

1 **BMI-1 extends proliferative potential of human bronchial epithelial cells whilst**  
2 **retaining their mucociliary differentiation capacity.**

3 Mustafa M. Munye<sup>1</sup>; Amelia Shoemark<sup>2</sup>; Robert A. Hirst<sup>3</sup>; Juliette M. Delhove<sup>1</sup>; Tyson  
4 V. Sharp<sup>4</sup>; Tristan R. McKay<sup>5</sup>; Christopher O'Callaghan<sup>1</sup>; Deborah L. Baines<sup>6</sup>;  
5 Steven J. Howe<sup>1</sup>; Stephen L. Hart<sup>1</sup>

6 **AFFILIATIONS**

7 <sup>1</sup> UCL Great Ormond Street Institute of Child Health, London, United Kingdom.

8 <sup>2</sup> Imperial College London, UK Electron Microscopy Dept, Royal Brompton and  
9 Harefield NHS Foundation Trust, London, UK.

10 <sup>3</sup> Primary Ciliary Dyskinesia Centre Department of Infection, Immunity and  
11 Inflammation, University of Leicester, Leicester, United Kingdom.

12 <sup>4</sup> Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of  
13 London, London, United Kingdom.

14 <sup>5</sup> School of Healthcare Science, Manchester Metropolitan University, Manchester,  
15 United Kingdom.

16 <sup>6</sup> Institute for Infection and Immunity, St George's, University of London, London,  
17 United Kingdom

18

19 **Correspondence should be addressed to M.M.M. (m.munye@ucl.ac.uk)**

20 **UCL Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, United**  
21 **Kingdom**

22 **ABBREVIATIONS LIST**

23

24 ALI = Air-Liquid Interface

25 BEGM = Bronchial Epithelial Growth Media

26 CBF = Cilia Beat Frequency

27 CFBE = Cystic Fibrosis Bronchial Epithelial

28 CRCs = Conditionally Reprogrammed Cells

29 DMEM = Dulbecco's Modified Eagle Medium

30 GFP = Green Fluorescent Protein

31 HBE = Human Bronchial Epithelial

32 hESCs = human Embryonic Stem Cells

33 hTERT = human Telomerase Reverse Transcriptase

34 iPSCs = induced Pluripotent Stem Cells

35  $I_{sc}$  = short circuit current

36 NHBE = Normal Human Bronchial Epithelial

37 ODA = Outer Dynein Arms

38 PBS = Phosphate Buffered Saline

39 PCD = Primary Ciliary Dyskinesia

40 ROCK = Rho-associated protein kinase

41 **ABSTRACT**

42 Air-liquid interface (ALI) culture of primary airway epithelial cells enables mucociliary  
43 differentiation providing an *in vitro* model of the human airway but their proliferative  
44 potential is limited. To extend proliferation, these cells were previously transduced  
45 with viral oncogenes or mouse *Bmi-1 + hTERT* but the resultant cell lines did not  
46 undergo mucociliary differentiation. We hypothesised that use of human *BMI-1* alone  
47 would increase the proliferative potential of bronchial epithelial cells while retaining  
48 their mucociliary differentiation potential. CF and non-CF bronchial epithelial cells  
49 were transduced by lentivirus with *BMI-1* then their morphology, replication kinetics  
50 and karyotype were assessed. When differentiated at ALI, mucin production, ciliary  
51 function and transepithelial electrophysiology were measured. Finally, shRNA  
52 knockdown of *DNAH5* in BMI-1 cells was used to model primary ciliary dyskinesia  
53 (PCD). BMI-1 transduced basal cells showed normal cell morphology, karyotype  
54 and doubling times despite extensive passaging. The cell lines underwent  
55 mucociliary differentiation when cultured at ALI with abundant ciliation and  
56 production of the gel-forming mucins MUC5AC and MUC5B evident. Cilia displayed  
57 a normal beat frequency and 9+2 ultrastructure. Electrophysiological characteristics  
58 of BMI-1 transduced cells were similar to un-transduced cells. shRNA knockdown of  
59 *DNAH5* in BMI-1 cells produced immotile cilia and absence of DNAH5 in the ciliary  
60 axoneme as seen in cells from patients with PCD. BMI-1 delayed senescence in  
61 bronchial epithelial cells, increasing their proliferative potential but maintaining  
62 mucociliary differentiation at ALI. We have shown these cells are amenable to  
63 genetic manipulation and can be used to produce novel disease models for research  
64 and dissemination.

- 65 Key words: air-liquid interface, airway model, lung, mucociliary differentiation,  
66 primary ciliary dyskinesia

## 67 INTRODUCTION

68 The ciliated epithelium lining the airways provides the first line of defence to inhaled  
69 pathogens and particles and plays a crucial role in many respiratory diseases. It is possible  
70 to remove respiratory epithelial cells from the nose or upper airways of donors by brushing  
71 and culture them in the laboratory on collagen-coated, semi-permeable membranes. The  
72 progenitor basal epithelial cells from the brushings cultured at Air-Liquid Interface (ALI)  
73 differentiate into a fully ciliated, pseudostratified epithelium closely resembling that found in  
74 the airway (3).

75 If cells are obtained from a donor with a lung disease, e.g., cystic fibrosis, primary ciliary  
76 dyskinesia (PCD), asthma and chronic obstructive pulmonary disease, these ALI cultures  
77 provide a surrogate model of the diseased lung for research into pathogenic mechanisms  
78 and for the development of new therapeutics(9, 14, 16). However, basal epithelial cells can  
79 only be passaged 2-3 times before they lose their proliferation and differentiation potential  
80 (6, 18). Thus, to establish the wider use of basal cells in ALI epithelial culture models,  
81 methods are required that enable basal cells to be cultured for longer, genetically  
82 engineered, expanded and stored easily prior to differentiation on ALI cultures. Such cells  
83 would also overcome ethical issues related to repeated brushing of volunteers.

84 Recent approaches to extend the utility of primary, basal epithelial cells involved culturing  
85 them with rho-associated protein kinase (ROCK) inhibitors on a layer of irradiated feeder  
86 cells to provide cell-derived growth factors (18, 27). The requirement for irradiated feeder  
87 cells makes the maintenance of basal cell cultures complex and time-consuming, difficult to  
88 scale up and may limit the use of this approach to specialist laboratories. Alternatively,  
89 induced pluripotent stem cells (iPSCs) and embryonic stem cells (hESCs) were differentiated  
90 into mature respiratory epithelial cells and used to generate a pseudostratified epithelium  
91 expressing CFTR (30). However, the process takes several weeks and often the resulting  
92 cultures are not suitable for disease modelling as they are contaminated with endodermal

93 cell types (31) and often present with karyotypic anomalies which may confound drug  
94 screening efforts.

95 Extended proliferative potential of primary human bronchial epithelial (HBE) cells  
96 was described by transduction of basal cells with the mouse polycomb complex  
97 protein *Bmi-1* and human telomerase reverse transcriptase (*hTERT*) (6). Unlike cells  
98 transformed with viral oncogenes, *Bmi-1+hTERT* cell lines had no chromosomal  
99 abnormalities and produced a pseudostratified epithelium on ALI but gave only  
100 sparse ciliogenesis. This limited differentiation capacity may be explained by reports  
101 that *hTERT*, following long-term growth in culture, up-regulates expression of the  
102 potent mitogen c-Myc, so promoting entry into the cell cycle (21) thereby impeding  
103 ciliogenesis.

104 We hypothesised that BMI-1 transduction alone may overcome these issues  
105 observed with *Bmi-1+hTERT*, to produce basal cells with the potential for extended  
106 proliferation that retain their differentiation capacity on ALI. In this study, *BMI-1*  
107 transduced primary basal epithelial cells from CF and healthy donors were  
108 investigated for their morphology, growth characteristics and karyotype. We also  
109 assessed the cells mucociliary differentiation potential at ALI along with their Na<sup>+</sup> and  
110 Cl<sup>-</sup> transport properties in Ussing chamber studies. We then demonstrate their use  
111 for the production of novel engineered disease models by shRNA knockdown of  
112 *DNAH5*, a gene associated with PCD, a ciliopathy with significant lung pathology  
113 resulting from abnormal mucociliary clearance. *BMI-1* transduction offers a facile  
114 method to greatly extend the utility of basal epithelial cells for translational and basic  
115 research.

116

## 117 **MATERIALS & METHODS**

### 118 **Materials**

119 Primary antibodies used in this study can be found in Table 1. Secondary antibodies  
120 for immunofluorescence were anti-IgG antibodies conjugated with AlexaFluor dyes  
121 (Invitrogen, Life Technologies). Secondary antibodies for Western blots were  
122 horseradish peroxidase-conjugated (HRP-conjugated) anti-IgG antibodies (Dako,  
123 Agilent Technologies).

### 124 **Collagen Coating**

125 Tissue culture flasks and transwells were coated for 1 hour at room temperature with  
126 1% (v/v) solution of a 3mg/mL bovine collagen solution (PureCol; Advanced  
127 Biomatrix) in phosphate buffered saline (PBS), then washed with distilled water and  
128 air-dried.

### 129 **Cell Culture**

130 HEK293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)  
131 supplemented with 10% (v/v) foetal bovine serum. Normal human bronchial epithelial  
132 (NHBE) cells, cystic fibrosis human bronchial epithelial cells (CFBE) cells were  
133 grown on collagen-coated plastic in bronchial epithelial growth media (BEGM;  
134 Lonza). All cells were grown at 37°C and 5% CO<sub>2</sub>. NHBE and CFBE cells were  
135 purchased from Lonza and Epithelix SàRL.

### 136 **Lentivirus Production and Transduction**

137 Full-length human BMI-1 cDNA was PCR cloned from pHR-EF1 $\alpha$ -BMI1-IRES-GFP  
138 plasmid(20) with *XhoI* and *BamHI* sites added and TOPO cloned into pCR4 TOPO  
139 vector before being subcloned into pLVX-Puro vector digested with *XhoI* and *BamHI*.  
140 Lentivirus was produced as previously described(20), concentrated by centrifugation

141 at 4,500 x g for 18 hours at 4°C, re-suspended in OptiMem and added to cell media  
142 to transduce NHBE and CFBE cells (Lonza) at passage 2.

### 143 **Doubling Time Analysis**

144 NHBE and NHBE BMI-1 cells at varying passage numbers were seeded at densities  
145 of 30,000 cells per well onto collagen-coated 12-well plates. Cells were detached  
146 using trypsin-EDTA following 1-4 days in culture and total cell numbers per well were  
147 counted using a haemocytometer. An online calculator was used to calculate the  
148 doubling time (Roth V. 2006 Doubling Time Computing, Available from:  
149 <http://www.doubling-time.com/compute.php>). Doubling times were calculated using  
150 the formula;

$$doubling\ time = \frac{duration \times \log(2)}{\log(final\ cell\ count) - \log(initial\ cell\ count)}$$

151 Where cell count values were mean cell count of 3 independent wells.

### 152 **Western Blotting**

153 Cells were lysed with Cell Extraction Buffer (Life Technologies), boiled in the  
154 presence of NuPage LDS Sample Buffer (Life Technologies) and loaded onto  
155 NuPage Novex 4-12% Bis-Tris gels (Life Technologies). Electrophoresis and protein  
156 transfer onto Immobilon-P polyvinylidene fluoride membranes were performed using  
157 standard protocols. Antibodies against BMI-1, p16Ink4a and GAPDH and  
158 appropriate HRP-conjugated secondary antibodies were used for probing with bands  
159 visualised using Pierce ECL Western Blotting Substrate (Life Technologies, Paisley,  
160 UK) and a UVIchemi chemiluminescence imaging system (UVItec).



161 **Air-liquid Interface (ALI) Culture**

162 Cells grown to ~80% confluence in T75 flasks were trypsinised, seeded at a density  
163 of 900,000 cells/cm<sup>2</sup> on Transwell inserts (Corning) and grown at an ALI as  
164 previously described(8). Cell were maintained at an ALI for 4 weeks before analyses  
165 were performed.

166 **Quantitative Reverse Transcription PCR (qRT-PCR)**

167 Unless indicated, all reagents for qRT-PCR were obtained from ThermoFisher. Total  
168 RNA was harvested from cells using RNeasy Mini Kit (Qiagen) and potential DNA  
169 impurities digested using DNase I enzyme (TURBO DNA-free kit). Purified RNA was  
170 reverse transcribed with 2.5U/μL murine leukaemia virus (MuLV) reverse  
171 transcriptase at 42°C for 1 hour in a reaction containing 1x GeneAmp PCR Gold  
172 Buffer, 1mM each dNTP, 5μM random hexamers, 5mM MgCl<sub>2</sub> and 1U/μL RNase  
173 inhibitor. The resulting cDNA was used in a qPCR reaction containing 1x Platinum  
174 Quantitative PCR SuperMix-UDG w/ROX and 1x TaqMan Gene Expression Assay  
175 primer/probe set (GAPDH primer/probe set Hs99999905\_m1; DNAH5 primer/probe  
176 set Hs00292485\_m1). The PCR reaction cycles used were 50°C for 2 minutes, 95°C  
177 for 10 minutes, 40 cycles of 95°C for 15 seconds and 60°C for 1 minute on an ABI  
178 PRISM 7000 Sequence Detection System (Applied Biosystems). Fluorescence data  
179 was collected at the end of each 60°C reaction and relative expression levels  
180 calculated using the delta-delta Ct (2- ΔΔCt) method(19).

181 **Immunofluorescence Staining and Confocal Microscopy**

182 Cells were fixed with 4% PFA for 10 minutes at room temperature, washed with PBS  
183 and permeabilised with PBS-Triton (PBS 0.1% (v/v) Triton-X100) for 10 minutes at  
184 room temperature before blocking, immunostaining and mounting on microscope  
185 slides as previously described(26). Images were obtained using an Inverted Zeiss

186 LSM 710 Confocal microscope with the appropriate excitation lasers selected for the  
187 dyes used.

### 188 **Fluorescence Microscopy**

189 Bright-field and fluorescence images were captured with a Nikon Digital Sight DS-  
190 QiMC video camera attached to a Nikon Eclipse Ti-U inverted microscope. Videos  
191 and images were processed using NIS Elements AR software (Nikon, v4.00.12).

### 192 **TEM for Cilia Ultrastructure**

193 Ciliated cells cultured at an ALI were scraped and cells washed off with 200 $\mu$ L  
194 warmed BEBM. Cells were fixed by addition of 2mL of 2.5% glutaraldehyde and  
195 stored at 4°C for at least 24 hours prior to further processing as previously  
196 described(24). Assessment of cilia ultrastructure was undertaken blinded by Dr  
197 Amelia Shoemark, a member of the PCD diagnostic service team at the Royal  
198 Brompton & Harefield NHS Foundation Trust, UK.

### 199 **High-Speed Video Microscopy**

200 High-speed video was recorded using a MotionPro X4 high-speed motion camera  
201 attached to a Nikon Eclipse Ti-U inverted microscope built with an environmental  
202 chamber. Videos were recorded at a frame rate of 500fps using Motion Studio  
203 software (IDT Vision, v2.11) with cells maintained at 37°C.

204 For cilia beat frequency (CBF) assessment, ALI cultures were washed twice with  
205 PBS to remove mucus that may have affected CBF. After washing, the cells were  
206 allowed to equilibrate at 37°C and 5% CO<sub>2</sub> for 20 minutes before video recording. At  
207 least four independent cultures per donor line were videoed with five areas recorded  
208 per culture, i.e., at least 20 videos were captured per donor line. To minimise bias

209 videos were recorded from the top, bottom, left, right and centre region of each  
210 culture and cilia beat-frequency assessed using CiliaFA software(25).

### 211 **Electrophysiology Studies**

212 Cells were grown at ALI for 4 weeks on Snapwell membranes (Corning) to enable  
213 mucociliary differentiation. Snapwells were then mounted on Ussing chambers and  
214 short circuit current ( $I_{sc}$ ) was measured as previously described (32). Briefly,  
215 monolayers were mounted in Ussing chambers in physiological salt solution  
216 consisting of 117mM NaCl, 25mM NaHCO<sub>3</sub>, 4.7mM KCl, 1.2mM MgSO<sub>4</sub>, 1.2mM  
217 KH<sub>2</sub>PO<sub>4</sub>, 2.5mM CaCl<sub>2</sub> and 11mM d-glucose. The solution was continuously  
218 circulated throughout the course of the experiment and maintained at 37°C whilst  
219 bubbled with 21% O<sub>2</sub> + 5% CO<sub>2</sub> premixed gas. Monolayers were first maintained  
220 under open-circuit conditions until transepithelial potential difference ( $V_t$ ) and  
221 resistance stabilised. The cells were then short-circuited by clamping  $V_t$  at 0 mV  
222 using a DVC-4000 voltage/current clamp, and  $I_{sc}$  was measured and recorded using  
223 a PowerLab computer interface. Every 30 seconds the preparations were returned to  
224 open-circuit conditions for 3 seconds so that the spontaneous  $V_t$  could be measured  
225 and trans-epithelial electrical resistance (TEER) calculated. Drugs were circulated in  
226 physiological salt solution and added in the order of amiloride (10  $\mu$ M, apical),  
227 forskolin (25  $\mu$ M, apical and basolateral) and GlyH-101 (10  $\mu$ M, apical).

228 **RESULTS**

229 ***Characterisation of BMI-1 transduced cells in submerged culture***

230 Primary NHBE cells maintained in submerged cultures displayed a  
231 characteristic cobblestone appearance (Figure 1a) but by passage 3 cells became  
232 elongated in appearance (white arrow; Figure 1b) and squamous differentiation was  
233 evident (black arrow; Figure 1b). In contrast, BMI-1 transduced NHBE cells (NHBE-  
234 BMI-1) maintained their cobblestone appearance following extensive passaging, for  
235 example at passage 11 (Figure 1c) and passage 17 (Figure 1d). However, squamous  
236 cells became evident following 25 passages (Figure 1e) after which the cells  
237 senesced, with no observable cell division for ten days. The cells maintained a  
238 normal diploid karyotype even at passage 23 (Figure 2).

239 BMI-1 down-regulates expression of the pro-senescent protein p16Ink4A. NHBE  
240 cells transduced with BMI-1 had low levels of p16Ink4A protein and high levels of  
241 BMI-1 (Figure 3a). Levels of BMI-1 in untransduced NHBE cells declined with an  
242 increase in passaging whilst levels of p16Ink4A increased and were higher in  
243 senesced, untransduced NHBE cells at passage 6 while BMI-1 expression was not  
244 evident by Western blot (Figure 3a).

245 SV40 large T-antigen or ROCK inhibition extends the replication potential of basal  
246 cells but alters the proliferation rate of the cells(4, 7, 12) therefore we assessed the  
247 doubling times of BMI-1 transduced cells at different passages (Figure 3b). We  
248 determined that untransduced cells at passage 2 had a doubling time of 1.18 days  
249 similar to BMI-1 transduced cells at passages 12 and 15 (doubling times of 1.25 and  
250 1.21 days respectively) although by passage 23 the doubling time had increased to  
251 1.49 days, consistent with observations of senescence at passage 25.

252 ***Differentiation of NHBE-BMI-1 Cells***

253 NHBE-BMI-1 basal cells were subsequently analysed for their differentiation  
254 potential when cultured at ALI. After 2 -3 weeks culture, both primary NHBE and  
255 NHBE-BMI-1 cells produced motile cilia (Video 1a and b respectively). NHBE-BMI-1  
256 cells maintained the ability to differentiate and produce cilia even at passage 15.

257 To quantify cilia function, we assessed cilia beat frequency of both primary and BMI-  
258 1 transduced NHBE and CFBE cells. Beating cilia from CFBE cells could not be  
259 detected, most likely due to the build-up of viscous mucus hindering cilia beating,  
260 until cultures were washed. As such, CFBE and NHBE cultures were washed twice  
261 prior to video recording and CBF analysis as detailed in the methods section.

262 CBF analysis of both primary and BMI-1 transduced NHBE and CFBE cells showed  
263 mean values within the normal range for respiratory cilia of 9-17Hz(25) (Figure 4a  
264 and b). Primary NHBE and NHBE-BMI-1 cells had a CBF of  $16.7 \pm 0.2$ Hz and  
265  $15.3 \pm 0.2$ Hz respectively (Figure 4a) and primary CFBE and CFBE-BMI-1 cells  
266 exhibited CBF values of  $12.9 \pm 0.3$ Hz and  $14.3 \pm 0.3$ Hz respectively.

267 Further evidence of differentiation was demonstrated by immuno-detection, in  
268 NHBE-BMI-1 cells, of the tight junction protein occludin (Figure 3c) and the mucins  
269 MUC5AC and MUC5B (Figure 4d, e). In addition, basal cells were present and  
270 indicated by p63 staining (Figure 4f) and BMI-1 protein was present in all nuclei  
271 (Figure 4g). The ciliary protein acetylated  $\alpha$ -tubulin was also detected by  
272 immunostaining and highlighted abundant ciliation (Figure 4h). Further analysis of  
273 the cilia in differentiated NHBE-BMI-1 cells by TEM showed that they had a normal  
274 9+2 ultrastructure with both inner and outer dynein arms present (Figure 4i, Table 2  
275 and Table 3).

276 ***Electrophysiology studies***

277 Primary HBE cells grown on ALI develop a trans-epithelial electrical  
278 resistance (TEER) with ion transport properties that can be measured by mounting of  
279 cultured epithelia on Ussing chambers and addition of drugs that can activate or  
280 inhibit specific cell surface ion channels. Cultures of primary NHBE cells from two  
281 different donors showed baseline TEER values of  $331.1 \pm 105.5 \Omega \cdot \text{cm}^2$  and  
282  $621.0 \pm 33.2 \Omega \cdot \text{cm}^2$  (Table 4) and primary CFBE cells developed TEER of  
283  $1307.9 \pm 36.6 \Omega \cdot \text{cm}^2$ . Similarly, *BMI-1* transduced NHBE and CFBE cells developed  
284 high TEER when grown at an ALI ( $1268.4 \pm 78.4 \Omega \cdot \text{cm}^2$  and  $917.6 \pm 165.3 \Omega \cdot \text{cm}^2$   
285 respectively; Table 4) demonstrating the cells retained their ability to form an  
286 electrically resistive epithelium.

287 Short circuit current ( $I_{sc}$ ) analysis in Ussing chambers of NHBE and CFBE cells  
288 revealed that both primary NHBE cells and passage 13 NHBE-BM-1 cells cultured at  
289 ALI also had similar electrophysiology. Amiloride ( $10 \mu\text{M}$ ), an inhibitor of the epithelial  
290  $\text{Na}^+$  channel ENaC reduced  $I_{sc}$  in all cultures, although the amiloride-sensitive  $I_{sc}$  was  
291 variable. Subsequent elevation of cellular cAMP with forskolin ( $25 \mu\text{M}$ ) increased  $I_{sc}$   
292 and this elevation was inhibited by the CFTR inhibitor Gly-H101 ( $10 \mu\text{M}$ ) (Figure 5 a,  
293 b). Thus, ENaC and CFTR-mediated ion transport was retained in NHBE-BMI-1  
294 cells. Primary CFBE cells and passage 17 CFBE BMI-1 cells cultured at ALI also  
295 exhibited amiloride-inhibitable  $I_{sc}$  but no response to either forskolin or GlyH-101 was  
296 observed, as expected due to the lack of CFTR in these cells (Figure 5 c, d). Thus,  
297 CFBE-BMI-1 cells, like NHBE-BMI-1, also maintain the  $\text{Na}^+$  and  $\text{Cl}^-$  ion transport  
298 characteristics of non-transduced primary CF cells.

299 ***Use of BMI-1 transduced cells to generate PCD cell models***

300 We next explored the potential use of the BMI-1 transduced NHBE cells to  
301 generate an in vitro model of PCD. The outer dynein arm protein *DNAH5* is the most  
302 commonly mutated gene but even so this is a rare disease and cells are often not  
303 readily available. Cells with *DNAH5* mutations lack the DNAH5 protein in the ciliary  
304 axoneme and have missing outer dynein arms (ODAs) (13). NHBE cells transduced  
305 with BMI-1 were additionally transduced with a DNAH5 shRNA lentiviral construct  
306 that also expresses green fluorescent protein (GFP).

307 *DNAH5* expression in shRNA-transduced cells was silenced by approximately 75%  
308 relative to untransduced cells (Figure 6a) while scrambled shRNA had no effect on  
309 *DNAH5* expression indicating silencing specificity.

310 NHBE-BMI-1 cells transduced with the two shRNAs were subsequently cultured at  
311 ALI to promote differentiation and ciliation. Following mucociliary differentiation,  
312 NHBE-BMI-1 GFP-positive cells, transduced with scrambled shRNA had motile cilia,  
313 (Video 2a) whereas GFP-positive *DNAH5* shRNA silenced cells had immotile cilia  
314 (Video 2b). However, in GFP negative cells (and by extension also *DNAH5* shRNA  
315 negative) motile cilia were still observed (Video 2c).

316 In untransduced NHBE BMI-1 cells and those GFP-positive cells transduced with the  
317 scrambled shRNA, DNAH5 was localised to the ciliary axoneme in all ciliated cells  
318 assessed as shown by co-localisation with acetylated  $\alpha$ -tubulin expression. In  
319 contrast, in DNAH5 shRNA transduced GFP-positive cells, only 2.9% (5/173) of  
320 ciliated cells had DNAH5 in the ciliary axoneme (Figure 6b and Table 5).

321

322

323 **DISCUSSION**

324 Airway diseases are a significant cause of morbidity and mortality. Mucociliary  
325 differentiation of primary airway epithelial cells using ALI culture methods provides  
326 an *in vitro* model that faithfully recapitulates the *in vivo* airway epithelium for the  
327 study of disease pathology and therapies. However, these cells can only be cultured  
328 for 2-3 passages before they lose their ability to differentiate(5). This has important  
329 practical, ethical and cost implications for research in the field. Traditional cell  
330 transformation methods, using viral oncogenes that promote entry into the cell cycle,  
331 produce immortal cell lines incapable of mucociliary differentiation most likely due to  
332 their inability to suspend cell division and allow cilia production and differentiation.

333 We have shown that prevention of cellular senescence by expression of *BMI-1*  
334 allows extended passaging of HBE cells from CF and non-CF donors. Western blot  
335 analysis highlighted that senescent primary NHBE cells had accumulated high levels  
336 of the pro-senescent protein p16<sup>Ink4a</sup> in agreement with other studies (1, 6, 20). *BMI-*  
337 *1* transduced cells, however, showed low levels of p16<sup>Ink4a</sup> thereby delaying cell  
338 senescence as reported previously(15).

339 In addition to exhibiting delayed senescence, *BMI-1* transduced cells retained their  
340 cell phenotype, karyotype, ion transport characteristics and mucociliary  
341 differentiation potential with abundant ciliation observed when cultured at ALI. Using  
342 chamber studies revealed that, like primary HBE cells, *BMI-1* transduced NHBE and  
343 CFBE cells formed electrically resistive cultures and the direction of change in  $I_{sc}$   
344 was as expected upon addition of amiloride, forskolin and the CFTR inhibitor Gly-  
345 H101. We note that baseline TEER values varied between HBE donors as did the  
346 magnitude of change in  $I_{sc}$  upon addition of amiloride, forskolin and the CFTR



347 inhibitor Gly-H101. Such variation has also been observed by Tosoni et al. (29) who  
348 recently demonstrated baseline TEER values ranged from 309 to 2963 $\Omega$ .cm<sup>2</sup> in ALI  
349 cultures generated from the cells of 18 healthy donors.

350 In agreement with our findings, Torr et al(28) recently demonstrated that  
351 transduction of basal cells, from different two donors, with human *BMI-1* alone  
352 extends the proliferative potential of NHBE cells whilst retaining their differentiation  
353 potential as demonstrated by immunostaining and scanning electron microscopy.  
354 Our study extends on these findings demonstrating that passaging capacity of  
355 diseased cells (CFBE) can also be extended using this method. Taken together this  
356 would suggest *BMI-1* transduction of bronchial epithelial cells permits extended  
357 passaging and mucociliary differentiation independent of donor and/or disease status  
358 although further studies are needed to confirm this.

359 *BMI-1* transduction did not immortalise the HBE cells in contrast to viral antigens  
360 such as the SV40 large T-antigen used to produce the 16HBE14o- cell line(5).  
361 However, *BMI* transduced cells could still be differentiated at 20-25 passages  
362 representing a significant advantage of this method over use of viral antigens. Using  
363 the ALI culture protocol outlined in the current study one can routinely obtain from 6-  
364 8 functional epithelial transwells in a 24-well ALI culture format per passage enabling  
365 the generation of a minimum of ~90-100 transwells from a single donor. This is  
366 significantly higher than the 10-15 epithelial transwells that can be generated with  
367 ~1x10<sup>6</sup> primary bronchial epithelial cells (typical quantity obtained from commercial  
368 providers) or brushing of the nasal turbinate of a single donor(29). Furthermore, sub-  
369 culturing of *BMI-1* transformed cells, as opposed to seeding ALI cultures, would  
370 enable banking of early passage cells and the potential to generate exponentially  
371 more functional epithelia at each passage.

372 Tosoni et al. (29) recently demonstrated that ALI cultures generated from different  
373 healthy donors can yield epithelia with vastly different physiological properties and  
374 drug responses. The *BMI-1* transduction protocol enables the generation of a large  
375 number of epithelia generated from donors with similar genetic backgrounds, or  
376 indeed from a single donor, allowing the study of disease pathophysiology in a  
377 manner that avoids the influence of genetic variability in cells from different donors.  
378 This highlights the potential for the development of personalised treatments using  
379 BMI-1 transduced cells.

380 In addition, an extended passaging capacity affords the opportunity for modification  
381 of HBE cells to create new models, to better understand disease and find novel  
382 treatments. As a proof of concept, we transduced NHBE BMI-1 cells with shRNA  
383 targeted against *DNAH5* in an attempt to create a model of PCD. The shRNA  
384 construct contained a GFP reporter to allow for selection of cells in which the *DNAH5*  
385 shRNA was expressed. Focussing on cells expressing GFP, we demonstrated loss  
386 of ciliary motility and absence of DNAH5 in the ciliary axoneme of cells transduced  
387 with the *DNAH5* targeted shRNA so mimicking the phenotype seen in patient  
388 cells(13). shRNA-mediated knockdown has been previously used to model PCD in  
389 otherwise healthy primary HBE cells (10, 11, 17) but these cells were not long lived  
390 so could not be used for further study to assess, for example, protein interactions or  
391 novel treatments. Gene addition, shRNA knockdown, or genome editing of BMI-1  
392 transduced HBE cells could therefore provide a more useful tool for the study of a  
393 number of airway diseases.

394 Recently the use of pharmacological Rho-kinase inhibition along with co-culture of  
395 HBE cells with irradiated feeder-layer fibroblasts has been described to allow  
396 indefinite passage of HBE cells whilst retaining the cells differentiation capacity when

397 placed at ALI (18, 27). However, studies where the mucociliary differentiation  
398 potential of CRCs have been assessed have not reported successful mucociliary  
399 differentiation beyond passage 11(2, 22, 27). Furthermore, CRC morphology and  
400 doubling times differ significantly to their parent cells with CRC cells being smaller  
401 and growing in colonies as well as showing faster proliferation rates(18, 27).  
402 Following viral transduction, *BMI-1* expressing NHBE and CFBE cells are cultured  
403 exactly as non-transformed primary cells, without the need for a feeder layer, a factor  
404 that is likely to aid in the rapid uptake of this method of transformation and  
405 dissemination of the resulting cell models between laboratories and in the  
406 maintenance of cells in biobanks.

407 In summary, here we have shown that *BMI-1* transduction delays senescence in  
408 HBE cells from healthy and CF donors whilst maintaining the cells mucociliary  
409 differentiation potential. We have undertaken extensive characterisation of the  
410 differentiated cells showing normal ciliary beat frequency and ciliary ultrastructure.  
411 Ussing chamber studies with BMI-1 transformed NHBE and CFBE cells showed that  
412 these cells exhibit similar Na<sup>+</sup> and Cl<sup>-</sup> ion transport characteristics to their respective  
413 primary cells, validating their use as models of CF. Furthermore, we have  
414 demonstrated how BMI-1- transduced cells can be engineered by further  
415 transduction with DNAH5 shRNA to recapitulate an in vitro disease model of primary  
416 ciliary dyskinesia, a valuable feature when studying rare diseases such as PCD  
417 where patient samples are difficult to obtain.

418

419

420 **REFERENCES**

- 421 1. **Brookes S, Rowe J, Gutierrez Del Arroyo A, Bond J, and Peters G.** Contribution  
 422 of p16(INK4a) to replicative senescence of human fibroblasts. *Exp Cell Res* 298: 549-559,  
 423 2004.
- 424 2. **Butler CR, Hynds RE, Gowers KH, Lee Ddo H, Brown JM, Crowley C, Teixeira**  
 425 **VH, Smith CM, Urbani L, Hamilton NJ, Thakrar RM, Booth HL, Birchall MA, De**  
 426 **Coppi P, Giangreco A, O'Callaghan C, and Janes SM.** Rapid Expansion of Human  
 427 Epithelial Stem Cells Suitable for Airway Tissue Engineering. *Am J Respir Crit Care Med*  
 428 194: 156-168, 2016.
- 429 3. **Chu Q, Tousignant JD, Fang S, Jiang C, Chen LH, Cheng SH, Scheule RK, and**  
 430 **Eastman SJ.** Binding and uptake of cationic lipid:pDNA complexes by polarized airway  
 431 epithelial cells. *Hum Gene Ther* 10: 25-36., 1999.
- 432 4. **Cozens AL, Yezzi MJ, Kunzelmann K, Ohrui T, Chin L, Eng K, Finkbeiner WE,**  
 433 **Widdicombe JH, and Gruenert DC.** CFTR expression and chloride secretion in polarized  
 434 immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 10: 38-47, 1994.
- 435 5. **Cozens AL, Yezzi MJ, Kunzelmann K, Ohrui T, Chin L, Eng K, Finkbeiner WE,**  
 436 **Widdicombe JH, and Gruenert DC.** CFTR Expression and Chloride Secretion in Polarized  
 437 Immortal Human Bronchial Epithelial Cells. *Am J Respir Cell Mol Biol* 10: 38-47, 1994.
- 438 6. **Fulcher ML, Gabriel SE, Olsen JC, Tatreau JR, Gentzsch M, Livanos E,**  
 439 **Saavedra MT, Salmon P, and Randell SH.** Novel human bronchial epithelial cell lines for  
 440 cystic fibrosis research. *Am J Physiol Lung Cell Mol Physiol* 296: 82-91, 2009.
- 441 7. **Fulcher ML, Gabriel SE, Olsen JC, Tatreau JR, Gentzsch M, Livanos E,**  
 442 **Saavedra MT, Salmon P, and Randell SH.** Novel human bronchial epithelial cell lines for  
 443 cystic fibrosis research. *Am J Physiol Lung Cell Mol Physiol* 296: L82-91, 2009.
- 444 8. **Hirst RA, Rutman A, Williams G, and Callaghan CO.** Ciliated Air-Liquid  
 445 Cultures as an Aid to Diagnostic Testing of Primary Ciliary Dyskinesia. *Chest* 138: 1441-  
 446 1447, 2010.
- 447 9. **Hirst RA, Rutman A, Williams G, and O'Callaghan C.** Ciliated air-liquid cultures  
 448 as an aid to diagnostic testing of primary ciliary dyskinesia. *Chest* 138: 1441-1447, 2010.
- 449 10. **Horani A, Brody SL, Ferkol TW, Shoseyov D, Wasserman MG, Ta-shma A,**  
 450 **Wilson KS, Bayly PV, Amirav I, Cohen-Cyberknoh M, Dutcher SK, Elpeleg O, and**  
 451 **Kerem E.** CCDC65 mutation causes primary ciliary dyskinesia with normal ultrastructure  
 452 and hyperkinetic cilia. *PLoS ONE* 8: e72299, 2013.
- 453 11. **Horani A, Ferkol TW, Shoseyov D, Wasserman MG, Oren YS, Kerem B, Amirav**  
 454 **I, Cohen-Cyberknoh M, Dutcher SK, Brody SL, Elpeleg O, and Kerem E.** LRRC6  
 455 mutation causes primary ciliary dyskinesia with dynein arm defects. *PLoS ONE* 8: e59436,  
 456 2013.
- 457 12. **Horani A, Nath A, Wasserman MG, Huang T, and Brody SL.** Rho-Associated  
 458 Protein Kinase Inhibition Enhances Airway Epithelial Basal-Cell Proliferation and Lentivirus  
 459 Transduction. *Am J Respir Cell Mol Biol* 49: 341-347, 2013.
- 460 13. **Hornef N, Olbrich H, Horvath J, Zariwala MA, Fliegauf M, Loges NT,**  
 461 **Wildhaber J, Noone PG, Kennedy M, Antonarakis SE, Blouin JL, Bartoloni L, Nusslein**  
 462 **T, Ahrens P, Griese M, Kuhl H, Sudbrak R, Knowles MR, Reinhardt R, and Omran H.**  
 463 DNAH5 Mutations Are a Common Cause of Primary Ciliary Dyskinesia With Outer Dynein  
 464 Arm Defects. *Am J Respir Crit Care Med* 174: 120-126, 2006.
- 465 14. **Hussain S, Ji Z, Taylor AJ, DeGraff LM, George M, Tucker CJ, Chang CH, Li**  
 466 **R, Bonner JC, and Garantzotis S.** Multiwalled Carbon Nanotube Functionalization with

467 High Molecular Weight Hyaluronan Significantly Reduces Pulmonary Injury. *ACS Nano* 10:  
468 7675-7688, 2016.

469 15. **Jacobs JJ, Kieboom K, Marino S, DePinho RA, and van Lohuizen M.** The  
470 oncogene and Polycomb-group gene *bmi-1* regulates cell proliferation and senescence  
471 through the *ink4a* locus. *Nature* 397: 164-168, 1999.

472 16. **Kesimer M, Kirkham S, Pickles RJ, Henderson AG, Alexis NE, Demaria G,  
473 Knight D, Thornton DJ, and Sheehan JK.** Tracheobronchial air-liquid interface cell  
474 culture: a model for innate mucosal defense of the upper airways? *Am J Physiol Lung Cell  
475 Mol Physiol* 296: L92-L100, 2009.

476 17. **Li Y, Yagi H, Onuoha EO, Damerla RR, Francis R, Furutani Y, Tariq M, King  
477 SM, Hendricks G, Cui C, Saydmohammed M, Lee DM, Zahid M, Sami I, Leatherbury  
478 L, Pazour GJ, Ware SM, Nakanishi T, Goldmuntz E, Tsang M, and Lo CW.** DNAH6  
479 and Its Interactions with PCD Genes in Heterotaxy and Primary Ciliary Dyskinesia. *PLoS  
480 Genet* 12: e1005821, 2016.

481 18. **Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, Timofeeva OA,  
482 Nealon C, Dakic A, Simic V, Haddad BR, Rhim JS, Dritschilo A, Riegel A, McBride A,  
483 and Schlegel R.** ROCK inhibitor and feeder cells induce the conditional reprogramming of  
484 epithelial cells. *Am J Pathol* 180: 599-607, 2012.

485 19. **Livak KJ, and Schmittgen TD.** Analysis of relative gene expression data using real-  
486 time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.

487 20. **McKay TR, Camarasa MV, Iskender B, Ye JP, Bates N, Miller D, Fitzsimmons  
488 JC, Foxler D, Mee M, Sharp TV, Aplin J, Brison DR, and Kimber SJ.** Human feeder cell  
489 line for derivation and culture of hESC/hiPSc. *Stem Cell Res* 7: 154-162, 2011.

490 21. **Milyavsky M, Shats I, Erez N, Tang X, Senderovich S, Meerson A, Tabach Y,  
491 Goldfinger N, Ginsberg D, Harris CC, and Rotter V.** Prolonged culture of telomerase-  
492 immortalized human fibroblasts leads to a premalignant phenotype. *Cancer Res* 63: 7147-  
493 7157, 2003.

494 22. **Reynolds SD, Rios C, Wesolowska-Andersen A, Zhuang Y, Pinter M, Happoldt  
495 C, Hill CL, Lallier SW, Cosgrove GP, Solomon GM, Nichols DP, and Seibold MA.**  
496 Airway Progenitor Clone Formation is Enhanced by Y-27632-dependent Changes in the  
497 Transcriptome. *Am J Respir Cell Mol Biol* 2016.

498 23. **Rousseau K, Wickstrom C, Whitehouse DB, Carlstedt I, and Swallow DM.** New  
499 monoclonal antibodies to non-glycosylated domains of the secreted mucins MUC5B and  
500 MUC7. *Hybridoma and Hybridomics* 22: 293-299, 2003.

501 24. **Shoemark A, Dixon M, Beales PL, and Hogg CL.** Bardet Biedl Syndrome Motile  
502 Ciliary Phenotype. *Chest* 147: 764-770, 2015.

503 25. **Smith CM, Djakow J, Free RC, Djakow P, Lonnen R, Williams G, Pohunek P,  
504 Hirst RA, Easton AJ, Andrew PW, and O'Callaghan C.** ciliaFA: a research tool for  
505 automated, high-throughput measurement of ciliary beat frequency using freely available  
506 software. *Cilia* 1: 14, 2012.

507 26. **Smith CM, Kulkarni H, Radhakrishnan P, Rutman A, Bankart MJ, Williams G,  
508 Hirst RA, Easton AJ, Andrew PW, and O'Callaghan C.** Ciliary dyskinesia is an early  
509 feature of respiratory syncytial virus infection. *Eur Respir J* 43: 485-496, 2014.

510 27. **Suprynowicz FA, Upadhyay G, Krawczyk E, Kramer SC, Hebert JD, Liu X,  
511 Yuan H, Chelvaraju C, Clapp PW, Boucher RC, Jr., Kamonjoh CM, Randell SH, and  
512 Schlegel R.** Conditionally reprogrammed cells represent a stem-like state of adult epithelial  
513 cells. *Proc Natl Acad Sci U S A* 109: 20035-20040, 2012.

514 28. **Torr E, Heath M, Mee M, Shaw D, Sharp TV, and Sayers I.** Expression of  
515 polycomb protein BMI-1 maintains the plasticity of basal bronchial epithelial cells.  
516 *Physiological Reports* 4: e12847, 2016.

- 517 29. **Tosoni K, Cassidy D, Kerr B, Land SC, and Mehta A.** Using Drugs to Probe the  
518 Variability of Trans-Epithelial Airway Resistance. *PLoS ONE* 11: e0149550, 2016.
- 519 30. **Wong AP, Bear CE, Chin S, Pasceri P, Thompson TO, Huan LJ, Ratjen F, Ellis**  
520 **J, and Rossant J.** Directed differentiation of human pluripotent stem cells into mature  
521 airway epithelia expressing functional CFTR protein. *Nat Biotechnol* 30: 876-882, 2012.
- 522 31. **Wong AP, and Rossant J.** Generation of Lung Epithelium from Pluripotent Stem  
523 Cells. *Curr Pathobiol Rep* 1: 137-145, 2013.
- 524 32. **Woollhead AM, Sivagnanasundaram J, Kalsi KK, Pucovsky V, Pellatt LJ, Scott**  
525 **JW, Mustard KJ, Hardie DG, and Baines DL.** Pharmacological activators of AMP-  
526 activated protein kinase have different effects on Na<sup>+</sup> transport processes across human lung  
527 epithelial cells. *Br J Pharmacol* 151: 1204-1215, 2007.

528

529

530 **AUTHOR CONTRIBUTIONS**

531 M.M.M., A.S., R.A.H., J.M.D. and D.L.B. contributed to data collection. All authors  
532 contributed to study design, data analysis, interpretation of the data and critical  
533 revision of the final manuscript. All authors approved the final version of the  
534 manuscript.

535 **GRANTS**

536 This study was funded by the Great Ormond Street Hospital Children's Charity  
537 (GOSHCC), the Child Health Research Appeal Trust (CHRAT) and supported by the  
538 National Institute for Health Research Biomedical Research Centre at Great Ormond  
539 Street Hospital for Children NHS Foundation Trust and University College London.

540 **DISCLOSURES**

541 The authors declare no competing financial interests.

542

543

544 **ADDITIONAL INFORMATION**

545 Supplementary videos available.



546 **FIGURE LEGENDS**

547 **Figure 1. BMI-1 maintains healthy cell morphology in 2D culture.**

548 The morphology of (a) NHBE cells at passage 1 and (b) passage 3 was observed  
549 under light microscopy and compared to NHBE BMI-1 cells after passages (c) 11, (d)  
550 17 and (e) 25. White arrows highlight elongated cells and black arrows highlight  
551 squamous cells. Scale bars are 100µm.

552 **Figure 2. Karyotype analysis of NHBE-BMI-1 cells.**

553 Karyotype of passage 23 NHBE-BMI-1 cells was undertaken by The Doctors  
554 Laboratory, London.

555 **Figure 3. Elevated p16<sup>Ink4a</sup> precedes senescence and BMI-1 functions by**  
556 **inhibiting p16<sup>Ink4a</sup> and retains a normal cell doubling time.**

557 (a) Western blot was used to assess levels of BMI-1 and p16<sup>Ink4A</sup> in serially  
558 passaged NHBE cells and BMI-1 transduced cells and (b) cell counting was used to  
559 determine the replication kinetics of NHBE and NHBE BMI-1 cells at varying  
560 passages. Growth curves are presented as percent of mean of day 1 cell count. Data  
561 are mean ± S.E.M. For each data point n=3 biological replicates.

562 **Figure 4. BMI-1 cells retain their mucociliary differentiation capacity.**

563 Extensively passaged BMI-1 transduced cells (passage 15) were differentiated on  
564 ALI and cilia beat frequency of (a) NHBE and (b) CFBE cells was determined using  
565 ciliaFA plugin(25) for ImageJ. Data are mean ± S.E.M; n= 4 independent ALI  
566 cultures, 5 fields videoed per culture. Immunostaining of NHBE-BMI-1 cells was used  
567 to show tight junction formation (occludin; c), mucin production (MUC5AC and  
568 MUC5B; d and e respectively), the presence of basal cells (p63+; f), widespread  
569 BMI-1 expression (BMI-1; g), and extensive ciliation (acetylated α-tubulin; h). TEM  
570 was used to determine cilia ultrastructure (i). Images are representative of 4  
571 independent ALI cultures per marker. Scale bars for c-h are 50µm and 100nm for i.

572 **Figure 5. BMI-1 cells form ALI cultures suitable for Ussing chamber studies.**

573 Representative Ussing chamber traces and changes in short-circuit current ( $I_{sc}$ ) in  
574 response to administration of amiloride (apical), forskolin (apical and basolateral) and  
575 GlyH-101 (apical) in primary and BMI-1 transduced (a and b) NHBE and (c and d)  
576 CFBE cells are shown. Data are mean ± S.E.M; n= at least 3 independent ALI  
577 cultures (see Table 4 for exact values).

578 **Figure 6. DNAH5 knockdown recapitulates PCD phenotype.**

579 (a) qRT-PCR was used to assess DNAH5 mRNA expression in NHBE-BMI-1 cells  
580 and NHBE BMI-1-transduced with lentivirus expressing either a scrambled or

581 *DNAH5*-targetting shRNA and grown in submerged 2D culture. \*\*P<0.01; one-way  
582 ANOVA with Bonferroni's post-test used to assess significance. Data are mean ±  
583 S.E.M. (b) Immunostaining for *DNAH5* and acetylated α-tubulin was used to assess  
584 the presence or absence of *DNAH5* in the ciliary axoneme of shRNA transduced and  
585 untransduced NHBE BMI-1 cells differentiated at ALI. Presence of GFP fluorescence  
586 denotes cells transduced with the GFP-shRNA construct and so expressing the  
587 shRNA. Scale bars are 20µm. Images are representative of 4 independent ALI  
588 cultures per condition.

589 **TABLES**

590 **Table 1. Primary antibodies used in this study.**

591

<b>Name</b>	<b>Supplier</b>	<b>Dilution WB/IF</b>
<b>Anit-MUC5AC</b>	Life Technologies	NA/1:100
<b>Anti-Acetylated <math>\alpha</math>-tubulin</b>	Sigma-Aldrich	NA/1:500
<b>Anti-BMI-1</b>	Life Technologies	1:200/1:100
<b>Anti-GAPDH</b>	Life Technologies	1:1000/1:500
<b>Anti-MUC5B</b>	Kind gift from Professor Dallas Swallow(23)	NA/neat
<b>Anti-Occludin</b>	Invitrogen, Life Technologies	NA/1:100
<b>Anti-p16<sup>INK4</sup></b>	Pharmingen, BD Biosciences	1:200/NA
<b>Anti-p63</b>	Invitrogen, Life Technologies	NA/1:100

592

593 **Table 2. Microtubule organisation of motile cilia.**

594

<b>Microtubule Organisation</b>	<b>Frequency (%)</b>
<b>Normal 9+2</b>	92.05
<b>Central Pair Defect</b>	0.66
<b>Disarranged</b>	3.31
<b>Other Defect</b>	3.97

595

596 **Table 3. Dynein arm presence in motile cilia.**

597

<b>Dynein Arms</b>	<b>Frequency (%)</b>
<b>ODA and IDA Present</b>	100.00
<b>ODA Only</b>	0.00
<b>IDA Only</b>	0.00
<b>ODA and IDA Absent</b>	0.00

598

599

600 **Table 4. Trans-epithelial electrical resistance (TEER) measurements.**

<b>Name</b>	<b>Passage</b>	<b>TEER (<math>\Omega \cdot \text{cm}^2 \pm \text{S.E.M}</math>)</b>	<b>n</b>
NHBE (AB053901)	P1	621.0 $\pm$ 33.2	5
NHBE (AB037501)	P1	331.1 $\pm$ 105.5	3
NHBE BMI-1	P13	1268.4 $\pm$ 78.4	4
CFBE	P2	1307.9 $\pm$ 36.6	5
CFBE BMI-1	P17	917.6 $\pm$ 165.3	6

601

602

603 **Table 5. DNAH5 localisation.**

604

<b>shRNA Target</b>	<b>Is DNAH5 located in ciliary axoneme?</b>	
	<b>Yes</b>	<b>No</b>
Untransduced	157	0
Scrambled	147	0
DNAH5	5	173

605

606













