Supplementary Data

Supplementary Methods

Cell Lines and Culture

Human Primary Endothelial Cells

Human umbilical vein endothelial cells (HUVECs, UZ Leuven, Leuven, Belgium) were freshly isolated from umbilical cords obtained from different donors under the ethical approval protocol S57123 (Ethics Committee Research UZ/KU Leuven) and with informed consent from all subjects, as previously described [1]. Unless specified, ECs were routinely cultured in endothelial cell basal medium (EGM2; containing 2% fetal bovine serum (FBS); PromoCell, Heidelberg, Germany) supplemented with endothelial cell growth medium supplement pack (ECGM-2; PromoCell, Heidelberg, Germany) and 100 IU/mL penicillin and 100 mg/mL streptomycin (Thermo Fisher Scientific, Geel, Belgium). Alternatively, M199 medium (1 mg/mL D-glucose, Thermo Fisher Scientific, Geel, Belgium) supplemented with 10% FBS (Thermo Fisher Scientific, Geel, Belgium), 2 mM L-glutamine (Thermo Fisher Scientific, Geel, Belgium), Endothelial Cell Growth Supplement (ECGS)/ Heparin (PromoCell, Heidelberg, Germany), 100 IU/mL penicillin and 100 mg/mL streptomycin was used. In all experiments, HUVECs were used as single-donor cultures and were used between passage 1 and 4. All experiments were performed with ECs from different donors. Unless otherwise indicated, experiments were done on sub-confluent ECs cultures and CFTRinh-(172) (Merck, Darmstadt, Germany) was used at 10 μM and added 24 h before cell lysis.

Blood Outgrowth Endothelial Cells

Blood outgrowth endothelial cells (BOECs) were freshly isolated from peripheral blood obtained from different healthy donors and CF patients (clinical information is shown in supplemental Table 4) under the ethical approval protocol S57123 (Ethics Committee Research UZ/KU Leuven) and with informed consent from all subjects. Our protocol is based on a published method with minor adaptations [2]. Briefly, six EDTA-

coated tubes (Vacutest Kima, Arzergrande, Italy) or ~54 mL of blood were collected by venipuncture per individual. Under sterile conditions, equal parts of the blood were transferred into three 50 mL conical tubes and diluted with Dulbecco's Phosphate-Buffered Saline (DPBS) (Thermo Fisher Scientific, Geel, Belgium) to a final volume of 50 mL per tube. Six new 50 mL conical tubes containing 12.5 mL of Ficoll-Paque™ PLUS density gradient centrifugation medium (GE Healthcare, Chicago, USA) were prepared (1 tube for every 25 mL of diluted blood, 6 tubes per donor), tilted to an angle of 20° with the work surface, and 25 mL of diluted blood was slowly added on top of the medium. Afterwards, the samples were centrifuged at 1000 g for 20 min in a cooled centrifuge (4°C) with the brake off. The buffy coat layer was collected using a pipette and transferred into two new 50 mL conical tubes and washed with DPBS in a final volume of 50 mL. The tubes were centrifuged at 540 g for 7 min at 4°C with the brake on. The supernatant was discarded and the cellular pellet was resuspended in 10 mL of EGM2 supplemented with ECGM-2 containing 20% heat-inactivated embryonic stem-cell FBS (Thermo Fisher Scientific, Geel, Belgium) hereafter called BOEC medium. Cells were centrifuged for 5 min at 300g at room temperature and the cell pellet resuspended in BOEC medium. A 6-well plate was coated with a 50 µg/mL type I collagen (Collagen Type I, rat tail; Merck, Darmstadt, Germany) solution in 0.02 M acetic acid in PBS for 30 minutes, and washed twice with DPBS before adding 2 mL of the cell suspension per well. The culture plate was put in the incubator at 37°C in a 95% air/ 5% CO_2 atmosphere saturated with H_2O. The next day the BOEC medium was refreshed and hereafter every 2-3 days until colonies started to form. The checking for colonies with a cobblestone-like morphology started on day 7 onward and was performed on a daily basis. The colonies appeared between days 8 and 25 and were further grown in BOEC medium until the colony reached a 50% confluency. The cells were then trypsinized with 0.25% trypsin-EDTA (Thermo Fisher Scientific, Geel, Belgium) and reseeded in a 0.1% gelatin-coated 25 cm^2 flask with full EGM2. The cells were grown until they reached 70-90% confluency and were then transferred to a 75 cm^2 flask. Hereafter, BOECs were cultured in EGM2 + 15% FBS. In all experiments,

BOECs were used as single-donor cultures and were used between passage 4 and 10. All experiments were performed with ECs from different donors. Unless otherwise indicated, experiments were done on sub-confluent ECs cultures. CFTR modulators VX-770 (3 μM) and VX-809 (3 μM) (Selleckchem, Houston, USA) were used as previously reported [3–5].

CFTR Mice

Animal housing and all experimental procedures were approved by the Institutional Animal Ethics Committee of the KU Leuven (Leuven, Belgium) under the project number P036/2019. Cftr^{tm1Unc}-Tg(FABPCFTR)1Jaw/J mice were used which re-express human CFTR in the gut under control of the FABP1 promoter, which prevents morbidity and mortality in CFTR knock-out mice due to acute intestinal obstructions [6,7].

Knockdown and Overexpression Strategy

cDNA for human CFTR was obtained from Origene (Rockville, USA). Lentiviral expression constructs were obtained by cloning the respective cDNAs into pRRLsinPPT.CMV.MCS MM WPRE-vector. To generate short hairpin RNA (shRNA) vectors against CFTR (two different shRNAs, labeled KD1 and KD2, were used), oligonucleotides were cloned into the pLKO-shRNA2 vector (No. PT4052-5; Takara Bio, Saint-Germain-en-Laye, France). CFTR KD1 and KD2 shRNA were from Origene (TRCN0000082964: CCGGCCTGGAATTGTCAGACATATACTCGAGTATATGTCTGACAATTCCAGGTTTTTG and TRCN0000082967: CCGGGCAGTACGATTCCATCCAGAACTCGAGTTCTGGATGGAATCGTACTGCTTTTTG; Rockville, USA). A nonsense scrambled shRNA sequence was used as a negative control. Lentiviral particles were produced in 293T cells as previously described [8]. For lentiviral transduction, a MOI of 10 was used throughout the study and results from the main figures display KD1 and KD2 constructs merged. MOI of 30

was only used for the initial screening (supplemental Figure 1). Transductions were performed on day 0 and after 24 h the cells were refed with fresh medium. All experiments were performed from day 3 or 4 onwards. Knockdown efficiency was monitored for each experiment at the protein level. For 3HA-CFTR localization studies, lentiviral vectors were used encoding 3HA-WT-CFTR under the control of the human CMV promoter (with the 3HA-tag inserted into the fourth extracellular loop of CFTR (described in Sharma *et al.* [9]) (*CFTR* cDNA sequence NCBI GenBank CCDS 5773.1)).

Bulk RNA Sequencing

RNA of control vs $CFTR^{KD}$ and vehicle vs CFTR(inh)-172 (Merck, Darmstadt, Germany) treated ECs was extracted using the TRIzol kit (Thermo Fisher Scientific, Geel, Belgium) following the manufacturer‘s instructions; quality and quantity were measured on a Nanodrop (Thermo Fisher Scientific, Geel, Belgium). Sequence libraries were prepared with the Lexogen QuantSeq 3' mRNA-Seq Library prep kit according to the manufacturer's protocol. Samples were indexed to allow for multiplexing. Library quality and size range was assessed using a Bioanalyzer (Agilent Technologies, Leuven, Belgium) with the DNA 1000 kit (Agilent Technologies, Leuven, Belgium) according to the manufacturer’s recommendations. Libraries were diluted to a final concentration of 2 nM and subsequently sequenced on an Illumina HiSeq4000 platform according to the manufacturers recommendations. Single-end reads of 50 bp length were produced with a minimum of 1M reads per sample. Quality control of raw reads was performed with FastQC v0.11.7. (available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc) [10]. Adapters were filtered with ea-utils fastq-mcf v1.05. (available online at: https://github.com/ExpressionAnalysis/ea-utils) [11]. Splice-aware alignment was performed with STAR v2.6.1b against the human reference genome hg38 using the default parameters [12]. Reads mapping to multiple loci in the reference genome were discarded. Resulting BAM alignment files were handled with Samtools v1.5. [13]. Quantification of reads per gene was performed with HT-seq Count v2.7.14. Raw

sequencing data are available in ArrayExpress under accession number E-MTAB-8599.

Gene Set Enrichment Analysis

Bio-informatic analysis was carried out by using the in-house developed BIOMEX software [14]. We used gene set enrichment analysis (GSEA) as implemented in the clusterProfiler package (version 3.6.0) to compare gene expression signatures between controls and CFTR knockdown or CFTR inhibitor-treated HUVEC samples. Gene set analysis was performed using a set of vascular related gene sets selected from the Molecular Signatures Database (MSigDB version 7.0 downloaded from http://bioinf.wehi.edu.au/software/MSigDB/), as previously described [15]. GSEA scores were calculated for sets with a minimum of 10 detected genes, all other parameters were default. Individual genes shown in heatmaps were selected out of relevant MSigDB gene lists whereafter they were filtered based on significant adjusted p-value and endothelial function.

RNA Isolation and Gene Expression Analysis

Total RNA was extracted with the PureLink RNA mini kit (Thermo Fisher Scientific, Geel, Belgium) according to the manufacturer's instructions; RNA quality and concentration were measured on a Nanodrop (Thermo Fisher Scientific, Geel, Belgium). cDNA synthesis was performed with the iScript cDNA synthesis kit (BioRad, Temse, Belgium) with a starting RNA material of 500 µg. RNA expression analysis was performed by Taqman quantitative RT-PCR using premade primer sets (Applied Biosystems, Carlsbad, CA and IDT, Belgium): HPRT Hs.PT.58.2145446 (NM_000194); VCAM1 Hs.PT.56a.24988808 (NM_001199834); ICAM1 Hs.PT.56a.4746364 (NM_000201); SELE Hs.PT.58.1165629 (NM_000450); IL8 Hs.PT.58.39926886.g (NM_000584); SQSTM1 Hs.PT.58.39829257 (NM_003900); MAP1LC3B Hs.PT.58.27295455.g (NM_022818); GCLC Hs.PT.58.246294 (NM_001498).

Immunoblotting

Protein extraction and immunoblot analysis were performed using a modified Laemmli sample buffer (125 mM Tris-HCl, pH 6.8 buffer containing 2% SDS and 10% glycerol) in the presence of protease and phosphatase inhibitors (Roche, Anderlecht, Belgium). Mitochondrial isolation was performed using the mitochondria isolation kit for cultured cells according to the manufacturer's instructions (Thermo Fisher Scientific, Geel, Belgium). Lysates were separated by SDS-PAGE under reducing conditions, transferred to a nitrocellulose membrane, and analyzed by immunoblotting. Primary antibodies used were: CFTR (MAB25031; R&D Systems, Abingdon, United Kingdom), p62 (P0067; Merck, Darmstadt, Germany), LC3B (ab51520; Abcam, Cambridge, United Kingdom), p-P70S6K (#9205; Cell Signaling, Frankfurt am Main, Germany), P70S6K (#9202; Cell Signaling, Frankfurt am Main, Germany), p-S6 (#4858; Cell Signaling, Frankfurt am Main, Germany), S6 (#2217; Cell Signaling, Frankfurt am Main, Germany), OPA1 (612606; R&D Systems, Abingdon, United Kingdom), DRP1 (NB110-55237; R&D Systems, Abingdon, United Kingdom) and Parkin1 (ab15954; Abcam, Cambridge, United Kingdom). Equal loading was verified by GAPDH immunoblotting (#2118; Cell Signaling, Frankfurt am Main, Germany) for total and cytosolic fractions and VDAC1 (sc390991; Santa Cruz, Heidelberg, Germany) for mitochondrial fractions. All antibodies were used in 5% bovine serum albumin (BSA) diluted in TBST buffer (Tris-buffered saline, 0.1% Tween 20). Appropriate secondary antibodies were from Cell Signaling Technology (Anti-Rabbit IgG HRP linked #7074; Anti-Mouse IgG HRP linked, #7076; Frankfurt am Main, Germany) in a 1:2000 dilution in TBST with 5% BSA. Signal was detected using the ECL and/or Femto systems (GE Healthcare, Chicago, USA) according to the manufacturer's instructions. Densitometric quantifications of bands were done with Fiji software (https://fiji.sc) [16].

Immunocytochemistry

All methods for histology and immunostainings have been previously described [1,17–20]. Cells grown for 24 h on coverslips were fixed with 4% PFA for 15 min at room temperature and processed for immunocytochemistry. In general, PBS-Triton 0.5%-2%BSA was used to permeabilize the cells or PBS-2%BSA where non-permeabilized cells were required. Primary antibodies were incubated with the cells overnight, and secondary antibodies were incubated for 2 h. On tissues, immunostainings were performed using the following primary antibodies: anti-CD31 (550274; BD Biosciences, Erembodegem, Belgium), anti-VE-cadherin (555289; BD Biosciences, Erembodegem, Belgium), anti-CFTR (MAB25031; R&D Systems, Abingdon, United Kingdom), anti-HA (11583816001; Roche, Anderlecht, Belgium), anti-vWF (ab6994; Abcam, Cambridge, United Kingdom). Sections were then incubated with the appropriate fluorescently conjugated secondary antibodies (Alexa 488, 546, 568, 633 or 647; Thermo Fisher Scientific, Geel, Belgium), or followed by amplification with the proper tyramide signal amplification systems when needed (Perkin Elmer, Zaventem, Belgium). Actin was counterstained with Alexa-568 Fluor conjugated phalloidin (Thermo Fisher Scientific, Geel, Belgium). Nuclei were counterstained with DAPI (Thermo Fisher Scientific, Geel, Belgium).

In Vitro Functional Assays

Proliferation

Endothelial proliferation was quantified by incubating the cells with 1 mCi/mL [^{3}H]-thymidine in full EGM2 as previously described [20] . Thereafter, cells were fixed with 100% ethanol for 15 min at 4°C, precipitated with 10% trichloroacetic acid for 10 min at 4°C, and lysed with 0.1 N NaOH. The amount of [^{3}H]-thymidine incorporated into DNA was measured by scintillation counting. CFTR(inh)-172 in 10 μM or vehicle (DMSO) were added to the medium 24 h before cell lysis. [^{3}H]-thymidine was added together with the supplement 6 h before cell lysis. The data were normalized to protein content, determined by BCA assay (Roche, Anderlecht, Belgium) immediately after the assay.

LDH Viability Assay

Cell survival was assessed by lactate dehydrogenase (LDH) release into the media using the Cytotoxicity Detection kit (Roche, Anderlecht, Belgium) as indicated in manufacturer's specifications, whereby low LDH release signifies low cell death and high survival. CFTR(inh)-172 at 10 μM or vehicle (DMSO) were added to the medium 24 h before cell lysis.

Scratch Wound Migration Assay

A scratch wound was applied on confluent EC monolayers (pretreated for 8 h with 1 μg/mL Mitomycin C to block proliferation) using a 20 μl tip, 24 h after seeding. After scratch wounding and photography (T0), the cultures were further incubated in fully supplemented EGM2 medium with CFTR(inh)-172 at 10 μM or vehicle (DMSO) for 18 h (until near closure was reached in the control condition) and photographed again (T18) using a Leica DMI6000 microscope (Leica Microsystems, Wetzlar, Germany). Migration was measured with the Fiji software (https://fiji.sc)[16] and is expressed as percentage of wound closure (gap area at T0 minus gap area at T18 in % of gap area at T0).

Trans-endothelial Electrical Resistance

25,000 ECs were seeded in growth medium on 6.5 mm 0.1% gelatin-coated polyester transwells, 0.4 μm pore size (Costar ref. 3470; Merck, Darmstadt, Germany). The trans-endothelial electrical resistance (TEER) was measured using the Endohm-6 electrode (World Precisions Instruments, Friedberg, Germany) connected to an EVOM2 Volt/Ohm meter (World Precisions Instruments, Friedberg, Germany). Gelatin-coated wells without cells were used to measure the intrinsic electrical resistance of the inserts and these values were then subtracted from the values measured in the presence of cells. Measurements were performed every day, taking at least 2 measurements per treatment. The medium was changed every day with fully supplemented EGM2 medium with CFTR(inh)-172 at 10 μM or vehicle (DMSO).

Staining and quantification of VE-cadherin junctions

VE-cadherin staining and quantification of junctional length was performed as previously described [21,22]. First, the total junctional length (100%) was determined by summing up all segments, then the sum of all continuous segments was calculated as the percentage of total junctional length. The percentage difference between total and continuous represents the discontinuous length. Reticulated fraction was determined with the formula reticulated length/total junctional length using the VE-cadherin staining [23]. Junctional lengths, reticulated and total junctional length were defined manually with the Fiji software (https://fiji.sc) [16]. For each condition, a minimum of 2 fields were quantified (20-40 cells per field on average) per condition. The intensity plot profile was created with Fiji to show overlapping signal between VE-cadherin and CD31, justifying the validity of using VE-cadherin to measure reticulated junctions.

Intracellular and Mitochondrial ROS Analysis

Intracellular and mitochondrial ROS levels were measured using CM-H_2DCFDA (Thermo Fisher Scientific, Geel, Belgium) and MitoSOX (Thermo Fisher Scientific, Geel, Belgium) according to the manufacturer's instructions. The ROS levels were determined by pre-incubation of the ECs for 30 min with the dye in serum free M199. The fluorescent intensity was measured by flow cytometry. CFTR(inh)-172 in 10 μM or vehicle (DMSO) were added to the medium 24 h before the addition of the fluorescent dye.

Leukocyte Adhesion Assay

Peripheral blood mononuclear cells (PBMC) were collected from healthy human volunteers, isolated by gradient centrifugation as described above and labelled with calcein according to the manufacturer's instructions (C1430, Thermo Fisher Scientific, Geel, Belgium). Confluent monolayers of HUVECs were grown in 35 mm high glass bottom ibidi dishes (Ibidi, Beloeil, Belgium) and incubated either with vehicle (DMSO), CFTR(inh)-172 (10 μM) or LPS (1 mg/mL) overnight (37°C, 5% CO_2). After this period, medium was removed and ECs were washed with PBS. The mononuclear cells were added (1.0 x 10^6/ibidi dish) and incubated for 60 min (37°C, 5% CO_2). Non-adherent

cells were removed by gently washing 5 times with PBS and cells were fixed using 4% PFA. Five fields of view per well (magnification 20X, Leica DMI6000B microscope, Wetzlar, Germany), randomly chosen, were analyzed, and the number of adherent leukocytes per field was determined.

Metabolism Assays

Oxygen Consumption Rate (OCR)

Cells were seeded at 50,000 cells per well in Seahorse XF24 tissue culture plates (Seahorse Bioscience Europe, Copenhagen, Denmark) the day before the assay. The measurement of oxygen consumption was performed at 10 min intervals (2 min mixing, 2 min recovery, 6 min measuring) for 3 h using the Seahorse XF24 analyzer. Inhibitors and substrates were used at the following concentrations: oligomycin (1.2 μM), antimycin (1 μM), FCCP (5 μM). At any condition, at least 4 consecutive measurements of OCR are done. The data were normalized to protein content, determined by BCA assay upon lysis of the cells (using RIPA buffer) immediately after the assay.

Detection of Glutathione Species and NADPH

Metabolites from ECs grown on a 6-well plate were extracted in 300 μL of a 80% methanol, containing 2 μM d27myristic acid extraction buffer. Extraction samples were then spun down in a cooled centrifuge for 15 min at 20.000 g and the supernatant was transferred to LC-MS vials. Targeted measurements of GSSG, GSH, NADP+, NADPH were performed using a Dionex UltiMate 3000 LC System (Thermo Fisher Scientific, Geel, Belgium) coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Geel, Belgium) operated in negative mode. Practically, 35 μL of sample was injected on a SeQuant ZIC/ pHILIC Polymeric column (Merck, Darmstadt, Germany). The gradient started with 20% of solvent B (10 mM NH_4-acetate in MQH_2O, pH 9.3) and 80% solvent A (LC-MS grade acetonitrile) and remained at 20% B until 2 min post injection. Next, a linear gradient to 80% B was carried out until 29 min. At 38 min the gradient returned to 40% B followed by a decrease to 20% B at 42 min. The

chromatography was stopped at 58 min. The flow was kept constant at 100 μL per min at the column was placed at 25°C throughout the analysis. The MS was operated both in targeted MS2 mode using a spray voltage of 3.5 kV, capillary temperature of 320°C, sheath gas at 10.0, auxiliary gas at 5.0. For the targeted MS2 mode, AGC was set at $2e^5$, maximum IT at 100 ms, a resolution of 17.500 and an isolation window of 1.2 m/z. Data collection was performed using Xcalibur software (Thermo Fisher Scientific, Geel, Belgium).

Transmission Electron Microscopy

Transmission electron microscopy was performed on a JEOL JEM1400 (JEOL Europe BV, Zaventem, Belgium) (VIB Bio Imaging Core, Leuven Platform). For TEM observations samples were fixed for 24 h with 2.5% glutaraldehyde at pH 7.3, buffered with 0.05 M sodium cacodylate. Prior to embedding in Agar 100 Resin (Agar Scientific, Stansted, UK) the material was post fixed in 2% OsO_4 (buffered with 0.05 M sodium cacodylate, pH 7.3) and dehydrated in a graded acetate series. Semi-thin (± 1 μm) sections were cut with a Reichert Jung Ultracut E microtome and stained with 0.1% thionin - 0.1% methylene blue. The ultra-thin (± 70 nm) sections, on copper grids, were stained with uranyl acetate and lead citrate. 7-20 pictures were taken from each condition.

Mouse Experiments

Leukocyte Infiltration Into the Lungs and Liver

Liver and lungs of 11-14 weeks old mice were perfused with PBS, thereafter they were dissected and fixed in 4% PFA overnight at 4°C, dehydrated, embedded in paraffin and sectioned into 7 µm-thin sections. Immunostaining of leukocytes was done with rat anti-Mouse CD45 antibody (30-F11; BD Biosciences, Erembodegem, Belgium) and staining of the endothelium was done with a goat anti-mouse CD105 antibody (AF1320; R&D Systems, Abingdon, United Kingdom). Sections were then incubated with an appropriate secondary antibody (Alexa-647; Jackson Immuno Research,

Uden, The Netherlands) and TSA Cy3 amplification (Perkin Elmer, Zaventem, Belgium). Imaging was performed using a Zeiss LSM 780 confocal microscope (Zeiss, Jena, Germany). Morphometric analysis of the area of $CD45^+$ dots in % of CD105 lung area was done using Fiji analysis software.

Flow Cytometry Analysis

Mice were first anesthetized using Ketamine/Xylazine (500 mL/mouse of Ketamine 0.65%; Xylazine 0.05% diluted in saline solution) and perfused with 10 mL PBS followed by perfusion with 5 mL of full DMEM-based digestion buffer containing 0.1% collagenase II (Thermo Fisher Scientific, Geel, Belgium), 0,1% collagenase I (Thermo Fisher Scientific, Geel, Belgium) and DNase I 0.075 mg/ml (Merck, Darmstadt, Germany). Lungs were dissected and placed into a gentleMACS dissociation tubes (Miltenyi, Leiden, The Netherlands) containing 5 mL of digestion buffer, and processed as per the manufacturer's instructions. The organs were incubated in a water bath at 37°C, 30 min with shaking every 10 min. Next, tissue was homogeneously dissociated and the reaction was stopped by adding 10 mL PBS/0.1% BSA. Subsequently, the cell suspension was spun down for 5 min at 300g before staining and flow cytometry analysis. The staining was performed using a mix containing CD34 (48-0341-80; Thermo Fisher Scientific, Geel, Belgium); CD31 (551262 & 561410; BD Biosciences, Erembodegem, Belgium), CD45 (45-0454-82; Thermo Fisher Scientific, Geel, Belgium), ICAM1 (550287; BD Biosciences, Erembodegem, Belgium) and VCAM1 (553330; BD Biosciences, Erembodegem, Belgium) antibodies. The vibrant dye (V35003; Thermo Fisher Scientific, Geel, Belgium) was used to gate for viable cells. After removing doublet cells, endothelial cells were identified based on viable cells with $CD45^-$ $CD31^+$ expression. Data were recorded by flow cytometry on the FACS AriaIII (BD Biosciences, Erembodegem, Belgium), and analyzed with the FlowJo 8.8.6 software (https://www.flowjo.com).

Quantification and Statistical Analysis

Data represent mean ± SEM of at least three independent experiments. Statistical significance was calculated by standard two-tailed t-test (with Welch's correction when variances were significantly different between groups), ANOVA (for multiple comparisons within one dataset), one-sample t-test (for comparisons to point normalized data) using Prism v8.2. Bio-informatic analysis was carried out by using the in-house developed BIOMEX software [14]. p-value < 0.05 was considered as statistically significant.

Supplemental References

1. De Bock K, Georgiadou M, Schoors S, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell*. 2013;154(3):651–663.
2. Martin-Ramirez J, Hofman M, van den Biggelaar M, Hebbel RP, Voorberg J. Establishment of outgrowth endothelial cells from peripheral blood. *Nat Protoc*. 2012;7(9):1709–1715.
3. Vonk AM, van Mourik P, Ramalho AS, et al. Protocol for Application, Standardization and Validation of the Forskolin-Induced Swelling Assay in Cystic Fibrosis Human Colon Organoids. *STAR Protoc.* 2020;100019.
4. Berkers G, van Mourik P, Vonk AM, et al. Rectal Organoids Enable Personalized Treatment of Cystic Fibrosis. *Cell Rep.* 2019;26(7):1701-1708.e3.
5. Ramalho AS, Fürstová E, Vonk AM, et al. Correction of CFTR function in intestinal organoids to guide treatment of Cystic Fibrosis. *Eur. Respir. J.* 2020;1902426.
6. Munder A, Wölbeling F, Kerber-Momot T, et al. Acute intratracheal Pseudomonas aeruginosa infection in cystic fibrosis mice is age-independent. *Respir. Res.* 2011;12(1):148.
7. Zhou L, Dey CR, Wert SE, et al. Correction of lethal intestinal defect in a mouse model of cystic fibrosis by human CFTR. *Science*. 1994;266(5191):1705–1708.
8. Wong BW, Wang X, Zecchin A, et al. The role of fatty acid beta-oxidation in lymphangiogenesis. *Nature*. 2016;
9. Sharma M, Pampinella F, Nemes C, et al. Misfolding diverts CFTR from recycling to degradation: quality control at early endosomes. *J. Cell Biol.* 2004;164(6):923–933.
10. Andrews S. FastQC: a quality control tool for high throughput sequence data. 2010;
11. Aronesty E. ea-utils : command-line tools for processing biological sequencing data. 2011;
12. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq

aligner. *Bioinformatics*. 2013;29(1):15–21.
13. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25(16):2078–2079.
14. Taverna F, Goveia J, Karakach TK, et al. BIOMEX: an interactive workflow for (single cell) omics data interpretation and visualization. *Nucleic Acids Res.* 2020;
15. Goveia J, Rohlenova K, Taverna F, et al. An Integrated Gene Expression Landscape Profiling Approach to Identify Lung Tumor Endothelial Cell Heterogeneity and Angiogenic Candidates. *Cancer Cell*. 2020;37(1):21-36.e13.
16. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat. Methods*. 2012;9(7):676–682.
17. Cantelmo AR, Conradi LC, Brajic A, et al. Inhibition of the Glycolytic Activator PFKFB3 in Endothelium Induces Tumor Vessel Normalization, Impairs Metastasis, and Improves Chemotherapy. *Cancer Cell*. 2016;30(6):968–985.
18. Vandekeere S, Dubois C, Kalucka J, et al. Serine Synthesis via PHGDH Is Essential for Heme Production in Endothelial Cells. *Cell Metab.* 2018;1–15.
19. Bruning U, Morales-Rodriguez F, Kalucka J, et al. Impairment of Angiogenesis by Fatty Acid Synthase Inhibition Involves mTOR Malonylation. *Cell Metab.* 2018;28(6):866-880.e15.
20. Kalucka J, Bierhansl L, Conchinha NV, et al. Quiescent Endothelial Cells Upregulate Fatty Acid β-Oxidation for Vasculoprotection via Redox Homeostasis. *Cell Metab.* 2018;28(6):881-894.e13.
21. Fraccaroli A, Pitter B, Taha AA, et al. Endothelial alpha-parvin controls integrity of developing vasculature and is required for maintenance of cell-cell junctions. *Circ. Res.* 2015;117(1):29–40.
22. Eelen G, Dubois C, Cantelmo AR, et al. Role of glutamine synthetase in angiogenesis beyond glutamine synthesis. *Nature*. 2018;561(7721):63–69.
23. Fernández-Martín L, Marcos-Ramiro B, Bigarella CL, et al. Crosstalk Between Reticular Adherens Junctions and Platelet Endothelial Cell Adhesion Molecule-1 Regulates Endothelial Barrier Function. *Arterioscler. Thromb. Vasc. Biol.* 2012;32(8):e90–e102.