(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 August 2020 (20.08.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/165352 A1

(51) International Patent Classification:
A61K 38/17 (2006.01) *C12Q 1/6883* (2018.01)
A61K 9/00 (2006.01) *C12N 15/67* (2006.01)
A61K 48/00 (2006.01) *A61P 11/00* (2006.01)
A61K 31/7105 (2006.01) *A61K 31/198* (2006.01)

(21) International Application Number:
PCT/EP2020/053774

(22) International Filing Date:
13 February 2020 (13.02.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
19 15 7210.6 14 February 2019 (14.02.2019) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2020/165352 A1

(54) Title: TREATMENT OF CILIOPATHIES

(57) Abstract: The present disclosure provides a pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy in a subject suffering of a ciliopathy, wherein the polyribonucleotide encodes a functional version of a protein a defect of which is associated with said ciliopathy, and wherein administration of said pharmaceutical composition to the respiratory system of said subject is effected when the subject shows an inflammation of the respiratory system. Further, the present disclosure relates to a method for analyzing the effect of a polyribonucleotide on ciliogenesis, wherein said polyribonucleotide encodes a protein involved in and/or required for ciliogenesis.

Treatment of ciliopathies

The present invention relates to a pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy in a subject suffering of a ciliopathy, wherein the polyribonucleotide encodes a functional version of a protein a defect of which is associated with said ciliopathy, and wherein administration of said pharmaceutical composition to the respiratory system of said subject is effected when the subject shows an inflammation of the respiratory system. The present invention further relates to a method for analyzing the effect of a polyribonucleotide on ciliogenesis, wherein said polyribonucleotide encodes a protein involved in and/or required for ciliogenesis.

Oxygen is vital for many multicellular organisms as it is crucial for the energy supply of the cells. In mammals, oxygen comprised in the air can enter the organism via the respiratory system, which refers to the organs involved in breathing. These include for example nose, throat, larynx, trachea, bronchi, and lungs. In the latter, gas from the environment is exchanged with gas comprised in the internal blood circulatory system, which transports the oxygen from the lungs to cells in different parts of the organism. However, besides the required oxygen also other components can enter the organism via the respiratory system such as irritating agents comprised in the air including pollutants, disease causing agents and pathogens such as viruses and bacteria. Hence, defense mechanisms exist such as cough reflexes and sneezing for expelling the irritating agent from the respiratory system. For expulsion, the irritating agent is embedded in viscous mucus that is secreted by epithelial cells in the respiratory system and then transported across the epithelial surface by ciliated cells.

Ciliated cells can transport mucus and irritating agents across the epithelial surface by a synchronized beat of mutiple motile cilia. Cilia are membrane-enclosed tubular structures that extend from the epithelial surface into the space of the respiratory system that is in contact with the environment. Within the cells, the axoneme of a cilium is anchored to a basal body via anchoring structures. An axoneme is a central bundle of microtubules in which nine outer doublet microtubules surround a central pair of singlet microtubules (i.e. in respiratory cilia). The outer doublet microtubules and the central pair of microtubules are connected by radial spokes. Each of the nine outer doublet microtubules consists of an A- and a B-tubulus with the doublets being circumferentially interconnected by a nexin-dynein regulatory complex. Inner dynein

arm and outer dynein arm are connected to each A-tubulus. These dynein arms contain motor proteins that can walk along the microtubules, which results in bending and thus, beating of the cilium (cf. e.g. Lodish et al, 2000, Molecular Cell Biology, 4th edition, New York: W. H. Freeman, Section 19.4). Investigations of ciliary structures using the model *Trypanosoma brucei* indicated that some of these structural components exhibit a rapid turnover, whereas skeletal components of the radial spokes, the central pair and the outer dynein arms are primarily incorporated at the distal end of the ciliary structure during development (Vincensini et al., Biol Cell, 2018, 110:1-15).

As the synchronized beating of ciliated cells is crucial for the transport of fluids across epithelial surfaces, perturbations of ciliary structures can cause serious disorders. Motility defects including a reduced amplitude and/or frequency of beating and/or asynchronicity can occur as a result of a reduction or loss of dynein arms, a disorganisation of the microtubule arrangement including mislocalization and changes in the total number of microtubules per axoneme and combinations thereof for example. As far as known, changes in these functional axonemal elements are caused by underlying genetic defects. These are mostly due to changes in the sequence of a gene that encodes an axonemal component including the ones mentioned above. As genes can be transcribed into polyribonucleotides such as mRNAs, which in turn can be translated into proteins, the DNA sequence of a gene affects the synthesis of the respective protein in view of amount and functionality. As ciliary disorders, i.e. ciliopathies, caused by sequence changes are inherited and thus, are associated with motility defects of cilia at all developmental stages including embryo or newborns, ciliopathies can have severe consequences for the organism.

A well-known example of a ciliary disorder is primary ciliary dyskinesia (PCD), a progressive disorder that is often associated with declining lung function. Thus, long-term treatments including for example chest percussions and postural drainage are required for enhancing mucus clearance with increasing frequency, and in severe cases even lung transplantation. Further, PCD patients suffer from recurrent infections in lungs and/or ears, often also from subfertility, hydrocephalus and body laterality, i.e. left-right axis, defects, as well as retinal and/or neurological problems. But despite the severity of most ciliopathies, no standardized effective strategies for treating ciliopathies like PCD exist so far. Current therapies are for example extrapolated from cystic fibrosis and have in most cases not even been validated for the specific ciliopathy to be treated, such as for example PCD. Hence, there is a need to have at hand solutions for being able to efficiently treat subjects suffering of a ciliopathy such as PCD.

The present application addresses the need for restoring ciliary function in subjects suffering of a ciliopathy, such as PCD, by providing the embodiments as recited in the claims.

In particular, the present invention relates to a pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy in a subject suffering of a ciliopathy, wherein the polyribonucleotide encodes a functional version of a protein a defect of which is associated with said ciliopathy, and wherein administration of said pharmaceutical composition to the respiratory system of said subject is effected when the subject shows an inflammation of the respiratory system.

The present invention is based on the finding that it is indeed possible to restore proper ciliary function by transfecting cells (which show a defect in a certain protein of a protein complex of the cilia and the defect of which leads to a loss of proper ciliary function) with polyribonucleotides which encode a functional version of said protein. However, it was also found that ciliated cells have to be transfected at an early stage during differentiation in order to achieve this effect. This leads to practical problems since the precursor cells of the epithelial cells which carry the cilia, i.e. basal cells, are not accessible for transfection via the airway system since they are located deeper down in the epithelium and are not exposed on the surface. According to the present invention, a transfection of the epithelial cells by administration of a polyribonucleotide is effected when the subject which suffers of a ciliopathy shows an inflammation of the respiratory system. During an inflammation of the respiratory system the airway epithelium shows lesions and wounds which make precursor cells of the ciliated cells accessible, i.e. the basal cells which have not yet started ciliogenesis. Transfecting these cells with a polyribonucleotide as described above which expresses a functional version of the respective protein allows to render these cells into cells which form functional cilia or at least partly functional cilia which can lead to a substantial alleviation of the respective symptoms.

In the context of the present invention, the term “ciliopathy” refers to diseases associated with and/or characterized by defects of ciliated cells. Thus, ciliopathies comprise disorders of ciliary structures, including ciliary anchoring structures, basal bodies to which ciliary structures are anchored to within a cell, and/or ciliary function. Examples of ciliopathies include PCD, Bardet-Biedl syndrome, Simpson-Golabi-Behmel syndrome (type 2), leber congenital amaurosis, nephronophthisis, cranioectodermal dysplasia (Sensenbrenner) (cf. e.g. Mitchison et al., 2017, Ultrastructural Pathology,41(6):415-427).

The term "ciliopathy" as used herein refers to a ciliopathy which is caused by a genetic defect in the DNA of a subject, e.g. the chromosomal or the mitochondrial DNA. Such a genetic defect may be caused by a mutation and can comprise loss, addition or exchange of a sequence part. Examples are copy number variation, presence/absence variation, deletion (full or partial), insertion, miss-sense mutation, nonsense mutation, splice site variation, or a combination thereof. Such changes in the DNA can lead to changes in the availability of the encoded protein such as a loss or a reduction of the amount of protein, or to a protein with altered function.

In a preferred embodiment the term "ciliopathy" refers to a disease connected with a defect in motile cilia. One example of such a ciliopathy is primary ciliary dyskinesia (PCD). PCD is a rare disease caused by dysfunction of motile cilia. PCD is heterogeneous at the genetic, functional and ultrastructural level. PCD is associated with impaired mucus transport and clearance. Subjects suffering from PCD show recurrent nasal congestions, sinus infections, ear infections, infertility, situs abnormalities such as situs inversus totalis and heterotaxy, also referred to as "situs ambiguous", and/or hydrocephalus. On the molecular level, PCD is associated in most cases with abnormalities in the structure, function, and biogenesis of cilia of the respiratory system. Examples for such abnormalities are absent or shortened dynein arms, defective central pair complex,radial spoke or nexin links. Such abnormalities and thus, ciliary motility defects associated with PCD is caused by mutations in genes encoding the respective components, in particular by mutations in genes listed in **Table 1**, wherein group "A" and "B" refer to genes with pathogenic mutations estimated to account for at least 1 % and less than 1 % of PCD cases, respectively (cf. Zariwala et al., GeneReviews®, 2007, updated 2015, Primary Ciliary Dyskinesia, editors Adam et al., Seattle (WA): University of Washington, Seattle; 1993-2018).

Hence, the polyribonucleotide comprised in the pharmaceutical composition according to the present invention is preferably a polyribonucleotide that can be translated into a functional version of a protein listed in **Table 1**.

More preferably, the polyribonucleotide comprised in the pharmaceutical composition according to the present invention is an mRNA that can be translated into a functional version of a protein selected from the group consisting of DNAH5, DNAH11, CCDC39, DNAI1, CCDC40, CCDC103, SPAG1, ZMYND10, ARMC4, CCDC151, DNAI2, RSPH1, CCDC114, RSPH4A, DNAAF1 (LRRC50), DNAAF2 (KTU), and LRRC6.

Table 1

Gene	Locus	Protein	Group
DNAH5	CILD3	DNAH5	A
DNAH11	CILD7	DNAH11	A
CCDC39	CILD14	CCDC39	A
DNAI1	CILD1	DNAI1	A
CCDC40	CILD15	CCDC40	A
CCDC103	CILD17	CCDC103	A
SPAG1	CILD28	SPAG1	A
ZMYND10	CILD22	ZMYND10	A
ARMC4	CILD23	ARMC4	A
CCDC151	CILD30	CCDC151	A
DNAI2	CILD9	DNAI2	A
RSPH1	CILD24	RSPH1	A
CCDC114	CILD20	CCDC114	A
RSPH4A	CILD11	RSPH4A	A
DNAAF1 (LRRC50)	CILD13	DNAAF1 (LRRC50)	A
DNAAF2 (KTU)	CILD10	DNAAF2 (KTU)	A
LRRC6	CILD19	LRRC6	A
C21orf59	CILD26	C21orf59	B
CCDC65 (DRC2)	CILD27	CCDC65 (DRC2)	B
CCNO	CILD29	CCNO	B
DNAAF3	CILD2	DNAAF3	B
DNAH1		DNAH1	B
DNAH8		DNAH8	B
DNAL1	CILD16	DNAL1	B
DRC1 (CCDC164)	CILD21	DRC1 (CCDC164)	B
DYX1C1	CILD25	DYX1C1	B
DNAAF5 (HEATR2)	CILD18	DNAAF5 (HEATR2)	B
HYDIN	CILD5	HYDIN	B
MCIDAS		MCIDAS	B
NME8 (TXNDC3)	CILD6	NME8 (TXNDC3)	B
RSPH3		RSPH3	B
RSPH9	CILD12	RSPH9	B

In some embodiments of any of the foregoing or other aspects and embodiments of the disclosure, the polyribonucleotide or modified polyribonucleotide comprises a primary sequence that is at least 85%, at least 90%, at least 92% or at least 95%

identical (e.g., at least 95, 96, 97, 98, 99 or 100% identical) to one or more of **SEQ ID NO: 1 or 5 to 11** (e.g., to the sequence set forth in **SEQ ID NO: 1 or 5 to 11**). In some embodiments, the polyribonucleotide is a modified polyribonucleotide having a level and/or type of modification selected from any such level and/or type set forth herein. In certain embodiments, the percent identity of a polyribonucleotide is measured only with respect to the CCDC40 coding sequence-portion of **SEQ ID NO: 1 or 5 to 11** (e.g., UTRs, other non-coding sequence and GFP or epitope tags are not considered when calculating percent identity). In certain embodiments of any of the foregoing, such polyribonucleotide (or modified polyribonucleotide) encodes a functional CCDC40 protein.

In some embodiments of any of the foregoing or other aspects and embodiments of the disclosure, the polyribonucleotide or modified polyribonucleotide comprises a primary sequence that is at least 85%, at least 90%, at least 92%, or at least 95% identical (e.g., at least 95, 96, 97, 98, 99 or 100% identical) to one or more of **SEQ ID NO: 2 or 12 to 14** (e.g., to the sequence set forth in **SEQ ID NO: 2 or 12 to 14**). In some embodiments, the polyribonucleotide is a modified polyribonucleotide having a level and/or type of modification selected from any such level and/or type set forth herein. In certain embodiments, the percent identity of a polyribonucleotide is measured only with respect to the CCDC39 coding sequence-portion of **SEQ ID NO: 2 or 12 to 14** (e.g., UTRs, other non-coding sequence and GFP tags or epitope tags are not considered when calculating percent identity). In certain embodiments of any of the foregoing, such polyribonucleotide (or modified polyribonucleotide) encodes a functional CCDC39 protein.

In some embodiments of any of the foregoing or other aspects and embodiments of the disclosure, the polyribonucleotide or modified polyribonucleotide comprises a primary sequence that is at least 85%, at least 90%, at least 92%, or at least 95% identical (e.g., at least 95, 96, 97, 98, 99 or 100% identical) to **SEQ ID NO: 4** (e.g., to the sequence set forth in **SEQ ID NO: 4**). In some embodiments, the polyribonucleotide is a modified polyribonucleotide having a level and/or type of modification selected from any such level and/or type set forth herein. In certain embodiments, the percent identity of a polyribonucleotide is measured only with respect to the MCIDAS coding sequence-portion of **SEQ ID NO: 4** (e.g., UTRs, other non-coding sequence and GFP or epitope tags are not considered when calculating percent identity). In certain embodiments of any of the foregoing, such polyribonucleotide (or modified polyribonucleotide) encodes a functional MCIDAS protein.

In certain embodiments, the disclosure provides pharmaceutical compositions comprising any of the foregoing polyribonucleotides. Moreover, any such polyribonucleotides (or pharmaceutical compositions) may be used in the any of the methods described herein.

In one embodiment of the pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy, said ciliopathy is associated with a defect in a coiled-coil domain containing 40 (CCDC40; cf. e.g. NCBI Reference Sequences NM_017950.4 and NP_060420.2 for human mRNA and protein CCDC40 sequence, respectively) protein or with a defect in a coiled-coil domain containing 39 (CCDC39; cf. e.g. NCBI Reference Sequences NM_181426.2 and NP_852091.1 for human mRNA and protein CCDC39 sequence, respectively) protein.
CCDC40 and CCDC39 build a complex that is located between radial spokes and A-tubuli. Defect versions of the CCDC40 or CCDC39 protein can be caused by mutations in the *CCDC40* gene or in the *CCDC39* gene such as insertions, deletions, nonsense and splice site mutations. In particular, a deletion of position 248 and a TGT insertion between positions 2824 and 2825 in the DNA sequence encoding the CCDC40 protein appear to be quite frequent. Defect versions of CCDC40 or CCDC39 give rise to defects in structures of the axoneme such as absent or eccentric central pairs, abnormal radial spokes and nexin links, an abnormal assembly of the dynein regulatory complex, and/or a reduction of inner dynein arms. Hence, the polyribonucleotide comprised in the pharmaceutical composition according to the present invention is preferably an mRNA that can be translated into a functional version of CCDC40 and or of CCDC39. Further information on proteins involved in ciliogenesis as well as genes and molecular pathways associated with ciliopathies can be found e.g. in the review article of Reiter and Leroux (Reiter and Leroux, 2017, Nat Rev Mol Cell Biol,18(9):533-547).

The pharmaceutical composition of the present invention is to be administered to a subject suffering from a ciliopathy. Herein a subject suffering from a ciliopathy, may also be referred to as a patient. Patients may show abnormal ciliary structure and/or function, and/or biogenesis defects that result in retention of mucus and bacteria in the respiratory tract. The diagnosis of a ciliopathy for a given subject may be based for example on clinical findings, molecular analyses and/or ciliary ultrastructural analyses of a biopsy of said subject as e.g. also reviewed in Goutaki et al. (Goutaki et al., 2016, Eur Respir J, 48(4):1081-1095).

In the context of the present invention, the term “polyribonucleotide” refers to a single-stranded sequence built up of adenosine, guanosine, cytidine, and/or uridine

residues (in modified or unmodified form, see below). Herein, the term "polyribonucleotide encoding a protein" refers to a polyribonucleotide which contains a coding region which encodes a protein, i.e. which can be translated into a sequence of amino acids. Thus, in the context of the present invention the term "polyribonucleotide encoding a protein" preferably refers to an mRNA, wherein an mRNA should be understood to mean any polyribonucleotide molecule which, if it comes into the cell, is suitable for the expression of a protein or is translatable into a protein.

Herein, the term "protein" encompasses any kind of amino acid sequence, i.e. chains of two or more amino acids which are each linked via peptide bonds. The term "protein" used in this context refers to any amino acid sequence of interest. Preferably, the encoded amino acid sequence is at least 5 amino acids long, more preferably at least 10 amino acids, even more preferably at least 50, 100, 200 or 500 amino acids. Thus, the term "protein" covers short peptides as well as polypeptides. As regards the function of the encoded protein, there is no limitation except that a defect variant of the protein is associated with a ciliopathy. Herein, the term "associated with" is intended to encompass the terms "causing", "being involved in" and/or "enhancing".

Herein, the term "a protein the defect of which" refers to a "defect protein" or "defect version of a protein" and thus, to a version of a protein with altered function compared to a functional version of said protein. However, the term may also encompass a version of said protein with a complete or partial lack of synthesis and thus, availability in the cell. In any case, the defect of the protein version results in a version of said protein that cannot fulfill the protein´s native function.

A version of a protein that fulfils its native function is referred to as "functional protein" or "functional version of a protein" herein, and is encoded by the same DNA sequence as the respective defect protein, but without the defect causing change in the DNA sequence and thus, without a mutation in the DNA sequence.
Hence, in the context of the present invention, the "functional protein" is preferably a building block of an A-tubulus, a B-tubulus, or a nexin-dynein regulatory complex, or a radial spoke, an inner dynein arm, and/or an outer dynein arm.

Thus, the term "the polyribonucleotide encodes a functional version of a protein a defect of which is associated with said ciliopathy" preferably refers to an mRNA that encodes a functional protein, which is involved in the structural organization of a ciliar axoneme, an axoneme anchoring structure or a basal body and fulfills its native

function. Thus, the polyribonucleotide according to the present invention preferably refers to an mRNA that encodes a functional protein, the presence of which in the cell of a subject suffering of a ciliopathy is needed or beneficial to moderate or prevent a manifestation of said ciliopathy that is associated with a defect of said protein as encoded by the DNA sequence of the cell or to alleviate the associated symptoms.

In addition, the polyribonucleotide employed according to the present invention may also comprise further functional regions and/or 3' or 5' non-coding regions. The 3' and/or 5' non-coding regions can be sequences which naturally flank the encoded protein or artificial sequences which contribute to the stabilization and/or regulation of said polyribonucleotide. Suitable sequences may be identified and investigated by routine experiments. Further, said polyribonucleotide can also have further functional regions and may be combined with regulatory elements and target sequences of micro-RNAs for example for spatial and temporal control the activity of the desired polyribonucleotide comprising a sequence which encodes a protein, i.e. for example with respect to specific cells or cell types and/or developmental stages or specific time frames.

The polyribonucleotide employed according to the present invention may comprise a partly or fully codon optimized sequence derived from the natural sequence to be used. Codon optimization refers to a technique which is applied to maximize protein expression by increasing the translational efficiency of the respective polyribonucleotide as in some cases codons exist that are preferentially used by some species for a given amino acid. Further, said polyribonucleotide might comprise further modifications to adjust and/or extend the duration of action. Said polyribonucleotide might also contain an m7GpppG cap, an internal ribosome entry site (IRES) and/or a polyA tail at the 3′ end and/or additional sequences for promoting translation.
In some embodiments of the present invention the polyribonucleotide employed according to the present invention may contain unmodified and modified nucleotides. The term "unmodified nucleotide" used herein refers to A, C, G and U nucleotides. The term "modified nucleotide" used herein refers to any naturally occurring or non-naturally occurring isomers of A, C, G and U nucleotides as well as to any naturally occurring or naturally occurring analogs, alternative or modified nucleotide or isomer thereof having for example chemical modifications or substituted residues. Modified nucleotides can have a base modification and/or a sugar modification. Modified nucleotides can also have phosphate group modifications, e.g., with respect to the five prime cap of an mRNA molecule. Modified nucleotides also include nucleotides that are synthesized post-transcriptionally by covalent modification of the nucleotides.

Further, any suitable mixture of non-modified and modified nucleotides is possible. A non-limiting number of examples of modified nucleotides can be found in the literature (e.g. Cantara et al., Nucleic Acids Res, 2011, 39(Issue suppl_1):D195-D201; Helm and Alfonzo, Chem Biol, 2014, 21(2):174-185; Carell et al., Angew Chem Int Ed Engl, 2012, 51(29):7110-31) and some preferable modified nucleotides are mentioned exemplarily in the following based on their respective nucleoside residue: 1-methyladenosine, 2-methylthio-N6-hydroxynorvalyl carbamoyladenosine, 2-methyladenosine, 2'-O-ribosylphosphate adenosine, N6-methyl-N6-threonylcarbamoyladenosine, N6-acetyladenosine, N6-glycinylcarbamoyladenosine, N6-isopentenyladenosine, N6-methyladenosine, N6-threonylcarbamoyladenosine, N6,N6-dimethyladenosine, N6-(cis-hydroxyisopentenyl)adenosine, N6-hydroxynorvalylcarbamoyladenosine, 1,2'-O-dimethyladenosine, N6,2'-O-dimethyladenosine, 2'-O-methyladenosine, N6,N6,2'-O-trimethyladenosine, 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine, 2-methylthio-N6-methyladenosine, 2-methylthio-N6-isopentenyladenosine, 2-methylthio-N6-threonyl carbamoyladenosine, N6-2-methylthio-N6-threonyl carbamoyladenosine, 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine, 7-methyladenosine, 2-methylthio-adenosine, 2-methoxy-adenosine, 2'-amino-2'-deoxyadenosine, 2'-azido-2'-deoxyadenosine, 2'-fluoro-2'-deoxyadenosine, 2-aminopurine, 2,6-diaminopurine, 7-deaza-adenosine, 7-deaza-8-aza-adenosine, 7-deaza-2-aminopurine, 7-deaza-8-aza-2-aminopurine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine; 2-thiocytidine, 3-methylcytidine, N4-acetylcytidine, 5-formylcytidine, N4-methylcytidine, 5-methylcytidine, 5-hydroxymethylcytidine, 5-hydroxycytidine, lysidine, N4-acetyl-2'-O-methylcytidine, 5-formyl-2'-O-methylcytidine, 5,2'-O-dimethylcytidine, 2-O-methylcytidine, N4,2'-O-dimethylcytidine, N4,N4,2'-O-trimethylcytidine, isocytidine, pseudocytidine, pseudoisocytidine, 2-thio-cytidine, 2'-methyl-2'-deoxycytidine, 2'-amino-2'-deoxycytidine, 2'-fluoro-2'-deoxycytidine, 5-iodocytidine, 5-bromocytidine, 2'-azido-2'-deoxycytidine, 2'-amino-2'-deoxycytidine, 2'-fluor-2'-deoxycytidine, 5-aza-cytidine, 3-methyl-cytidine, 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-l-methyl-pseudoisocytidine, 4-thio-l-methyl-1-deaza-pseudoisocytidine, 1-methyl-l-deaza-pseudoisocytidine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, 4-methoxy-l-methyl-pseudoisocytidine, zebularine,5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine; 1-methylguanosine, N2,7-dimethylguanosine, N2-methylguanosine, 2'-O-ribosylphosphate guanosine, 7-methylguanosine, hydroxywybutosine, 7-aminomethyl-7-deazaguanosine, 7-cyano-7-deazaguanosine, N2,N2-dimethylguanosine, N2,7,2'-O-trimethylguanosine, N2,2'-O-dimethylguanosine, 1,2'-O-dimethylguanosine, 2'-O-methylguanosine, N2,N2,2'-O-trimethylguanosine,

N2,N2J-trimethylguanosine, Isoguanosine, 4-demethylwyosine, epoxyqueuosine, undermodified hydroxywybutosine, methylated undermodified hydroxywybutosine, isowyosine, peroxywybutosine, galactosyl-queuosine, mannosyl-queuosine, queuosine, archaeosine, wybutosine, methylwyosine, wyosine, 7-aminocarboxypropyldemethylwyosine, 7-aminocarboxypropylwyosine, 7-aminocarboxypropylwyosinemethylester, 7-deaza-guanosine, 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine, 6-thio-7-methyl-guanosine, 7-methylinosine, 6-methoxy-guanosine, 1-methylguanosine, 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, N2,N2-dimethyl-6-thio-guanosine, N1-methylguanosine, 2'-amino-3'-deoxyguanosine, 2'-azido-2'-deoxyguanosine, 2'-fluoro-2'-deoxyguanosine, 2-thiouridine, 3-(3-amino-3-carboxypropyl)uridine, 3-methyluridine, 4-thiouridine, 5-methyl-2-thiouridine, 5-methylaminomethyluridine, 5-carboxymethyluridine, 5-carboxymethylaminomethyluridine, 5-hydroxyuridine, 5-methyluridine, 5-taurinomethyluridine, 5-carbamoylmethyluridine, 5-(carboxyhydroxymethyl)uridine methyl ester, dihydrouridine, 5-methyldihydrouridine, 5-methylaminomethyl-2-thiouridine, 5-(carboxyhydroxymethyl)uridine, 5-(carboxyhydroxymethyl)-2'-O-methyluridine methyl ester, 5-(isopentenylaminomethyl)uridine, 5-(isopentenylaminomethyl)-2-thiouridine, 3,2'-O-dimethyluridine, 5-carboxymethylaminomethyl-2'-O-methyluridine, 5-carbamoylhydroxymethyluridine, 5-carbamoylmethyl-2'-O-methyluridine, 5-carbamoylmethyl-2-thiouridine, 5-methoxycarbonylmethyl-2'-O-methyluridine, 5-(isopentenylaminomethyl)-2'-O-methyluridine, 5,2'-O-dimethyluridine, 2'-O-methyluridine, 2'-O-methyl-2-thiorudine, 2-thio-2'-O-methyluridine, uridine 5-oxyacetic acid, 5-methoxycarbonylmethyluridine, uridine 5-oxyacetic acid methyl ester, 5-methoxyuridine, 5-aminomethyl-2-thiouridine, 5-carboxymethylaminomethyl-2-thiouridine, 5-methylaminomethyl-2-selenouridine, 5-methoxycarbonylmethyl-2-thiouridine, 5-taurinomethyl-2-thiouridine, pseudouridine, 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine, 1-methylpseudouridine, 3-methylpseudouridine, 2'-O-methylpseudouridine, 5-formyluridine, 5-aminomethyl-2-geranyluridine, 5-taurinomethyluridine, 5-iodouridine, 5-bromouridine, 2'-methyl-2'-deoxyuridine, 2'-amino-2'-deoxyuridine, 2'-azido-2'-deoxyuridine, 2'-fluoro-2'-deoxyuridine, inosine, 1-methylinosine, 1,2'-O-dimethylinosine, 2'-O-methylinosine, 5-aza-uridine, 2-thio-5-aza-uridine, 4-thio-pseudouridine, 2-thio-pseudouridine, 5-carboxymethyl-uridine, 1-carboxymethyl-pseudouridine, 5-propynyl-uridine, 1-propynyl-pseudouridine, 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine, 1-taurinomethyl-4-thio-uridine, 5-methyl-uridine, 1-methyl-pseudouridine, 4-thio-l-methyl-pseudouridine, 2-thio-l-methyl-pseudouridine, 1-methyl-l-deaza-pseudouridine, 2-thio-1-methyl-l-deaza-pseudouridine, dihydropseudouridine, 2-thio-

dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, 1,2'-O-dimethyladenosine, 1,2'-O-dimethylguanosine, 1,2'-O-dimethylinosine, 2,8-dimethyladenosine, 2- methylthiomethylenethio-N6-isopentenyl-adenosine, 2-geranylthiouridine, 2-lysidine, 2-methylthio cyclic N6-threonylcarbamoyladenosine, 2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine, 2-methylthio-N6-hydroxynorvalylcarbamoyladenosine, 2-methylthio-N6-threonylcarbamoyladenosine, 2-selenouridine, 2-thio-2'-O-methyluridine, 2'-O-methyladenosine, 2'-O-methylcytidine, 2'-O-methylguanosine, 2'-O-methylinosine, 2'-O-methylpseudouridine, 2'-O-methyluridine, 2'-O-methyluridine 5-oxyacetic acid methyl ester, 2'-O-ribosyladenosinephosphate, 2'-O-ribosylguanosinephosphate, 3,2'-O-dimethyluridine, 3-(3-amino-3-carboxypropyl)-5,6-dihydrouridine, 3-(3-amino-3-carboxypropyl)pseudouridine, 5,2'-O-dimethylcytidine, 5,2'-O-dimethyluridine, 5-(carboxyhydroxymethyl)-2'-O-methyluridine methyl ester, 55-(isopentenylaminomethyl)-2'-O-methyluridine, 5-aminomethyl-2-geranylthiouridine, 5-aminomethyl-2-selenouridine, 5-aminomethyluridine, 5-carbamoylmethyl-2'-O-methyluridine, 5-carboxyhydroxymethyluridine, 5-carboxymethyl-2-thiouridine, 5-carboxymethylaminomethyl-2-geranylthiouridine, 5-carboxymethylaminomethyl-2-selenouridine, 5-carboxymethylaminomethyl-2'-O-methyluridine, 5-cyanomethyluridine, 5-formyl-2'-O-methylcytidine, 5-methoxycarbonylmethyl-2'-O-methyluridine, 5-methylaminomethyl-2-geranylthiouridine, 7-aminocarboxypropyl-demethylwyosine, 7-methylguanosine, 8-methyladenosine, N2,2'-O-dimethylguanosine, N2,7,2'-O-trimethylguanosine, N2,7-dimethylguanosine, N2,N2,2'-O-trimethylguanosine, N2,N2,7-trimethylguanosine, N2,N2,7-trimethylguanosine , N4,2'-O-dimethylcytidine, N4,N4,2'-O-trimethylcytidine, N4,N4-dimethylcytidine, N4-acetyl-2'-O-methylcytidine, N6,2'-O-dimethyladenosine, N6,N6,2'-O-trimethyladenosine, N6-formyladenosine, N6-hydroxymethyladenosine, agmatidine, 2-methylthio cyclic N6-threonylcarbamoyladenosine, glutamyl-queuosine, guanosine added to any nucleotide, guanylylated 5' end , hydroxy-N6-threonylcarbamoyladenosine; most preferably pseudo-uridine, N1-methyl-pseudo-uridine, 2´-fluoro-2´-deoxycytidine, 5-iodocytidine, 5-methylcytidine, 2-thiouridine, 5-iodouridine and/or 5-methyl-uridine.

Furthermore, the term “modified nucleotide” comprises nucleotides containing isotopes such as deuterium. The term "isotope" refers to an element having the same number of protons but different number of neutrons resulting in different mass numbers. Thus, isotopes of hydrogen for example are not limited to deuterium, but include also tritium. Furthermore, the polyribonucleotide can also contain isotopes of other elements including for example carbon, oxygen, nitrogen and phosphor. It is

also possible that modified nucleotides are deuterated or contain another isotope of hydrogen or of oxygen, carbon, nitrogen or phosphor.

The total number of modified nucleotide types in the polyribonucleotide can be 0, 1, 2, 3, or 4. Hence, in some embodiments, at least one nucleotide of one nucleotide type, e.g. at least one U nucleotide, can be a modified nucleotide. In some embodiments, at least one nucleotide of in total two nucleotide types, e.g. at least one U nucleotide and at least one C nucleotide, can be a modified nucleotide. In some embodiments, at least one nucleotide of in total three nucleotide types, e.g. at least one G nucleotide, at least one U nucleotide and at least one C nucleotide, can be a modified nucleotide. In some embodiments, at least one nucleotide of all four nucleotide types can be a modified nucleotide. In all these embodiments one or more nucleotides per nucleotide type can be modified, the percentage of said modified nucleotides of per nucleotide type being 0%, 2.5%, 5%, 7.5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 100%.
In some embodiments, the total percentage of modified nucleotides comprised in the mRNA molecules to be purified is 0%, 2.5%, 5 %, 7.5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 100%.

Hence, the polyribonucleotide can for example be characterized in that 0.5 to 50%, preferably 5 to 50% of the U nucleotides and 5 to 50% of the C nucleotides are modified. Said modified U nucleotides are preferably 5-ioduridine and said modified C nucleotides are preferably 5-iodcytidine.
In some embodiments, the polyribonucleotide can be characterized in that 15 to 25% of the U nucleotides and 3 to 15%, preferably 5 to 15% of the C nucleotides are modified, wherein said modified U nucleotides are preferably 5-methyluridine and said modified C nucleotides are preferably 5-iodcytidine.
In some embodiments, the polyribonucleotide can be characterized in that 30 to 50% of the U nucleotides and 10 to 20% of the C nucleotides are modified, wherein said modified U nucleotides are preferably 5-ioduridine and said modified C nucleotides are preferably 5-iodcytidine.
In some embodiments, the polyribonucleotide can be characterized in that 30 to 50% of the U nucleotides and 5 to 15% of the C nucleotides are modified, wherein said modified U nucleotides are preferably 5-ioduridine and said modified C nucleotides are preferably 5-iodcytidine.
In some embodiments, the polyribonucleotide can be characterized in that 0.5 to 5% of the U nucleotides and 25 to 35% of the C nucleotides are modified, wherein said modified U nucleotides are preferably 2-thiouridine and said modified C nucleotides are preferably 5-methylcytidine.

The polyribonucleotide can for example also be characterized in that 50 to 100%, preferably 100%, of the U nucleotides are modified. Said modified U nucleotides are preferably N1-methyl-pseudo-uridine.

As described above, according to the present invention, the administration of the pharmaceutical composition to the patient suffering of a ciliopathy is effected when the subject shows an inflammation of the respiratory system. In the context of the present invention, the term "inflammation" refers to cellular responses to insults including infection, trauma, and hypersensitivity. An "inflammation of the respiratory system" refers to inflammatory responses in the respiratory system, especially in, but without limitation to, nose, pharynx, larynx, trachea, bronchi and/or lung. Inflammatory responses in the respiratory system can for example be caused by irritating agents such as pathogens, toxins, pollutants, and/or allergens. During inflammation specific cell types are activated that can release for example cytokines and mediators to modify activities of other cells. These processes are. e.g., described in Iwasaki et al. (Iwasaki et al., 2017, Nat Rev Immunol, 17(1):7-20).
Thus, in one embodiment the subject suffering from a ciliopathy to which the polyribonucleotide is to be administered has been subjected, prior to treatment, to an assay in order to determine whether the subject suffers from an inflammation of the respiratory system and wherein the subject has been positively determined to have an inflammation of the respiratory system.

Preferably, the inflammation of the respiratory system is an acute inflammation. An acute inflammation can occur over seconds, minutes, hours, and days, but might not occur over longer periods. Thus, an acute inflammation is an inflammation that occurs in a time range up to 4 weeks, preferably in a time frame of less than 3 weeks. It can be determined by routine lab methods based on a locally increased blood flow, a locally increased permeability of the capillaries, and/or increased numbers of neutrophils, macrophages and/or lymphocytes. More information about markers of airway inflammation in primary ciliary dyskinesia can be found, e.g., in Zihlif et al. (Zihlif et al., 2006, Pediatr Pulmonol, 41(6):509-14).
Generally, inflammation can be classified as either acute or chronic. Acute inflammation is the response of the body to a harmful stimulus and characterized e.g. by the increased move of granulocytes to the affected tissue. The classical signs of inflammation are heat, pain, redness, swelling, and loss of function. Ciliopathies such as, PCD are an inherited disorders and as such - if untreated - a permanent, lifelong stimulus caused by a loss of function, resulting in a permanent and as such chronic kind of inflammation, typically not showing the above symptoms (beside loss of function). Further examples for diseases associated with chronic inflammation are

e.g.: hay fever, periodontal disease, atherosclerosis, and osteoarthritis. Nonetheless, patients suffering from such diseases can in addition get an acute inflammation e.g. through receiving an additional harmful stimulus and as a consequence one or more of the classical symptoms like classical signs of inflammation are heat, pain, redness, swelling, and additional loss of function.

Thus, in one embodiment the subject suffering from a ciliopathy to which the polyribonucleotide is to be administered has been subjected prior to treatment to an assay in order to determine whether the subject suffers from an acute inflammation of the respiratory system and wherein the subject has been positively determined to have an acute inflammation of the respiratory system.

Alternatively or in addition, the inflammation of the respiratory system refers to an exacerbation of inflammation, preferably an acute exacerbation of inflammation. Exacerbation refers to the worsening of a disease or an increase in its symptoms. It is best investigated in the context of chronic obstructive pulmonary disease (COPD) since exacerbations result in a decrease of a patient's quality of life, accelerate the decline of lung function, and contribute substantially to disease-related costs.
In case of a clinical trial a respiratory system exacerbation could be defined as follows: "A respiratory system exacerbation is defined in a trial as either respiratory tract symptoms leading to start of systemic antibiotic treatment, irrespective of results of bacterial culture, or as a decline in forced expiratory volume in one second (FEV1) % predicted equal to or above 10 percentage points relative to the average of FEV1% predicted at screening and randomization, whether or not antibiotics are prescribed. The occurrence of exacerbations can be assessed by patient interview, physical examination and spirometry. At each trial visit, and at any extra contacts with the trial sites attributable to exacerbations, the participants can be interviewed regarding symptoms and concomitant medication since last contact with the trial site. The interview can be supplemented by a weekly patient diary on symptoms and antibiotics. A physical examination reviewing the participants' general condition, vital signs, ears, heart and lungs can be performed at all visits." (c.f. e.g. Kobbernagel et al., 2016, BMC Pulmonary Medicine, 16:104).

Hence, according to a preferred embodiment, the pharmaceutical composition comprises an mRNA for use in treating a ciliopathy in a subject suffering of a ciliopathy, wherein the mRNA encodes a functional version of a ciliary structure protein a defect of which is associated with said ciliopathy, and wherein administration of said pharmaceutical composition to the respiratory system of said subject suffering of said ciliopathy is effected when the subject suffering of a

ciliopathy shows an acute inflammation, preferably an acute exacerbation, of the respiratory system.
Thus, in one embodiment the subject suffering from a ciliopathy to which the polyribonucleotide is to be administered has been subjected, prior to treatment, to an assay in order to determine whether the subject suffers from an acute inflammation, preferably an acute exacerbation, of the respiratory system and wherein the subject has been .positively determined to have a chronic inflammation, preferably an acute exacerbation, of the respiratory system.

The presence or absence of an inflammation of the respiratory system of a subject suffering of a ciliopathy can be determined by routine procedures, e.g., by analyzing a blood sample or by determining whether the patient suffers from a running nose or the like.
In the respiratory system, inflammations are generally caused by infections, in particular viral or bacterial infections. Thus, the presence or absence of an inflammation in a ciliopathy patient can, preferably, be assessed by determining the presence or absence of an infection, preferably an acute infection. Preferably, the infection is a viral and/or bacterial infection.
Acute infections are characterized by auscultation findings, purulent cough, infiltrates, hemoptysis, fever, increase in blood inflammation parameters (c-reactive protein (CRP) >200 mg/ml, blood sedimentation <100 mm/h). Chronic infections are further characterized by migrating infiltrates, antibiotic resistance, persistent general symptoms and moderately increased blood inflammation markers (CRP 50-100 mg/ml, blood sedimentation <50 mm/h) (Klinische Pneumonologie, 1. Aufl. 2014 Georg Thieme Verlag KG, ISBN 978-3-13-129751-8; Jaroszewski et al., 2012, Thorac Surg Clin, 22(3):301-24).

In the context of the present invention, the term “respiratory system” comprises organs involved in breathing such as nose, pharynx, larynx, trachea, bronchi and lungs. In particular, the respiratory system can also be referred to as respiratory tract in case of some mammals including humans, herein also referred to as subjects. Herein, the terms “respiratory system” and “respiratory tract” are used interchangeably. The respiratory tract can be divided into the upper respiratory tract and the lower respiratory tract. The upper respiratory tract includes the nose comprising nasal cavity, nasal conchae, nasal vestibulae and nasal passages; paranasal sinuses; the pharynx, and the portion of the larynx above the vocal folds (cords). The lower respiratory tract includes the portion of the larynx below the vocal folds, trachea, bronchi and bronchioles. Herein, the lungs are included in the lower

respiratory tract and comprise respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli.

In the context of the present invention, the term "pharmaceutical composition" refers to a composition comprising at least a polyribonucleotide according to the present invention for administration to a subject in order to treat a ciliopathy. The polyribonucleotide is preferably included in an effective amount, i.e. an amount sufficient to induce a detectable therapeutic response in the subject to which the pharmaceutical composition is to be administered. The pharmaceutical composition of the invention can be in the form of a sterile aqueous or non-aqueous solution, suspension or emulsion or aerosol. Preferably, the pharmaceutical composition is in a form which allows administration to the respiratory system e.g. via inhalation, nebulization, via a spray or droplets, e.g. a nasal spray or nasal droplets.
This is advantageous for the patients as an administration using a spray, droplets, a nebulizer or by inhalation can easily be done by the patient, is comfortable to transport and thus, easily available for the patient without restricting any freedom of action.

In a preferred embodiment the pharmaceutical composition comprises an mRNA that can be translated into a functional version of a protein selected from the group consisting of DNAH5, DNAH11, CCDC39, DNAI1, CCDC40, CCDC103, SPAG1, ZMYND10, ARMC4, CCDC151, DNAI2, RSPH1, CCDC114, RSPH4A, DNAAF1 (LRRC50), DNAAF2 (KTU), and LRRC6, and is administered to a subject suffering from a PCD caused by a defect of said protein by using a spray, droplets, a nebulizer and/or by inhalation. More preferably the protein is CCDC40 and/or CCDC39.

The pharmaceutical composition can comprise a pharmaceutically acceptable carrier, i.e. chemical compounds, materials, ingredients, and/or compositions, which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Thus, a pharmaceutically acceptable carrier is an inactive substance formulated alongside the pharmaceutically active substance for facilitating its handling in view of dosage, adsorption, solubility or pharmacokinetic considerations. Examples of suitable pharmaceutical acceptable carriers are well known in the art and include phosphate buffered saline solutions, buffer, water, emulsions, such as oil/water emulsions, various types of wetting agents, and sterile solutions. In particularly, aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Examples of non-

aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and organic esters such as ethyl oleate. Further examples of pharmaceutically acceptable carriers include but are not limited to saline, Ringer's solution and dextrose solution, citrate, phosphate, and other organic acids; salt-forming counter-ions, e.g. sodium and potassium; low molecular weight (> 10 amino acid residues) polypeptides; proteins, e.g. serum albumin, or gelatine; hydrophilic polymers, e.g. polyvinylpyrrolidone; amino acids such as histidine, glutamine, lysine, asparagine, arginine, or glycine; carbohydrates including glucose, mannose, or dextrins; monosaccharides; disaccharides; other sugars, e.g. sucrose, mannitol, trehalose or sorbitol; chelating agents, e.g. EDTA; non-ionic surfactants, e.g., polyoxyethylene sorbitan monolaurate, available on the market with the commercial name Tween, propylene glycol, Pluronics or polyethylene glycol; antioxidants including methionine, ascorbic acid and tocopherol; and/or preservatives, e.g. octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, e.g. methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol). Suitable pharmaceutically acceptable carriers and their formulations are described in greater detail in Remington's Pharmaceutical Sciences, 17th ed., 1985, Mack Publishing Co. Furthermore, preservatives, stabilizers and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases, nanosystems or liposomes, and the like.

The pharmaceutical composition of the present invention may be administered to a patient via a large range of classes of forms of administration known to the skilled person to be suitable for administration to the respiratory system, such as the use of sprays, droplets, inhalators, nebulizers and the like. Dose and duration of action depend on the function which said polyribonucleotide is to fulfil and have to be deliberately adjusted in each case. The duration of action will be as long as possible for example, if said polyribonucleotide is used, as is the case here, for the chronic therapy of a disease due to a deficient gene, i.e. changed DNA sequence. The duration may also be adjusted to a specific time window.

In one embodiment the pharmaceutical composition is administered at least once a week. This is advantageous for ensuring an efficient and persisting effect of the treatment of said PCD. Preferably the pharmaceutical composition is administered on a weekly basis for at least 2 weeks, more preferably for at least 3 weeks and even more preferably for at least 4 weeks. Alternatively, the pharmaceutical composition may be administered twice a week for at least 1 week, preferably for at least 2 weeks, more preferably for at least 3 weeks and even more preferably for at least 4

weeks. In one embodiment, if there is a need for further treatment after an initial treatment for four weeks as described above, the treatment is carried out on a weekly basis. Alternatively to the weekly administration, administration may be switched to administration once a month for a longer period of time, e.g. for at least two months, preferably for at least 3 months, more preferably for at least 4 month, even more preferably for at least 5 months and most preferably for at least 6 months.

In one preferred embodiment the pharmaceutical composition is administered to the respiratory system of the subject after the subject inhaled an appropriate solution, preferably a mucolytic agent, such as a hypertonic saline or a solution of N-acetylcysteine (NAC), or washed their nasal cavities and/or sinus with an appropriate solution, preferably a mucolytic agent, such as a hypertonic saline or N-acetylcysteine (NAC), in order to remove mucus and potentially shedded airway epithelial cells. Thus, it is preferred that the pharmaceutical composition is administered to the respiratory system of the subject after the subject inhaled an appropriate solution, preferably a mucolytic agent, such as a hypertonic saline or a solution of N-acetylcysteine (NAC), and coughed up mucus located on epithelial cells. This is especially advantageous for exposing epithelial cells before administering the pharmaceutical composition and thus, for enhancing transfection efficacy of the polyribonucleotide.
Thus, in one embodiment the subject suffering from a ciliopathy to which the polyribonucleotide is to be administered is a subject which has been subjected, prior to treatment, by inhaling an appropriate solution, preferably a mucolytic agent, such as a hypertonic saline or a solution of N-acetylcysteine (NAC), or to washing their nasal cavities and/or sinus with an appropriate solution, preferably a mucolytic agent, such as a hypertonic saline or N-acetylcysteine (NAC).
Such a step aims at physically removing mucus from the respiratory system of the subject prior to the administration of the polyribonucleotide.
In addition, such a subject is preferably a subject which has been subjected, prior to treatment, to an assay in order to determine whether the subject suffers from an inflammation, preferably an acute inflammation or exacerbation of inflammation of the respiratory system and wherein the subject has been positively determined to have an inflammation, preferably an acute inflammation or exacerbation of inflammation, of the respiratory system.

In preferred embodiments, the concentration of NAC in said solution is between 3% and 20%, preferably between 5% and 15% more preferably between 8% and 12%, most preferably it is 10%. The percentage is based on weight/weight. Preferably, a solution containing NAC also contains sodium edetate (with edetate referring to

ethylendiamin tetra acetate) and/or sodium hydroxide in pharmaceutically acceptable concentrations. Preferably, the solution is an aqueous solution.

In some embodiments of the pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy, the pharmaceutical composition further comprises a mucolytic agent, such as N-acetylcysteine (NAC) and/or a hypertonic solution comprising sodium chloride. Both, NAC that acts as an expectorant and a hypertonic solution comprising sodium chloride are advantageous for reducing retention of viscous mucus in subjects suffering of PCD. This can reduce the risk of infections of the respiratory system, and thus, additional stress for the patient.
In preferred embodiments, the pharmaceutical composition further comprises NAC in a concentration as indicated above.
In preferred embodiments, the pharmaceutical composition further comprises a hypertonic solution comprising sodium chloride in a concentration between 2% and 8%, preferably between 3% and 7%, more preferably between 4% and 7%.
In preferred embodiments, the pharmaceutical composition further comprises NAC in a concentration between 3% and 20%, preferably between 5% and 15% more preferably between 8% and 12%, most preferably it is 10%, and/or a hypertonic solution comprising sodium chloride in a concentration between 2% and 8%, preferably between 3% and 7%, more preferably between 4% and 7%.

As stated above, in a preferred embodiment of the pharmaceutical composition according to the present invention said pharmaceutical composition comprises an mRNA that can be translated into a functional version of a protein selected from the group consisting of DNAH5, DNAH11, CCDC39, DNAI1, CCDC40, CCDC103, SPAG1, ZMYND10, ARMC4, CCDC151, DNAI2, RSPH1, CCDC114, RSPH4A, DNAAF1 (LRRC50), DNAAF2 (KTU), and LRRC6, preferably CCDC40 and/or CCDC39. Such a pharmaceutical composition is also referred to as "first pharmaceutical composition" in the following.

The multiciliate differentiation and DNA synthesis associated cell cycle (MCIDAS) protein is a transcriptional regulator protein that is specifically required for multiciliate cell differentiation which includes ciliogenesis of multiple motile cilia (cf. e.g. NCBI Reference Sequences NM_001190787.1 and NP_001177716.1 for human mRNA and protein MCIDAS sequence, respectively; or an optimized polyribonucleotide sequence as shown in **SEQ ID NO: 4**).
Hence, in some embodiments of the pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy, said pharmaceutical composition

further comprises a polyribonucleotide encoding a functional version of a multiciliate differentiation and DNA synthesis associated cell cycle (MCIDAS) protein.

In some embodiments, the pharmaceutical composition is a first pharmaceutical composition as described above that is administered together with a second pharmaceutical composition comprising a polyribonucleotide encoding an MCIDAS protein.

Both pharmaceutical compositions, i.e. a first pharmaceutical composition as defined above further comprising a polyribonucleotide encoding an MCIDAS protein or a first pharmaceutical composition that is administered together with a second pharmaceutical composition comprising a polyribonucleotide encoding an MCIDAS protein, are advantageous as the administration of a pharmaceutical composition according to the present invention is especially effective in restoring ciliary cell structure and function, when the pharmaceutical composition is administered to cells before and/or during ciliogenesis. Further, both are preferably administered to a patient by using a nasal spray, and/or a nebulizer, and/or by inhalation, preferably at least once a week and/or for at least 4 weeks.

In some embodiments, the pharmaceutical composition comprises a polyribonucleotide that can be translated into a functional version of two or more proteins. This can be done e.g. using a multicistronic polyribonucleotide that is a single polyribonucleotide encoding two or more different proteins. Designing such a multicistronic polyribonucleotide using e.g. 2A peptides is well described in the literature. As an example, Liu et al. demonstrated the use of 2A peptides to generate multicistronic mRNAs (Liu et al., 2017, Scientific Reports, 7:2193). These 2A peptides can further be combined e.g. with a furin cleavage site to remove the additional amino acids (part of 2A peptide) which get appended onto the first protein in sequence as a consequence of ribosome skipping (functional feature of 2A peptide). As an example, the use of furin cleavage with 2A peptides has been described e.g. by Chng et al. (Chng et al., 2015 MAbs, 7(2):403-412). Thus, a single polyribonucleotide can be designed which encodes for two or more, preferably two therapeutic proteins (e.g. CCDC40 and MCIDAS) where the coding regions of the two therapeutic proteins are separated by a 2A peptide (as described in the above citation).

Hence, in some embodiments, the same applies as described for the embodiments above except that the pharmaceutical composition comprises a polyribonucleotide that can be translated into functional versions of two or more, preferably two proteins. Thus, in some embodiments, the pharmaceutical composition as described in the embodiments above comprises a multicistronic polyribonucleotide comprising a sequence that can be translated into a functional version of a protein selected from

the group consisting of DNAH5, DNAH11, CCDC39, DNAI1, CCDC40, CCDC103, SPAG1, ZMYND10, ARMC4, CCDC151, DNAI2, RSPH1, CCDC114, RSPH4A, DNAAF1 (LRRC50), DNAAF2 (KTU), and LRRC6, preferably CCDC40 and/or CCDC39, and a sequence that can be translated into a functional version of a MCIDAS protein.

In some embodiments, the pharmaceutical composition comprises a polyribonucleotide that can be translated into a functional version of an MCIDAS protein and that comprises an optimized polyribonucleotide sequence as shown in **SEQ ID NO: 4**, and a polyribonucleotide that can be translated into a functional version of a CCDC40 and/or CCDC39 protein and that comprises an optimized polyribonucleotide sequence as shown in **SEQ ID NO: 1** (or **SEQ ID NO: 5 to 11**) **and/or SEQ ID NO: 2** (or **SEQ ID NO: 12 to 14**). Preferably, it is administered to a patient by using a nasal spray, and/or a nebulizer, and/or by inhalation, preferably at least once a week and/or for at least 4 weeks.

Alternatively or additionally to MCIDAS, GemC1, FoxJ1, and/or E2f4VP16 can be used for example. GemC1 is specifically expressed in ciliated epithelia and is a central regulator of ciliogenesis. It has been reported that ectopic expression of GemC1 was sufficient to induce early steps of multiciliogenesis in airway epithelial cells ex vivo by upregulating MCIDAS and FoxJ1, two key transcriptional regulators of multiciliogenesis (Arbi M et al., 2016, EMBO reports, 17(3):400-413). Moreover, it was reported in the same study that GemC1 can transactivate MCIDAS and FoxJ1 upstream regulatory sequences directly. E2f4VP16 refers to a form of E2f4 that contains a generic activation domain from HSV1 VP16 and can have a positive effect on the activation of the expression of key genes associated with multiciliogenesis (Kim S et al., Scientific Reports, 2018, 8:12369).

Hence, in some embodiments of the pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy, said pharmaceutical composition is a first pharmaceutical composition as described above and comprises further at least one of the following: a polyribonucleotide encoding a functional version of an MCIDAS protein, a polyribonucleotide encoding a functional version of a GemC1 protein, a polyribonucleotide encoding a functional version of a FoxJ1 protein, and/or a polyribonucleotide encoding a functional version of an E2f4VP16 protein.
In some embodiments of the pharmaceutical composition, a first pharmaceutical composition as described above can be administered together with a second pharmaceutical composition comprising at least one of the following: a polyribonucleotide encoding a functional version of an MCIDAS protein, a

polyribonucleotide encoding a functional version of a GemC1 protein, a polyribonucleotide encoding a functional version of a FoxJ1 protein, and/or a polyribonucleotide encoding a functional version of an E2f4VP16 protein.
Both pharmaceutical compositions, i.e. a first pharmaceutical composition as defined above further comprising a polyribonucleotide encoding an MCIDAS, GemC1, FoxJ1, and/or E2f4VP16 protein or a first pharmaceutical composition that is administered together with a second pharmaceutical composition comprising a polyribonucleotide encoding an MCIDAS, GemC1, FoxJ1, and/or E2f4VP16 protein, are advantageous as the administration of a pharmaceutical composition according to the present invention is especially effective in restoring ciliatary cell structure and function, when the pharmaceutical composition is administered to cells before the cells initiate ciliogenesis or while they are in ciliogenesis. Further, both are preferably administered to a patient by using a nasal spray, and/or a nebulizer, and/or by inhalation, preferably at least once a week and/or for at least 4 weeks.

As stated above, in some embodiments, the same applies as described for the embodiments above except that the pharmaceutical composition comprises a polyribonucleotide that can be translated into functional versions of two or more, preferably two proteins. Thus, in some embodiments, the pharmaceutical composition as described in the embodiments above comprises a multicistronic polyribonucleotide.

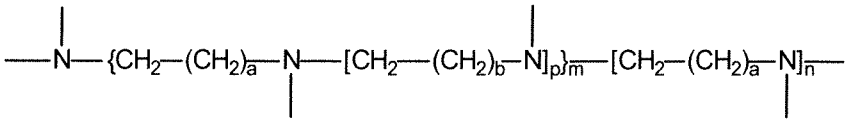
The pharmaceutical composition may comprise compounds which facilitate transfection of cells with polyribonucleotides. Examples of such compounds are those disclosed in WO 2014/20723.

The pharmaceutical composition can further comprise one or more agent(s) or one or more reagent(s) for delivering and/or introducing the RNA into a target cell or a target tissue. In particular, it is envisaged that this/these agent(s) or reagent(s) support(s) the delivering and/or introducing the RNA into the cell or tissue. This/these agent(s) or reagent(s) may be administered together with the RNA. The RNA to be delivered/introduced may also be coupled with (e.g. covalently bound to or complexed with) or uncoupled with (for example only admixed with) this/these agent(s(or reagent(s). Respective agents or reagents are known in the art (e.g. Tavernier, J Control Release 150(3) (2011), 238-47) and are, for example, selected from the group consisting of lipids and liposomes, micelles, polymers and dendrimers, among others. Particular examples of respective agents or reagents are GL67, EDMPC, DOTAP (l,2-dioleyl-3-trimethylammonium propane), DODAP (1,2-dioleyl- 3-dimethylammonium propane), DOTMA (l,2-di-0-octadecenyl-3-

trimethylammonium propane), XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[l,3]-dioxolane) and MC3 (((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate), ALNY- 100 ((3 aR,5s,6aS)-N,N-dimethyl-2,2- di((9Z, 12Z)-octadeca-9, 12-dienyl)tetrahydro-3aH-cyclopenta[d] [1 ,3]dioxol-5- amine)), NC98-5 (4,7, 13-tris(3-oxo-3-(undecylamino)propyl)-Nl,N16-diundecyl- 4,7, 10, 13-tetraazahexadecane-l, 16-diamide), C12-200, DLin-KC2 -DMA, DODAP, l,2-distearyloxy-N,N-dimethyl-3- aminopropane or "DSDMA", l,2-dioleyloxy-N,N-dimethyl-3-aminopropane or "DODMA", l,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane or "DLinDMA", 1,2- dilinolenyloxy-N,N-dimethyl-3-aminopropane or "DLenDMA", N-dioleyl-N,N- dimethylammonium chloride or "DODAC", N,N-distearyl-N,N-dimethylammonium bromide or "DDAB", N-(l,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide or "DMRIE", 3-dimethylamino-2-(cholest-5-en-3-beta- oxybutan-4-oxy)-l-(ci s,cis-9, 12-octadecadienoxy)propane or "CLinDMA", 2-[5'- (cholest-5-en-3-beta-oxy)-3'-oxapentoxy)-3-dimethy l-l-(cis,cis-9', 1-2'- octadecadienoxy)propane or "CpLinDMA", N,N-dimethyl-3,4-dioleyloxybenzylamine or "DMOBA", l,2-N,N'-dioleylcarbamyl-3-dimethylaminopropane or "DOcarbDAP", 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine or "DLinDAP", l,2-N,N'-Dilinoleylcarbamyl-3-dimethylaminopropane or "DLincarbDAP", l,2-Dilinoleoylcarbamyl-3-dimethylaminopropane or "DLinCDAP", 2,2-dilinoleyl-4-dimethylaminomethyl- [l,3]-dioxolane or "DLin-K-DMA", 2,2-dilinoleyl-4-dimethylaminoethyl-[l,3]- dioxolane or "DLin-K-XTC2-DMA", or mixtures thereof (Heyes, J Controlled Release 107 (2005), 276-287; Morrissey, Nat. Biotechnol. 23(8) (2005), 1003-1007; WO2005/121348). Further examples are DC- Chol (N,N-dimethyl-N-ethylcarboxamidocholesterol), l,4-bis(3-N-oleylamino- propyl)piperazine (Gao, Biochem. Biophys. Res. Comm. 179 (1991), 280; Wolf, et al BioTechniques 23 (1997), 139; U.S. Pat. No. 5,744,335). Further examples are LIPOFECTIN (DOTMA:DOPE) (Invitrogen, Carlsbad, Calif), LIPOFECTAMINE (DOSPA:DOPE) (Invitrogen), LIPOFECTAMINE2000. (Invitrogen), FUGENE, TRANSFECTAM (DOGS), and EFFECTENE. Further examples are modified and unmodified polyacrylates, polyalkycyanoacrylates, polylactide, polylactide-polyglycolide copolymers, polycaprolactones, dextran, albumin, gelatin, alginate, collagen, chitosan, cyclodextrins, polylysin, polyarginine, oligo/polyamines and polyethylenimine.

The agents or reagents may be oligomers, polymers or lipidoids. They may comprise oligo(alkylene amine) moieties like, for example, the characteristic oligo(alkylene amine) moieties as described in PCT/EP2014/063756. In particular, the agents or reagents may be the oligomers, polymers or lipidoids as described in PCT/EP2014/063756. One main characteristic of these particular agents or reagents

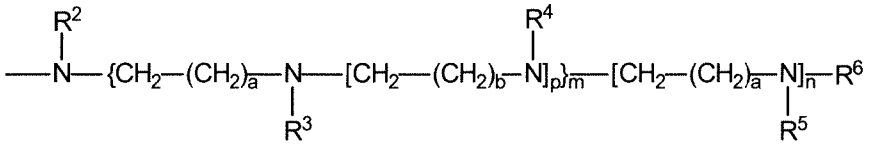
is that they contain a following common structural entity of formula (I):



(I)

Such agents or reagents may be (a component comprising) an oligo(alkylene amine) selected from:

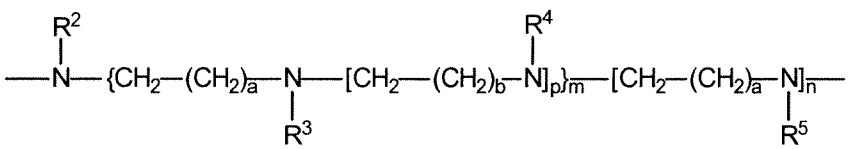
a) an oligomer or polymer comprising a plurality of groups of formula (II) as a side chain and/or as a terminal group:



(II)

wherein the variables a, b, p, m, n and R^2 to R^6 are defined as follows, independently for each group of formula (II) in a plurality of such groups:
a is 1 and b is an integer of 2 to 4; or a is an integer of 2 to 4 and b is 1,
p is 1 or 2,
m is 1 or 2; n is 0 or 1 and m+n is ≥ 2; and
R^2 to R^5 are, independently of each other, selected from hydrogen; a group $-CH_2-CH(OH)-R^7$, $-CH(R^7)-CH_2-OH$, $-CH_2-CH_2-(C=O)-O-R^7$, $-CH_2-CH_2-(C=O)-NH-R^7$ or $-CH_2-R^7$ wherein R^7 is selected from C3-C18 alkyl or C3-C18 alkenyl having one C-C double bond; a protecting group for an amino group; and a poly(ethylene glycol) chain;
R^6 is selected from hydrogen; a group $-CH_2-CH(OH)-R^7$, $-CH(R^7)-CH_2-OH$, $-CH_2-CH_2-(C=O)-O-R^7$, $-CH_2-CH_2-(C=O)-NH-R^7$ or $-CH_2-R^7$ wherein R^7 is selected from C3-C18 alkyl or C3-C18 alkenyl having one C-C double bond; a protecting group for an amino group; $-C(NH)-NH_2$; a poly(ethylene glycol) chain; and a receptor ligand,
and wherein one or more of the nitrogen atoms indicated in formula (II) may be protonated to provide a cationic group of formula (II);

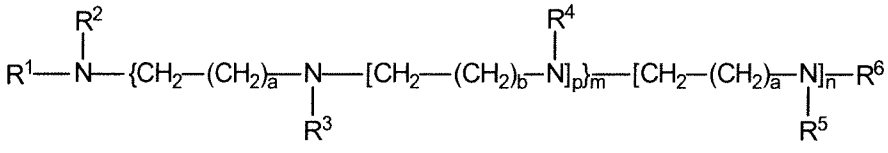
b) an oligomer or polymer comprising a plurality of groups of formula (III) as repeating units:



(III)

wherein the variables a, b, p, m, n and R^2 to R^5 are defined as follows, independently for each group of formula (III) in a plurality of such groups:
a is 1 and b is an integer of 2 to 4; or a is an integer of 2 to 4 and b is 1,
p is 1 or 2,
m is 1 or 2; n is 0 or 1 and m+n is ≥ 2; and
R^2 to R^5 are, independently of each other, selected from hydrogen; a group $-CH_2-CH(OH)-R^7$, $-CH(R^7)-CH_2-OH$, $-CH_2-CH_2-(C=O)-O-R^7$ or $-CH_2-CH_2-(C=O)-NH-R^7$ or $-CH_2-R^7$ wherein R^7 is selected from C3-C18 alkyl or C3-C18 alkenyl having one C-C double bond; a protecting group for an amino group; $-C(NH)-NH_2$; and a poly(ethylene glycol) chain;
and wherein one or more of the nitrogen atoms indicated in formula (III) may be protonated to provide a cationic group of formula (III); and

c) a lipidoid having the structure of formula (IV):



(IV)

wherein the variables a, b, p, m, n and R^1 to R^6 are defined as follows:
a is 1 and b is an integer of 2 to 4; or a is an integer of 2 to 4 and b is 1,
p is 1 or 2,
m is 1 or 2; n is 0 or 1 and m+n is ≥ 2; and
R^1 to R^6 are independently of each other selected from hydrogen; a group $-CH_2-CH(OH)-R^7$, $-CH(R^7)-CH_2-OH$, $-CH_2-CH_2-(C=O)-O-R^7$, $-CH_2-CH_2-(C=O)-NH-R^7$ or $-CH_2-R^7$ wherein R^7 is selected from C3-C18 alkyl or C3-C18 alkenyl having one C-C double bond; a protecting group for an amino group; $-C(NH)-NH_2$; a poly(ethylene glycol) chain; and a receptor ligand; provided that at least two residues among R^1 to R^6 are a group $-CH_2-CH(OH)-R^7$, $-CH(R^7)-CH_2-OH$, $-CH_2-CH_2-(C=O)-O-R^7$, $-CH_2-CH_2-(C=O)-NH-R^7$ or $-CH_2-R^7$ wherein R^7 is selected from C3-C18 alkyl or C3-C18 alkenyl having one C-C double bond;
and wherein one or more of the nitrogen atoms indicated in formula (IV) may be protonated to provide a cationic lipidoid of formula (IV).

Preferably, such agents or reagents may be (a component comprising) an oligo(alkylene amine) selected from a) and b), wherein

a) is an oligomer or polymer comprising a plurality of groups of formula (IIa) as a side chain and/or as a terminal group:

$$-NR^2\{CH_2-(CH_2)_a-NR^3-CH_2-(CH_2)_b-NR^4\}_m-[CH_2-(CH_2)_a-NR^5]_n-R^6 \qquad \text{(IIa)},$$

wherein a, b, m, n, and R^2 to R^6 are defined as described above, and wherein one or more of the nitrogen atoms indicated in formula (IIa) may be protonated to provide a cationic oligomer or polymer structure; and

b) is an oligomer or polymer comprising a plurality of groups of formula (IIIa) as repeating units:

$$-NR^2\{CH_2-(CH_2)_a-NR^3-CH_2-(CH_2)_b-NR^4\}_m-[CH_2-(CH_2)_a-NR^5]_n- \quad \text{(IIIa)},$$

wherein a, b, m, n, and R^2 to R^5 are defined as described above, and wherein one or more of the nitrogen atoms indicated in formula (IIIa) may be protonated to provide a cationic oligomer or polymer structure.

Furthermore, such agents or reagents may be (a component comprising) an oligo(alkylene amine) selected from a lipidoid having the structure of formula (IVa):

$$R^1-NR^2\{CH_2-(CH_2)_a-NR^3-CH_2-(CH_2)_b-NR^4\}_m-[CH_2-(CH_2)_a-NR^5]_n-R^6 \quad \text{(IVa)},$$

wherein a, b, m, n, and R^1 to R^6 are defined as described above, and wherein one or more of the nitrogen atoms indicated in formula (IVa) may be protonated to provide a cationic lipidoid.

As to such agents or reagents, in formula (II), (IIa), (III), (IIIa), (IV) or (IVa) n may be 1; or m may be 1 and n may be 1.

Further, as to such agents or reagents, in formula (II), (IIa), (III), (IIIa), (IV) or (IVa) a may be 1 and b may be 2; or a may be 2 and b may be 1.

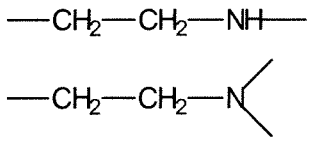
In some embodiments, the oligomer, polymer or lipidoid may be a cationic (e.g. protonated) oligomer, polymer or lipidoid.

One non-limiting example of such an oligomer, polymer or lipidoid to be employed is a cationic lipid which was prepared by mixing 100mg N,N′-Bis(2-aminoethyl)-1,3-propanediamine (0.623mmol) with 575.07mg 1,2-Epoxydodecane (3.12mmol, (N-1) eq. where N is 2x amount of primary amine plus 1x amount secondary amine per oligo(alkylene amine)) and mixed for 96h at 80°C under constant shaking. Such an oligomer, polymer or lipidoid is also referred to as lipidoid “C12-(2-3-2)”.

An agent or reagent, in particular a polymer, to be employed may be a copolymer, in particular a statistical copolymer. Such a copolymer may be a copolymer which contains a statistical/random arrangement of alkylene amine repeating units of alternating length (e.g. in contrast to a less preferred polymer which contains analogous arrangements of alkylene amine repeating units of non-alternating length).

The copolymer may be a cationic (e.g. protonated) copolymer. Copolymers to be employed are known in the art and are, for example, described in EP 14 19 9439.2, WO 01/00708, EP-A1 1 198 489 and CA-A1 2,377,207.

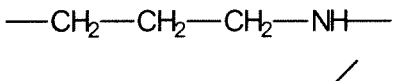
In particular, the copolymer may be a statistical copolymer comprising a plurality of repeating units (a) independently selected from repeating units of the following formulae (a1) and (a2):



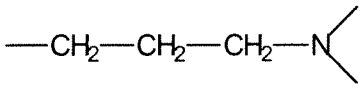
(a1)

(a2), and

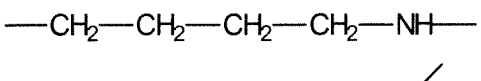
a plurality of repeating units (b) independently selected from repeating units of the following formulae (b1) to (b4):



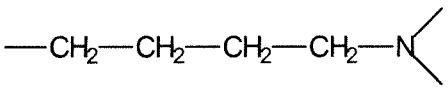
(b1)



(b2)



(b3)



(b4)

wherein the molar ratio of the sum of the repeating units (a) to the sum of the repeating units (b) lies within the range of 0.7/1.0 to 1.0/0.7, and

wherein one or more of the nitrogen atoms of the repeating units (a) and/or (b) contained in the copolymer may be protonated to provide a cationic copolymer.

The copolymer may be a statistical copolymer, wherein any repeating units (a) and any repeating units (b) are statistically distributed in the copolymer macromolecule. It is typically obtained from the copolymerization of a mixture of monomers yielding, during the polymerization reaction, the repeating units (a) with monomers yielding, during the polymerization reaction, the repeating units (b). Preferably, the copolymer is a random copolymer wherein any repeating units (a) and any repeating units (b) are randomly distributed in the polymer macromolecule.

Such a copolymer can be a linear, branched or dendritic copolymer. As will be understood by the skilled reader, a repeating unit of the formula (a1), (b1) or (b3) with two valencies (i.e. open bonds to neighboring units) leads to a propagation of the

copolymer structure in a linear manner. Thus, a linear copolymer may comprise repeating units of formula (a1) and one or more types of the repeating units of formulae (b1) and (b3), but no repeating units of formula (a2), (b2) or (b4). As will be further understood, the presence of a repeating unit of formula (a2), (b2) or (b4) with three valencies provides a branching point in the copolymer structure. Thus, a branched copolymer comprises one or more types of the repeating units of formulae (a2), (b2) and (b4), and may further comprise one or more types of the repeating units of formulae (a1), (b1) and (b3).

Such a copolymer may comprise a plurality of repeating units (a) independently selected from repeating units of formulae (a1) and (a2) defined above, and a plurality of repeating units (b) independently selected from repeating units of formulae (b1) to (b4) defined above. Preferred are copolymers comprising a plurality of repeating units (a) independently selected from repeating units of formulae (a1) and (a2) defined above, and a plurality of repeating units (b) independently selected from repeating units of formulae (b1) and (b2) defined above.

Preferbaly, such a copolymer is a branched copolymer comprising one or more types of repeating units selected from repeating units (a2), (b2) and (b4), and which optionally further comprises one or more types of the repeating units of formulae (a1), (b1) and (b3), and in particular a copolymer which comprises repeating units of the formula (a2) and one or more type of the repeating units of formulae (b2) and (b4), and which optionally further comprises one or more types of the repeating units of formulae (a1), (b1) and (b3). In line with the above, a more preferred copolymer is thus a branched copolymer which comprises repeating units of the formula (a2) and repeating units of formula (b2), and which optionally further comprises one or more types of the repeating units of formulae (a1) and (b1).

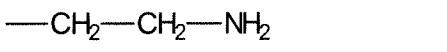
In the copolymers, the total number of the repeating units (a) and repeating units (b) is typically 20 or more, preferably 50 or more and more preferably 100 or more. Typically, the total number of the repeating units (a) and repeating units (b) is 10,000 or less, preferably 5,000 or less, more preferably 1,000 or less.

Furthermore, it is preferred for the copolymers that the repeating units (a) and (b) account for 80 mol% or more, more preferably 90 mol% or more of all repeating units in the copolymer. Further preferred are copolymers wherein repeating units (a) selected from (a1) and (a2) and repeating units (b) selected from (b1) and (b2) account for 80 mol% or more, more preferably 90 mol% or more of all repeating units in the copolymer. It is most preferred that all of the repeating units in the copolymer

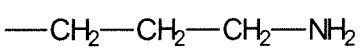
are repeating units (a) or (b), in particular that all of the repeating units in the copolymer are repeating units (a) selected from (a1) and (a2) or repeating units (b) selected from (b1) and (b2).

The weight average molecular weight of the copolymer, as measured e.g. via size exclusion chromatography relative to linear poly(ethylene oxide) standards, generally ranges from 1,000 to 500,000 Da, preferably from 2,500 to 250,000 Da and more preferably 5,000-50,000 less.

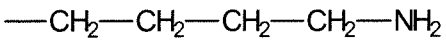
The terminal groups of such a copolymer typically comprise one or more types of groups (c) independently selected from groups of the formulae (c1) to (c3) below, preferably from groups of the formulae (c1) and (c2) below:



(c1)



(c2)



(c3).

Preferably, the terminal groups in the copolymer consist of one or more types of groups (c) independently selected from groups of the formulae (c1) to (c3) below, preferably from groups of the formulae (c1) and (c2). As will be understood by the skilled person, the number of terminal groups depends on the structure of the copolymer. While a linear copolymer has only two terminals, larger numbers of terminal groups are contained in a branched, in particular in a dendritic copolymer. As will be further understood, also one or more of the nitrogen atoms of the terminal groups (c) contained in the copolymer may be protonated to provide a cationic copolymer.

In the copolymer, the molar ratio of the sum of the repeating units (a) to the sum of the repeating units (b) lies within the range of 0.7/1.0 to 1.0/0.7, and preferably within the range of 0.8/1.0 to 1.0/0.8. This molar ratio can be determined, e.g., via NMR. It will thus be understood that the ratio is usually determined for a plurality of macromolecules of the copolymer, and typically indicates the overall ratio of the sum of repeating units (a) to the sum of repeating units (b) in the plurality of macromolecules.

As indicated above, one or more of the nitrogen atoms of the copolymer may be protonated to result in a copolymer in a cationic form, typically an oligocationic or

polycationic form. It will be understood that the primary, secondary, or tertiary amino groups in the repeating units (a) or (b) or in the terminal groups (c) can act as proton acceptors, especially in water and aqueous solutions, including physiological fluids. Thus, such copolymers typically have an overall positive charge in an aqueous solution at a pH of below 7.5. An aqueous solution, as referred to herein, is a solution wherein the solvent comprises 50 % (vol./vol.) or more, preferably 80 or 90 % or more, and most preferably 100 % of water. Also, if the compositions are in contact with a physiological fluid having a pH of below 7.5, including e.g. blood and lung fluid, they typically contain repeating units (a) and (b) wherein the nitrogen atoms are protonated. The pK_a values of the copolymers used in the compositions can be determined by acid-base titration using an automated pK_a titrator. The net charge at a given pH value can then be calculated e.g. from the Henderson–Hasselbach equation. Any charge may be shared across several of the basic centres and cannot necessarily be attributed to a single point. Typically, in solutions at physiological pH, the copolymers used in the compositions comprise repeating units with amino groups in protonated state and repeating units with amino groups in unprotonated state.

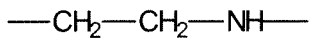
However, as will be understood by the skilled reader, the copolymers as well as the compositions may also be provided as a dry salt form which contains the copolymer in a cationic form.

As will be further understood, counterions (anions) for the positive charges of protonated amino groups in compositions comprising the copolymer and nucleic acid, in particular RNA, preferably single-stranded RNA such as mRNA, are typically provided by anionic moieties contained in the nucleic acid. If the positively charged groups are present in excess compared to the anionic moieties in the nucleic acid, positive charges may be balanced by other anions, in particular those typically encountered in physiological fluids, such as Cl^- or HCO_3^-.

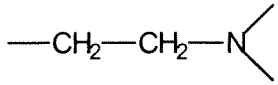
In line with the above, a preferred copolymer is a random copolymer, wherein

80 mol% or more of all repeating units, more preferably all repeating units, are formed by

a plurality of repeating units (a) independently selected from repeating units of the following formulae (a1) and (a2):

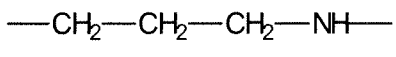


(a1)

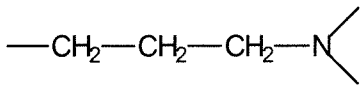


(a2), and

a plurality of repeating units (b) independently selected from repeating units of the following formulae (b1) and (b2):



(b1)

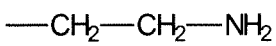


(b2),

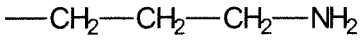
wherein the molar ratio of the sum of the repeating units (a) to the sum of the repeating units (b) lies within the range of 0.7/1.0 to 1.0/0.7, more preferably within the range of 0.8/1.0 to 1.0/0.8;

wherein the terminal groups of the copolymer are formed by

groups (c) independently selected from groups of the formulae (c1) and (c2):



(c1)



(c2); and

wherein one or more of the nitrogen atoms of the repeating units (a) and/or (b) and/or of the terminal groups (c) contained in the copolymer may be protonated to provide a cationic copolymer. It is further preferred that the copolymer is a branched copolymer, comprising units (a2) and (b2), optionally together with units (a1) and/or (b1).

The copolymers can be conveniently prepared with procedures analogous to those known for the preparation of polyalkyleneimines, such as branched or linear polyethyleneimine (PEI). It will be understood that the monomers used for the production of the copolymers will have to be adjusted accordingly. Herein, it has been found that the monomers can be conveniently reacted in a quantitative manner, such that the ratio of the units (a) and (b) in the copolymer can be adjusted by adjusting the monomer ratio accordingly in the monomer mixture subjected to polymerization. While polyethyleneimine can be prepared e.g. via ring-opening polymerization of aziridine, the copolymers can be prepared via ring opening polymerization of a monomer mixture comprising or consisting of aziridine, azetidine and, where applicable pyrrolidine, or, in preferred embodiments, of aziridine and azetidine. It will be understood that the expression “where applicable” refers to the presence or absence of repeating units (b3) and (b4) or terminal groups (c3) which would be formed by the pyrrolidine. The ring opening polymerization of the non-substituted cyclic amines usually leads to branched copolymers. Linear copolymers can be prepared, e.g., via polymerization of suitable N-substituted aziridines, N-substituted azetidines and N-substituted pyrrolidines, or N-substituted aziridines and N-substituted azetidines, which may be followed e.g. by a hydrolytic cleavage of N-

substituents attached to the resulting polyalkyleneimine chain, e.g. in analogy to the procedure published in Katrien F. Weyts, Eric J. Goethals, New synthesis of linear polyethyleneimine, Polymer Bulletin, 1988, 19(1):13-19. Dendrimers can be synthesized e.g. according to the method described in Yemul et al, Colloid and Polymer Science, 2008, 286(6-7):747-752, Synthesis and characterization of poly(ethylenimine) denrimers.

For the preparation of a dendrimer (or dendritic copolymer), synthetic strategies can be analogously applied which are known for the production of polyethyleneimine or polypropyleneamine dendrimers. Polypropylenimine dendrimers can be synthesized from acrylonitrile building blocks using a repetitive sequence of a Michael addition to a primary amine, followed by a heterogeneously catalyzed hydrogenation (Newkome and Shreiner Poly(amidoamine), polypropylenimine, and related dendrimers and dendrons possessing different 1→2 branching motifs: An overview of the divergent procedures. Polymer 49 (2008) 1-173; De Brabander-Van Den Berg et al. Large-scale production of polypropylenimine dendrimers, Macromolecular Symposia (1994) 77 (1) 51–62). Polyethylenimine dendrimers can be produced using a repetitive sequence of a Michael addition of a vinyl bromide building block to a primary amine followed by a conversion of alkylbromide to amine using a Gabriel amine synthesis method (Yemul & Imae, Synthesis and characterization of poly(ethyleneimine) dendrimers, Colloid Polym Sci (2008) 286:747–752). Hence the person skilled in the art will be able to produce not only dendrimers with strictly alternating layers of e.g. propylenimine and ethylenimine can be produced. Similarly dendrimer generations with layers comprising or consisting of random compositions of repeating units of formula (a2), (b2) and (b4) and preferably repeating units (a2) and (b2) can be generated.
The ring opening polymerization of aziridine and azetidine, or of aziridine, azetidine and pyrrolidine, can be carried out in solution, e.g. in water. The total monomer concentration is not particularly limited, typical concentrations range from 10% wt/wt to 80% wt/wt, preferably 30% wt/wt to 60% wt/wt. Typically, the polymerization is initiated by protons, such that it is preferred to add a Brønsted acid, in particular a mineral acid such as sulphuric acid to the reaction system. Small amounts of acid are generally sufficient, such as 0.001 to 0.01 equivalents, based on the total concentration of monomers. The reaction proceeds at convenient rates e.g. in the temperature range of 50 to 150°C, in particular 90 to 140°C. In these ranges, higher molecular weight copolymers are usually at higher temperatures, and lower molecular weight copolymers at lower temperatures.

In principle, a lipidoid is a preferred agent or reagent to be employed, in particular as

compared to an oligomer and, more particular particular, to a polymer.

Further examples of the one or more agent(s) or one or more reagent(s) for delivering and/or introducing the RNA into a target cell or a target tissue are the liposomal transfection reagents (LTR'S) and magnetic particles (MPs) as described herein elsewhere.

One particular mode for delivering and/or introducing the RNA into target cells or target tissue is transfection. Hence, the RNA to be employed can be envisaged to be transfected (into (target) cells or tissue), to be delivered/administered via transfection and/or to be prepared for transfection. Means and methods for transfecting RNA are well known in the art and are, for example, described in Tavernier (loc. cit.), Yamamoto (Eur J Pharm Biopharm. 71(3) (2009), 484-9) and Kormann (Nat Biotechnol. 29(2) (2011), 154-7). Particular modes of transfection are lipofection, magnetofection or magnetolipofection.

Hence, the RNA to be employed may be prepared for lipofection, prepared to be transfected by lipofection, delivered/introduced via lipofection and/or administered via lipofection.

Thus, the pharmaceutical composition may (further) comprise at least one lipid or liposomal transfection reagent or enhancer (LTR; liposomal transfection reagent). The RNA to be employed may be comprised in, complexed with and/or delivered by the LTR. In particular, the RNA to be employed may be comprised in and/or delivered by (respective) lipofection complexes comprising the RNA and the LTR. The pharmaceutical composition may (further) comprise the lipofection complexes.

LTRs are known in the art and are, for example, distributed by OzBiosciences, Marseille, France. LTRs to be employed may be selected from the group consisting of the above-described agents or reagents for delivering and/or introducing the RNA into a target cell or a target tissue. For example, such LTRs may be lipids or lipidoids, preferably cationic lipids or cationic lipidoids, like the lipidoids as disclosed in PCT/EP2014/063756 (e.g. C12-(2-3-2), the lipids as disclosed in EP2285772 (e.g. Dogtor) and the lipopolyamines as disclosed in EP1003711 (e. g. DreamFect™ and DreamFect Gold™). A particular LTR may be selected from the group consisting of

(i) C12-(2-3-2);
(ii) DreamFect™, preferably DreamFect Gold™ (DF™/DF-Gold™; OzBiosciences, Marseille, France);
(iii) Dogtor (OzBiosciences, Marseille, France); and

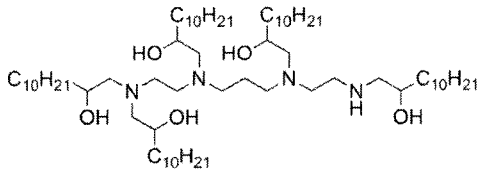
(iv) Lipofectamine like, for example, Lipofectamine 2000 (Invitrogene, CA, USA).

In principle, Dogtor is a preferred, DreamFect™ is a more preferred and DF-Gold™ and C12-(2-3-2) are even more preferred LTR(s).
LTRs like Dogtor are, for example, described in EP2285772. LTRs like DF™ or DF-Gold™ are, for example, described in EP1003711. In principle, the oligomers, polymers or lipidoids as disclosed in PCT/EP2014/063756, the particular cationic lipids as disclosed in EP2285772 and the particular lipopolyamines as disclosed in EP1003711 are preferred LTRs. LTRs like C12-(2-3-2) and DF-Gold™ are most preferred.

Non-limiting examples of lipofection complexes are DF-Gold™/RNA lipoplexes and C12-(2-3-2)/RNA lipoplexes.

C12-(2-3-2) is a particularly preferred LTR having the structure shown in formula (V) (cf. Jarzębińska et al., Angew Chem Int Ed Engl., 2016; 55(33):9591-5):

C12-(2-3-2)

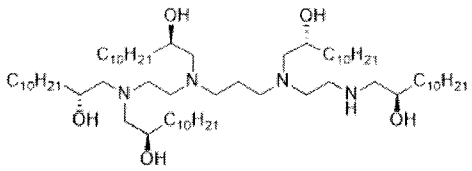


formula (V)

C12-(2-3-2) is preferably prepared as described e.g. in WO 2016/075154 A1, EP 3013964, and Jarzębińska et al. (Angew Chem Int Ed Engl., 2016;55(33):9591-5). The cationic lipidoid can be prepared by mixing N1-(2-aminoethyl)-N3-(2-((3,4-dimethoxybenzyl)amino)ethyl)propane-1,3-diamine (8.9 g, 1 eq., 28.67 mmol) with 1,2-Epoxydodecane (42.27, 8 eq., 229.4 mmol) and mixed for 24 h at 80 °C under constant shaking followed by purification and removal of the 3,4-dimethoxybenzyl protection group.

Different isomers, of C12-(2-3-2) can be used such as a racemate, an S-isomer, and/or an R-isomer. Preferably, C12-(2-3-2) is used as pure R-isomer and has the structure shown in formula (VI). For obtaining pure R-isomers of C12-(2-3-2) it can be prepared as described above for C12-(2-3-2) using the R-isomer of 1,2-Epxoydodecane for synthesis.

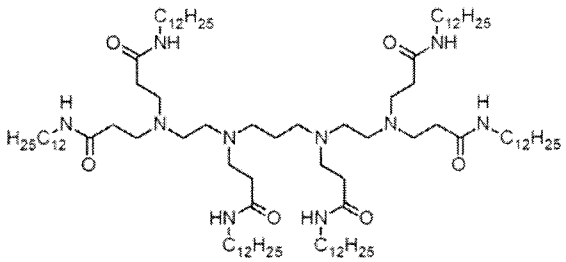
formula (VI)



Hence, in preferred embodiments the pharmaceutical composition comprises a polyribonucleotide for use in treating a ciliopathy and further comprises a lipidoid having the structure shown in formula (V), preferably as shown in formula (VI).

A further particularly preferred LTR is a cationic lipidoid having formula (VII), herein also referred to as "dL_P" which can be synthesized via reaction of N,N'-Bis(2-aminoethyl)-1,3-propanediamine with N-Dodecylacrylamide using boric acid as catalyst. For the reaction, the mixture can be stirred at 100 °C under microwave irradiations.

Hence, in further preferred embodiments the pharmaceutical composition comprises a polyribonucleotide for use in treating a ciliopathy and further comprises a lipidoid having having the structure shown in formula (VII).



formula (VII)

Furthermore, the pharmaceutical composition comprises a cationic lipidoid having formula (V), (VI) and/or (VII), preferably dL_P and/or C12-(2-3-2), more preferably dL_P and/or the R-isomer of C12-(2-3-2), comprised in a formulation as described in the following. In particular, the herein described agents and reagents for delivering and/or introducing the RNA into a target cell or a target tissue and the herein described LTRs may be combined with one or more (e.g. two, three or four) further lipid(s) (like, for example, cholesterol, DPPC, DOPE and/or PEG-lipids (e.g. DMPE-PEG, DMG-PEG2000)). These further lipids may support the desired function of the agents/reagents and LTRs (support and/or increase the delivering and/or introducing of RNA into the cell or tissue and improve transfection efficiency, respectively) and function as respective "helper lipids". Particular examples of such "helper lipids" are cholesterol, DPPC, DOPE and/or PEG-lipids (e.g. DMPE-PEG, DMG-PEG (e.g.

DMG-PEG2000). The further lipids (e.g. "helper lipids") may also be part(s) of the herein disclosed complexes/particles. The skilled person is readily in the position to prepare complexes/particles in accordance with the invention. Examples of further lipids (e.g. "helper lipids") are also known in the art. The skilled person is readily in the position to choose suitable further lipids (e.g. "helper lipids") and ratios of the agents/reagents/LTRs and the further lipids (e.g. "helper lipids"). Such ratios may be molar ratios of 1-4 : 1-5, 3-4 : 4-6, about 4 : about 5, about 4 : about 5.3 of agents/reagents/ LTRs : further lipid(s) (the more narrow ranges are preferred). For example, the agents/reagents/LTRs may be combined with three further lipids, like DPPC, cholesterol, and DMG-PEG2000, at a molar ratio of 8 : 5.3 : 4.4 : 0.9, respectively, or, more particular, 8 : 5.29 : 4.41 : 0.88, respectively.

Preferably, dL_P and/or C12-(2-3-2), more preferably dL_P and/or the R-isomer of C12-(2-3-2), is generated as described above and used with helper lipids DPPC and cholesterol and PEG-lipid DMG-PEG2000 at the molar ratios 8:5.29:4.41:0.88 for formulating lipoid particles.
A composition in which the R-isomer of C12-(2-3-2) (formula VI) is formulated with the lipids DPPC and cholesterol and PEG-lipid DMG-PEG2000 at the molar ratios 8:5.29:4.41:0.88 is also referred herein as "LF92". A composition in which the dL_P (formula VII) is formulated with the lipids DPPC and cholesterol and PEG-lipid DMG-PEG2000 at the molar ratios 8:5.29:4.41:0.88 is also referred herein as "LF111".
As also exemplarily described e.g. in WO 2016/075154 A1, EP 3013964, and Zhang et al. (TERMIS, 2019, Tissue Engineering: Part A, Vol. 25, Numbers 1 and 2), dL_P and/or C12-(2-3-2) can be used as a non-viral vector, to make a stable lipoplex with mRNA molecules, based on electrostatic interaction between the positive amino groups of lipidoid and negative phosphate groups of mRNA molecules (Anderson, Human Gene Therapy 14, 2003, 191-202). To stabilized the lipoplex structure and reduce the leakage, dL_P and/or C12-(2-3-2), more preferably dL_P and/or the R-isomer of C12-(2-3-2), can be supplied with two helper lipids entitled 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol (Anderson, Drug Delivery 11, 2004, 33-39; Liang, Journal of Colliod and Interface Science 278, 2004, 53-62). At the end, 1,2-Dimyristoyl-*sn*-glycerol, methoxypolyethylene Glycol (DMG-PEG) 2kD (DMG-PEG2000) is to be added to the lipid mix to provide a PEGylated liposome. It is already well known that PEGylation improves the physico-chemical characteristic of liposome formulation by increasing water solubility, protecting from enzymatic degradation, and limiting immunogenic and antigenic reactions (Milla, Current Drug Metabolism 13, 2012, 105-119). Final N/P ratios for entire ethanoic lipid mixture are to be 8 : 5.29 : 4.41 : 0.88 standing for molar ratios of amino group of dL_P and/or C12-(2-3-2) / DPPC / cholesterol / DMG-PEG2000, respectively, to one phosphate

group of mRNA molecule.

Hence, in preferred embodiments the pharmaceutical composition comprises a polyribonucleotide for use in treating a ciliopathy further comprises dL_P and/or C12-(2-3-2), preferably dL_P and/or the R-isomer of C12-(2-3-2), formulated with DPPC, cholesterol, and DMG-PEG2000.
Moreover, in particularly preferred embodiments the pharmaceutical composition comprises a polyribonucleotide for use in treating a ciliopathy further comprises dL_P and/or C12-(2-3-2), preferably dL_P and/or the R-isomer of C12-(2-3-2), formulated with DPPC, cholesterol, and DMG-PEG2000 with final N/P ratios for entire ethanoic lipid mixture of 8 : 5.29 : 4.41 : 0.88 for molar ratios of amino group of dL_P and/or C12-(2-3-2) / DPPC / cholesterol / DMG-PEG2000, respectively, to one phosphate group of mRNA molecule.

R-isomers of C12-(2-3-2) formulated with DPPC, cholesterol, and DMG-PEG2000 as stated above are also referred to as LF92 formulation.
Hence, in particularly preferred embodiments of the pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy, said pharmaceutical composition further comprises an LF92 formulation.
dL_P formulated with DPPC, cholesterol, and DMG-PEG2000 as stated above are also referred to as LF111 formulation.
Hence, in particularly preferred embodiments of the pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy, said pharmaceutical composition further comprises an LF111 formulation.

In a preferred embodiment of the pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy, said pharmaceutical composition further comprises an LF92 and/or LF111 formulation. LF92 and/or LF111 refers to a carrier formulation that is used for cell transfection. This is advantageous as the use of LF92 and/or LF111 is associated with high transfection efficiencies and thus, ensures that the polyribonucleotide encoding a functional version of a protein a defect of which is associated with the ciliopathy can efficiently enter a cell, preferably an undifferentiated ciliary cell or basal cell, and thus restore ciliated cell function in the subject suffering of PCD.

In a particularly preferred embodiment, the pharmaceutical composition comprises an LF92 and/or LF111 formulation, at least one of the following: an mRNA that can be translated into a functional version of a MCIDAS protein, an mRNA that can be translated into a functional version of a GemC1 protein, an mRNA that can be

translated into a functional version of a FoxJ1 protein, an mRNA that can be translated into a functional version of a E2f4VP16 protein; and an mRNA that can be translated into a functional version of CCDC40 and/or CCDC39. Preferably, such a composition is administered to a patient by using a spray, droplets and/or a nebulizer, and/or by inhalation, preferably at least once a week and/or for at least 4 weeks.
Hence, in some embodiments, the same applies as described for the embodiments above except that the pharmaceutical composition comprises a polyribonucleotide that can be translated into functional versions of two or more, preferably two proteins. Thus, in some embodiments, the pharmaceutical composition as described in the embodiments above comprises a multicistronic polyribonucleotide.

The present invention also relates to a method of treating a ciliopathy in a subject suffering of a ciliopathy, comprising the administration of a pharmaceutical composition comprising a polyribonucleotide wherein the polyribonucleotide encodes a functional version of a protein a defect of which is associated with said ciliopathy, and wherein said pharmaceutical composition is administered to the respiratory system of said subject and is effected when the subject shows an inflammation of the respiratory system. As regards the possible embodiments and preferred embodiments of the pharmaceutical composition and its ways and time of administration, the same applies as has been set forth herein above in connection with the pharmaceutical composition according to the present invention.

The present invention also relates to a pharmaceutical composition comprising a polyribonucleotide encoding a protein a defect of which is associated with a ciliopathy and N-acetylcysteine (NAC), a hypertonic solution comprising sodium chloride, and/or an LF92 and/or LF111 formulation.
As regards the pharmaceutical composition and its components, the same applies as described above in connection with the pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy in a subject suffering of a ciliopathy. Moreover, also the other features of such a pharmaceutical composition can be as described above.
Hence, in a preferred embodiment, the pharmaceutical composition can further comprise an mRNA that can be translated into a functional version of MCIDAS.

The present disclosure also relates to a polyribonucleotide encoding human CCDC40 as shown in any of **SEQ ID NO: 1 or 5 to 11**.
In some embodiments of any of the foregoing or other aspects and embodiments of the disclosure, the polyribonucleotide or modified polyribonucleotide encodes human CCDC40 (e.g., functional human CCDC40) and comprises a primary sequence that

is at least 85%, at least 90%, at least 92%, or at least 95% identical (e.g., at least 95, 96, 97, 98, 99 or 100% identical) to one or more of **SEQ ID NO: 1 or 5 to 11** (e.g., to the sequence set forth in **SEQ ID NO: 1 or 5 to 11**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' untranslated region (UTR), a CYBA 5' UTR, a Kozak element followed by a codon optimized sequence encoding a functional version of a human CCDC40 protein, a CYBA 3' UTR, and a poly(A) tail (cf. e.g. **SEQ ID NO: 1**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' untranslated region (UTR), a Kozak element followed by a codon optimized sequence encoding a functional version of a human CCDC40 protein, and a poly(A) tail (cf. e.g. **SEQ ID NO: 5**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' untranslated region (UTR), an additional U nucleotide, a TISU element followed by a codon optimized sequence encoding a functional version of a human CCDC40 protein, and a poly(A) tail (cf. e.g. **SEQ ID NO: 6**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' untranslated region (UTR), a hAg 5' UTR, a Kozak element followed by a codon optimized sequence encoding a functional version of a human CCDC40 protein, and a poly(A) tail (cf. e.g. **SEQ ID NO: 7**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' untranslated region (UTR), a human CMV IE9 5' UTR, a Kozak element followed by a codon optimized sequence encoding a functional version of a human CCDC40 protein, a human Growth hormone 3' UTR, and a poly(A) tail (cf. e.g. **SEQ ID NO: 8**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' untranslated region (UTR), a Kozak element, an EGFP encoding sequence, a G4S spacer followed by a codon optimized sequence encoding a functional version of a human CCDC40 protein, and a poly(A) tail (cf. e.g. **SEQ ID NO: 9**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' untranslated region (UTR), a Kozak element followed by a codon optimized sequence encoding a functional version of a human CCDC40 protein, a G4S spacer, an EGFP encoding sequence, and a poly(A) tail (cf. e.g. **SEQ ID NO: 10**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' untranslated region (UTR), a CYBA 5' UTR, a Kozak element followed by a HA tag, a G4S spacer, a codon optimized sequence encoding a functional version of a human CCDC40 protein, a T2A peptide, a sequence encoding tdTomato, a CYBA 3' UTR, and a poly(A) tail (cf. e.g. **SEQ ID NO: 11**).

In some embodiments, the polyribonucleotide is a modified polyribonucleotide having a level and/or type of modification selected from any such level and/or type set forth herein.

In certain embodiments, the percent identity of a polyribonucleotide is measured only with respect to the CCDC40 coding sequence-portion of **SEQ ID NO: 1 or 5 to 11** (e.g., UTRs, other non-coding sequence and GFP or epitope tags are not considered when calculating percent identity, and the polyribonucleotide may or may not contain such regions).

In certain embodiments of any of the foregoing, such polyribonucleotide (or modified polyribonucleotide) encodes a functional CCDC40 protein.

The present disclosure also relates to a polyribonucleotide encoding human CCDC39 as shown in any of **SEQ ID NO: 2 or 12 to 14**.

In some embodiments of any of the foregoing or other aspects and embodiments of the disclosure, the polyribonucleotide or modified polyribonucleotide encodes human CCDC39 and comprises a primary sequence that is at least 85%, at least 90%, at least 92% or at least 95% identical (e.g., at least 95, 96, 97, 98, 99 or 100% identical) to one or more of **SEQ ID NO: 2 or 12 to 14** (e.g., to the sequence set forth in **SEQ ID NO: 2 or 12 to 14**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' UTR, a CYBA 5' UTR, a Kozak element followed by a codon optimized sequence encoding a functional version of a human CCDC39 protein, a CYBA 3' UTR, and a poly(A) tail (cf. e.g. **SEQ ID NO: 2**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' UTR, a Kozak element followed by a codon optimized sequence encoding a functional version of a human CCDC39 protein, and a poly(A) tail (cf. e.g. **SEQ ID NO: 12**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' UTR, an additional U nucleotide, a TISU element followed by a codon optimized sequence encoding a functional version of a human CCDC39 protein, and a poly(A) tail (cf. e.g. **SEQ ID NO: 13**).

In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' UTR, a SP30 as 5' UTR (i.e. a random sequence of 30 nucleotides), a Kozak element followed by a codon optimized sequence encoding a functional version of a human CCDC39 protein, and a poly(A) tail (cf. e.g. **SEQ ID NO: 14**).

In some embodiments, the polyribonucleotide is a modified polyribonucleotide having a level and/or type of modification selected from any such level and/or type set forth herein.

In certain embodiments, the percent identity of a polyribonucleotide is measured only with respect to the CCDC39 coding sequence-portion of **SEQ ID NO: 2 or 12 to 14** (e.g., UTRs, other non-coding sequence and GFP or epitope tags are not considered when calculating percent identity, and the polyribonucleotide may or may not contain such regions).
In certain embodiments of any of the foregoing, such polyribonucleotide (or modified polyribonucleotide) encodes a functional CCDC39 protein.

The present disclosure also relates to a polyribonucleotide encoding human MCIDAS as shown in **SEQ ID NO: 4**. In some embodiments of any of the foregoing or other aspects and embodiments of the disclosure, the polyribonucleotide or modified polyribonucleotide encodes human MCIDAS and comprises a primary sequence that is at least 85%, at least 90%, at least 92% or at least 95% identical (e.g., at least 95, 96, 97, 98, 99 or 100% identical) to **SEQ ID NO: 4** (e.g., to the sequence set forth in **SEQ ID NO: 4**). In certain embodiments, said polyribonucleotide contains at its 5' end a part of the T7 promoter, Ethris' minimal 5' UTR, a Kozak element followed by a codon optimized sequence encoding a functional version of a human MCIDAS protein, an optional 3' UTR that also functions as reverse primer binding site for PCR based template production as well as a poly(A) tail. In some embodiments, the polyribonucleotide is a modified polyribonucleotide having a level and/or type of modification selected from any such level and/or type set forth herein. In certain embodiments, the percent identity of a polyribonucleotide is measured only with respect to the MCIDAS coding sequence-portion of **SEQ ID NO: 4** (e.g., UTRs, other non-coding sequence and GFP or epitope tags are not considered when calculating percent identity, and the polyribonucleotide may or may not contain such regions). In certain embodiments of any of the foregoing, such polyribonucleotide (or modified polyribonucleotide) encodes a functional MCIDAS protein.

For purposes of determining percentage identity of a first sequence relative to a second sequence, an analog (e.g., methylcytidine) matches cytidine, etc. In certain embodiments, the term "primary sequence" may be used to refer to a polynucleotide sequence without regard to whether or the level of modification, such that a primary sequence identical to CUCUCUA would include that sequence regardless of whether any or all of the recited nucleotides are modified (e.g., analogs of any more or more of C, U and A may be present and would be considered the same primary sequence). In certain embodiments, percent identity is only determined by reference to the portion of a given listed sequence corresponding to the coding sequence for, for example, CCDC40 or CCDC39. While in other embodiments, the percent identity is determined by reference to both the coding sequence and one or more non-coding

sequences. In certain embodiments, the percent identity is determinated across the entire length of a listed sequence (e.g., by reference to the entire length of a sequence listed in the sequence listing herein).

The present invention furthermore relates to a method for analyzing the effect of a polyribonucleotide on ciliogenesis, wherein said polyribonucleotide encodes a protein involved in and/or required for ciliogenesis, said method comprising the steps of:

(a) obtaining a nose brush of a subject having a ciliopathy, said nose brush comprising undifferentiated basal cells and differentiated ciliated cells,
(b) culturing the cells obtained from step (a) as a submerse cell culture for obtaining undifferentiated basal cells and dedifferentiated ciliated cells,
(c) culturing undifferentiated basal cells and dedifferentiated ciliated cells obtained from step (b) as an air liquid interface cell culture and performing an air lift,
(d) transfecting cells obtained from step (c) with a polyribonucleotide encoding a protein involved in and/or required for ciliogenesis,
(e) culturing the transfected cells obtained from step (d) for obtaining differentiated ciliated cells, and
(f) determining the effect of said polyribonucleotide on ciliogenesis using a lactate dehydrogenase measurement, a NucGreen assay, a high speed video microscopy, a ciliary beat frequency measurement, a mucociliary clearance assay, and/or immunofluorescence staining.

For obtaining cells of the respiratory epithelium of a patient, a nose brush is advantageous as it is easy to handle and the procedure fast and painless for the patient. Cells obtained from a nose brush comprise undifferentiated basal cells and differentiated ciliated cells that can be cultured as a submerse cell culture. By culturing the cells as submerse cell culture undifferentiated basal cells and dedifferentiated ciliated cells can be obtained as the submerse conditions induce a dedifferentiation of epithelial cells. The thus obtained dedifferentiated ciliated cells as well as the undifferentiated basal cells of the nose brush are then to be cultured as an air liquid interface (ALI) cell culture.

An air liquid interface cell culture of step (c) refers to a cell culture that is characterized in that the basal surface of the cells is in contact with a liquid culture medium, whereas the apical surface is exposed to air. Cells can be seeded for example onto a permeable membrane of a cell culture insert, which is initially supplied with culture medium to both the apical and basal compartments. This can also be referred to as “submerse cell culture”. Once confluence is reached, the cells

are then subjected to an "air-lift" step, where the medium is supplied only to the basal chamber. This mimics the conditions found in, for example, the respiratory system and induces cell differentiation including ciliogenesis in undifferentiated ciliary cells. Thus, cells mimicking the epithelium of the respiratory system including, for example, basal and ciliated cells can be obtained for further investigation *in vitro*.

The obtained epithelium mimicking cells can then be transfected with a polyribonucleotide encoding a protein involved in and/or required for ciliogenesis. Thus, said polyribonucleotide is introduced into the cells which can be done artificially by viral infection or means other than viral infection to express the exogenous polyribonucleotide in the cells. The transfected cells are then cultured for obtaining differentiated ciliated cells from basal and undifferentiated ciliated cells. Thus, the effect of a polyribonucleotide such as an mRNA on ciliogenesis can be determined *in vitro* while preserving an environment of the cells that mimics the epithelium of the respiratory system as it can be found for example in a subject.

The effect of a polyribonucleotide on ciliogenesis can be determined by various methods known to a person skilled in the art. These methods include for example a lactate dehydrogenase measurement, a NucGreen assay, a high speed video microscopy, a ciliary beat frequency measurement, a mucociliary clearance assay, and/or immunofluorescence staining. Thus, a lactate dehydrogenase measurement (LDH) can be performed to determine the amount of LDH released in the environment surrounding the cells. The respective assay can be a colorimetric assay, wherein the amount of released LDH is measured with an enzymatic reaction which converts for example iodonitrotetrazolium or a tetrazolium salt into a red color formazan. Thus, the assay can be easily read out by spectroscopy.
Alternatively or additionally, a NucGreen assay can be performed. NucGreen is a permanent green fluorescing nucleic acid stain upon binding to nucleic acids and can be used to investigate toxicity-related effects of transfection for example. Alternatively or additionally, immunofluorescence staining of the protein encoded by the transfected polyribonucleotide can be performed to determine its amount and cellular localization including co-localization with other proteins. For determining the amount of the expressed protein, it is also possible to use Western Blot technology.
Furthermore, ciliary structural defects or lack of cilia can be detected for example using specialised microscopy. Screening tests include measurement of nasal nitric oxide production rate, ciliary motility by high speed video microscopy of nasal cells, or *in vivo* tests including a saccharin test for example. More specific diagnosis requires for example examination of cilia by immunofluorescence and transmission electron microscopy.

Alternatively or additionally, ciliary beat frequency measurement can be applied to investigate beat frequency, number of beating cilia and ciliated cells, and beating synchronicity using high speed video microscopy. The same applies to mucociliary clearance assays that can be performed to determine the rate of mucus clearance which is affected by ciliary function.

As regards the polyribonucleotide to be employed in the described method, the same applies as described above in connection with the pharmaceutical composition of the present invention for use in treating a ciliopathy. Moreover, also the other features of such a polyribonucleotide can be as described above.
Furthermore, the polyribonucleotide might contain a sequence encoding a marker. Examples include tdTomato (as comprised e.g. in **SEQ ID NO: 3**), green fluorescent protein (GFP), and enhanced GFP (eGFP) as described in the appended examples together with respective detection methods. This is especially advantageous for validating a successful transfection of a cell with the polyribonucleotide.

In some embodiments of the method for analyzing the effect of a polyribonucleotide on ciliogenesis, the cells are transfected within 0 to 12 days, preferably within 0 to 11 days, more preferably within 0 to 6 days, even more preferably 0 to 4 days and most preferably 0 to 48 hours after the air lift is performed in step (c). This is advantageous as the air lift step induces ciliogenesis of basal and undifferentiated ciliary cells.

In some embodiments of the method for analyzing the effect of a polyribonucleotide on ciliogenesis, the cells are transfected with a polyribonucleotide, preferably an mRNA, encoding a protein involved in and/or required for ciliogenesis using a lipidoid having the structure shown in formula (V), (VI), and/or (VII).

In some embodiments of the method for analyzing the effect of a polyribonucleotide on ciliogenesis, the cells are cultured in steps (b) to (e) using Medium G. The composition of Medium G is typically as shown in **Table 2**).

Table 2

Substance	**Volume**	**Final concentration**
DMEM/Ham's F12	100 mL	
Ultroser G	2 mL	2 %
Fetal Clone II	2 mL	2 %
Insulin	100 µL	2.5 µg/mL
Bovine Brain Extract	250 µL	22.5 µg/mL
Transferrin	100 µL	2.5 µg/mL

Hydrocortisone	20 µL	20 nM
3,3',5-Triiodo-L-thyronine	3.33 µL	500 nM
Epinephrine	10 µL	1.5 µM
Retinoic Acid	10 µL	10 nM
Phosphorylethanolamine	5 µL	250 nM
Ethanolamine	100 µL	250 nM
DAPT	10 µL	1 µM

In some embodiments of the method for analyzing the effect of a polyribonucleotide on ciliogenesis, the cells are cultured in steps (b) to (e) using one of the media shown in **Table 3**. The media shown in **Table 3** are some commercially available media on the market that can be used to grow ALI culture.

Table 3

Supplier	**Growth Phase**	**Differentiation Phase**
LONZA	ALI-G	ALI-D
StemCell	ALI-Ex	ALI-M
Epithelix	MucilAir	MucilAir
PELOBiotech	Pelo	Pelo

In some embodiments of the method for analyzing the effect of a polyribonucleotide on ciliogenesis, the nose brush further comprises fibroblasts and wherein growth of said fibroblasts is inhibited in steps (b) to (e). This is advantageous as fibroblasts grow faster compared to basal and undifferentiated ciliary cells and can thus hamper the investigation of the effect of a polyribonucleotide on ciliogenesis as described above.
Before transferring the cells into the flask of the stationary phase, fibroblasts can be separated from the rest of the cells due to a short incubation time of 1-2 h. During that time, fibroblasts can settle down and adhere to the cell flask. All other cells remain in the media as suspension cells and can be easily transferred afterwards to the "stationary phase flask".

The method according to the present invention preferably results in the identification of a polyribonucleotide that has a positive effect on ciliogenesis and that can be used to restore ciliary cell structure in function and thus, in a pharmaceutical composition according to the second aspect and its use in treating a ciliopathy in a subject suffering of a ciliopathy according to the first aspect of the invention. Thus, as regards features of the polyribonucleotide, the same applies as described above in

connection with the pharmaceutical composition of the present invention for the use in treating a ciliopathy. Moreover, also the other features of such a polyribonucleotide can be as described above.

Figure 1: Translation efficiency of CCDC40 mRNA. 2/1.4/0.3/0.2/0.05x10^6 HEK293 cells were seeded in 6-well plates. 24 h after seeding cells were transfected with different CCDC40 constructs (ETH031T06-T10, 2.5 µg/9.5 cm^2) using Lipofectamine2000. Cells lysis was performed 6, 24, 48, 72 and 144 h after transfection. 50 µg of total protein lysate were analyzed with SDS-PAGE and Western Blot. CCDC40 was detected using Anti-CCDC40 Antibody (HPA022974) from Atlas Antibodies (1:2000). GAPDH served as a loading control. GFP served as a transfection control.

Figure 2: Translation efficiency of CCDC40 mRNA constructs in BEAS-2B, RPMI2650, and HEK293 cells: 2x10^6 HEK293, 7.5x10^5 BEAS-2B and 5x10^5 RPMI 2650 cells were seeded in 6-well plates. 24 h after seeding cells were transfected with different CCDC40 constructs (2.5 µg/9.5 cm^2) using Lipofectamine2000. Cells lysis was performed 6 h after transfection. Protein lysates were analyzed with SDS-PAGE and Western Blot (HEK293: 50 µg, RPMI 2650: 20 µg, BEAS-2B: 30 µg of total lysate). CCDC40 was detected using Anti-CCDC40 Antibody (HPA022974) from Atlas Antibodies (1:2000).

Figure 3: High speed video microscopy (HSVM) results after LF92/CCDC40 transfection. Patient derived ALI cultures were transfected every other day with 3 µg LF92/CCDC40. Prior transfection and every 24 h after transfection, videos (20 per insert) were taken and CFB (ciliary beat frequency) was calculated using the SAVA (Sisson-Ammons video analysis) Software. Allover 16 transfections (1 month) were performed. Measurement was done at 37 °C using the 40x maginification. Calculated are the mean values of the ciliary beat frequency measurements (Whole Field Analysis, WFA).

Figure 4: Mucociliary clearance (MCC) shown as Z-Projection and Polargraph. CCDC40 patient ALI culture was transfected with CCDC40 mRNA/LF92 at a concentration of 3 µg/insert, 18 d upon air-lift. ALI cultures were maintained in Medium G during the experiment. Transfections (TFs) were performed once a week for 4 weeks (= 4x TF). Mucociliary Clearance (MCC) was measured using 0.5 µm fluorescent beads at 20x magnification. 30 s videos of different areas were taken and analyzed with the Polargraph software from Nikon one week after the last TF. One exemplary picture is shown.

Figure 5: Mucociliary clearance (MCC) shown as Z-Projection (upper left) and Polargraph (upper right) for a tdTomato and Polargraph of a healthy (lower right) control. Upper row: Cells from patient with CCDC40 mutation in ALI culture were transfected with tdTomato mRNA/LF111 at a concentration of 3 µg/insert, 18 d upon air-lift. ALI cultures were maintained in Medium G during the experiment. Transfections (TFs) were performed once a week for 4 weeks (= 4x TF). Mucociliary Clearance (MCC) was measured using 0.5 µm fluorescent beads at 30x magnification. 20 s videos of different areas were taken and analyzed with the Polargraph software from Nikon one week after the last TF. One exemplary video was shown. Lower right: Particle tracking of healthy WT ALI culture. MCC was measured using 0.5 µm fluorescent beads at 20x magnification. 20 s videos of different areas were taken and analyzed with the Polargraph software from Nikon one week after the last TF. One exemplary picture and two analyzed ROIs are shown.

Figure 6: Cells from patient with CCDC40 mutation in ALI culture were transfected either with tdTomato mRNA/LF111 or with CCDC40 mRNA/LF92 at a dose of 3 µg/insert, 18 d upon air-lift. ALI cultures were maintained in Medium G during the experiment. Transfections (TFs) were performed once a week for 4 weeks (= 4x TF). As a control, WT ALI from healthy nose brushes were used. Samples for cryo-embedding were prepared one week after the last TF. CCDC40 mRNA and tdTomato mRNA treated ALI membranes were cut at 20 µm and stained with anti-GAS8 (red), anti-acetylated Tubulin (green) and DAPI (blue). Pictures were taken with the confocal microscope. One exemplary image is shown. Scale bar represents 10 µm.

Figure 7: Cells from patient with CCDC40 mutation in ALI culture were transfected either with tdTomato mRNA/LF111 or with CCDC40 mRNA/LF92 at a dose of 3 µg/insert, 18 d upon air-lift. ALI cultures were maintained in Medium G during the experiment. Transfections (TFs) were performed once a week for 4 weeks (= 4x TF). As a control, ALI from healthy controls were used. Samples for cryo-embedding were prepared one week after the last TF. CCDC40 mRNA and tdTomato mRNA treated ALI membranes were cut at 20 µm and stained with anti-DNALI1 (red), anti-acetylated Tubulin (green) and DAPI (blue). Pictures were taken with the confocal microscope. One exemplary image is shown. Scale bar represents 10 µm.

Figure 8: Cells from patient with CCDC40 mutation in ALI culture were transfected either with tdTomato mRNA/LF111 or with CCDC40 mRNA/LF92 at a dose of 3 µg/insert, 18 d upon air-lift. ALI cultures were maintained in Medium G during the experiment. Transfections (TFs) were performed once a week for 4 weeks (= 4x TF).

As a control, ALI from healthy controls were used. Samples for cryo-embedding were prepared one week after the last TF. CCDC40 mRNA and tdTomato mRNA treated ALI membranes were cut at 20 µm and stained with anti-CCDC39 (1:200, red), anti-acetylated Tubulin (1:10.000, green) and DAPI (blue). Pictures were taken with the confocal microscope. One exemplary image is shown. Magnification 40x.

Figure 9: CCDC39 protein (110 kDa) expression in HEK-293 & BEAS-2B, 6 h & 24 h after transfection. 1.4x10^5 HEK-293 cells and 3.5x10^5 BEAS-2B cells were seeded in 6-well plates and transfected with CCDC39-RNA (ETH047T02, minimal 5´UTR, Lipofectamine MessengerMax; Ratio 1:1.5). After 6h and 24h cells were lysed with M-PER and with Triton X-100 buffer. 50 µg of protein lysate were used for Western blot analysis. As a control, lysate of untransfected (UT) and EGFP transfected cells were used. High dose = 0.5 µg/cm^2, low dose = 0.25 µg/cm^2.

Figure 10:
CCDC39 protein (110 kDa) expression in untreated ALI cultures. Two inserts were extracted using the axonemal extraction protocol. 30, 10 and 5 µL of protein lysate were used for Western blot analysis. Active Area: Insert 39.9 = 71.25%, Insert 41.2 = 59.88%

Figure 11:
CCDC39 protein (110 kDa) expression in 16HBE14o- after treatment with proteasome inhibitor. 6.0x10^5 16HBE14o- cells were seeded in 6-well plates and transfected with CCDC39-RNA (Lipofectamine MessengerMax; Ratio 1:1.5). After 6 h cells were lysed with M-PER buffer. 20 µg of protein lysate were used for Western blot analysis. As a control, lysate of untransfected (UT) and EGFP transfected cells were used. High dose = 0.5 µg/cm^2, low dose = 0.25 µg/cm^2. RNA: ETH047T02: minimal 5´UTR, ETH047T03: TISU, ETH047T04: CYBA, ETH047T05: SP30.

Figure 12: CCDC39 protein (110 kDa) expression in 16HBE14o- after treatment with proteasome inhibitor. 6.0x10^5 16HBE14o- cells were seeded in 6-well plates and transfected with CCDC39-RNA (ETH047T03, TISU 5´UTR, Lipofectamine MessengerMax; Ratio 1:1.5). After 24 h cells were lysed with M-PER buffer. 20 µg of protein lysate were used for Western blot analysis. As a control, lysate of untransfected (UT) and EGFP transfected cells were used. High dose = 0.5 µg/cm^2, low dose = 0.25 µg/cm^2.

Other aspects and advantages of the invention will be described in the following examples, which are given for purposes of illustration and not by way of limitation. Each publication, patent, patent application or other document cited in this application is hereby incorporated by reference in its entirety.

Examples

Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting.

I. Material and Methods

Materials, Devices, Software, and Test system used

Materials are listed in **Table 4**.

Table 4

Substance/Consumable/Chemical	**Supplier**	**Cat #**
DPBS (w/o Mg2+/Ca2+)	Thermo Fisher Scientific	14190-169
LHC-9 medium	Thermo Fisher Scientific	12677019
DMEM (1x) - GlutaMAX™ Supplement	Thermo Fisher Scientific	21885
BEAS-2B	ATCC	CRL 9609
HEK 293	DSMZ	ACC305
Trypsin inhibitor	Thermo Fisher Scientific	R007100
RPMI 2650	DSMZ	ACC207)
Fibronectin	Merck Millipore	FC010
Collagen	Corning Incorporated	354236
Pierce™ Bovine Serum Albumin	Thermo Fisher Scientific	23209
DPBS (with Mg2+/Ca2+), for air-brush	Thermo Fisher Scientific	14040133
Lipofectamine® 2000	Thermo Fisher Scientific	11668027
Corning® Cell Scrapers	Corning Incorporated	CORN3010
Protease inhibitor cOmplete	Sigma Aldrich	11873580001
Pierce™ BCA Protein Assay Kit	Thermo Fisher Scientific	23225
Bolt LDS Sample Buffer (4x)	Thermo Fisher Scientific	B0007
Bolt Sample Reducing Agent (10x)	Thermo Fisher Scientific	B0009
Precision Plus Protein™ Dual Color Standards	BIO-RAD	161-0374
Trans-Blot Turbo Transfer Pack Mini	BIO-RAD	1704156

0.2µm PVDF		
Luminata Crescendo Western HRP Substrate	Merck Millipore	WBLUR0500
GAPDH (D16H11)XP® Rabbit mAb	Cell Signaling	5174
Anti-CCDC40 antibody	Sigma Aldrich	HPA022974
Goat anti-rabbit IgG-HRP	Santa Cruz	sc-2004
Celletta™brush cell collector	Engelbrecht Medizin-und Labortechnik	9100060
Ultroser G	Pall Life Sciences	15950
Gelatine	Caelo	01704140
NaCl	Carl Roth	0601.1
TRIS	Carl Roth	AE15.3
TRIS/HCl	Carl Roth	A9090.3
EDTA	Carl Roth	X986.1
NaOH (32%)	Carl Roth	T196.1
FBS (submerse culture)	Thermo Fisher Scientific	10500-064
Penicillin-Streptomycin	Thermo Fisher Scientific	15140-122
Collagen I, rat tail	Gibco	A1048301
Acetic acid 100 %	Carl Roth	3738.1
Corning Transwell	Costar	3470
Trypsin/EDTA 1x	Sigma Aldrich	T3924
Collagenase type 4	Worthington Biochemical Company	LS004188
Antibiotics /Antimycotics 100 x	Thermo Scientific	15240
PneumaCult ALI medium	STEMCELL Technologies	5001
Sucrose (CAS# 57-50-1)	Sigma Aldrich	S1888
Lactate Dehydrogenase Cytotoxicity Assay Kit	Pierce Biotechnology	88954
NucGreen™ Dead 488 ReadyProbes® Reagent	Life Technologies	R37109
NaCl 0.9 % Mini-Plasco® connect (Isotonic Saline Solution)	BBraun	9511711
FBS (ALI culture)	Thermo Fisher Scientific	A3160801
Shandon Cryomatrix™ Frozen Embedding Medium	Thermo Fisher Scientific	6769006
Superfrost Ultra Plus slides	Thermo Fisher Scientific	J1800AMNZ
Paraformaldehyde	Sigma Aldrich	P6148

Goat serum	Abcam	Ab7481
BSA IgG free protease free	Jackson Immunoresearch	001-000-161
Skim milk (Blotting grade)	Carl Roth	T145.2
Hoechst33342	Thermo Fisher Scientific	H3570
monoclonal anti-acetylated alpha-tubulin antibody	Sigma Aldrich	T6793
polyclonal anti-CCDC40 antibody	Proteintech	25049-1-AP
polyclonal anti-CCDC39 antibody	Atlas antibodies	HPA035364
Alexa Fluor 546 antibody	Thermo Fisher Scientific	A11035
Alexa Fluor 488 antibody	Thermo Fisher Scientific	A11029
Insulin	Sigma Aldrich	I9278
Bovine Brain Extract	Lonza	CC-4098
Transferrin	Sigma Aldrich	T8158
Hydrocortisone	Sigma Aldrich	H4001
3,3',5-Triiodo-L-thyronine sodium sal	Sigma Aldrich	T6397
Epinephrine	Sigma Aldrich	E4642
Retinoic Acid	Sigma Aldrich	R2625
Phosphorylethanolamine	Sigma Aldrich	P0503
Ethanolamine	Sigma Aldrich	E0135
DAPT	Tocris	2634
PBS (w/o Mg2+/Ca2+)	Thermo Fisher Scientific	14190-94
10x PBS (with Mg2+/Ca2+)	Thermo Fisher Scientific	AM9625
Triton-X 100 (ALI culture)	Sigma Aldrich	T8787
Triton X-100 (submerse culture)	Sigma Aldrich	T9284
DMEM/Ham's F-12 1:1	Invitrogen	11039
Ultroser G	Pall Life Sciences	15950-017
Circular plasmid	Ethris	
BstBI	New England BioLabs	
Chloroform	Sigma Aldrich	288306
Ethanol	Sigma Aldrich	34852-M
ARCA cap analogue	Jena Biosciences	
Cytidine-5´-triphosphate	Jena Biosciences	
5-Iodocytidine-5´-triphosphate	Jena Biosciences	
Uridine-5´-triphosphate	Jena Biosciences	
5-Iodouridine-5´-triphosphate	Jena Biosciences	
Cytidine-5´-triphosphate	Jena Biosciences	
5-Iodocytidine-5´-triphosphate	Jena Biosciences	

Uridine-5´-triphosphate	Jena Biosciences	
5-Methyl-uridine-5´-triphosphate	Jena Biosciences	
DNaseI	Thermo Fisher Scientific	
Ammonium acetate	Sigma	
Quick dephosphorylation Kit	New England BioLabs	
Poly(A) polymerase	New England BioLabs	
β-Mercaptoethanol	Sigma-Aldrich	M3148
6-Well Plate	Omnilab	CORN3506
BCA Protein Assay Kit	Thermo Fisher Scientific	23225
Bolt Antioxidant	Thermo Fisher Scientific	BT0005
Bolt™ 4-12% SDS-PAGE gel	Thermo Fisher Scientific	NW04120BOX
Bolt™ MES SDS Running buffer (20x)	Thermo Fisher Scientific	B0002
cOmplete, EDTA-free Protease Inhibitor Cocktail	Sigma-Aldrich	11873580001
DNase I Solution (2500 U/mL)	Thermo Fisher Scientific	90083
DPBS	Life Technologies	14040133
DTT	Sigma Aldrich	646563
EDTA	Carl Roth	8040.3
Heat inactivated FBS	Thermo Fisher Scientific	10500064
HEPES	Carl Roth	HN78.2
LHC-9 Medium	Thermo Fisher Scientific	12680013
Luminata Classico Western HRP substrate	Merck Millipore	WBLUC0500
MEM GlutaMax	Thermo Fisher Scientific	41090028
M-PER™ buffer	Thermo Fisher Scientific	78501
MgSO4	Carl Roth	T888.1
Pen/Strep	Thermo Fisher Scientific	15140122
SDS 10%	Thermo Fisher Scientific	24730020
Sodium deoxycholate	Sigma-Aldrich	D6750-10G
SuperSignalTM West Festo	Thermo Fisher Scientific	34095
T175 Flasks	Corning/Omnilab	CORN431080
T75 Flasks	Corning/Omnilab	CORN430641
Trans-Blot® Turbo™ Mini PVDF Transfer Packs	Bio-Rad	1704156
Triton X-100	Sigma-Aldrich	T9284-100ML
TRYPSIN–EDTA (0.05%)	Thermo Fisher Scientific	25300054
Lipofectamine® MessengerMAX™ Transfection Reagent	Thermo Fisher Scientific	LMRNA003

Aqua bidest.	Kerndl	22501
ALI membranes	Sigma-Aldrich	CLS3470
Luminata Forte Western HRP substrate	Merck Millipore	WBLUF0500
MMRB (cf. **Table 18**)		
Liquid nitrogen		
WFI	B Braun	3703444
T7 RNA Polymerase	Thermo Fisher Scientific	
Inorganic Pyrophosphatase	Thermo Fisher Scientific	
RNAse Inhibitor	Thermo Fisher Scientific	
HCL	Roth	4625.2
T175 Flasks (CORN431080)	Corning/Omnilab	
T75 Flasks (CORN430641)	Corning/Omnilab	

Devices are listed in **Table 5**.

Table 5

Device	**Supplier**
Tecan Infinite® 200 PRO plate reader	Tecan
Thermomixer® C	Eppendorf
Power PAC 300	BIO-RAD
Trans-Blot® Turbo™ Transfer System	BIO-RAD
ChemiDoc™ XRS System	BIO-RAD
TriStar2 Multimode Reader LB 942	Berthold Technologies, Bad Wildbad, Germany
High-speed video microscopy	Ammons Engineering, Mt Morris, MI, USA
Nikon Eclipse Ti-S	Nikon MEA53300
ELWD 40x S Plan Fluor o	Nikon, MEA48430
Minitube SC300 heating system	Minitube International AG
Superfrost Ultra Plus slides	Thermo Fisher Scientific
100 MWCO cut of filter	Sartorius
Cryostat Microm HM560	Microm
ChemiDoc	Bio-Rad
Countess Cell Counting Device (C10281)	Thermo Fisher Scientific
Fluorescence microscope (DMi8)	Leica
Mini Gel Tank	Thermo Fisher Scientific
Trans-Blot® Turbo™ Transfer System	Bio-Rad

Device	Supplier
Lysing Matrix A Tubes	MP Biomedicals
MP FastPrep-24 (HOM-1)	MP Biomedicals
96-well black microplates (655090)	Greiner

Software is listed in **Table 6**.

Table 6

Software	Provider
Magellan™- Data Analysis Software	Tecan
Image Lab™	BIO-RAD
Sisson-Ammons Video Analysis (SAVA software)	Ammons Engineering, Mt Morris, MI, USA
Megaplus camera model ES 310 turbo	Redlake Inc., USA
NIS-Elements Basic Research	Nikon
Nikon NIS Elemets AR Software	Nikon

The test system is listed in **Table 7**.

Table 7

Test System	Species	Strain
HEK 293	human primary embryonal kidney	DSMZ no.: ACC305
RPMI 2650	anaplastic squamous cell carcinoma	DSMZ no.: ACC207
BEAS-2B	bronchial epithelium	ATCC no.: CRL 9609

I. Submerse cell culture

1. Culturing

Cell lines were cultivated in a cell incubator under humidified atmosphere at 37 °C and 5 % CO_2 content. All reagents and solutions were heated to 37 °C in a water bath before usage.

Confluent cells (about 90 %) were first washed with 20 mL DPBS (w/o Mg^{2+}/Ca^{2+}) to remove dead cells. To detach the cells 2 mL of Trypsin/EDTA solution (0.05 %) was added per flask. Cells were then incubated at 37 °C until detachment of cells occurs. 8 mL of the respective medium was used to stop the Trypsin. Because LHC-9 medium was almost serum-free at least an equal volume of Trypsin inhibitor was added to the detached BEAS-2B cells to inactivate trypsinisation. The cell solution

had to be centrifuged afterwards for 5 minutes (min) at 1100 revolutions per minute (rpm) to remove the trypsin inhibitor again. The pellet was then resolved in medium. For passaging, the cells were split in different ratios according to the next use.

HEK 293 (DSMZ no.: ACC305)

This established cell line was from a human primary embryonal kidney transformed by adenovirus type 5 (AD 5). It was cultured in Dulbecco's Modified Eagle Medium (DMEM) - GlutaMAX™ Supplement with 10 % heat inactivated (h.i.) fetal calf serum (FBS) and 1 % penicillin/streptomycin (P/S). This cell line was split two times a week.

RPMI 2650 (DSMZ no.: ACC207)

This cell line had its origin from the pleural effusion of a 52-year-old man with anaplastic squamous cell carcinoma of the nasal septum. It was chosen because of the epitheloid ciliated morphology and the similarity to the bronchiolar epithelium. It was also cultured in Dulbecco's Modified Eagle Medium (DMEM) - GlutaMAX™ Supplement with 10 % heat inactivated (h.i.) fetal calf serum (FBS) + 1 % penicillin/streptomycin (P/S) + 1x Non-essential-amino-acid solution (NEAA). This cell line was split 1-2 times a week.

BEAS-2B (ATCC no.: CRL 9609)

BEAS-2B cells were derived from normal bronchial epithelium obtained from autopsy of non-cancerous individuals. Cells were then infected with a replication-defective SV40/adenovirus 12 hybrid and cloned. The used culture medium was LHC-9 with modification w/o additives. They were also split 1-2 times a week depending on the confluence.
The flasks used for BEAS-2B cells were coated with a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL collagen and 0.01 mg/mL bovine serum albumin dissolved in LHC-9. The mixture was added at a ratio of 0.2 mL per cm^2 surface area. Afterwards, incubation at 37 °C for at least 6 h was necessary. Prior to the addition of cells, the flasks were washed three times with Dulbecco's phosphate-buffered saline (DPBS) without Mg^{2+}/Ca^{2+}.

2. In vitro transcription

To generate templates for *in vitro* transcription, circular plasmids were linearized by restriction digestion with *BstBI* and further purified by chloroform ethanol precipitation.
mRNA was produced using a standard *in vitro* transcription mix (including indicated modified triphosphate nucleotides) containing T7 RNA polymerase, inorganic

pyrophosphatase, and RNase inhibitor. Co-transcriptional capping was achieved by addition of an ARCA cap analogue. For *in vitro* transcription of chemically modified RNA, 7.5 % of Cytidine-5´-Triphosphate were replaced by 5-Iodocytidine-5´-Triphosphate and 30 % Uridine-5´-Triphosphate were replaced by 5-Iodouridine-5´-Triphosphate (Jena Biosciences), respectively (cf. e.g **SEQ ID NO: 1**). In another chemically modified RNA production setup 3 % of Cytidine-5´-Triphosphate were replaced by 5-Iodocytidine-5´-Triphosphate and 15 % Uridine-5´-Triphosphate were replaced by 5-Methyl-Uridine-5´-Triphosphate (Jena Biosciences), respectively (cf. e.g **SEQ ID NO: 2**). Residual template DNA was digested using DNaseI. Subsequently mRNA was purified by ammonium acetate precipitation followed by a washing step using 70 % ethanol.
Dephosphorylation of residual uncapped mRNA was carried out using *a Quick dephosphorylation Kit* followed by purification via ammonium acetate precipitation followed by a washing step using 70 % ethanol and ultrafiltration using a 100 MWCO cut of filter.
mRNA was further polyadenylated by using a poly(A) polymerase. Again mRNA was purified by ammonium acetate precipitation followed by a washing step using 70 % ethanol and ultrafiltration using a 100 MWCO cut of filter. Poly(A) length was determined by capillary gel electrophoresis to be between 100 and 250 nucleotides.

In particular, the CCDC40 mRNA constructs investigated in this experiment comprised an 5' ARCA cap and PPA and were the following: with Ethris' minimal UTR(ETH031T06; T06; **SEQ ID NO: 5**), with TISU 5' UTR but without 3' UTR (ETH031T07; T07; **SEQ ID NO: 6**), with hAg 5' UTR but without 3' UTR (ETH031T08; T08; **SEQ ID NO: 7**), with CYBA 5' and 3' UTR (ETH031T0; T09; **SEQ ID NO: 1**), and with 5' UTR from human CMV IE9 and 3' UTR from human Growth hormone (ETH031T0; T10; **SEQ ID NO: 8**).

3. Transfection

To provide at least 90 % confluency, cells were seeded in 6-well plates 24 h before transfection. Cell counts differed according to the time point of read-out and cell type (compare **Table 8**).

Table 8

	cells/well x10^6		
Time	**HEK 293**	**RPMI 2650**	**BEAS-2B**
6 h	2	0.6	0.75
24 h	1.4	0.4	0.5
48 h	1	0.3	-

72 h	0.75	0.2	-
144 h	0.125	0.05	-

Transfection was performed using different CCDC40 mRNA constructs as well as mRNA encoding for enhanced green fluorescent protein (eGFP) as a transfection control. Transfection was performed using Lipofectamine® 2000 Prior to the transfection culture medium was exchanged to provide optimal conditions for the cells. The lipoplex reaction required an mRNA/WFI mix as well as a Lipofectamine® 2000/medium mix. 125 µL of each mix was used with volume ratio of 1:2 from mRNA to Lipofectamine® 2000 as the mRNAs showed a stock concentration of 1 µg/µl. Different mRNA concentrations were used and the mix was adjusted accordingly (see **Table 9** for an exemplary pipetting scheme).

Table 9

µg RNA/well	**RNA in µL**	**+WFI in µL**	**Lipofectamine® 2000 in µL**	**+serumfree medium in µL**
5	5	120	10	115
2.5	2.5	122.5	5	120
1	1	124	2	123

After preparing both mixes the mRNA/WFI Mix was added to the Lipofectamine®2000/Medium Mix and the solution incubated for ≥ 5 min before adding it dropwise to the seeded cells. To obtain a better viability of the cells, an additional medium change was performed 4 h after transfection.

4. CCDC40 Western blot

a. Sampling for Western blot

For cell lysis the culture medium from transfection was removed and the cells were washed with DPBS (without Mg^{2+}/Ca^{2+}) and then scraped off using Corning® Cell Scrapers. The cells were lysed with 100 µL of a Lysis-Buffer solution [10x TritonX-100 lysis buffer (250 mM Tris-HCl, 1 % TritonX-100, pH: 7.8) was adjusted to 1x in WFI and complemented with protease inhibitor cOmplete] before samples were frozen at -20 °C.

Total protein concentration was determined using Pierce™ BCA Protein Assay Kit (BCA assay). The assay was performed according to the manufacturers' protocol. Briefly, a 1:50 BCA Working Reagent (WR) dilution was prepared. 5 µL of each sample were transferred to a 96-well plate (flat bottom, transparent) and 200 µL WR

were added per well. The last step was an incubation at 37 °C for 30 min. Results were measured on a Tecan Infinite® 200 PRO plate reader using Magellan™- Data Analysis Software.

b. Preparation of NET-Gelatine

Table 10

Substance	Final concentration
TRIS/HCl, pH 7.5	0.2 M
EDTA, pH 8.0	0.05 M
Triton-X100	0.5 % (v/v)
NaCl	1.16 M
Gelatine	25 g/L

c. SDS-PAGE and Western blot

To perform sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) the lysates were mixed with 5 µL Bolt LDS Sample Buffer (4x) and 2 µL Bolt Sample Reducing Agent (10x) and then heated to 70 °C for 10 min and 350 rpm in the Thermomixer® C. To enable comparison between the samples on one gel, the same amount of total protein was loaded. 7 µL of the marker (Precision Plus Protein™ Dual Color Standards) were filled into the first pocket of each gel, to enable the accurate protein size estimation afterwards. The gel tank was then connected to the power supply (Power PAC 300) for 30 min at 1 A. Subsequently the cassette was opened with a spatula and the gel was then moved to the blotting station.

The Transfer System (Trans-Blot® Turbo™ Transfer System, 30 min, 25 V, 1 A) and suitable membranes (Trans-Blot Turbo Transfer Pack Mini 0.2 µm PVDF) were used for blotting according to the manufacturer's instructions. By scrolling a roller over the "sandwich", any bubbles which could interfere the protein transfer were eliminated. To block free binding sites, the membrane was put into an NET-gelatine and shaken for 60 min on a plate shaker. The gel was cut at the 75 kD band to detect CCDC40 and Glycerinaldehyd-3-phosphat-Dehydrogenase (GAPDH) at the same time. Antibodies were diluted with NET-gelatine (Anti-CCDC40 1:2000 in 6 mL, GAPDH 1:10000 in 10 mL NET-gelatine) and the membranes were incubated with their corresponding antibody on a plate shaker over night at 4 °C. GAPDH functions as a loading control. A list of employed antibodies was shown in **Table 11**. The next day the membrane was washed three times with NET-gelatine for 10 min to remove antibodies that were non-specifically bound or residual. The secondary antibody Goat anti-rabbit IgG-HRP was diluted 1:10,000 with NET-gelatine. After a 60 min

incubation on a plate shaker at room temperature (RT), the membranes were washed again three times for 10 min with NET-gelatine. The horseradish peroxidase HRP conjugated secondary antibody could be detected via a molecular imager (ChemiDoc™ XRS System). Therefore, 5 mL of Luminata Crescendo Western HRP Substrate was added on the gel and incubated for 5 min. The last picture that was not yet overexposed and the colorimetric picture were then merged and illustrated with the Image Lab™ Software. In addition, the band intensities were determined with Image Lab™ afterwards.

Table 11

Name	Manufacturer	Usage
GAPDH (D16H11)XP® Rabbit mAb	Cell Signalling	Primary antibody
Anti-CCDC40	SIGMA-ALDRICH	Primary antibody
Goat anti-rabbit IgG-HRP	SCBT	Secondary antibody

II. ALI culture

1. Sampling of Human Respiratory Epithelial Cells (hREC)

a. Nasal brushing

RPMI medium was heated to room temperature and the brush was wet with sterile isotonic saline solution. A Celletta™brush cell collector with protective tip was applied. After asking the patient to clean his nose, he had to sit in a chair with the head against a wall to hold the head. The brush was rubbed a few times rapidly against the medial and superior side of the inferior nasal meatus using rotatory and linear movements. When taking the brush out it was immediately put in a collecting tube (15 mL cornicle) with 5 ml pre-warmed RPMI medium. The brush was vigorously shaken within the tube for at least 40 times to detach the cells from the brush.

b. Stationary culture

The tube was spun at 900 rpm for 5 min at room temperature. After discarding the supernatant, the pellet was resolved in UG medium [DMEM/Ham's F-12 1:1 with 5 mL sterile 100x anibiotics/mycotics and 10 mL sterile Ultroser G]. 5 mL medium were used for a T25 and 25 mL for a T75 flask.

Having transferred the cells to the cell culture flask, the cells were incubated at 37 °C in a humidified incubator with 5 % CO_2 over night. Cells should get attached after 20-24 h. UG medium was replaced 3 times a week (Monday, Wednesday and Friday).

2. Generation and transfection of ALI culture

a. Coating of transwell inserts (0.33 cm²)

Following plates were employed for ALI culture: Corning Transwell with clear polyester-membrane (0.4 µm pores, 6.5 mm diameter of inserts, 24-well plates). Rat-tail collagen was diluted 1:5 in acetic acid and 250 µL were loaded on the apical side of each insert. After incubating the plates over night at room temperature, the remaining liquid was aspirated with a pasteur pipette and then the plate left to dry with an open lid for at least 5 min (sterile). Finally, inserts were washed twice with DPBS (w/o Ca^{2+}/Mg^{2+}) and then dried over night at room temperature (sterile). Inserts were stored at 4 °C for up to one month.

b. Cell expansion of hREC as submerse culture (stationary culture)

Cells were fed every 2 to 3 days (Monday, Wednesday and Friday) with 15 mL pre-warmed UG medium. Care had to be taken not to add new medium directly onto the collagen layer to prevent damage of the collagen layer and detachment of the cells. As soon as cells were 80 % confluent or collagen layer began to detach, cells were transferred to ALI filters (approximately after 3 weeks in submerse culture).

c. Preparation of Medium G

Different ALI media were tested, but the self-made Medium G was preferred.
This medium was prepared for one week but needed to be stored at 4 °C. Batches were frozen in aliquots based on upcoming experiment size. **Table 2** shows the composition of Medium G.

d. Seeding and air-lift of hREC (ALI culture)

1. Aspirate UG medium, wash once with wash medium [DMEM/Ham's F-12 1:1 with 1x sterile antibiotics/antimycotics (AA)] or DPBS (w/o Ca^{2+}/Mg^{2+})

2. In case of coated flasks with self-made rat-tail collagen:
    a. Dissolve collagen layer by adding 9 mL collagenase (1x) and incubate for 45 to 60 min at 37 °C
    b. Transfer cells into a 15 mL cornicle tube and centrifuge at 1000 rpm, room temperature for 5 min
    c. Wash cells with wash buffer (1000 rpm, room temperature, for 5 min)

d. Add 2 mL Trypsin EDTA (1x) and pipette gently up and down to separate the cells, incubate cells at 37 °C and shake the cornicle until the cell clusters disintegrate (approximately 5 minutes)

In case of coated flasks with collagen purchased from gibco:

a. Add 3 ml Trypsin EDTA (1x) to the cell culture flask until the cells detach (approximately 5 min)

3. Stop trypsin by adding 0.5 mL FBS and 5 mL wash medium
4. Transfer cells into a 15 ml falcon and wash cells twice with wash medium (1000 rpm, room temperature, for 5 min)
5. Resuspend pellet in appropriate amount of ALI medium (1-2 mL, depends on pellet size) and count the cells using cell counter
6. Add 250 µL of the cell suspension (400,000 cells/mL) into each collagen coated transwell with 500 µL ALI medium in the basolateral side
7. Incubate cells at 37 °C in a humidified, 5 % CO_2 incubator over night

Day 3-5: ALI-Growth

8. Check inserts under the microscope consistently
9. Feed cells with ALI medium every day until 80 - 100 % confluence (3 - 4 days)

ALI-Differentiation

10. Airlift cells when 80 -100 % confluency is reached
    a. Aspirate ALI medium from both, the apical and basolateral side
    b. Add 500 µL fresh ALI medium to the basolateral compartment
    c. Incubate cells at 37 °C in a humidified, 5 % CO_2 incubator
11. Wash cells with preheated wash medium (apical) once a week and feed cells with ALI medium every 2-3 days (or Monday – Wednesday – Friday)

e. Transfection of hREC

The formulations (LF111-mRNA) were diluted in 2 % sucrose to a final concentration of 3 µg/insert (= 9 µg/cm²). To remove mucus, the inserts were washed with 200 µL DPBS (w/o Ca^{2+}/Mg^{2+}) on the apical side and incubated for 20 min at 37 °C (Epithelix ALI) or 200 µL DPBS (w/o Ca^{2+}/Mg^{2+}) on the apical side and incubated for 5 min at room temperature (hREC, UKM) prior transfection. Afterwards DPBS (w/o Ca^{2+}/Mg^{2+}) from the apical surface was removed by gentle aspiration without damaging the epithelium. Directly after the mucus wash, the cells were washed again with WFI (100 µL) to remove traces of DPBS (w/o Ca^{2+}/Mg^{2+}). 25 µL of the formulation was added on the apical side of the insert to allow cellular uptake. Removal of carrier was

performed after 6 h of incubation, cells were washed once with 200 µL DPBS (w/o Ca^{2+}/Mg^{2+}) and culture was maintained as ALI without liquid on the apical side.

3. Read-out assays

Various read-out assays are employed. LDH measurement and NucGreen assay were used to investigate toxicity-related effects of transfection. Ciliary beat frequency measurements (CBF) were needed to examine the manner of ciliary beating with regard to beat frequency and percentage of ciliated area. If ciliary beating leads to a directed flow it can be studied using the Mucociliary Clearance (MCC) Assay. Immunofluorescence (IF) stainings for CCDC40, its heterodimer partner CCDC39 and the cilia marker acetylated alpha-tubulin were utilized to detect if, after treatment, CCDC40 protein or its partner CCDC39 can be detected in the cilia. In addition, proteins of the dynein regulator complex (DRC) like GAS8 and DNALI1 were stained. The DRC is anchored by CCDC40/CCDC39 and only present if CCDC40/CCDC39 are correctly integrated into the cilia axoneme.

a. LDH measurement

The LDH concentration is measured using the Pierce™ Lactate Dehydrogenase Cytotoxicity Assay Kit. The procedure is performed as described in the manufacturer's protocol. The ALI cultures were incubated on the apical side with 100 µL DPBS (with Mg^{2+}/Ca^{2+}) for 30 min at 37 °C and 5 % CO_2. 50 µL of the LDH reaction mix and 50 µL of the incubation medium were mixed utilizing a 96-well format and incubated protected from light at room temperature for 30 min adding 50 µL stop solution. The absorbance of the formazan was measured at 490 nm and 680 nm using a microplate reader (TriStar2 Multimode Reader LB 942). The 680 nm value was substracted from the 490 nm for background normalization. A double determination for each insert was carried out.

b. Ciliary Beat Frequency (CBF) measurement

Ciliary beat frequency (CBF) analyses of hRECs were performed by high-speed video microscopy and Sisson-Ammons Video Analysis. Videos (125 fps, 640×480 pixel resolution) were recorded using a Megaplus camera model ES 310 turbo attached to an inverted phase-contrast microscope equipped with an ELWD 40x S Plan Fluor objective under physiological conditions, by maintaining the temperature at 36 °C by a Minitube SC300 heating system.

c. Mucociliary Clearance Assay (MCC, fluorescence beads)

ALI medium and DPBS (w/o Ca^{2+}/Mg^{2+}) were pre-warmed to room temperature and the plate of the microscope was heated to 37 °C. ALI inserts were transferred to a new plate and washed apically twice with pre-warmed ALI medium (alternative DPBS w/o Ca^{2+}/Mg^{2+}). 100 µL ALI medium was added to the apical compartment before recording 20 videos per insert (40x magnification, Ph2) to get an overall impression of the insert (SAVA software). Red fluorescent particles with a diameter of 0.5 µm were diluted 1:1000 in DPBS (w/o Ca^{2+}/Mg^{2+}) and vortexed for at least 1 min. 10 µL of the particle dilution were added to 100 µL of ALI medium, vortexed and 110 µL added to the apical compartment. While handling the beads, it was important to vortex before each step to prevent agglutination. Moreover, work with dimmed light was necessary to avoid bleaching.

The flow of the fluorescent particles was recorded with the NIS-Elements Basic Research software. Movement was recorded over 20-30 s using a 20x magnifcation (Ph1) while exciting the particles with a 488 nm laser. The exposure time had to be adjusted to the number of recorded frames per second (fps). For 7.5 fps the exposure time was set to 100 ms and for 15 fps to 50 ms. The exact observation area was analyzed with SAVA (20x magnifcation, Ph1) to visualize ciliary movement and 3D structures of the cell layer.

For evaluation and data analysis, Nikon NIS Elemets AR Software, NIS PLUG-IN <ADVANCED 2D TRACKING> <AR> (see above NIS Elements AR Tracking Modul) is employed.

d. IF of ALI culture

Embedding of ALI culture

Prior membrane preparation, the ALI insert was washed twice with DPBS (w/o Mg^{2+}/Ca^{2+}, 200 µL, 1 min, room temperature) to remove the mucus. The following membrane preparation as carried out according to the following protocol (see below).

The transwell filter membrane was cut out using a scalpel or a syringe before transferring the membrane into a petri dish with 3 mL DPBS (Ca^{2+}/Mg^{2+}). The membrane was cut into equal pieces (4-6) and cryomolds were prepared with Shandon Cryomatrix™ Frozen Embedding Medium (intermediate size). Membrane pieces were put into the cryomatrix and arranged in parallel to each other before cryomolds were incubated on dry ice until the cryomatrix hardens. Cryomolds were stored at -80 °C for at least 2 h before cutting at cryostat.

Cutting of ALI membranes

ALI membranes embedded in Shandon Cryomatrix™ were cut using the cryostat Microm HM560. Therefore, the cryostat was pre-cooled (2-3 h prior cutting) to -20 °C (table at -21°C and chamber at -25°C). The ALI membrane containing cryomolds was transferred from -80°C to -20°C for at least 1 h. Cutting of the membrane was performed at 20 µm, depending on the handling conditions of the membrane. The membrane slices were transferred to Superfrost Ultra Plus slides, dried for at least 1 h at room temperature and stored at -80 °C over night or until use at -80 °C.

Staining procedure

For applied antibodies, see **Table 12**.

Day 1:

1. Wash with 1x DPBS (Ca^{2+}/Mg^{2+}) for 5 min at room temperature
2. Fixation: 4 % PFA/1x DPBS (Ca^{2+}/Mg^{2+}) for 15 min at room temperature
3. Wash 3x DPBS (Ca^{2+}/Mg^{2+}) (2x quick, 1x 5 min)
4. Permeabilization:
    a. 0.5 % Triton X-100 für 5 min or
    b. 0.2 % Triton X-100 für 10 min (depends on the used antibody)
5. Wash 3x DPBS (Ca^{2+}/Mg^{2+}) (2x quick, 1x 5 min) at room temperature
6. Blocking: 5 % normal goat serum + 2 % BSA in DPBS (Ca^{2+}/Mg^{2+}) or 5 % skim milk in DPBS (Ca^{2+}/Mg^{2+}) → 2 h at room temperature
7. 1st antibody: 200 µl over night at 4°C

Day 2:

8. Wash for 45 min with DPBS (Ca^{2+}/Mg^{2+}) (3x 15min)
9. 2nd antibody: 200 µl for 1 h at room temperature
10. Hoechst33342 1:1000 in DPBS (Ca^{2+}/Mg^{2+}) for 10 min at room temperature in the dark
11. Wash 6x 5 min with DPBS (Ca^{2+}/Mg^{2+})
12. Mount cover slips with Dako antifade

Table 12

Name	Species	Dilution	Reactivity	Cat. No.
monoclonal anti-acetylated alpha-tubulin	mouse IgG2b, clone 6-11B-1	1:10000	human	T6793

polyclonal anti-CCDC40	rabbit IgG	1:200	human	25049-1-AP
polyclonal anti-CCDC39	rabbit, affinity purified	1:300	human	HPA035364
polyclonal anti-GAS8	rabbit IgG	1:200	Human	HPA041311
polyclonal anti-DNALI1	rabbit, affinity isolated	1:200	human	HPA028305
Alexa Fluor 546	goat anti-rabbit IgG (H+L)	1:1000	human	A11035
Alexa Fluor 488	goat anti-mouse IgG (H+L)	1:1000	human	A11029
DAPI	-	1:000	-	62249

e. Air-brush on ALI culture

An air-brush model was employed to generate differentiating ALI culture. The protocol was established after Crespin et al. 2011 (Approaches to Study Differentiation and Repair of Human Airway Epithelial Cells).

Cells were shortly washed on the apical side with pre-warmed DPBS (Mg^{2+}/Ca^{2+}). The compressor connected to the air-brush was set to 1 kg/cm^2 by running the air-brush and turning the small wheel. The air-brush might need to run for some time to stay at a constant value. Then the small reservoir on top of the air-brush was filled with DPBS (Mg^{2+}/Ca^{2+}) and the insert put to a new plate without basal medium. The air-brush was started by pressing to the first pressure point and waiting until the pressure on the compressor stays constant. Than the air-brush was put vertically on top of the insert and the lever of the air-brush was pressed very little beyond the first pressure point [disperses the DPBS (Mg^{2+}/Ca^{2+}) from the reservoir] for 2 s. Following, the apical side of the insert was shortly washed with pre-warmed DPBS (Mg^{2+}/Ca^{2+}) and the insert was put into fresh medium applicable for ALI differentiation. The wound was checked by microscopic observation. If it was too small (less than 1/3 of insert area) or the cells in the wound only slightly detached, the procedure was repeated. Cultures were incubated in a humidified atmosphere at 37 °C, 5 % CO_2 and cultured as ALI.

III. Tagged CCDC40 mRNAs

The mRNAs under study are shown in **Table 13**.

Table 13

RNA-ID	ORF	5´ UTR	3´ UTR	Modified NTP I [%]	Modified NTP I [Name]	Modified NTP II [%]	Modified NTP II [Name]
ETH 031T28 (**SEQ ID NO: 9**)	eGFP-hCCDC40	minimal	no	15	5-Methyl-UTP	3	5-Iodo-CTP
ETH 031T30 (**SEQ ID NO: 10**)	hCCDC40-eGFP	minimal	no	15	5-Methyl-UTP	3	5-Iodo-CTP
ETH 031T26 (**SEQ ID NO: 11**)	HA-CCDC40-T2A-tdTomato	CYBA	CYBA	15	5-Methyl-UTP	3	5-Iodo-CTP

a. Cell Culture

HEK-293 cells are cultivated in MEM GlutaMAX™, 10 % FBS, 100 U/ml P/S (= cultivation medium) at 37 °C, 5 % CO_2.
Before seeding, cells are washed with DPBS and detached using Trypsin. After trypsinisation, trypsin is deactivated using cultivation medium containing FBS. Therefore, an equal or greater volume of culture medium is used. After centrifugation for 5 min at 1100x g and resuspension in normal growth media, cells are counted using a Countess Cell Counting Device.

b. In vitro transcription

To generate templates for *in vitro* transcription, circular plasmids were linearized by restriction digestion with *BstBI* and further purified by chloroform ethanol precipitation.

mRNA was produced using a standard *in vitro* transcription mix containing T7 RNA polymerase, inorganic pyrophosphatase, and RNase inhibitor. Co-transcriptional capping was achieved by addition of an ARCA cap analogue. For *in vitro* transcription of chemically modified RNA, 7.5 % of cytidine-5´-triphosphate were replaced by 5-iodocytidine-5´-triphosphate and 30 % uridine-5´-triphosphate were replaced by 5-iodouridine-5´-triphosphate, respectively. In another *SNIM*®RNA production setup 3 % of cytidine-5´-triphosphate was replaced by 5-iodocytidine-5´-triphosphate and 15 % uridine-5´-triphosphate was replaced by 5-methyl-uridine-5´-triphosphate, respectively. Residual template DNA was digested using DNaseI. Subsequently mRNA was purified by ammonium acetate precipitation followed by a washing step using 70 % ethanol.

Dephosphorylation of residual uncapped mRNA was carried out using *a Quick dephosphorylation Kit* followed by purification via ammonium acetate precipitation followed by a washing step using 70 % ethanol and ultrafiltration using a 100 MWCO cut of filter.

mRNA was further polyadenylated by using a poly(A) polymerase. Again mRNA was purified by ammonium acetate precipitation followed by a washing step using 70 % ethanol and ultrafiltration using a 100 MWCO cut of filter. Poly(A) length was determined by capillary gel electrophoresis to be between 100 and 250 nucleotides.

c. Seeding, transfection and readout

Cells were seeded in 96-well black microplates (25,000 cells per well) 24 h before treatment. 24 h after seeding fresh medium was added to each well (100 µL). mRNA was transfected using Lipofectamine® MessengerMAX™ in a RNA to Lipofectamine ratio of 1:1.5 (w/v). 250 ng/96-well (≙ 758 ng/cm^2) eGFP-CCDC40 (ETH031T28 or ETH031T30) or HA-CCDC40-T2A-tdTomato (ETH031T26) mRNA was employed. All RNAs were having a stock concentration of 1 mg/mL. For lipoplex formation mRNA was diluted in aqua bidest.. Lipofectamine® MessengerMAX™ was diluted in SFM (serum-free medium) and was mixed by pipetting. After incubation of 10 min at RT, the RNA solution was added to the Lipofectamine® MessengerMAX™ solution, mixed and incubated for another 5 min at RT. Afterwards, the mix was diluted two-fold in SFM before adding the Lipoplex solution to the wells. In **Table 14** an example for one RNA was calculated.

Table 14

ng RNA/well	RNA [µL]	H2O [µL]	MM [µL]	SFM [µL]	Total volume added to cells [µL]
250	4.5	108	6.76	105.76	25

6 h and 24 h post transfection, transfection efficiency was examined by fluorescence microscopy, pictures were taken at 10x magnification.

IV. CCDC39

The mRNA (ETH047T02; **SEQ ID NO: 12**) under study encoded hCCDC39 and comprised Ethris minimal UTR. In total, 15% of uridine-5'-triphosphates were replaced by 5-methyl-uridine-5'-triphosphates, and in total 3 % of cytidine-5'-triphosphates by 5-iodo-cytidine-5'-triphosphates. Antibodies used are shown in **Table 15**.

Table 15

Antibody Type	Antibody Number	Name	Supplier	Cat. No
First	207	CCDC39	atlas antibodies	HPA035364
First	219	Actin	abcam	ab8227
Second	Goat Anti-Rabbit IgG H&L (HRP)	Abcam	ab205718	Goat Anti-Rabbit IgG H&L (HRP)

1. CCDC39 Transfection and Western blot in submerse cell culture

a. Cell Culture

BEAS-2B cells were cultivated in LHC-9 media in coated flasks. For details see I 1 above. Experiments were performed in uncoated plates.

HEK-293 cells were cultivated in MEM GlutaMax media, supplemented with heat inactivated FBS and penicillin/streptomycin (P/S).

Before seeding, cells were washed with DPBS and detached using Trypsin. After trypsinisation, trypsin was deactivated using either a trypsin inhibitor for BEAS-2B or cultivation medium containing FBS for HEK-293. Therefore, an equal or greater volume of trypsin inhibitor/culture medium was used. After centrifugation for 5 min at

1100x g and resuspension in normal growth media, cells were counted using a Countess Cell Counting Device. Considering a seeding volume of 2 mL and a 6-well plate format, $5.0x10^5$ seeded BEAS-2B cells and $1.4x10^6$ seeded HEK-293 cells were obtained.

b. Transfection

Cells were seeded in their respective density 24 h before treatment. 24 h after seeding 2 mL of fresh medium was added to each well. mRNA was transfected using Lipofectamine® MessengerMAX™ in a RNA to Lipofectamine ratio of 1:1.5 (w/v). All RNAs were having a stock concentration of 1 mg/mL. For lipoplex formation mRNA was diluted in acqua bidest.. Lipofectamine® MessengerMAX™ was diluted in SFM and was mixed by pipetting. After incubation of 10 min at RT, the RNA solution was added to the Lipofectamine® MessengerMAX™ solution, mixed and incubated for another 5 min at RT. Afterwards, the Lipoplex solution was added to the wells. In **Table 16** an example for transfection is calculated for one 6-well plate.

Table 16

	ng/well	**RNA [µL]**	**H2O [µL]**	**MM [µL]**	**SFM [µL]**	**Total volume added to cells [µL]**
High dose	5000	5.0	120	7.5	117.5	250
Lowe dose	2500	2.5	122.5	3.75	121.25	250

c. Cell lysis

To obtain optimal cell lysis, two different lysis buffers were compared, a Triton X-100 buffer and the commercially available lysis buffer M-PER™, both of them complemented with protease inhibitor cOmplete, EDTA-free, and 40 µL/mL DNase I Solution (in a mixture of 23:1:1). Therefore, cells were seeded in a 6-well plate and treated for 6 h and 24 h. After treatment, cells were harvested. Therefore, plates were washed once using 1 mL DPBS. To remove the cells from the plate another 1 mL of DPBS was added and cells were scraped from plates and moved to Eppendorf tubes. To remove DPBS, cells were centrifuged at 6250x g for 2 min at 4 °C. Cell lysis was performed by adding 200 µL of the respective buffer. To ensure complete lysis the cells were incubated on ice for 30 min. After lysis, BCA Assay was performed to determine the total protein concentration using a BCA Protein Assay Kit according to the manufacturer's instructions with the following changes:

- 200 µL working reagent were added to 5 µL cell lysate
- Samples and standard were measured in triplicate

- Before incubation of samples at 37 °C for 30 minutes, the plate was shaken for 30 seconds at 450 rpm

d. Sample preparation

The samples are mixed with 5 µL Bolt® LDS Sample buffer and 2 µL Bolt® Sample Reducing Agent and then heated for 10 min at 70 °C before SDS-PAGE was performed. 30 µg of total protein was used for SDS-PAGE and Bolt™ 4-12% SDS-PAGE gel, 10 well was employed.

e. SDS Page and Blotting method

Trans-Blot® Turbo™ System Transfer was used. SDS-PAGE was performed applying 200 V for 40 min. Transfer was done using the TransBlot® TurboTM Transfer System for 30 minutes.

f. Antibody and blocking method

After transfer, the membranes were blocked at RT for 1 h. NET-gelatine was used as blocking reagent. Antibody HPA035364 from atlas antibodies was used for the detection of CCDC39, and antibody ab8227 from abcam for the detection of Actin. Membranes were cut at about 60 kDa before the antibodies were added. The membranes were incubated overnight at 4 °C with the primary antibodies. After three washes (10 min each) with blocking solution at RT, horseradish peroxidase-conjugated secondary antibody, was added at RT for 1 h (diluted 1:20 000). The membranes were washed again three times with blocking solution 10 min each at RT.

g. Chemiluminescent Signal Development

Signals were visualized with a chemiluminescent substrate kit (Luminata Crescendo, Classico or Forte Western HRP substrate, depending on the intensity of the signal) and the ChemiDocTM MP System.

2. Western blot of endogenous CCDC39 in differentiated ALI culture

a. Cell lysis

250 µL ALI lysis buffer (for details of the composition cf. **Table 17**) was added per insert and incubated on ice for 15 min. Before, during (every 5 min) and at the end of

incubation, ALI lysis buffer was pipetted back and forth. After transfer into eppendorf tubes and rinsing of each insert with 150 µL ALI lysis buffer, samples were vortexed thoroughly. At this step, samples could optionally be stored at -20 °C until further processing.

Table 17

Substance	Stock	Final	For 10 ml
TritonX-100	10 %	1 %	1 ml
NaCl	1.5 M	150 mM	1 ml
SDS	1 %	0.1 %	1 ml
Tris	250 mM	50 mM	2 ml
Protease inhibitor cOmplete	25 x	1x	0.4 ml
WFI			4.5 ml

b. Separation of axonemal lysate from whole cell lysate

Samples were added to Lysing Matrix A Tubes (tubes were washed before with 70 % EthOH and garnet is removed). The tubes were fastened and frozen in liquid nitrogen for 20 seconds while moving the tube. Tubes were quickly inserted into MP FastPrep-24 (HOM-1), settings: 6.5 m/s, 3x 1 min. Subsequently, tubes were left to condense on ice for 15 minutes before transferring the liquid to fresh Eppendorf tubes and spinning at 1100 rpm, 4 °C, for 20 minutes. The supernatant was transferred to new Eppendorf tubes and pellet and supernatant were stored at -20 °C until further processing.

c. Preparation of axonemal extract lysate: High-salt extraction of ciliary axonemal protein

Each pellet was resuspended in 50 µl MMRB + 0.1 % Triton X-100 (for details of the composition of MMRB + 0.1% TritonX-100 see **Table 18**) and incubated on ice for 30 minutes (with occasional gentle vortexing). After spinning at 14,000 rpm at 4 °C for 10 minutes, the supernatant was transferred to new Eppendorf tubes and stored at -20 °C until further processing.

Table 18

Substance	Stock	Final	Volume for 1 ml
HMEN (cf. **Table 19**)	1x	-	982 µl

DTT (e.g. Sigma 646563)	1 M	2 mM	2 µl
β-Mercaptoethanol (e.g. Sigma M3148)	100 %	70 mM	5 µl
TritonX-100	10 %	0.1 %	10 µl
Protease inhibitor cOmplete (Roche/Sigma 11873580001)	25x	1x	40 µl

Table 19

Substance	**Final**
HEPES, pH 7.4	30 mM
MgSO4	5 mM
EDTA	0.1 mM
NaCl	625 mM

d. Preparation of lysates for Western blot

Samples were thawed on ice and 30 µl of sample were transferred to new Eppendorf tubes. 12 µL LDS sample buffer and 5 µl 10x Reducing Agent were added before heating the samples at 70 °C, 350 rpm for 10 minutes. 5, 10 and 30 µl were applied per well to a Bolt™ 4-12% SDS-PAGE gel, 10 well.

e. SDS Page and Blotting method: cf. IV 1 e

f. Antibody and blocking method: cf. IV 1 f

g. Chemiluminescent Signal Development: cf. IV 1 g

II. Results

CCDC40 expression was determined in HEK293 cells after 6 h, 24 h, 48 h, 72 h and 144 h post-transfection. In particular, 2/1.4/0.3/0.2/0.05x10^6 cells were seeded in 6-well plates and cells were transfected 24 h after seeding with different CCDC40 constructs (2.5 µg/9.5 cm^2) using Lipofectamine® 2000. The CCDC40 mRNA constructs used in this experiment were T06 to T10 (**SEQ ID NO: 1**, and **SEQ ID NO: 5** to **SEQ ID NO: 8**). Cell lysis was performed 6, 24, 48, 72 and 144 h after transfection and 50 µg of total protein lysate were analyzed with SDS-PAGE and Western Blot.

As it can be seen in **Figure 1**, a peak translation efficiency was detectable 6 h post-transfection of CCDC40 mRNAs in HEK cells.

The next experiment was performed as described above except that in this case, $2x10^6$ HEK293, $7.5x10^5$ BEAS-2B and $5x10^5$ RPMI 2650 cells were seeded in 6-well plates and protein lysates analyzed with SDS-PAGE and Western Blot (HEK293: 50 µg, RPMI 2650: 20 µg, BEAS-2B: 30 µg of total lysate). CCDC40 was detected using Anti-CCDC40 Antibody (HPA022974) from Atlas Antibodies (1:2000).
Here, it could be observed that T09 (CYBA; **SEQ ID NO: 1**) and T10 (human CMV IE9 and 3' UTR from human Growth hormone **SEQ ID NO: 8**) UTRs led to the highest translation efficiency 6 h post-transfection in BEAS-2B, RPMI2650 and HEK293 cells (**Figure 2**).

Two experiments were then performed to investigate restoration of cilia motility in patient derived ALI cultures. In particular, in the first experiment a non-differentiated ALI culture (data not shown) was used (ciliary cells had a deletion of exon 1 and 2 resulting in very short and less motile cilia), whereas in the second experiment a differentiated ALI culture was used. In both cases, the ALI culture was transfected with 3 µg LF92/CCDC40 (ETH031T09; **SEQ ID NO: 1**) every other day for 1 month for a total of 16 transfections. As readout a high speed video microscopy (HSVM) was performed every 24 h and immune fluorescence immunocytochemistry (IF-ICC). Prior transfection and every 24 h after transfection, videos (20 per insert) were taken and CFB (ciliary beat frequency) was calculated using the SAVA Software. Allover 16 transfections (1 month) were performed. Measurement was done at 37°C using the 40x magnification. Calculated are the mean values of the cilia beat frequency (CBF).

Repeated transfection of LF92/CCDC40 was well tolerated in patient derived fully differentiated ALI cultures. The CCDC40 protein could be detected in naturally occurring subcellular region, i.e. cilia, of airway epithelial cells after 22 d by IF-ICC and cilia motility increased after LF92/CCDC40 transfection until day 18 to ~50% of normal cilia beat frequency as it can be seen in **Figure 3** for the experiment with the differentiated ALI culture.

As described in the Material and Methods section, an undifferentiated ALI culture was obtained using Medium G and transfections (TF) started 18 days post air-lift. Transfections (TFs) of CCDC40 patient ALI culture with CCDC40 mRNA/LF92 were performed once a week for 4 weeks (= 4x TF). Mucociliary clearance (MCC) was measured using 0.5 µm fluorescent beads at 20x magnification. 30 s videos of

different areas are taken and analyzed with the Polargraph software from Nikon one week after the last TF.
Importantly, restoration of cilia beating in CCDC40 patient ALI after four weekly CCDC40 mRNA/LF92 transfections could be detected. Moreover, cilia beating was synchronized, which allowed a directed particle transport as it can be seen in **Figure 4**. For comparison, particle transport is shown in **Figure 5** in case of a control, i.e. fourfold tdTomato mRNA/LF111 transfection, and a healthy control.

Thus, successful CCDC40 mRNA/LF92 TF of patient ALI culture with LF92, as indicated by a comparison with the tdTomato control, during the differentiation phase of the ciliary cells resulted in successful cilia growth, successful cilia beating, successful directed particle transport, and successful protein detection (CCDC39 as binding partner of CCDC40 + GAS8/DNALI1 as part of the DRC complex). Moreover, tdTomato control inserts did not show any of the above mentioned effects.

Figure 6 demonstrates successful incorporation of GAS8 protein within the axonemes after repeated patient ALI treatment with CCDC40 mRNA as in healthy controls which is absent in control (tdTomato mRNA) treated patient ALIs. **Figure 7** demonstrates successful incorporation of DNALI-1 protein within the axonemes after repeated patient ALI treatment with CCDC40 mRNA as in healthy controls which is absent in control (tdTomato mRNA) treated patient ALIs. **Figure 8** demonstrates successful incorporation of CCDC39 protein within the axonemes after repeated patient ALI treatment with CCDC40 mRNA as in healthy controls which is absent in control (tdTomato mRNA) treated patient ALIs.

To summarize, cilia motility could not be restored to a significant level when using the differentiated ALI culture, but partially restored when using the differentiated ALI culture.

With respect to the CCDC39 experiments, CCDC39 proteins could be detected by Western Blot analyses 6 h and 24 h after transfection in HEK-293 and BEAS-2B cells as it can be seen in **Figure 9**. As shown in **Figure 11**, CCDC39 (ETH047T03; **SEQ ID NO: 13**) expression could be detected after 6 h in 16HBE14o- using Proteasome Inhibitor. The same experiment was also performed for ETH047T02 (**SEQ ID NO: 12**), ETH047T04 (**SEQ ID NO: 2**), and ETH047T05 (**SEQ ID NO: 14**) with comparable results. As shown in **Figure 12**, CCDC39 (ETH047T03; **SEQ ID NO: 13**) expression could also be detected after 24 h in 16HBE14o- using Proteasome Inhibitor.

Exemplary sequences described in the application are provided below. The disclosure provides, in some embodiments, polyribonucleotides comprising, for example, the UTR sequences set forth in **SEQ ID NO: 1 or 2**, or a sequence at least 95%, 96%, 97%, 98%, or 99% identical to such sequences, or a polyribonucleotide sequence, such as an mRNA, corresponding to or encoded by any of the foregoing. In certain embodiments of any of the foregoing, the polynucleotide or polyribonucleotide is modified (e.g., comprises nucleotide analogues, as described herein).

SEQ ID NO: 1

CCDC40 sequence with CYBA 5' and 3' UTR (ETH031T09):

Part of the T7 promoter, **Ethris' minimal 5' UTR**, *CYBA 5' UTR*, ***Kozak element***, start codon (AUG),codon optimized sequence encoding a functional version of a human CCDC40 protein, stop codon (UGA), CYBA ***3' UTR***, part of restriction site for BstBI, ^{polyA}: **tail of "A" produced via post polyadenylation of mRNA**

GGGAGA**C***CGCGCCUAGCAGUGUCCCAGCCGGGUUCGUGUCGCC***GCCACC**AUGGCUGAAC
CUGGCGGAGCCGCCGGAAGAUCCCACCCUGAAGAUGGCUCUGCCAGCGAGG
GCGAGAAAGAGGGCAACAACGAGAGCCACAUGGUGUCCCCCCCAGAGAAGGA
CGACGGCCAGAAAGGCGAAGAGGCCGUGGGCUCUACCGAGCACCCUGAGGA
AGUGACCACACAGGCCGAGGCCGCCAUUGAAGAGGGCGAGGUGGAAACAGAG
GGCGAAGCCGCUGUGGAAGGCGAAGAGGAAGCCGUGUCUUACGGCGACGCC
GAGAGCGAGGAAGAGUACUACUACACCGAGACAAGCAGCCCCGAGGGCCAGA
UCUCUGCCGCCGAUACCACCUACCCCUACUUCAGCCCCCCUCAGGAACUGCC
UGGGGAAGAGGCCUACGAUAGCGUGUCCGGCGAAGCUGGCCUGCAGGGCUU
UCAGCAGGAAGCCACAGGCCCUCCCGAGAGCCGGGAAAGAAGAGUGACAAGC
CCCGAGCCUAGCCACGGCGUGCUGGGACCAUCUGAGCAGAUGGGCCAAGUG
ACCUCUGGCCCUGCUGUGGGCAGACUGACAGGCAGCACAGAGGAACCUCAG
GGCCAGGUGCUGCCUAUGGGAGUGCAGCACCGGUUCAGACUGAGCCACGGC
AGCGACAUCGAGAGCAGCGACCUGGAAGAGUUCGUCAGCCAGGAACCCGUGA
UCCCUCCUGGCGUGCCAGAUGCCCAUCCCAGGGAAGGCGAUCUGCCCGUGU
UCCAGGACCAGAUCCAGCAGCCCUCUACCGAAGAGGGGGCUAUGGCCGAGAG
AGUGGAAAGCGAGGGCUCCGACGAAGAAGCCGAGGACGAGGGAUCUCAGCU
GGUGGUGCUGGACCCCGACCACCCUCUGAUGGUGCGGUUUCAGGCCGCCCU
GAAGAACUACCUGAACCGGCAGAUCGAGAAGCUGAAACUGGACCUGCAGGAA
CUGGUGGUGGCCACAAAGCAGAGCAGAGCCCAGAGACAGGAACUGGGCGUG
AACCUGUACGAGGUGCAGCAGCAUCUGGUGCAUCUGCAGAAGCUGCUGGAAA
AGAGCCACGACCGGCACGCCAUGGCCAGCUCUGAGCGCAGACAGAAAGAGGA
AGAACUGCAGGCCGCCAGAGCCCUGUACACCAAGACAUGCGCCGCUGCCAAC

GAGGAACGGAAGAAGCUGGCUGCCCUGCAGACCGAGAUGGAAAACCUGGCUC
UGCACCUGUUCUACAUGCAGAAUAUCGACCAGGACAUGCGGGACGACAUCAG
AGUGAUGACCCAGGUCGUGAAGAAGGCCGAGACAGAGAGAAUCCGGGCCGAG
AUUGAGAAGAAAAAGCAGGACCUGUACGUGGACCAGCUGACCACCAGGGCCC
AGCAGCUGGAAGAGGAUAUCGCCCUGUUCGAGGCCCAGUACCUGGCCCAGG
CCGAAGAUACCCGGAUCCUGAGAAAGGCCGUGUCCGAGGCCUGCACCGAGAU
CGAUGCCAUCAGCGUGGAAAAGCGGCGGAUCAUGCAGCAGUGGGCCAGCAG
CCUCGUGGGCAUGAAGCACAGAGAUGAGGCCCACCGGGCCGUGCUGGAAGC
UCUGAGAGGCUGUCAGCACCAGGCCAAGAGCACCGACGGCGAGAUCGAGGC
CUACAAGAAAUCCAUCAUGAAGGAAGAGGAAAAGAACGAGAAACUGGCCAGCA
UCCUGAACAGAACCGAAACCGAGGCCACCCUGCUGCAGAAACUGACCACCCA
GUGCCUGACCAAACAGGUGGCCCUGCAGUCCCAGUUCAACACCUACAGACUG
ACCCUGCAGGACACCGAGGACGCCCUGAGUCAGGAUCAGCUGGAACAGAUGA
UUCUGACCGAGGAACUGCAGGCUAUCCGGCAGGCCAUUCAGGGGGAGCUGG
AACUGCGGAGAAAGACCGACGCCGCCAUCAGAGAGAAGCUGCAGGAACACAU
GACCAGCAACAAGACCACCAAGUACUUCAACCAGCUGAUUCUGCGCCUGCAG
AAAGAAAAGACCAACAUGAUGACACACCUGAGCAAGAUCAACGGCGACAUUGC
CCAGACCACCCUGGACAUCACCCACACCAGCAGCAGACUGGACGCCCACCAG
AAAACCCUGGUGGAACUGGACCAGGAUGUGAAGAAAGUGAACGAGCUGAUCA
CCAACAGCCAGAGCGAGAUCAGCCGGCGGACCAUCCUGAUCGAGAGAAAGCA
GGGCCUGAUCAACUUCCUGAACAAACAGCUGGAAAGAAUGGUGUCCGAGCUG
GGCGGCGAGGAAGUGGGACCUCUGGAACUGGAAAUCAAGCGGCUGAGCAAG
CUGAUCGACGAGCACGACGGCAAGGCCGUGCAGGCUCAAGUGACAUGGCUG
CGGCUGCAGCAGGAAAUGGUCAAAGUGACCCAGGAACAGGAAGAACAGCUGG
CCUCCCUGGACGCCAGCAAGAAAGAACUGCACAUCAUGGAACAGAAAAAGCU
GCGGGUGGAAAGCAAGAUCGAGCAGGAAAAAAAAGAACAGAAAGAAAUCGAGC
ACCACAUGAAGGACCUGGACAACGACCUGAAGAAACUGAAUAUGCUGAUGAAC
AAGAACCGCUGCUCCAGCGAAGAACUGGAACAGAACAACAGAGUGACCGAGA
ACGAGUUCGUGCGGAGCCUGAAGGCCAGCGAGCGGGAAACCAUCAAGAUGCA
GGACAAGCUGAACCAGCUGUCCGAGGAAAAAGCCACACUGCUGAACCAGCUG
GUGGAAGCCGAGCACCAGAUCAUGCUGUGGGAGAAGAAGAUCCAGCUGGCCA
AAGAAAUGCGGAGCAGCGUGGACAGCGAGAUCGGCCAGACCGAAAUCAGAGC
CAUGAAGGGCGAGAUCCACCGGAUGAAAGUGCGGCUGGGACAGCUGCUGAAA
CAGCAGGAAAAGAUGAUCCGGGCCAUGGAACUGGCCGUGGCCAGACGGGAAA
CCGUGACAACCCAGGCUGAGGGCCAGCGGAAGAUGGACAGAAAGGCCCUGAC
CCGGACCGACUUCCACCACAAGCAGCUGGAACUGAGGCGGAAGAUCCGGGAC
GUGCGGAAGGCCACCGAUGAGUGCACAAAGACAGUGCUGGAACUGGAAGAGA
CACAGCGGAACGUGUCCUCCAGCCUGCUGGAAAAACAGGAAAAGCUGAGCGU
GAUCCAGGCCGACUUCGACACCCUGGAAGCUGACCUGACAAGACUGGGAGCC
CUGAAAAGACAGAACCUGUCCGAGAUCGUGGCACUGCAGACCCGGCUGAAAC
AUCUGCAGGCUGUGAAAGAGGGACGCUACGUGUUCCUGUUCAGAUCCAAGCA
GUCUCUGGUGCUGGAAAGACAGCGGCUGGACAAGCGGCUGGCACUGAUUGC
CACCAUCCUGGAUAGAGUGCGCGACGAGUACCCACAGUUCCAGGAAGCACUG
CACAAGGUGUCCCAGAUGAUCGCCAACAAGCUGGAAUCCCCUGGCCCCAGCU

GA*CCUCGCCCCGGACCUGCCCUCCCGCCAGGUGCACCCACCUGCAAUAAAUGCAGCGAAGCC GGGA*UUCG-polyA

SEQ ID NO: 2

CCDC39 sequence with CYBA UTRs (ETH047T04):

Part of the T7 promoter, **Ethris' minimal 5' UTR**, CYBA *5' UTR*, ***Kozak element***, start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC39 protein, stop codon (UGA), CYBA ***3' UTR***, part of restriction site for BstBI, poly(A) tail

GGGAGA**C***CGCGCCUAGCAGUGUCCCAGCCGGGUUCGUGUCGCC***GCCACC**AUGAGCAGCG
AGUUUCUGGCCGAACUGCACUGGGAGGACGGCUUCGCUAUUCCCGUGGCCA
ACGAGGAAAACAAGCUGCUGGAAGAUCAGCUGAGCAAGCUGAAGGACGAGAG
AGCCUCUCUGCAGGACGAGCUGAGAGAGUACGAGGAACGGAUCAACAGCAUG
ACCAGCCACUUCAAGAACGUGAAGCAAGAGCUGAGCAUCACCCAGAGCCUGU
GCAAGGCCAGAGAGAGAGAAACCGAGAGCGAGGAACACUUCAAGGCUAUCGC
CCAGCGCGAGCUGGGAAGAGUGAAGGAUGAGAUCCAGCGGCUGGAAAACGA
GAUGGCCAGCAUCCUGGAAAAGAAGUCCGACAAAGAGAACGGCAUCUUCAAG
GCCACACAGAAGCUGGACGGCCUGAAGUGCCAGAUGAACUGGGAUCAGCAGG
CCCUGGAAGCCUGGCUGGAAGAGUCUGCCCACAAGGAUUCUGACGCCCUGAC
ACUGCAGAAGUACGCCCAGCAGGACGACAACAAGAUCCGGGCUCUGACCCUG
CAGCUGGAAAGACUGACCCUGGAAUGCAACCAGAAGCGGAAGAUCCUGGACA
ACGAGCUGACCGAGACAAUCAGCGCCCAGCUGGAACUGGAUAAGGCCGCUCA
GGACUUCAGAAAGAUCCACAACGAGCGGCAAGAACUGAUCAAGCAGUGGGAG
AACACCAUCGAGCAGAUGCAGAAACGCGACGGCGACAUCGACAACUGCGCCC
UGGAACUCGCCCGGAUCAAGCAAGAGACACGCGAGAAAGAGAACCUGGUCAA
AGAGAAGAUCAAGUUCCUCGAGUCCGAGAUCGGCAACAACACCGAGUUCGAG
AAGCGGAUCAGCGUGGCCGACAGAAAGCUGCUGAAGUGCAGAACCGCCUACC
AGGACCACGAGACAAGCCGGAUUCAGCUCAAGGGCGAGCUGGAUUCUCUGAA
GGCCACCGUGAACAGAACCAGCAGCGAUCUGGAAGCCCUGCGGAAGAACAUC
AGCAAGAUCAAGAAGGACAUCCACGAGGAAACCGCCAGGCUGCAGAAAACAAA
GAACCACAAUGAGAUCAUCCAGACCAAGCUGAAAGAGAUCACCGAAAAGACCA
UGAGCGUGGAAGAGAAGGCCACAAACCUGGAAGAUAUGCUCAAAGAGGAAGA
GAAAGACGUCAAAGAGGUGGACGUUCAACUGAACCUGAUUAAGGGCGUGCUG
UUCAAGAAGGCCCAAGAGCUGCAGACCGAAACCAUGAAGGAAAAGGCCGUCC
UGUCUGAGAUCGAGGGCACCAGAUCUAGCCUGAAGCACCUGAACCAUCAGCU
GCAGAAGCUCGACUUCGAGACACUGAAGCAGCAAGAGAUCAUGUACAGCCAG
GAUUUCCACAUCCAGCAGGUCGAGCGGCGGAUGUCUAGACUGAAGGGCGAG
AUCAACUCCGAGGAAAAACAGGCCCUCGAGGCCAAGAUCGUGGAACUGAGAA
AGAGCCUCGAAGAGAAGAAGUCUACCUGCGGCCUGCUGGAAACCCAGAUUAA
GAAGCUGCACAACGACCUGUACUUCAUCAAGAAAGCCCACAGCAAGAACAGCG
ACGAGAAGCAGAGCCUGAUGACCAAGAUCAAUGAGCUGAACCUGUUCAUCGA

UCGGAGCGAAAAAGAGCUGGACAAGGCCAAGGGCUUCAAGCAGGACCUGAUG
AUCGAGGACAACCUGCUGAAGCUGGAAGUGAAGCGGACCAGAGAGAUGCUGC
ACAGCAAGGCCGAGGAAGUGCUGUCUCUGGAAAAGCGGAAGCAGCAGCUGUA
CACCGCCAUGGAAGAGAGAACCGAAGAGAUCAAGGUGCACAAGACCAUGCUG
GCUUCCCAGAUCAGAUACGUGGACCAAGAGCGCGAGAACAUCUCCACCGAGU
UUAGAGAGAGACUGUCCAAGAUCGAGAAGCUGAAGAACCGCUACGAGAUCCU
GACCGUCGUGAUGCUGCCUCCUGAGGGCGAAGAGGAAAAGACCCAGGCCUAC
UACGUGAUCAAGGCAGCCCAAGAAAAAGAGGAACUCCAGAGAGAAGGCGACU
GCCUGGACGCCAAGAUUAACAAGGCCGAAAAAGAAAUCUACGCCCUCGAGAA
CACCCUGCAGGUCCUGAACAGCUGCAACAACAACUACAAGCAGAGCUUCAAGA
AAGUCACCCCUAGCUCCGACGAGUACGAGCUGAAGAUUCAGCUGGAAGAACA
GAAAAGAGCCGUGGACGAGAAGUACAGAUACAAGCAGCGGCAGAUCAGAGAG
CUGCAAGAGGAUAUCCAGAGCAUGGAAAACACCCUGGACGUGAUCGAGCACC
UGGCCAACAACGUGAAAGAGAAGCUGUCCGAGAAACAGGCCUACAGCUUUCA
GCUGUCCAAAGAGACAGAGGAACAGAAGCCCAAACUGGAACGCGUGACCAAG
CAGUGCGCCAAGCUGACAAAAGAGAUCCGGCUGCUGAAAGACACCAAGGACG
AAACCAUGGAAGAACAAGACAUCAAGCUGCGCGAGAUGAAGCAGUUCCACAAA
GUGAUCGACGAGAUGCUGGUGGACAUCAUUGAAGAGAACACAGAGAUCCGCA
UCAUCCUGCAGACCUAUUUCAGCAGAGCGGCCUGGAACUGCCUACCGCCUC
UACAAAGGGCAGCAGACAGAGCAGCAGAUCCCCUAGCCACACAAGCCUGAGC
GCCAGAAGCUCUAGAAGCACCAGCACCUCUACCAGCCAGUCCAGCAUUAAGG
UGCUGGAACUCAAGUUCCCCGCCAGCUCUAGCCUCGUGGGAAGCCCUUCUAG
ACCUAGCAGCGCCUCUAGCAGCUCCAGCAACGUGAAGUCCAAGAAAGCUCC
AAGUGA***ccUcgccccggaccUgcccUcccgccaggUgcacccaccUgcaaUaaaUgcagcg AAGCCGGGA***UUCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 3

tdTomato:

Part of the T7 promoter, **Ethris' minimal 5' UTR**, vector backbone sequence (pVAX Vektor: Life Technologies), ***Kozak element, start codon (AUG),*** codon optimized sequence encoding a functional version of a tdTomato protein, ***stop codon (UAG)*** , part of restriction site for NotI, *part of the vector backbone sequence (pVAX Vektor: Life Technologies)*

GGGAGA**C**CCAAGCUGGCUAGCGUUUAAACUUAAGCUUGGUACCGCGGGCCCGGGAUCCACCGGUC***GCCACC***AU
GGUGAGCAAGGGCGAGGAGGUCAUCAAAGAGUUCAUGCGCUUCAAGGUGCG
CAUGGAGGGCUCCAUGAACGGCCACGAGUUCGAGAUCGAGGGCGAGGGCGA
GGGCCGCCCCUACGAGGGCACCCAGACCGCCAAGCUGAAGGUGACCAAGGG
CGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCCCCCAGUUCAUGUACGG
CUCCAAGGCGUACGUGAAGCACCCCGCCGACAUCCCCGAUUACAAGAAGCUG
UCCUUCCCCGAGGGCUUCAAGUGGGAGCGCGUGAUGAACUUCGAGGACGGC
GGUCUGGUGACCGUGACCCAGGACUCCUCCCUGCAGGACGGCACGCUGAUC

UACAAGGUGAAGAUGCGCGGCACCAACUUCCCCCCGACGGCCCCGUAAUGC
AGAAGAAGACCAUGGGCUGGGAGGCCUCCACCGAGCGCCUGUACCCCCGCG
ACGGCGUGCUGAAGGGCGAGAUCCACCAGGCCCUGAAGCUGAAGGACGGCG
GCCACUACCUGGUGGAGUUCAAGACCAUCUACAUGGCCAAGAAGCCCGUGCA
ACUGCCCGGCUACUACUACGUGGACACCAAGCUGGACAUCACCUCCCACAAC
GAGGACUACACCAUCGUGGAACAGUACGAGCGCUCCGAGGGCCGCCACCACC
UGUUCCUGGGGCAUGGCACCGGCAGCACCGGCAGCGGCAGCUCCGGCACCG
CCUCCUCCGAGGACAACAACAUGGCCGUCAUCAAAGAGUUCAUGCGCUUCAA
GGUGCGCAUGGAGGGCUCCAUGAACGGCCACGAGUUCGAGAUCGAGGGCGA
GGGCGAGGGCCGCCCCUACGAGGGCACCCAGACCGCCAAGCUGAAGGUGAC
CAAGGGCGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCCCCCAGUUCAU
GUACGGCUCCAAGGCGUACGUGAAGCACCCCGCCGACAUCCCCGAUUACAAG
AAGCUGUCCUUCCCCGAGGGCUUCAAGUGGGAGCGCGUGAUGAACUUCGAG
GACGGCGGUCUGGUGACCGUGACCCAGGACUCCUCCCUGCAGGACGGCACG
CUGAUCUACAAGGUGAAGAUGCGCGGCACCAACUUCCCCCCGACGGCCCCG
UAAUGCAGAAGAAGACCAUGGGCUGGGAGGCCUCCACCGAGCGCCUGUACCC
CCGCGACGGCGUGCUGAAGGGCGAGAUCCACCAGGCCCUGAAGCUGAAGGA
CGGCGGCCACUACCUGGUGGAGUUCAAGACCAUCUACAUGGCCAAGAAGCCC
GUGCAACUGCCCGGCUACUACUACGUGGACACCAAGCUGGACAUCACCUCCC
ACAACGAGGACUACACCAUCGUGGAACAGUACGAGCGCUCCGAGGGCCGCCA
CCACCUGUUCCUGUACGGCAUGGACGAGCUGUACAAGUAG*GCGGCCAAUUCUGCAGAA*
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
*AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA*GCGGCC

SEQ ID NO: 4

MCIDAS sequence with Ethris' minimal 5' UTR and optional 3' UTR:

Part of the T7 promoter, **Ethris' minimal 5' UTR**, ***Kozak element,*** start codon (AUG), codon optimized sequence encoding a functional version of a human MCIDAS protein, stop codon (UGA), ***optional 3' UTR*** (also functions as reverse primer binding site for PCR based template production) , restriction site for NheI, poly(A) tail

GGGAGA***CGCCACC***AUGCAAGCUUGUGGCGGCGGAGCUGCUGGAAGAAGGGCCU
UCGAUAGCAUCUGCCCCAACCGGAUGCUUGCCCUUCCUGGUAGAGCCCUGCU
GUGCAAGCCUGGCAAGCCCGAGAGAAAGUUCGCCCCUCCAAGAAAGUUCUUC
CCCGGCUGUACUGGCGGCAGCCCUGUGUCUGUGUAUGAGGACCCUCCUGAU
GCCGAGCCUACAGCUCUGCCUGCUCUGACCACAAUCGACCUGCAGGAUCUGG
CCGAUUGCAGCUCUCUGCUGGGAUCUGAUGCUCCUCCUGGCGGAGAUCUGG
CUGCCUCUCAGAAUCACAGCCACCAGACAGAGGCCGACUUCAACCUGCAAGA
CUUCCGGGACACCGUGGACGACCUGAUCAGCGAUAGCAGCAGCAUGAUGAGC
CCCACUCUGGCCAGCGGCGAUUUCCCAUUCAGCCCCUGUGACAUCAGCCCUU
UCGGCCCUUGUCUGAGCCCUCCACUGGAUCCUAGAGCACUGCAGAGCCCACC
UCUGAGGCCUCCAGAUGUUCCUCCACCUGAGCAGUACUGGAAAGAGGUGGCC

GACCAGAACCAGAGAGCACUGGGCGACGCUCUGGUGGAAAACAACCAGCUGC
ACGUGACCCUGACACAGAAGCAAGAAGAGAUCGCCAGCCUGAAAGAACGGAA
UGUGCAGCUGAAAGAGCUGGCCUCCAGGACAAGACACCUGGCCAGUGUGCU
GGACAAGCUGAUGAUCACCCAGAGCAGAGAUUGCGGAGCCGCCGCUGAACCU
UUUCUGCUGAAGGCCAAGGCCAAGAGAAGCCUGGAAGAACUGGUGUCUGCCG
CCGGACAGGAUUGCGCUGAAGUGGAUGCCAUCCUGCGCGAGAUCAGCGAGA
GAUGUGAUGAGGCCCUGCAGAGCAGGGACCCCAAAAGACCUAGACUGCUGCC
CGAGCCUGCCAACACCGAUACCAGACCUGGAAAUCUGCACGGCGCCUUCAGA
GGCCUGAGAACCGAUUGCUCUAGAAGCGCCCUGAACCUGAGCCACAGCGAAC
UCGAAGAAGGCGGCAGCUUCAGCACCCGGAUCAGAAGCCACAGCACCAUCAG
AACCCUGGCCUUUCCACAGGGCAACGCCUUCACAAUCAGAACCGCCAACGGC
GGCUACAAGUUCAGAUGGGUGCCAAGCUGAGCUAGC***CACCGGGCAAUACGA***
GCUCAAGCCAGUCUCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 5

Sequence with Ethris' minimal 5' UTR (ETH031T06):

Part of the T7 promoter, **Ethris' minimal 5' UTR**, ***Kozak element,*** start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC40 protein, stop codon (UGA), part of restriction site for BstBI, polyA: **tail of "A" produced via post polyadenylation of mRNA**

GGGAGA**C*GCCACC***AUGGCUGAACCUGGCGGAGCCGCCGGAAGAUCCCACCCUG
AAGAUGGCUCUGCCAGCGAGGGCGAGAAAGAGGGCAACAACGAGAGCCACAU
GGUGUCCCCCCCAGAGAAGGACGACGGCCAGAAAGGCGAAGAGGCCGUGGG
CUCUACCGAGCACCCUGAGGAAGUGACCACACAGGCCGAGGCCGCCAUUGAA
GAGGGCGAGGUGGAAACAGAGGGCGAAGCCGCUGUGGAAGGCGAAGAGGAA
GCCGUGUCUUACGGCGACGCCGAGAGCGAGGAAGAGUACUACUACACCGAGA
CAAGCAGCCCCGAGGGCCAGAUCUCUGCCGCCGAUACCACCUACCCCUACUU
CAGCCCCCCUCAGGAACUGCCUGGGGAAGAGGCCUACGAUAGCGUGUCCGG
CGAAGCUGGCCUGCAGGGCUUUCAGCAGGAAGCCACAGGCCCUCCCGAGAG
CCGGGAAAGAAGAGUGACAAGCCCCGAGCCUAGCCACGGCGUGCUGGGACCA
UCUGAGCAGAUGGGCCAAGUGACCUCUGGCCCUGCUGUGGGCAGACUGACA
GGCAGCACAGAGGAACCUCAGGGCCAGGUGCUGCCUAUGGGAGUGCAGCAC
CGGUUCAGACUGAGCCACGGCAGCGACAUCGAGAGCAGCGACCUGGAAGAGU
UCGUCAGCCAGGAACCCGUGAUCCCUCCUGGCGUGCCAGAUGCCCAUCCCAG
GGAAGGCGAUCUGCCCGUGUUCCAGGACCAGAUCCAGCAGCCCUCUACCGAA
GAGGGGGCUAUGGCCGAGAGAGUGGAAAGCGAGGGCUCCGACGAAGAAGCC
GAGGACGAGGGAUCUCAGCUGGUGGUGCUGGACCCCGACCACCCUCUGAUG
GUGCGGUUUCAGGCCGCCCUGAAGAACUACCUGAACCGGCAGAUCGAGAAGC
UGAAACUGGACCUGCAGGAACUGGUGGUGGCCACAAAGCAGAGCAGAGCCCA
GAGACAGGAACUGGGCGUGAACCUGUACGAGGUGCAGCAGCAUCUGGUGCA

UCUGCAGAAGCUGCUGGAAAAGAGCCACGACCGGCACGCCAUGGCCAGCUCU
GAGCGCAGACAGAAAGAGGAAGAACUGCAGGCCGCCAGAGCCCUGUACACCA
AGACAUGCGCCGCUGCCAACGAGGAACGGAAGAAGCUGGCUGCCCUGCAGAC
CGAGAUGGAAAACCUGGCUCUGCACCUGUUCUACAUGCAGAAUAUCGACCAG
GACAUGCGGGACGACAUCAGAGUGAUGACCCAGGUCGUGAAGAAGGCCGAGA
CAGAGAGAAUCCGGGCCGAGAUUGAGAAGAAAAAGCAGGACCUGUACGUGGA
CCAGCUGACCACCAGGGCCCAGCAGCUGGAAGAGGAUAUCGCCCUGUUCGAG
GCCCAGUACCUGGCCCAGGCCGAAGAUACCCGGAUCCUGAGAAAGGCCGUGU
CCGAGGCCUGCACCGAGAUCGAUGCCAUCAGCGUGGAAAAGCGGCGGAUCAU
GCAGCAGUGGGCCAGCAGCCUCGUGGGCAUGAAGCACAGAGAUGAGGCCCA
CCGGGCCGUGCUGGAAGCUCUGAGAGGCUGUCAGCACCAGGCCAAGAGCAC
CGACGGCGAGAUCGAGGCCUACAAGAAAUCCAUCAUGAAGGAAGAGGAAAAG
AACGAGAAACUGGCCAGCAUCCUGAACAGAACCGAAACCGAGGCCACCCUGC
UGCAGAAACUGACCACCCAGUGCCUGACCAAACAGGUGGCCCUGCAGUCCCA
GUUCAACACCUACAGACUGACCCUGCAGGACACCGAGGACGCCCUGAGUCAG
GAUCAGCUGGAACAGAUGAUUCUGACCGAGGAACUGCAGGCUAUCCGGCAGG
CCAUUCAGGGGGAGCUGGAACUGCGGAGAAAGACCGACGCCGCCAUCAGAGA
GAAGCUGCAGGAACACAUGACCAGCAACAAGACCACCAAGUACUUCAACCAGC
UGAUUCUGCGCCUGCAGAAAGAAAAGACCAACAUGAUGACACACCUGAGCAA
GAUCAACGGCGACAUUGCCCAGACCACCCUGGACAUCACCCACACCAGCAGC
AGACUGGACGCCCACCAGAAAACCCUGGUGGAACUGGACCAGGAUGUGAAGA
AAGUGAACGAGCUGAUCACCAACAGCCAGAGCGAGAUCAGCCGGCGGACCAU
CCUGAUCGAGAGAAAGCAGGGCCUGAUCAACUUCCUGAACAAACAGCUGGAA
AGAAUGGUGUCCGAGCUGGGCGGCGAGGAAGUGGGACCUCUGGAACUGGAA
AUCAAGCGGCUGAGCAAGCUGAUCGACGAGCACGACGGCAAGGCCGUGCAG
GCUCAAGUGACAUGGCUGCGGCUGCAGCAGGAAAUGGUCAAAGUGACCCAGG
AACAGGAAGAACAGCUGGCCUCCCUGGACGCCAGCAAGAAAGAACUGCACAU
CAUGGAACAGAAAAAGCUGCGGGUGGAAAGCAAGAUCGAGCAGGAAAAAAAA
GAACAGAAAGAAAUCGAGCACCACAUGAAGGACCUGGACAACGACCUGAAGAA
ACUGAAUAUGCUGAUGAACAAGAACCGCUGCUCCAGCGAAGAACUGGAACAG
AACAACAGAGUGACCGAGAACGAGUUCGUGCGGAGCCUGAAGGCCAGCGAGC
GGGAAACCAUCAAGAUGCAGGACAAGCUGAACCAGCUGUCCGAGGAAAAAGC
CACACUGCUGAACCAGCUGGUGGAAGCCGAGCACCAGAUCAUGCUGUGGGAG
AAGAAGAUCCAGCUGGCCAAAGAAAUGCGGAGCAGCGUGGACAGCGAGAUCG
GCCAGACCGAAAUCAGAGCCAUGAAGGGCGAGAUCCACCGGAUGAAAGUGCG
GCUGGGACAGCUGCUGAAACAGCAGGAAAAGAUGAUCCGGGCCAUGGAACUG
GCCGUGGCCAGACGGGAAACCGUGACAACCCAGGCUGAGGGCCAGCGGAAG
AUGGACAGAAAGGCCCUGACCCGGACCGACUUCCACCACAAGCAGCUGGAAC
UGAGGCGGAAGAUCCGGGACGUGCGGAAGGCCACCGAUGAGUGCACAAAGA
CAGUGCUGGAACUGGAAGAGACACAGCGGAACGUGUCCUCCAGCCUGCUGGA
AAAACAGGAAAAGCUGAGCGUGAUCCAGGCCGACUUCGACACCCUGGAAGCU
GACCUGACAAGACUGGGAGCCCUGAAAAGACAGAACCUGUCCGAGAUCGUGG
CACUGCAGACCCGGCUGAAACAUCUGCAGGCUGUGAAAGAGGGACGCUACGU
GUUCCUGUUCAGAUCCAAGCAGUCUCUGGUGCUGGAAAGACAGCGGCUGGA
CAAGCGGCUGGCACUGAUUGCCACCAUCCUGGAUAGAGUGCGCGACGAGUAC

CCACAGUUCCAGGAAGCACUGCACAAGGUGUCCCAGAUGAUCGCCAACAAGC
UGGAAUCCCCUGGCCCCAGCUGAUUCG-polyA

SEQ ID NO: 6

Sequence with TISU 5' UTR (ETH031T07):

Part of the T7 promoter, **Ethris' minimal 5' UTR with additional U nucleotide**, ***TISU element,*** start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC40 protein, stop codon (UGA), part of restriction site for BstBI, **polyA: tail of "A" produced via post polyadenylation of mRNA**

GGGAGA**CU*GCCAAG***AUGGCUGAACCUGGCGGAGCCGCCGGAAGAUCCCACCCU
GAAGAUGGCUCUGCCAGCGAGGGCGAGAAAGAGGGCAACAACGAGAGCCACA
UGGUGUCCCCCCCCAGAGAAGGACGACGGCCAGAAAGGCGAAGAGGCCGUGG
GCUCUACCGAGCACCCUGAGGAAGUGACCACACAGGCCGAGGCCGCCAUUGA
AGAGGGCGAGGUGGAAACAGAGGGCGAAGCCGCUGUGGAAGGCGAAGAGGA
AGCCGUGUCUUACGGCGACGCCGAGAGCGAGGAAGAGUACUACUACACCGAG
ACAAGCAGCCCCGAGGGCCAGAUCUCUGCCGCCGAUACCACCUACCCCUACU
UCAGCCCCCCCUCAGGAACUGCCUGGGGAAGAGGCCUACGAUAGCGUGUCCG
GCGAAGCUGGCCUGCAGGGCUUUCAGCAGGAAGCCACAGGCCCUCCCGAGA
GCCGGGAAAGAAGAGUGACAAGCCCCGAGCCUAGCCACGGCGUGCUGGGAC
CAUCUGAGCAGAUGGGCCAAGUGACCUCUGGCCCUGCUGUGGGCAGACUGA
CAGGCAGCACAGAGGAACCUCAGGGCCAGGUGCUGCCUAUGGGAGUGCAGC
ACCGGUUCAGACUGAGCCACGGCAGCGACAUCGAGAGCAGCGACCUGGAAGA
GUUCGUCAGCCAGGAACCCGUGAUCCCUCCUGGCGUGCCAGAUGCCCAUCC
CAGGGAAGGCGAUCUGCCCGUGUUCCAGGACCAGAUCCAGCAGCCCUCUACC
GAAGAGGGGGCUAUGGCCGAGAGAGUGGAAAGCGAGGGCUCCGACGAAGAA
GCCGAGGACGAGGGAUCUCAGCUGGUGGUGCUGGACCCCGACCACCCUCUG
AUGGUGCGGUUUCAGGCCGCCCUGAAGAACUACCUGAACCGGCAGAUCGAGA
AGCUGAAACUGGACCUGCAGGAACUGGUGGUGGCCACAAAGCAGAGCAGAGC
CCAGAGACAGGAACUGGGCGUGAACCUGUACGAGGUGCAGCAGCAUCUGGU
GCAUCUGCAGAAGCUGCUGGAAAAGAGCCACGACCGGCACGCCAUGGCCAGC
UCUGAGCGCAGACAGAAAGAGGAAGAACUGCAGGCCGCCAGAGCCCUGUACA
CCAAGACAUGCGCCGCUGCCAACGAGGAACGGAAGAAGCUGGCUGCCCUGCA
GACCGAGAUGGAAAACCUGGCUCUGCACCUGUUCUACAUGCAGAAUAUCGAC
CAGGACAUGCGGGACGACAUCAGAGUGAUGACCCAGGUCGUGAAGAAGGCCG
AGACAGAGAGAAUCCGGGCCGAGAUUGAGAAGAAAAAGCAGGACCUGUACGU
GGACCAGCUGACCACCAGGGCCCAGCAGCUGGAAGAGGAUAUCGCCCUGUUC
GAGGCCCAGUACCUGGCCCAGGCCGAAGAUACCCGGAUCCUGAGAAAGGCCG
UGUCCGAGGCCUGCACCGAGAUCGAUGCCAUCAGCGUGGAAAAGCGGCGGA
UCAUGCAGCAGUGGGCCAGCAGCCUCGUGGGCAUGAAGCACAGAGAUGAGG
CCCACCGGGCCGUGCUGGAAGCUCUGAGAGGCUGUCAGCACCAGGCCAAGA
GCACCGACGGCGAGAUCGAGGCCUACAAGAAAUCCAUCAUGAAGGAAGAGGA

AAAGAACGAGAAACUGGCCAGCAUCCUGAACAGAACCGAAACCGAGGCCACCC
UGCUGCAGAAACUGACCACCCAGUGCCUGACCAAACAGGUGGCCCUGCAGUC
CCAGUUCAACACCUACAGACUGACCCUGCAGGACACCGAGGACGCCCUGAGU
CAGGAUCAGCUGGAACAGAUGAUUCUGACCGAGGAACUGCAGGCUAUCCGGC
AGGCCAUUCAGGGGGAGCUGGAACUGCGGAGAAAGACCGACGCCGCCAUCA
GAGAGAAGCUGCAGGAACACAUGACCAGCAACAAGACCACCAAGUACUUCAAC
CAGCUGAUUCUGCGCCUGCAGAAAGAAAAGACCAACAUGAUGACACACCUGA
GCAAGAUCAACGGCGACAUUGCCCAGACCACCCUGGACAUCACCCACACCAG
CAGCAGACUGGACGCCCACCAGAAAACCCUGGUGGAACUGGACCAGGAUGUG
AAGAAAGUGAACGAGCUGAUCACCAACAGCCAGAGCGAGAUCAGCCGGCGGA
CCAUCCUGAUCGAGAGAAAGCAGGGCCUGAUCAACUUCCUGAACAAACAGCU
GGAAAGAAUGGUGUCCGAGCUGGGCGGCGAGGAAGUGGGACCUCUGGAACU
GGAAAUCAAGCGGCUGAGCAAGCUGAUCGACGAGCACGACGGCAAGGCCGUG
CAGGCUCAAGUGACAUGGCUGCGGCUGCAGCAGGAAAUGGUCAAAGUGACCC
AGGAACAGGAAGAACAGCUGGCCUCCCUGGACGCCAGCAAGAAAGAACUGCA
CAUCAUGGAACAGAAAAAGCUGCGGGUGGAAAGCAAGAUCGAGCAGGAAAAA
AAAGAACAGAAAGAAAUCGAGCACCACAUGAAGGACCUGGACAACGACCUGAA
GAAACUGAAUAUGCUGAUGAACAAGAACCGCUGCUCCAGCGAAGAACUGGAA
CAGAACAACAGAGUGACCGAGAACGAGUUCGUGCGGAGCCUGAAGGCCAGCG
AGCGGGAAACCAUCAAGAUGCAGGACAAGCUGAACCAGCUGUCCGAGGAAAA
AGCCACACUGCUGAACCAGCUGGUGGAAGCCGAGCACCAGAUCAUGCUGUGG
GAGAAGAAGAUCCAGCUGGCCAAAGAAAUGCGGAGCAGCGUGGACAGCGAGA
UCGGCCAGACCGAAAUCAGAGCCAUGAAGGGCGAGAUCCACCGGAUGAAAGU
GCGGCUGGGACAGCUGCUGAAACAGCAGGAAAAGAUGAUCCGGGCCAUGGAA
CUGGCCGUGGCCAGACGGGAAACCGUGACAACCCAGGCUGAGGGCCAGCGG
AAGAUGGACAGAAAGGCCCUGACCCGGACCGACUUCCACCACAAGCAGCUGG
AACUGAGGCGGAAGAUCCGGGACGUGCGGAAGGCCACCGAUGAGUGCACAAA
GACAGUGCUGGAACUGGAAGAGACACAGCGGAACGUGUCCUCCAGCCUGCUG
GAAAAACAGGAAAAGCUGAGCGUGAUCCAGGCCGACUUCGACACCCUGGAAG
CUGACCUGACAAGACUGGGAGCCCUGAAAAGACAGAACCUGUCCGAGAUCGU
GGCACUGCAGACCCGGCUGAAACAUCUGCAGGCUGUGAAAGAGGGACGCUAC
GUGUUCCUGUUCAGAUCCAAGCAGUCUCUGGUGCUGGAAAGACAGCGGCUG
GACAAGCGGCUGGCACUGAUUGCCACCAUCCUGGAUAGAGUGCGCGACGAG
UACCCACAGUUCCAGGAAGCACUGCACAAGGUGUCCCAGAUGAUCGCCAACA
AGCUGGAAUCCCCUGGCCCCAGCUGAUUCG-polyA

SEQ ID NO: 7

Sequence with hAg 5' UTR but without 3' UTR (ETH031T08):

Part of the T7 promoter, **Ethris' minimal 5' UTR**, hAg *5'UTR*, ***Kozak element***, start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC40 protein, stop codon (UGA), part of restriction site for BstBI, polyA: **tail of "A" produced via post polyadenylation of mRNA**

GGGAGA*CUCUUCUGGUCCCCACAGACUCAGAGAGAAC**GCCACC*AUGGCUGAACC
UGGCGGAGCCGCCGGAAGAUCCCACCCUGAAGAUGGCUCUGCCAGCGAGGG
CGAGAAAGAGGGCAACAACGAGAGCCACAUGGUGUCCCCCCCAGAGAAGGAC
GACGGCCAGAAAGGCGAAGAGGCCGUGGGCUCUACCGAGCACCCUGAGGAA
GUGACCACACAGGCCGAGGCCGCCAUUGAAGAGGGCGAGGUGGAAACAGAG
GGCGAAGCCGCUGUGGAAGGCGAAGAGGAAGCCGUGUCUUACGGCGACGCC
GAGAGCGAGGAAGAGUACUACUACACCGAGACAAGCAGCCCCGAGGGCCAGA
UCUCUGCCGCCGAUACCACCUACCCCUACUUCAGCCCCCCUCAGGAACUGCC
UGGGGAAGAGGCCUACGAUAGCGUGUCCGGCGAAGCUGGCCUGCAGGGCUU
UCAGCAGGAAGCCACAGGCCCUCCCGAGAGCCGGGAAAGAAGAGUGACAAGC
CCCGAGCCUAGCCACGGCGUGCUGGGACCAUCUGAGCAGAUGGGCCAAGUG
ACCUCUGGCCCUGCUGUGGGCAGACUGACAGGCAGCACAGAGGAACCUCAG
GGCCAGGUGCUGCCUAUGGGAGUGCAGCACCGGUUCAGACUGAGCCACGGC
AGCGACAUCGAGAGCAGCGACCUGGAAGAGUUCGUCAGCCAGGAACCCGUGA
UCCCUCCUGGCGUGCCAGAUGCCCAUCCCAGGGAAGGCGAUCUGCCCGUGU
UCCAGGACCAGAUCCAGCAGCCCUCUACCGAAGAGGGGGCUAUGGCCGAGAG
AGUGGAAAGCGAGGGCUCCGACGAAGAAGCCGAGGACGAGGGAUCUCAGCU
GGUGGUGCUGGACCCCGACCACCCUCUGAUGGUGCGGUUUCAGGCCGCCCU
GAAGAACUACCUGAACCGGCAGAUCGAGAAGCUGAAACUGGACCUGCAGGAA
CUGGUGGUGGCCACAAAGCAGAGCAGAGCCCAGAGACAGGAACUGGGCGUG
AACCUGUACGAGGUGCAGCAGCAUCUGGUGCAUCUGCAGAAGCUGCUGGAAA
AGAGCCACGACCGGCACGCCAUGGCCAGCUCUGAGCGCAGACAGAAAGAGGA
AGAACUGCAGGCCGCCAGAGCCCUGUACACCAAGACAUGCGCCGCUGCCAAC
GAGGAACGGAAGAAGCUGGCUGCCCUGCAGACCGAGAUGGAAAACCUGGCUC
UGCACCUGUUCUACAUGCAGAAUAUCGACCAGGACAUGCGGGACGACAUCAG
AGUGAUGACCCAGGUCGUGAAGAAGGCCGAGACAGAGAGAAUCCGGGCCGAG
AUUGAGAAGAAAAAGCAGGACCUGUACGUGGACCAGCUGACCACCAGGGCCC
AGCAGCUGGAAGAGGAUAUCGCCCUGUUCGAGGCCCAGUACCUGGCCCAGG
CCGAAGAUACCCGGAUCCUGAGAAAGGCCGUGUCCGAGGCCUGCACCGAGAU
CGAUGCCAUCAGCGUGGAAAAGCGGCGGAUCAUGCAGCAGUGGGCCAGCAG
CCUCGUGGGCAUGAAGCACAGAGAUGAGGCCCACCGGGCCGUGCUGGAAGC
UCUGAGAGGCUGUCAGCACCAGGCCAAGAGCACCGACGGCGAGAUCGAGGC
CUACAAGAAAUCCAUCAUGAAGGAAGAGGAAAAGAACGAGAAACUGGCCAGCA
UCCUGAACAGAACCGAAACCGAGGCCACCCUGCUGCAGAAACUGACCACCCA
GUGCCUGACCAAACAGGUGGCCCUGCAGUCCCAGUUCAACACCUACAGACUG
ACCCUGCAGGACACCGAGGACGCCCUGAGUCAGGAUCAGCUGGAACAGAUGA
UUCUGACCGAGGAACUGCAGGCUAUCCGGCAGGCCAUUCAGGGGGAGCUGG
AACUGCGGAGAAAGACCGACGCCGCCAUCAGAGAGAAGCUGCAGGAACACAU
GACCAGCAACAAGACCACCAAGUACUUCAACCAGCUGAUUCUGCGCCUGCAG
AAAGAAAAGACCAACAUGAUGACACACCUGAGCAAGAUCAACGGCGACAUUGC
CCAGACCACCCUGGACAUCACCCACACCAGCAGCAGACUGGACGCCCACCAG
AAAACCCUGGUGGAACUGGACCAGGAUGUGAAGAAAGUGAACGAGCUGAUCA
CCAACAGCCAGAGCGAGAUCAGCCGGCGGACCAUCCUGAUCGAGAGAAAGCA
GGGCCUGAUCAACUUCCUGAACAAACAGCUGGAAAGAAUGGUGUCCGAGCUG
GGCGGCGAGGAAGUGGGACCUCUGGAACUGGAAAUCAAGCGGCUGAGCAAG

CUGAUCGACGAGCACGACGGCAAGGCCGUGCAGGCUCAAGUGACAUGGCUG
CGGCUGCAGCAGGAAAUGGUCAAAGUGACCCAGGAACAGGAAGAACAGCUGG
CCUCCCUGGACGCCAGCAAGAAAGAACUGCACAUCAUGGAACAGAAAAAGCU
GCGGGUGGAAAGCAAGAUCGAGCAGGAAAAAAAAGAACAGAAAGAAAUCGAGC
ACCACAUGAAGGACCUGGACAACGACCUGAAGAAACUGAAUAUGCUGAUGAAC
AAGAACCGCUGCUCCAGCGAAGAACUGGAACAGAACAACAGAGUGACCGAGA
ACGAGUUCGUGCGGAGCCUGAAGGCCAGCGAGCGGGAAACCAUCAAGAUGCA
GGACAAGCUGAACCAGCUGUCCGAGGAAAAAGCCACACUGCUGAACCAGCUG
GUGGAAGCCGAGCACCAGAUCAUGCUGUGGGAGAAGAAGAUCCAGCUGGCCA
AAGAAAUGCGGAGCAGCGUGGACAGCGAGAUCGGCCAGACCGAAAUCAGAGC
CAUGAAGGGCGAGAUCCACCGGAUGAAAGUGCGGCUGGGACAGCUGCUGAAA
CAGCAGGAAAAGAUGAUCCGGGCCAUGGAACUGGCCGUGGCCAGACGGGAAA
CCGUGACAACCCAGGCUGAGGGCCAGCGGAAGAUGGACAGAAAGGCCCUGAC
CCGGACCGACUUCCACCACAAGCAGCUGGAACUGAGGCGGAAGAUCCGGGAC
GUGCGGAAGGCCACCGAUGAGUGCACAAAGACAGUGCUGGAACUGGAAGAGA
CACAGCGGAACGUGUCCUCCAGCCUGCUGGAAAAACAGGAAAAGCUGAGCGU
GAUCCAGGCCGACUUCGACACCCUGGAAGCUGACCUGACAAGACUGGGAGCC
CUGAAAAGACAGAACCUGUCCGAGAUCGUGGCACUGCAGACCCGGCUGAAAC
AUCUGCAGGCUGUGAAAGAGGGACGCUACGUGUUCCUGUUCAGAUCCAAGCA
GUCUCUGGUGCUGGAAAGACAGCGGCUGGACAAGCGGCUGGCACUGAUUGC
CACCAUCCUGGAUAGAGUGCGCGACGAGUACCCACAGUUCCAGGAAGCACUG
CACAAGGUGUCCCAGAUGAUCGCCAACAAGCUGGAAUCCCCUGGCCCCAGCU
GAUUCG-polyA

SEQ ID NO: 8

Sequence with human CMV IE9 5' UTR and human Growth hormone 3' UTR (ETH031T10):

Part of the T7 promoter, **Ethris' minimal 5' UTR**, human CMV IE9 *5' UTR*, ***Kozak element***, start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC40 protein, stop codon (UGA), human Growth hormone ***3' UTR***, part of restriction site for BstBI, **polyA: tail of "A" produced via post polyadenylation of mRNA**

GGGAGA***C****CAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAG*
ACACCGGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCG
*GAUUCCCCGUGCCAAGAGUGACUCACCGUCCUUGACACG****GCCACC***AUGGCUG
AACCUGGCGGAGCCGCCGGAAGAUCCCACCCUGAAGAUGGCUCUGCCAGCGA
GGGCGAGAAAGAGGGCAACAACGAGAGCCACAUGGUGUCCCCCCCAGAGAAG
GACGACGGCCAGAAAGGCGAAGAGGCCGUGGGCUCUACCGAGCACCCUGAG
GAAGUGACCACACAGGCCGAGGCCGCCAUUGAAGAGGGCGAGGUGGAAACAG
AGGGCGAAGCCGCUGUGGAAGGCGAAGAGGAAGCCGUGUCUUACGGCGACG
CCGAGAGCGAGGAAGAGUACUACUACACCGAGACAAGCAGCCCCGAGGGCCA

GAUCUCUGCCGCCGAUACCACCUACCCCUACUUCAGCCCCCCUCAGGAACUG
CCUGGGGAAGAGGCCUACGAUAGCGUGUCCGGCGAAGCUGGCCUGCAGGGC
UUUCAGCAGGAAGCCACAGGCCCUCCCGAGAGCCGGGAAAGAAGAGUGACAA
GCCCCGAGCCUAGCCACGGCGUGCUGGGACCAUCUGAGCAGAUGGGCCAAG
UGACCUCUGGCCCUGCUGUGGGCAGACUGACAGGCAGCACAGAGGAACCUCA
GGGCCAGGUGCUGCCUAUGGGAGUGCAGCACCGGUUCAGACUGAGCCACGG
CAGCGACAUCGAGAGCAGCGACCUGGAAGAGUUCGUCAGCCAGGAACCCGUG
AUCCCUCCUGGCGUGCCAGAUGCCCAUCCCAGGGAAGGCGAUCUGCCCGUG
UUCCAGGACCAGAUCCAGCAGCCCUCUACCGAAGAGGGGGCUAUGGCCGAGA
GAGUGGAAAGCGAGGGCUCCGACGAAGAAGCCGAGGACGAGGGAUCUCAGC
UGGUGGUGCUGGACCCCGACCACCCUCUGAUGGUGCGGUUUCAGGCCGCCC
UGAAGAACUACCUGAACCGGCAGAUCGAGAAGCUGAAACUGGACCUGCAGGA
ACUGGUGGUGGCCACAAAGCAGAGCAGAGCCCAGAGACAGGAACUGGGCGU
GAACCUGUACGAGGUGCAGCAGCAUCUGGUGCAUCUGCAGAAGCUGCUGGAA
AAGAGCCACGACCGGCACGCCAUGGCCAGCUCUGAGCGCAGACAGAAAGAGG
AAGAACUGCAGGCCGCCAGAGCCCUGUACACCAAGACAUGCGCCGCUGCCAA
CGAGGAACGGAAGAAGCUGGCUGCCCUGCAGACCGAGAUGGAAAACCUGGCU
CUGCACCUGUUCUACAUGCAGAAUAUCGACCAGGACAUGCGGGACGACAUCA
GAGUGAUGACCCAGGUCGUGAAGAAGGCCGAGACAGAGAGAAUCCGGGCCGA
GAUUGAGAAGAAAAAGCAGGACCUGUACGUGGACCAGCUGACCACCAGGGCC
CAGCAGCUGGAAGAGGAUAUCGCCCUGUUCGAGGCCCAGUACCUGGCCCAG
GCCGAAGAUACCCGGAUCCUGAGAAAGGCCGUGUCCGAGGCCUGCACCGAGA
UCGAUGCCAUCAGCGUGGAAAAGCGGCGGAUCAUGCAGCAGUGGGCCAGCA
GCCUCGUGGGCAUGAAGCACAGAGAUGAGGCCCACCGGGCCGUGCUGGAAG
CUCUGAGAGGCUGUCAGCACCAGGCCAAGAGCACCGACGGCGAGAUCGAGG
CCUACAAGAAAUCCAUCAUGAAGGAAGAGGAAAAGAACGAGAAACUGGCCAGC
AUCCUGAACAGAACCGAAACCGAGGCCACCCUGCUGCAGAAACUGACCACCC
AGUGCCUGACCAAACAGGUGGCCCUGCAGUCCCAGUUCAACACCUACAGACU
GACCCUGCAGGACACCGAGGACGCCCUGAGUCAGGAUCAGCUGGAACAGAUG
AUUCUGACCGAGGAACUGCAGGCUAUCCGGCAGGCCAUUCAGGGGGAGCUG
GAACUGCGGAGAAAGACCGACGCCGCCAUCAGAGAGAAGCUGCAGGAACACA
UGACCAGCAACAAGACCACCAAGUACUUCAACCAGCUGAUUCUGCGCCUGCA
GAAAGAAAAGACCAACAUGAUGACACACCUGAGCAAGAUCAACGGCGACAUUG
CCCAGACCACCCUGGACAUCACCCACACCAGCAGCAGACUGGACGCCCACCA
GAAAACCCUGGUGGAACUGGACCAGGAUGUGAAGAAAGUGAACGAGCUGAUC
ACCAACAGCCAGAGCGAGAUCAGCCGGCGGACCAUCCUGAUCGAGAGAAAGC
AGGGCCUGAUCAACUUCCUGAACAAACAGCUGGAAAGAAUGGUGUCCGAGCU
GGGCGGCGAGGAAGUGGGACCUCUGGAACUGGAAAUCAAGCGGCUGAGCAA
GCUGAUCGACGAGCACGACGGCAAGGCCGUGCAGGCUCAAGUGACAUGGCU
GCGGCUGCAGCAGGAAAUGGUCAAAGUGACCCAGGAACAGGAAGAACAGCUG
GCCUCCCUGGACGCCAGCAAGAAAGAACUGCACAUCAUGGAACAGAAAAAGC
UGCGGGUGGAAAGCAAGAUCGAGCAGGAAAAAAAAGAACAGAAAGAAAUCGAG
CACCACAUGAAGGACCUGGACAACGACCUGAAGAAACUGAAUAUGCUGAUGAA
CAAGAACCGCUGCUCCAGCGAAGAACUGGAACAGAACAACAGAGUGACCGAG
AACGAGUUCGUGCGGAGCCUGAAGGCCAGCGAGCGGGAAACCAUCAAGAUGC

AGGACAAGCUGAACCAGCUGUCCGAGGAAAAAGCCACACUGCUGAACCAGCU
GGUGGAAGCCGAGCACCAGAUCAUGCUGUGGGAGAAGAAGAUCCAGCUGGC
CAAAGAAAUGCGGAGCAGCGUGGACAGCGAGAUCGGCCAGACCGAAAUCAGA
GCCAUGAAGGGCGAGAUCCACCGGAUGAAAGUGCGGCUGGGACAGCUGCUG
AAACAGCAGGAAAAGAUGAUCCGGGCCAUGGAACUGGCCGUGGCCAGACGGG
AAACCGUGACAACCCAGGCUGAGGGCCAGCGGAAGAUGGACAGAAAGGCCCU
GACCCGGACCGACUUCCACCACAAGCAGCUGGAACUGAGGCGGAAGAUCCGG
GACGUGCGGAAGGCCACCGAUGAGUGCACAAAGACAGUGCUGGAACUGGAAG
AGACACAGCGGAACGUGUCCUCCAGCCUGCUGGAAAAACAGGAAAAGCUGAG
CGUGAUCCAGGCCGACUUCGACACCCUGGAAGCUGACCUGACAAGACUGGGA
GCCCUGAAAAGACAGAACCUGUCCGAGAUCGUGGCACUGCAGACCCGGCUGA
AACAUCUGCAGGCUGUGAAAGAGGGACGCUACGUGUUCCUGUUCAGAUCCAA
GCAGUCUCUGGUGCUGGAAAGACAGCGGCUGGACAAGCGGCUGGCACUGAU
UGCCACCAUCCUGGAUAGAGUGCGCGACGAGUACCCACAGUUCCAGGAAGCA
CUGCACAAGGUGUCCCAGAUGAUCGCCAACAAGCUGGAAUCCCCUGGCCCCA
GCUGA***CGGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCUG***
GAAGUUGCCACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGC
AUCUUCG-polyA

SEQ ID NO: 9

ETH031T28: N terminal EGFP tag-CCDC40

Part of the T7 promoter, **Ethris' minimal 5' UTR**, ***Kozak element***, start codon (AUG), sequence encoding an *EGFP* protein, G4S spacer, codon optimized sequence encoding a functional version of a human CCDC40 protein, stop codon (UGA), part of restriction site for BstBI, poly(A) tail

GGGAGA***CGCCACC***AUG*GUGAGCAAGGGCGAGGAGCUGUUCACCGGGGUGGUGC*
CCAUCCUGGUCGAGCUGGACGGCGACGUAAACGGCCACAAGUUCAGCGUGU
CCGGCGAGGGCGAGGGCGAUGCCACCUACGGCAAGCUGACCCUGAAGUUCA
UCUGCACCACCGGCAAGCUGCCCGUGCCCUGGCCCACCCUCGUGACCACCCU
GACCUACGGCGUGCAGUGCUUCAGCCGCUACCCCGACCACAUGAAGCAGCAC
GACUUCUUCAAGUCCGCCAUGCCCGAAGGCUACGUCCAGGAGCGCACCAUCU
UCUUCAAGGACGACGGCAACUACAAGACCCGCGCCGAGGUGAAGUUCGAGGG
CGACACCCUGGUGAACCGCAUCGAGCUGAAGGGCAUCGACUUCAAGGAGGAC
GGCAACAUCCUGGGGCACAAGCUGGAGUACAACUACAACAGCCACAACGUCU
AUAUCAUGGCCGACAAGCAGAAGAACGGCAUCAAGGUGAACUUCAAGAUCCG
CCACAACAUCGAGGACGGCAGCGUGCAGCUCGCCGACCACUACCAGCAGAAC
ACCCCCAUCGGCGACGGCCCCGUGCUGCUGCCCGACAACCACUACCUGAGCA
CCCAGUCCGCCCUGAGCAAAGACCCCAACGAGAAGCGCGAUCACAUGGUCCU
GCUGGAGUUCGUGACCGCCGCCGGGAUCACUCUCGGCAUGGACGAGCUGUA
*CAAG*GGCGGAGGGGCAGCGCUGAACCUGGCGGAGCCGCCGGAAGAUCCCACCCUG
AAGAUGGCUCUGCCAGCGAGGGCGAGAAAGAGGGCAACAACGAGAGCCACAU

GGUGUCCCCCCCAGAGAAGGACGACGGCCAGAAAGGCGAAGAGGCCGUGGG
CUCUACCGAGCACCCUGAGGAAGUGACCACACAGGCCGAGGCCGCCAUUGAA
GAGGGCGAGGUGGAAACAGAGGGCGAAGCCGCUGUGGAAGGCGAAGAGGAA
GCCGUGUCUUACGGCGACGCCGAGAGCGAGGAAGAGUACUACUACACCGAGA
CAAGCAGCCCCGAGGGCCAGAUCUCUGCCGCCGAUACCACCUACCCCUACUU
CAGCCCCCCUCAGGAACUGCCUGGGGAAGAGGCCUACGAUAGCGUGUCCGG
CGAAGCUGGCCUGCAGGGCUUUCAGCAGGAAGCCACAGGCCCUCCCGAGAG
CCGGGAAAGAAGAGUGACAAGCCCCGAGCCUAGCCACGGCGUGCUGGGACCA
UCUGAGCAGAUGGGCCAAGUGACCUCUGGCCCUGCUGUGGGCAGACUGACA
GGCAGCACAGAGGAACCUCAGGGCCAGGUGCUGCCUAUGGGAGUGCAGCAC
CGGUUCAGACUGAGCCACGGCAGCGACAUCGAGAGCAGCGACCUGGAAGAGU
UCGUCAGCCAGGAACCCGUGAUCCCUCCUGGCGUGCCAGAUGCCCAUCCCAG
GGAAGGCGAUCUGCCCGUGUUCCAGGACCAGAUCCAGCAGCCCUCUACCGAA
GAGGGGGCUAUGGCCGAGAGAGUGGAAAGCGAGGGCUCCGACGAAGAAGCC
GAGGACGAGGGAUCUCAGCUGGUGGUGCUGGACCCCGACCACCCUCUGAUG
GUGCGGUUUCAGGCCGCCCUGAAGAACUACCUGAACCGGCAGAUCGAGAAGC
UGAAACUGGACCUGCAGGAACUGGUGGUGGCCACAAAGCAGAGCAGAGCCCA
GAGACAGGAACUGGGCGUGAACCUGUACGAGGUGCAGCAGCAUCUGGUGCA
UCUGCAGAAGCUGCUGGAAAAGAGCCACGACCGGCACGCCAUGGCCAGCUCU
GAGCGCAGACAGAAAGAGGAAGAACUGCAGGCCGCCAGAGCCCUGUACACCA
AGACAUGCGCCGCUGCCAACGAGGAACGGAAGAAGCUGGCUGCCCUGCAGAC
CGAGAUGGAAAACCUGGCUCUGCACCUGUUCUACAUGCAGAAUAUCGACCAG
GACAUGCGGGACGACAUCAGAGUGAUGACCCAGGUCGUGAAGAAGGCCGAGA
CAGAGAGAAUCCGGGCCGAGAUUGAGAAGAAAAAGCAGGACCUGUACGUGGA
CCAGCUGACCACCAGGGCCCAGCAGCUGGAAGAGGAUAUCGCCCUGUUCGAG
GCCCAGUACCUGGCCCAGGCCGAAGAUACCCGGAUCCUGAGAAAGGCCGUGU
CCGAGGCCUGCACCGAGAUCGAUGCCAUCAGCGUGGAAAAGCGGCGGAUCAU
GCAGCAGUGGGCCAGCAGCCUCGUGGGCAUGAAGCACAGAGAUGAGGCCCA
CCGGGCCGUGCUGGAAGCUCUGAGAGGCUGUCAGCACCAGGCCAAGAGCAC
CGACGGCGAGAUCGAGGCCUACAAGAAAUCCAUCAUGAAGGAAGAGGAAAAG
AACGAGAAACUGGCCAGCAUCCUGAACAGAACCGAAACCGAGGCCACCCUGC
UGCAGAAACUGACCACCCAGUGCCUGACCAAACAGGUGGCCCUGCAGUCCCA
GUUCAACACCUACAGACUGACCCUGCAGGACACCGAGGACGCCCUGAGUCAG
GAUCAGCUGGAACAGAUGAUUCUGACCGAGGAACUGCAGGCUAUCCGGCAGG
CCAUUCAGGGGGAGCUGGAACUGCGGAGAAAGACCGACGCCGCCAUCAGAGA
GAAGCUGCAGGAACACAUGACCAGCAACAAGACCACCAAGUACUUCAACCAGC
UGAUUCUGCGCCUGCAGAAAGAAAAGACCAACAUGAUGACACACCUGAGCAA
GAUCAACGGCGACAUUGCCCAGACCACCCUGGACAUCACCCACACCAGCAGC
AGACUGGACGCCCACCAGAAAACCCUGGUGGAACUGGACCAGGAUGUGAAGA
AAGUGAACGAGCUGAUCACCAACAGCCAGAGCGAGAUCAGCCGGCGGACCAU
CCUGAUCGAGAGAAAGCAGGGCCUGAUCAACUUCCUGAACAAACAGCUGGAA
AGAAUGGUGUCCGAGCUGGGCGGCGAGGAAGUGGGACCUCUGGAACUGGAA
AUCAAGCGGCUGAGCAAGCUGAUCGACGAGCACGACGGCAAGGCCGUGCAG
GCUCAAGUGACAUGGCUGCGGCUGCAGCAGGAAAUGGUCAAAGUGACCCAGG
AACAGGAAGAACAGCUGGCCUCCCUGGACGCCAGCAAGAAAGAACUGCACAU

CAUGGAACAGAAAAAGCUGCGGGUGGAAAGCAAGAUCGAGCAGGAAAAAAA
GAACAGAAAGAAAUCGAGCACCACAUGAAGGACCUGGACAACGACCUGAAGAA
ACUGAAUAUGCUGAUGAACAAGAACCGCUGCUCCAGCGAAGAACUGGAACAG
AACAACAGAGUGACCGAGAACGAGUUCGUGCGGAGCCUGAAGGCCAGCGAGC
GGGAAACCAUCAAGAUGCAGGACAAGCUGAACCAGCUGUCCGAGGAAAAAGC
CACACUGCUGAACCAGCUGGUGGAAGCCGAGCACCAGAUCAUGCUGUGGGAG
AAGAAGAUCCAGCUGGCCAAAGAAAUGCGGAGCAGCGUGGACAGCGAGAUCG
GCCAGACCGAAAUCAGAGCCAUGAAGGGCGAGAUCCACCGGAUGAAAGUGCG
GCUGGGACAGCUGCUGAAACAGCAGGAAAAGAUGAUCCGGGCCAUGGAACUG
GCCGUGGCCAGACGGGAAACCGUGACAACCCAGGCUGAGGGCCAGCGGAAG
AUGGACAGAAAGGCCCUGACCCGGACCGACUUCCACCACAAGCAGCUGGAAC
UGAGGCGGAAGAUCCGGGACGUGCGGAAGGCCACCGAUGAGUGCACAAAGA
CAGUGCUGGAACUGGAAGAGACACAGCGGAACGUGUCCUCCAGCCUGCUGGA
AAAACAGGAAAAGCUGAGCGUGAUCCAGGCCGACUUCGACACCCUGGAAGCU
GACCUGACAAGACUGGGAGCCCUGAAAGACAGAACCUGUCCGAGAUCGUGG
CACUGCAGACCCGGCUGAAACAUCUGCAGGCUGUGAAAGAGGGACGCUACGU
GUUCCUGUUCAGAUCCAAGCAGUCUCUGGUGCUGGAAAGACAGCGGCUGGA
CAAGCGGCUGGCACUGAUUGCCACCAUCCUGGAUAGAGUGCGCGACGAGUAC
CCACAGUUCCAGGAAGCACUGCACAAGGUGUCCCAGAUGAUCGCCAACAAGC
UGGAAUCCCCUGGCCCCAGCUGAUUCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 10

ETH031T30: C terminal EGFP tag-CCDC40

Part of the T7 promoter, **Ethris‘ minimal 5’ UTR**, ***Kozak element***, start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC40 protein, G4S spacer, sequence encoding an *EGFP* protein, stop codon (UGA), part of restriction site for BstBI, poly(A) tail

GGGAGA***CGCCACC***AUGGCUGAACCUGGCGGAGCCGCCGGAAGAUCCCACCCUG
AAGAUGGCUCUGCCAGCGAGGGCGAGAAAGAGGGCAACAACGAGAGCCACAU
GGUGUCCCCCCCAGAGAAGGACGACGGCCAGAAAGGCGAAGAGGCCGUGGG
CUCUACCGAGCACCCUGAGGAAGUGACCACACAGGCCGAGGCCGCCAUUGAA
GAGGGCGAGGUGGAAACAGAGGGCGAAGCCGCUGUGGAAGGCGAAGAGGAA
GCCGUGUCUUACGGCGACGCCGAGAGCGAGGAAGAGUACUACUACACCGAGA
CAAGCAGCCCCGAGGGCCAGAUCUCUGCCGCCGAUACCACCUACCCCUACUU
CAGCCCCCCUCAGGAACUGCCUGGGGAAGAGGCCUACGAUAGCGUGUCCGG
CGAAGCUGGCCUGCAGGGCUUUCAGCAGGAAGCCACAGGCCCUCCCGAGAG
CCGGGAAAGAAGAGUGACAAGCCCCGAGCCUAGCCACGGCGUGCUGGGACCA
UCUGAGCAGAUGGGCCAAGUGACCUCUGGCCCUGCUGUGGGCAGACUGACA
GGCAGCACAGAGGAACCUCAGGGCCAGGUGCUGCCUAUGGGAGUGCAGCAC
CGGUUCAGACUGAGCCACGGCAGCGACAUCGAGAGCAGCGACCUGGAAGAGU

UCGUCAGCCAGGAACCCGUGAUCCCUCCUGGCGUGCCAGAUGCCCAUCCCAG
GGAAGGCGAUCUGCCCGUGUUCCAGGACCAGAUCCAGCAGCCCUCUACCGAA
GAGGGGGCUAUGGCCGAGAGAGUGGAAAGCGAGGGCUCCGACGAAGAAGCC
GAGGACGAGGGAUCUCAGCUGGUGGUGCUGGACCCCGACCACCCUCUGAUG
GUGCGGUUUCAGGCCGCCCUGAAGAACUACCUGAACCGGCAGAUCGAGAAGC
UGAAACUGGACCUGCAGGAACUGGUGGUGGCCACAAAGCAGAGCAGAGCCCA
GAGACAGGAACUGGGCGUGAACCUGUACGAGGUGCAGCAGCAUCUGGUGCA
UCUGCAGAAGCUGCUGGAAAAGAGCCACGACCGGCACGCCAUGGCCAGCUCU
GAGCGCAGACAGAAAGAGGAAGAACUGCAGGCCGCCAGAGCCCUGUACACCA
AGACAUGCGCCGCUGCCAACGAGGAACGGAAGAAGCUGGCUGCCCUGCAGAC
CGAGAUGGAAAACCUGGCUCUGCACCUGUUCUACAUGCAGAAUAUCGACCAG
GACAUGCGGGACGACAUCAGAGUGAUGACCCAGGUCGUGAAGAAGGCCGAGA
CAGAGAGAAUCCGGGCCGAGAUUGAGAAGAAAAAGCAGGACCUGUACGUGGA
CCAGCUGACCACCAGGGCCCAGCAGCUGGAAGAGGAUAUCGCCCUGUUCGAG
GCCCAGUACCUGGCCCAGGCCGAAGAUACCCGGAUCCUGAGAAAGGCCGUGU
CCGAGGCCUGCACCGAGAUCGAUGCCAUCAGCGUGGAAAAGCGGCGGAUCAU
GCAGCAGUGGGCCAGCAGCCUCGUGGGCAUGAAGCACAGAGAUGAGGCCCA
CCGGGCCGUGCUGGAAGCUCUGAGAGGCUGUCAGCACCAGGCCAAGAGCAC
CGACGGCGAGAUCGAGGCCUACAAGAAAUCCAUCAUGAAGGAAGAGGAAAAG
AACGAGAAACUGGCCAGCAUCCUGAACAGAACCGAAACCGAGGCCACCCUGC
UGCAGAAACUGACCACCCAGUGCCUGACCAAACAGGUGGCCCUGCAGUCCCA
GUUCAACACCUACAGACUGACCCUGCAGGACACCGAGGACGCCCUGAGUCAG
GAUCAGCUGGAACAGAUGAUUCUGACCGAGGAACUGCAGGCUAUCCGGCAGG
CCAUUCAGGGGGAGCUGGAACUGCGGAGAAAGACCGACGCCGCCAUCAGAGA
GAAGCUGCAGGAACACAUGACCAGCAACAAGACCACCAAGUACUUCAACCAGC
UGAUUCUGCGCCUGCAGAAAGAAAAGACCAACAUGAUGACACACCUGAGCAA
GAUCAACGGCGACAUUGCCCAGACCACCCUGGACAUCACCCACACCAGCAGC
AGACUGGACGCCCACCAGAAAACCCUGGUGGAACUGGACCAGGAUGUGAAGA
AAGUGAACGAGCUGAUCACCAACAGCCAGAGCGAGAUCAGCCGGCGGACCAU
CCUGAUCGAGAGAAAGCAGGGCCUGAUCAACUUCCUGAACAAACAGCUGGAA
AGAAUGGUGUCCGAGCUGGGCGGCGAGGAAGUGGGACCUCUGGAACUGGAA
AUCAAGCGGCUGAGCAAGCUGAUCGACGAGCACGACGGCAAGGCCGUGCAG
GCUCAAGUGACAUGGCUGCGGCUGCAGCAGGAAAUGGUCAAAGUGACCCAGG
AACAGGAAGAACAGCUGGCCUCCCUGGACGCCAGCAAGAAAGAACUGCACAU
CAUGGAACAGAAAAAGCUGCGGGUGGAAAGCAAGAUCGAGCAGGAAAAAAAA
GAACAGAAAGAAAUCGAGCACCACAUGAAGGACCUGGACAACGACCUGAAGAA
ACUGAAUAUGCUGAUGAACAAGAACCGCUGCUCCAGCGAAGAACUGGAACAG
AACAACAGAGUGACCGAGAACGAGUUCGUGCGGAGCCUGAAGGCCAGCGAGC
GGGAAACCAUCAAGAUGCAGGACAAGCUGAACCAGCUGUCCGAGGAAAAAGC
CACACUGCUGAACCAGCUGGUGGAAGCCGAGCACCAGAUCAUGCUGUGGGAG
AAGAAGAUCCAGCUGGCCAAAGAAAUGCGGAGCAGCGUGGACAGCGAGAUCG
GCCAGACCGAAAUCAGAGCCAUGAAGGGCGAGAUCCACCGGAUGAAAGUGCG
GCUGGGACAGCUGCUGAAACAGCAGGAAAAGAUGAUCCGGGCCAUGGAACUG
GCCGUGGCCAGACGGGAAACCGUGACAACCCAGGCUGAGGGCCAGCGGAAG
AUGGACAGAAAGGCCCUGACCCGGACCGACUUCCACCACAAGCAGCUGGAAC

UGAGGCGGAAGAUCCGGGACGUGCGGAAGGCCACCGAUGAGUGCACAAAGA
CAGUGCUGGAACUGGAAGAGACACAGCGGAACGUGUCCUCCAGCCUGCUGGA
AAAACAGGAAAAGCUGAGCGUGAUCCAGGCCGACUUCGACACCCUGGAAGCU
GACCUGACAAGACUGGGAGCCCUGAAAAGACAGAACCUGUCCGAGAUCGUGG
CACUGCAGACCCGGCUGAAACAUCUGCAGGCUGUGAAAGAGGGACGCUACGU
GUUCCUGUUCAGAUCCAAGCAGUCUCUGGUGCUGGAAAGACAGCGGCUGGA
CAAGCGGCUGGCACUGAUUGCCACCAUCCUGGAUAGAGUGCGCGACGAGUAC
CCACAGUUCCAGGAAGCACUGCACAAGGUGUCCCAGAUGAUCGCCAACAAGC
UGGAAUCCCCUGGCCCCAGCGGCGGAGGGGGCAGC*GUGAGCAAGGGCGAGGAGCUG
UUCACCGGGGUGGUGCCCAUCCUGGUCGAGCUGGACGGCGACGUAAACGGC
CACAAGUUCAGCGUGUCCGGCGAGGGCGAGGGCGAUGCCACCUACGGCAAG
CUGACCCUGAAGUUCAUCUGCACCACCGGCAAGCUGCCCGUGCCCUGGCCCA
CCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUCAGCCGCUACCCCGA
CCACAUGAAGCAGCACGACUUCUUCAAGUCCGCCAUGCCCGAAGGCUACGUC
CAGGAGCGCACCAUCUUCUUCAAGGACGACGGCAACUACAAGACCCGCGCCG
AGGUGAAGUUCGAGGGCGACACCCUGGUGAACCGCAUCGAGCUGAAGGGCA
UCGACUUCAAGGAGGACGGCAACAUCCUGGGGCACAAGCUGGAGUACAACUA
CAACAGCCACAACGUCUAUAUCAUGGCCGACAAGCAGAAGAACGGCAUCAAG
GUGAACUUCAAGAUCCGCCACAACAUCGAGGACGGCAGCGUGCAGCUCGCCG
ACCACUACCAGCAGAACACCCCCAUCGGCGACGGCCCCGUGCUGCUGCCCGA
CAACCACUACCUGAGCACCCAGUCCGCCCUGAGCAAAGACCCCAACGAGAAG
CGCGAUCACAUGGUCCUGCUGGAGUUCGUGACCGCCGCCGGGAUCACUCUC
GGCAUGGACGAGCUGUACAAG*UGAUUCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 11

ETH031T26: N terminal HA Tag-CCDC40-T2A peptide-tdTomato with 5‘ and 3‘ CYBA UTRs

Part of the T7 promoter, **Ethris‘ minimal 5' UTR**, CYBA *5' UTR*, ***Kozak element***, start codon (AUG), *HA Tag*, G4S spacer, codon optimized sequence encoding a functional version of a human CCDC40 protein,, **T2A peptide**, *tdTomato*, stop codon (UGA), CYBA ***3' UTR***, part of restriction site for BstBI, poly(A) tail

GGGAGA**CCGCGCCUAGCAGUGUCCCAGCCGGGUUCGUGUCGCC*****GCCACC***AUG*UA
CCCCUACGACGUGCCCGACUACGCC*GGCGGAGGGGGCAGCGCUGAACCUGGCGGAGCCGCCGGA
AGAUCCCACCCUGAAGAUGGCUCUGCCAGCGAGGGCGAGAAAGAGGGCAACA
ACGAGAGCCACAUGGUGUCCCCCCCAGAGAAGGACGACGGCCAGAAAGGCGA
AGAGGCCGUGGGCUCUACCGAGCACCCUGAGGAAGUGACCACACAGGCCGA
GGCCGCCAUUGAAGAGGGCGAGGUGGAAACAGAGGGCGAAGCCGCUGUGGA
AGGCGAAGAGGAAGCCGUGUCUUACGGCGACGCCGAGAGCGAGGAAGAGUA
CUACUACACCGAGACAAGCAGCCCCGAGGGCCAGAUCUCUGCCGCCGAUACC
ACCUACCCCUACUUCAGCCCCCCUCAGGAACUGCCUGGGGAAGAGGCCUACG

AUAGCGUGUCCGGCGAAGCUGGCCUGCAGGGCUUUCAGCAGGAAGCCACAG
GCCCUCCCGAGAGCCGGGAAAGAAGAGUGACAAGCCCCGAGCCUAGCCACGG
CGUGCUGGGACCAUCUGAGCAGAUGGGCCAAGUGACCUCUGGCCCUGCUGU
GGGCAGACUGACAGGCAGCACAGAGGAACCUCAGGGCCAGGUGCUGCCUAU
GGGAGUGCAGCACCGGUUCAGACUGAGCCACGGCAGCGACAUCGAGAGCAG
CGACCUGGAAGAGUUCGUCAGCCAGGAACCCGUGAUCCCUCCUGGCGUGCC
AGAUGCCCAUCCCAGGGAAGGCGAUCUGCCCGUGUUCCAGGACCAGAUCCAG
CAGCCCUCUACCGAAGAGGGGGCUAUGGCCGAGAGAGUGGAAAGCGAGGGC
UCCGACGAAGAAGCCGAGGACGAGGGAUCUCAGCUGGUGGUGCUGGACCCC
GACCACCCUCUGAUGGUGCGGUUUCAGGCCGCCCUGAAGAACUACCUGAACC
GGCAGAUCGAGAAGCUGAAACUGGACCUGCAGGAACUGGUGGUGGCCACAAA
GCAGAGCAGAGCCCAGAGACAGGAACUGGGCGUGAACCUGUACGAGGUGCA
GCAGCAUCUGGUGCAUCUGCAGAAGCUGCUGGAAAAGAGCCACGACCGGCAC
GCCAUGGCCAGCUCUGAGCGCAGACAGAAAGAGGAAGAACUGCAGGCCGCCA
GAGCCCUGUACACCAAGACAUGCGCCGCUGCCAACGAGGAACGGAAGAAGCU
GGCUGCCCUGCAGACCGAGAUGGAAAACCUGGCUCUGCACCUGUUCUACAUG
CAGAAUAUCGACCAGGACAUGCGGGACGACAUCAGAGUGAUGACCCAGGUCG
UGAAGAAGGCCGAGACAGAGAGAAUCCGGGCCGAGAUUGAGAAGAAAAAGCA
GGACCUGUACGUGGACCAGCUGACCACCAGGGCCCAGCAGCUGGAAGAGGA
UAUCGCCCUGUUCGAGGCCCAGUACCUGGCCCAGGCCGAAGAUACCCGGAUC
CUGAGAAAGGCCGUGUCCGAGGCCUGCACCGAGAUCGAUGCCAUCAGCGUG
GAAAAGCGGCGGAUCAUGCAGCAGUGGGCCAGCAGCCUCGUGGGCAUGAAG
CACAGAGAUGAGGCCCACCGGGCCGUGCUGGAAGCUCUGAGAGGCUGUCAG
CACCAGGCCAAGAGCACCGACGGCGAGAUCGAGGCCUACAAGAAAUCCAUCA
UGAAGGAAGAGGAAAAGAACGAGAAACUGGCCAGCAUCCUGAACAGAACCGAA
ACCGAGGCCACCCUGCUGCAGAAACUGACCACCCAGUGCCUGACCAAACAGG
UGGCCCUGCAGUCCCAGUUCAACACCUACAGACUGACCCUGCAGGACACCGA
GGACGCCCUGAGUCAGGAUCAGCUGGAACAGAUGAUUCUGACCGAGGAACUG
CAGGCUAUCCGGCAGGCCAUUCAGGGGGAGCUGGAACUGCGGAGAAAGACC
GACGCCGCCAUCAGAGAGAAGCUGCAGGAACACAUGACCAGCAACAAGACCA
CCAAGUACUUCAACCAGCUGAUUCUGCGCCUGCAGAAAGAAAAGACCAACAUG
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UCACCCACACCAGCAGCAGACUGGACGCCCACCAGAAAACCCUGGUGGAACU
GGACCAGGAUGUGAAGAAAGUGAACGAGCUGAUCACCAACAGCCAGAGCGAG
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GACCUCUGGAACUGGAAAUCAAGCGGCUGAGCAAGCUGAUCGACGAGCACGA
CGGCAAGGCCGUGCAGGCUCAAGUGACAUGGCUGCGGCUGCAGCAGGAAAU
GGUCAAAGUGACCCAGGAACAGGAAGAACAGCUGGCCUCCCUGGACGCCAGC
AAGAAAGAACUGCACAUCAUGGAACAGAAAAAGCUGCGGGUGGAAAGCAAGAU
CGAGCAGGAAAAAAAAGAACAGAAAGAAAUCGAGCACCACAUGAAGGACCUGG
ACAACGACCUGAAGAAACUGAAUAUGCUGAUGAACAAGAACCGCUGCUCCAGC
GAAGAACUGGAACAGAACAACAGAGUGACCGAGAACGAGUUCGUGCGGAGCC
UGAAGGCCAGCGAGCGGGAAACCAUCAAGAUGCAGGACAAGCUGAACCAGCU
GUCCGAGGAAAAAGCCACACUGCUGAACCAGCUGGUGGAAGCCGAGCACCAG

AUCAUGCUGUGGGAGAAGAAGAUCCAGCUGGCCAAAGAAAUGCGGAGCAGCG
UGGACAGCGAGAUCGGCCAGACCGAAAUCAGAGCCAUGAAGGGCGAGAUCCA
CCGGAUGAAAGUGCGGCUGGGACAGCUGCUGAAACAGCAGGAAAAGAUGAUC
CGGGCCAUGGAACUGGCCGUGGCCAGACGGGAAACCGUGACAACCCAGGCU
GAGGGCCAGCGGAAGAUGGACAGAAAGGCCCUGACCCGGACCGACUUCCACC
ACAAGCAGCUGGAACUGAGGCGGAAGAUCCGGGACGUGCGGAAGGCCACCG
AUGAGUGCACAAAGACAGUGCUGGAACUGGAAGAGACACAGCGGAACGUGUC
CUCCAGCCUGCUGGAAAAACAGGAAAAGCUGAGCGUGAUCCAGGCCGACUUC
GACACCCUGGAAGCUGACCUGACAAGACUGGGAGCCCUGAAAAGACAGAACC
UGUCCGAGAUCGUGGCACUGCAGACCCGGCUGAAACAUCUGCAGGCUGUGAA
AGAGGGACGCUACGUGUUCCUGUUCAGAUCCAAGCAGUCUCUGGUGCUGGA
AAGACAGCGGCUGGACAAGCGGCUGGCACUGAUUGCCACCAUCCUGGAUAGA
GUGCGCGACGAGUACCCACAGUUCCAGGAAGCACUGCACAAGGUGUCCCAGA
UGAUCGCCAACAAGCUGGAAUCCCCUGGCCCCAGCGGCAGCGGC<u>GAGGGCAGAGG</u>
<u>CAGCCUGCUGACCUGCGGCGACGUGGAGGAGAACCCCGGCCCC</u><u>AUG</u>*<u>GUGAGCAAGGGCGAGGAGG</u>*
<u>UCAUCAAAGAGUUCAUGCGCUUCAAGGUGCGCAUGGAGGGCUCCAUGAACGG</u>
<u>CCACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCAC</u>
<u>CCAGACCGCCAAGCUGAAGGUGACCAAGGGCGGCCCCCUGCCCUUCGCCUG</u>
<u>GGACAUCCUGUCCCCCCCAGUUCAUGUACGGCUCCAAGGCGUACGUGAAGCAC</u>
<u>CCCGCCGACAUCCCCGAUUACAAGAAGCUGUCCUUCCCCGAGGGCUUCAAGU</u>
<u>GGGAGCGCGUGAUGAACUUCGAGGACGGCGGUCUGGUGACCGUGACCCAGG</u>
<u>ACUCCUCCCUGCAGGACGGCACGCUGAUCUACAAGGUGAAGAUGCGCGGCAC</u>
<u>CAACUUCCCCCCCGACGGCCCCGUAAUGCAGAAGAAGACCAUGGGCUGGGAG</u>
<u>GCCUCCACCGAGCGCCUGUACCCCCGCGACGGCGUGCUGAAGGGCGAGAUC</u>
<u>CACCAGGCCCUGAAGCUGAAGGACGGCGGCCACUACCUGGUGGAGUUCAAGA</u>
<u>CCAUCUACAUGGCCAAGAAGCCCGUGCAACUGCCCGGCUACUACUACGUGGA</u>
<u>CACCAAGCUGGACAUCACCUCCCACAACGAGGACUACACCAUCGUGGAACAG</u>
<u>UACGAGCGCUCCGAGGGCCGCCACCACCUGUUCCUGGGGCAUGGCACCGGC</u>
<u>AGCACCGGCAGCGGCAGCUCCGGCACCGCCUCCUCCGAGGACAACAACAUGG</u>
<u>CCGUCAUCAAAGAGUUCAUGCGCUUCAAGGUGCGCAUGGAGGGCUCCAUGAA</u>
<u>CGGCCACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGG</u>
<u>CACCCAGACCGCCAAGCUGAAGGUGACCAAGGGCGGCCCCCUGCCCUUCGCC</u>
<u>UGGGACAUCCUGUCCCCCCAGUUCAUGUACGGCUCCAAGGCGUACGUGAAGC</u>
<u>ACCCCGCCGACAUCCCCGAUUACAAGAAGCUGUCCUUCCCCGAGGGCUUCAA</u>
<u>GUGGGAGCGCGUGAUGAACUUCGAGGACGGCGGUCUGGUGACCGUGACCCA</u>
<u>GGACUCCUCCCUGCAGGACGGCACGCUGAUCUACAAGGUGAAGAUGCGCGG</u>
<u>CACCAACUUCCCCCCCGACGGCCCCGUAAUGCAGAAGAAGACCAUGGGCUGG</u>
<u>GAGGCCUCCACCGAGCGCCUGUACCCCCGCGACGGCGUGCUGAAGGGCGAG</u>
<u>AUCCACCAGGCCCUGAAGCUGAAGGACGGCGGCCACUACCUGGUGGAGUUCA</u>
<u>AGACCAUCUACAUGGCCAAGAAGCCCGUGCAACUGCCCGGCUACUACUACGU</u>
<u>GGACACCAAGCUGGACAUCACCUCCCACAACGAGGACUACACCAUCGUGGAA</u>
<u>CAGUACGAGCGCUCCGAGGGCCGCCACCACCUGUUCCUGUACGGCAUGGAC</u>
<u>GAGCUGUACAAG</u><u>UGA</u>***<u>CCUCGCCCCGGACCUGCCCUCCCGCCAGGUGCACCC</u>***
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AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAA

SEQ ID NO: 12

ETH047T02 (CCDC39 with Ethris' minimal UTR):

Part of the T7 promoter, **Ethris' minimal 5' UTR**, ***Kozak element***, start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC39 protein, stop codon (UGA), part of restriction site for BstBI, poly(A) tail

GGGAGA***CGCCACC***AUGAGCAGCGAGUUUCUGGCCGAACUGCACUGGGAGGACG
GCUUCGCUAUUCCCGUGGCCAACGAGGAAAACAAGCUGCUGGAAGAUCAGCU
GAGCAAGCUGAAGGACGAGAGAGCCUCUCUGCAGGACGAGCUGAGAGAGUAC
GAGGAACGGAUCAACAGCAUGACCAGCCACUUCAAGAACGUGAAGCAAGAGC
UGAGCAUCACCCAGAGCCUGUGCAAGGCCAGAGAGAGAGAAACCGAGAGCGA
GGAACACUUCAAGGCUAUCGCCCAGCGCGAGCUGGGAAGAGUGAAGGAUGAG
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AGAAGCGGAAGAUCCUGGACAACGAGCUGACCGAGACAAUCAGCGCCCAGCU
GGAACUGGAUAAGGCCGCUCAGGACUUCAGAAAGAUCCACAACGAGCGGCAA
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GGCAACAACACCGAGUUCGAGAAGCGGAUCAGCGUGGCCGACAGAAAGCUGC
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CCCCUAGCCACACAAGCCUGAGCGCCAGAAGCUCUAGAAGCACCAGCACCUC
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AAAA

SEQ ID NO: 13

RNA encoding for human CCDC39 sequence and contains **TISU element** as 5' UTR (**ETH047T03**)

Part of the T7 promoter, **Ethris' minimal 5' UTR with additional U nucleotide**, ***TISU element***, start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC39 protein, stop codon (UGA), part of restriction site for BstBI, poly(A) tail

GGGAGA**CU*GCCAAG***AUGAGCAGCGAGUUUCUGGCCGAACUGCACUGGGAGGAC
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CAAGAUCCGGGCUCUGACCCUGCAGCUGGAAAGACUGACCCUGGAAUGCAAC
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AGGGCGAGCUGGAUUCUCUGAAGGCCACCGUGAACAGAACCAGCAGCGAUCU
GGAAGCCCUGCGGAAGAACAUCAGCAAGAUCAAGAAGGACAUCCACGAGGAA
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UGAACCUGAUUAAGGGCGUGCUGUUCAAGAAGGCCCAAGAGCUGCAGACCGA
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AGCAAGAGAUCAUGUACAGCCAGGAUUUCCACAUCCAGCAGGUCGAGCGGCG
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SEQ ID NO: 14

ETH047T05: SP30; RNA encoding for human CCDC39 and containing ***random sequence of 30 nucleotides*** (***SP30***) which was used as 5' UTR

Part of the T7 promoter, **Ethris' minimal 5' UTR, *SP30* as 5' UTR**, ***Kozak element***, start codon (AUG), codon optimized sequence encoding a functional version of a human CCDC39 protein, stop codon (UGA), part of restriction site for BstBI, poly(A) tail

GGGAGA***CAUUGAAAUUUAUCUCUUGUGUUGUGGUCGCGCCACC***AUGAGCAGCGAGUUU
CUGGCCGAACUGCACUGGGAGGACGGCUUCGCUAUUCCCGUGGCCAACGAG
GAAAACAAGCUGCUGGAAGAUCAGCUGAGCAAGCUGAAGGACGAGAGAGCCU
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CCACUUCAAGAACGUGAAGCAAGAGCUGAGCAUCACCCAGAGCCUGUGCAAG
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CCAAGCUGACAAAAGAGAUCCGGCUGCUGAAAGACACCAAGGACGAAACCAU
GGAAGAACAAGACAUCAAGCUGCGCGAGAUGAAGCAGUUCCACAAAGUGAUC
GACGAGAUGCUGGUGGACAUCAUUGAAGAGAACACAGAGAUCCGCAUCAUCC
UGCAGACCUAUUUCAGCAGAGCGGCCUGGAACUGCCUACCGCCUCUACAAA
GGGCAGCAGACAGAGCAGCAGAUCCCCUAGCCACACAAGCCUGAGCGCCAGA
AGCUCUAGAAGCACCAGCACCUCUACCAGCCAGUCCAGCAUUAAGGUGCUGG
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CAGCGCCUCUAGCAGCUCCAGCAACGUGAAGUCCAAGAAAAGCUCCAAGUGA
UUCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Claims

1. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy in a subject suffering of a ciliopathy, wherein the polyribonucleotide encodes a functional version of a protein a defect of which is associated with said ciliopathy, and wherein administration of said pharmaceutical composition to the respiratory system of said patient is effected when the patient shows an inflammation of the respiratory system.

2. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy according to any of the preceding claims, wherein said ciliopathy is primary ciliary dyskinesia (PCD).

3. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy according to any of the preceding claims, wherein said ciliopathy is associated with a defect in a coiled-coil domain containing 40 (CCDC40) protein and/or with a defect in a coiled-coil domain containing 39 (CCDC39) protein.

4. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy according to any of the preceding claims, wherein the presence or absence of an inflammation of the respiratory system of a subject suffering of a ciliopathy is determined by analyzing a blood sample and/or by analyzing the amount of exhaled nitric oxide.

5. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy according to any of the preceding claims, wherein administration of the pharmaceutical composition comprises administration using a nasal spray and/or a nebulizer and/or by inhalation.

6. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy according to any of the preceding claims, wherein the pharmaceutical composition is administered at least once a week and/or for at least 4 weeks.

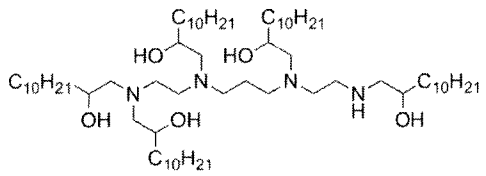
7. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy according to any of the preceding claims, wherein the pharmaceutical

composition further comprises N-acetylcysteine (NAC) and/or a hypertonic solution comprising sodium chloride.

8. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy according to any of the preceding claims, wherein said pharmaceutical composition further comprises a polyribonucleotide encoding a multiciliate differentiation and DNA synthesis associated cell cycle (MCIDAS) protein and/or wherein said pharmaceutical composition is a first pharmaceutical composition that is administered together with a second pharmaceutical composition comprising a polyribonucleotide encoding an MCIDAS protein.

9. A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy according to any of the preceding claims, wherein said pharmaceutical composition further comprises a lipidoid having the structure shown in formula (V):

C12-(2-3-2)



formula (V)

10. A pharmaceutical composition comprising a polyribonucleotide encoding a protein for a defect of which is associated with a ciliopathy and N-acetylcysteine (NAC), a hypertonic solution comprising sodium chloride, and/or an LF92 formulation.

11. A method for analyzing the effect of a polyribonucleotide on ciliogenesis, wherein said polyribonucleotide encodes a protein involved in and/or required for ciliogenesis, said method comprising the steps of:
    (a) obtaining a nose brush of a subject having a ciliopathy, said nose brush comprising undifferentiated basal cells and differentiated ciliated cells,
    (b) culturing the cells obtained from step (a) as a submerse cell culture for obtaining undifferentiated basal cells and dedifferentiated ciliated cells,
    (c) culturing undifferentiated basal cells and dedifferentiated ciliated cells obtained from step (b) as an air liquid interface cell culture and performing an air lift,
    (d) transfecting cells obtained from step (c) with a polyribonucleotide encoding a protein involved in and/or required for ciliogenesis,
    (e) culturing the transfected cells obtained from step (d) for obtaining differentiated ciliated cells, and

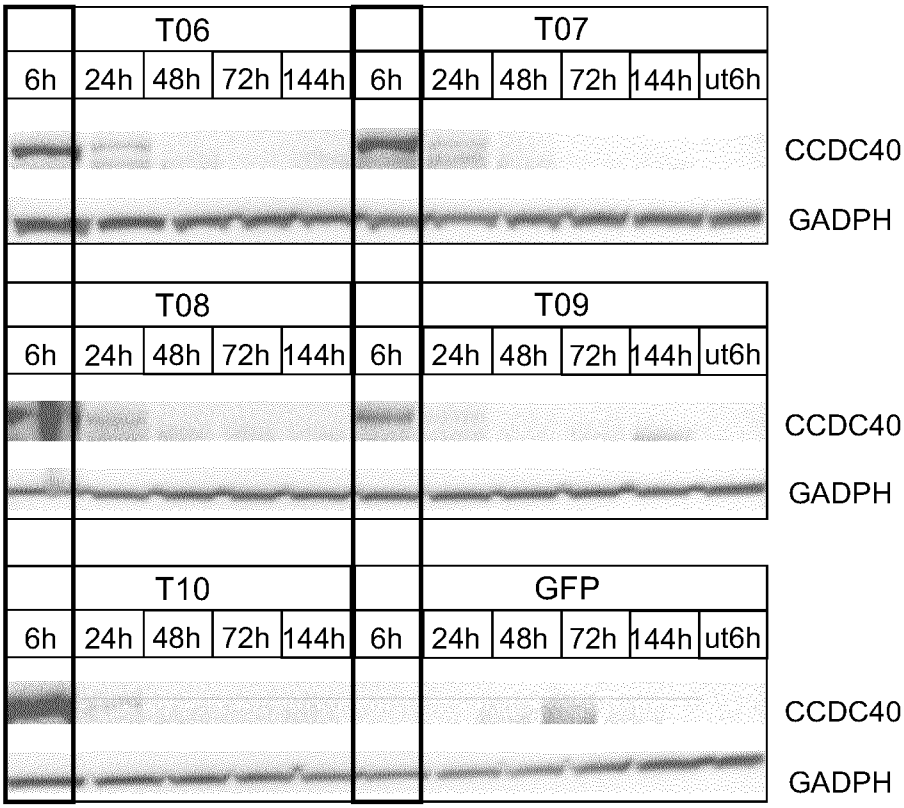
(f) determining the effect of said polyribonucleotide on ciliogenesis using a lactate dehydrogenase measurement, a NucGreen assay, a high speed video microscopy, a ciliary beat frequency measurement, a mucociliary clearance assay, and/or immunofluorescence staining.

12. The method according to claim 11, wherein the cells are transfected within 0 to 48 hours after the air lift is performed in step (c).

13. The method according to claim 11 or 12, wherein the cells are transfected with a polyribonucleotide encoding a protein involved in and/or required for ciliogenesis using a lipidoid having the structure shown in formula (V).

14. The method according to any of claims 11 to 13, wherein the cells are cultured in steps (b) to (e) using Medium G.

15. The method according to any of claims 11 to 14, wherein the nose brush further comprises fibroblasts and wherein growth of said fibroblasts is inhibited in steps (b) to (e).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T08
6h
24h
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Figure 1

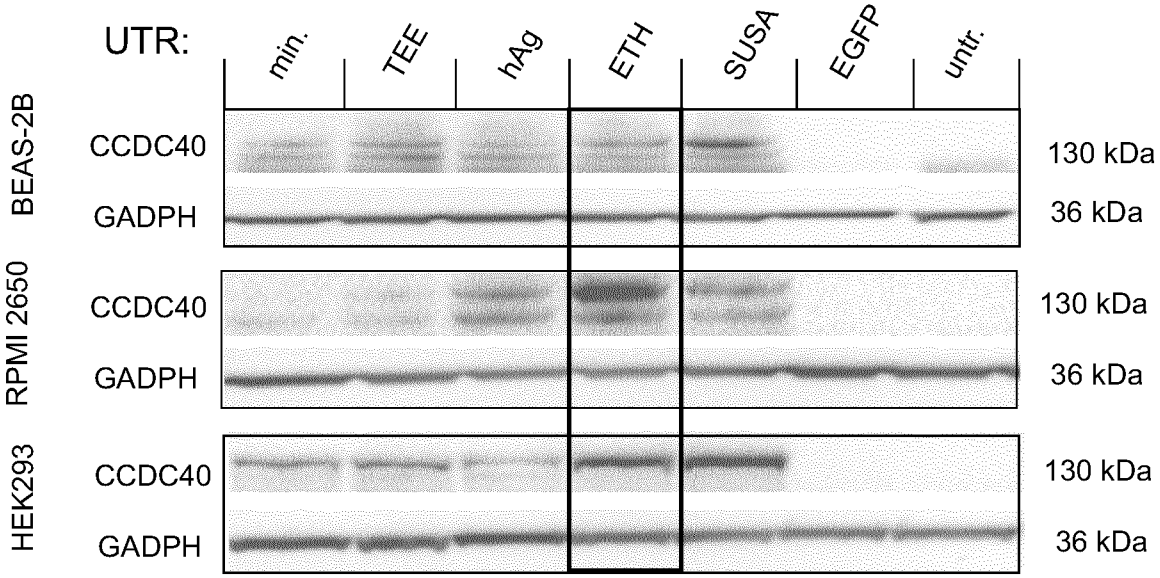


Figure 2

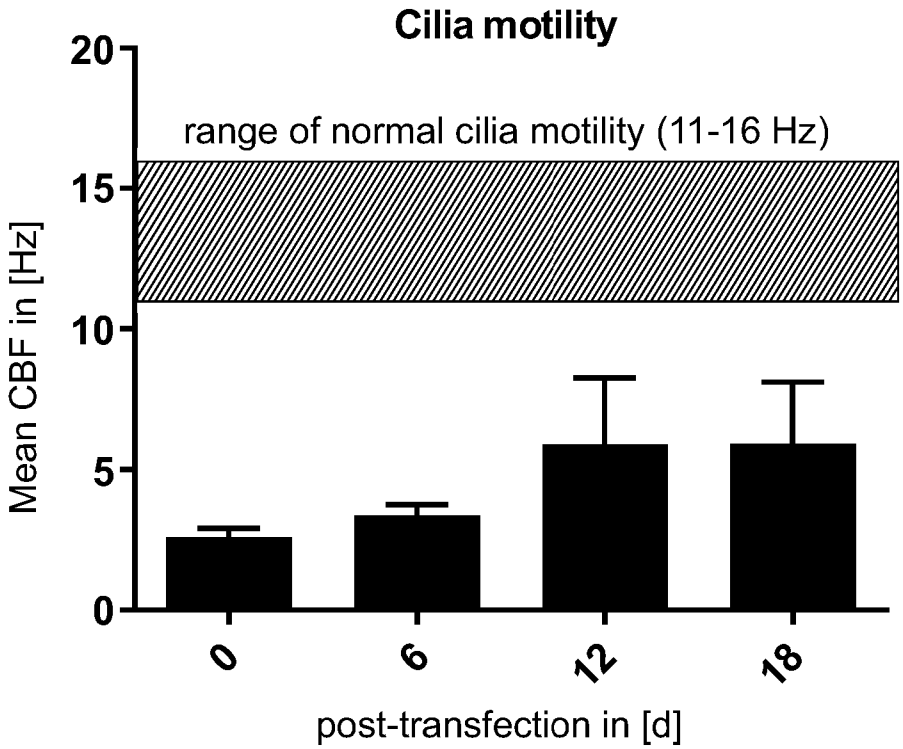


Figure 3

Figure 4

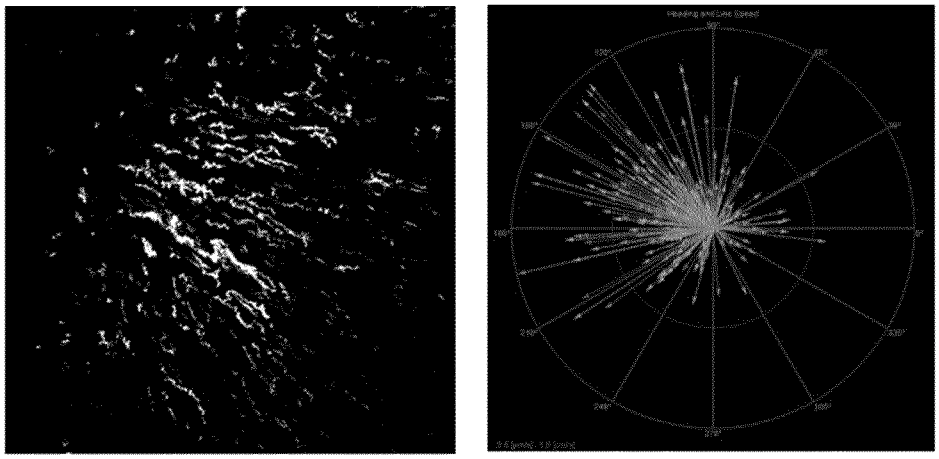


Figure 5

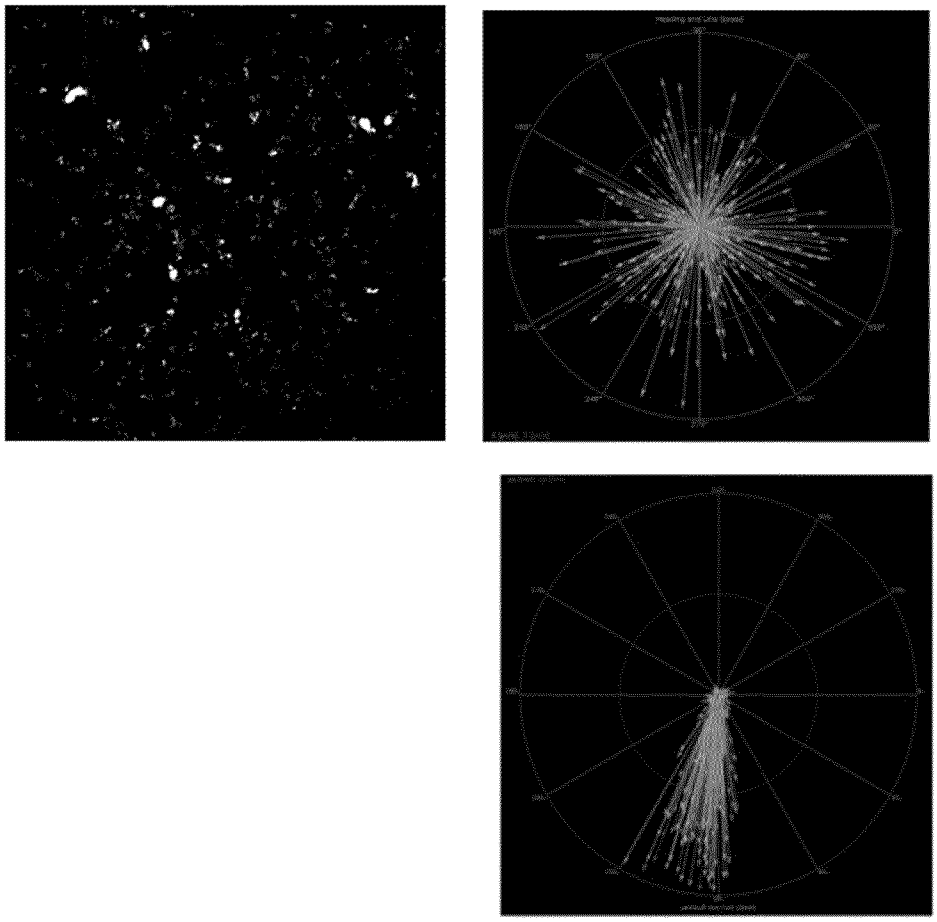
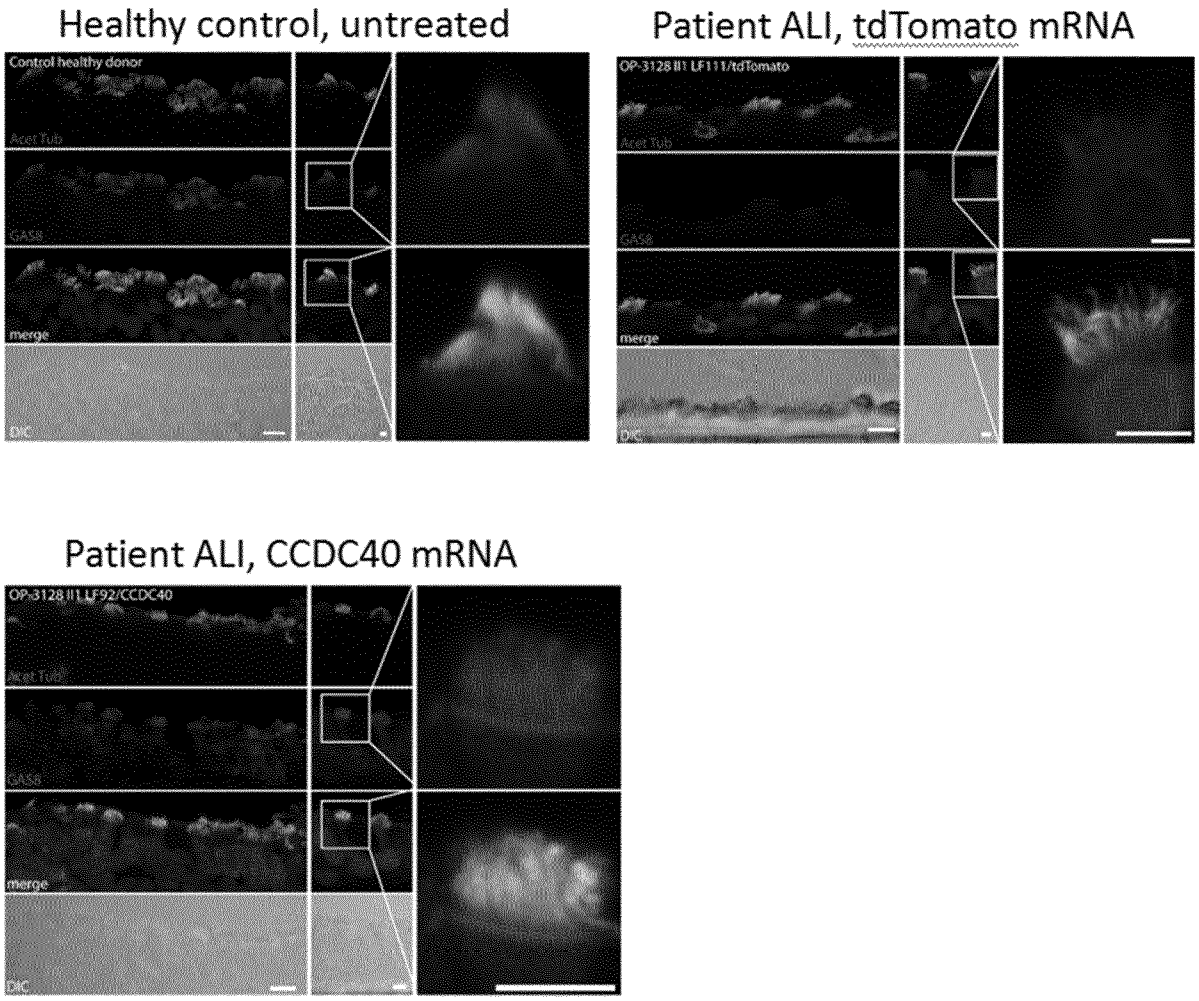


Figure 6


Healthy control, untreated
Control healthy donor
Acet Tub
GAS8
merge
DIC
Patient ALI, tdTomato mRNA
OP-3128 II1 LF111/tdTomato
Acet Tub
GAS8
merge
DIC
Patient ALI, CCDC40 mRNA
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Acet Tub
GAS8
merge
DIC

Figure 7

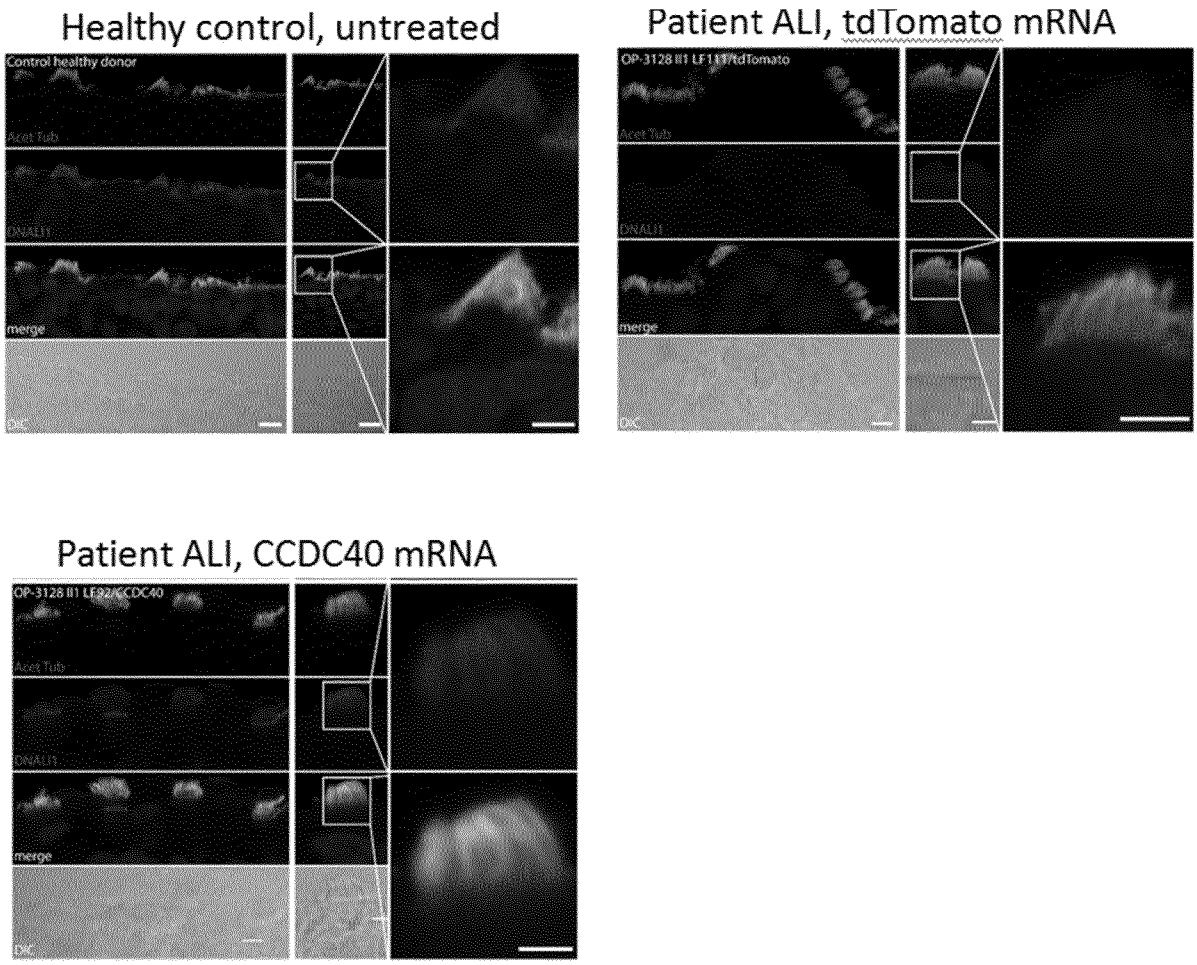
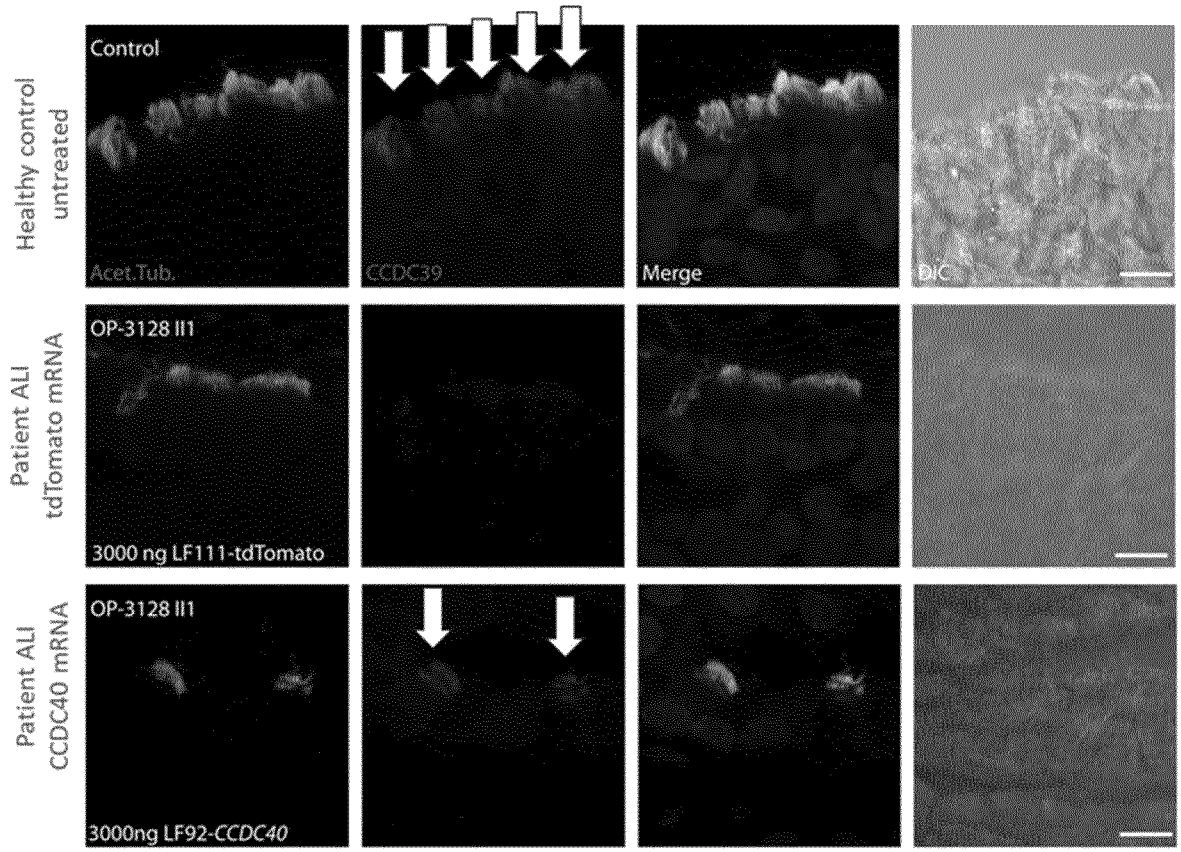

Healthy control, untreated
Control healthy donor
Acet Tub
DNALI1
merge
DIC
Patient ALI, tdTomato mRNA
Acet Tub
DNALI1
merge
DIC
Patient ALI, CCDC40 mRNA
Acet Tub
DNALI1
merge
DIC

Figure 8


Healthy control untreated
Control
Acet.Tub.
CCDC39
Merge
DIC
Patient ALI tdTomato mRNA
OP-3128 II1
3000 ng LF111-tdTomato
Patient ALI CCDC40 mRNA
OP-3128 II1
3000ng LF92-CCDC40

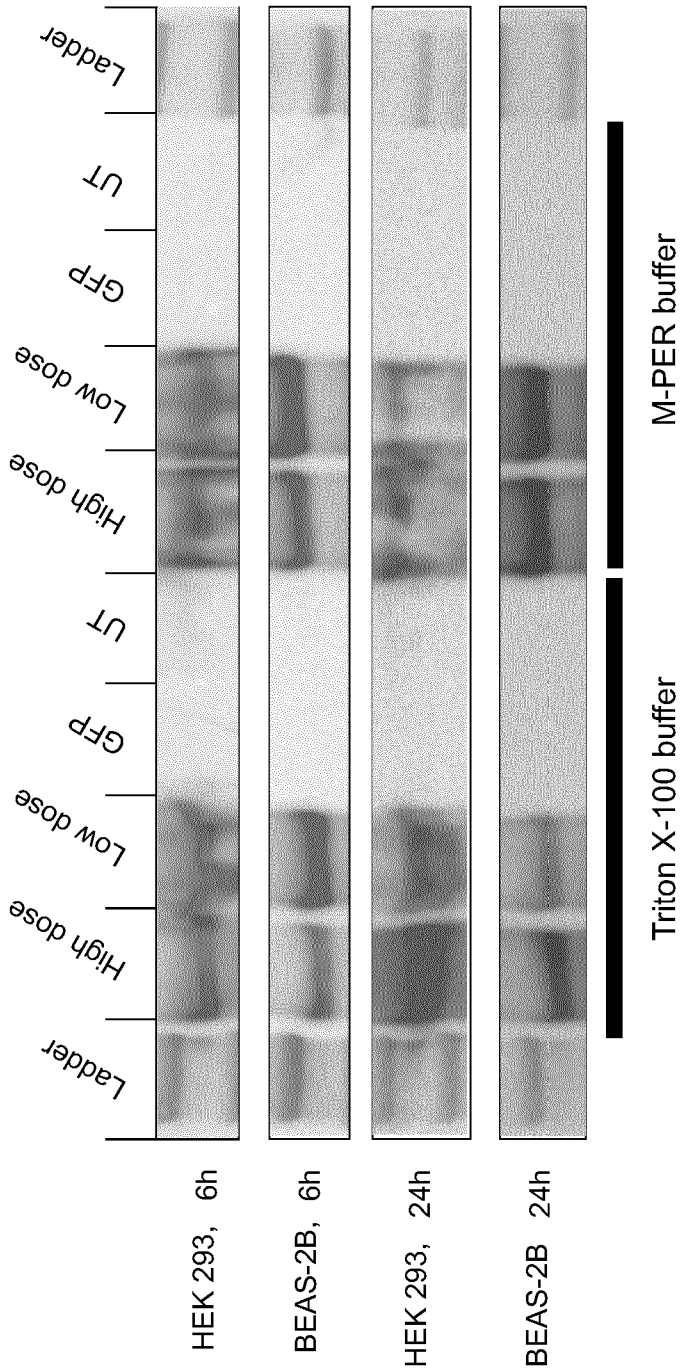

Ladder
High dose
Low dose
GFP
UT
Ladder
High dose
Low dose
GFP
UT
Triton X-100 buffer
M-PER buffer
HEK 293, 6h
BEAS-2B, 6h
HEK 293, 24h
BEAS-2B 24h

Figure 9

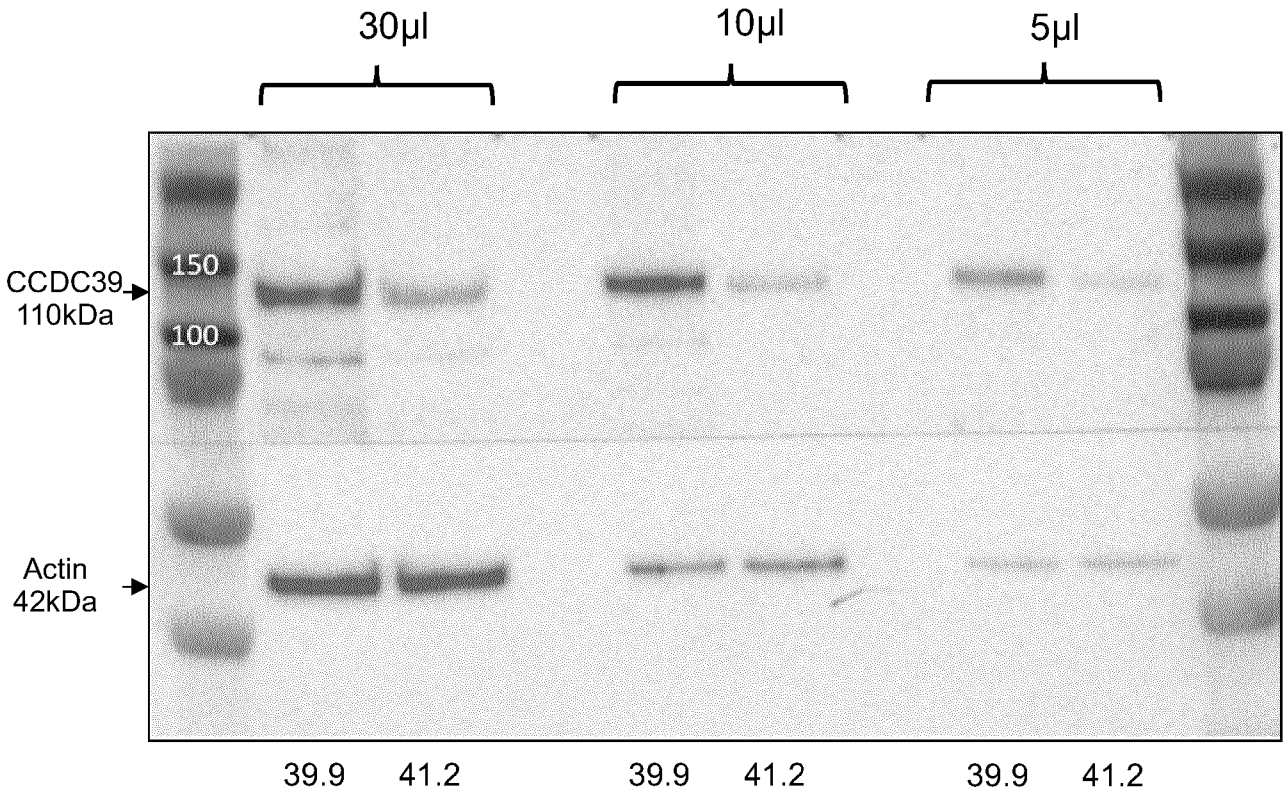


Figure 10

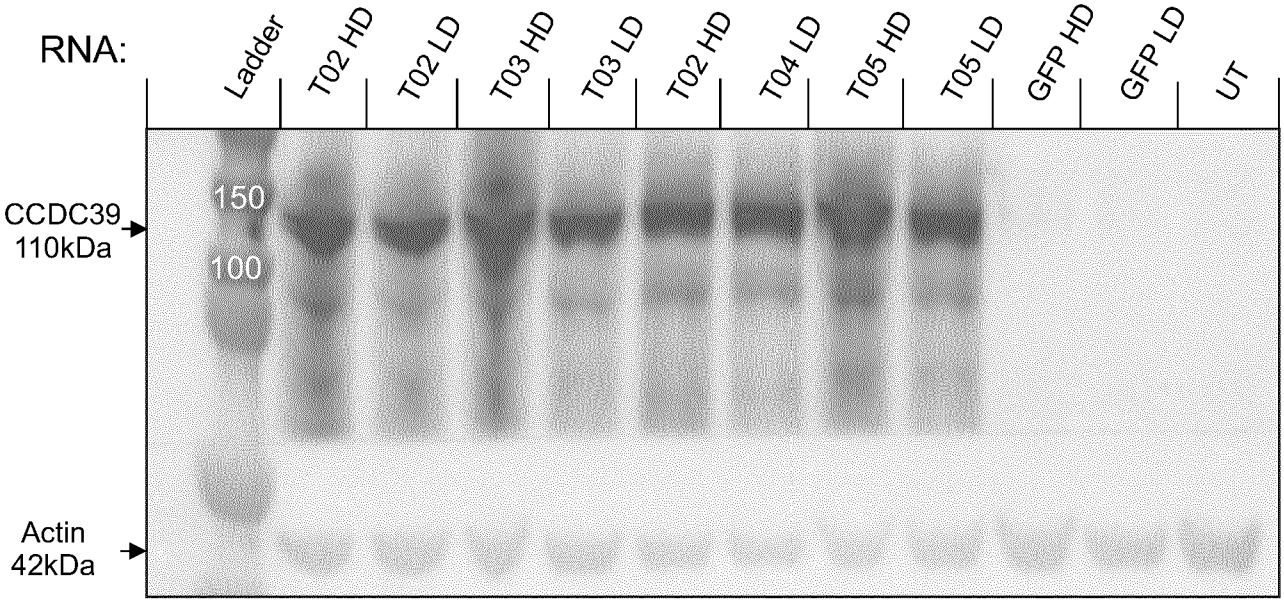


Figure 11

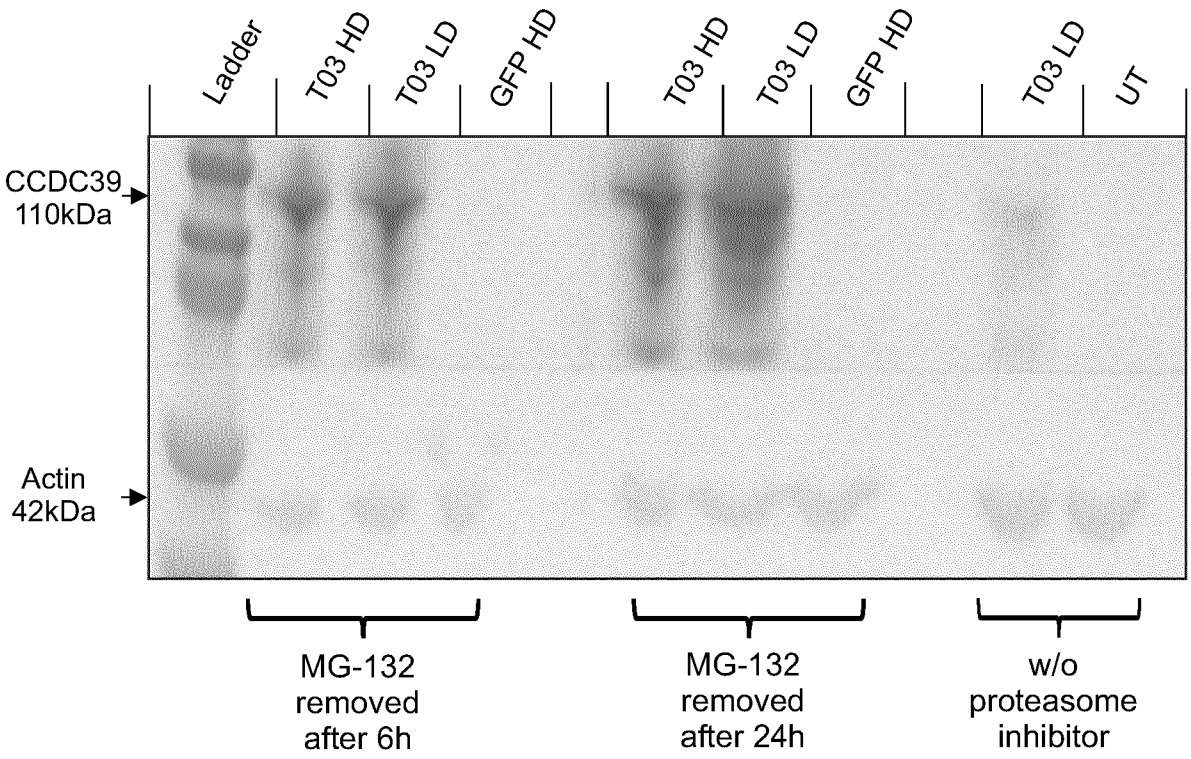

Ladder
T03 HD
T03 LD
GFP HD
T03 HD
T03 LD
GFP HD
T03 LD
UT
CCDC39
110kDa
Actin
42kDa
MG-132
removed
after 6h
MG-132
removed
after 24h
w/o
proteasome
inhibitor

Figure 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/053774

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61K9/00 A61K48/00 A61K31/7105 C12Q1/6883
C12N15/67 A61P11/00 A61K31/198
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C40B C12Q C12N A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/205767 A1 (TRANSCRIPTX INC [US]) 30 November 2017 (2017-11-30) paragraphs [0004], [0061], [0092], [0139] - [0142], [0216]; example 9	1-10
X	WO 2018/203092 A1 (UCL BUSINESS PLC [GB]) 8 November 2018 (2018-11-08) page 3, line 5 - line 10 page 5, line 1 - line 33	1-10
X	US 2015/126589 A1 (GEIGER JOHANNES [DE] ET AL) 7 May 2015 (2015-05-07) paragraphs [0001], [0018], [0025]; claim 28	1-10

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 June 2020	02/07/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pilling, Stephen

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZIHLIF NADWA ET AL: "Markers of airway inflammation in primary ciliary dyskinesia studied using exhaled breath condensate.", PEDIATRIC PULMONOLOGY JUN 2006, vol. 41, no. 6, June 2006 (2006-06), pages 509-514, XP002792982, ISSN: 8755-6863 abstract ----- TERRÉ BERTA ET AL: "GEMC1 is a critical regulator of multiciliated cell differentiation.", THE EMBO JOURNAL 02 05 2016, vol. 35, no. 9, 2 May 2016 (2016-05-02), pages 942-960, XP002792983, ISSN: 1460-2075 abstract	1-10
A	----- Mustafa Mohamed Munye: "Towards gene therapy for primary ciliary dyskinesia (Thesis)", Institute of Child Health, University College London , July 2014 (2014-07), pages 1-304, XP002799489, London Retrieved from the Internet: URL:https://discovery.ucl.ac.uk/id/eprint/1435816/2/Thesis%20FINAL%20submitted.pdf [retrieved on 2020-06-22] Section 1.2, 1.2.3, 1.3.2, 5.1,2.2.47,2.2.48,3.3.1.3,3.3.3,3.4	11-15
A	----- MUNYE MUSTAFA M ET AL: "Novel Human Bronchial Epithelial Cell Lines for the Study of Gene Therapy Approaches To Treat Primary Ciliary Dyskinesia", MOLECULAR THERAPY, vol. 21, no. Suppl. 1, June 2013 (2013-06) , page S168, XP9521131, & 16TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-GENE-AND-CELL-THERAPY (ASGCT); SALT LAKE CITY, UT, USA; MAY 15 -18, 2013 abstract ----- -/--	11-15

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MUNYE M ET AL: "Towards gene therapy for primary ciliary dyskinesia", CILIA, BIOMED CENTRAL LTD, LONDON, UK, vol. 1, no. Suppl 1, 16 November 2012 (2012-11-16), page P109, XP021123150, ISSN: 2046-2530, DOI: 10.1186/2046-2530-1-S1-P109 abstract ----- JEREMY C MCINTYRE ET AL: "Gene therapy rescues cilia defects and restores olfactory function in a mammalian ciliopathy model", NATURE MEDICINE, vol. 18, no. 9, 1 September 2012 (2012-09-01), pages 1423-1428, XP055707286, New York ISSN: 1078-8956, DOI: 10.1038/nm.2860 abstract ----- GABRIELLE WHEWAY ET AL: "An siRNA-based functional genomics screen for the identification of regulators of ciliogenesis and ciliopathy genes", NATURE CELL BIOLOGY, vol. 17, no. 8, 1 August 2015 (2015-08-01) , pages 1074-1087, XP55707232, GB ISSN: 1465-7392, DOI: 10.1038/ncb3201 abstract ----- HORANI AMJAD ET AL: "Genetics and biology of primary ciliary dyskinesia", PAEDIATRIC RESPIRATORY REVIEWS, W.B. SAUNDERS, AMSTERDAM, NL, vol. 18, 11 September 2015 (2015-09-11), pages 18-24, XP029446039, ISSN: 1526-0542, DOI: 10.1016/J.PRRV.2015.09.001 abstract "PCD Genetics" on page 20 ----- JUNEKEHLET MARTHIN ET AL: "Patient-specific three-dimensional explant spheroids derived from human nasal airway epithelium: a simple methodological approach for ex vivo studies of primary ciliary dyskinesia", CILIA, BIOMED CENTRAL LTD, LONDON, UK, vol. 6, no. 1, 23 March 2017 (2017-03-23), pages 1-9, XP021243370, DOI: 10.1186/S13630-017-0049-5 abstract ----- -/--	11-15 11-15 11-15 11-15 11-15

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOON KIM ET AL: "Functional genomic screen for modulators of ciliogenesis and cilium length", NATURE, vol. 464, no. 7291, 15 April 2010 (2010-04-15), pages 1048-1051, XP055182068, ISSN: 0028-0836, DOI: 10.1038/nature08895 abstract -----	11-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2020/053774

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-10

A pharmaceutical composition comprising a polyribonucleotide for use in treating a ciliopathy in a subject suffering of a ciliopathy, wherein the polyribonucleotide encodes a functional version of a protein a defect of which is associated with said ciliopathy, and wherein administration of said pharmaceutical composition to the respiratory system of said patient is effected when the patient shows an inflammation of the respiratory system

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2. claims: 11-15

A method for analyzing the effect of a polyribonucleotide on ciliogenesis, wherein said polyribonucleotide encodes a protein involved in and/or required for ciliogenesis, said method comprising the steps of:(a) obtaining a nose brush of a subject having a ciliopathy, said nose brush comprising undifferentiated basal cells and differentiated ciliated cells,(b) culturing the cells obtained from step (a) as a submerse cell culture for obtaining undifferentiated basal cells and dedifferentiated ciliated cells,(c) culturing undifferentiated basal cells and dedifferentiated ciliated cells obtained from step (b) as an air liquid interface cell culture and performing an air lift,(d) transfecting cells obtained from step (c) with a polyribonucleotide encoding a protein involved in and/or required for ciliogenesis,(e) culturing the transfected cells obtained from step (d) for obtaining differentiated ciliated cells, and(f) determining the effect of said polyribonucleotide on ciliogenesis using a lactate dehydrogenase measurement, a NucGreen assay, a high speed video microscopy, a ciliary beat frequency measurement, a mucociliary clearance assay, and/or immunofluorescence staining.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2020/053774

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017205767 A1	30-11-2017	AU 2017271665 A1	17-01-2019
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		KR 20190045403 A	02-05-2019
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		ZA 201408184 B	28-10-2015