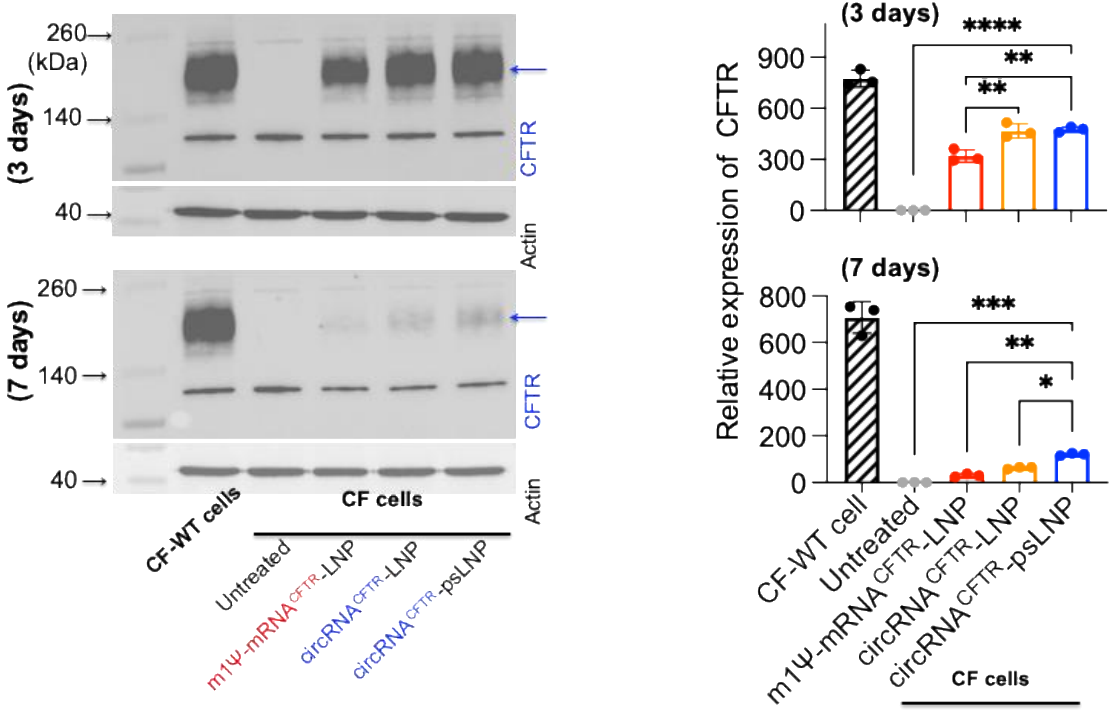
circRNA-CFTR outperformed mRNA-CFTR for CFTR expression in CFBE41o- cells



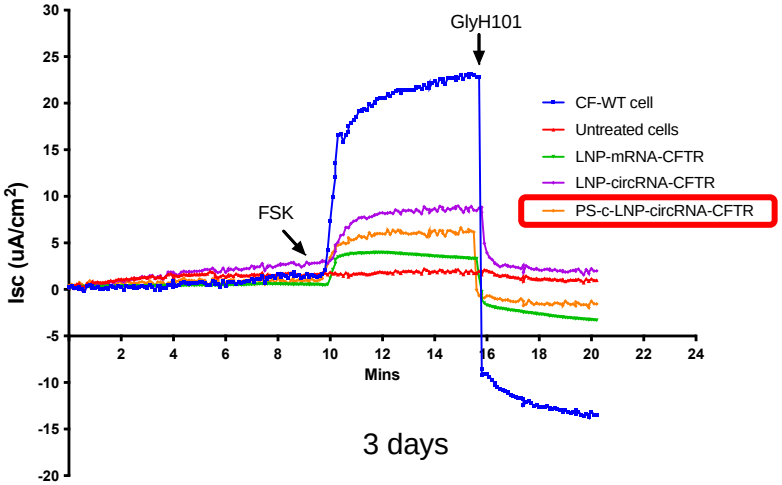
CF-WT cells:
CF cells stably transfected with an Epstein-Barr virus (EBV)-based episomal expression vector, pCEP4b.

CFBE41o- CF cells: derived from a patient homozygous for the F508del mutation.

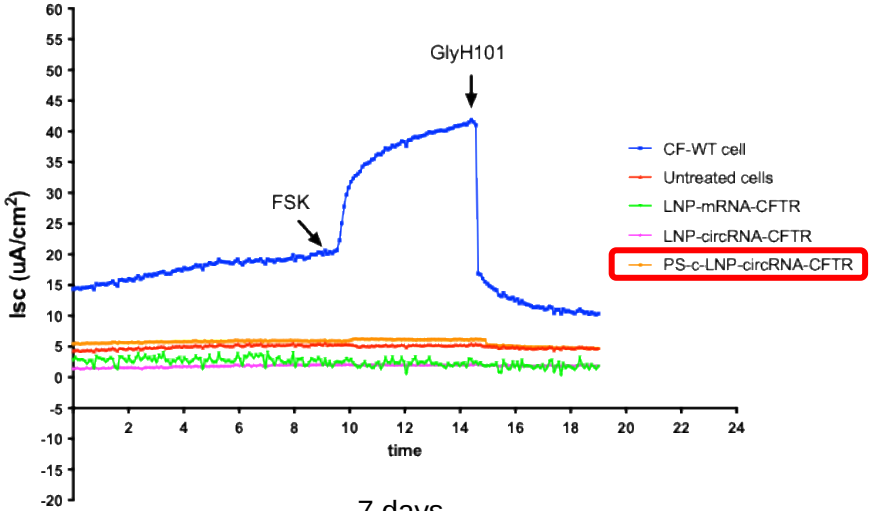
circRNA-CFTR outperformed mRNA-CFTR for CFTR expression in CFBE41o- cells (WB)



circRNA-CFTR outperformed mRNA-CFTR for CFTR expression in CFBE41o- cells (Ussing chamber: chloride function experiment)



3 days



7 days

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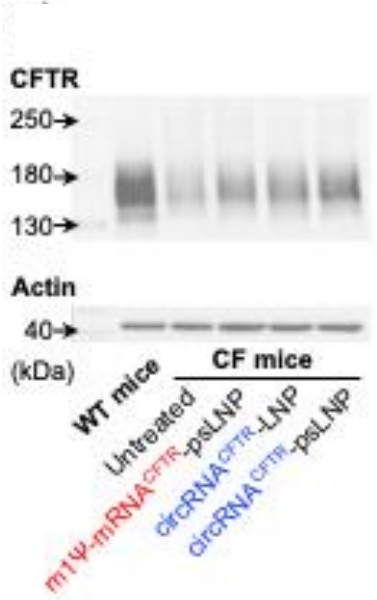
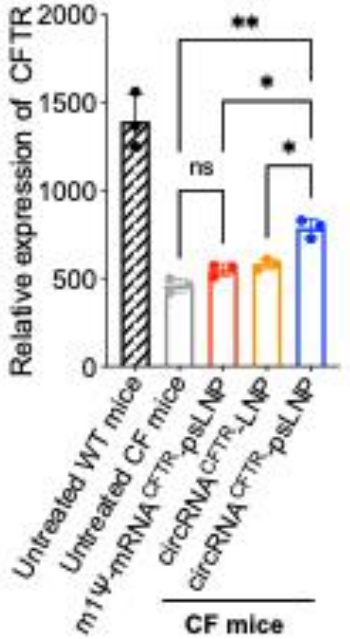
4: Evaluate **c**ircRNA-CFTR LNPs in CF mouse model (ongoing)

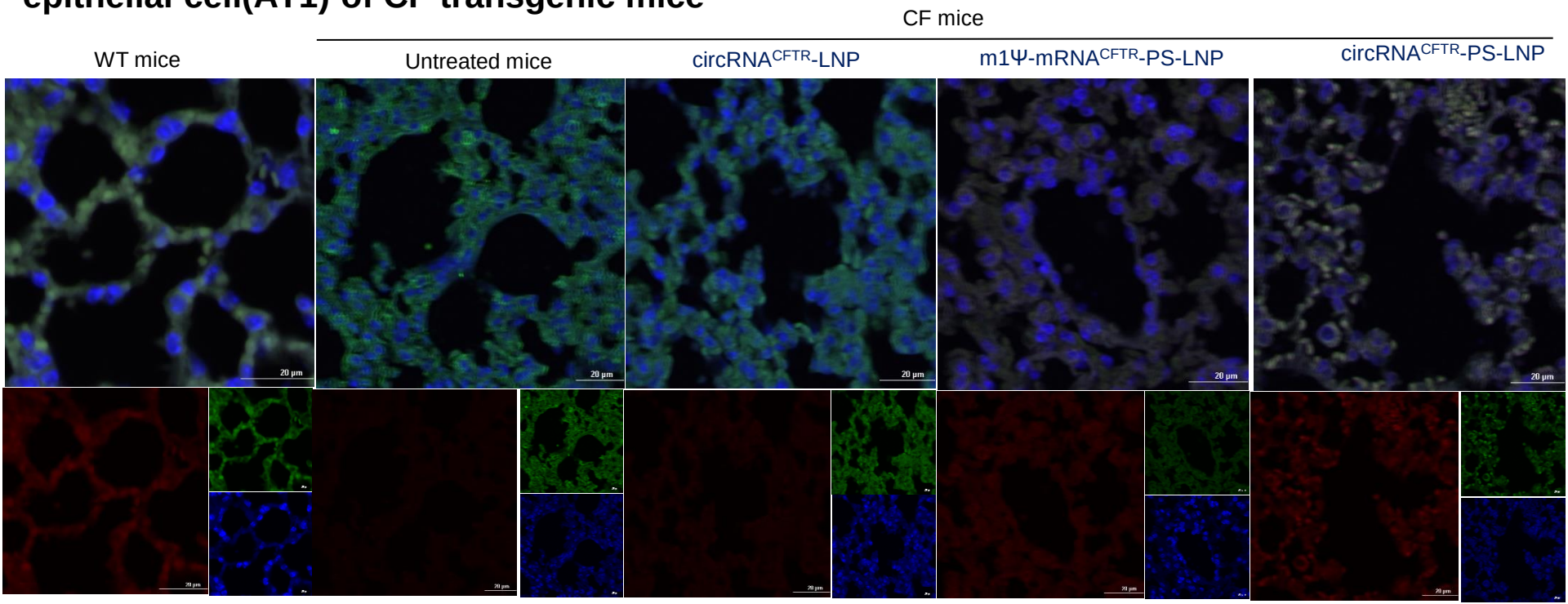
Pulmonary delivery of circRNA-CFTR LNPs resulted in CFTR expression in the lung of CF transgenic mice





- Mice*: Cftr/tm1Unc* Tg(FABPCFTR)1 Jaw/J double-transgenic CF KO mice.
- **2ug** circRNA-CFTR LNPs were subject to **intratracheal injection** into mice, prior to tissue collection **7 days** later.

Pulmonary delivery of circRNA-CFTR LNPs resulted in CFTR expression in the alveolar epithelial cell(AT1) of CF transgenic mice



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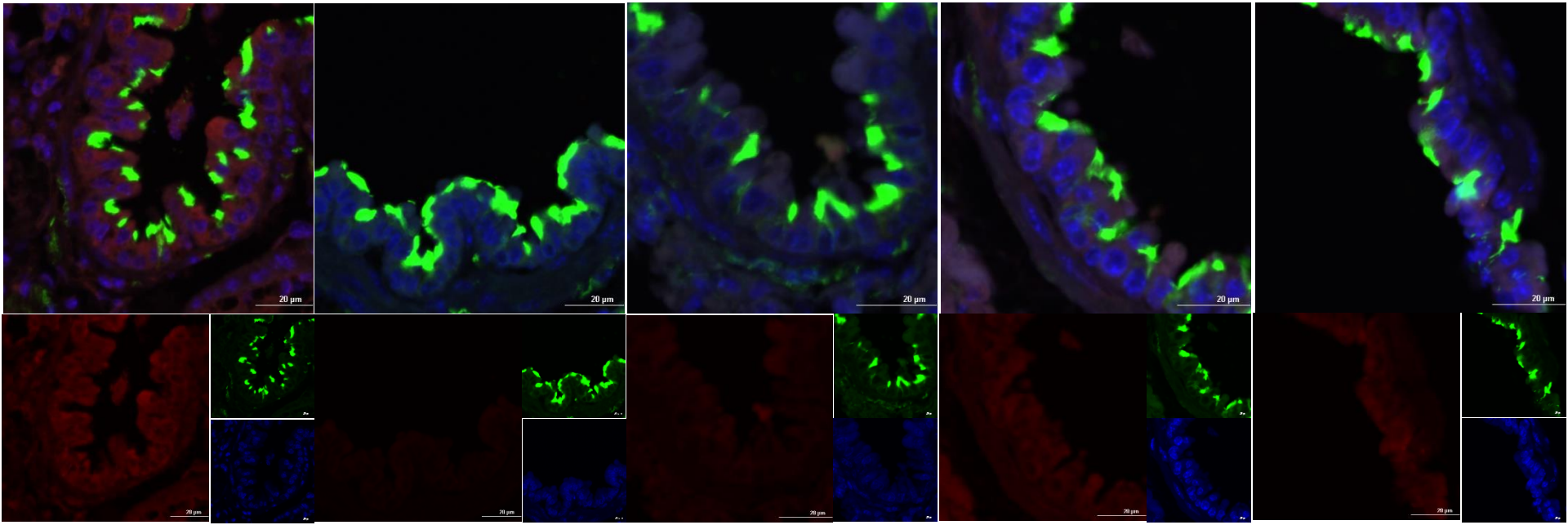
HOP; CFTR; DAPI (nuclei).

Pulmonary delivery of circRNA-CFTR LNPs resulted in CFTR expression in the Ciliated cell of CF transgenic mice

CF mice

WT mice | Untreated mice | circRNA^{CFTR}-LNP | m1Ψ-mRNA^{CFTR}-PS-LNP | circRNA^{CFTR}-PS-LNP



- Mice*: Cftr/tm1Unc* Tg(FABPCFTR)1 Jaw/J double-transgenic CF KO mice. **Tubulin; CFTR; DAPI (nuclei).**
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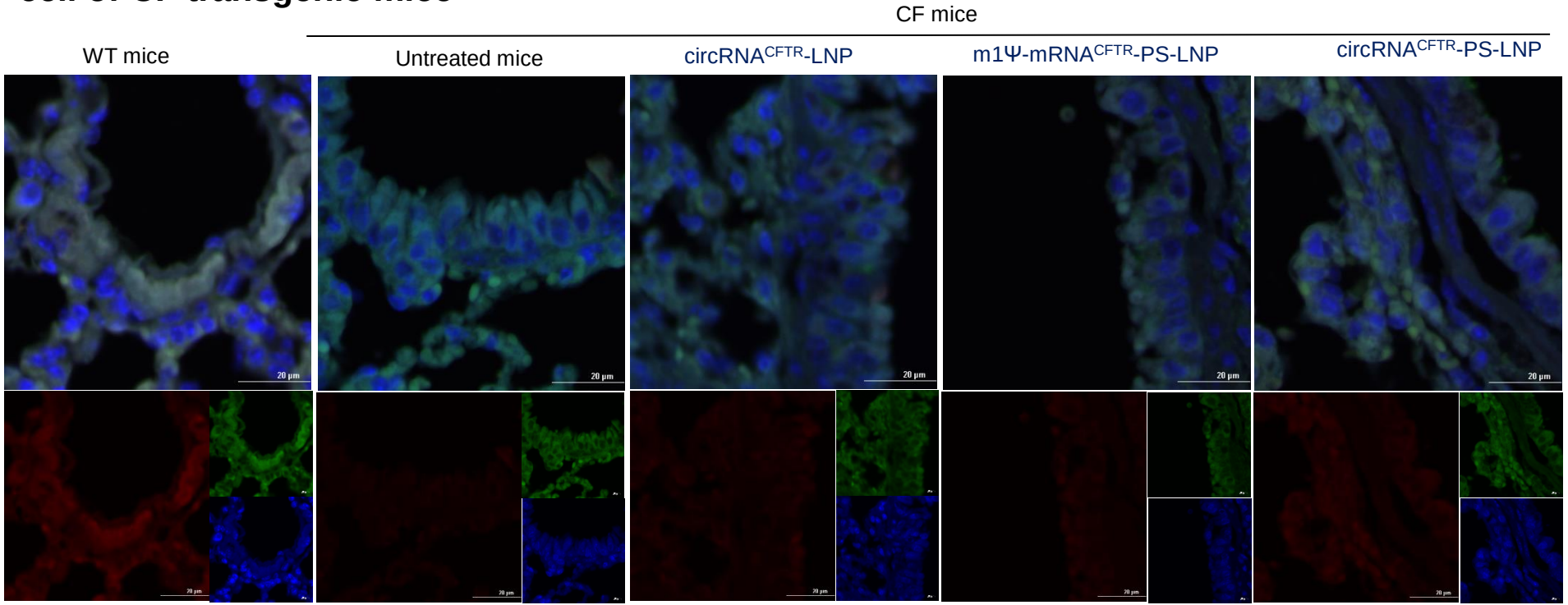
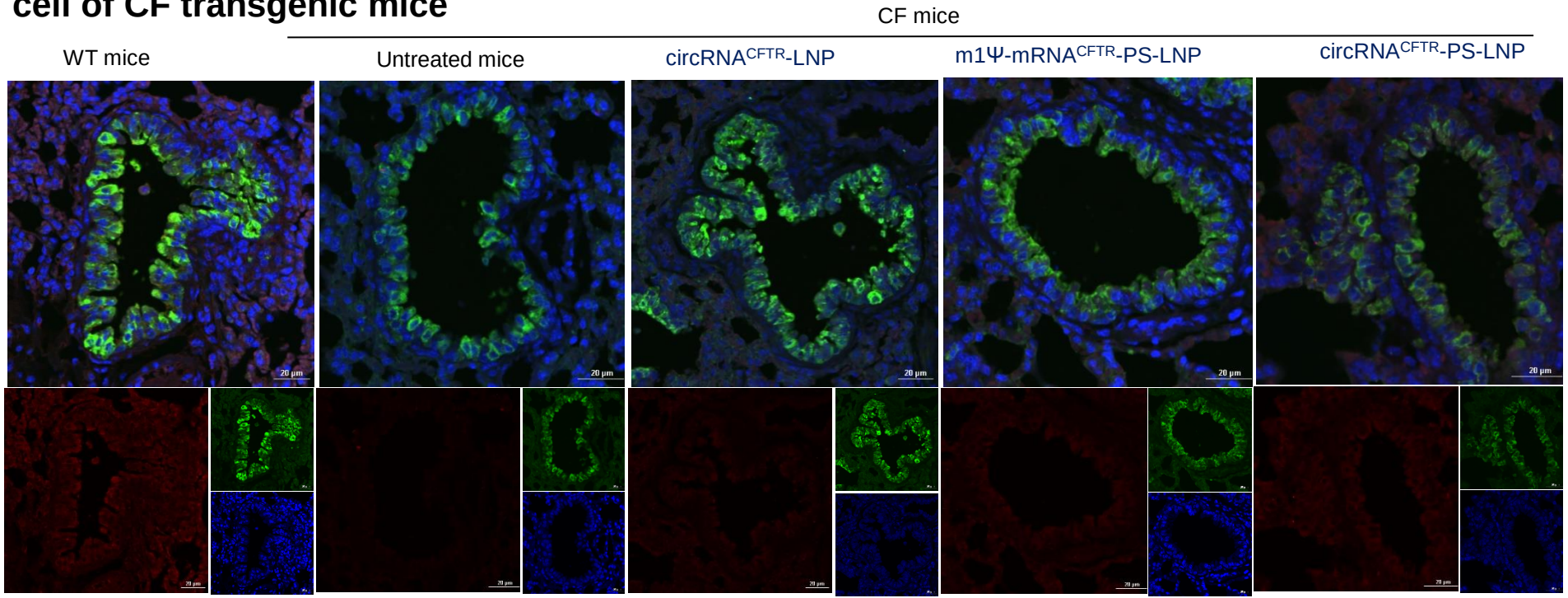
Pulmonary delivery of circRNA-CFTR LNPs resulted in CFTR expression in the Club cell of CF transgenic mice



- Mice*: Cftr/tm1Unc* Tg(FABPCFTR)1 Jaw/J double-transgenic CF KO mice. **SCGB1A1; CFTR; DAPI (nuclei).**
- **2ug** circRNA-CFTR LNPs were subject to **intratracheal injection** into mice, prior to tissue collection **7 days** later.

Pulmonary delivery of circRNA-CFTR LNPs resulted in CFTR expression in the Goblet cell of CF transgenic mice



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Mucin; CFTR; DAPI (nuclei).

Thank You

Zhu Lab

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guizhiz@med.umich.edu
@gzzhujoy
https://www.guizhizhu.org