

# Nebulized LNP-formulated DNAI1 mRNA Therapy to Restore Mucociliary Clearance for the Treatment of Primary Ciliary Dyskinesia

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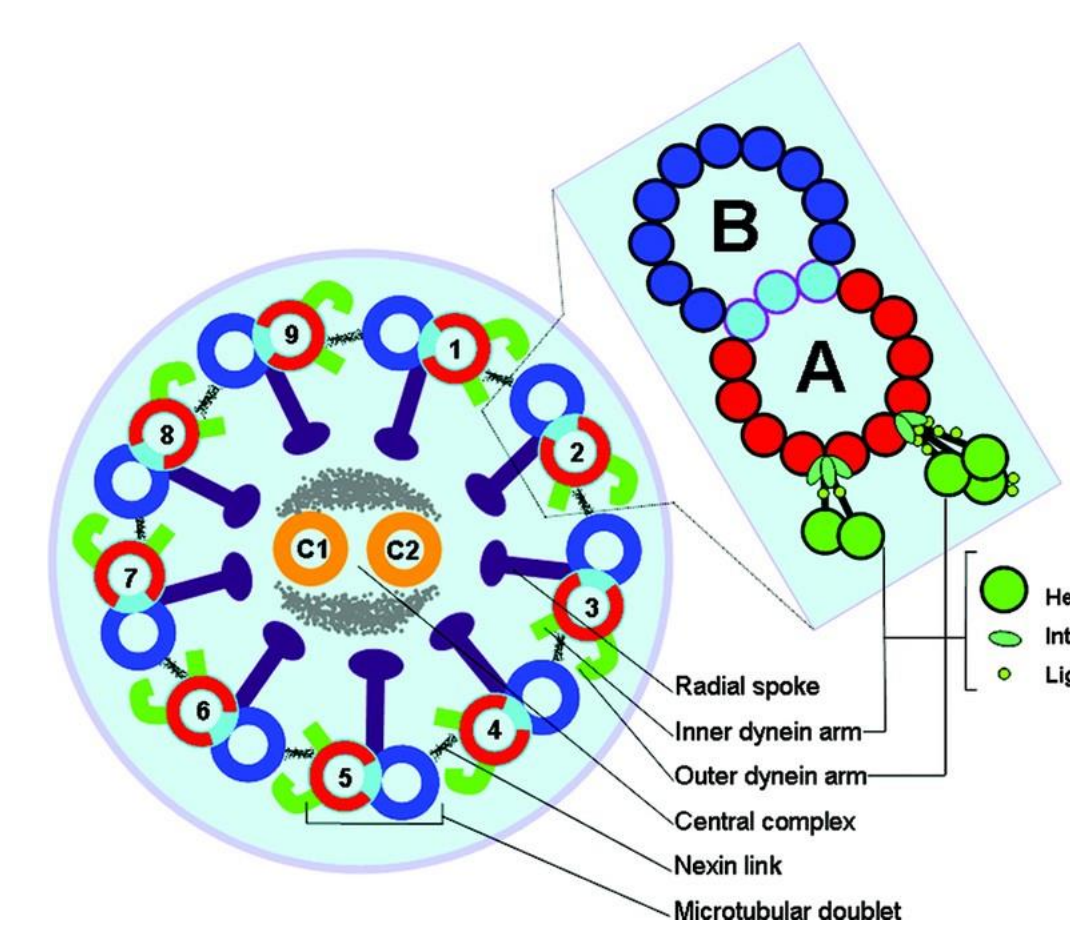
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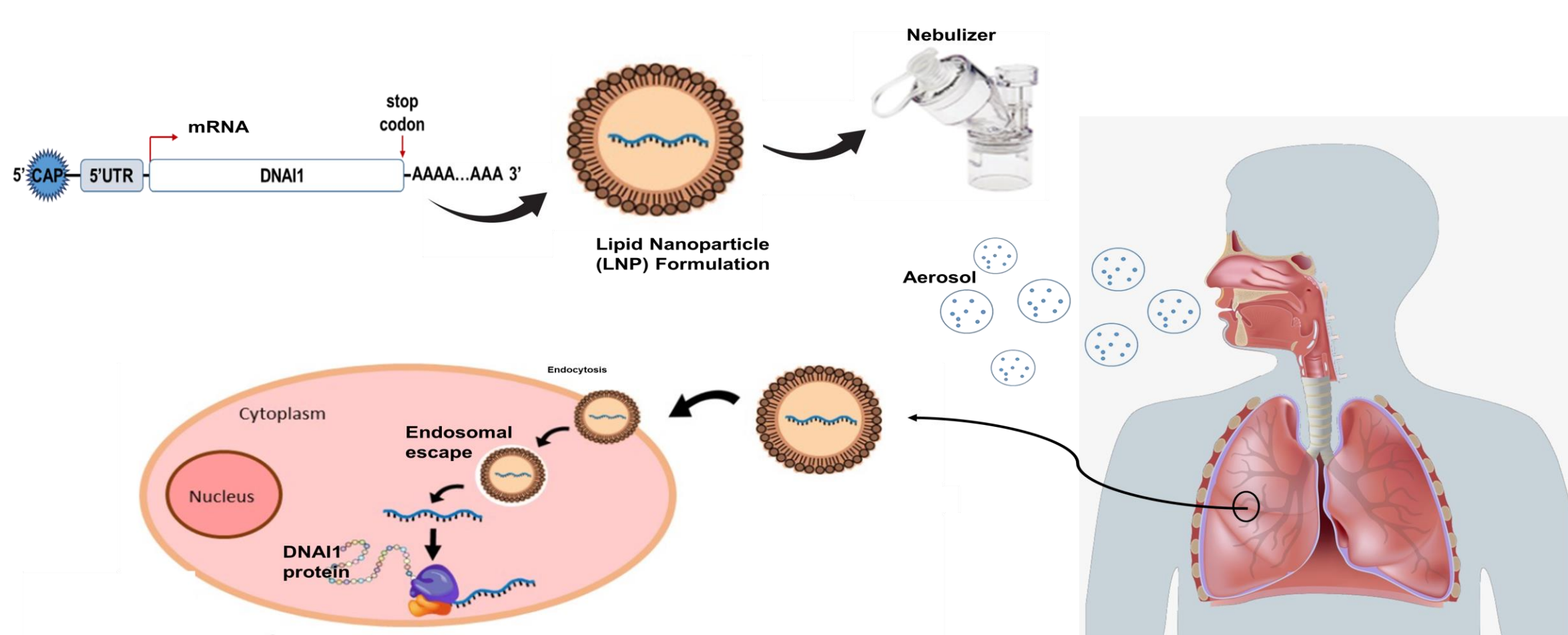
## INTRODUCTION

Primary ciliary dyskinesia (PCD) is a rare genetic respiratory disease caused by inherited mutations in more than 40 different genes. Bi-allelic mutations in any of the PCD genes result in loss of ciliary activity and mucociliary clearance. People with PCD suffer from recurrent respiratory tract infections and inflammation leading to bronchiectasis with varying severity. Currently, there are no disease-modifying therapies available, and treatments are limited to palliative care for the management of symptoms. Thus, there is a clear unmet medical need for therapeutic approaches to treat the underlying causes of PCD.

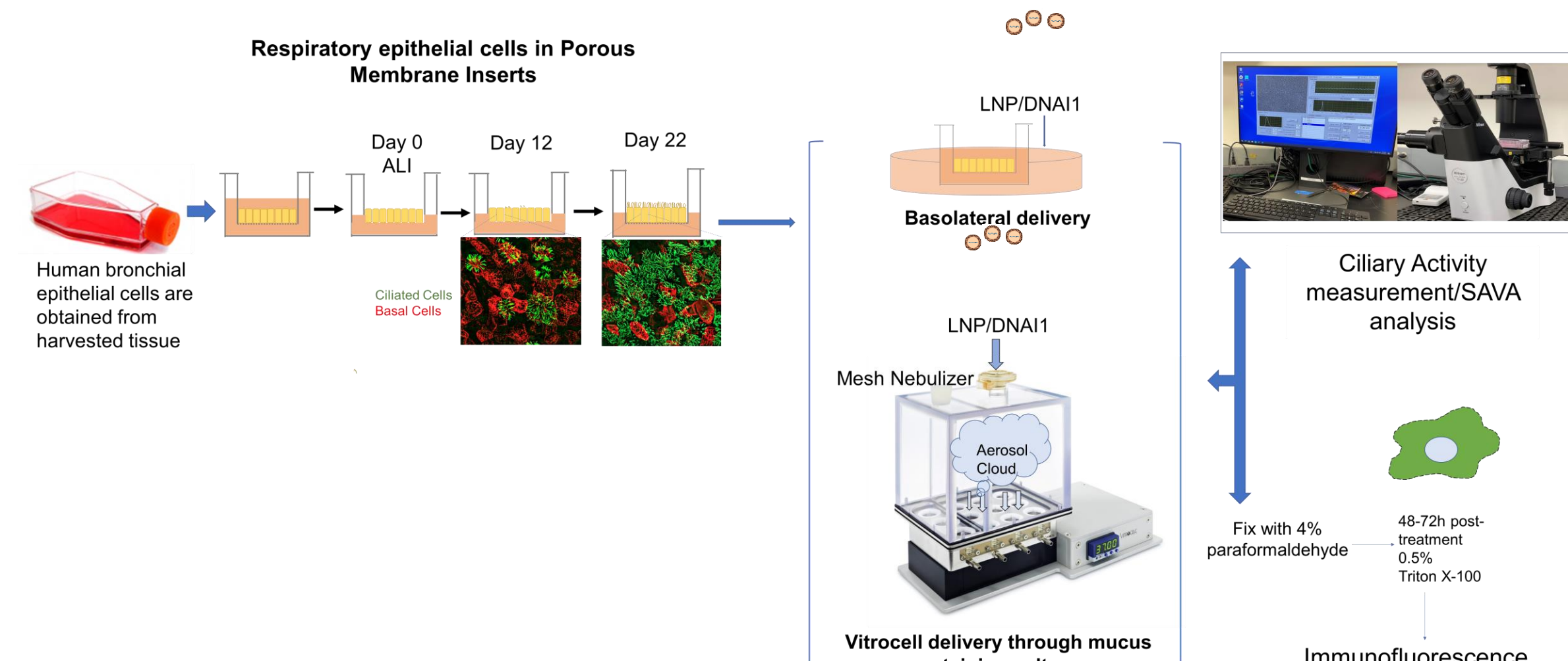


DNAI1 (699 amino acids), dynein axonemal intermediate-chain 1 protein, is an integral component of the outer dynein arm complex that is essential for ciliary movement. Dynein arms are located along the length of central microtubule A/B doublets and hydrolyze ATP to generate ciliary movement. DNAI1 is expressed in ciliated cells lining the airways of the nasal cavity, middle ear, paranasal sinuses, lower respiratory tract, fallopian tubes, and ventricles in the brain.

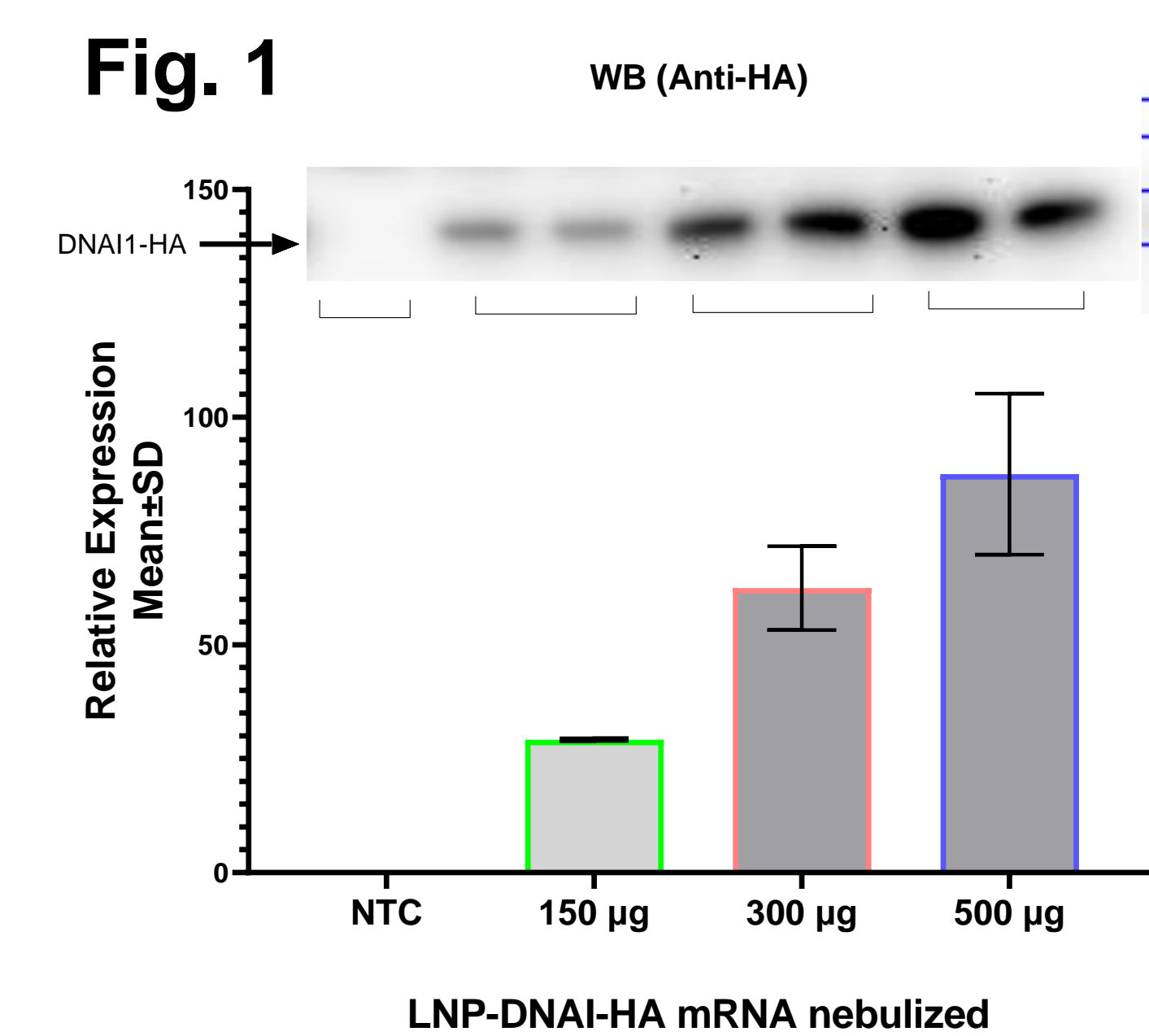
ReCode Therapeutics is developing an mRNA-based therapy for the treatment of PCD caused by mutations in *DNAI1*. The *DNAI1* mRNA is sequence optimized and formulated in a proprietary 5-component lipid nanoparticle (LNP). The formulated mRNA is nebulized and delivered as an aerosol directly into the airway. Using knockdown human bronchial epithelial (hBE) cultures and knockout mouse tracheal cultures we show that ReCode's LNP can restore ciliary activity.



## Primary human bronchial epithelial cell (hBEC) cultures at the air-liquid-interface (ALI)



## LNP-DNAI1 mRNA delivered as an aerosol leads to dose-dependent DNAI1 protein expression



Well-differentiated DNAI1 knockdown (KD) hBE cultures were nebulized with a single dose of LNP-DNAI1-HA mRNA using the Vitrocell exposure chamber.

Fig. 1) WB intensity normalized to total protein in treated cultures after 24 h.

Fig. 2

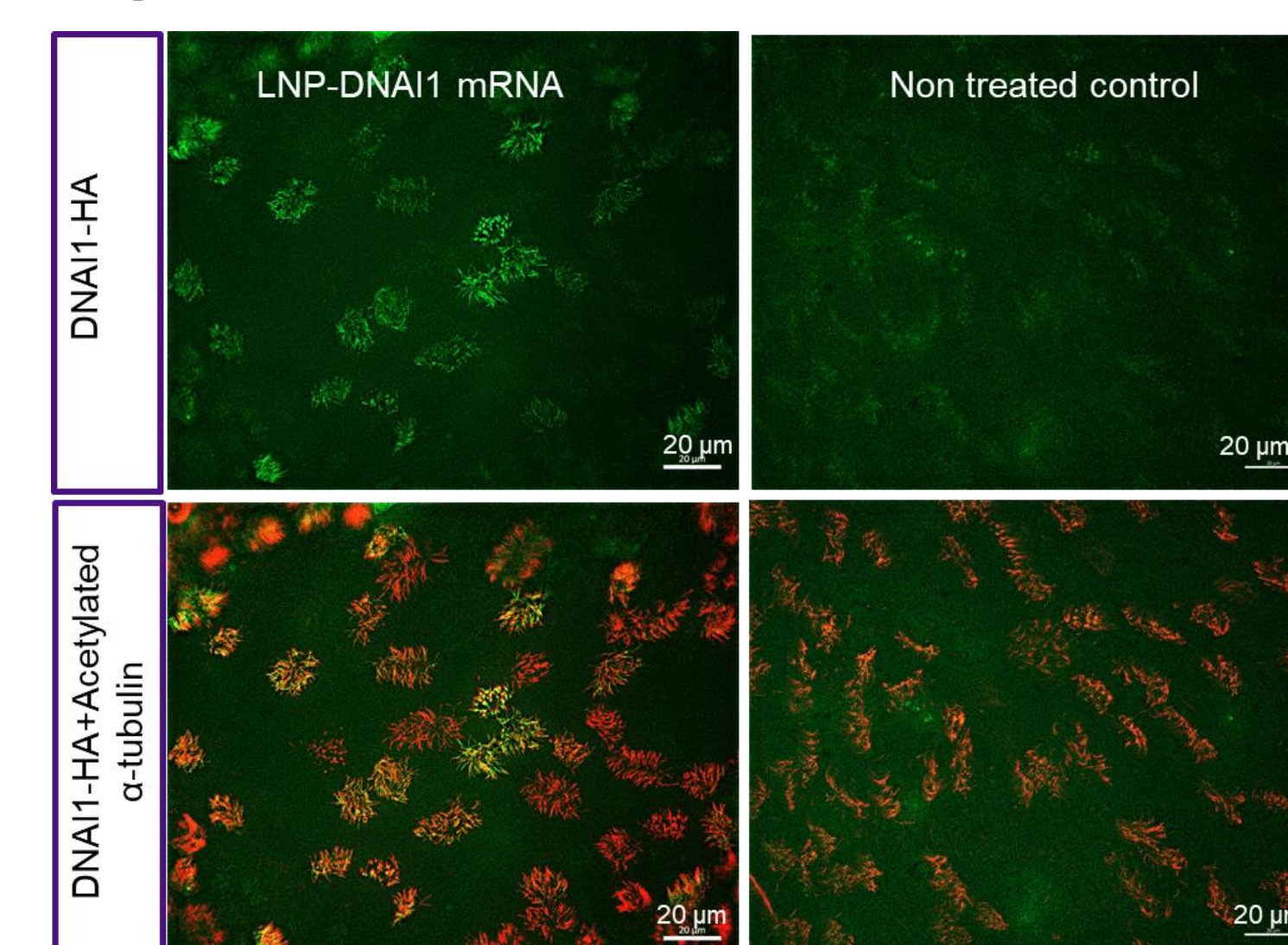
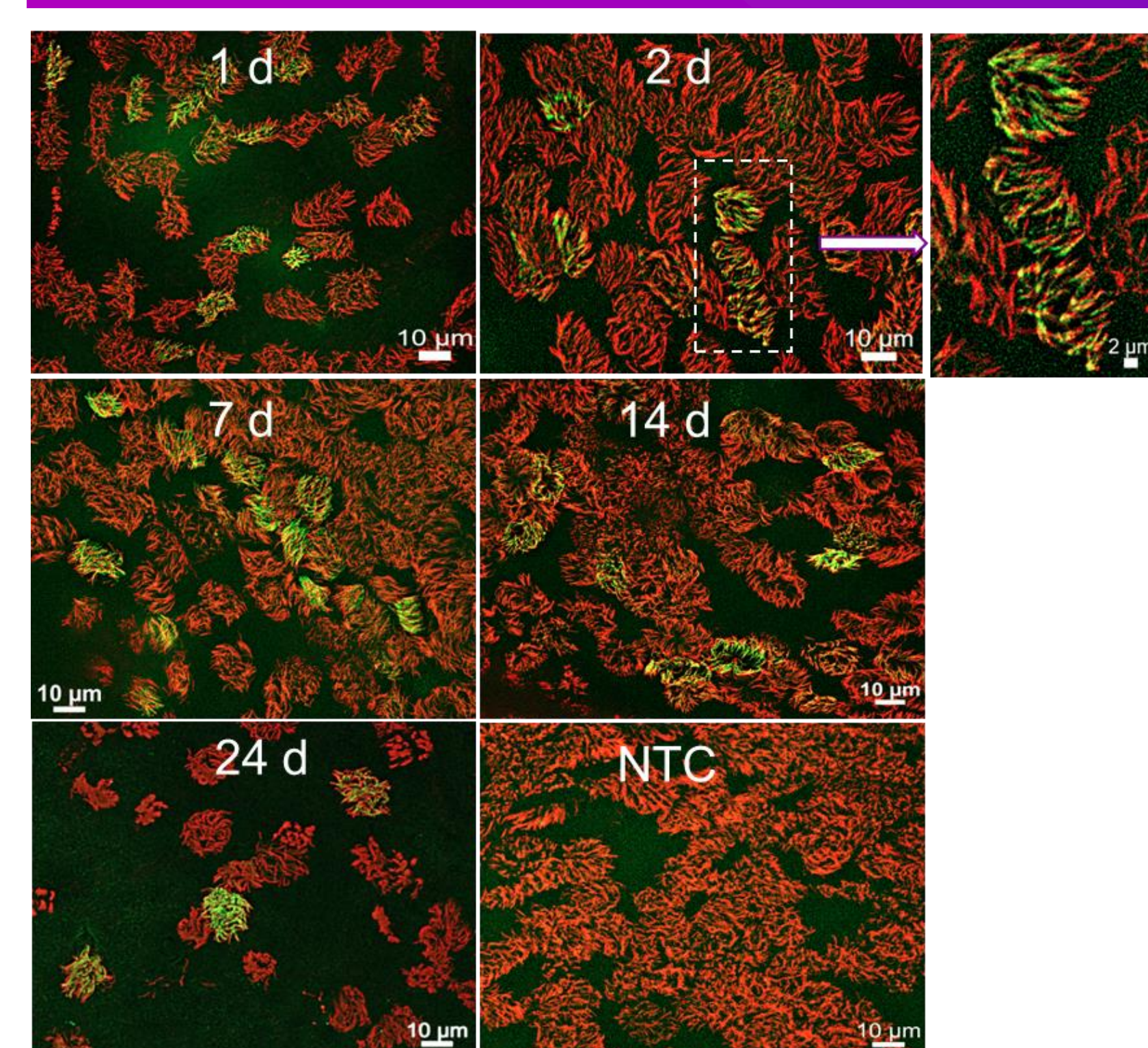


Fig. 2) Immunofluorescence microscopy images after 72 h. *DNAI1-HA* protein stained with anti-HA Ab (and anti rabbit Alexa Flour 488) and colocalized with cilia axoneme stained with TUBA antibody (anti mouse Alexa Flour 647).

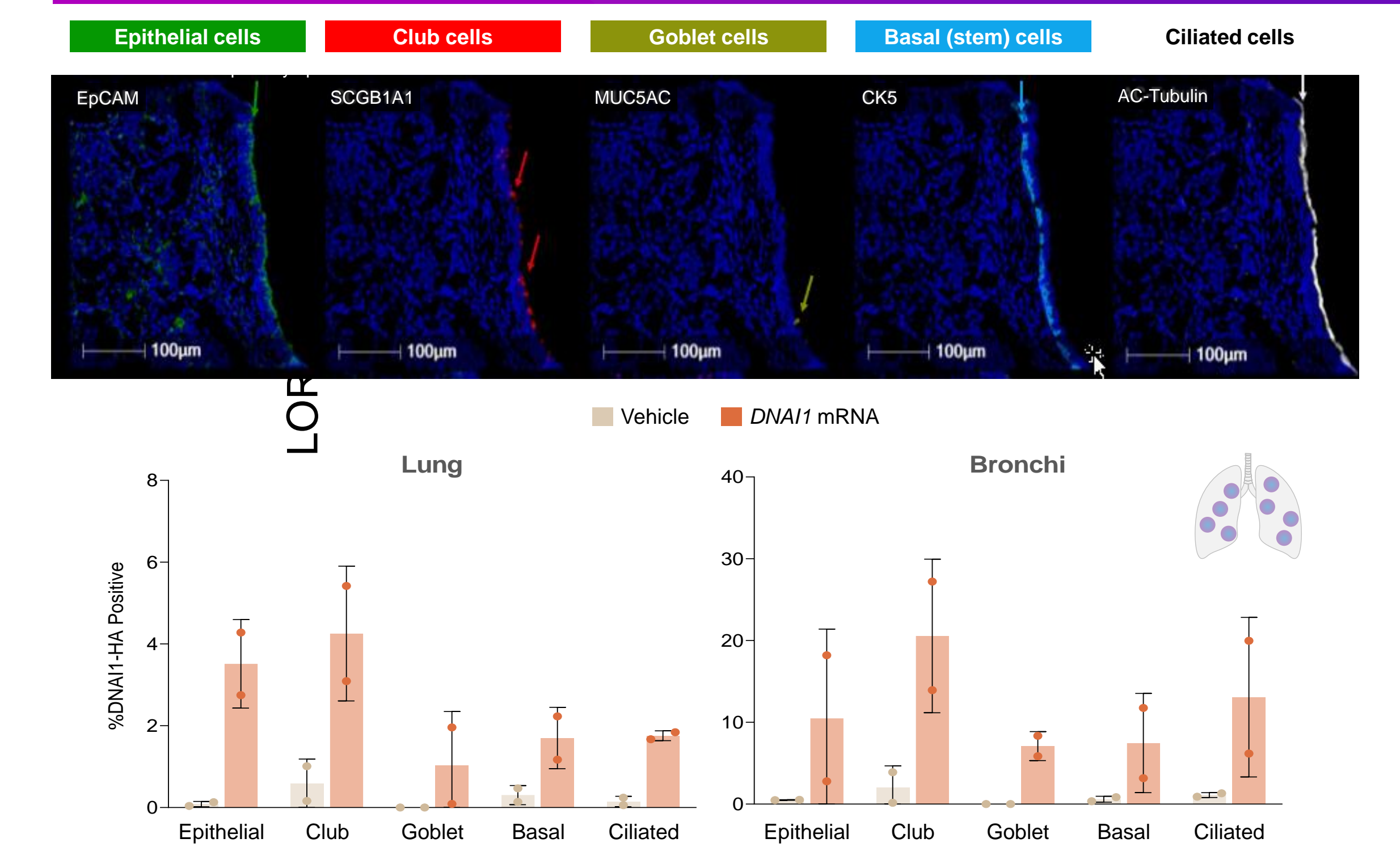
## Newly-made HA-tagged DNAI1 protein incorporated into axoneme is detectable 24 days after single delivery



Panels on the left show time-dependent DNAI1-HA protein colocalized with TUBA in axonemes of ciliated cells in hBE cultures differentiated in trans-membrane inserts. Pictures were taken using Zeiss Microscope, Axio observer 7 at 63x in two channels (488 nm, 647 nm)

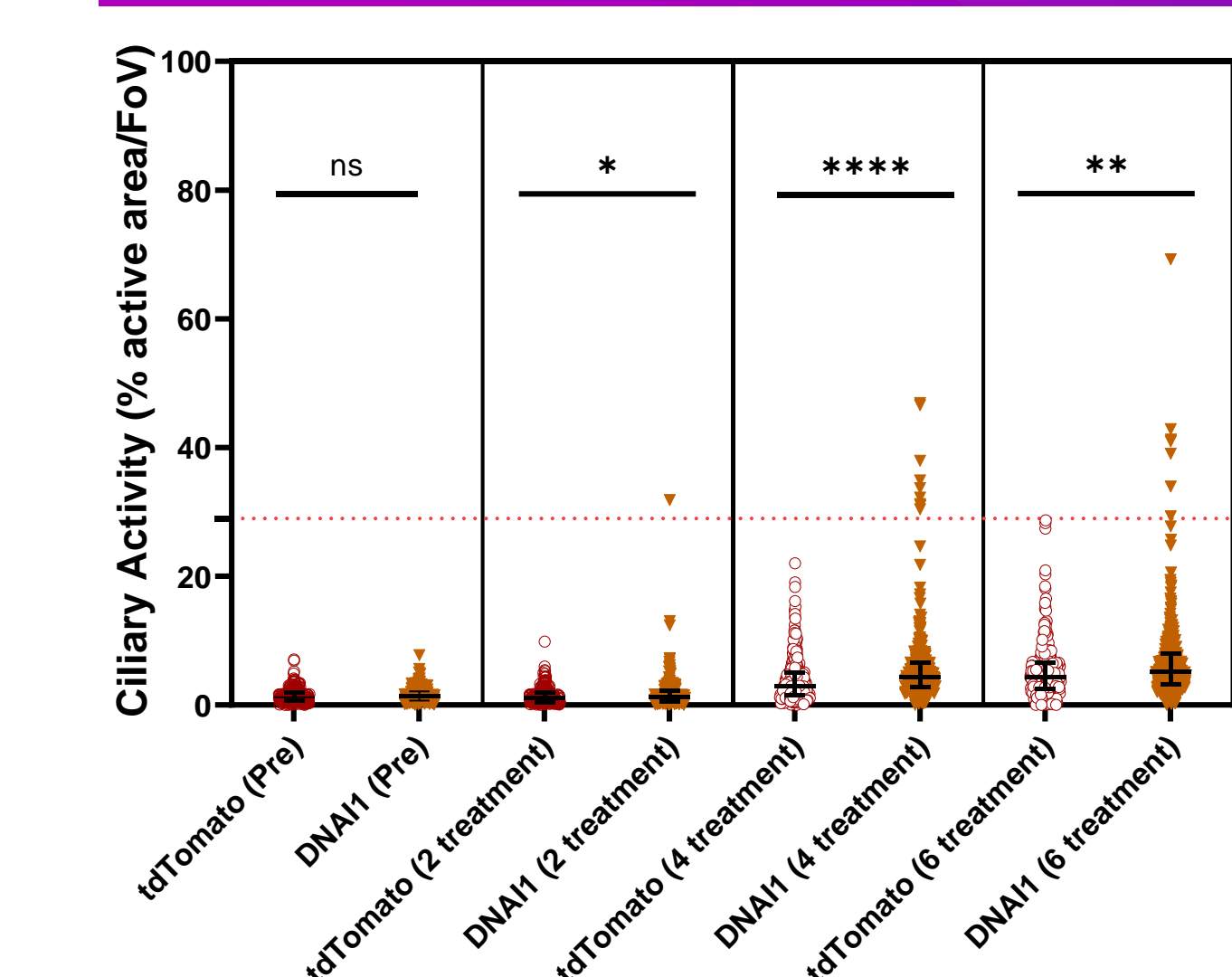
Well-differentiated DNAI1 knockdown hBE cultures were treated with a single dose of LNP-DNAI1-HA mRNA. Incorporation of DNAI1-HA protein in axoneme was detected by immunofluorescence microscopy as described above. Multiple inserts were dosed, 2 cultures were taken out and fixed at each time points. DNAI1-HA protein incorporated along the length of cilia and could be observed as early as 24 h after treatment and remains detectable until the last time point (24 d). Integration peaked between 48 h and 72 h after treatment.

## Newly-made DNAI1-HA protein in NHPs



Single 0.4 mg/kg administration of inhaled LNP-formulated DNAI1-HA mRNA. Lung and bronchial sections collected from two non-human primates (NHPs, 1M/1F) 6 hrs after dosing

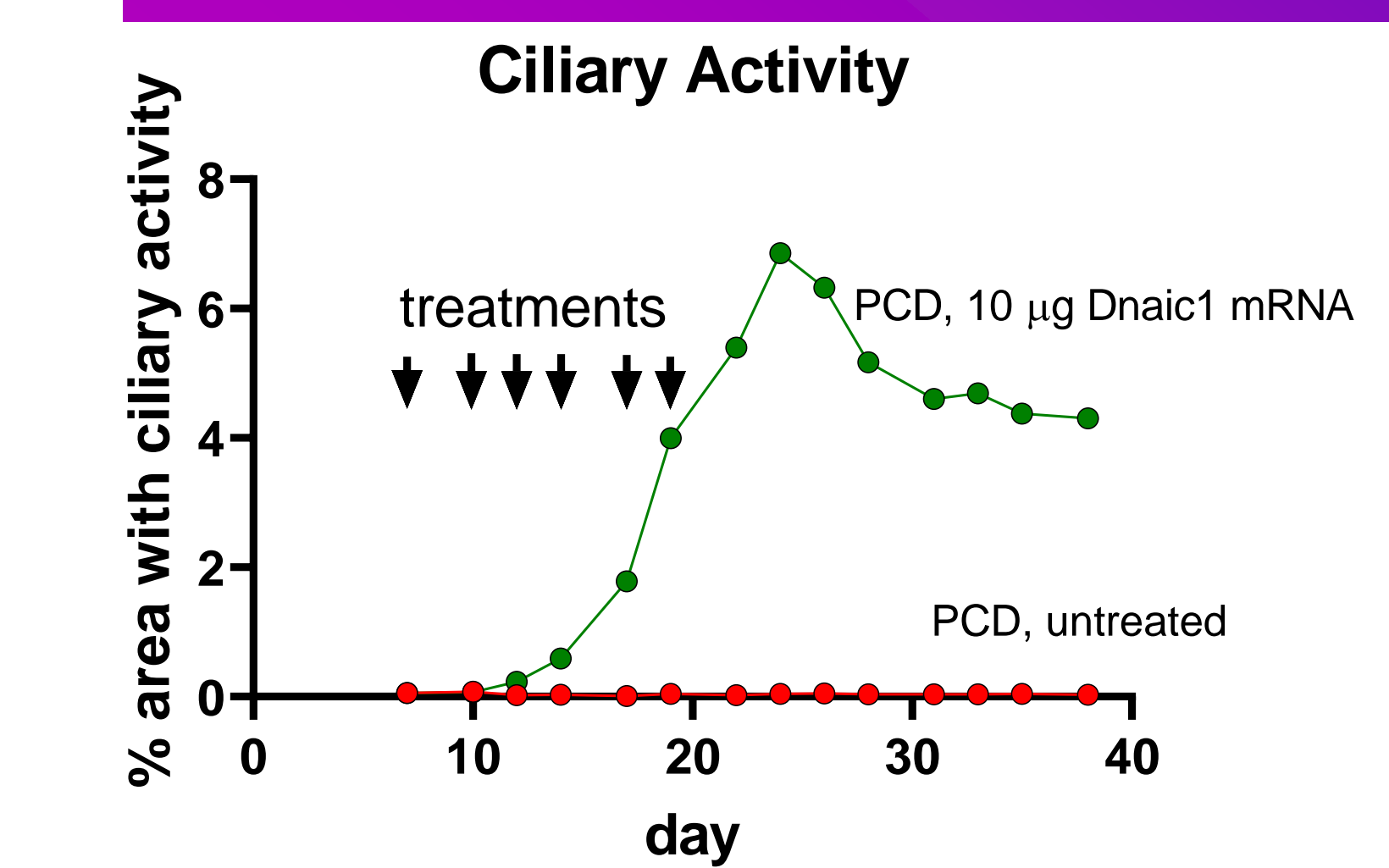
## Aerosolized DNAI1 mRNA rescues ciliary activity in KD-hBE ALI cultures



DNAI1 mRNA treated cultures higher levels of ciliary activity after 2, 4 and 6 treatments compared to tdTomato mRNA treated cultures.

Increased activities were statistically significant as determined by Welch's t-test. \* =  $P < 0.05$ , \*\* =  $P = 0.001$ , \*\*\*\* =  $P < 0.0001$ , ns = not significant ( $P > 0.05$ ).

## Prolonged rescue of ciliary activity in knock-out primary tracheal mouse ALI cultures



Ciliary activity in treated *Dnaic1* KO cells remained above 20% of normal (more than 50% of max) 21 days after the last treatment (the last timepoint assessed)

## CONCLUSION

- Newly-translated DNAI1 protein incorporates throughout the ciliary axoneme of human ciliated cells and can be detected for 24 days after treatment.
- Newly-made DNAI1 protein is detected in ciliated cells, as well as in club and basal cells (precursors of ciliated cells) in hBEs and Non-Human Primates
- LNP-formulated DNAI1 mRNA delivered as an aerosol rescues ciliary function in cell-based PCD models in the presence of mucus.
- Ciliary function in cell-based knock-out mouse PCD models rescued by LNP-formulated *Dnaic1* persists for weeks after the last treatment.