



## Early View

Original Research Article

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Please cite this article as: von Hardenberg S, Maier P, Christian L, *et al.* Pleuroparenchymal fibroelastosis in a monogenic mitochondrial DNA depletion syndrome. *Eur Respir J* 2026; in press (<https://doi.org/10.1183/13993003.00791-2026>).

This manuscript has recently been accepted for publication in the *European Respiratory Journal*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.

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# Pleuroparenchymal fibroelastosis in a monogenic mitochondrial DNA depletion syndrome

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**Take Home Message:**

This study provides evidence that DGUOK dysfunction represents a monogenic cause associated with pleuroparenchymal fibroelastosis and suggests mitochondrial dysfunction as a potential key mechanism underlying its pathogenesis.

## Abstract

**Background:** Pleuroparenchymal fibroelastosis (PPFE) is a rare, most often idiopathic fibrotic lung disease with poor prognosis, mainly affecting adults. Biallelic pathogenic variants in the deoxyguanosine kinase gene (*DGUOK*) cause a mitochondrial DNA (mtDNA) depletion syndrome, predominantly affecting infants with severe hepatic and neurological disease. Neither detailed pulmonary manifestations nor a pulmonary-dominant phenotype has been reported.

**Methods:** We describe nine patients with PPFE and *DGUOK*-associated mitochondriopathy. Clinical, radiological, histopathological, and genetic data were systematically collected from all patients. Functional studies, single-nucleus RNA sequencing (snRNAseq), immunofluorescence staining, transmission electron microscopy, respiratory chain enzyme activity assays and mtDNA recovery experiment were conducted on patient-derived fibroblasts, muscle or lung tissues. mtDNA content quantification was performed on whole genome sequencing data.

**Results:** All patients (ages 5–36 years) carried biallelic pathogenic *DGUOK* variants, including an unreported intronic alteration in seven. All presented with progressive dyspnea, weight loss and some with pneumothoraces. Chest computed tomography and lung biopsies demonstrated features of PPFE. snRNAseq of lung tissue from four pediatric patients identified Aberrant Basaloid cells at the fibrotic edge, together with a transcriptional signature of nuclear DNA damage response. Ultrastructural analysis confirmed mitochondrial abnormalities in alveolar type 2 and Aberrant Basaloid cells.

**Conclusion:** This study provides evidence that mitochondrial dysfunction can contribute to early-onset PPFE independent of age, with a DNA damage response signature in aberrant epithelial cell populations previously described only in adult fibrotic lung disease. *DGUOK* deficiency and other hereditary mitochondrial disorders should be considered in children and young adults presenting with PPFE of unknown origin.

## Introduction

Pleuroparenchymal fibroelastosis (PPFE) is a rare fibrotic lung disease histologically characterized by subpleural intraalveolar fibroelastosis, usually with upper lobe predominance [1]. Typical clinical findings are progressive dyspnea, chronic nonproductive cough, weight loss, platythorax, deep sternal notch and a restrictive ventilatory defect [2, 3]. The disease often progresses rapidly, which is reflected in a poor prognosis with 5-year survival rates ranging from 20-60% [4, 5]. PPFE is categorized as idiopathic (iPPFE) or secondary (sPPFE) [2]: iPPFE is considered a specific disease entity among other idiopathic interstitial pneumonias (IIP) in the American Thoracic Society/European Respiratory Society (ATS/ERS) statement on the classification of IIPs [6]. While PPFE has been increasingly described in adults, there are only very few children reported, mostly after chemotherapy or following bone marrow transplantation [3, 7, 8]. Since the first description in 2004, growing evidence from familial cases of iPPFE and the detection of pathogenic variants in telomerase genes in some patients suggests a hereditary contribution [1, 7, 9-14]. However, to date, no specific monogenic cause has been identified.

There is growing evidence that acquired mitochondrial dysfunction contributes to the pathogenesis of fibrosing lung diseases [15-19]. Moreover, the association of pulmonary fibrosis with Acadian Fanconi syndrome, a rare mitochondriopathy caused by pathogenic *NDUFAF6* variants, suggests that mitochondrial dysfunction may directly drive pulmonary fibrosis [20].

Deoxyguanosine kinase (DGUOK) is a key enzyme of the mitochondrial deoxyribonucleoside triphosphate (dNTP) salvage pathway, required for mitochondrial DNA (mtDNA) synthesis [21]. Reduced or absent DGUOK activity disrupts the mitochondrial dNTP pool, leading to a mtDNA depletion syndrome [22]. DGUOK deficiency follows an autosomal recessive inheritance pattern, classically presenting in newborns and infants with severe hepatic and neurological involvement [23, 24]. In addition, cases of isolated hepatic disease in children and late-onset myopathy in adults have been described [24, 25]. In a recently published review of 173 patients with DGUOK deficiency, respiratory involvement was reported in 13% of cases; however, no

consistent pattern of respiratory involvement has been described [26]. Additionally, one case with an unspecified interstitial lung disease was reported in a case series of 24 individuals [27], and a pneumothorax in a separate case report [28]. Pulmonary hypertension has also been associated with DGUOK deficiency in some cases [24, 29].

Here, we describe five pediatric and four adult patients with *DGUOK*-associated mitochondriopathy and PPFE, representing a distinct phenotype compared to ‘classical’ features of DGUOK deficiency. Our findings support the relevance of mitochondrial dysfunction in the pathophysiology of the DGUOK-associated PPFE and highlight the importance of a comprehensive diagnostic evaluation - including genetic workup - in patients with diffuse lung disease, especially when the disease is considered idiopathic and manifest during childhood, adolescence, or early adulthood.

## **Material and Methods**

### **Study information, Genetic and Functional Analyses**

We report nine patients with genetically confirmed DGUOK deficiency. Clinical data were collected from medical records and treating physicians, and all participants (or their legal guardians) provided written informed consent in accordance with the Declaration of Helsinki. The study was approved by the local ethics committee of Hannover Medical School (Ethics approval nos. 2923-2015, 1969-2013, and 12241-BO-K 2026). Genetic diagnoses were established by whole-exome sequencing (P4–P5) or whole-genome sequencing (P1–P3 and P6–P9) using standard protocols, followed by bioinformatic analysis, variant annotation, and interpretation according to ACMG guidelines [30].

Mitochondrial function was assessed by respiratory chain enzyme activity assays and Seahorse extracellular flux analysis. DGUOK protein expression was evaluated by western blot, and the effect of deoxyribonucleoside supplementation on mtDNA recovery following depletion was assessed by qPCR in patient-derived fibroblasts. Lung tissue specimens were examined by routine histology and transmission electron microscopy. Single-nucleus RNA

sequencing was performed on formalin-fixed lung tissue. Cell states of interest were validated by multiplex immunofluorescence and in situ RNA hybridization.

Details of genetic, transcriptomic and cellular analyses are provided in the Supplementary Appendix.

## Results

### Clinical phenotype

A total of nine patients from four families were included. P1, P2, and P3 originate from one family (F1), with P1 and P2 being siblings and P3 a distant cousin. P4 and P5 come from different families (F2 and F3), while P6–P9 are siblings (F4) (Figure 1A–D). Age at onset of respiratory symptoms varied between 0 (P5) and 28 (P6) years. At time of first presentation, leading features were exertional dyspnea, chronic cough, a deepened suprasternal notch (Figure 1), platythorax and low body weight. All patients had a restrictive ventilatory defect and chest CT's showed typical features of PPFE. Six patients presented with histories of pneumothorax (Figure 1, Figure S1, Supplementary Appendix and Table 1). Lung biopsies (n=4, P1–P4) revealed alveolar fibroelastosis (AFE), the histological pattern underlying PPFE (Figure 1I, J). In those, treatment with methylprednisolone-pulses, prednisolone and nintedanib was attempted, without apparent benefit, and all discontinued nintedanib due to side effects (nausea, vomiting, and further weight loss). Two patients underwent liver transplantation at the age of 2 years (P3, F1) and 7 months (P5, F3), respectively, due to acute liver failure. In contrast to the other seven cases, these two showed psychomotor developmental delay over time. All other patients did not have clinical signs of liver disease or neurodevelopmental delay, even though liver enzymes were slightly elevated in five patients. Extrapulmonary organ manifestations were fatigue (n=4), muscle weakness (n=6), peripheral polyneuropathy with predominantly axonal damage (n=5), and proteinuria (n=4). P1 underwent lung transplantation (LuTx) seven months after diagnosis of PPFE. P3 and P4 were also evaluated for LuTx but were considered unsuitable because of severe cachexia and rapidly progressive immobilizing polyneuropathy. P3 died 1.5 years and P4 three years after onset of respiratory symptoms due

to terminal respiratory failure. P5 was not evaluated for LuTx and died at five years of age due to cardio-respiratory failure. The remaining patients are still alive at last follow-up. Details are shown in Figure 1, Table 1 and Table S3, Supplementary Appendix.

### **Germline biallelic loss-of-function variants in *DGUOK***

We performed whole exome or whole genome sequencing in nine patients and identified biallelic germline variants in *DGUOK* (ENST00000264093.9). In P1, P2 (F1), P4 (F2), and P6-P9 (F4) the same intronic homozygous variant (c.592-26A>G p.(Val198Alafs\*4)) was detected (Figure S2, Supplementary Appendix, exemplified for P1 and P2). P3 (F1) carries this intronic variant in trans with a nonsense variant (c.313C>T p.(Arg105\*)) whereas P5 (F3) is homozygous for a four-base-pair duplication (c.763\_766dup p.(Phe256\*)). Accordingly, the clinical phenotype of P1, P2, P4, P6-P9 differs from that of P3 and P5 with no liver involvement and with respiratory symptoms as the initial manifestation (Clinical phenotype and Table S3, Supplementary Appendix). All tested healthy relatives were either heterozygous carriers of one of the familial variants or wild type (Figure 1).

The variants c.313C>T p.(Arg105\*) and c.763\_766dup p.(Phe256\*) are predicted to lead to premature stop codons with subsequent mRNA degradation via nonsense-mediated decay (NMD), while the intronic c.592-26A>G variant was predicted to cause aberrant splicing based upon the splicing site prediction program spliceAI score of 0.5 and a branch point predicted value of 5.4. RNA sequencing revealed that exon 5 skipping occurs in many, but not all, blood-derived transcripts (Figure 2B). In lung tissue, by contrast, this variant results in skipping of exon 5 in all transcripts. Sanger cDNA sequencing confirmed that exon 5 skipping causes a frameshift (Figure S2, Supplementary Appendix), introducing a premature stop codon in exon 6 (p.(Val198Alafs\*4)), potentially resulting in NMD. At the protein level, *DGUOK* expression was absent or markedly reduced in all patient-derived samples (Figure 2C). In contrast, all other published intronic variants (Figure S2, Supplementary Appendix) do not appear to result in the distinct phenotype described here.

## **Reduced blood mtDNA and tissue-specific respiratory chain dysfunction**

Quantification of the average mtDNA revealed a clear reduction in mtDNA levels in the blood of patients carrying the homozygous variant, consistent with a mitochondrial DNA depletion syndrome (Figure 2B). In contrast, respiratory chain enzyme activities in patient-derived fibroblasts (P1, P3, P4, P6) revealed values within the normal reference ranges for complexes I+III, IV (COX), and V (ATP synthase). The activities of complexes II+III were at the lower limit of the reference interval or slightly below it (P4, P6). Consistently, Seahorse mitochondrial stress testing showed no statistically significant differences in maximal oxygen consumption rate (OCR) between patient-derived fibroblasts and controls, despite a trend toward higher OCR in patient cells (Figure S3, Supplementary Appendix). While mitochondrial respiratory chain function was preserved in fibroblasts, muscle tissue from patient P3 exhibited a tissue-specific defect characterized by reduced activities of complexes II–IV and complete loss of cytochrome c oxidase (COX) activity, despite normal citrate synthase activity, indicating preserved mitochondrial content. These findings demonstrate a tissue-specific manifestation of mitochondrial respiratory chain dysfunction (Figure S3, Supplementary Appendix).

## **Deoxyribonucleoside Supplementation Rescues Mitochondrial DNA Depletion**

Building on previous findings [31], we investigated whether supplementation with exogenous deoxynucleosides (ATGC) enhances mtDNA recovery in patient-derived fibroblasts (P1, P4, and P6) compared to controls. ATGC supplementation increased mtDNA copy number in all three DGUOK-deficient fibroblast cultures compared with unsupplemented cells, whereas only minimal changes were observed in control fibroblasts (Figure 2D). On average, mtDNA levels increased by 245% in DGUOK-deficient fibroblasts following ATGC supplementation, compared with only a 25% increase in control fibroblasts.

## **Emergence of Aberrant Basaloid Cells in patients with PPFE and DGUOK-associated mitochondriopathy**

Single-nucleus RNA sequencing (snRNAseq) was performed on six lung samples from four pediatric patients (P1-P4) and control samples identified. Amongst epithelial cells, Aberrant basaloid cells were identified in DGUOK patients as a population expressing basal cell (KRT17 and TP63), mesenchymal (ITGAV, ITGB6, CDH2), injury- (CTSE, PTGS2) and senescence-associated genes (CDKN2B, GDF15) (Figure 3A–G; Figure S4 and Table S4, Supplementary Appendix). Their proposed precursors, the intermediate (iAT2/ATi) cells, were likewise identified [32]. iAT2 and Aberrant Basaloid cells frequencies were significantly increased in DGUOK patients compared to controls (8.3% vs 0.9 %,  $p_{\text{adj}} < 0.005$  and 6.1 % vs 0.5 %,  $p_{\text{adj}} < 0.002$ , respectively; Figure 3C).

Comparing gene expression across epithelial cell types, AT2 cells exhibited the strongest differential gene expression, with over 2,000 genes significantly altered between patients and controls. Specifically, nuclear-encoded genes involved in DNA double-strand break response (*ATM*, *RAD50*, *RNF8*) as well as mitochondrial matrix proteins (*MTERF1*, *FASTKD2*, *FASTKD1*, *PRIMPOL*), were upregulated (Figure 3D; Table S5, Supplementary Appendix).

Additional alterations were observed across endothelial, mesenchymal, myeloid, and lymphoid compartments (Figure S5-S6 and Table S5, Supplementary Appendix). In particular, a relative expansion of profibrotic and elastofibrotic fibroblasts was detected.

Immunofluorescence stains confirmed the presence of Aberrant Basaloid cells, which form a monolayer at the fibrotic edge, surrounded by iAT2 cells (Figure 4A-H).  $\gamma$ H2AX immunostaining validated the snRNAseq findings, indicating increased DNA double-strand breaks in DGUOK-deficient AT2 and Aberrant Basaloid cells but not in cells from healthy controls. (Figure 4I–K).

## **Electron microscopy reveals tissue-specific mitochondrial degeneration in DGUOK-deficient lungs**

Electron microscopy of lung tissue revealed numerous, enlarged mitochondria characterized by a loss of mitochondrial matrix as well as swelling and degeneration of their cristae in AT2

and Aberrant Basaloid cells (Figure 4L-O; Figure S7, Supplementary Appendix). No alterations were observed in skin fibroblasts (Figure S8, Supplementary Appendix), underscoring the tissue-selective impact of DGUOK deficiency.

## Discussion

In this study, we present the first pulmonary phenotype of mitochondrial DNA depletion syndrome. We demonstrate that a homozygous intronic variant in *DGUOK* causes a clinically distinct, later-onset pulmonary phenotype, that differs from the classical early-onset hepatocerebral presentation of DGUOK-associated mitochondriopathy. We identify Aberrant Basaloid cells in pediatric fibrotic lung tissue, linking their emergence to cell injury rather than age and extending their relevance from adult fibrotic lung disease to childhood-onset disease. These cells likely represent a downstream consequence of DGUOK dysfunction, reflecting a nonspecific response to epithelial injury rather than a primary effect of the genetic defect.

In our cohort, seven patients carrying the intronic homozygous c.592-26A>G variant exhibited no evidence of severe neonatal, infantile, or late-onset hepatocerebral involvement [26, 28], and remained symptom-free at least until adolescence. All patients presented with respiratory symptoms and weight loss as the predominant manifestations at disease onset. This phenotype likely reflects partial preservation of *DGUOK* function, allowing sufficient residual protein activity to maintain hepatic function, while chronically reduced *DGUOK* levels may result in progressive involvement of other organ systems over time, particularly the lung. Pulmonary disease may therefore emerge long after the classical hepatocerebral phenotype, especially in patients with hypomorphic variants and may extend beyond DGUOK deficiency to other mitochondrial disorders. We recommend considering *DGUOK* deficiency and other inherited mitochondrial disorders in children, adolescents, and young adults with PPFE of unknown cause, and screening for PPFE in patients with established mitochondrial disease. Several observations support residual DGUOK activity in combination with marked tissue tropism: oxidative phosphorylation and mitochondrial morphology were preserved in patient-derived fibroblasts despite impaired respiratory chain function in skeletal muscle, while lung

tissue showed complete *DGUOK* exon 5 skipping, mtDNA damage, and mitochondrial degeneration. Similar to the adult myopathic phenotype of *DGUOK* deficiency, a substantial proportion of our patients presented with muscle weakness. Shortness of breath has been described as a leading symptom in a patient with the isolated myopathic phenotype, but was attributed to the underlying myopathy and, consequently, no pulmonary evaluation was performed [33].

Although genotype–phenotype correlations in *DGUOK* deficiency remain incomplete, available data indicate that early-onset disease is associated with more severe loss of *DGUOK* function, whereas late-onset phenotypes likely reflect residual enzymatic activity. In the largest published cohort [24], most patients with neonatal or infantile disease carried at least one truncating or splice-site variant, while the remainder harbored biallelic missense variants predicted to impair protein function.

To date, neither detailed analyses of respiratory symptoms nor a defined pulmonary phenotype have been reported in *DGUOK* deficiency or in any other hereditary mitochondrial disorder [24, 26]. However, an unspecified respiratory involvement was noted in 13% of 173 subjects in a large *DGUOK*-deficient cohort [26] or in a patient harboring two intronic variants, who presented with early-onset liver disease and an unspecified interstitial lung disease [27], similar to our patient P3. In our cohort, a retrospective review identified previously unrecognized PPFE in P5 who died from progressive respiratory failure and severe pulmonary hypertension with suprasystemic pressure at a young age. Together, these observations suggest that pulmonary manifestations of *DGUOK* deficiency are overlooked when liver involvement dominates the clinical presentation, and that pulmonary hypertension, reported in approximately 20% of patients [34], may in some cases be secondary to underlying PPFE. Thus, further pathogenic variants associated with PPFE remain to be identified.

We identify Aberrant Basaloid cells - previously described only in adult, age-associated fibrosing lung disease - for the first time in a pediatric cohort, establishing that this population arises independently of aging [32, 35-37]. Their emergence is accompanied by DNA damage: AT2 cells showed upregulation of nuclear-encoded genes for the DNA double-strand break

response and mitochondrial transcription, consistent with impaired mtDNA maintenance and ROS accumulation, alongside enlarged, swollen mitochondria with lysis of cristae. These findings position mitochondrial dysfunction as a primary driver of PPFE pathogenesis rather than an acquired contributor. AT2 cells may be particularly susceptible to mitochondrial dysfunction because of their high metabolic demands for surfactant synthesis and epithelial regeneration. Impaired mtDNA maintenance is expected to promote reactive oxygen species generation, which may activate TGF- $\beta$  signaling and drive epithelial injury, the emergence of aberrant basaloid cells, and profibrotic remodeling. The characteristic upper-lobe subpleural distribution of PPFE may further reflect regional susceptibility related to increased mechanical strain, reduced perfusion, and lower oxygen tension.

As there are currently no effective drugs available for DGUOK deficiency, lung transplantation is the only treatment option in some but not all patients. Consistent with previous studies, we demonstrate that deoxynucleoside supplementation restores mtDNA content in DGUOK-deficient fibroblasts, providing proof-of-concept for a potential therapeutic strategy that warrants further evaluation in vivo.

This study has several limitations. The cohort is small, reflecting the rarity of DGUOK deficiency, and follow-up was limited, precluding conclusions on the natural history of the pulmonary disease. Because pulmonary involvement may have remained clinically silent for years before diagnosis, the PPFE observed here may represent the end stage of a longstanding, slowly progressive process. Thus, a degree of selection bias toward more severe disease in our cohort cannot be excluded, underscoring the need for future prospective observational studies to detect early clinical changes and abnormalities. Most importantly, the pathogenic sequence proposed above - from mtDNA depletion to alveolar epithelial injury and fibrotic remodeling - remains inferential, as we did not test causality directly. Mechanistic studies in animal models and in disease-relevant in vitro systems will be required to establish whether DGUOK deficiency drives PPFE by this route. Furthermore, comprehensive comparative analyses with non-DGUOK PPFE and other fibrosing interstitial lung diseases limits conclusions regarding the disease specificity of these findings.

In summary, our findings show that mitochondrial DNA depletion syndromes can manifest as primary fibrosing lung disease and suggest that pulmonary involvement is an underrecognized feature of the mtDNA depletion syndrome spectrum, extending beyond the traditional early-onset phenotypes. Furthermore, our findings underscore the necessity of comprehensive molecular analysis and genetic counseling in cases of unexplained, early onset fibrotic lung disease. Importantly, restoration of mtDNA content by deoxynucleoside supplementation in patient-derived fibroblasts provides proof-of-concept for a potential therapeutic strategy and warrants further evaluation in vivo.

## Acknowledgements

We would like to thank the families for their participation. We thank Dr. Jens Dingemann, Dr. Julia Brendel and Dr. Fabio Ius for their dedicated surgical care of the patients. We thank Duygu Halibryam for her Turkish translation and assistance. We thank Caterina Garone and Peter Freisinger for the constructive discussion on DGUOK deficiency. We thank Andreas Schmiedl for his assistance in electron microscopy.

## Contributors

SvH, NS, JS and PM contributed to the study conception and design. Experiments and data collection were performed by SvH, NS, JCS, PM, AMD, DJ, AH, ADu, TH, LC. Patient recruitment, clinical assessment and sample collection were conducted by NS, JCS, PM, DS, BS, NJ, LH. WES, WGS, snRNAseq analysis, bioinformatics and/or data analysis were performed by SvH, NS, JCS, PM, LK, MS, DJ, KP, JR, AS, WH, BS. Supervision was provided by NDD, BA. The first draft of the manuscript was written by SvH, NS, JCS, PM, LK. All authors reviewed and approved the manuscript.

## Data sharing

The datasets generated and analyzed for this study are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the datasets should be directed to the corresponding author.

## Declaration of interests

JCS reports consulting fees and honoraria from Boehringer Ingelheim, Merck/MSD, GSK, AOP Health, PureTech Health, Ferrer and Vicore Pharma; support for meetings from Boehringer Ingelheim; and he has IP on basal cell-targeted therapies in IPF. PM reports personal honoraria for a lecture from Boehringer Ingelheim, Germany. AH reports consulting fees from Alexion, BMS, Galapagos, Johnson & Johnson, Kyverna, Merck Serono, and Sanofi;

honoraria for lectures or other educational activities from AbbVie, Medscape, Merck Serono, Pfizer, Roche, and Sanofi; and participation on boards for Kyverna, BMS, and Alexion and engagements with in DGN (German Society of Neurology), DMSG (German Multiple Sclerosis Society), and DGKN (German Society for Clinical Neurophysiology. MG reports institutional funding from the Deutsche Forschungsgemeinschaft and Boehringer Ingelheim, Germany, and participation on Advisory board for Boehringer Ingelheim (InpedILD study), with receipt of honoraria. TH reports a grant from the Peter-Max-Müller Stiftung; speaking fees from ARGEX and Alexion; and travel support from Merck (Charcot Meeting). JR reports honoraria from Boehringer Ingelheim, AstraZeneca, and CSL Behring. KP provided support for Sanger sequencing of cDNA. NS reports consulting fees and honoraria for lectures from Boehringer Ingelheim and Infectopharm, participation on Advisory board for Boehringer Ingelheim (InpedILD study); and serves as President of the German-speaking Society for Pediatric Pneumology (GPP). NJ reports honoraria from Orphalan and Sanofi; travel support from ESPGHAN and ELITA; advisory board memberships in ELITA and ERN Rare Liver; and engagements with Hepatology Committee ESPGHAN, RARE Liver ERN, ERN Transplantchild, and ELTR/ELITA. DR reports grants/contracts from RACOON-RESCUE and RACOON-INCLUDED, funded by the Federal Ministry of Research, Technology, and Space. BS reports institutional funding and research collaboration with GlaxoSmithKline; consulting fees from Boehringer Ingelheim, AstraZeneca, and GlaxoSmithKline; honoraria for lectures from Boehringer Ingelheim, AstraZeneca, and GlaxoSmithKline; and travel support from Boehringer Ingelheim. All other authors report no conflicts of interest.

## **Funding**

JCS is supported by the Else Kröner-Fresenius-Stiftung (2023\_EKCS.18) and the German Center for Lung Research (FKZ 82DZL002C1 & FKZ 82DZLT82C1).

PM is supported by the Clinician Scientist Program TITUS, funded by Hannover Medical School and the Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH, member of the German Center for Lung Research).

**Table 1. Demographic and Clinical Characteristics of Nine Patients with DGUOK Deficiency**

|                                          | P1             | P2             | P3             | P4 | P5 | P6             | P7 | P8 | P9 |
|------------------------------------------|----------------|----------------|----------------|----|----|----------------|----|----|----|
| Male                                     | +              | -              | +              | -  | -  | +              | +  | +  | -  |
| Parental consanguinity                   | +              | +              | +              | -  | +  | +              | +  | +  | +  |
| Age at onset                             | 15             | 15             | 2              | 13 | 0  | 28             | 23 | 20 | 19 |
| Organ first involved (Pu / Li)           | Pu             | Pu             | Li             | Pu | Li | Pu             | Pu | Pu | Pu |
| DGUOK variants                           | A              | A              | B              | A  | C  | A              | A  | A  | A  |
| FU time since onset                      | 3              | 2              | 7              | 3  | 5  | 8              | 10 | 8  | 3  |
| <b>General symptoms</b>                  |                |                |                |    |    |                |    |    |    |
| BMI <15                                  | +              | -              | +              | +  | +  | -              | +  | -  | -  |
| Fatigue                                  | +              | +              | +              | +  | /  | +              | +  | -  | -  |
| Muscle weakness                          | +              | -              | +              | +  | /  | +              | +  | +  | -  |
| <b>Respiratory symptoms and findings</b> |                |                |                |    |    |                |    |    |    |
| Exertional dyspnea                       | +              | +              | +              | +  | +  | +              | +  | +  | +  |
| History of pneumothorax                  | +              | +              | +              | +  | -  | -              | +  | +  | -  |
| Suprasternal notch                       |                | +              | +              | +  | /  | +              | +  | +  | +  |
| Chronic cough                            | +              | -              | +              | +  | -  | +              | +  | -  | -  |
| Restrictive ventilatory defect           | +              | +              | +              | +  | +  | +              | +  | +  | +  |
| FVC 40% predicted                        | +              | +              | +              | +  | /  | +              | /  | /  | /  |
| Hypoxemia at rest                        | -              | -              | -              | -  | +  | -              | /  | /  | /  |
| Lung biopsy performed                    | +              | +              | +              | +  | -  | -              | -  | -  | -  |
| Histological AFE pattern                 | +              | +              | +              | +  | /  | /              | /  | /  | /  |
| <b>Chest CT Features</b>                 |                |                |                |    |    |                |    |    |    |
| Predom. UL involvement                   | +              | +              | +              | +  | +  | +              | +  | +  | +  |
| Pleural & septal thickening              | +              | +              | +              | +  | +  | +              | +  | +  | +  |
| Subpleural reticular opacities           | +              | +              | +              | +  | +  | +              | +  | +  | +  |
| Traction bronchiectasis                  | +              | +              | -              | +  | -  | +              | +  | -  | -  |
| Ground glass opacities                   | +              | -              | +              | +  | +  | +              | +  | +  | -  |
| Honeycombing                             | -              | -              | -              | -  | -  | -              | -  | -  | -  |
| Platythorax                              | +              | -              | -              | +  | -  | +              | +  | +  | -  |
| Tracheal deviation                       | +              | +              | -              | +  | +  | +              | +  | +  | -  |
| <b>Extrapulmonary manifestations</b>     |                |                |                |    |    |                |    |    |    |
| Liver affection                          | -              | -              | +              | -  | +  | -              | -  | -  | -  |
| Liver Tx                                 | -              | -              | +              | -  | +  | -              | -  | -  | -  |
| Pulmonary hypertension                   | -              | -              | -              | -  | +  | -              | -  | -  | -  |
| Arterial Hypertension                    | -              | -              | -              | -  | -  | +              | -  | -  | -  |
| Developmental delay                      | -              | -              | +              | -  | +  | -              | -  | -  | -  |
| Hyporeflexia                             | /              | +              | +              | /  | /  | +              | /  | /  | /  |
| Peripheral neuropathy                    | +              | +              | +              | +  | -  | +              | -  | -  | -  |
| Proteinuria                              | + <sup>a</sup> | + <sup>a</sup> | + <sup>a</sup> | -  | /  | + <sup>b</sup> | -  | -  | -  |
| Renal insufficiency                      | -              | -              | -              | -  | -  | +              | -  | -  | -  |
| <b>Status</b>                            |                |                |                |    |    |                |    |    |    |
| Alive                                    | +              | +              | -              | -  | -  | +              | +  | +  | +  |
| LuTx                                     | +              | E              | E              | E  | -  | E              | -  | -  | -  |
| Age at death [years]                     | /              | /              | 9              | 16 | 5  | /              | /  | /  | /  |

+: Yes. -: No. / : Not available. E: LuTx Evaluation. Pu: Pulmo. Li : Liver, FU: Follow-up

A: c.592-26A>G/c.592-26A>G; B: c.592-26A>G/c.313C>T; C: c.763\_766dup/ c.763\_766dup

<sup>a</sup>mild/transient, not further specified. <sup>b</sup>predominantly albuminuria

## References

1. Frankel SK, Cool CD, Lynch DA, Brown KK. Idiopathic pleuroparenchymal fibroelastosis: description of a novel clinicopathologic entity. *Chest*. 2004;126(6):2007-13.
2. Suzuki Y. Idiopathic pleuroparenchymal fibroelastosis: A review of the previous literature and current knowledge. *Respir Investig*. 2025;63(1):127-37.
3. Nguyen HN, Das S, Gazzaneo MC, Melicoff E, Mallory GB, Eldin KW, et al. Clinico-radiologic features of pleuroparenchymal fibroelastosis in children. *Pediatr Radiol*. 2019;49(9):1163-70.
4. Enomoto Y, Nakamura Y, Satake Y, Sumikawa H, Johkoh T, Colby TV, et al. Clinical diagnosis of idiopathic pleuroparenchymal fibroelastosis: A retrospective multicenter study. *Respir Med*. 2017;133:1-5.
5. Nakamura Y, Mori K, Enomoto Y, Kono M, Sumikawa H, Johkoh T, et al. Prognostic and Clinical Value of Cluster Analysis in Idiopathic Pleuroparenchymal Fibroelastosis Phenotypes. *J Clin Med*. 2021;10(7).
6. Ryerson CJ, Adegunsoye A, Piciocchi S, Hariri LP, Khor YH, Wijsenbeek MS, et al. Update of the international multidisciplinary classification of the interstitial pneumonias: an ERS/ATS statement. *Eur Respir J*. 2025;66(6).
7. Atag E, Bas Ikizoglu N, Gokdemir Y, Erdem Eralp E, Kiyan G, Yilmazbayhan D, et al. An adolescent with idiopathic pleuroparenchymal fibroelastosis. Case report. *Arch Argent Pediatr*. 2018;116(1):e147-e50.
8. Gerstle K, Tanager K, Hegde S, Chung JH, Husain AN, Applebaum MA, et al. Pleuroparenchymal fibroelastosis in an 8-year old treated for neuroblastoma. *Pediatr Pulmonol*. 2020;55(3):E1-e4.
9. Kinoshita Y, Utsunomiya T, Koide Y, Wada K, Ueda Y, Yoshida Y, et al. Familial pleuroparenchymal fibroelastosis: an entity to be recognized. *Respir Med Res*. 2022;82:100954.

10. Jaula H, Mattila L, Lappi-Blanco E, Salonen J, Vähänikkilä H, Ahvenjärvi L, et al. Clinical, radiological and histopathological features of patients with familial pulmonary fibrosis. *Respir Res.* 2024;25(1):239.
11. Baqir M, LeMahieu AM, Hartman TE, Farah WH, Yi EE, Ryu JH. Pleuroparenchymal fibroelastosis: review of 81 cases. *Expert Rev Respir Med.* 2026;20(2):217-26.
12. Alrehaili G, Kemppainen J, Kalra S, Pinto EVF, Moua T, Yi ES, et al. Genetic Testing Goes Beyond Imaging and Histological Evaluation in Pleuroparenchymal Fibroelastosis. *Lung.* 2024;202(2):151-6.
13. Newton CA, Batra K, Torrealba J, Kozlitina J, Glazer CS, Aravena C, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J.* 2016;48(6):1710-20.
14. Borie R, Tabèze L, Thabut G, Nunes H, Cottin V, Marchand-Adam S, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. *Eur Respir J.* 2016;48(6):1721-31.
15. Zhang X, Wang Y, Guo X, Xiao Y, Wan W, Zou H, et al. Mitochondrial dysfunction in fibrotic diseases: Research progress and MSC-exos therapy. *Exp Mol Pathol.* 2025;143:104983.
16. Liu S, Xi Q, Li X, Liu H. Mitochondrial dysfunction and alveolar type II epithelial cell senescence: The destroyer and rescuer of idiopathic pulmonary fibrosis. *Front Cell Dev Biol.* 2025;13:1535601.
17. Wang J, Wu H, Gao J, Zhang T. Mitochondrion in Pulmonary Disease: Small Organelle but Big Function. *Small Methods.* 2026;10(2):e2500674.
18. Bueno M, Lai YC, Romero Y, Brands J, St Croix CM, Kamga C, et al. PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. *J Clin Invest.* 2015;125(2):521-38.
19. Rangarajan S, Bernard K, Thannickal VJ. Mitochondrial Dysfunction in Pulmonary Fibrosis. *Ann Am Thorac Soc.* 2017;14(Supplement\_5):S383-s8.

20. Hartmannová H, Piherová L, Tauchmannová K, Kidd K, Acott PD, Crocker JFS, et al. Acadian variant of Fanconi syndrome is caused by mitochondrial respiratory chain complex I deficiency due to a non-coding mutation in complex I assembly factor NDUFAF6. *Human Molecular Genetics*. 2016;25(18):4062-79.
21. Cámara Y, González-Vioque E, Scarpelli M, Torres-Torronteras J, Caballero A, Hirano M, et al. Administration of deoxyribonucleosides or inhibition of their catabolism as a pharmacological approach for mitochondrial DNA depletion syndrome. *Hum Mol Genet*. 2014;23(9):2459-67.
22. Dimmock DP, Zhang Q, Dionisi-Vici C, Carrozzo R, Shieh J, Tang LY, et al. Clinical and molecular features of mitochondrial DNA depletion due to mutations in deoxyguanosine kinase. *Hum Mutat*. 2008;29(2):330-1.
23. Freisinger P, Fütterer N, Lankes E, Gempel K, Berger TM, Spalinger J, et al. Hepatocerebral mitochondrial DNA depletion syndrome caused by deoxyguanosine kinase (DGUOK) mutations. *Arch Neurol*. 2006;63(8):1129-34.
24. Manzoni E, Carli S, Gaignard P, Schlieben LD, Hirano M, Ronchi D, et al. Deoxyguanosine kinase deficiency: natural history and liver transplant outcome. *Brain Commun*. 2024;6(3):fcae160.
25. Ronchi D, Garone C, Bordoni A, Gutierrez Rios P, Calvo SE, Ripolone M, et al. Next-generation sequencing reveals DGUOK mutations in adult patients with mitochondrial DNA multiple deletions. *Brain*. 2012;135(Pt 11):3404-15.
26. Keshavan N, Rahman S. Natural history of deoxyguanosine kinase deficiency. *Mol Genet Metab*. 2024;143(1-2):108554.
27. Vara R, Pinon M, Fratter C, Hegarty R, Hadzic N. Hepatic presentations of mitochondrial DNA depletion syndrome in children: A single tertiary liver centre experience. *J Inherit Metab Dis*. 2023;46(4):634-48.
28. Ramboux A, Poncelet A, Clapuyt P, Scheers I, Sokal E, Reding R, et al. Deoxyguanosine kinase deficiency and recurrent spontaneous pneumothorax: a case report. *J Med Case Rep*. 2023;17(1):413.

29. Grabhorn E, Tsiakas K, Herden U, Fischer L, Freisinger P, Marquardt T, et al. Long-term outcomes after liver transplantation for deoxyguanosine kinase deficiency: a single-center experience and a review of the literature. *Liver Transpl.* 2014;20(4):464-72.
30. Taviglian SV, Harrison SM, Boucher KM, Biesecker LG. Fitting a naturally scaled point system to the ACMG/AMP variant classification guidelines. *Hum Mutat.* 2020;41(10):1734-7.
31. Dombi E, Marinaki T, Spingardi P, Millar V, Hadjichristou N, Carver J, et al. Nucleoside supplements as treatments for mitochondrial DNA depletion syndrome. *Front Cell Dev Biol.* 2024;12:1260496.
32. Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, et al. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci Adv.* 2020;6(28):eaba1983.
33. Komal FNU, Moretti PM, Shaibani AI. Deoxyguanosine kinase mutation producing juvenile-onset mitochondrial myopathy. *Neurol Genet.* 2018;4(5):e269.
34. Muto Y, Sekine A, Hagiwara E, Komatsu S, Baba T, Oda T, et al. Clinical characteristics of pulmonary hypertension in patients with pleuroparenchymal fibroelastosis. *Respir Investig.* 2022;60(4):543-50.
35. Adams TS, Schupp JC, Balayev A, Khoury J, Justet A, Nikola F, et al. Alveolar epithelial cell plasticity and injury memory in human pulmonary fibrosis. *bioRxiv.* 2025.
36. Ruwisch J, Cazes A, Leiber LM, Borie R, Neubert L, Christian L, et al. The pleuroparenchymal fibroelastosis atlas reveals aberrant cell states and their zonation as an alternate roadmap to lung fibrosis. *Science Advances.* 2026;12(19):eaeb5967.
37. Leiber LM, Christian L, Neubert L, Ruwisch J, Yilmaz H, Plucinski EKJ, et al. Aberrant and Ectopic Cell Populations in Restrictive Allograft Syndrome after Lung Transplantation. *European Respiratory Journal.* 2026:2500537.

## Figure 1. Genetic and clinical features of patients with DGUOK-associated mitochondriopathy

Panel A-D shows the pedigrees of four unrelated families. Double lines denote consanguinity, filled symbols indicate affected individuals with biallelic *DGUOK* variants and half-filled symbols indicate heterozygous carriers. Genotypes are shown below the symbols. Squares and circles represent males and females, respectively. A slash denotes deceased individuals. Roman numerals indicate generations, whereas Arabic numerals denote individuals within each generation. Representative chest CT images of patients are presented in Panel E-H with P4 (E, F) and P6 (G, H), showing predominantly apical, subpleural and septal thickening (red arrows), traction bronchiectasis (red dashed arrows), pneumothorax (red asterisk) and tracheal deviation (yellow asterisk). Panel I shows a deepened suprasternal notch, characteristic for fibrotic contraction of the upper lung lobes in P2. The hands of the patients show no signs of digital clubbing (P6) but severe atrophy of the small hand muscles (Panel J). Panels K–N illustrate the characteristics and rapid progression of PPFE in patient P1. At diagnosis, Panels K and L show a video-assisted thoracoscopic surgery (VATS) biopsy with Hematoxylin and Eosin (HE) and Elastica van Gieson (EvG) staining, respectively, revealing a sharply demarcated subpleural area with an alveolar fibroelastosis (AFE) pattern, highlighted by green boxes. Panel L illustrates the characteristic AFE features, including prominent black-stained elastic fibers in the areas of the former alveolar walls and the former alveolar lumina filled with pink-stained collagen. Panels M and N show HE and EvG staining of the explanted lung obtained 8 months later, demonstrating advanced fibrotic remodeling, highlighted by yellow arrows.

## Figure 2. Functional characterization of the deep intronic DGUOK variant.

Panel A depicts RNA-seq read coverage tracks for blood and lung tissue of P1 and a control (Ctrl). Red arcs indicate splice junctions. The exon–intron structure of the gene is displayed at the bottom, with exons 5–7 indicated. In P1 the variant c.592-26A>G in *DGUOK* leads to exon 5 skipping in the majority of blood-derived RNA reads compared to Ctrl, while lung-derived

RNA of P1 shows exclusive exon 5 skipping. Panel B illustrates the amount of mitochondrial DNA (mtDNA) in patients carrying the homozygous variant c.592-26A>G in *DGUOK* (Pat) and a control group (Ctrl). Each point represents an individual sample, and the distribution width indicates the density of the data. The normalized mean coverage of mtDNA is significantly higher in the control group compared to the case group, as indicated by the triple asterisk (\*\*\*), denoting a highly significant difference ( $p < 0.001$ ). The black bars represent the median and interquartile range. Panel C represents western blot showing *DGUOK* expression in control (Ctrl) and patient's material (Fibroblasts (FB) of P1, P4, P6, Lung of P1, P3). Vinculin served as a loading control. Panel D shows the effect of deoxynucleoside supplementation on mtDNA recovery in patient-derived fibroblasts. Relative mtDNA copy number was determined by qPCR following treatment with (+ddC +ATCG) or without (+ddC -ATCG) exogenous deoxynucleosides. Deoxynucleoside supplementation substantially increased mtDNA recovery in patient-derived fibroblasts (P1, P4, and P6), while only minor changes were observed in controls. Data represent two independent experiments.

**Figure 3. snRNAseq reveals AT2 transition to Aberrant Basaloid cells in *DGUOK*-associated PPFE**

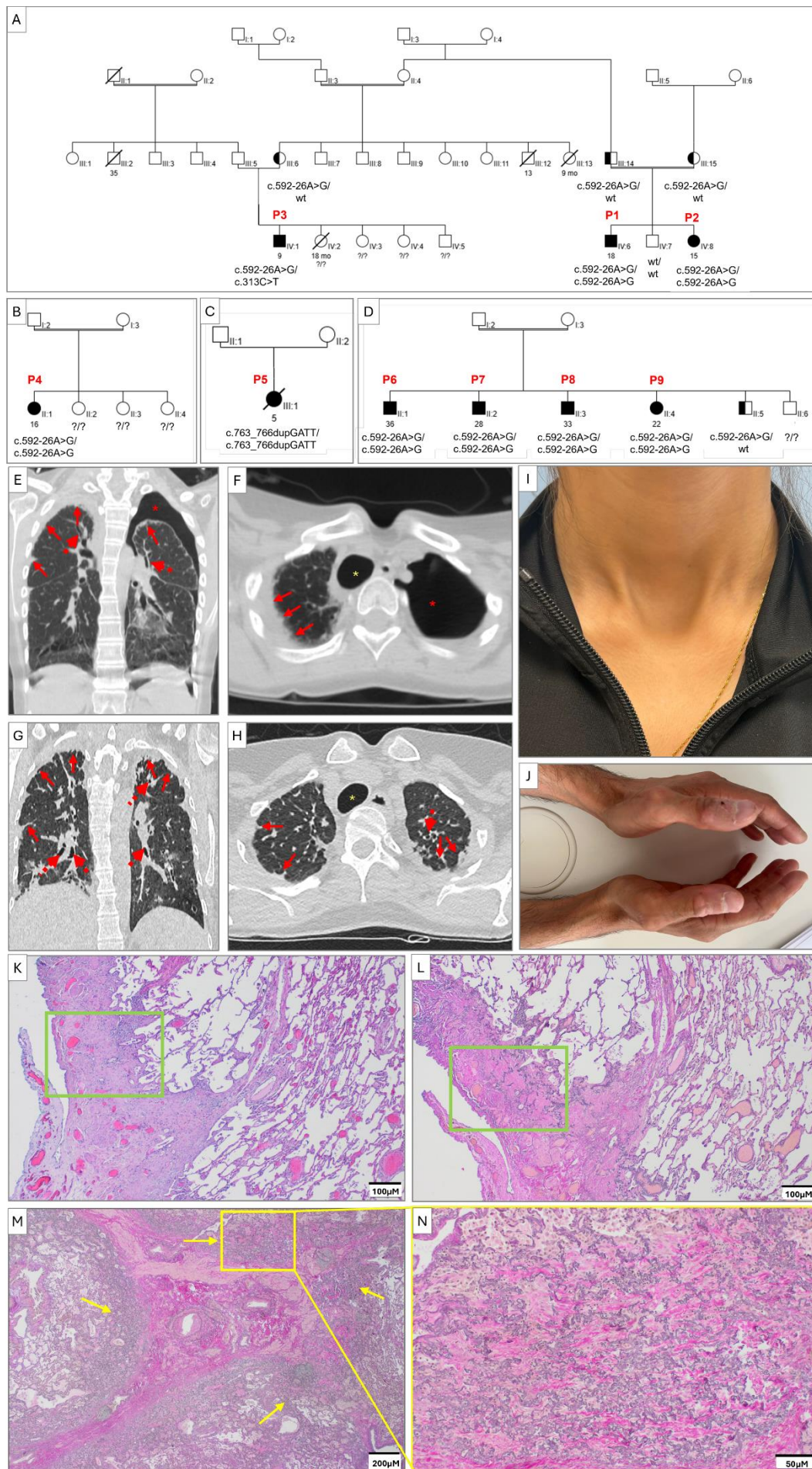
Panel A schematically illustrates the steps of the snRNAseq experiments. Six lung tissue samples from four patients were analyzed. Lung tissue samples from 16 subjects with a physiologic appearance served as controls. Nuclei were isolated and sorted by DAPI-based cell sorting, then encapsulated in droplets with barcoded beads. Within each droplet, transcripts were labeled with cell-specific barcodes and unique molecular identifiers (UMIs), and the resulting libraries were sequenced. Cell populations of interest were localized by multiplex immune fluorescence (IF) staining and IF microscopy. Panel B shows three Uniform Manifold Approximation and Projections (UMAPs) of epithelial cells, annotated by cell type, disease, and subject. Panel C shows significant differences in the relative frequencies of epithelial cell types between patients with PPFE and *DGUOK*-associated mitochondriopathy and controls, including the presence of intermediate cells (iAT2) and Aberrant Basaloid cells

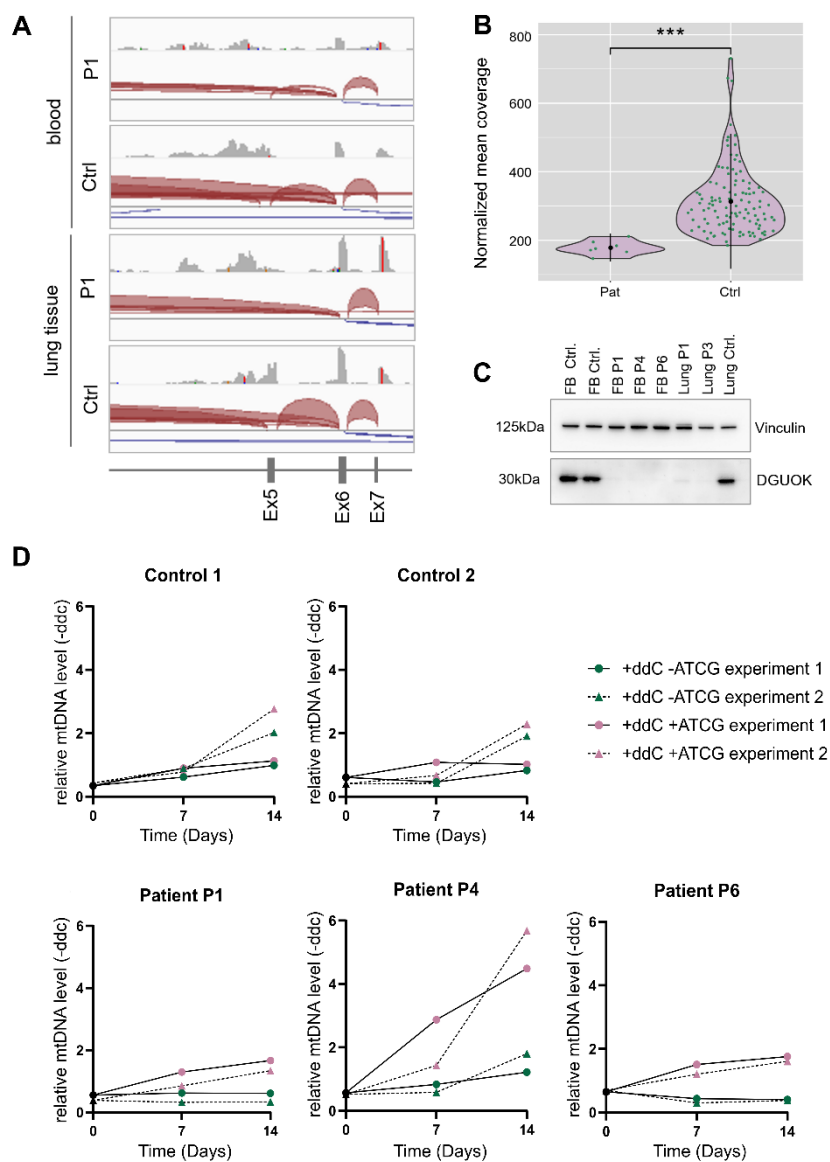
(Aberr. Basaloid). Box plots show the median, interquartile range, and full range of values, Asterisks indicate statistical significance (\* $p < 0.05$ ). Panel D shows a volcano plot of changes in gene expression between control and disease, with positive values indicating higher expression in disease. Genes involved in mitochondrial matrix proteins (green) and in DNA double strand break response (purple) show significantly increased expression. Panels E depicts the pseudotime trajectory from AT2 via intermediate cells (iAT2) to Aberrant Basaloid cells. In Panel F, a generalized additive model illustrates the genes changing from AT2 to Aberrant Basaloid cells as a function of pseudotime, while Panel G shows a heatmap of the corresponding genes, with each column representing a single nucleus randomly sampled from fixed intervals.

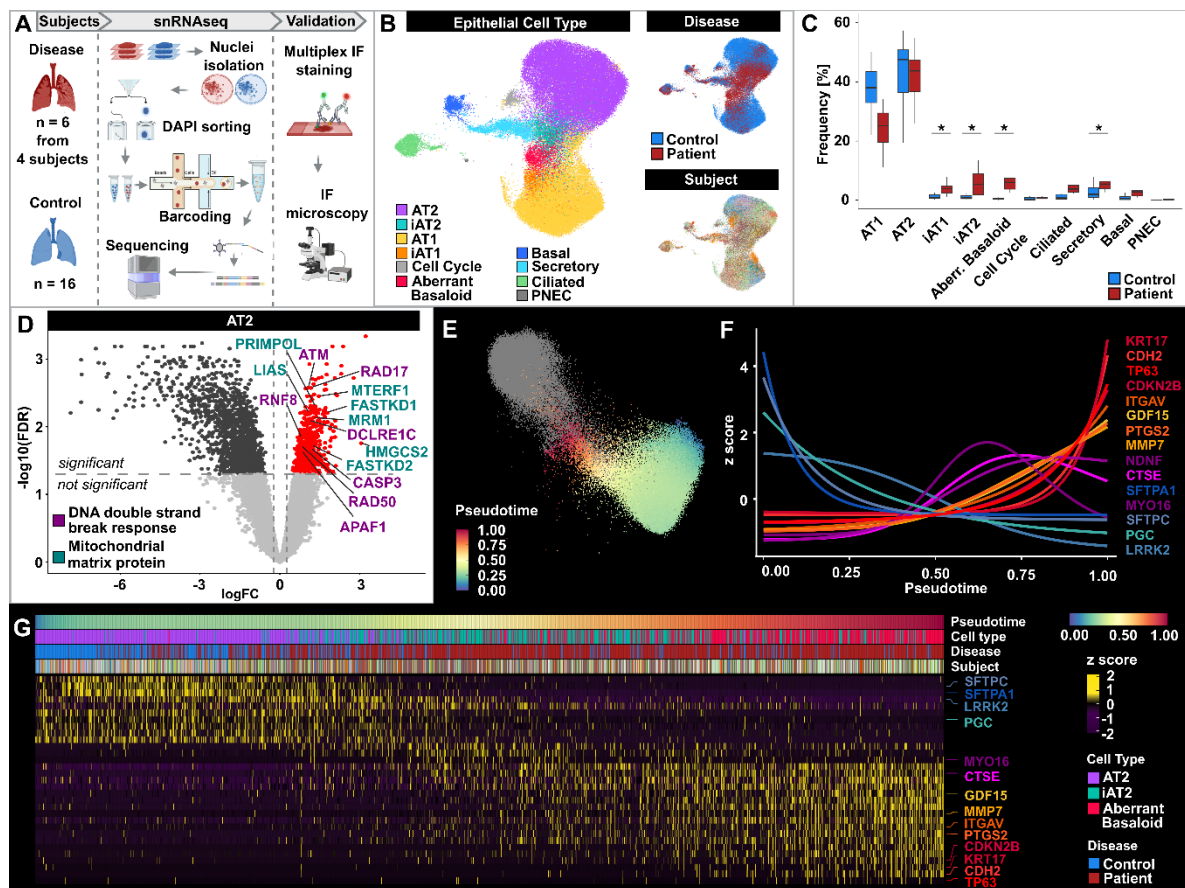
#### **Figure 4. Aberrant Basaloid cells are localized at the fibro-alveolar remodeling edge**

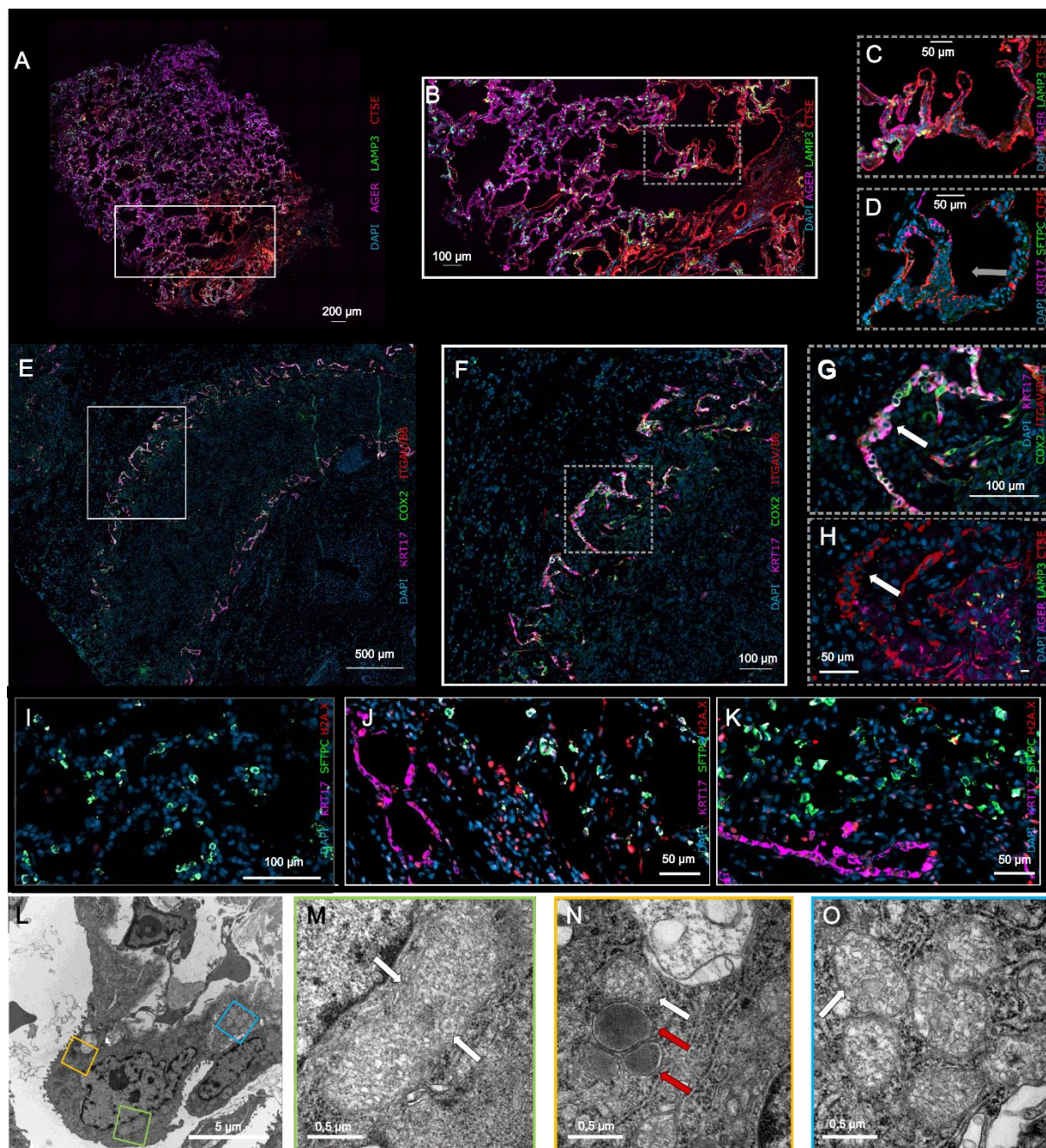
Panel A to H show representative immunofluorescence stains of lung tissue from two pediatric patients (Panel A-D: cryobiopsy of P4; Panel E-H: lung explant of P1) with PPFE and DGUOK-associated mitochondriopathy. Intermediate cells (iAT2) as precursor cells of Aberrant Basaloid cells are found expressing CTSE but not KRT17, as indicated by the gray arrow in Panel D. Aberrant Basaloid cells localize at the fibro-alveolar remodeling edge, highlighted by white arrows in Panels G and H. They were characterized by positivity for CTSE, COX2, ITGAV/ITGB6, and KRT17, and by absence of expression of AGER, a specific marker for AT1 cells, and SFTPC and LAMP3, a specific marker for AT2 cells. Panel A, B, C and H show LAMP3 (green), CTSE (red), AGER (pink). Panel D depicts SFTPC (green), CTSE (red) and KRT17 (pink). Panel E, F and G demonstrate COX2 (green), ITGAV/B6 (red) and KRT17 (pink). Nuclei are stained with DAPI. Panels I–K show nuclear phospho-histone H2AX ( $\gamma$ H2AX) immunostaining in healthy controls (Panel I) and DGUOK patients (Panels J and K).  $\gamma$ H2AX staining was absent in healthy controls, whereas DGUOK patients showed prominent nuclear  $\gamma$ H2AX-positive foci, indicative of DNA double-strand breaks, particularly in Aberrant Basaloid cells (KRT17-positive) and AT2 cells (SFTPC-positive). Panel L shows an overview of the ultrastructure of an Aberrant Basaloid cell and panels M to O some details with a focus on the

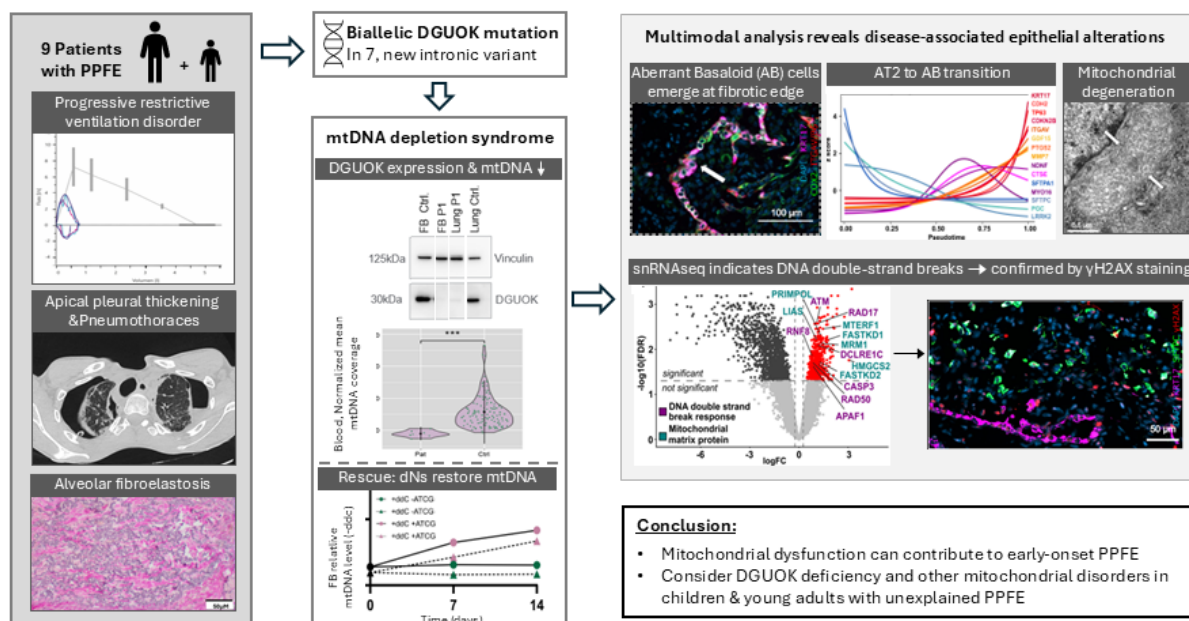
mitochondria (white arrows). These are enlarged and characterized by a loss of mitochondrial matrix as well as swelling and lysis/ degeneration of their cristae (Panels M-O). The red arrows in Panel N highlight electron dense bodies as a typical feature of Aberrant Basaloid cells.











## **Supplementary Appendix**

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## **I. Supplementary methods**

### **Patient cohort, ethics approval, and consent to participate**

Nine patients with genetically confirmed DGUOK-deficiency (five children and four adults) have been included. Data on symptoms and clinical courses were obtained from clinical records and treating physicians. The study adhered to the principles of the Declaration of Helsinki. Patients provided assent, and their parents provided informed consent for the use of data and biomaterials, either through inclusion in the European Registry for childhood interstitial lung disease (chILD-EU Registry), via the Broad Consent of the German Center for Lung Research (DZL), or as part of the treatment contract of Hannover Medical School. DZL Broad consent and enrollment in chILD-EU registry was approved by the local ethics committee of the Hannover Medical School, Germany (Ethical vote 2923-2015, 1969-2013 and 12241-BO-K-2026, respectively).

### **Whole exome or genome sequencing and variant interpretation**

Whole-exome sequencing (WES) (P4, P5) or whole-genome sequencing (WGS) (P1, P2, P3, P6-P9) was performed at Hannover Medical School on DNA extracted from peripheral blood samples collected in ethylenediaminetetraacetic acid (EDTA) tubes. DNA extraction, library preparation, and sequencing were conducted using standard protocols. Bioinformatic analyses, including alignment, variant calling, annotation, filtering, prioritization, and evaluation, were performed using our center-specific pipeline. Sequencing reads were aligned to the human reference genome (GRCh38), and variant calling was conducted according to established best practices. Variant interpretation incorporated allele frequency data and computational prediction tools, including phyloP, SIFT, PolyPhen-2, FATHMM, CADD, and REVEL. Identified variants were further assessed using public databases, including the Leiden Open Variation Database ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>), and the Genome Aggregation Database (gnomAD; <https://gnomad.broadinstitute.org/>). The classification of sequence variants was performed in accordance with the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG)<sup>31</sup>.

### **Whole blood RNA sequencing analysis**

Whole blood collected in PAXgene Blood RNA tubes (PreAnalytics GmbH, Switzerland) and lung tissue preserved in RNAlater (ThermoFisher) were used for whole-transcriptome analysis. Total RNA was extracted according to the respective manufacturers' protocols. RNA quality and quantity were assessed by spectrophotometry. RNA libraries were prepared for sequencing using Takara SMART-Seq stranded total RNA Kit and processed on an Illumina NovaseqXplus platform with standard paired-end runs. Downstream data analysis included

alignment to the GRCh19 reference genome, expression quantification, and pathway enrichment analysis using established bioinformatic pipelines (STAR version 2.7, Center for Human Genetics, Germany, Tübingen). Sashimi plots and coverage profiles were generated using the IGV Genome Browser v2.18.1.

### **Cell culture**

3 mm punch biopsies of patient P1, P3 and P4 as well as the healthy mother of P1/ P2 and the healthy father of P4 were obtained under local anesthesia and transported in sterile PBS or cell culture media with antibiotics. The tissue was minced under sterile conditions and cultured in DMEM with 10% fetal bovine serum (Sigma-Aldrich), 1 % sodium pyruvate (Gibco) and 1 % penicillin-streptomycin (Gibco) at 37°C with 5% CO<sub>2</sub>. Low passages of fibroblasts cultures migrating from the explant were expanded and used for further experiments.

### **Western Blot Analysis**

Protein lysates were prepared from fibroblasts using the RIPA lysis buffer system (Santa Cruz). After electrophoresis, proteins were transferred onto an Amersham Protran nitrocellulose membrane (Sigma/Merck, Darmstadt, Germany). The membrane was blocked for 1 h in non-fat milk and incubated with primary polyclonal antibody (anti-DGUOK Invitrogen Immunogen PA5-63419, anti-Vinculin #18799 Cell signaling) overnight at 4°C and secondary antibody (Goat anti rabbit IgG-HRP #7074 Cell signaling, Goat anti rabbit IgG-HRP, abcam #ab6721) at room temperature for 2 h, respectively.

### **Respiratory chain enzyme analysis in fibroblasts and muscle tissue**

Muscle biopsies of P3 for respiratory chain enzyme analysis were snap-frozen immediately after collection by plunging the fresh tissue into liquid nitrogen and stored at –80°C to preserve mitochondrial enzyme activity. Fibroblasts of P1, P3 and P4 were cultured in T25 flask and then plated on Petri dishes with a diameter of 2 cm. Fibroblasts were sonicated to expose respiratory chain enzymes. Respiratory chain complexes (complex I+III, complex II+III, cytochrome c oxidase and ATP-synthase as well as citrate synthase) were assayed as described before [1]. A postnuclear supernatant of the skeletal muscle biopsy was prepared and ATP-synthase assayed as described previously [2]. Complex I+III, complex II+III and cytochrome c-oxidase as well as citrate synthase enzyme activities were measured using a slight modification of the methods described above for fibroblasts.

### **Mitochondrial DNA content calculation**

The calculation of the mean coverage of the mitochondrial chromosome and the autosomes of each sample was performed with PanDepth [3] by using the Whole genome sequencing (WGS)-cram files of the seven patients with the same homozygous pathogenic variant (c.592-26A>G, P1, P2, P4, P6-P9) and of 115 randomly chosen controls. WGS was performed on blood samples from the respective individuals. Mitochondrial DNA content calculation was done according to the formula  $2 \times (\text{chrM coverage}) / (\text{genome coverage})$  [4] using bc (<https://www.gnu.org/software/bc/>). Visualization was done with the ggplot2 and ggsignif packages on R version 4.5.2.

### **Mitochondrial DNA depletion and recovery with deoxyribonucleosides and mtDNA quantification**

The assay was performed as already described by Dombi et al. [5]. Regular fetal calf serum was replaced throughout by dialyzed fetal calf serum (dFCS). Control and DGUOK-deficient fibroblasts were cultured in high-glucose DMEM (#D5796, Merck) supplemented with penicillin-streptomycin (#P0781 Merck), and 0.1% dFCS (#A3382001 Life Technologies). Cells were initially seeded in T25 flasks at  $2 \times 10^6$  cells per flask and preconditioned for 24 h in 10% dFCS before being switched to 0.1% dFCS (d -13). After additional 3 days (d-10) cells were treated for 10 days with 20  $\mu$ M 2'-3'-dideoxycytidine (ddC, #PHR3266 Merck) with refreshing the ddC containing medium on day -7 and -4. 1  $\times$  T25 flask/cell culture was used as untreated control. 10 days after ddC treatment start, the cells from the untreated flasks and 1 ddC-treated flask were trypsinated and transferred to liquid nitrogen after pelleting the cells (300xg, 5 min) to generate reference samples (d0). The remaining flasks were then used as untreated controls or treated with a deoxynucleoside mixture (dA+dT+dG+dC, 50  $\mu$ M each, #D7400, #T1895, #D7145, #D3897, Merck). Treated and untreated cells were again harvested 7 and 14 days after dA+dT+dG+dC treatment started. The dA+dT+dG+dC-containing medium was refreshed for the non-harvested cells on day 3, 7 and 10.

ddC, dC and dA were dissolved in water and sterile-filtered through a PES membrane. dG was dissolved in 1M ammonia solution and dT in DMSO. dT was sterile-filtered through a PTFE membrane. Cells were tested for mycoplasma contamination before and after the experiment. Total cellular DNA was isolated using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich). qPCR was performed using TaqMan™ ROX Probe Master Mix dTTP Blue (#UF-RPMT-B0701, Eurogentec) and primers and probes (customer specific, Sigma) shown in Supplemental Table Sx, specific for mitochondrial DNA and the single copy nuclear gene B2M. Quantification was performed using the  $2^{-\Delta\Delta C_t}$  method.

**Supplemental Table S1: Primer and Probe Sequences**

| <b>Primer Sequences</b> |                                          |
|-------------------------|------------------------------------------|
| Mito_F                  | 5' CCCTAAAACCCGCCACATCT                  |
| Mito_R                  | 5' GAGCGATGGTGAGAGCTAAGGT                |
| Nuc_F                   | 5' TGCTGTCTCCATGTTTGATGTATCT             |
| Nuc_R                   | 5' TCTCTGCTCCCCACCTCTAAGT                |
| <b>Probe Sequences</b>  |                                          |
| Mito_P                  | 5' VIC-CCATCACCCCTCTACATCACCGCCC-TAMRA   |
| Nuc_P                   | 5' 6-FAM-CAGGTTGCTCCACAGGTAGCTCTAG-TAMRA |

**Transcriptomic Analyses****1. Nuclei isolation from FFPE tissue for Chromium Fixed RNA Profiling**

From each formalin-fixed paraffin-embedded (FFPE) tissue block, three sections with a thickness of 50  $\mu\text{m}$  were prepared and placed into Miltenyi C-tubes. Deparaffinization was performed at room temperature by immersing each specimen in xylene for three consecutive 10-minute treatments. For each specimen and wash, 1 mL of xylene was used. Tissue was rehydrated through an ethanol series using 100% ethanol, 70% ethanol, 50% ethanol, and 30% ethanol. Post rehydration, each specimen was washed thrice with 1X phosphate-buffered saline (PBS) containing 0.5 mM calcium chloride. The Tissue Digestion Buffer (TDB, see table Y) was pre-warmed at 37°C for 10 minutes before use. Two milliliters of TDB were added to each specimen in C-tubes, before loading onto a gentleMACS Octo Dissociator and processed using the following program: (1) incubation at 37°C for 45 minutes; (2) clockwise rotation at 2,000 rpm for 30 seconds at 37°C; (3) counterclockwise rotation at 2,000 rpm for 30 seconds at 37°C; and (4) removal of the C-tubes. Finally, the C-tubes were briefly centrifuged at 300  $\times$  g for approximately 30 seconds to collect all nuclei at the bottom of the tube, before resuspension in the supernatant. The dissociated tissues were filtered through 70  $\mu\text{m}$  mesh filters (PluriSelect #43-50070-51) to remove debris and undissociated tissue fragments. To maximize recovery, each C-tube was first rinsed with 1 mL of 1X PBS, which was passed through the filter, and the filter itself was subsequently rinsed with an additional 1 mL of 1X PBS. Filtrates from both steps were collected in the same tube as the corresponding dissociated tissue. The specimens were centrifuged at 850  $\times$  g for 5 minutes at 4°C, and supernatants were carefully removed without disturbing the pellets. Each pellet was resuspended in 1 mL of chilled Quenching Buffer (content of 10X Genomics, #1000781) and subsequently passed through a 20  $\mu\text{m}$  filter (PluriSelect #43-10020-50) to remove debris and any remaining undissociated cells. Nuclei quality and count was assessed using a LUNA-FL™ Dual Fluorescence Cell Counter with the Acridine Orange/Propidium Iodide (AO/PI) Cell Viability Kit (BioCat, #F23001-LG). Nuclei suspensions were gently mixed by pipetting, and 18  $\mu\text{L}$  of per suspension was combined with 2  $\mu\text{L}$  of AO/PI. LUNA™ reusable slides were loaded with 10  $\mu\text{L}$  of this mixture per chamber. All nuclei suspensions were stored overnight at 4°C with 10% enhancer (content of 10X Genomics, #1000781) prior to Flow-Activated Nuclei

Sorting (FANS): Enhancer was thawed at 65°C for 10 minutes, briefly vortexed, and spun down. Before use, it was kept warm and inspected to ensure no precipitate was present. Pre-warmed Enhancer was then added to each nuclei suspension at a ratio of 1:10 and gently mixed by pipetting.

**Supplemental Table S2: Buffers used for snRNA-sequencing:**

| Buffer | Compound                                    | Manufacturer (Catalog Number) | Stock concentration                | Final concentration |
|--------|---------------------------------------------|-------------------------------|------------------------------------|---------------------|
| TDB    | Liberase™ TM Research Grade                 | Merck/ Roche (5401127001)     | Lyophilized, prepared as 1 mg/mL   | 250 µg/mL           |
|        | Collagenase D from Clostridium histolyticum | Merck/ Roche (11088882001)    | Lyophilized, prepared as 100 mg/mL | 2.5 mg/mL           |
|        | Calcium Chloride Solution                   | Jena Bioscience (BU-103)      | 2.5 M                              | 0.5 mM              |
|        | PBS - Phosphate-Buffered Saline             | Invitrogen™ (AM9624)          | 10X                                | 1X                  |
|        | Protector RNase-Inhibitor 10 000 U          | Merck/ Roche (3335402001)     | 40 U/µL                            | 0.04 U/µL           |
|        | Nuclease-free H2O                           | Invitrogen™ (AM9932)          | N/A                                | To 2 mL             |

## 2. Isolation of DAPI<sup>+</sup> Single Nuclei by Fluorescence-Activated Nuclei Sorting (FANS)

Immediately prior to sorting, nuclei suspensions were stained with 4',6-diamidino-2-phenylindole (DAPI, Thermo Scientific™ #62248), with final concentrations ranging from 1 to 10 µg/mL and adjusted as needed to achieve optimal staining. Sorting was carried out using cell sorters equipped with a 100 µm nozzle at 35 psi (FACSAria III Fusion, FACSAria IIu, Becton-Dickinson, or MoFlo XDP, Beckman-Coulter) in the central research facility Cell Sorting of Hannover Medical School. For each specimen, 5 × 10<sup>5</sup> DAPI<sup>+</sup> single nuclei were collected into individual 15-mL tubes, each containing 1 mL of 0.5X PBS with 0.02% BSA (Sigma-Aldrich, #126615-25ML). Intact single nuclei were isolated by flow cytometry based on light scattering characteristics and DNA content, as indicated by DAPI fluorescence. A tight gate was applied in the DAPI channel to exclude nuclei with suboptimal DNA content and debris. Doublets were removed in subsequent gating steps using scatter pulse parameters, and the main population was further refined using an FSC-SSC gate. Reanalysis of the nuclei from each specimen confirmed a homogeneous population. Most specimens yielded the desired 300,000 nuclei, besides one sample of patient 2 and one of 3, of which all sorted nuclei were used for further analysis. The sorted single nuclei suspensions were kept on ice until processing with the Chromium Fixed RNA Profiling Reagent Kits for Multiplexed Samples.

## 3. Chromium Fixed RNA Profiling of Multiplexed Samples

Sorted DAPI<sup>+</sup> single nuclei were immediately processed following the User Guide CG000527, Rev B (Chromium Fixed RNA Profiling Reagent Kits for Multiplexed Samples), as briefly

summarized below. Hybridization was performed in PCR tube-strips at 42°C for 19h. Due to exact determination of nuclei count, samples were pooled after hybridization for a pooled wash in Post-Hyb Wash Buffer B (contains 10X Genomics, #2001308 and #2000482). After the third rinsing step, samples were resuspended in 100 µL and filtered through a 20 µm filter. Nuclei concentration was determined thrice, with 2 µL of sample pool, 16 µL of ddH<sub>2</sub>O and 2 µL of AO/PI of which 10 µL were counted. GEM-X FX Chip was loaded according to instructions with a target nuclei recovery of 320,000. The chip was loaded into the Chromium X and GEMs were transferred immediately after instrument run. GEM incubation, GEM recovery and pre-amplification were performed on the same day, DNA cleanup and library preparation the next day. cDNA libraries were stored at -20°C until sequencing.

#### **4. Sequencing & Data processing of snRNAseq data**

Libraries were sequenced on an Illumina NovaSeqX platform, with a sequencing configuration of 28 base pairs for Read1, 90 base pairs for Read2, and 10 base pairs for Index5 and Index7. Then, base calls were converted to reads using the Cell Ranger software (8.0.1) and its bcl2fastq implementation ("mkfastq"). Reads were aligned to the human reference genome GRCh38 (GENCODE v32/Ensembl 98, "GRCh38-2020-A") and the Chromium Human Transcriptome Probe Set \_v1.0.1 for GRCh38-2020-A. Count matrices were analyzed using the Seurat pipeline (R package Seurat v5.4.0 in R v4.4.3), following recommendations for scRNAseq data processing and integration <https://satijalab.org/seurat/>). The newly generated data from 6 samples from 4 DGUOK patients, 2 pediatric control and one adult control subjects was merged with 13 previously generated high quality control samples, generated with the same technology (GSE284081, GSE301982). Low quality filtering was conducted in an iterative adaptive fashion per sample. Nuclei with > 5% mitochondrial counts, < 300 total counts, and < 200 features were filtered out prior to downstream analysis. UMI counts were normalized by applying a scale factor of 10,000 UMIs per cell. This was followed by natural log transformation with a pseudocount of 1 in accordance with methods previously published by our group and others. UMI counts were normalized using a scale factor of 10,000 UMIs per cell, followed by natural log transformation with a pseudocount of 1. The most variable genes were identified using Seurat's FindVariableGenes (nFeatures = 3000) function. Integration was performed using IntegrateLayers (method = scVIIntegration).

#### **5. Integration, dimensionality reduction, clustering, cell type annotation of snRNAseq data**

Louvain clustering was then performed, and for visualization, nuclei and their graph-based embeddings were projected into a low-dimensional space using Uniform Manifold Approximation and Projection (UMAP). Clusters lacking differential gene expression were

merged. Cell lineages (i.e., “Epithelial,” “Stromal,” “Immune”) were then assigned based on lineage-specific marker gene expression. This procedure was repeated twice for each subset, incorporating graph embedding and cluster analysis to identify multiplets and cellular debris, which were removed prior to downstream analyses. The cleaned lineage-specific datasets were subsequently integrated using scVI integration, followed by final dimensionality reduction and graph embedding as described above. Cell types were identified through cluster-specific marker genes as determined by the Wilcoxon rank-sum tests implemented in Seurat’s FindMarkers function, comparing all cells within each cluster to all other cells in the dataset. To account for multiple testing, p-values were adjusted using Bonferroni correction method, with adjusted p-values < 0.05 considered statistically significant. Final cell type annotations were assigned in a supervised manner by comparing the DEGs of each cluster together with the expression of established marker genes. Differential gene expression between DGUOK and control was performed using a pseudobulk approach. Raw counts were summed per cell type per subject, and differential expression was tested using edgeR’s quasi-likelihood framework, with TMM normalization. A two-group model was fitted per cell type, with control as the reference, and results were aggregated across all cell types.

## **6. Trajectory inference and gene expression dynamics analysis**

Downstream trajectory analyses were carried out in Python v3.11.11 using Scanpy v1.11.5 and Palantir v1.4.3. For reconstruction of the aberrant basaloid trajectory [6], cells were reclustered with the Leiden algorithm (resolution = 0.7). Trajectory topology was then inferred with Partition-based Graph Abstraction (PAGA), using Leiden clusters as grouping variables. Edges with low confidence were removed by applying a connectivity threshold of 0.25, and the resulting abstracted graph was used to initialize a force-directed layout for visualization. To focus specifically on the alveolar epithelial differentiation axis, AT1, AT2, Aberrant Basaloid, iAT1, and iAT2 populations were subsetted for pseudotime inference. Trajectory modeling was performed in Palantir using graph-based diffusion without imputation. Aberrant Basaloid cells were designated as the early progenitor/root state, while AT1 and AT2 populations were set as terminal fates. Representative root and terminal cells were selected based on maximal within-cluster connectivity in the kNN graph. Palantir was run with these defined early and terminal states (knn = 20, 2500 waypoints), producing pseudotime values, differentiation entropy, and fate probabilities toward AT1 and AT2 endpoints. Cells were assigned to terminal fates using a probability threshold of >0.5, and fate trajectories were visualized through graph embeddings and Palantir branch plots. Final fate labels were further refined to ensure lineage consistency: cells annotated as AT1 or iAT1 were restricted to the AT1 branch, and cells annotated as AT2 or iAT2 were restricted to the AT2 branch. To support robust modelling of gene expression dynamics, each fate trajectory was downsampled to a maximum of 1000 cells

per epithelial subtype. Pseudotime values were unity normalized and inverted so that Aberrant Basaloid cells mapped to early pseudotime, while terminal AT1 and AT2 states corresponded to late pseudotime. Gene expression dynamics along each trajectory were modelled using tradeSeq v1.20.0. For each fate branch, 2000 highly variable genes were selected, and generalized additive models were fit with negative binomial regression (fitGAM) using three knots. Association testing was then performed to identify genes with significant variation across pseudotime, with Benjamini–Hochberg correction applied to control the false discovery rate (adjusted  $p < 0.05$ ).

## 7. Spatial Validation

The procedure has been described previously [7-9]. Slides were prepared as 2  $\mu\text{m}$  thick tissue sections. Rehydration was done through a xylene (Carl Roth, Cat. CN80.1) and ethanol (Otto Fischer, Cat. 27690) series. Tissue decrosslinking was performed at 95°C in ddH<sub>2</sub>O with 1 % Antigen Unmasking Solution (Vector Laboratories, Cat. H3301) for 20 min. Slides were cooled down to RT for 20 min in 1X PBS (Chemsolute, Cat. 8418). Blocking of unspecific binding sites was reduced through incubation with 2.5% v/v normal horse serum (Vector Laboratories, Cat. S-2012) for 20 min. Incubation with primary antibodies was done for 1 h and tissue was rinsed twice with PBS for 2 min afterwards. DyLight™ 488 Anti-mouse-IgG and DyLight™ 594 Anti-rabbit-IgG secondary antibodies, both contained in the VectaFluor™ Duet Immunofluorescence Double Labeling Kit (Vector Laboratories, Cat. DK-8828), were applied to the slides for 1 h with a subsequent 1X PBS washing step as before. For three primary antibody panels, slides were incubated with pre-conjugated antibodies for 1 h, followed by a 1X PBS wash.

**Supplemental Table S3: Antibodies and respective dilutions used for multiple immunohistofluorescent antigen staining:**

| Target                  | Clonality (Clone)   | Dilution     | Manufacturer (Catalog No.)                                                            |
|-------------------------|---------------------|--------------|---------------------------------------------------------------------------------------|
| AGER                    | Monoclonal (A11)    | 1:25         | Santa Cruz Biotechnology Inc. (sc-80652)                                              |
| COX2                    | Monoclonal (COX229) | 1:100        | Invitrogen (35-8200)                                                                  |
| CTSE                    | Polyclonal          | 1:100        | Merck/Sigma-Aldrich (HPA012940)                                                       |
| ITGAVB6                 | Monoclonal EM05201) | 1:50         | Merck/Sigma-Aldrich (ZRB1104)                                                         |
| KRT17                   | Monoclonal (E-4)    | 1:25         | Santa Cruz Biotechnology Inc. (sc-393002 AF647)                                       |
| LAMP3                   | Polyclonal          | 1:200        | Biotechnne (AF4087)                                                                   |
| SFTPC                   | Monoclonal (H-8)    | 1:100        | Santa Cruz Biotechnology Inc. (sc-518029)                                             |
| $\gamma$ H2AX           | Monoclonal (20E3)   | 1:100        | Cell Signaling Technology (9718S)                                                     |
| Mouse-IgG<br>Rabbit-IgG | Polyclonal          | Ready to use | VectaFluor Duet Immunofluorescence Double Labeling Kit: Vector Laboratories (DK-8828) |

The Vector TrueView Autofluorescence Quenching Kit (Vector Laboratories, Cat. SP-8400-15) was applied to each slide according to manufacturer's instructions to reduce background fluorescence, with 1X PBS rinsing afterward. DAPI-containing Antifade Mounting Medium

(Vector Laboratories, Cat. H-1800-10) was used for coverslipping. Slides were stored in the dark at 4°C until imaging.

## **8. RNAscope: RNA in situ hybridization**

The procedure has been described previously [7-9]. Slides were prepared as 2 µm thick tissue sections and incubated for 1 h at 60°C in an oven. Slides were rehydrated through 2x 5 min incubation in xylene and 2x 2 min incubation in 100% ethanol. Slides were dried in an oven for 5 min at 60°C. Afterwards, slides were treated with Hydrogen Peroxide (ACD, Cat. 322000) for 10 min at RT. Slides were rinsed in ddH<sub>2</sub>O by dipping the slides in and out ten times, twice. Slides were immersed in a pre-heated 1x target retrieval buffer (ACD, Cat. 322000) at 95°C for 30 min, twice rinsed in ddH<sub>2</sub>O for 1 min, and transferred to 100% ethanol for 1 min. Slides were removed from ethanol and ethanol was removed by drying for 5 min at 60°C. A hydrophobic barrier around the tissue was created using the intended pen (Vector Laboratory, Cat. H-4000). Tissue was digested using RNAscope Protease Plus (ACD, Cat. 322381) for 30 min at 40°C. Here and in between subsequent incubation periods, the slides were rinsed with 1x RNAscope Wash Buffer (ACD, Cat. 310091) for 2 min, twice. RNAscope probes (Bio-Techne, Cat. 401891, 401891-C2, 476341-C3) were mixed in the ratio 50:1:1 (C1:C2:C3) and pre-heated to 40°C for 10 min. The probe mix was applied to the slides for a 2 h at 40°C. For an optional overnight stopping point at RT, 5x SSC (Life Technologies, Cat. AM9770) was applied to the slides. The next day, incubations with RNAscope Multiplex FL v2 Amp 1, 2 and 3 were done in order at 40°C for 30 min, 30 min and 15 min, respectively. The Opal fluorophores (Akoya Biosciences, Cat. FP1487001KT, FP1488001KT, FP1495001KT, FP1497001KT) were reconstituted according to manufacturer's instructions and diluted 1:1000 in TSA Buffer (ACD, Cat. 322809). The fluorescence signal was developed for each channel independently through the following procedure: Incubation with RNAscope Multiplex FL v2 HRP-C1 (provided in ACD, Cat. 323110) for 15 min at 40°C, incubation with one specific Opal Fluorophore assigned to the channel for 30 min at 40°C, and incubation with RNAscope Multiplex FL v2 HRP-Blocker (provided in ACD, Cat. 323110) for 15 min at 40°C. Autofluorescence was quenched using the TrueBlack Lipofuscin Autofluorescence Quenching Solution (Biotium, Cat. B-23007) diluted 19:1 in 70% Ethanol for 1 min. Slides were stained with DAPI (ACD, part of Cat. 323110) for 15 s. Coverslipping was done with Prolong Gold Antifade Mounting Medium (Thermo Fisher Scientific, Cat. P36930). Slides were stored at 4°C in the dark until imaging.

## **9. Immunofluorescence Microscopy**

Slides were imaged using the Zeiss Axio Scan 7 equipped with a 20x Plan-Apochromat 20x/0.8 27M objective. Emission was filtered through 96 HE BFP 450/40, 38 eGFP 525/50, 43 HE DsRed 605/70, 26 Alexa Fluor 660 685/50, and 50 Cy 5 690/50 broad pass filters.

### **Mitochondrial Respiration Analysis Using the Agilent Seahorse HS mini Analyzer**

Mitochondrial respiration was assessed in fibroblasts of patient P1, P3 and P4 using the Agilent Seahorse HS mini Analyzer according to the manufacturer's instructions. Cells (20,000/well) were seeded in standard culture medium (see section cell culture) in Seahorse XFp microplates and incubated overnight. Sensor cartridges were hydrated overnight in sterile distilled water at 37 °C without CO<sub>2</sub>. On the day of the assay, cells were washed and equilibrated for 1 hour in Seahorse XF assay medium (XF DMEM with 10 mM glucose, 1 mM pyruvate, and 2 mM glutamine, pH 7.4, Agilent Technologies, US) at 37°C in a non-CO<sub>2</sub> incubator. Sensor cartridges were transferred into calibrant solution and also incubated for 1 hour prior to the assay at 37°C without CO<sub>2</sub>. The Seahorse XFp Cell Mito Stress Test Kit (Agilent Technologies, US) was performed according to manufacturer's protocol by sequential injection of oligomycin (1.5 µM), FCCP (2 µM), and rotenone/antimycin A (0.5 µM each). Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured in three cycles at baseline and after each subsequent injection. Data were analyzed using Seahorse Analytics software to calculate mitochondrial parameters like basal respiration, maximal respiration, proton leak, spare capacity, ATP production and non-mitochondrial respiration and normalized to cell number. Experiments were conducted in at least three independent replicates.

### **Lung biopsy and histology**

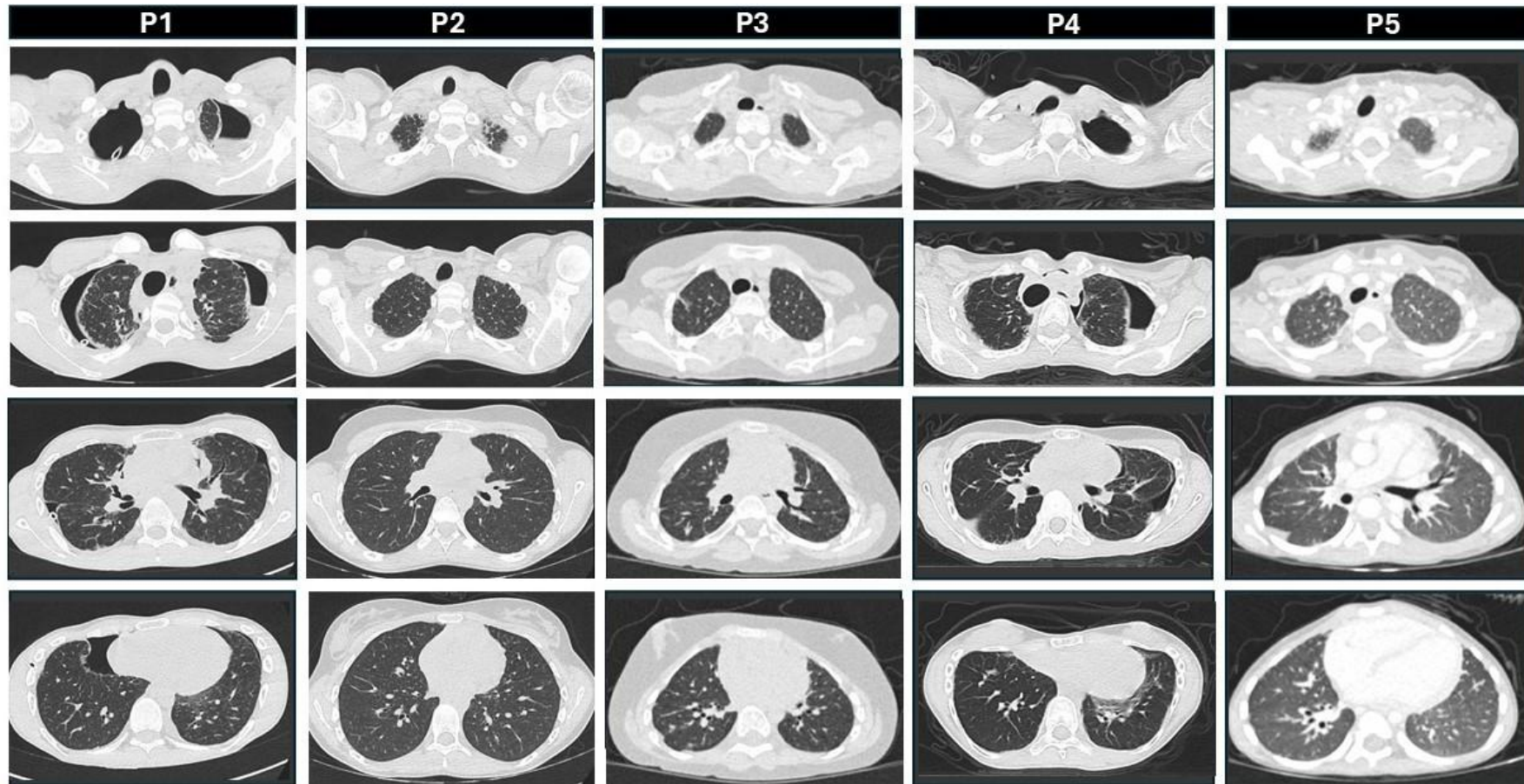
Lung tissue biopsies have been sampled by video-assisted thoracoscopic surgery from patients P1, P2, P3 and P4, and by transbronchial cryobiopsy from patients P3 and P4. In addition, tissue obtained from the explanted lung of patient P1 was used. Samples were formalin fixed, paraffin embedded, and stained with hematoxylin-eosin, periodic acid–Schiff, and Masson's trichrome staining. In addition, samples were taken for transmission electron microscopy from severely affected areas (periphery of lung explant) and areas that appeared from a macroscopically point of view unaffected (more central region of the lung). Fixation and processing of these samples was performed in analogue to the cell pellets and is outlined in the next section.

## **Electron microscopy**

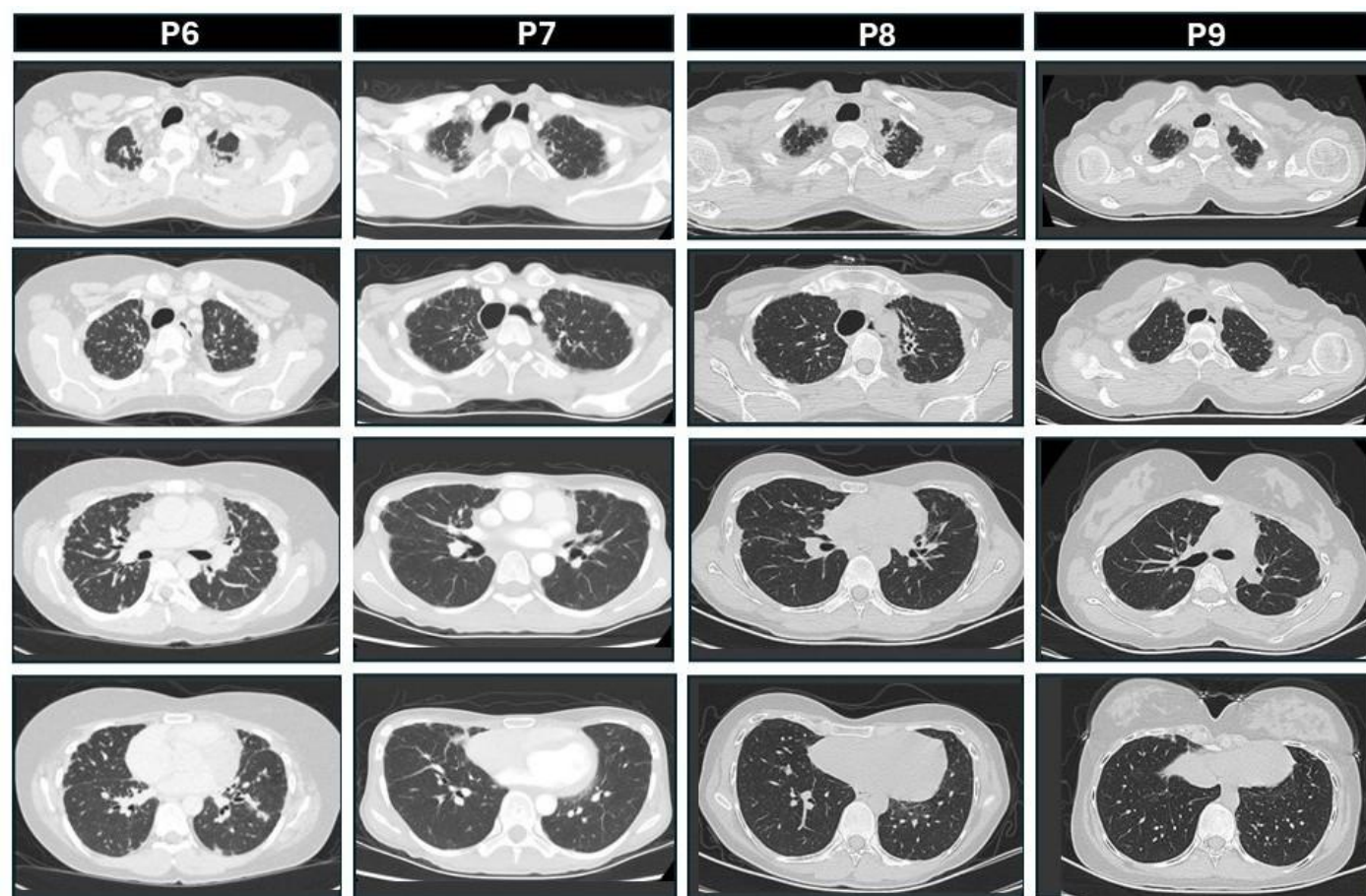
Fibroblasts from patients P1, P3, and P4, as well as from two healthy relatives (the mother of P1 and the father of P4), were expanded in T75 flasks and harvested by trypsinization at 90% confluence. The cell pellets were fixed by a solution of 1.5% glutaraldehyde, 1.5% paraformaldehyde in 0.15 M HEPES buffer, treated with half-saturated uranylacetate solution, post-fixed with 4% OsO<sub>4</sub> and dehydrated using a series of ascending acetone-concentrations from 70% to 100%. Finally, cell pellets were embedded in Epoxy resin. Ultrathin sections of 60 nm thickness were cut using an ultramicrotome (Leica, RM2265, Nussloch, Germany) and finally investigated by transmission electron microscopy (Morgagni, FEI, Eindhoven, The Netherlands) equipped with a digital camera (Olympus Soft Imaging Solution, Münster, Germany).

## II. Supplementary Figures

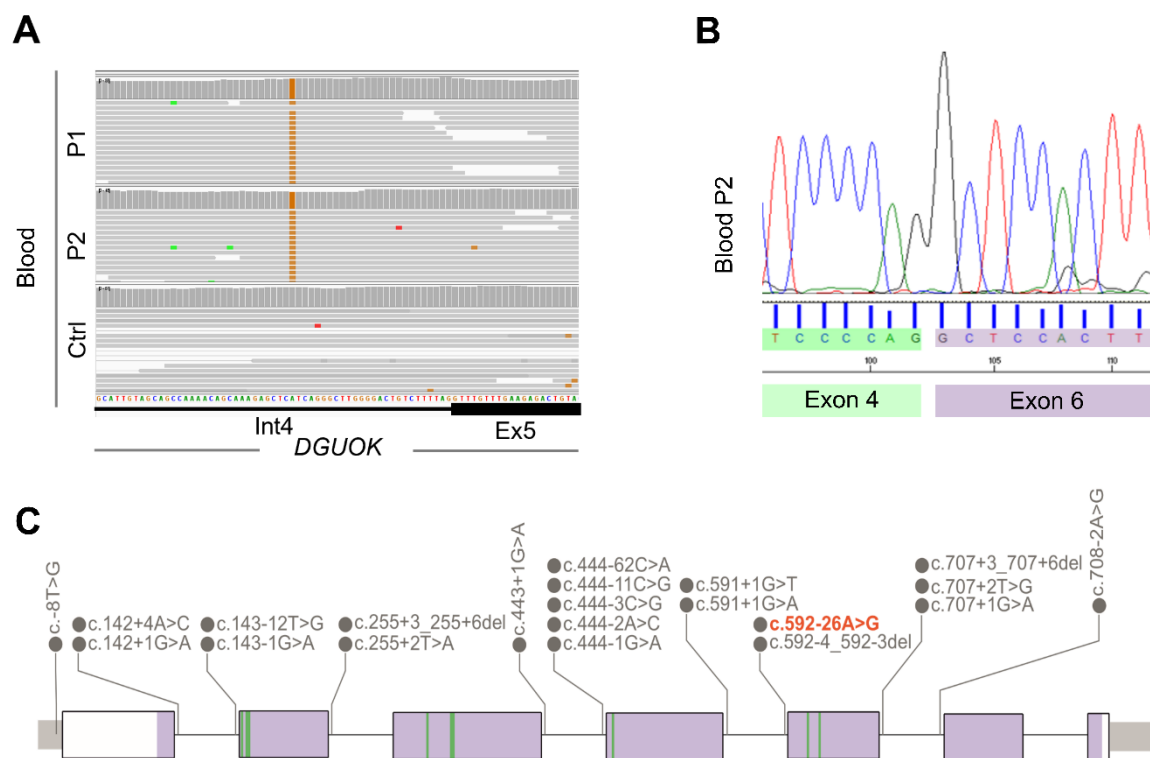
**Supplementary Figure S1. Axial chest CT images of all nine patients with DGUOK-associated PPFE at the apical, suprahilar, hilar, and infrahilar levels**



Supplementary Figure S1 (continued)



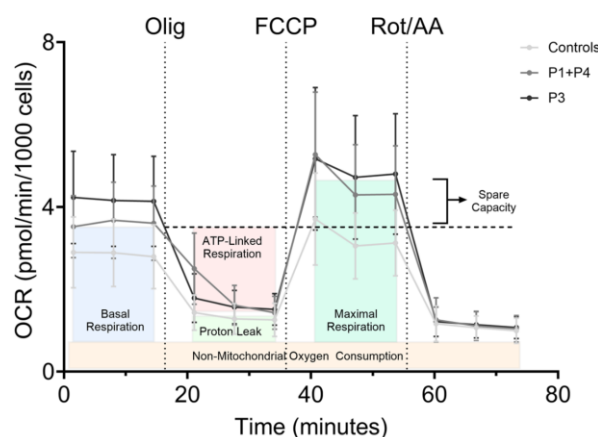
## Supplementary Figure S2. Genetic characterization of the *DGUOK* variant



Panel A shows an Integrative Genomics Viewer (IGV) screenshot from WGS analysis of peripheral blood from P1 and P2 highlighting an adenine-to-guanine substitution present in all reads (homozygous occurrence), located 26 base pairs upstream of the intron–exon boundary between intron 4 and exon 5. The control sample (Ctrl) shows no deviation from the reference sequence at this position. In panel B, Sanger sequencing of blood-derived cDNA from P2 demonstrates aberrant splicing caused by the *DGUOK* c.592-26A>G variant, resulting in complete exon 5 skipping and direct joining of exon 4 to exon 6. Panel C represents the *DGUOK* gene structure with previously reported intronic and splice-region variants above the gene model. Exons are shown as purple boxes and introns as connecting lines. The intronic variant c.592-26A>G, identified in this study, is highlighted in red. Green bars indicate substrate-binding sites.

## Supplementary Figure S3. Analysis of Mitochondrial Respiration and Enzyme Activity

**A**

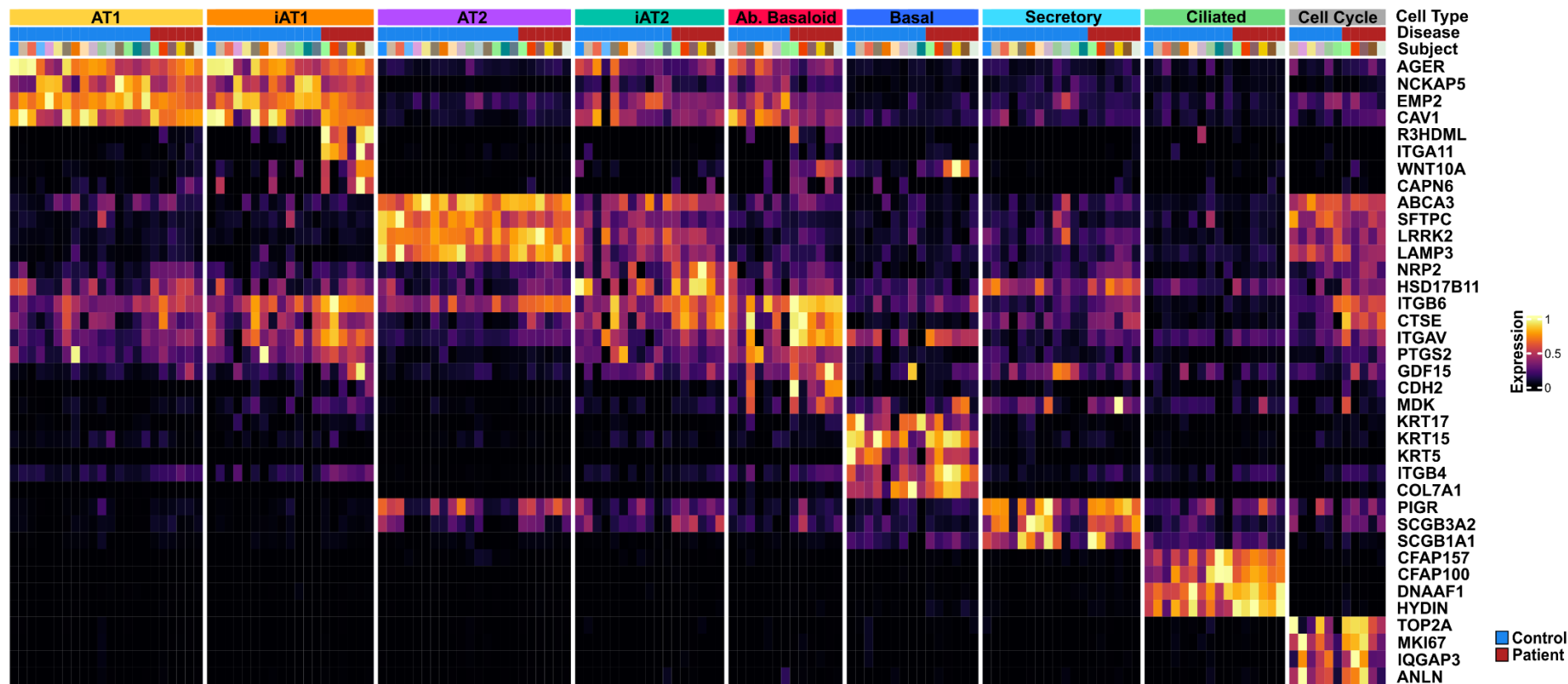


**B**

|                  | FB - P1 | FB - P3 | FB - P4 | FB – Ref. range | Muscle - P3 | Muscle - Ref. range |
|------------------|---------|---------|---------|-----------------|-------------|---------------------|
| Komplex I+III    | 92      | 79      | 56      | 61-157          | 26          | 18-88               |
| Komplex II+III   | 7       | 4       | 2       | 4-13            | 3           | 5-83                |
| Komplex IV       | 64      | 40      | 41      | 27-92           | 0           | 13-94               |
| Komplex V        | 170     | 113     | 133     | 71-241          | 315         | 200-860             |
| Citrate synthase | 77      | 82      | 69      | 50-155          | 79          | 61-114              |

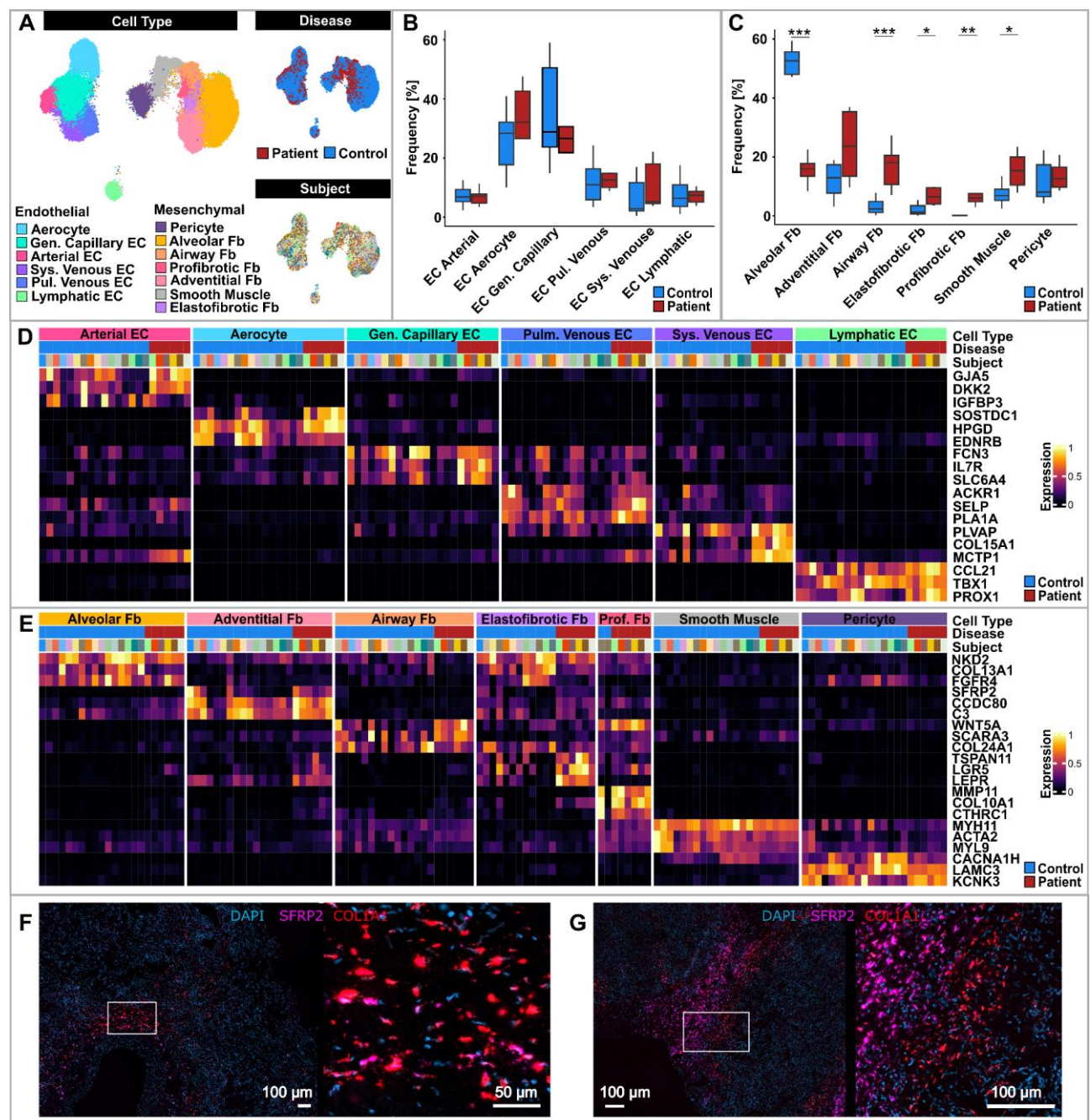
Panel A: Oxygen consumption rate (OCR; pmol O<sub>2</sub>/min/1000 cells) in primary fibroblasts from controls (light grey), P1 combined with P4 (grey), and severely affected P3 (black) was determined by Cell Mito Stress test on a Seahorse XF HS mini extracellular flux analyzer. Three measurement cycles were taken: one for basal respiration (without any injection), as well as after each injection of Olig (Oligomycin), FCCP (Carbonylcyanide-4-(trifluoromethoxy)phenylhydrazone) and Rot/AA (Rotenone / Antimycin A). After each assay cell number was determined and data was normalized to OCR/1000 cells. All data are presented as mean and SD from at least three independent experiments in triplicates (number of pooled wells: controls n=12, P1+P4 n=8+3, P3 n=9). Panel B: Respiratory chain complex activities in cultured skin fibroblasts (FB) from patients 1, 3 and 4 and respiratory chain complex activities in a muscle specimen from patient 3. Citrate synthase as a mitochondrial marker was in the reference range. Activities are in nmol/min per mg protein.

Supplementary Figure S4. Transcriptional changes in epithelial cells



This heat map presents the normalized gene-expression values annotated by epithelial cell type and disease status.

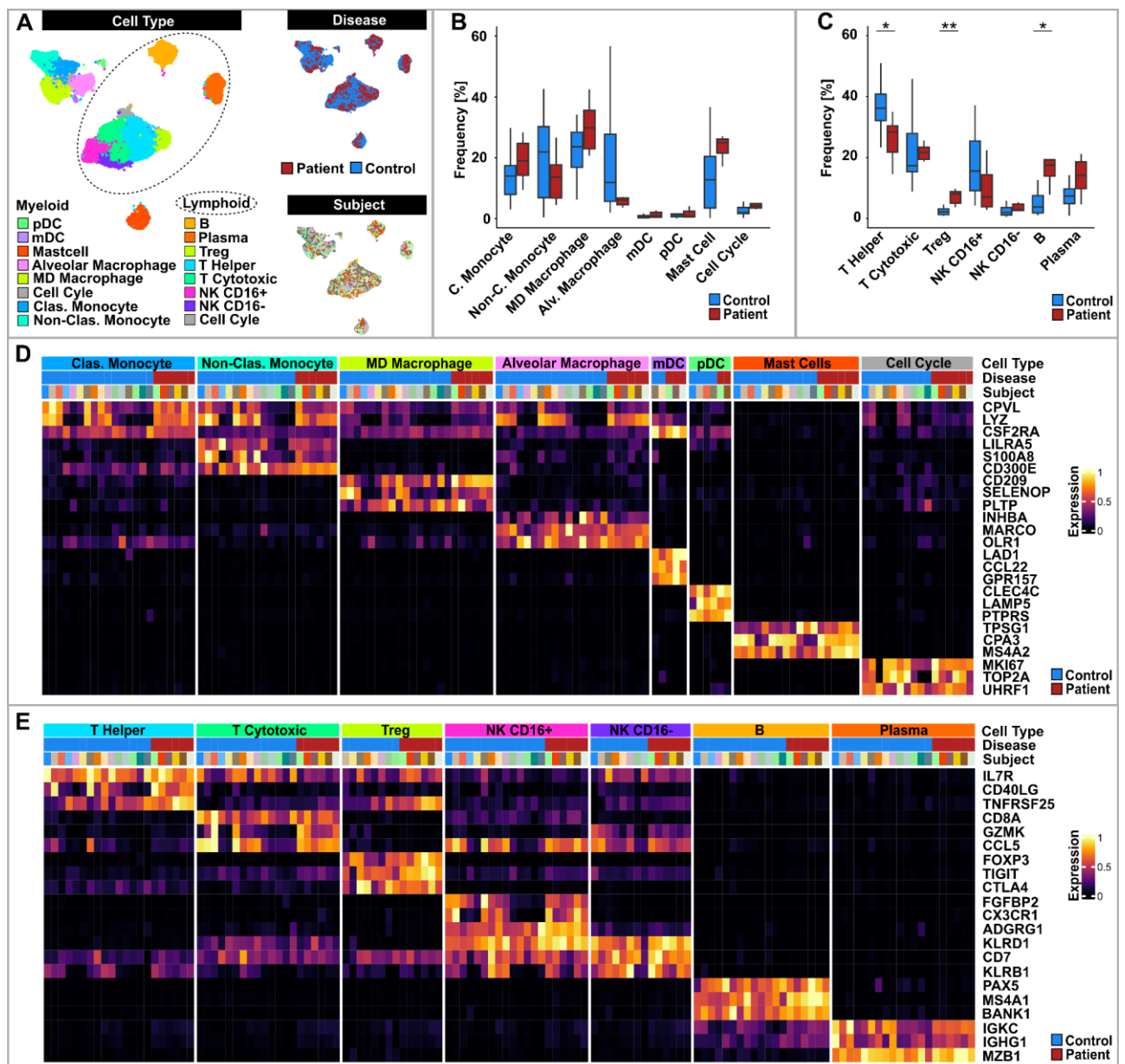
# **Supplementary Figure S5. Expansion of Profibrotic and Elastofibrotic Fibroblasts with associated transcriptional changes**



Panel A shows three UMAPs of endothelial and mesenchymal cells, annotated by cell type, disease, and subject. Panel B depicts the relative frequencies of endothelial cell types between PPFE in DGUOK-associated mitochondriopathy and controls, showing no significant differences. Box plots show the median, interquartile range, and full range of values. Asterisks indicate statistical significance (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Panel C illustrates the significant relative increase in Airway Fibroblasts (Fb), Elastofibrotic Fb, Profibrotic Fb, and Smooth Muscle cells, accompanied by a decrease in Alveolar Fb. Box plots show the median,

interquartile range, and full range of values Asterisks indicate statistical significance (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Panels D and E show heat maps of normalized gene-expression values, annotated by cell type and disease status. Panels F and G depict COL1A1-positive fibroblasts adjacent to Aberrant Basaloid cells, with SFRP2-positive fibroblasts, some of which co-express COL1A1.

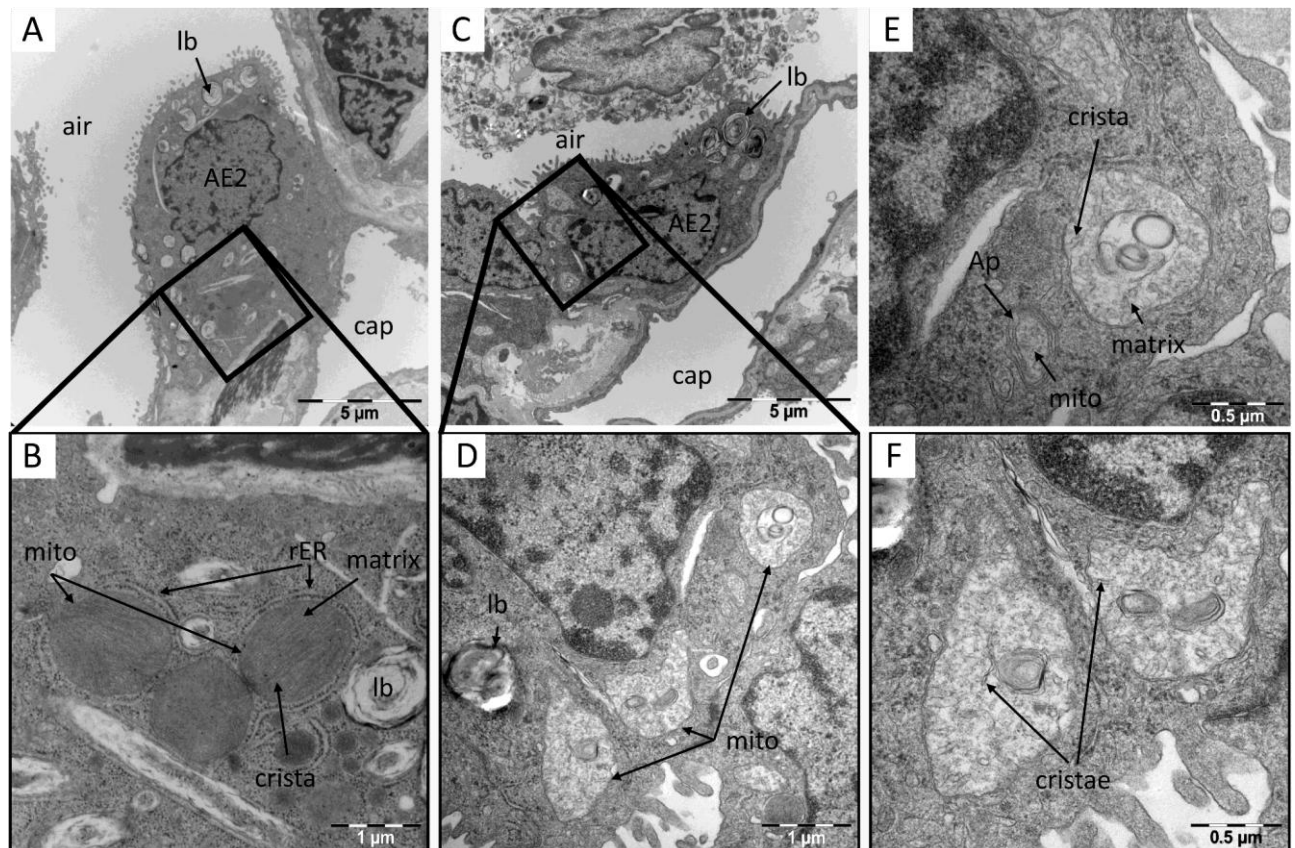
## Supplementary Figure S6. Change in lung resident immune frequencies and gene expression



Panel A shows three UMAPs of myeloid and lymphoid cells, annotated by cell type, disease, and subject. Panel B depicts the relative frequencies of myeloid cell types between PPFE in DGUOK-associated mitochondriopathy and controls, showing no significant differences. Box plots show the median, interquartile range, and full range of values Asterisks indicate statistical

significance (\* $P < 0.05$ ; \*\* $P < 0.01$ ). Panel C illustrates a significant relative increase in Tregs, and B cells with decrease in T Helper cells. Box plots show the median, interquartile range, and full range of values. Asterisks indicate statistical significance (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Panels D and E show heat maps of normalized gene-expression values, annotated by cell type and disease status in myeloid and lymphoid cell lineages, respectively.

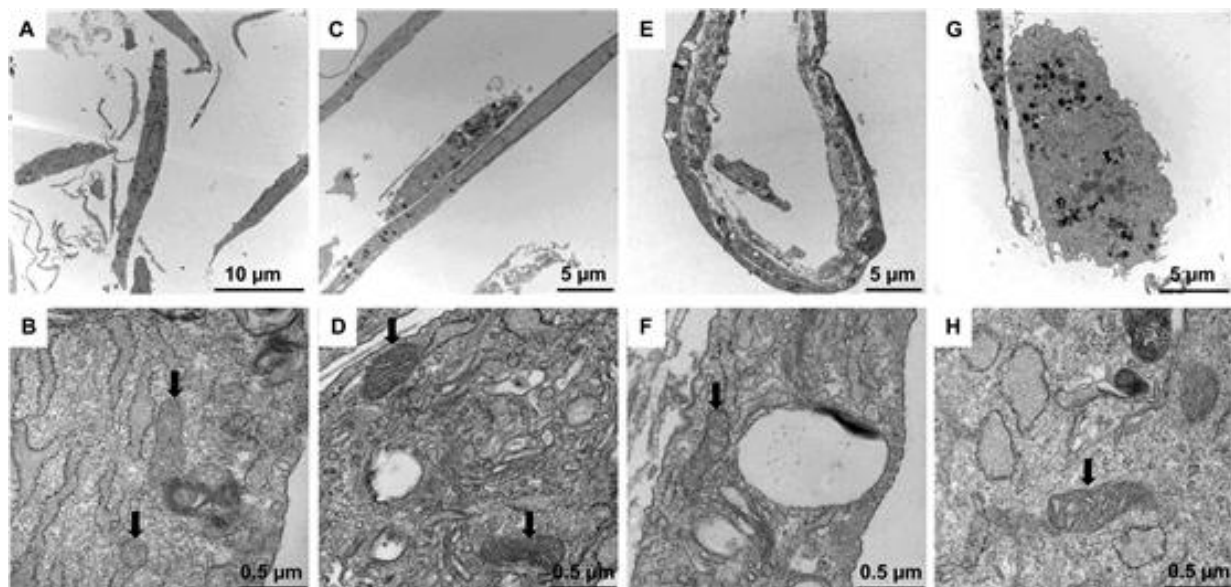
### Supplementary Figure S7. Degenerated mitochondria in AT2 cells



Panel A demonstrates an overview of an AT2 cell in a region with maintained acinar microarchitecture and absence of fibrosis of a lung explant diagnosed as Idiopathic Pulmonary Fibrosis. In Panel B details are illustrated showing enlarged mitochondria with maintained mitochondrial ultrastructure as characterized by two layers of biomembranes forming the inner and outer mitochondrial membrane, slim and densely packed cristae and in the space between the cristae electron dense mitochondrial matrix. Near to the mitochondria rough endoplasmic reticulum (rER) and a lamellar body (lb) can be found. Panel C illustrates a representative AT2 cell in a region of the lung of maintained acinar microarchitecture and absence of fibrosis in a lung explant suffering from DGUOK-deficiency. Panels D-F show details of the ultrastructure of the AT2 cell with a focus on the mitochondria (mito). In general, these are enlarged with diameters of 1  $\mu\text{m}$  and more. While the enveloping double layer of biomembranes is maintained, there is a rarefaction of normal appearing slim cristae which can be identified as remnants at best. Other cristae appear to be dilatated, fragmented and thus subject of

cristolysis. Moreover, loss of the mitochondrial matrix can be seen in many mitochondria. Although the process of mitophagy is a very rare event in AT2 cells an autophagosome (Ap) containing a fragment of an mitochondrium (mito) is demonstrated in Panel E.

**Supplementary Figure S8. Ultrastructure of cultured fibroblasts do not show differences between *DGUOK* deficiency and healthy controls**



The upper row demonstrates overview images of the ultrastructure of cultured fibroblast and the lower row details at higher magnification with a focus on mitochondria (arrows). Panels A-F show fibroblasts of patients suffering from *DGUOK*-deficiency while Panel G and H are from a healthy control. Immediately after removal from the incubator, cultures of fibroblast were fixed. The culture medium was replaced by a solution of 1.5% glutaraldehyde, 1.5% paraformaldehyde in 0.15 M HEPES buffer. In general, the sizes of the mitochondria were in all patients in a normal range. No signs of mitochondrial degeneration were observed. The cristae were slim and regularly arranged. The mitochondrial matrix was electron dense in all cases.

## Supplementary

### III. Supplementary Tables

#### Supplementary Table S3. Clinical characteristics

|                                    | Patient 1                                    | Patient 2                                    | Patient 3                | Patient 4                                    | Patient 5                     | Patient 6                   | Patient 7                   | Patient 8                                | Patient 9                   |
|------------------------------------|----------------------------------------------|----------------------------------------------|--------------------------|----------------------------------------------|-------------------------------|-----------------------------|-----------------------------|------------------------------------------|-----------------------------|
| Sex                                | Male                                         | Female                                       | Male                     | Female                                       | Female                        | Male                        | Male                        | Male                                     | Female                      |
| Ethnicity                          | Arab                                         | Arab                                         | Arab                     | Caucasian                                    | NA                            | Turkish                     | Turkish                     | Turkish                                  | Turkish                     |
| Country of family origin           | Syria                                        | Syria                                        | Syria                    | Turkey                                       | NA                            | Turkey                      | Turkey                      | Turkey                                   | Turkey                      |
| Parental consanguinity             | yes                                          | Yes                                          | Yes                      | No                                           | Yes                           | Yes                         | Yes                         | Yes                                      | Yes                         |
| Primary manifestation              | Insufficient weight gain, exertional dyspnea | Insufficient weight gain, exertional dyspnea | Liver failure            | Insufficient weight gain, exertional dyspnea | Liver failure                 | Exertional dyspnea          | Exertional dyspnea          | Exertional dyspnea and muscle complaints | Exertional dyspnea          |
| Age at onset                       | 15 years                                     | 15 years                                     | 2 years                  | 13 years                                     | 3 months                      | 28 years                    | 23 years                    | 20 years                                 | 19 years                    |
| Age at first evaluation            | 17 years                                     | 15 years                                     | 2 years                  | 14 years                                     | 3 months                      | 36 years                    | 33 years                    | 27 years                                 | 22 years                    |
| DGUOK variants (ENST00000264093.9) | c.592-26A>G/<br>c.592-26A>G                  | c.592-26A>G/<br>c.592-26A>G                  | c.592-26A>G/<br>c.313C>T | c.592-26A>G/<br>c.592-26A>G                  | c.763_766dup/<br>c.763_766dup | c.592-26A>G/<br>c.592-26A>G | c.592-26A>G/<br>c.592-26A>G | c.592-26A>G/<br>c.592-26A>G              | c.592-26A>G/<br>c.592-26A>G |
| <b>Liver involvement</b>           |                                              |                                              |                          |                                              |                               |                             |                             |                                          |                             |
| Cholestasis                        | No                                           | No                                           | Yes                      | No                                           | Yes                           | No                          | No                          | No                                       | No                          |
| Jaundice                           | No                                           | No                                           | Yes                      | No                                           | Yes                           | No                          | No                          | No                                       | No                          |
| Coagulopathy                       | No                                           | No                                           | Yes                      | No                                           | Yes                           | No                          | No                          | No                                       | No                          |
| Hepatomegaly                       | No                                           | yes                                          | No                       | No                                           | Yes                           | No                          | No                          | No                                       | No                          |
| Ascites                            | No                                           | No                                           | Yes                      | No                                           | Yes                           | No                          | No                          | No                                       | No                          |
| Hematemesis                        | No                                           | No                                           | No                       | No                                           | No                            | No                          | No                          | No                                       | No                          |
| Portal hypertension                | No                                           | No                                           | Yes                      | No                                           | Yes                           | No                          | No                          | No                                       | No                          |
| Hepatocellular carcinoma           | No                                           | No                                           | No                       | No                                           | No                            | No                          | No                          | No                                       | No                          |
| Liver transplantation              | No                                           | No                                           | Yes                      | No                                           | Yes                           | No                          | No                          | No                                       | No                          |
| <b>Renal involvement</b>           |                                              |                                              |                          |                                              |                               |                             |                             |                                          |                             |
| Proteinuria                        | Yes                                          | Yes                                          | Yes                      | No                                           | NA                            | Yes                         | No                          | No                                       | No                          |
| Renal insufficiency                | No                                           | No                                           | No                       | No                                           | No                            | Yes                         | No                          | No                                       | No                          |
| <b>Skeletal muscle involvement</b> |                                              |                                              |                          |                                              |                               |                             |                             |                                          |                             |
| Muscle weakness                    | Yes                                          | No                                           | Yes                      | Yes                                          | NA                            | Yes                         | Yes                         | Yes                                      | No                          |
| Truncal weakness                   | Yes                                          | No                                           | Yes                      | Yes                                          | NA                            | No                          | No                          | No                                       | No                          |
| Muscle pain                        | No                                           | No                                           | No                       | No                                           | NA                            | Yes                         | Yes                         | Yes                                      | No                          |

|                                       |              |           |                              |              |            |            |      |         |      |
|---------------------------------------|--------------|-----------|------------------------------|--------------|------------|------------|------|---------|------|
| Muscle atrophy                        | No           | No        | No                           | No           | NA         | Both hands | Yes  | Yes     | No   |
| <b>Respiratory involvement</b>        |              |           |                              |              |            |            |      |         |      |
| Respiratory distress / dyspnea        | Yes          | Yes       | Yes                          | Yes          | Yes        | Yes        | Yes  | Yes     | No   |
| History of pneumothorax               | Yes, chronic | Yes       | Yes, persistent after biopsy | Yes, chronic | No         | No         | Yes  | Yes     | No   |
| Chronic cough                         | Yes          | No        | Yes                          | Yes          | No         | Yes        | Yes  | No      | No   |
| Initial FVC in L (% of predicted)     | 0.9 (19)     | 1.16 (39) | 0.34 (12)                    | 0.55L (16)   | NA         | 1.18 (25)  | NA   | NA      | NA   |
| FEV1 in L (% of predicted)            | 0.77 (19)    | 1.16 (43) | NA                           | NA           | NA         | 1.10 (28)  | NA   | NA      | NA   |
| DLCO in % of predicted                | NA           | 53%       | NA                           | NA           | NA         | 34         | NA   | NA      | NA   |
| <b>Cardiovascular involvement</b>     |              |           |                              |              |            |            |      |         |      |
| Cardiomyopathy                        | No           | No        | No                           | No           | No         | No         | No   | No      | No   |
| Pulmonary hypertension                | No           | No        | No                           | No           | Yes        | No         | No   | No      | No   |
| Arterial Hypertension                 | No           | No        | No                           | No           | No         | Yes        | No   | No      | No   |
| <b>Gastrointestinal involvement</b>   |              |           |                              |              |            |            |      |         |      |
| Vomiting                              | No           | No        | Yes                          | No           | Yes        | No         | No   | No      | No   |
| <b>Weight or growth abnormalities</b> |              |           |                              |              |            |            |      |         |      |
| Height [cm]                           | 170          | 150       | 87                           | 158.5        | 66         | 173        | 170  | 178     | 165  |
| Weight [kg]                           | 40           | 37        | 10                           | 30.8         | 5          | 61         | 40   | 55      | 42   |
| Age at height & weight measurement    | 17           | 15        | 2.75                         | 15           | 0.8        | 36         | 33   | 27      | 22   |
| BMI                                   | 13.8         | 16.4      | 13.2                         | 12.3         | 11.4       | 20.4       | 13.8 | 17.4    | 15.4 |
| <b>Ocular involvement</b>             |              |           |                              |              |            |            |      |         |      |
| Ophthalmoplegia                       | No           | No        | No                           | No           | No         | No         | No   | No      | No   |
| Ptosis                                | No           | No        | No                           | No           | No         | No         | No   | No      | No   |
| Visual impairment                     | No           | No        | Yes, RE                      | No           | No         | No         | No   | Yes, RE | No   |
| <b>Neurological involvement</b>       |              |           |                              |              |            |            |      |         |      |
| Nystagmus                             | No           | No        | No                           | No           | No         | No         | No   | No      | No   |
| Global developmental delay            | No           | No        | Yes                          | No           | Yes        | No         | No   | No      | No   |
| Seizures/abnormal EEG                 | No           | No        | No                           | No           | No         | No         | No   | No      | No   |
| Hyporeflexia                          | NA           | Yes       | Yes                          | NA           | NA         | Yes        | NA   | NA      | NA   |
| Encephalopathy                        | No           | No        | No                           | No           | Yes, in LF | No         | No   | No      | No   |
| Peripheral neuropathy                 | Yes          | Yes       | Yes                          | Yes          | No         | Yes        | No   | No      | No   |
| Strabismus                            | No           | No        | No                           | No           | NA         | No         | No   | No      | No   |
| Dysphagia                             | No           | No        | Yes                          | No           | NA         | No         | Yes  | No      | No   |

|                                      |             |                          |              |          |                                          |       |       |                                        |       |
|--------------------------------------|-------------|--------------------------|--------------|----------|------------------------------------------|-------|-------|----------------------------------------|-------|
| <b>Other symptoms</b>                |             |                          |              |          |                                          |       |       |                                        |       |
| Suprasternal notch                   | yes         | yes                      | yes          | Yes      | NA                                       | Yes   | Yes   | Yes                                    | Yes   |
| Fatigue                              | yes         | Yes                      | Yes          | Yes      | NA                                       | Yes   | Yes   | No                                     | No    |
| Other                                |             | Mild hypo-<br>thyroidism | Osteoporosis |          | PHA, therapy-<br>resistant<br>dermatitis |       | CVI   | Frequent<br>headaches,<br>Gynecomastia |       |
| <b>Laboratory findings</b>           |             |                          |              |          |                                          |       |       |                                        |       |
| Elevated Lactate                     | Yes         | No                       | Yes, in LF   | Yes      | Yes                                      | No    | NA    | NA                                     | NA    |
| Low blood glucose                    | No          | No                       | Yes          | No       | Yes                                      | No    | No    | No                                     | No    |
| Elevated ALT                         | No          | No                       | No           | Yes      | Yes                                      | No    | Yes   | Yes                                    | No    |
| Elevated AST                         | No          | Yes                      | Yes          | Yes      | Yes                                      | No    | Yes   | Yes                                    | No    |
| Elevated AFP                         | NA          | NA                       | Yes          | NA       | NA                                       | NA    | NA    | NA                                     | NA    |
| Elevated GGT                         | Yes         | Yes                      | Yes          | Yes      | Yes                                      | No    | No    | No                                     | NA    |
| Elevated tyrosine                    | NA          | No                       | No           | NA       | Yes                                      | NA    | NA    | NA                                     | NA    |
| Elevated ammonia                     | NA          | NA                       | Yes          | NA       | Yes                                      | NA    | NA    | NA                                     | NA    |
| Elevated ferritin                    | No          | No                       | No           | No       | Yes                                      | No    | NA    | NA                                     | No    |
| Low albumin                          | No          | No                       | Yes          | No       | Yes                                      | No    | No    | No                                     | NA    |
| Elevated alanine                     | NA          | No                       | No           | NA       | Yes                                      | NA    | NA    | NA                                     | NA    |
| Elevated CK                          | No          | Yes                      | No           | Yes      | Yes                                      | No    | Yes   | Yes                                    | Yes   |
| Elevated AP                          | NA          | No                       | No           | No       | Yes                                      | No    | No    | No                                     | NA    |
| Elevated methionine                  | NA          | No                       | No           | No       | Yes                                      | NA    | NA    | NA                                     | NA    |
| Elevated NT-proBNP                   | No          | No                       | No           | No       | Yes                                      | No    | NA    | NA                                     | NA    |
| <b>Status</b>                        |             |                          |              |          |                                          |       |       |                                        |       |
| Current status                       | Alive, LuTx | Alive                    | Deceased     | Deceased | Deceased                                 | Alive | Alive | Alive                                  | Alive |
| If deceased, age at<br>death [years] | NA          | NA                       | 9            | 16       | 5                                        | NA    | NA    | NA                                     | NA    |

RE: Refractive Error; LF: Liver failure; PHA: Pseudohypoaldosteronism; CVI: Chronic Venous Insufficiency

## REFERENCES

1. Lücke T, Höppner W, Schmidt E, Illsinger S, Das AM. Fabry disease: reduced activities of respiratory chain enzymes with decreased levels of energy-rich phosphates in fibroblasts. *Mol Genet Metab*. 2004;82(1):93-7.
2. Das AM, Byrd DJ. Regulation of the mitochondrial ATP-synthase in skeletal muscle from children--a new diagnostic tool. *J Inherit Metab Dis*. 1996;19(2):137-9.
3. Yu H, Shi C, He W, Li F, Ouyang B. PanDepth, an ultrafast and efficient genomic tool for coverage calculation. *Brief Bioinform*. 2024;25(3).
4. Ding J, Sidore C, Butler TJ, Wing MK, Qian Y, Meirelles O, et al. Assessing Mitochondrial DNA Variation and Copy Number in Lymphocytes of ~2,000 Sardinians Using Tailored Sequencing Analysis Tools. *PLoS Genet*. 2015;11(7):e1005306.
5. Dombi E, Marinaki T, Spingardi P, Millar V, Hadjichristou N, Carver J, et al. Nucleoside supplements as treatments for mitochondrial DNA depletion syndrome. *Front Cell Dev Biol*. 2024;12:1260496.
6. Adams TS, Schupp JC, Balayev A, Khoury J, Justet A, Nikola F, et al. Alveolar epithelial cell plasticity and injury memory in human pulmonary fibrosis. *bioRxiv*. 2025.
7. Christian L, Yilmaz H, Ruwisch J, Giercke L, Seeliger B, Kamp JC, et al. Spatial Transcriptomics Uncovers Hybrid, Pro-Inflammatory and Pro-Fibrotic Cellular Niches in Pulmonary Granuloma of Patients with Chronic Sarcoidosis. *American Journal of Respiratory and Critical Care Medicine*. 2026.
8. Ruwisch J, Cazes A, Leiber LM, Borie R, Neubert L, Christian L, et al. The pleuroparenchymal fibroelastosis atlas reveals aberrant cell states and their zonation as an alternate roadmap to lung fibrosis. *Science Advances*. 2026;12(19):eaeb5967.
9. Leiber LM, Christian L, Neubert L, Ruwisch J, Yilmaz H, Plucinski EKJ, et al. Aberrant and Ectopic Cell Populations in Restrictive Allograft Syndrome after Lung Transplantation. *European Respiratory Journal*. 2026:2500537.