Data Supplement

Higher throughput drug screening for rare respiratory diseases: readthrough therapy in primary ciliary dyskinesia

Dani Do Hyang Lee (1*), Daniela Cardinale (1*), Ersilia Nigro (2), Colin R. Butler (2), Andrew Rutman (3), Mahmoud R. Fassad (5, 6), Robert A. Hirst (3), Dale Moulding (7), Alexander Agrotis (8), Elisabeth Forsythe (5), Daniel Peckham (4), Evie Robson (4), Claire M. Smith (1), Satyanarayana Somavarapu (9), Philip L. Beales (5), Stephen L. Hart (5), Sam M. Janes (2), Hannah M. Mitchison (5), Robin Ketteler (8), Robert E. Hynds (2,10[#]) and Christopher O'Callaghan (1,3[#])

(1) Respiratory, Critical Care & Anesthesia, UCL Great Ormond Street Institute of Child Health, London, U.K.
(2) Lungs for Living Research Centre, UCL Respiratory, Division of Medicine, University College London, London, U.K.
(3) Centre for PCD Diagnosis and Research, Department of Respiratory Sciences, University of Leicester, Leicester, U.K.
(4) Leeds Institute for Medical Research, University of Leeds, Leeds, U.K.
(5) Ciliary Disease Section, Genetics and Genomic Medicine Research and Teaching Department, UCL Great Ormond Street Institute of Child Health, London, U.K.
(6) Department of Human Genetics, Medical Research Institute, Alexandria University, Alexandria, Egypt.
(7) Developmental Biology and Cancer, UCL Great Ormond Street Institute of Child Health, London, U.K.
(8) MRC Laboratory for Molecular Cell Biology, University College London, London, U.K.
(9) Department of Pharmaceutics, UCL School of Pharmacy, University College London, U.K.
(10) UCL Cancer Institute, University College London, U.K.

***** indicates equal contribution

[#] Corresponding Authors
Professor Christopher O'Callaghan (cocallaghan@ucl.ac.uk)
Respiratory, Critical Care & Anesthesia, Great Ormond Street Children's Hospital and UCL Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, U.K.

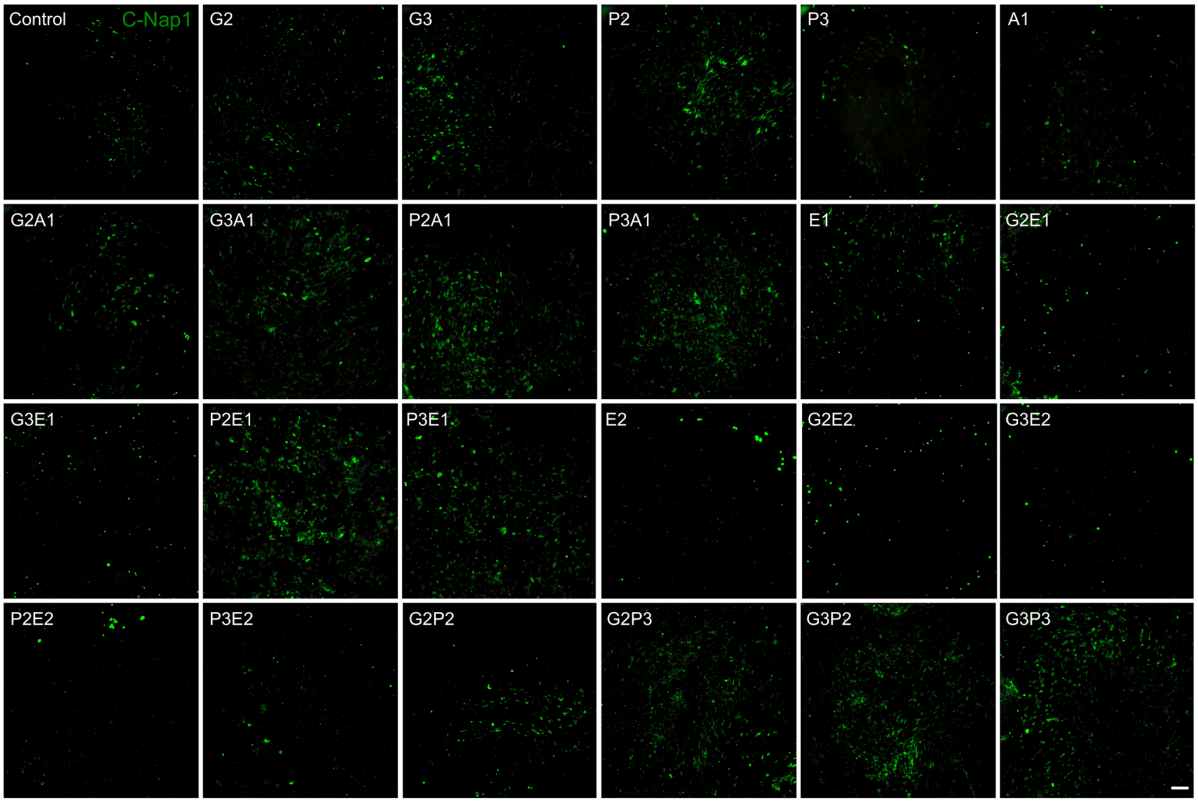
Dr. Robert E. Hynds (rob.hynds@ucl.ac.uk)
UCL Respiratory, University College London, 5 University Street, London, WC1E 6JF, U.K.

Airway Disease	Patient	Culture Passage Number			
		BEGM	3T3+Y	24-t	96-t
	Healthy 1	2	2	3	3
	Healthy 2	2	4		
	Healthy 3	2	3	3	3
	Healthy 4	2	2	4	4
	PCD 1	2	2		
	PCD 2	2	2		
	PCD 3	2	2		
	PCD 4	2	2		
	PCD 5	2	2	4	4
	PCD 6	2	2		
	PCD 7	2	2		
	PCD 8	2	2		
	PCD 9	2	2		
	PCD 10 PCD 11 PCD 12 RGMC	2	2	 3 3	 3 3

Supplementary Table S1: Passage number of cells used in experiments. 24-t and 96-t refer to air-liquid interface cultures in 24- and 96-well transwell inserts, respectively.

Primer	Forward	Reverse
MCIDAS (N-terminal)	5'-GGAGGCAGGAGGCACAATG-3'	5'-GGAGCGAACTTCCTCTCCG-3'
MCIDAS (C-terminal)	5'-CCTCGGTGCTGGATAAGCTG-3'	5'-CTCCTCCAGGCTCCTTTTGG-3'
GAPDH	5'-TGCACCACCAACTGCTTAGC-3'	5'-GGCATGGACTGTGGTCATGAG-3'

Supplementary Table S2: Primers used for MCIDAS qPCR.

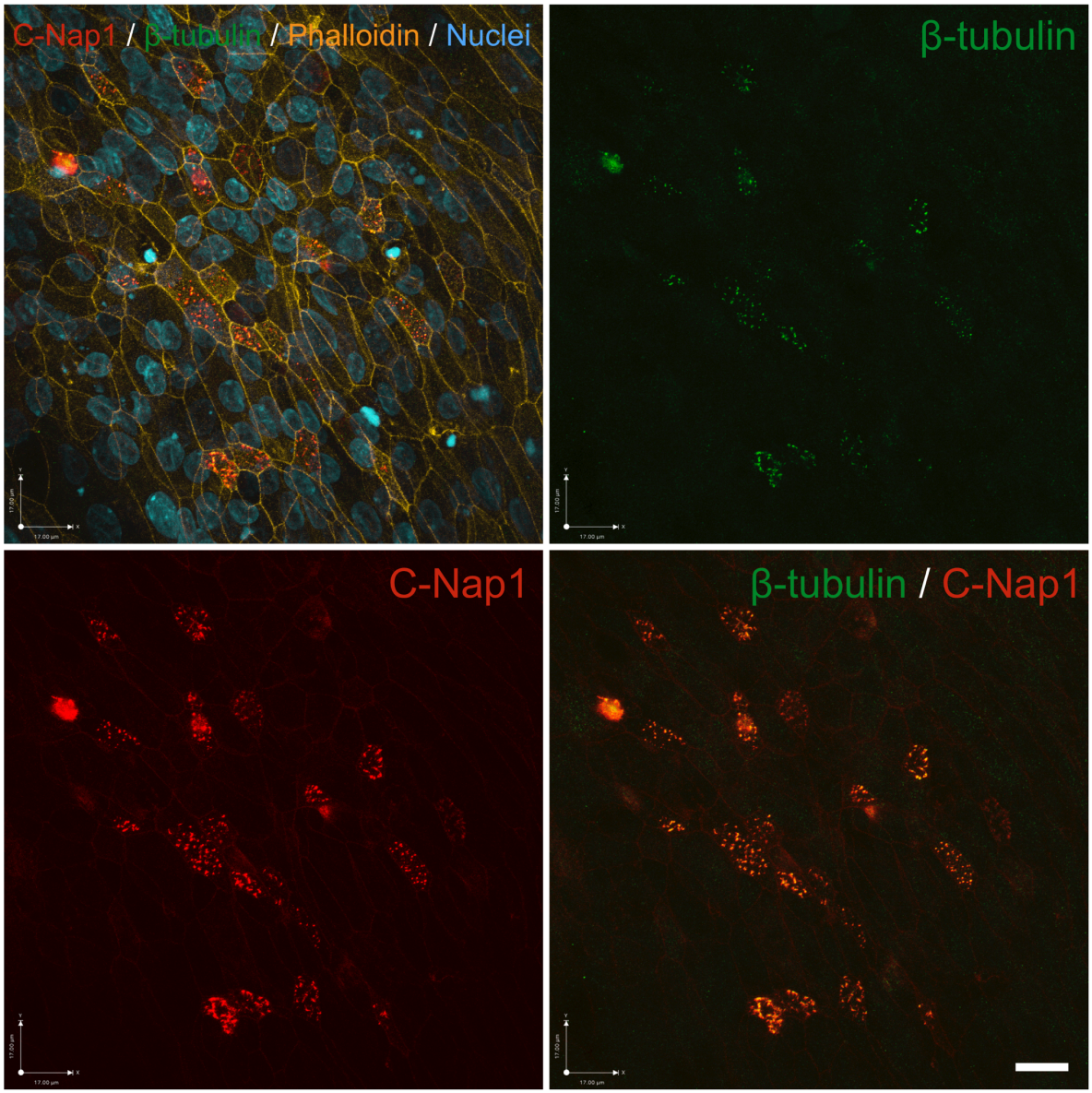


Supplementary Figure S1: Confocal scanning images from screening of RGMC cells treated with read-through drugs in ALI cultures.

C-Nap1 staining (green), Z-stack projection, max intensity. Scale bar = 200 µm.

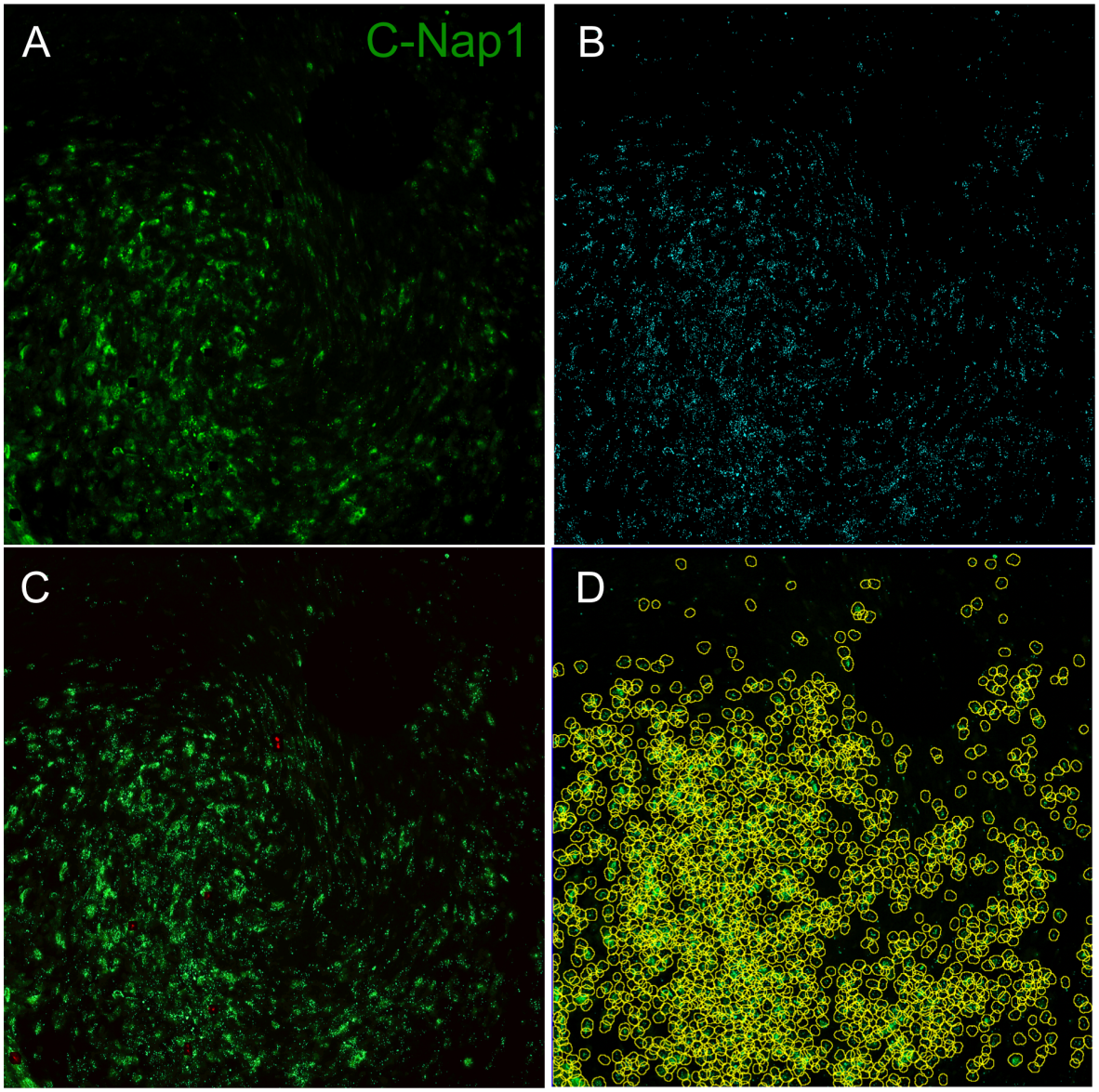
Supplementary Figure S2. Western blots of multicillin in healthy volunteers at ALI.

Cells were collected at different time points (days) after transfer into air-liquid interface culture. SDS page of nuclear extracts from cultures from two different healthy volunteers, showing multicillin expression compared to cofilin or histone deacetylase (HDAC-1).



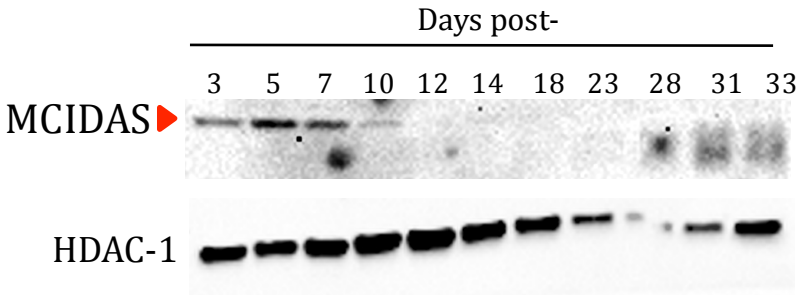
Supplementary Figure S3: Formation of basal bodies in healthy donor cells in 96 transwell air-liquid interface cultures.

Immunofluorescence images demonstrating the colocalization of C-Nap1 (red) basal body marker with the β-tubulin (green) cilia marker in healthy cells grown at ALI in 96 transwell for 15 days. Nuclei are in blue (DAPI) and F-actin in orange (phalloidin). Scale bar = 17 µm.



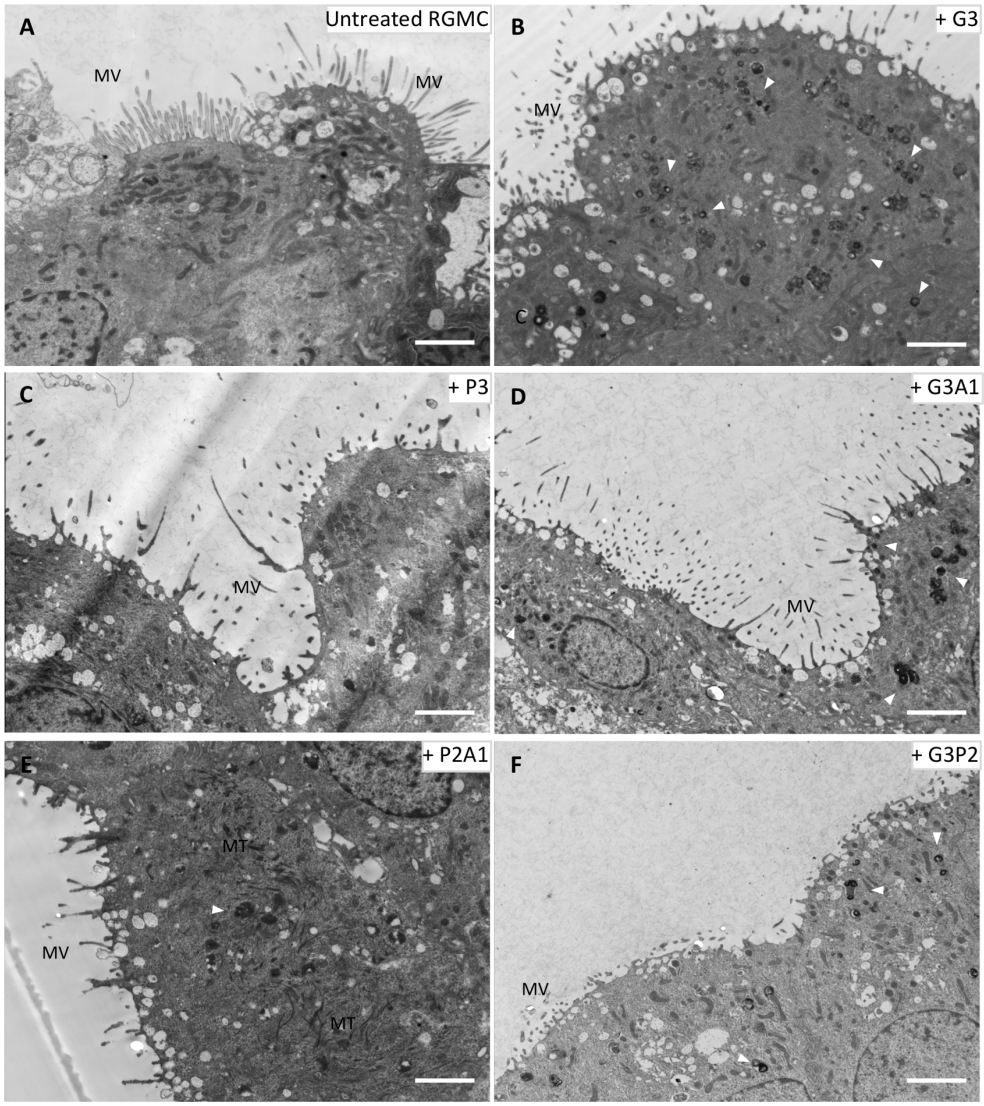
Supplementary Figure S4: Analysis of basal bodies in cultured primary human nasal epithelial cells at air-liquid interface using ImageJ.

Stacks from confocal scanning of 96 transwell plate were analysed in ImageJ. (A) original image (C-Nap1 staining (green), Z-stack projection, max intensity) . (B) Selected brightest points, with a radius of 4 pixels. (C) Over-saturated areas where excluded from analysis (red areas). (D) clusters of points (i.e. C-Nap1 staining; minimum of 4 points per cluster at a distance of 15 pixels). Scale bars = 200 µm.



Supplementary Figure S5. Long exposure Western blot of multicillin in untreated RGMC cells at air-liquid interface (ALI).

RGMC cells were collected at different time points after transfer into air-liquid interface culture. SDS page of nuclear extracts from cultures showing multicillin expression are shown compared to histone deacetylase (HDAC-1).



Supplementary Figure S6: Transmission electron microscopy of basal body precursors following readthrough therapy in cells from a patient with *MCIDAS*-mutated RGMC ciliopathy.

The different panels show representative low magnification images of RGMC cells untreated (A) and treated with different drugs combinations: Gentamicin 100 μg/ml (B, G3); Ataluren 10 μg/ml (C, P3); Gentamycin 100 μg/ml and Amlexanox 1.5 μg/ml (D, G3A1); Ataluren 5 μg/ml and Amlexanox 1.5 μg/ml (E, P2A1), Gentamycin 100 μg/ml and Ataluren 5 μg/ml (F, G3P2). Cells cultured at ALI were fixed at day 12 after air-lifiting. As seen in the untreated control these cells present long microvilli (MV) and not cilia. After drugs treatment precursors of basal bodies can be identified: electron-dense deuterosomes are indicated with arrowheads, we can distinguish some more structured centrioles (labelled with C) and microtubules agglomerations (MT). Scale bar = 2 μm.