**Supporting Information**

**Lung Tissue-Optimized Gene Editing in Human Cystic Fibrosis Models Following Topical Application of Lipid Nanoparticles**

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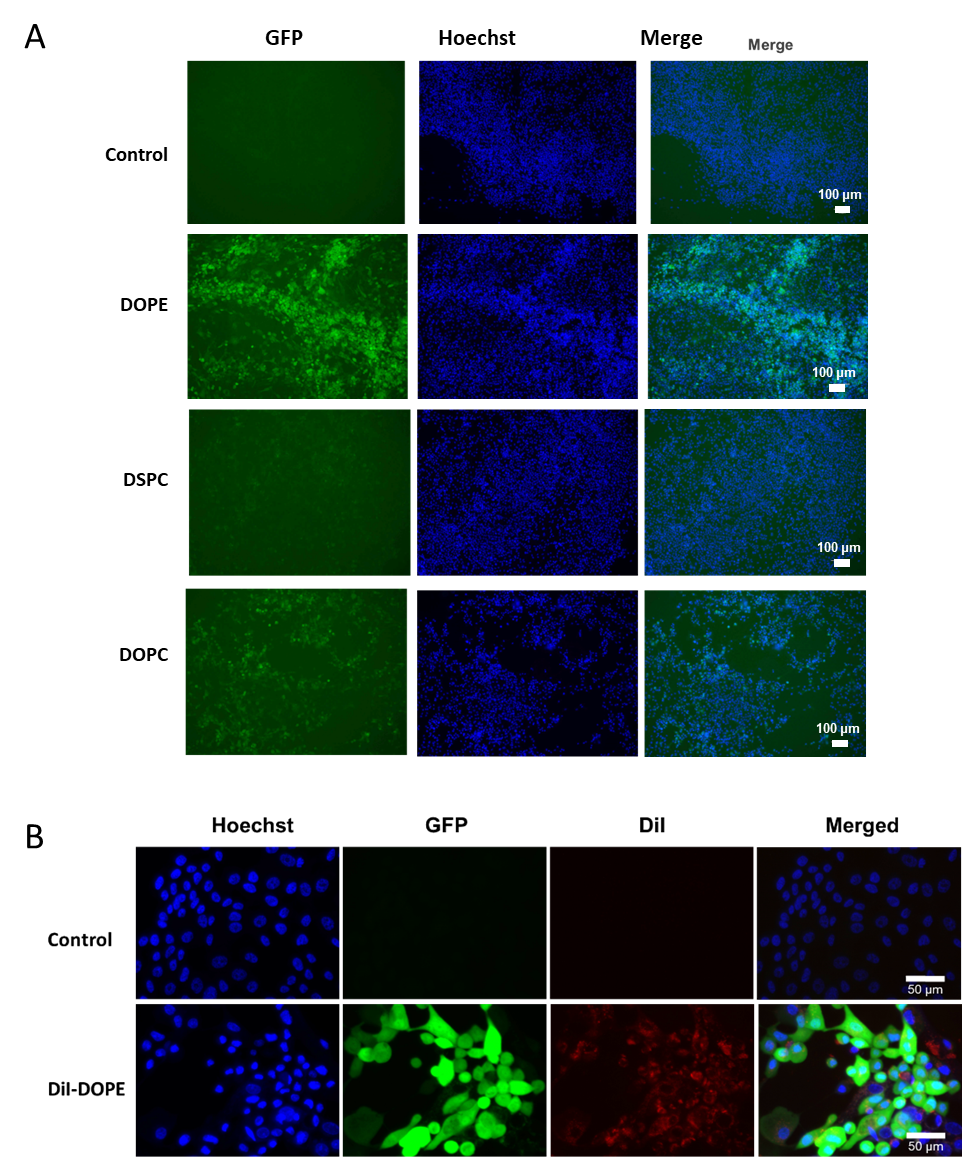
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**Supplementary Figures**

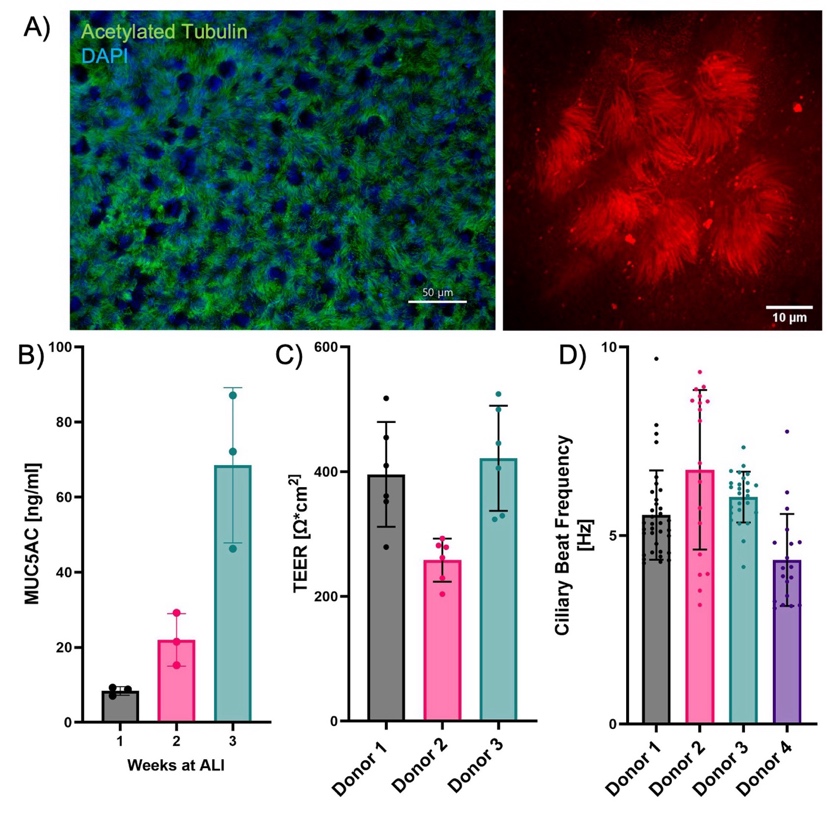
A screenshot of a graph

Description automatically generated with medium confidence

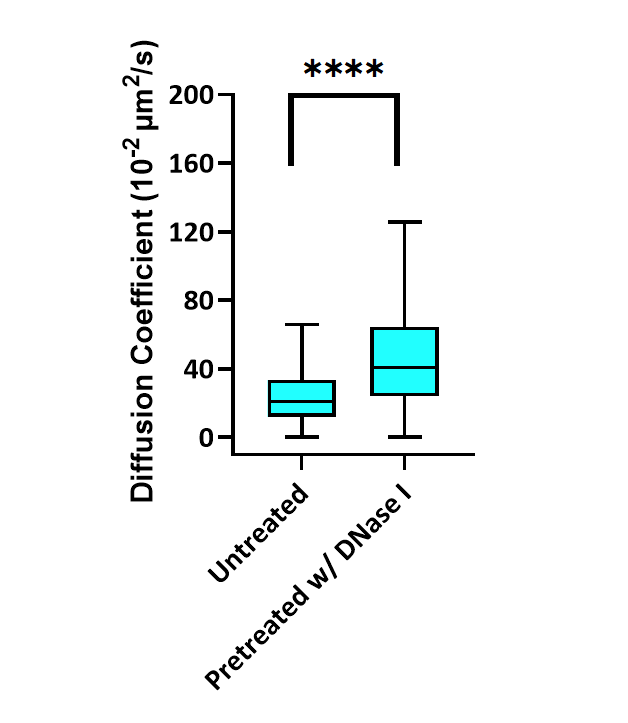
**Figure S1.** The transfection efficacy of luciferase-mRNA loaded LNPs with different helper lipids (DSPC, DOPC and DOPE) in primary human bronchial epithelial (NHBE) cells after 24 hours. The transfection efficacy increased with increasing mRNA concentration (0.1 – 1 ug/mL) and was ApoE dependent.

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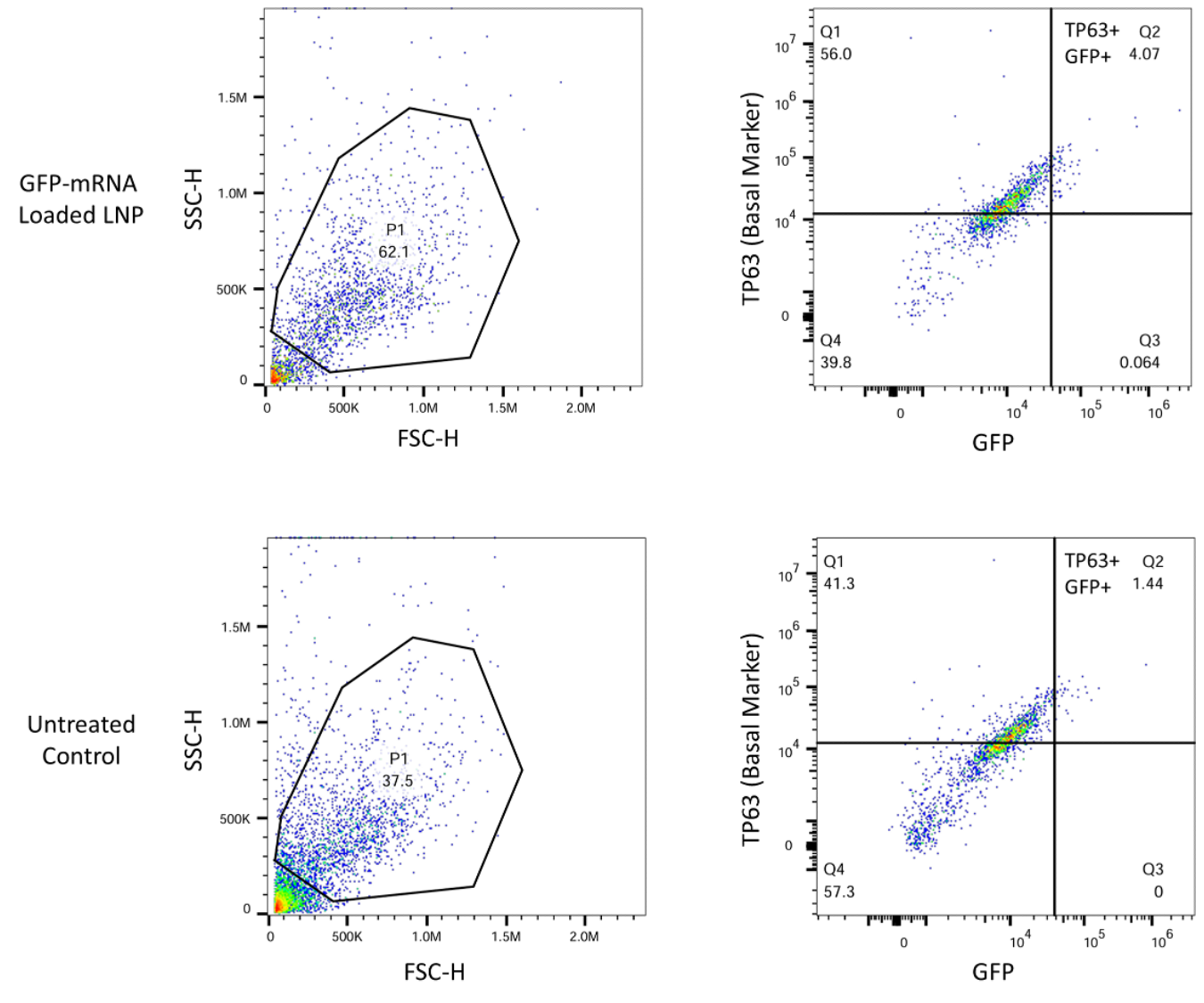
**Figure S2.** A) Zoom out representative fluorescence microscopy images comparing functional GFP expression after transfection of NHBE cells with DOPE-, DSPC-, and DOPC-LNP. Blue represents the nuclei stained with Hoechst; green= functional GFP expression. B)Representative fluorescence microscopy images showing the transfection efficiency and uptake of DiI-labeled DOPE-LNP loaded with GFP-mRNA over 24h. Blue represents the nuclei stained with Hoechst; green is indicative of GFP expression; red represents DiI-labeled LNP.



**Figure S3. A)** Left: Overview IF staining against acetylated tubulin representing the ciliated cells of human 3D bronchial epithelial models. Right: Close-up of the ciliated cells captured using spinning disc microscopy. **B)** MUC5AC concentrations of the apically collected mucus from 3D bronchial epithelial models over a 3-weeks differentiation period determined via ELISA. **C)** TEER values and **D)** ciliary beet frequencies (CBF) of 3D bronchial epithelial models after 3 weeks of differentiation generated from primary human bronchial epithelial cells of different donors.



**Figure S4.** LNP diffusion coefficient in untreated and DNAse I pre-treated 2% mucin samples as determined by spinning disc microscopy and multi particle tracking analysis.



**Figure S5.** Flow cytometry analysis of CF patient-derived 3D bronchial epithelial models 48h after topical application of GFP mRNA-loaded LNP H. Following model dissociation, the cells were sorted for TP63 as a marker of basal cells and GFP to indicate functional mRNA expression.

**Supplementary Tables**

**Table S1.** Top 15 regulated genes for the unmodified mRNA vs. untreated group. Genes are sorted by FDR (adjusted p-value). Genes with missing annotations are excluded.

| **ENSEMBL** | **Gene** | **Description** | **Log2FC** | **p-value** | **FDR** |
| --- | --- | --- | --- | --- | --- |
| ENSG00000135114 | OASL | 2’-5’-oligoadenylate synthetase like | 6.33 | < 2e-16 | < 2e-16 |
| ENSG00000138755 | CXCL9 | C-X-C motif chemokine ligand 9 | 8.11 | < 2e-16 | < 2e-16 |
| ENSG00000119922 | IFIT2 | interferon induced protein with tetratricopeptide repeats 2 | 6.57 | < 2e-16 | 1.7e-14 |
| ENSG00000169245 | CXCL10 | C-X-C motif chemokine ligand 10 | 8.38 | < 2e-16 | 5.7e-14 |
| ENSG00000169248 | CXCL11 | C-X-C motif chemokine ligand 11 | 7.50 | < 2e-16 | 3.6e-13 |
| ENSG00000162654 | GBP4 | guanylate binding protein 4 | 4.30 | 1.7e-15 | 5.6e-12 |
| ENSG00000081041 | CXCL2 | C-X-C motif chemokine ligand 2 | 2.74 | 4.7e-15 | 1.4e-11 |
| ENSG00000271503 | CCL5 | C-C motif chemokine ligand 5 | 9.08 | 1.4e-14 | 3.4e-11 |
| ENSG00000185745 | IFIT1 | interferon induced protein with tetratricopeptide repeats 1 | 5.87 | 1.5e-14 | 3.4e-11 |
| ENSG00000119917 | IFIT3 | interferon induced protein with tetratricopeptide repeats 3 | 4.68 | 1.9e-14 | 3.8e-11 |
| ENSG00000136688 | IL36G | interleukin 36 gamma | 3.59 | 4.1e-14 | 7.5e-11 |
| ENSG00000143867 | OSR1 | odd-skipped related transcription factor 1 | -1.22 | 5.9e-14 | 9.8e-11 |
| ENSG00000169429 | CXCL8 | C-X-C motif chemokine ligand 8 | 2.62 | 1.1e-13 | 1.7e-10 |
| ENSG00000185338 | SOCS1 | suppressor of cytokine signaling 1 | 4.58 | 3.1e-13 | 4.4e-10 |
| ENSG00000118503 | TNFAIP3 | TNF alpha induced protein 3 | 2.88 | 3.7e-13 | 4.9e-10 |

**Table S2.** Top 15 regulated genes for the modified mRNA vs. untreated group. Genes are sorted by FDR (adjusted p-value). Genes with missing annotations are excluded.

| **ENSEMBL** | **Gene** | **Description** | **Log2FC** | **p-value** | **FDR** |
| --- | --- | --- | --- | --- | --- |
| ENSG00000197614 | MFAP5 | microfibril associated protein 5 | 2.43 | 2.0e-09 | 2.8e-05 |
| ENSG00000183347 | GBP6 | guanylate binding protein family member 6 | 0.45 | 3.7e-09 | 2.8e-05 |
| ENSG00000175662 | TOM1L2 | target of myb1 like 2 membrane trafficking protein | 0.66 | 5.9e-08 | 0.0003 |
| ENSG00000163694 | RBM47 | RNA binding motif protein 47 | 0.52 | 3.7e-07 | 0.0014 |
| ENSG00000182795 | C1orf116 | chromosome 1 open reading frame 116 | 0.52 | 3.3e-06 | 0.0078 |
| ENSG00000197632 | SERPINB2 | serpin family B member 2 | 1.43 | 3.3e-06 | 0.0078 |
| ENSG00000078401 | EDN1 | endothelin 1 | 1.88 | 3.6e-06 | 0.0078 |
| ENSG00000125733 | TRIP10 | thyroid hormone receptor interactor 10 | 0.41 | 8.3e-06 | 0.0158 |
| ENSG00000102226 | USP11 | ubiquitin specific peptidase 11 | 0.56 | 1.1e-05 | 0.0180 |
| ENSG00000136688 | IL36G | interleukin 36 gamma | 2.66 | 1.2e-05 | 0.0180 |
| ENSG00000082146 | STRADB | STE20 related adaptor beta | 0.43 | 1.4e-05 | 0.0180 |
| ENSG00000154358 | OBSCN | obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF | -0.61 | 1.4e-05 | 0.0180 |
| ENSG00000101384 | JAG1 | jagged canonical Notch ligand 1 | -0.82 | 3.0e-05 | 0.0335 |
| ENSG00000115461 | IGFBP5 | insulin like growth factor binding protein 5 | -2.15 | 3.1e-05 | 0.0335 |
| ENSG00000167767 | KRT80 | keratin 80 | 1.27 | 3.4e-05 | 0.0338 |

**Table S3.** Top 15 regulated genes for the RNP vs. untreated group. Genes are sorted by FDR (adjusted p-value). Genes with missing annotations are excluded.

| **ENSEMBL** | **Gene** | **Description** | **Log2FC** | **p-value** | **FDR** |
| --- | --- | --- | --- | --- | --- |
| ENSG00000244734 | HBB | hemoglobin subunit beta | 5.96 | 1.2e-12 | 2.7e-08 |
| ENSG00000140931 | CMTM3 | CKLF like MARVEL transmembrane domain containing 3 | 0.52 | 1.0e-06 | 0.012 |
| ENSG00000161643 | SIGLEC16 | sialic acid binding Ig like lectin 16 | -5.63 | 6.4e-06 | 0.048 |
| ENSG00000177076 | ACER2 | alkaline ceramidase 2 | -0.43 | 7.8e-05 | 0.352 |
| ENSG00000139266 | MARCHF9 | membrane associated ring-CH-type finger 9 | 0.49 | 0.00011 | 0.352 |
| ENSG00000167771 | RCOR2 | REST corepressor 2 | 1.06 | 0.00022 | 0.623 |
| ENSG00000125931 | CITED1 | Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 1 | -1.91 | 0.00028 | 0.684 |
| ENSG00000137413 | TAF8 | TATA-box binding protein associated factor 8 | -0.32 | 0.00034 | 0.684 |
| ENSG00000203943 | SAMD13 | sterile alpha motif domain containing 13 | 3.04 | 0.00056 | 0.957 |
| ENSG00000134539 | KLRD1 | killer cell lectin like receptor D1 | -3.80 | 0.00059 | 0.957 |
| ENSG00000237641 | RPL28P2 | ribosomal protein L28 pseudogene 2 | -4.18 | 0.00077 | 1.000 |
| ENSG00000166263 | STXBP4 | syntaxin binding protein 4 | -0.34 | 0.00103 | 1.000 |
| ENSG00000115844 | DLX2 | distal-less homeobox 2 | -2.04 | 0.00104 | 1.000 |
| ENSG00000234498 | RPL13AP20 | ribosomal protein L13a pseudogene 20 | -1.02 | 0.00114 | 1.000 |
| ENSG00000185839 | AK2P1 | adenylate kinase 2 pseudogene 1 | 2.56 | 0.00173 | 1.000 |