# SUPPLEMENTAL MATERIAL

## Expanded Methods

*Cell culture.* De-identified primary human small PASMC and PAEC were harvested from lungs explanted from patients with hereditary PAH with a BMPR2 mutation or idiopathic PAH, undergoing transplantation, or from unused donor lungs (as controls). Supplemental Tables I-IV indicate demographics and other characteristics related to hemodynamic assessments and PAH medications. The lungs were obtained though the Pulmonary Hypertension Breakthrough Initiative (PHBI) funded by NIH (R24 HL123767) and the Cardiovascular Medical Research and Education Fund (CMREF; UL 1RR024986). Informed consent was obtained through the PHBI network at the Transplant Procurement Centers at Allegheny General Hospital, Baylor College of Medicine, Cleveland Clinic, Stanford University, University of Alabama and Vanderbilt University, and de-identified patient data were obtained from the Data Coordinating Center at the University of Michigan.

PASMC and PAEC were isolated and cultured from small pulmonary arteries <1mm in diameter, and were used between passages 3-8, but at the same passage in each experiment. PASMC were isolated and cultured in Smooth Muscle Growth Medium-2 containing 5% fetal bovine serum (FBS) (Lonza, Indianapolis, IN), and PAEC were cultured in Endothelial Cell Medium (Sciencell, Carlsbad, CA) as previously described[51]. Cells were maintained in 95% air and 5% $CO_2$ at 37°C. Starvation medium was basal medium plus 0.2% FBS. The cells were routinely tested for mycoplasma, and only mycoplasma negative cells were used.

Isolated PA and aorta from WT and KO mice were processed under a dissecting microscope in cold HBSS (GIBCO). The adventitial tissues were removed from the arteries, and the EC layer was denuded by rubbing the luminal surface with a cotton swab as described[52]. The SMC layers of the aorta were isolated and frozen in -80ºC for RNA extraction. The SMC layer from the PAs were digested in 1mg/mL collagenase (Sigma, #C9722) and 75μg/mL elastase (Worthington, #2292) in HBSS at 37 ºC for 25min as described before[53]. PASMC were spun down at 1,200 RPM for 5min and seeded in T25 flasks with SMC culture medium.

*Animal models. Aldh1a3*$^{-/-}$ floxed embryos of a mixed (50%) C57BL/6-(50%) 129/Sv genetic background were obtained from Dr. Norbert B. Ghyselinck of Institut de Génétique et de Biologie Moléculaire et Cellulaire in France. SMC-specific *Aldh1a3*$^{-/-}$ mice were created by crossing non-inducible SM22-Cre mice (C57BL/6 background) with *Aldh1a3*$^{-/-}$ floxed mice. Wild-type littermates were used as controls. The number of mice per experiment is indicated in the figure legends. Male mice 7-8 weeks of age were housed in hypoxia (10% $O_2$) for three weeks, or in room air for three weeks. RVSP, RVH, cardiac function and output, and pulmonary artery acceleration time (PAAT) were measured, all according to methods previously published by our group[9]. The heart and lungs were perfused with PBS, the left lung fixed, and sections embedded in paraffin for immunohistochemistry and immunofluorescence. The right lung was snap-frozen in liquid $N_2$ and kept at -80°C.

*siRNA transfections.* siRNA transfections were carried out according to a modification of a previous publication[54]. Control, ALDH1A3 (Ambion, s32), NFYA (Ambion, HSS107167), KAT2B (Ambion, s16895), PKM (Ambion, s10575), IDH1 (Ambion, s7121), DLD (Ambion, s4116) and β-catenin siRNA

(Ambion, s438), ON-TARGETplus control were transfected into subconfluent PASMC on culture plates. Plates were washed with PBS and transfected with 10mL OPTI-MEM (Invitrogen) containing 2µl Lipofectamine RNAiMAX (Invitrogen) and 25nM siRNA. Six hours later the transfection medium was replaced with normal growth medium.

*Plasmid transfections*. To generate ALDH1A3 and NFYA overexpressing donor control PASMC, TransIT-2020 Transfection Reagent (Mirus Bio, Madison, WI, MIR 5400) was used with the pPB-mCherry/Puro-EF1A/hALDH1A3:T2A:hNFYA, and the pRP-hyPBase-mCherry/Puro-CAG vectors (VectorBuilder, Chicago, IL). In brief, total 1.9µg of DNA vectors (0.3µg of pRP-hyPBase with 1.6µg of pPB-mCherry/Puro-EF1A/hALDH1A3:T2A:hNFYA or 0.3µg of pRP-hyPBase with 1.6µg of pPB-hyPBase-mCherry/Puro-CAG) and 5.7µL of TransIT-2020 reagent were mixed in 0.2 mL Opti-MEM). This solution was incubated for 20min at room temperature and then added to donor control PASMC in a 6 well plate with 1.8mL SMC culture medium for the transfection process. Medium was changed to fresh SMC culture medium after 48h. The transfected cells were allowed to proliferate for 4h, and then the cell culture medium was replaced with fresh medium with 0.75µg/mL of puromycin to select stable clones expressing ALDH1A3 and NFYA. Medium was changed after 48h of selection. Three weeks later, the cells were collected and assessed for *ALDH1A3* and *NFYA* mRNA by qPCR and used in further experiments.

*Immunoblotting.* PASMC were synchronized by serum-starvation (0.2% FBS) for 48h, then cultured with 5% FBS for 72h. Cells were trypsinized and washed with cold PBS, then lysed in boiling lysis buffer (10mM Tris-HCI and 1% SDS) and boiled at 95°C for 5 min before centrifugation. Protein concentration was determined using the BCA assay. Lysates were separated by SDS-PAGE and transferred to a PVDF

membrane. The membranes were incubated with primary antibodies as listed below in 5% BSA. Appropriate secondary antibodies were used. Normalization for total protein of anti-ALDH1A3 (1:1000, Abgent, Cat# AP7847a), anti-NFYA (1:1000, Santa Cruz Technology, Cat# sc-17753), anti-KAT2b (1:1000, Santa Cruz Technology, Cat# sc-13124), anti-PKM2 (1:2000, Cell Signaling Technology, Cat# 4053), anti-DLD (1:1000, ThermoFisher, Cat# PAS-70397), anti-IDH1 (1:1000, Cell Signaling Technology, Cat# 8137), anti-TTK (1:1000, Santa Cruz Technology, Cat# sc-376842), anti-CNNB1(1:1000, Santa Cruz Technology, Cat#248) or anti-H3K27ac (1:1000, Cell Signaling Technology, Cat# 610153) was carried out by re-probing the membrane with an antibody against α-Tubulin (1:5000, Sigma-Aldrich, Cat#T9026) or GAPDH (1:2000, EMD Millipore, Cat#2302). Signal was detected by Clarify ECL kit and BioRad ChemiDoc XRS system (BioRad) according to manufacturer's instructions. Densitometric quantifications were performed using NIH Image J software.

*Nuclear and cytoplasmic fractions.* Nuclear and cytosoplasmic fractions were isolated using NE-PER Nuclear and Cytoplasmic Extraction Reagents (ThermoFisher Scientific, Waltham, MA). Briefly, cells were washed in ice-cold 1xPBS, trypsinized, and then centrifuged at 500g for 5min. Halt protease and phosphatase inhibitor (ThermoFisher Scientific) were added before processing each fraction.

*Immunoprecipitation.* Nuclear extracts were diluted four times with low detergent buffer (20mM HEPES, pH 7.9, 1mM EDTA, 0.2% NP-40, Halt protease and phosphatase inhibitor) prior to immunoprecipitation. Equal protein concentrations were determined by BCA assay as described above. Diluted nuclear extracts or undiluted whole cell extracts were incubated with 2µg of specific antibodies overnight at 4°C with rotation. The next day, 10µl of Dynabeads Protein-G (Invitrogen) were added to cell extracts containing antibodies, and incubated for 4h at 4°C with rotation. After incubation, beads were washed three times in

ice-cold wash buffer (50mM Tris-HCl, pH 7.5, 150mM NaCl, 1mM EDTA, 0.05% NP-40) on a magnetic rack. Proteins were eluted in acid using 24µl of IgG elution buffer (Thermo Scientific) at room temperature for 15 min under gentle shaking. Final elution was resuspended in 4x NuPage LDS Sample Buffer (Novex) containing Bond Breaker TCEP Solution (1:4 diluted, ThermoFisher Scientific).

*Immunofluorescence.* Formaldehyde-fixed, paraffin-embedded human or mouse lung tissue sections were deparaffined and rehydrated. Antigen retrieval was performed by boiling the sections in 0.25mM EDTA. Sections were incubated with 3% hydrogen peroxide to block endogenous peroxidase, washed, and blocked with 5% BSA. Then the sections were incubated with primary antibodies against SM22α (1:400; Abcam, Cambridge, CA) and ALDH1A3 (1:100, Abgent, San Diego, CA) or PCNA(1:400, Cell Signaling Technology, Danvers MA) , PKM2 (1:200, Cell Signaling Technology, Danvers MA), NFYA (1:100, Santa Cruz Technology, CA) and active ß-catenin (1:100, Millipore, Massachusetts, MA) overnight at 4°C. The next day, sections were washed three times with PBS and then incubated with the secondary antibody (Alexa Fluor 488-labeled anti-rabbit 1:800, Alexa Fluor 594 labeled ant-mouse 1:800, Invitrogen) for 20min at room temperature. Sections were washed three times with PBS, mounted with Vectashield containing DAPI (Vector Laboratories, Burlingame, CA) and stored at 4°C until analysis. Confocal analysis wad performed using a confocal laser-scanning microscope (FV1000, Olympus, Center Valley, PA).

*Seahorse assay.* Glycolytic capacity was measured using the XF Glycolysis Stress Test with an XF24 extracellular flux analyzer (Seahorse Bioscience, Agilent Technologies, Cat# 103020-100), and baseline mitochondrial function and mitochondrial stress responses were measured by oxygen consumption rate (OCR) (Seahorse Bioscience, Agilent Technologies, Cat# 103015-100), following the manufacturer's

instructions and as described previously[51]. Briefly, cells were trypsinized after 72h of serum stimulation followed by 48h serum starvation; 100,000 cells per well were seeded on the Seahorse Assay plate for three hours. For extracellular acidification rate (ECAR) measurements, cells were washed in glucose-free XF base medium containing 2mM L-Glutamine at pH 7.35. ECAR was determined after serial injections with 5mM D-Glucose, 1μM Oligomycin and 100mM 2-Deoxyglucose. Aerobic glycolysis is defined as ECAR in response to Oligomycin after glucose injection. Glycolytic reserve is defined as $\Delta ECAR_{Oligomycin-Glucose}$. Non-glycolytic acidification is ECAR after 2-Deoxyglucose injection. For OCR measurements, cells were washed with XF assay medium containing 10mM Glucose, 1mM Sodium-Pyruvate and 2mM L-Glutamine set to pH 7.40. OCR was measured at baseline and following consecutive injections of 1μM Oligomycin, 1μM FCCP and a mix of 1μM Rotenone+1μM Antimycin A. Following manufacturer's instructions, maximal mitochondrial respiration was determined as OCR following FCCP injection. Pare respiration capacity was defined as ΔOCR (FCCP-baseline) and mitochondrial ATP production as ΔOCR (Baseline-Oligomycin). The measurements were normalized to cell number; the number of cells was monitored before and after the study and judged to be similar.

*Proliferation assays*. Proliferation of PASMC was assessed by cell counts and MTT assays. Forty-eight hours following transfection in serum starvation (0.2% FBS), PASMC were exposed to 5% FBS for 72h, and cell growth was assessed in 48-well plates for the MTT cell proliferation assay (American Type Culture Collection (ATCC), Manassas, Virginia, Cat#30-1010k). Cell number was verified by cell count as shown in supplementary figures.

*Caspase assay*. Survival of PASMC and PAEC were assessed by the Caspase 3/7 assay, modified from a previously published protocol[54]. PASMC or PAEC were seeded in a 96-well plates (10,000 cells per well)

in SMC or EC culture medium, and allowed to adhere overnight. Cells were then washed with PBS and incubated with 100μL SMC or EC medium with 0.2% FBS for 12h. Cells were then incubated for 1h in 100μL of Caspase 3/7 Luciferase Reagent Mix (Promega, Madison, WI #G8093), and total luminescence was measured in a plate reader (BioTek Synergy H1 Hybrid Reader, Winooski, VT).

*Flow cytometry cell cycle analysis.* Cell cycle analysis was performed using the FxCycle PI/RNase Staining Solution (Thermo Fisher Scientific, #F10797) according to the manufacturer's protocol. In brief, pre-chilled 70% ethanol was used to fix the cells in -20°C for 20min. Cells were stained in 0.5mL of FxCycle PI/RNase staining solution for 30min at room temperature before proceeding to flow cytometry.

*Reverse transcription qPCR (RT-qPCR).* Total RNA was extracted and purified using the Quick-RNA MiniPrep Kit (Zymo Research, Irvine, CA). The quantity and quality of RNA was determined by using a spectrophotometer. RNA was reverse transcribed using the High Capacity RNA to cDNA Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. RT-qPCR was performed using 1μL of 5μM Powerup SYBR green PCR Master Mix (Applied Biosystem, Cat# A25742), 2μL of $dH_2O$ and 2μL of cDNA sample in a 10μL reaction. Each measurement was carried out in duplicate using a CFX384 Real-Time System (Bio-Rad). The PCR conditions were: 95°C for 2min, followed by 40 cycles of 95°C for 15s, and 60°C for 60s. Primer sequences were designed using PrimerBank and are listed in the Table VI in the Supplementary Materials. Expression levels of genes was normalized to the expression level of *β-actin*.

Primers used for mice tissues are list in Table VI in the Data Supplement.

*Acetyl-CoA quantification.* Acetyl-CoA was measured by PicoProbe Acetyl-CoA Assay Kit (Abcam, Cat# 87546) following the manufacturer's instructions. Briefly, after 72h of serum stimulation preceded by 48h serum starvation of PASMC with or without *ALDH1A3* siRNA. Three $x10^6$ cells were harvested and suspended in the assay buffer. The cell lysates were homogenized using a Nuclear and Cytosolic Fraction kit as described above in the '*Nuclear and cytoplasmic fractions'* section of the Methods. The supernatants of the nuclear fraction were collected after centrifugation at 14,000g for 10min at 4°C. Lysates were then deproteinized by perchloric acid and neutralized with KOH. The PicoProbe assay was performed in 96-well clear bottom, black wall plates and the fluorescence was quantified using Infinite 200Pro (TECAN, Switzerland).

*Acetaldehyde quantification.* Nuclear and cytosolic fractions were isolated as described above. Briefly, clear sample fractions obtained in duplicate were transferred into separate wells of a clear, flat-bottom 96-well plate. Working reagent and standards (BioAssay Systems, Cat# EACT-100) were freshly prepared immediately before the assay. The working reagent was added to the standards and sample wells, mixed briefly and thoroughly, then incubated for 30min at room temperature. Optical density was read at 565nm.

*RNA-seq sample preparation and analysis*. RNA was extracted using the RNeasy Mini Kit (QIAGEN, Hilden, Germany). Libraries were prepared using the TruSeq Stranded Total RNA Library Prep Kit (Illumina, San Diego, CA) and subjected to sequencing on 1-2 Illumina HiSeq 2000 lanes to obtain an average of approximately 100-150 million uniquely mapped reads for each sample (Stanford Personalized Medicine Sequencing Core). RNA-seq reads were aligned to human genome hg38 by Hisat2[55]. Then the transcriptome was annotated to the annotation UCSC hg38 by FeatureCounts (Walter and Eliza Hall Institute of Medical Research, http://bioinf.wehi.edu.au/featureCounts/)[56]. The differential expression analyses were implemented by DESeq2 (Bioconductor,

http://bioconductor.org/packages/release/bioc/html/DESeq2.html)[57]. The count tables were uploaded in DESeq2 and normalized by the variance stabilizing transformation (VST) method. The differential expression analyses were implemented by the likelihood ratio test (LRT) of DESeq2, and the FDR cutoff was 0.01 and the foldchange cutoff was 1.2. Motif enrichment analyses were implemented by HOMER[58]. Functional enrichment analyses were implemented by GeneAnswers (Bioconductor, https://www.bioconductor.org/packages/release/bioc/html/GeneAnswers.html) package.

*ChIP-Seq (chromatin immunoprecipitation followed by sequencing) and Analysis.* Cells were trypsinized and cross-linked with 1% formaldehyde (EMD Millipore) for 10min at room temperature. To neutralize formaldehyde, 2M glycine (ThermoFisher Scientific) was added and incubated for 5min at room temperature. The cells were washed with ice cold PBS twice, snap-frozen and stored at -80°C. For ChIP-DNA preparation, cells were thawed by adding PBS and incubated at 4°C by rotating. To extract cell nuclei, cells were treated with hypotonic buffer for 10 min on ice, then were homogenized using a glass homogenizer. Nuclear pellets were resuspended in RIPA buffer (Millipore) and incubated for 30min on ice. Chromatin was sheared with SFX250 Sonifier (Branson, Bunbury, CT) and immunoprecipitated with H3K27ac antibody (Cell signaling Technology) at 4°C overnight on a nutator. For the input sample, 100µl of sheared nuclear lysate was removed and stored overnight at 4°C. The second day, protein A/G agarose beads (Millipore) were added to the chromatin-antibody complex and incubated for one hour at 4°C on a nutator and then the beads were eluted with SDS buffer (Santa Cruz Biotechnology) and incubated at 65°C for 10min. Supernatant containing ChIP-DNA was reverse cross-linked by incubating overnight at 65°C. The third day, ChIP-DNA was treated with RNase A (Qiagen) and proteinase K (ThermoFisher Scientific) and then purified. The ChIP-DNA samples were end repaired and A-tailed. The Illumina TruSeq adapters (Illumina, San Diego, CA) were ligated and size-selected from the gel before PCR amplification. PCR

products were purified and size selected in the gel again. The final purified samples were sequenced on HiSeq4000 (Illumina).

ChIP-seq data were aligned to the human genome (hg19) by Bowtie (John Hopkins University, http://bowtie-bio.sourceforge.net/index.shtml)[59] with only uniquely aligned reads kept. All duplicate reads were removed by SAMtools (Genome Research Limited, http://www.htslib.org/)[60]. The pipeline for the preprocess is available online (https://github.com/ny-shao/chip-seq_preprocess). The differential enrichment analysis was implemented by DiffReps (Icahn School of Medicine at Mount Sinai, https://github.com/shenlab-sinai/diffreps)[61]. The data visualization of ChIP-seq was performed with IGV Genome Browser (Broad Institute, https://www.broadinstitute.org/igv/)[62] and ngs.plot (Icahn School of Medicine at Mount Sinai, https://github.com/shenlab-sinai/ngsplot)[63]. Motif enrichment analyses were carried out with HOMER (University of California San Diego, http://homer.ucsd.edu/homer/) and the function enrichment analyses, by the GeneAnswer package.

*ChIP-qPCR.* Samples were prepared as described above for ChIP-Seq with NFYA (Diagenode, Cat# C15310261) or active-β-catenin (BD Transduction Laboratories, Cat# 05-665) antibodies. Input DNA (before immunoprecipitation) and immunoprecipitated DNA samples were subjected to real-time PCR analysis. Primer sequences were designed using PrimerBank and are listed in the Supplementary Materials. Expression levels of bound sequences were normalized to the input DNA of those sequences. Primers are listed in the Table VIII in the Data Supplement.

*Statistics.* All data are expressed as arithmetical mean ±SEM. Multiple group comparisons were calculated using one-way ANOVA. Multiple group comparisons with multiple treatments were calculated using

repeated measures two-way ANOVA followed by Bonferroni analysis. Statistical differences were assessed by either the unpaired two-tailed Student's test when the groups were independent, or by the paired Student's between when the same biological sample was being assessed at a different time or with a different treatment in the same experiment. A p-value of $<0.05$ was considered significant. The number of experiments, animals per group, and the statistical test used are indicated in the figure legends or in the appropriate text.

*Study approval*. The Animal Care Committee of Stanford University approved all protocols, in keeping with the regulation s of the American Physiological Society. Procurement of the tissues from human subjects is approved by the Administrative Panel on Human Subjects in Medical Research at Stanford University (IRB #350, Panel 6). Written informed consent was received from participants prior to inclusion in the study.

*Data and Software Availability.* RNAseq and ChIP-seq data will be deposited to GEO.

# Supplemental Tables

## Table I. Characteristics of Donors (Unused Donor Lungs)

| ID | Age (year) | Number | Race | Ethnicity | Cause of Death |
|---|---|---|---|---|---|
| Donor 1 | 57 | F | White | Non-Hispanic | Acute Myocardial Infarction |
| Donor 2 | 45 | M | White | Non-Hispanic | Anoxia |
| Donor 3 | 43 | F | White | Unknown | Cerebrovascular Stroke |
| Donor 4 | 24 | M | White | Non-Hispanic | Cerebrovascular Stroke/Intracranial Hemorrhage |
| Donor 5 | 33 | F | White | Non-Hispanic | Head Trauma, Blunt Injury |
| Donor 6 | 1 | M | White | Non-Hispanic | Anoxia/ Drowning |
| Donor 7 | 46 | F | White | Non-Hispanic | Cerebrovascular/Stroke ICH |
| Donor 8 | 46 | M | Asian | Unknown | Cerebrovascular/Stroke ICH |
| Donor 9 | 36 | F | White | Non-Hispanic | Subarachnoid Hemorrhage |
| Donor 10 | 28 | F | White | Non-Hispanic | Motor Vehicle Accident-Anoxia |
| Donor 11 | 56 | F | White | Non-Hispanic | Cerebrovascular Accident |
| Donor 12 | 26 | M | White | Non-Hispanic | Gunshot wound to the head |

## Table II. Summary Demographics of Donors (Unused Donor Lungs)

| Gender | Number | Age Median (range) | Age (Mean ± SD) | Race (n) | Ethnicity (n) |
|---|---|---|---|---|---|
| Female | 7 | 43.0 (28-57) | 42.7±11.2 | White 7 | Non-Hispanic 6 Unknown 1 |
| Male | 5 | 26.0 (1-45) | 28.4±18.4 | White 4 Asian 1 | Non-Hispanic 4 Unknown 1 |

## Table III. Characteristics of PAH Patients

| ID | Age (y) | Sex | Race | Ethnicity | Diagnosis | BMPR2 Mut.* | PAP† s/d/m | PVR‡ (WU) | 6MW# (m) | PAH Medications |
|---|---|---|---|---|---|---|---|---|---|---|
| PAH 1 | 27 | F | White | Non-Hispanic | HPAH | Yes | 110/49/69 | 12.11 | 359.7 | sildenafil, treprostinil, bosentan, iloprost |
| PAH 2 | 37 | M | White | Non-Hispanic | HPAH | Yes | 119/51/77 | 14.22 | 309 | sildenafil, sitaxsentan, ambrisentan, epoprostenol, Imatinib, treprostinil |
| PAH 3 | 33 | F | White | Non-Hispanic | HPAH | Yes | 87/29/48 | 9.74 | 288 | bosentan, treprostinil, sildenafil, epoprostenol |
| PAH 4 | 35 | M | White | Non-Hispanic | HPAH | Yes | 65/34/48 | 8.16 | 154 | bosentan, epoprostenol |
| PAH 5 | 25 | M | White | Hispanic or Latino | IPAH | No | 65/15/36 | N/A | 510.5 | epoprostenol, sildenafil, treprostinil |
| PAH 6 | 56 | F | White | Non-Hispanic | IPAH | No | 83/39/57 | 11.4 | 137.2 | sildenafil, ambrisentan, treprostinil |

| ID | Age (y) | Sex | Race | Ethnicity | Diagnosis | BMPR2 Mut.* | PAP† s/d/m | PVR‡ (WU) | 6MW# (m) | PAH Medications |
|---|---|---|---|---|---|---|---|---|---|---|
| PAH 7 | 40 | M | White | Hispanic or Latino | IPAH | No | 118/49/64 | 73 | 420 | sildenafil, ambrisentan, treprostinil |
| PAH 8 | 11 | F | White | Non-Hispanic | IPAH | No | 112/78 /95 | N/A | 243.8 | sildenafil, ambrisentan, epoprostenol treprostinil |
| PAH 9 | 39 | F | Unknown | Hispanic or Latino | IPAH | No | 112/45/69 | 14.97 | 262.1 | sildenafil, bosentan iloprost, epoprostenol |
| PAH 10 | 16 | F | White | Non-Hispanic | IPAH | N/A | NA/NA/95 | NA | 102.4 | sildenafil, SC treprostinil |
| PAH 11 | 53 | M | White | Non-Hispanic | IPAH | N/A | 56/15/33 | 3.86 | 77.7 | sildenafil, tadalafil, macitentan, epoprostenol |
| PAH 12 | 33 | F | Black or African American | Non-Hispanic | FPAH | Yes | 75/33/48 | 15.57 | 326.1 | epoprostenol, bosentan, sildenafil, treprostinil |
| PAH 13 | 15 | F | White | Non-Hispanic | IPAH | No | 175/66/102 | 25.24 | 387 | sildenafil, epoprostenol |
| PAH 14 | 25 | F | Asian | Non-Hispanic | IPAH | No | 134/56/87 | 20.96 | 201 | epoprostenol, sildenafil, bosentan |
| PAH 15 | 41 | F | Unknown | Hispanic or Latino | IPAH | No | 72/21/43 | N/A | 335.3 | treprostinil, bosentan, sildenafil |
| PAH 16 | 24 | M | White | Non-Hispanic | IPAH | No | 113/70/88 | 21.48 | 182 | sildenafil, epoprostenol, bosentan |
| PAH 17 | 53 | F | White | Non-Hispanic | APAH | No | 110/45/68 | 15.99 | 335 | sildenafil, bosentan, treprostinil, iloprost |
| PAH 18 | 28 | F | White | Non-Hispanic | APAH | No | 68/33/49 | 13.46 | 85.3 | Iloprost, sildenafil, bosentan, epoprostenol, treprostinil |

**BMPR2 Mut., BMPR2 mutation*
*†PAP s/d/m, Pulmonary Artery Pressure (systolic/diastolic/mean); value closest to transplant*
*‡PVR, Pulmonary vascular resistance*
*#6MW, distance walked during six minutes; value closest to transplant*

## Table IV. Summary Demographics of PAH Patients

| Gender | Number | Age Median (range) | Age (Mean ± SD) | Race (n) | Ethnicity (n) |
|---|---|---|---|---|---|
| Female | 12 | 30.5 (11-56) | 31.4±14.2 | White 8<br>Asian 1<br>Unknown 2<br>Black or African American 1 | Non-Hispanic 10<br>Hispanic or Latino 2 |
| Male | 6 | 36.0 (24-53) | 35.7±10.7 | White 6 | Non-Hispanic 4<br>Hispanic or Latino 2 |

## Table V. Origin of Cells and Tissues used in Each Experiment

| Figure | Cells |
|---|---|
| Figure 1A | PASMC from Donors 1-9, and PAH 1-12 |
| Figure 1 B-C | PASMC from Donors 3-5, 7-9, and PAH 1, 2, 4, 8, 10, 11 |
| Figure 1 D-E | PASMC from Donors 3, 7, 8, and PAH 1, 2, 8 |
| Figure 1F | Tissues from Donors 1, 9-12 and PAH 12, 15, 16, 17, 18 |
| Figure 2A | PASMC from Donors 3, 5, 6 |
| Figure 3 A-E, G | PASMC from Donors 3, 7, 8, and PAH 1, 2, 8 |
| Figure 3 F, H | PASMC from PAH 1, 2, 8 |
| Figure 4 B, H | PASMC from Donors 3, 7, 8, and PAH 1, 2, 8 |
| Figure 4 D, E, G | PASMC from PAH 1, 2, 8 |
| Figure 4 H | PASMC from PAH 1 |
| Figure 5 A-C, G, H | PASMC from PAH 1, 2, 8 |
| Figure 5 D | PASMC from PAH 1 |
| Figure 5 E-F | PASMC from Donors 3, 7, 8, and PAH 1, 2, 8 |
| Figure 6 A-B | PASMC from PAH 1 |
| Figure 6C | PASMC from PAH 1, 2, 8 |
| Figure 7A | PASMC from Donors 3, 7, 8 |
| Figure 7 B-D, G | PASMC from Donors 3, 7, 8, and PAH 1, 2, 8 |
| Figure 7F | PASMC from PAH 1 |
| Supplemental Figure IA-C | PASMC from Donors 1-9, and PAH 1-12 |
| Supplemental Figure ID-F | PASMC from Donors 3, 7, 8, and PAH 1, 2, 8 |
| Supplemental Figure IG-J | Tissues from Donor 7 and PAH 1, 2, 3, 12, 13, 14 |
| Supplemental Figure IK | PAEC from Donors 1, 3, 6 and PAH 2, 6, 7, 12 |
| Supplemental Figure IL | PAEC from Donors 1, 3, 6 and PAH 2, 6, 12 |
| Supplemental Figure III | PASMC from PAH 1, 2, 8 |
| Supplemental Figure IVA, IVC | PASMC from PAH 1 |
| Supplemental Figure IVB | PASMC from Donors 3, 7, 8, and PAH 1, 2, 8 |
| Supplemental Figure IVD, IVE | PASMC from PAH 1, 2, 8 |
| Supplemental Figure IVF | PASMC from PAH 1 |
| Supplemental Figure VA-E | PASMC from PAH 1, 2, 8 |
| Supplemental Figure VA-F | PASMC from Donor 8 |
| Supplemental Figure VI | PASMC from PAH 1 |

## Table VI. Human primers used for qPCR

| Gene | Forward primer | Reverse Primer |
|---|---|---|
| ALDH1A3 | GAATGGCACGAATCCAAGAG | CACGTCGGGCTTATCTCCT |
| NFYA | ATGTGGTCAATTCAGGAGGGA | ATTGTTTGGCATTCACGTAGAGA |
| β-actin | CATGCCATCCTGCGTCTGGA | CCGTGGCCATCTCTTGCTCG |
| PKM2 | ATAACGCCTACATGGAAAAGTGT | TAAGCCCATCATCCACGTAGA |

| Gene | Forward primer | Reverse Primer |
|---|---|---|
| DLD | CACTGCTACGAAAGCTGATGG | TAACTTCTGAACCCGTGGCTA |
| IDH1 | AGAAGCATAATGTTGGCGTCA | CGTATGGTGCCATTTGGTGATT |
| CCNA2 | GGATGGTAGTTTTGAGTCACCAC | CACGAGGATAGCTCTCATACTGT |
| CCNB1 | TTGGGGACATTGGTAACAAAGTC | ATAGGCTCAGGCGAAAGTTTTT |
| CDC20 | GCTTTGAACCTGAACGGTTTTG | TCTGGCGCATTTTGTGGTTTT |
| TTK | TCATGCCCATTTGGAAGAGTC | CCACTTGGTTTAGATCCAGGC |
| MAD2L1 | GGACTCACCTTGCTTGTAACTAC | GATCACTGAACGGATTTCATCCT |

## Table VII. Mice primers used for qPCR

| Gene | Forward primer | Reverse Primer |
|---|---|---|
| Aldh1a3 | CAGCAATTTCCTCCCATCCG | CCTCCTAGCTCCAGTGTGAC |
| β-actin | CCAACCGCGAGAAGATGA | CCAGAGGCGTACAGGGATAG |

## Table VIII. Primers used for ChIP-qPCR

| Primer ID | Sequence |
|---|---|
| DLD NFYA biding-F | CCTAAAGCATTCCCACCACCA |
| DLD NFYA biding-R | CTACCGCACGAGGCGTT |
| IDH1 NFYA biding-F | GGGTAGGTCCGAGCTTTTCC |
| IDH1 NFYA biding-R | GAATTGGCGTGTGGCGATTG |
| PKM2 NFYA biding-F | GATTTATGGGTTCCGGGGCA |
| PKM2 NFYA biding-R | GCTCTCCCCAACTTTCCGTT |
| CCNA2 NFYA biding-F | ACGTCATTCAAGGCGACAGG |
| CCNA2 NFYA biding-R | GCCAGTTTGTTTCTCCCTCCT |
| CCNB1 NFYA biding-F | GTCACCTTCCAAAGGCCACT |
| CCNB1 NFYA biding-R | CACCTGGAGAGCAGTGAAGC |
| CDC20 NFYA biding-F | CGTGATAGCTGAGACTTTCCCC |
| CDC20 NFYA biding-R | GAGCGCCTATTGGCTCCTT |
| TTK NFYA biding-F | CCGTCGCACAAGAACCAATC |
| TTK NFYA biding-R | CGTTTGAATTTCCCCGTCGC |
| ALDH1A3 β-catenin biding-F | CCGCTTTGATCGCGAGTG |
| ALDH1A3 β-catenin biding-R | TCAAAGCCAGACTCGTCCAC |
| NFYA β-catenin biding-F | CGCCTGCGTATTCCTTTCAC |
| NFYA β-catenin biding-R | CCCTTCGGAGGAGCAACG |

## Supplemental Excel Files

**Excel File I. List of Genes for the Pathways in Figure 1A**

**Excel File II. List of Genes for the Pathways in Figure 3A**

## Supplemental Figures

*See following pages*

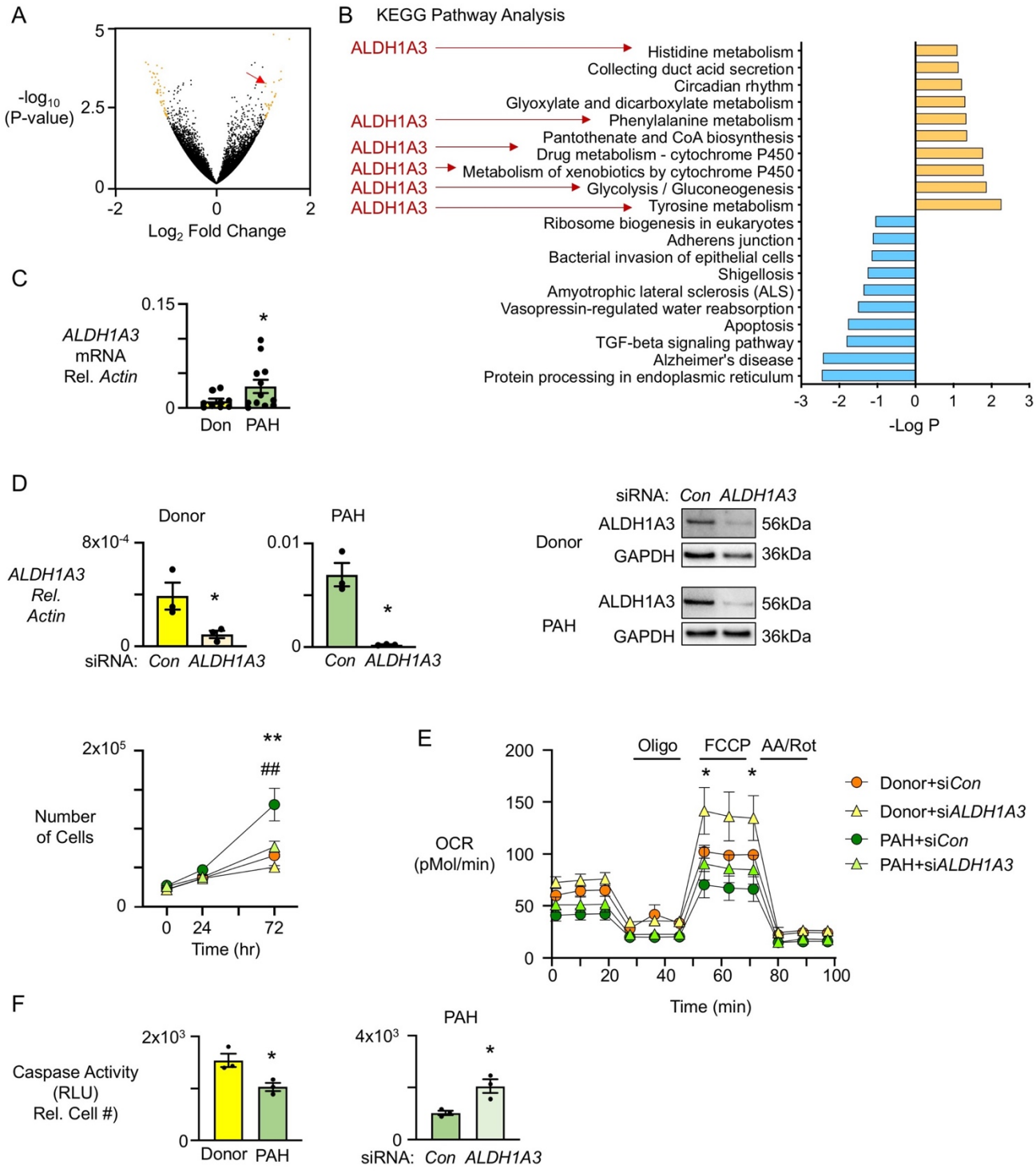


**Figure I (Panels A-F). ALDH1A3 is highly expressed in PAH PASMC.**

*Panels G-L on the next page, followed by the figure legend.*

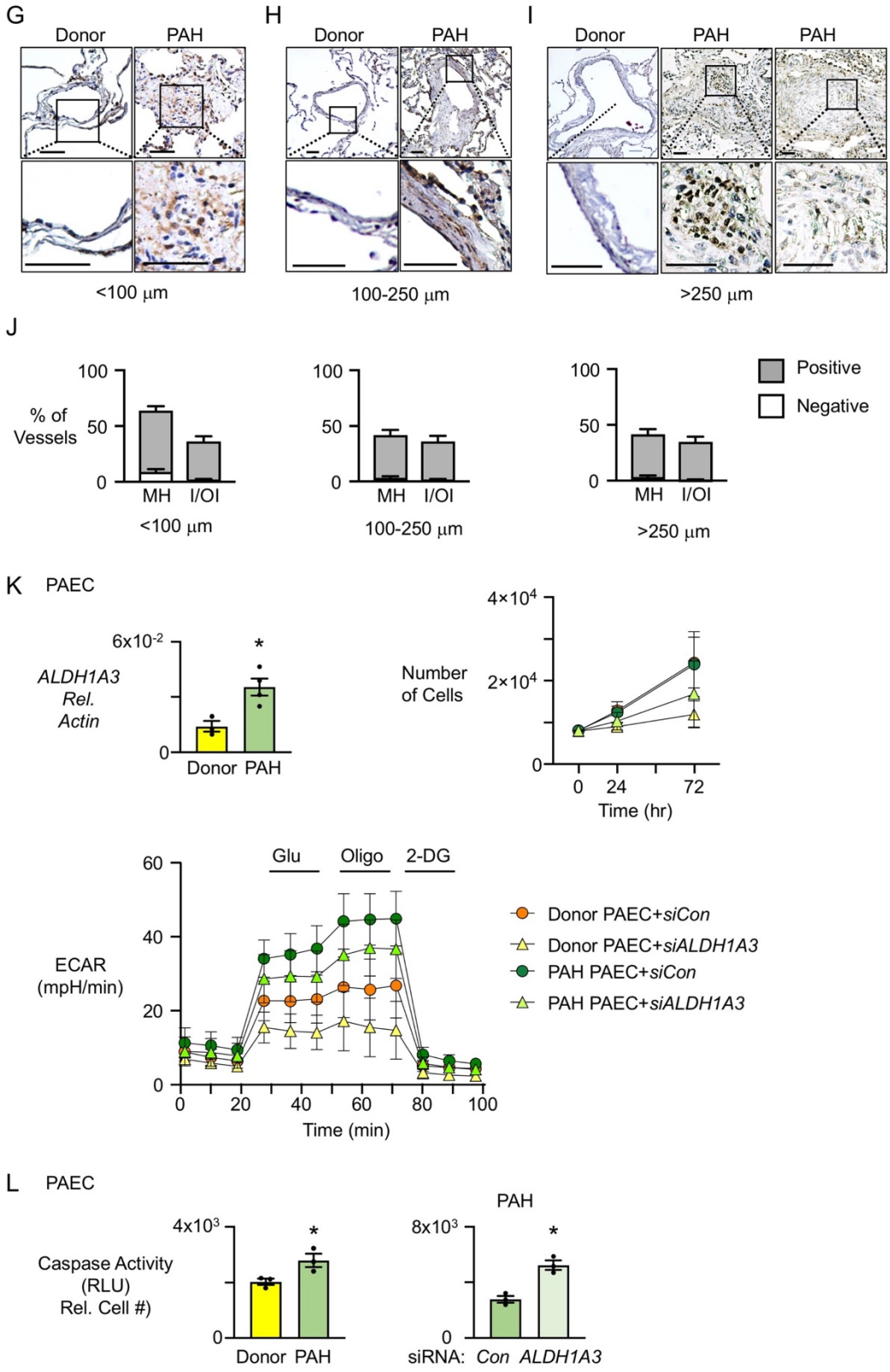


**Figure I (Panels G-L). ALDH1A3 is highly expressed in PAH PASMC.**

*See legend on the next page*

**Figure I. ALDH1A3 is highly expressed in PAH PASMC.**

**(A)** Volcano plot of significantly changed genes by RNA-seq in nine donor control vs. 12 PAH PASMC. with the position of the *ALDH1A3* transcript indicated by the Asterix. **(B)** Top ten enriched pathways from KEGG analysis ranked by p value and categories to which *ALDH1A3* is linked. Yellow and blue indicate pathways of up- or down-regulated genes, respectively. **(C)** qPCR mRNA levels of *ALDH1A3* in PASMC used in (A). *p<0.05 by unpaired Student t test.

**(D)** PASMC from Donor controls and PAH patients were transfected with non-targeting (Con) siRNA or *ALDH1A3* targeting siRNA. Left, *ALDH1A3* mRNA expression, *p<0.05, paired Student t test, and on the right, representative immunoblots of ALDH1A3 expression with GAPDH, following 48h of serum starvation and 72h serum stimulation in culture. Below: Cells were synchronized by culture for 48h under serum starvation (0 hr), then cultured under serum stimulation and counted at 24, 48 and 72 hours. n=3, **p<0.01, Donor siCon vs PAH siCon; ##p<0.01 PAH siCon vs PAH si*ALDH1A3*, at the indicated time point, by repeated measures two-way ANOVA followed by Bonferroni analysis.

**(E)** Oxygen consumption rate (OCR) in response to oligomycin (Oligo), fluoro-carbonyl cyanide phenylhydrazone (FCCP) and rotenone with antimycin A (AA/Rot). The measurements were normalized to cell number. Data represent mean±SEM; *p<0.05, n=3, Control siCon vs PAH siCon, by repeated measures 2-way ANOVA and Bonferroni post-test.

**(F)** Apoptosis assessed by Caspase-Glo 3/7 assay in PASMC from donor control and PAH, *p<0.05, n=3, unpaired student t test; PAH PASMC with control and ALDH1A3 siRNA, *p<0.05, n=3 paired student test.

**(G-J)** Representative immunohistochemistry for ALDH1A3 (brown) and hematoxylin counterstain (blue-purple) in **(G)** small peripheral PA (<100μm), **(H)** PA 100-250μm, and **(I)** large PA (>250μm) from one Donor and two PAH patients. Scale bars =50μM. **(J)** Pulmonary tissues from patients with PAH were analyzed for histopathologic signs of vascular disease in relation to ALDH1A3 protein expression. Vascular disease was scored as medial hypertrophy (MH), neointimal hyperplasia or occlusion including vessels with plexogenic features (I/Ol).

**(K)** Level of ALDH1A3 mRNA normalized to β-actin in PAEC from donor control and PAH patients. *p<0.05, n=3, unpaired student t-test (on the left); cell number of PAEC from donor control and PAH patients with control and ALDH1A3 siRNA, after serum starvation (0.2% FBS) (0h), serum stimulation (10% FBS) for 24h and 72h (on the right); ECAR under baseline conditions and in response to glucose (Glu), oligomycin (Oligo), and 2-deoxyglucose (2-DG), in PAEC from donor control and PAH patients with control or ALDH1A3 siRNA. Data represent mean±SEM, n=3, by repeated measures 2-way ANOVA and Bonferroni post-test (below).

**(L)** Apoptosis assessed by Caspase-Glo 3/7 assay in PAEC from donor control and PAH patients, *p<0.05, n=3, unpaired student t test (on the left); PAH PAEC with control and ALDH1A3 siRNA, *p<0.05, n=3 paired student test (on the right).

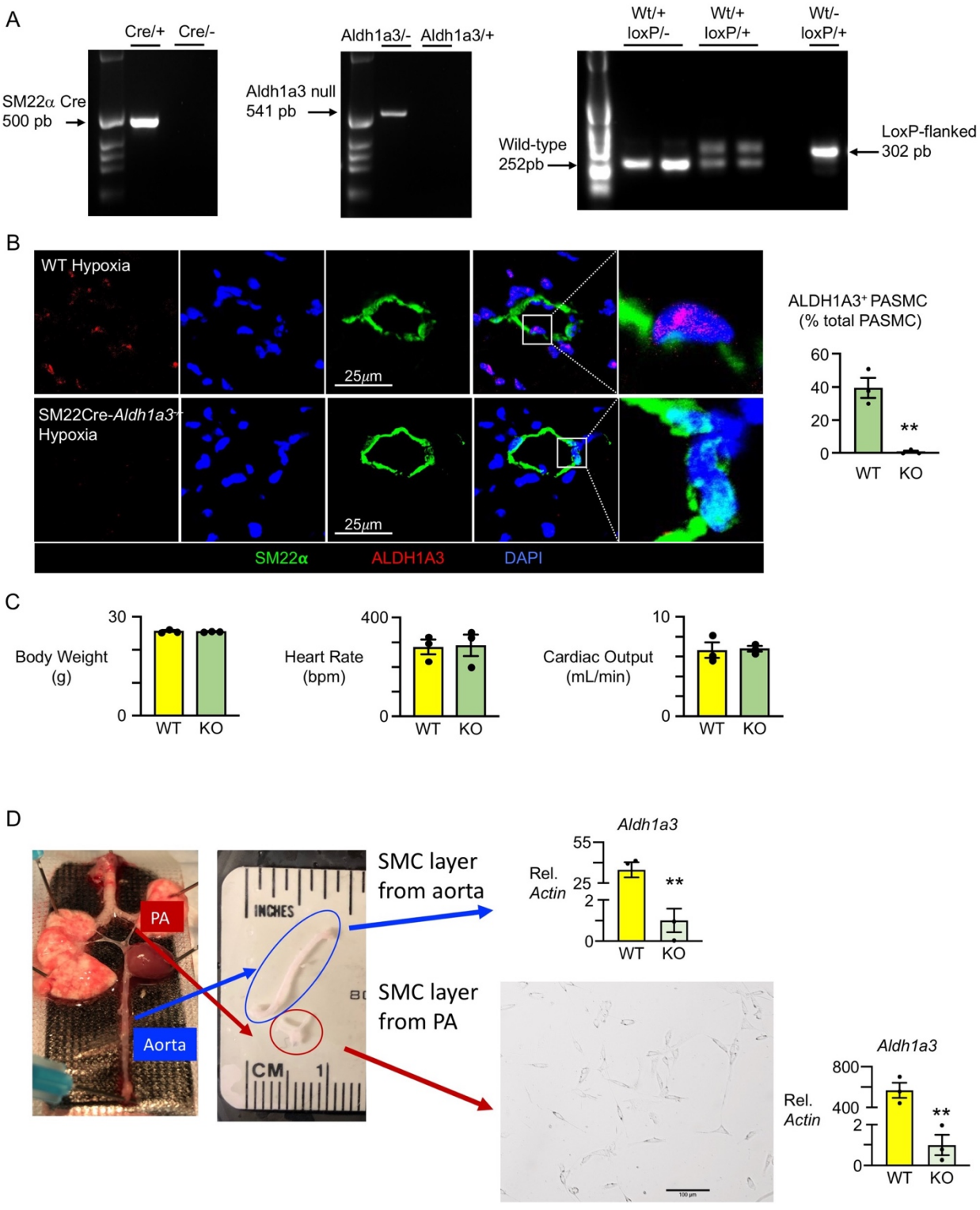


**Figure II (Panels A-D). Preserved proliferation and glycolysis in PASMC from littermate control (WT) vs. *SM22α-Aldh1a3*$^{-/-}$ (KO) mice.**

*Panels E, F, G on the next page, followed by the figure legend*

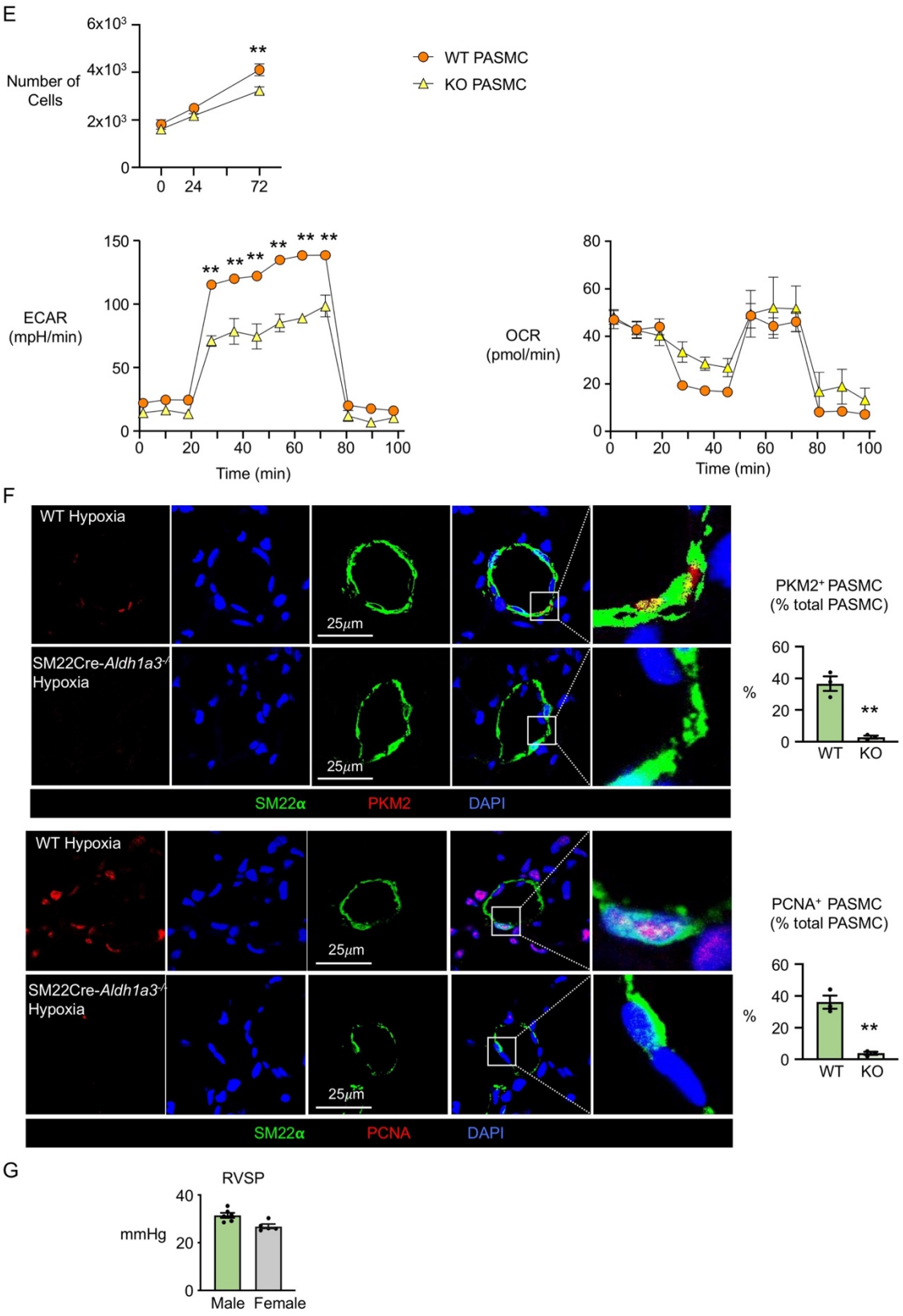


**Figure II (Panels E-G). Preserved proliferation and glycolysis in PASMC from littermate control (WT) vs. *SM22α-Aldh1a3*$^{-/-}$ (KO) mice.** *See legend on the next page*

**Figure II. Preserved proliferation and glycolysis in PASMC from littermate control (WT) vs. *SM22α-Aldh1a3*$^{-/-}$ (KO) mice.**

**(A)** PCR analysis of tail genomic DNA from mice with indicated SM22***α*** Cre-*Aldh1a3* genotype.

**(B)** SM22***α***(green), ALDH1A3 (red) fluorescent staining of pulmonary arteries from WT and KO mice under hypoxia and statistical analysis, n=3, **p<0.01, unpaired t-test.

**(C)** Body weight, heart rate and cardiac output of KO and littermate WT mice. n=3 males chosen at random as representative in each group.

**(D)** mRNA level of *Aldh1a3* in aorta and PASMC from WT and KO mice detected by qPCR, **p<0.01, n=3, unpaired student t test.

**(E)** Number of PASMC from WT and KO mice, following serum starvation (0.2% FBS) (0h), serum stimulation (10%FBS) for 24h and 72h; ECAR under baseline conditions and in response to glucose (Glu), oligomycin (Oligo), and 2-deoxyglucose (2-DG), in PASMC from WT and KO mice, **p<0.01, n=3; OCR in response to oligomycin (Oligo), fluoro-carbonyl cyanide phenylhydrazone (FCCP) and rotenone with antimycin A (AA/Rot). The measurements were normalized to cell number. Data represent mean±SEM; by repeated measures 2-way ANOVA and Bonferroni post-test.

**(F)** Top: SM22***α***(green), PKM2 (red) fluorescent staining of pulmonary arteries from WT and KO mice under hypoxia, with statistical analysis of n=3, **p<0.01, unpaired t-test; Bottom: SM22***α*** (green), PCNA (red) fluorescent staining of pulmonary arteries from WT and KO mice under hypoxia and statistical analysis, n=3, **p<0.01, unpaired t-test.

**(G)** Right Ventricle Systolic Pressure (RVSP) in six male mice and five female WT mice after three weeks hypoxia.

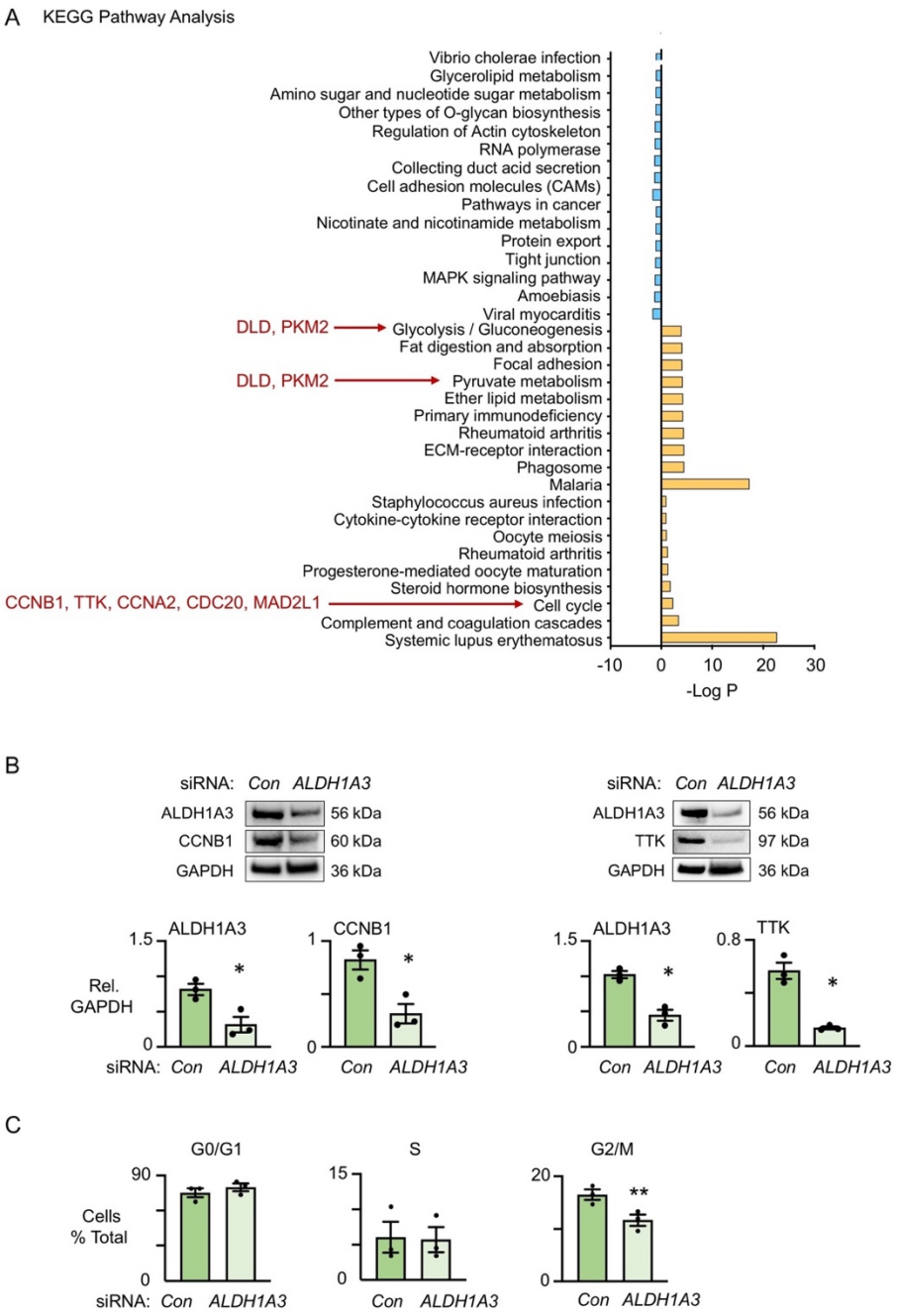


**Figure III. ALDH1A3 inhibition decreases cell cycle in PAH PASMC.**

**(A)** Enriched pathways by KEGG analysis from RNA-seq data of three PAH PASMC with non-targeting (Con) vs. *ALDH1A3* siRNA ranked by p value. Yellow and blue indicate pathways of up- or down-regulated genes, respectively.

**(B)** Representative immunoblots with quantification below of cyclin B1 (left, CCNB1) and TTK (right) protein expression relative to GAPDH in PAH PASMC with Con or *ALDH1A3* siRNA. n=3. *p<0.05 by Student t test.

**(C)** The fraction of cells in G0/G1, S and G2/M phase, detected by flow cytometry cell cycle analysis using propidium iodide DNA staining of PAH PASMC with control and *ALDH1A3* siRNA, n=3, **p<0.01, paired student t-test.

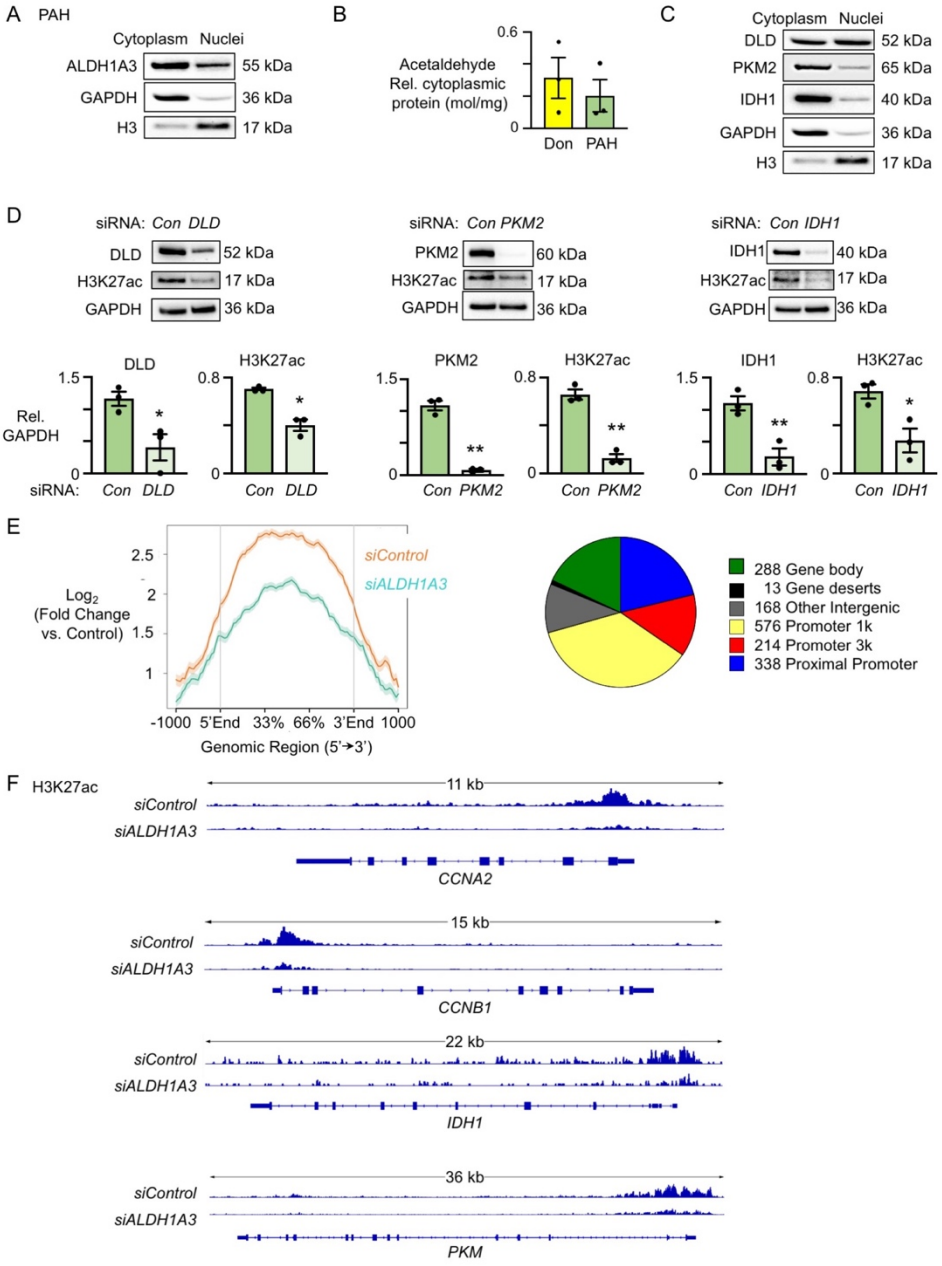


**Figure IV. ALDH1A3 inhibition decreases metabolic and cell cycle genes through H3K27ac**.
**(A)** Immunoblots of ALDH1A3 in cytoplasmic and nuclear fractions of PAH PASMC. **(B)** Levels of acetaldehyde in cytoplasm of PASMC of three donor control and three PAH patients, normalized to cytoplasmic protein (mol/mg)., no significant differences by unpaired Student t test. **(C)** Immunoblots of DLD, PKM2, and IDH1 in cytoplasmic and nuclear fractions of PAH PASMC. **(D)** Immunoblot and quantification of H3K27ac in PASMC of three PAH patients, treated with nontargeting (Con) or *DLD* siRNA (left panel); Con or *PKM2* siRNA (middle panel) and Con or *IDH1* siRNA (right panel). *p<0.05, **p<0.01 by paired Student t test. **(E)** The motif of decreased H3K27ac peak density and a pie chart of H3K37ac distribution of motifs with decreased H3K27ac peaks in PAH PASMC treated with *ALDH1A3* siRNA. **(F)** The H3K27ac density of *CCNA2*, *CCNB1*, *IDH1* and *PKM2* in PAH PASMC treated with Con vs. ALDH1A3 siRNA visualized in the IGV genome browser.

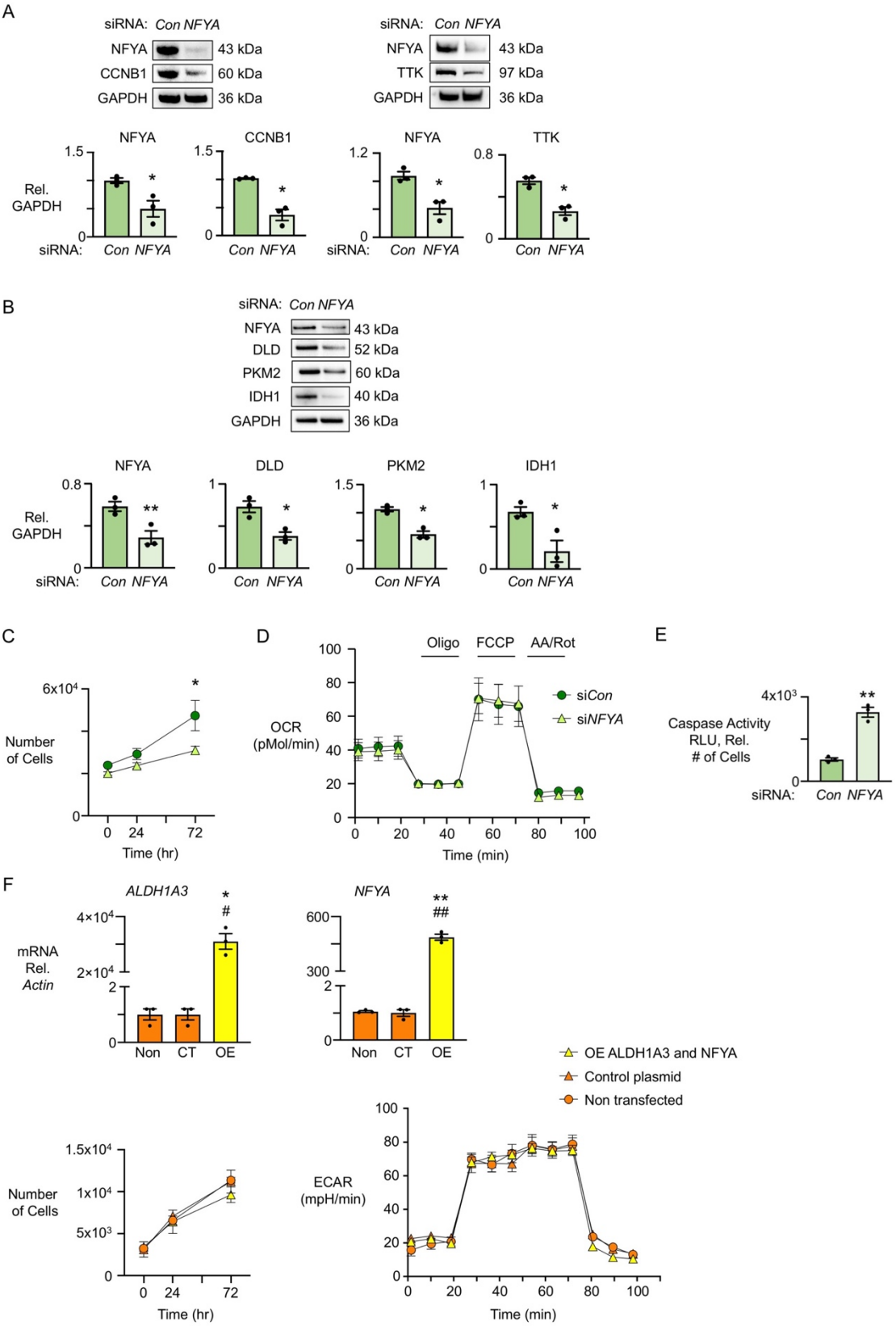


**Figure V. NFY inhibition decreases cell cycle genes**.
*See legend on the next page*

**Figure V. NFY inhibition decreases cell cycle genes**.

**(A)** Representative immunoblots of CCNB1 and TTK relative to GAPDH in PAH PASMC treated with nontargeting (Con) siRNA or *NFYA* siRNA, with quantification.

**(B)** Representative immunoblots of DLD, PKM2 and IDH1 relative to GAPDH in PASMC of three PAH patients treated with Con or *NFYA* siRNA, and quantification below., *p<0.05, **p<0.01 by paired Student t test. in A and B.

**(C)** PASMC from three PAH patients transfected with non-targeting (Con) siRNA or NFYA targeting siRNA. Cells were synchronized by culture for 48h under serum starvation (0hr), then cultured under serum stimulation. Cells were counted at 24h, 48h and 72h. *p<0.05, at the indicated time point, by repeated measures two-way ANOVA followed by Bonferroni analysis.

**(D)** Oxygen consumption rate (OCR) in response to oligomycin (Oligo), fluoro-carbonyl cyanide phenylhydrazone (FCCP) and rotenone with antimycin A (AA/Rot) in PAH PASMC with control and *NFYA* siRNA. The measurements were normalized to cell number. Data represent mean±SEM, n=3, by repeated measures 2-way ANOVA and Bonferroni post-test.

**(E)** Apoptosis assessed by Caspase-Glo 3/7 assay in PAH PASMC with control and NFYA siRNA, **p<0.01, n=3 paired student test.

**(F)** Level of *ALDH1A3* and *NFYA* mRNA, normalized to β-actin in PASMC from Donor controls transfected with control plasmid (CT) and with plasmid overexpressing ALDH1A3 and NFYA (OE), *p<0.05, OE vs. non-transfected cells (Non), #p<0.05, OE vs. CT, n=3, 1 way ANOVA (top); cell number of these three groups, after serum starvation (0.2% FBS) (0h), serum stimulation (10%FBS) for 24h and 72h (bottom left); ECAR under baseline conditions and in response to glucose (Glu), oligomycin (Oligo), and 2-deoxyglucose (2-DG), in these three groups. Data represent mean±SEM, n=3, by repeated measures 2-way ANOVA and Bonferroni post-test (bottom right).

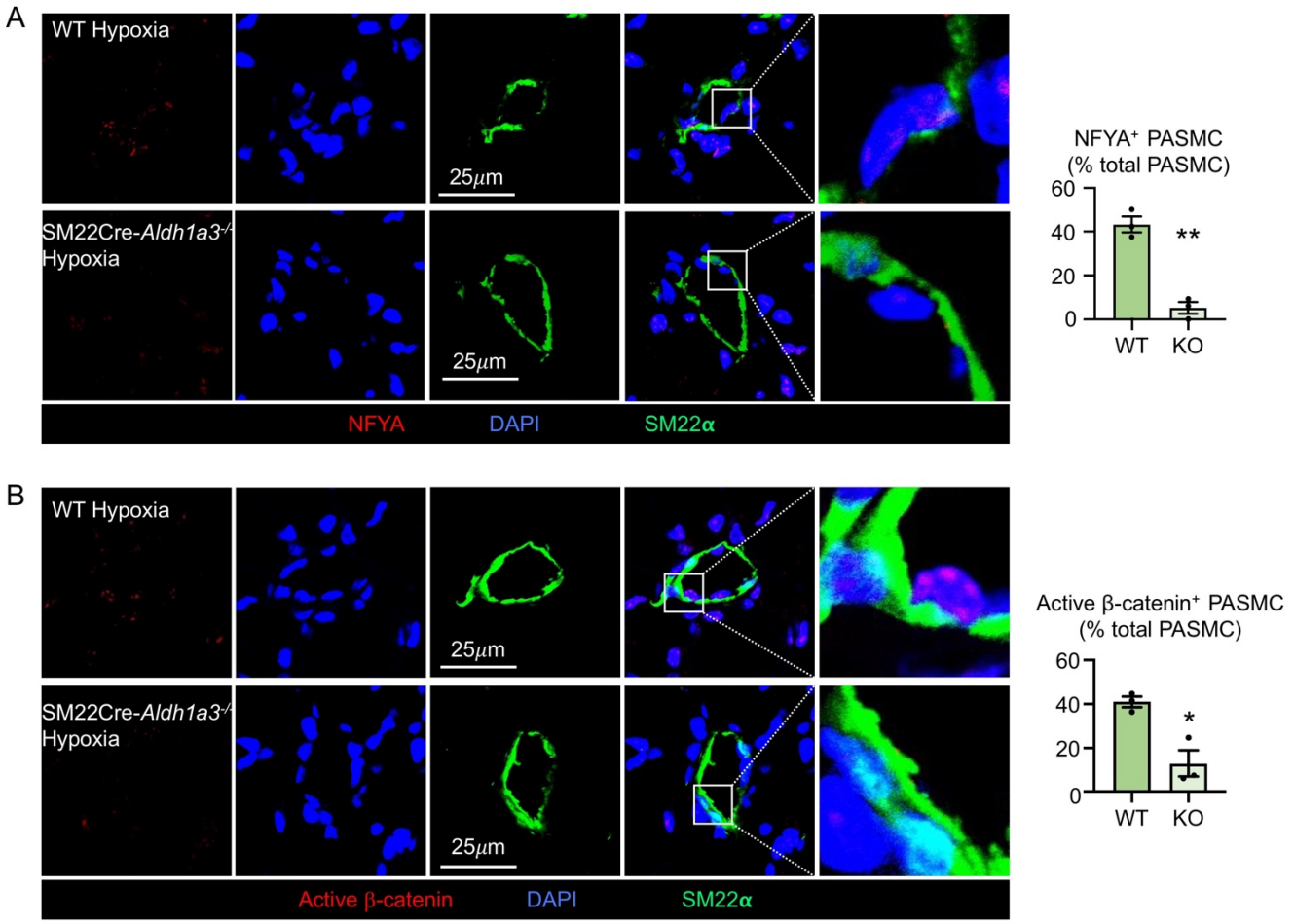


**Figure VI. Expression of markers in *SM22α-Aldh1a3*$^{-/-}$ (KO) and WT littermate mice**.

**(A)** SM22***α*** (green), NFYA (red) fluorescent staining of pulmonary arteries from WT and *SM22α-Aldh1a3*$^{-/-}$(KO) mice under hypoxia with statistical analysis, n=3, **p<0.01, unpaired t-test.

**(B)** SM22***α***(green), active β-catenin (red) fluorescent staining of pulmonary arteries from WT and KO mice under hypoxia and statistical analysis, n=3, *p<0.05, unpaired t-test.