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Methods

Isolated blood-free perfused and ventilated mouse lung

The model of isolated ventilated and blood-free perfused mouse lungs has been described previously [1]. Briefly, a switch from 21% O_2 (5.3% CO_2, rest N_2) in the ventilator gas supply to 1% O_2 (5.3% 5.3% CO_2, rest N_2) was used to induce HPV in the blood-free perfused mouse lungs. Repetitive hypoxic ventilation manoeuvers (10 min) were alternated with normoxic ventilation periods (15 min). Repetitive hypoxia-independent pulmonary vasoconstriction was induced by bolus injection of the thromboxane A2 mimetic U46619 (final concentration in the perfusate 190 nM) into the pulmonary arterial line. Increasing doses 0.01-1.00µM of MitoQ or decyl-TPP^+ as a control were added to the perfusate 5 minutes before each hypoxic ventilation period, or 5 minutes before bolus injection of U46619 which was used as hypoxia-independent vasoconstrictive stimulus. ΔPAP (increase in pulmonary arterial pressure) was calculated by subtracting the peak increase of PAP for each individual intervention (hypoxic ventilation or application of U46619) from the baseline PAP prior to the intervention. All gas concentrations were calculated for sea level conditions.

For ROS measurements, CMH was added 5 minutes before the hypoxic ventilation and lung homogenate taken 5 minutes after start of the hypoxic ventilation.

Isolation of pulmonary artery smooth muscle cells (PASMC)

PASMC were isolated from pulmonary precapillary arteries and cultured as described previously [2]. Briefly, the pulmonary artery was cannulated by a handmade cannula and M199 (Medium 199, Invitrogen, Carlsbad, USA) growth medium containing 5 mg/ml glow melting point agarose, 5mg/ml Fe_3O_4, 1% penicillin, and 1% streptomycin was injected into rats (12 ml) and mice (3 ml). In this mixture, iron particles do not pass through the capillaries

and therefore only precapillary arteries were filled with the rapidly solidifying agarose and iron. Lung tissue was minced with scissors for 5 min in 1ml PBS (phosphate buffered saline). The tissue mixture was then suspended in 10ml PBS in a falcon tube, which was placed in a magnetic holder. The pulmonary arteries containing the iron particles and agarose accumulate on the tubing walls. The supernatant was aspirated, and the arteries, after rinsing 3 times with PBS, were transferred into Petri dishes containing 10ml of M199 with 80U/ml collagenase and were then incubated at 37°C for 60 min. The tissue mixture was disrupted by drawing it through 15 and 18 gauge needles 5-6 times each. The resulting suspension containing the medial layer of the arteries attached to iron particles was placed in clear plastic tubes and rinsed three times with M199 containing 10% FCS (fetal calf serum) in the magnetic holder, as described above. The medial layer of the pulmonary artery attached to iron was finally re-suspended in medium, transferred to culture flasks and incubated at 37°C in the cell incubator for 4 to 5 days. Upon reaching 80% confluence, the grown cells were trypsinized and divided into the fresh culture flasks.

Isolation of primary lung fibroblast (LF)

Primary lung fibroblasts were isolated as described [3]. Briefly, mouse lungs were minced and incubated in 2mg/ml preheated collagenase P (Hoffmann-La Roche, Basel, Switzerland) for 45min at 37°C. Afterwards, the tissue mixture was disrupted by drawing it through 15 and 18 gauge needles 5-6 times each, filtered through a 40µm strainer and centrifuged at 4°C for 8min at 400g. The cell pellet was re-suspended in 5 ml 10% FBS, DMEM (Thermo Fisher Scientific, Waltham, USA). LF from passage 1 were used for experiments.

Measurement of ROS release by electron spin resonance spectroscopy

Intracellular and extracellular concentrations of ROS and reactive nitrogen species (RNS) were determined in PASMC and tissues by means of an ESR spectrometer (EMXmicro, Bruker, Rheinstetten, Germany), using the spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH, 0.5 mM) [4, 5] or 1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (CPH, 0.5mM) (Noxygen, Elzach, Germany). The superoxide portion of ROS/RNS was determined by subtracting the ESR signal of the samples incubated with 50 Units/ml polyethylen-glycol conjugated superoxide dismutase (pSOD) from the signal of the CMH-only samples. Incubation and measurements were performed in a specific buffer for ESR measurements (99.0 mM NaCl, 4.69 mM KCl**,** 2.5 mM $CaCl_2$ x $2H_2O$, 1.2 mM $MgSO_4$ x $7H_2O$, 25 mM $NaHCO_3$, 1.03 mM KH_2PO_4, 5.6 mM D(+) Glucose, 20 mM Na-HEPES, 25 µM deferoxamine, 5 µM diethyldithiocarbamate). Approximately 100,000 precapillary mouse PASMC from passage 1 were used per sample. Incubation was started at the time point corresponding to the addition of pSOD. After 90 minutes, MitoQ, or alternatively decyl-TPP^+ was added in doses of 0.1 and 0.5µM, followed by the addition of CMH after another 5 minutes, followed by an incubation period of 30 minutes. For the hypoxic samples, hypoxia was applied by exposing the PASMC for 5 minutes (acute hypoxic experiments) to a 37 °C warm atmosphere containing 1% O_2 (rest N_2) within a hypoxic glove chamber (Coy Laboratory Products, Coy Drive, USA). Normoxic samples, which were handled in parallel, were incubated in 21% O_2 at 37°C. After the end of the incubation period, all samples were immediately frozen in liquid nitrogen. In order to avoid reoxygenation, the hypoxic samples were frozen directly within the hypoxic glove chamber.

For chronic hypoxic experiments, PASMC were incubated for 5 days in a 37 °C warm atmosphere containing 1% O_2 (5% CO_2, rest N_2). Afterwards, the measurement of superoxide concentration was performed as described above. All manipulations were performed in a hypoxic glove chamber under continuous hypoxic conditions.

To investigate the superoxide levels within lung and heart tissues in chronic hypoxia, mice exposed to hypoxia (10% O_2) for 4 weeks were sacrificed under hypoxia and superoxide concentration was measured by ESR microscopy as described above.

For isolated lung experiments only CMH and not the CMH minus CMH/pSOD signal was used, as perfusion of the lungs with pSOD for 2h prior to measurement was not possible. However, the CMH signal represents the total ROS/RNS signal without specification for superoxide [4, 5].

X-band (9.65 GHz) ESR spectra of the frozen samples were recorded by the EMXmicro spectrometer using the following acquisition parameters: G-factor 2.0063, center field ~3366 G, modulation amplitude 2.999 G, receiver gain 50 dB and an attenuation of 20 dB (resulting in a microwave power of 2mW).

Chronic hypoxic exposure, pulmonary arterial banding, treatment with MitoQ and determination of PH

For induction of chronic hypoxia-induced PH, mice were kept under normobaric hypoxia (10% O_2) in a ventilated chamber for 28 days. Banding of the main pulmonary artery (PAB) was performed as described previously [6]. During chronic hypoxic incubation and after PAB, mice were treated with MitoQ or decyl-TPP^+, dissolved in tapped water, at a concentration of 50mg/kg/day by gavage[7]. Control mice were kept in a normoxic chamber and also treated with MitoQ or TPP^+. Development of PH was determined by measurement of right ventricular systolic pressure (RVSP), pulmonary vasculature remodeling, and right ventricular (RV) hypertrophy, as described previously[8]. Global and right heart function were measured by transthoracic echocardiography [9].

ROS measurement by the fluorescent dye MitoSOX and the fluorescent protein $HyPer_{cyto}$

For acute hypoxic experiments, mitochondrial ROS and cytosolic H_2O_2 concentrations were measured in PASMC by MitoSOX and $HyPer_{cyto}$, respectively. 5µM MitoSOX (Invitrogen, Carlsbad, USA) was used for detection of mitochondrial ROS as described [2]. Prior to the acute hypoxic exposure precapillary PASMC were incubated for 1 h in 5µM MitoSOX diluted in HRB buffer (HRB; 136.4 mM NaCl, 5.6 mM KCl, 1 mM $MgCl_2$, 2.2 mM $CaCl_2$, 10 mM Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], 5 mM glucose, pH 7.4) at 37°C. After accumulation in mitochondria, MitoSOX becomes fluorescent upon oxidation by different ROS [10]. For intracellular H_2O_2 detection, the coding information for the H_2O_2-sensitive, enhanced yellow fluorescent protein variant $HyPer_{cyto}$ (cytosolic hydrogen peroxide sensor) from Evrogen Company (Moscow, Russian Federation) [11] was subcloned under the control of the EF-1α (human elongation factor-1 alpha) enhancer/promoter into the pWPXL plasmid (distributed by Addgene, Boston, USA) and packed with a second-generation lentivirus transduction system with pMD2.G, as the envelope and psPAX2, as a packing vector (Addgene, Boston, USA). Lentiviral transduction was carried out with a titer of at least 1×10^7 particles according to established protocols (see http://tronolab.epfl.ch/ for more details). $HyPer_{cyto}$ has two excitation peaks with maxima at 420 nm and 500 nm, and one emission peak with a maximum at 516 nm. H_2O_2 increases the excitation at 500nm and decreases the excitations at 420nm of the $HyPer_{cyto}$ construct, allowing ratiometric measurements. Fluorescent signals for both MitoSOX and $Hyper_{cyto}$ were collected from individual PASMC.

Acute hypoxia was induced by switching from normoxic perfusion buffer (with a flow of 1 ml/min) to hypoxic buffer in a closed perfusion chamber (PeCon, Germany). For control experiments the normoxic perfusion buffer was switched to a second normoxic perfusion

buffer. Fluorescent signals were analyzed using a Polychrome II monochromator and IMAGO CCD camera (Till Photonics, Germany) coupled to an inverted microscope (IX70; Olympus, Germany).

To measure the level of mitochondrial ROS concentration in chronic hypoxia, PASMC were incubated for 4 days in 1% O_2, and subsequently split under hypoxia (1% O_2) within a hypoxic glove chamber (Coy Laboratory, USA). 30.000 PASMC were seeded into 96 multiwell plates. The next day, the PASMC were incubated with 5µM MitoSOX (Sigma-Aldrich, Germany) for 20 min at 37 °C. After the incubation, cells were washed twice with PBS under hypoxic atmosphere. The plates were sealed air-tight and moved to the microplate reader. The fluorescence signal from wells was measured using a microplate reader (Tecan Group Ltd., Männedorf, Swiss). Excitation and emission wavelength was set to 405/580nm. The experiments were performed in a light protected environment.

To measure H_2O_2, PASMC were infected with $HyPer_{cyto}$ lentivirus and afterwards transferred into the hypoxic chamber (1% O_2) for 5 days. H_2O_2 was measured in a closed perfusion chamber (PeCon, Germany) which was assembled and sealed under hypoxia in the hypoxic gloves box. Excitation and emission parameters of $HyPer_{cyto}$ were used as described above.

Proliferation assay

Proliferation of precapillary mouse PASMC was evaluated by determination of the ratio of proliferating cells labeled by 5-ethynyl-uridine (EdU) to the total cell number labeled by Hoechst staining as described previously [8]. Chronic hypoxia was simulated by incubation of PASMCs for 5 days in hypoxic atmosphere (1% O_2). Normoxic experiments were conducted in an atmosphere of 21% O_2. MitoQ or TPP^+ in doses of 0.1 and 0.5µM were applied during the whole time period of normoxic/hypoxic incubation.

DNA damage

DNA damage was evaluated by measurement of 8-hydroxyguanosine levels using DNA Damage Competitive ELISA (Thermo Fisher Scientific, Waltham, USA) according to the instructions of the manual. 8-hydroxyguanosine, which is the oxidative derivative of guanosine, is characteristic of DNA damage. PASMC from passage 1 were seeded into a 6-well plate and incubated for 5 days in 1% O_2 (chronic hypoxia) or in 21% O_2 (normoxia). Afterwards, cell growth medium (see above) was collected, PASMC were homogenized in phosphate-buffered saline with 1x protease inhibitor cocktail (Sigma-Aldrich, Germany) using the bead homogenizer Precellys[24] (Omni International, Kennesaw, USA), and DNA was extracted using QuickExtract™ DNA Extraction Solution (Epicentre, USA). DNA concentration was measured by NanoDrop (PeqLab, Erlangen, Germany) and protein concentration was measured by RC DC™ Protein Assay (Bio-Rad, Hercules, USA) according manual. Afterwards DNA and protein concentration were normalized.

Determination of the antioxidative capacity

PASMC from passage 1 were seeded into 6-well plates and incubated for 5 min or 5 days in 1% O_2 (hypoxia) or in 21% O_2 (normoxia). Afterwards, PASMC were homogenized in phosphate-buffered saline with 1x protease inhibitor cocktail (Sigma-Aldrich, Germany) using the bead homogenizer Precellys[24] (Omni International, Kennesaw, USA). PASMC homogenates were normalized for protein concentration, which was measured using the RC DC™ protein assay (Bio-Rad, Hercules, USA). SOD (Thermo Fisher Scientific, Waltham, USA), Catalase (Abcam, Cambridge, UK) and total antioxidant capacity (Abcam, Cambridge, UK) were measured according to the manufacturer's instructions.

Western blot

Cell lysates were separated on a 10 % SDS (sodium dodecyl sulfate) polyacrylamide gel, followed by electrotransfer to a 0.45µm PVDF (polyvinylidene fluoride) membrane (Pall Corporation, Dreieich, Germany). After blocking with 5% non-fat dry milk in TBS-T buffer (Tris Buffer Saline + 0.1% Tween 20) for 1 hour, the membrane was incubated overnight at room temperature with one of the following antibodies: anti-SOD1 (rabbit antibody, dilution 1:1000, Abcam Cambridge, UK), anti-SOD2 (mouse antibody, dilution 1:1000, Abcam Cambridge, UK), anti-Catalase (rabbit antibody, dilution 1:1000, Abcam Cambridge, UK), anti-PDK1 (rabbit antibody, dilution 1:1000, Cell Signaling Technology, Inc. Danvers, USA), anti-LDHA (rabbit antibody, dilution 1:1000, Cell Signaling Technology, Inc. Danvers, USA), anti-GPX2, 4 (rabbit antibody, dilution 1:200, Abcam Cambridge, UK), anti-GPX3 (rabbit antibody, dilution 1:1000, Novus Biologicals, LLC) and monoclonal mouse anti-β-actin (dilution 1:50000, Sigma-Aldrich, St. Louis, USA). After washing the membranes in TBS-T buffer, specific immunoreactive signals were detected by enhanced chemiluminescence (Bi-Rad, Hercules, USA) using a proprietary secondary antibody coupled to horseradish-peroxidase diluted 1:5000.

RNA isolation and real-time polymerase chain reaction (PCR)

Total RNA (1µg), extracted from isolated PASMC or lung homogenate by RNeasy Micro Kit (Qiagen N.V., Hilden, Germany) was reverse-transcribed using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, USA). As housekeeping genes β2M (β2-microglobulin) was used.

PCR analysis of Mitochondrial DNA copy number

PASMC from passage 1 were seeded into 6 well plates and incubated for 5 days in 1% O_2 (hypoxia) or in 21% O_2 (normoxia). DNA was extracted using QuickExtract™ DNA Extraction Solution (Epicentre, USA). Afterwards, real-time PCR (polymerase chain reaction) was performed using a master mix for RT PCR (iTaq SYBR Green supermix with ROX, Bio-Rad, Hercules, USA) and Mx3000P qPCR Systems (Agilent Technologies, Santa Clara, USA). Primers for mitochondrial NADH-ubiquinone oxidoreductase chain 1 (ND1) and genomic hexokinase 2 (HK2) were the following:

ND1 f	CTAGCAGAAACAAACCGGGC
ND1 r	CCGGCTGCGTATTCTACGTT
HK2 f	GCCAGCCTCTCCTGATTTTAGTGT
HK2 r	GGGAACACAAAAGACCTCTTCTGG

Determination of pO_2, pCO_2 and pH

Values of pO_2, pCO_2 and pH were measured in the perfusate of isolated lung experiments, which was collected from the left ventricle (which corresponds to the outflow line of the lung), in cell growth medium from PASMC exposed for 5 days to 1% O_2 and in ESR buffer after 5 min exposure of PASMC to 1% O_2. pO_2 was determined by a LICOX CMP tissue oxygen pressure monitor (GMS, Mielkendorf, Switzerland), pCO_2 and pH by a Rapidlab Blood Gas Analyzer 348 (Siemens, Erlangen, Germany) according to the manufacturer's instructions. Moreover, pO_2 was determined in the closed perfusion chamber by a LICOX CMP tissue oxygen pressure monitor (GMS, Mielkendorf, Switzerland).

Figure S1

Pulmonary vasoconstriction in isolated mouse lungs in the different experimental groups prior to substance application

(A) Increase of pulmonary arterial pressure (ΔPAP) during acute hypoxic ventilation (1% O_2, 5.3% CO_2, rest N_2) or (B) after bolus injection of the the thromboxane mimetic U46619 in the different experimental groups (MitoQ, TPP^+, control) in absence of MitoQ or TPP^+.

n=5-6 isolated lungs each.

Figure S1

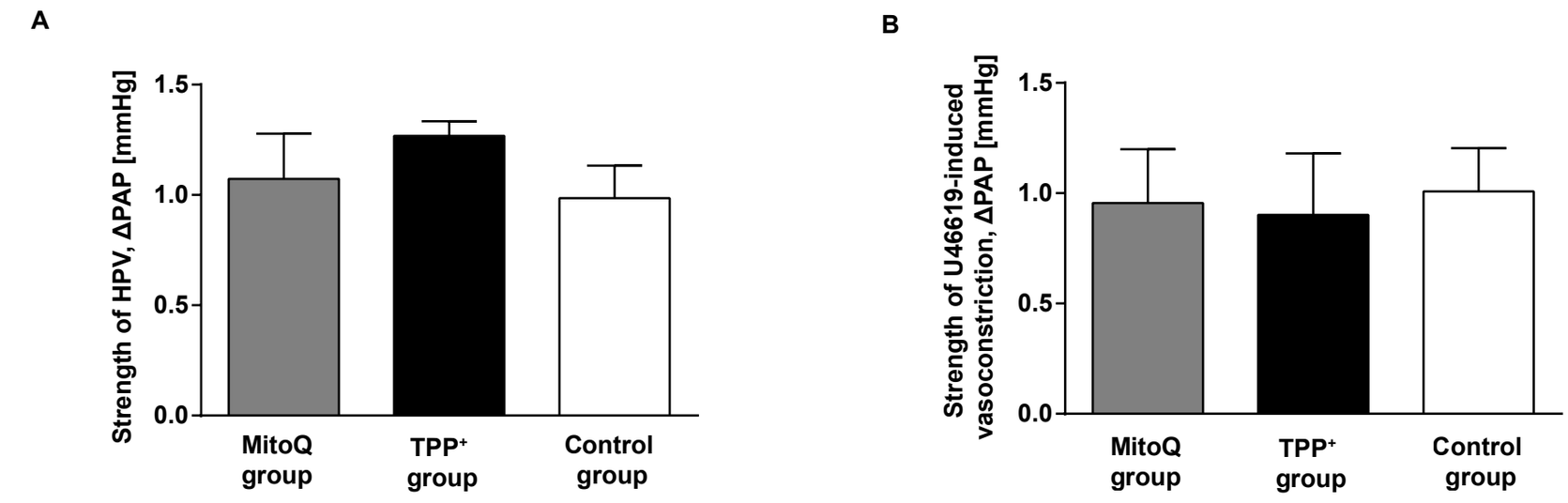

A
Strength of HPV, ΔPAP [mmHg]
1.5
1.0
0.5
0.0
MitoQ
TPP+
Control
group
B
Strength of U46619-induced vasoconstriction, ΔPAP [mmHg]
1.5
1.0
0.5
0.0
MitoQ
TPP+
Control
group

Figure S2

Levels of pO_2, pCO_2 and pH under different hypoxic conditions

(A) pO_2 values, (B) pCO_2 values and (C) pH, determined in the perfusate collected from the left ventricle in isolated ventilated and blood-free perfused mouse lungs during repetitive ventilation with normoxic (21%, 5% CO_2 rest N_2) and hypoxic (1% O_2, 5% CO_2 , rest N_2) gas.

D) Time course of pO_2 in the closed chamber of the fluorescence microscope, perfused with Hepes-Ringer buffer pre-gassed with N_2.

(E) pO_2, pCO_2, and (F) pH in the ESR buffer and cell growth medium during exposure of PASMC to an atmosphere containing 1% O_2 (rest N2) for 5 min and to an atmosphere of 1% O_2 (5% CO2, rest N2) for 5 days, respectively.

Figure S2

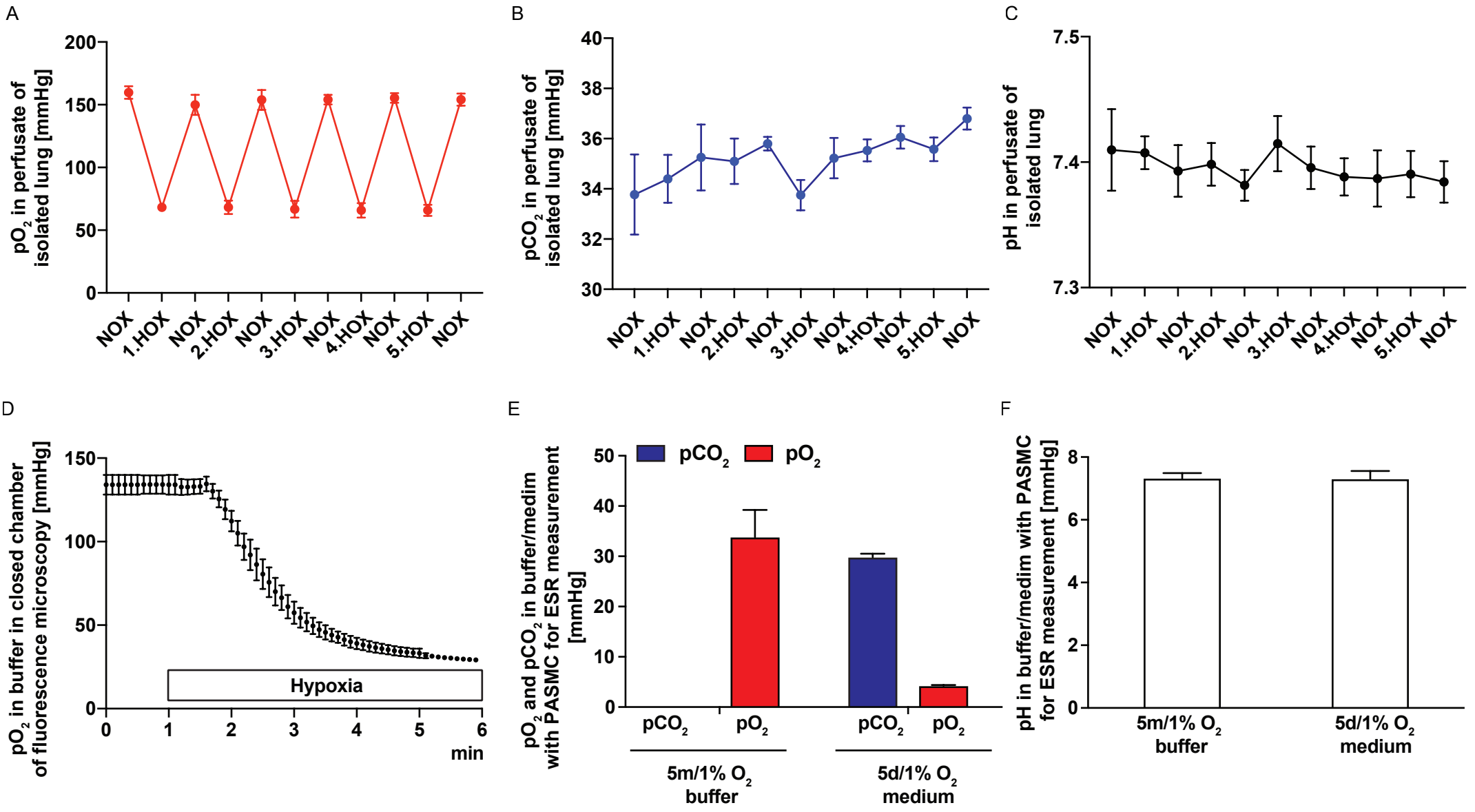
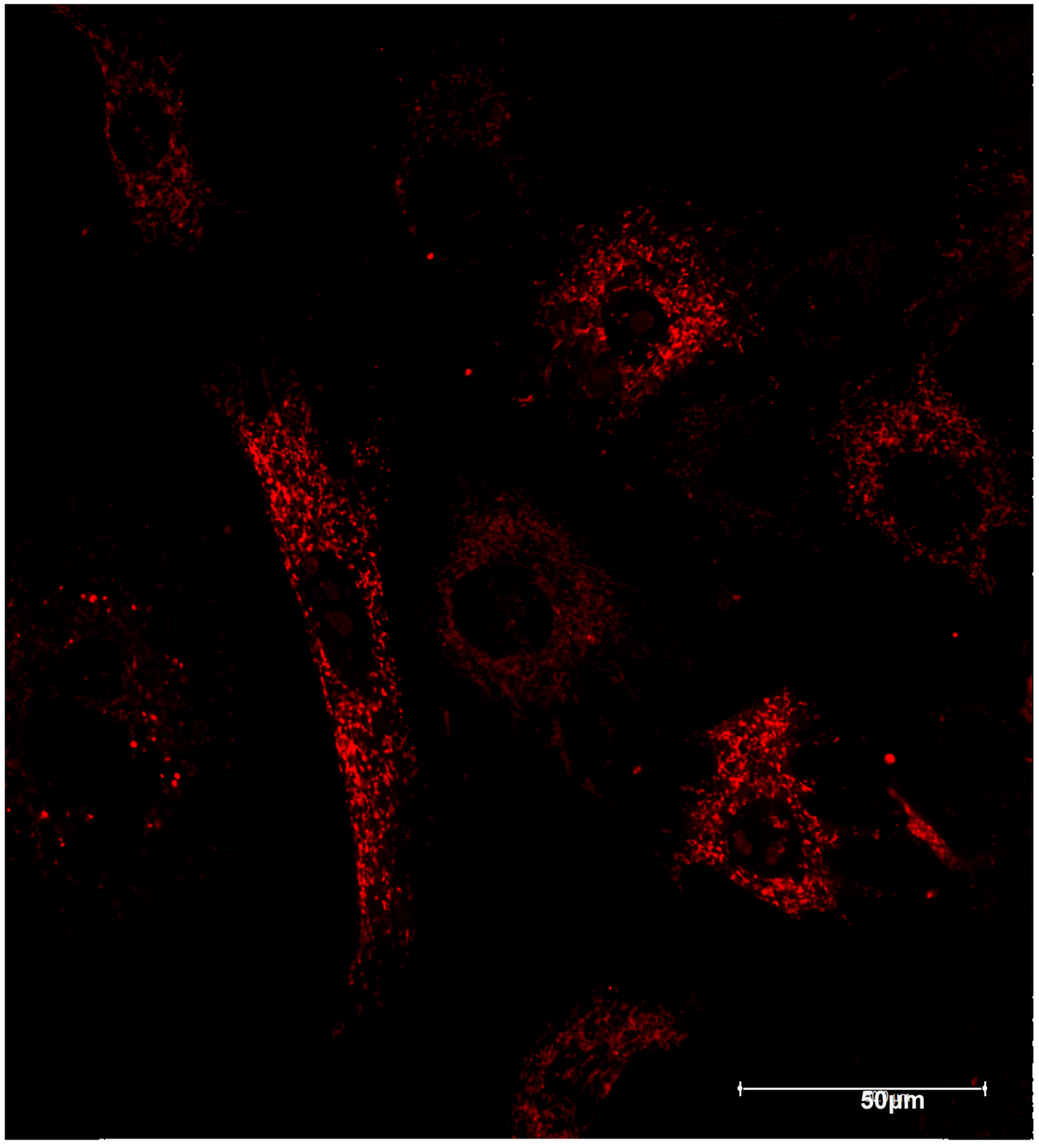

A
pO_2 in perfusate of isolated lung [mmHg]
200
150
100
50
0
NOX
1.HOX
NOX
2.HOX
NOX
3.HOX
NOX
4.HOX
NOX
5.HOX
NOX
B
pCO_2 in perfusate of isolated lung [mmHg]
40
38
36
34
32
30
NOX
1.HOX
NOX
2.HOX
NOX
3.HOX
NOX
4.HOX
NOX
5.HOX
NOX
C
pH in perfusate of isolated lung
7.5
7.4
7.3
NOX
1.HOX
NOX
2.HOX
NOX
3.HOX
NOX
4.HOX
NOX
5.HOX
NOX
D
pO_2 in buffer in closed chamber of fluorescence microscopy [mmHg]
150
100
50
0
Hypoxia
0
1
2
3
4
5
6
min
E
pO_2 and pCO_2 in buffer/medim with PASMC for ESR measurement [mmHg]
pCO_2
pO_2
50
40
30
20
10
0
pCO_2
pO_2
pCO_2
pO_2
5m/1% O_2 buffer
5d/1% O_2 medium
F
pH in buffer/medim with PASMC for ESR measurement [mmHg]
8
6
4
2
0
5m/1% O_2 buffer
5d/1% O_2 medium

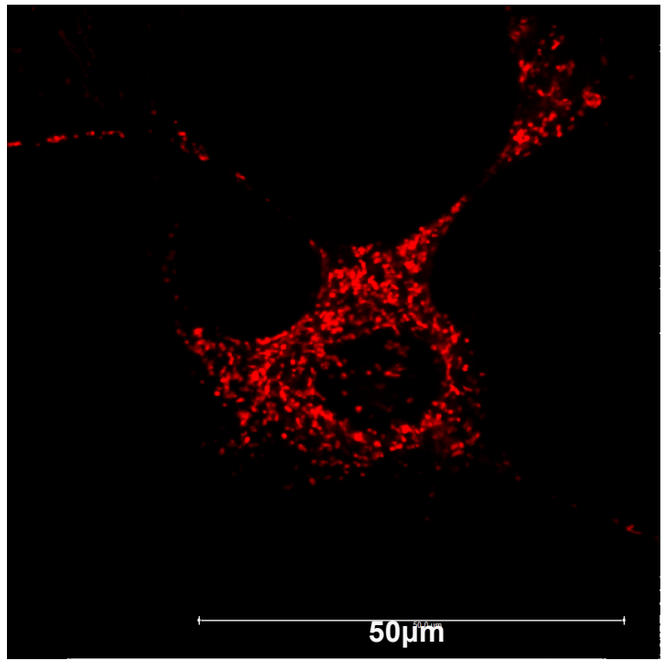
Figure S3

Representative pictures of PASMC stained with MitoSOX

PASMC were incubated with 5μM MitoSOX diluted in Hepes-Ringer buffer (HRB) for 15 min. After incubation, PASMC were washed three times with HRB buffer and the fluorescent signal was analyzed by a Leica TCS SP5 confocal microscope (Leica Microsystems, Wetzlar, Germany).

Figure S3





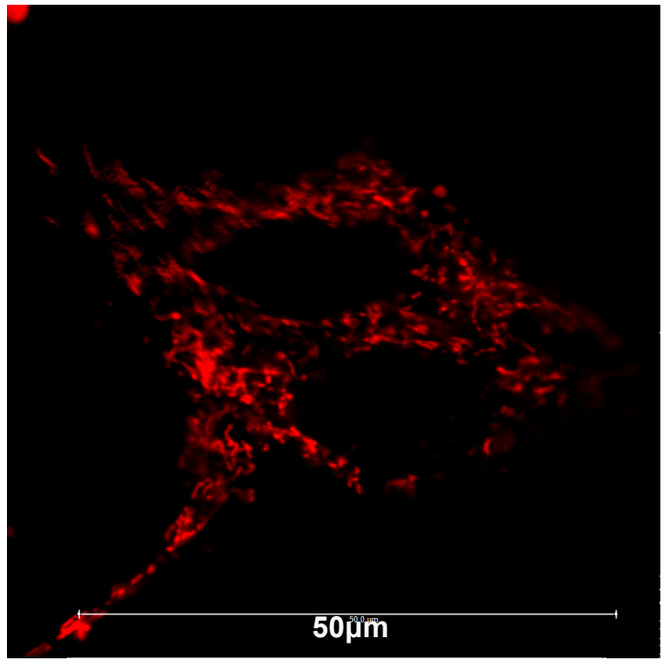


Figure S4

Effect of TPP^+ on development of pulmonary hypertension

(A) Right ventricular systolic pressure (RVSP), (B) cardiac output (CO), (C) tricuspid annular plane systolic excursion (TAPSE), (D) ratio of RV mass to the mass of left ventricle plus septum (RV/[LV+Septum]), (E) right ventricular wall thickness (RVWT), (F) right ventricular internal diameter (RVID) and (G) right ventricular outflow tract diameter (RVOTD) in TPP^+ and untreated (control) mice exposed for 4 weeks to chronic hypoxia (4w HOX) or normoxia (NOX). n=5-8 animals per group, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001 and †† p<0.01, ††† p<0.001 interaction by two-way ANOVA with Tukey post hoc test.

H) Superoxide concentration in RV homogenate from TPP^+ and untreated (control) mice exposed for 4 weeks to chronic hypoxia (4w HOX) or normoxia (NOX). The superoxide concentration was measured by ESR spectroscopy as difference in the CMH signal with or without pegylated superoxide dismutase (ΔpSOD)**.** Data are normalized to RV mass (g). n=3-5 * p<0.05 by two-way ANOVA with Tukey post.

Figure S4

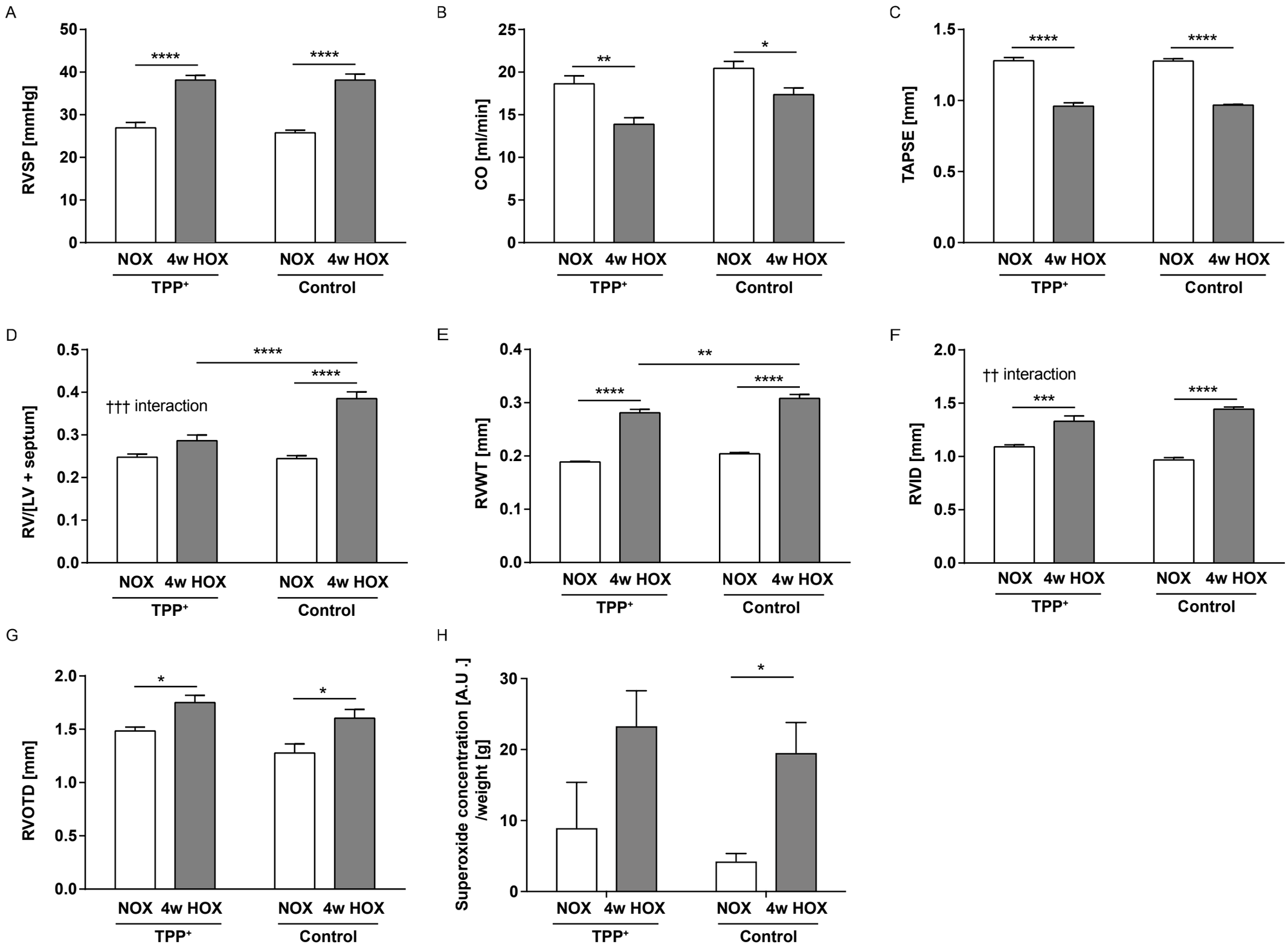

A
RVSP [mmHg]
50
40
30
20
10
0
****
****
NOX
4w HOX
NOX
4w HOX
TPP+
Control
B
CO [ml/min]
25
20
15
10
5
0
**
*
NOX
4w HOX
NOX
4w HOX
TPP+
Control
C
TAPSE [mm]
1.5
1.0
0.5
0.0
****
****
NOX
4w HOX
NOX
4w HOX
TPP+
Control
D
RV/[LV + septum]
0.5
0.4
0.3
0.2
0.1
0.0
†††
interaction
****
****
NOX
4w HOX
NOX
4w HOX
TPP+
Control
E
RVWT [mm]
0.4
0.3
0.2
0.1
0.0
**
****
****
NOX
4w HOX
NOX
4w HOX
TPP+
Control
F
RVID [mm]
2.0
1.5
1.0
0.5
0.0
††
interaction
***
****
NOX
4w HOX
NOX
4w HOX
TPP+
Control
G
RVOTD [mm]
2.0
1.5
1.0
0.5
0.0
*
*
NOX
4w HOX
NOX
4w HOX
TPP+
Control
H
Superoxide concentration [A.U.]
/weight [g]
30
20
10
0
*
NOX
4w HOX
NOX
4w HOX
TPP+
Control

Figure S5

Expression and activity of antioxidative enzymes in chronic hypoxic lung tissue or pulmonary arterial smooth muscle cells

(A) mRNA expression of various enzymes of the antioxidative system in lung homogenate from mice exposed to 10% O_2 for 4 weeks and in (B) PASMC exposed for 5 days to 1% O_2. (C) Protein level of GPX 2, 3, 4 and (D) SOD1/2 in PASMC exposed to 1% O_2 for 5 days. (E) SOD activity and (F) catalase activity in cell lysates from PASMC exposed to 1% O_2 for 5min or 5 days. (G) Total antioxidative capacity in cell homogenates from PASMC exposed to 1% O_2 for 5 days.

n=4 per group, *p<0.05, **p<0.01, *** p<0.001 and **** p<0.0001 compared to normoxic control by Student's *t* test with Welsh's correction.

Figure S5

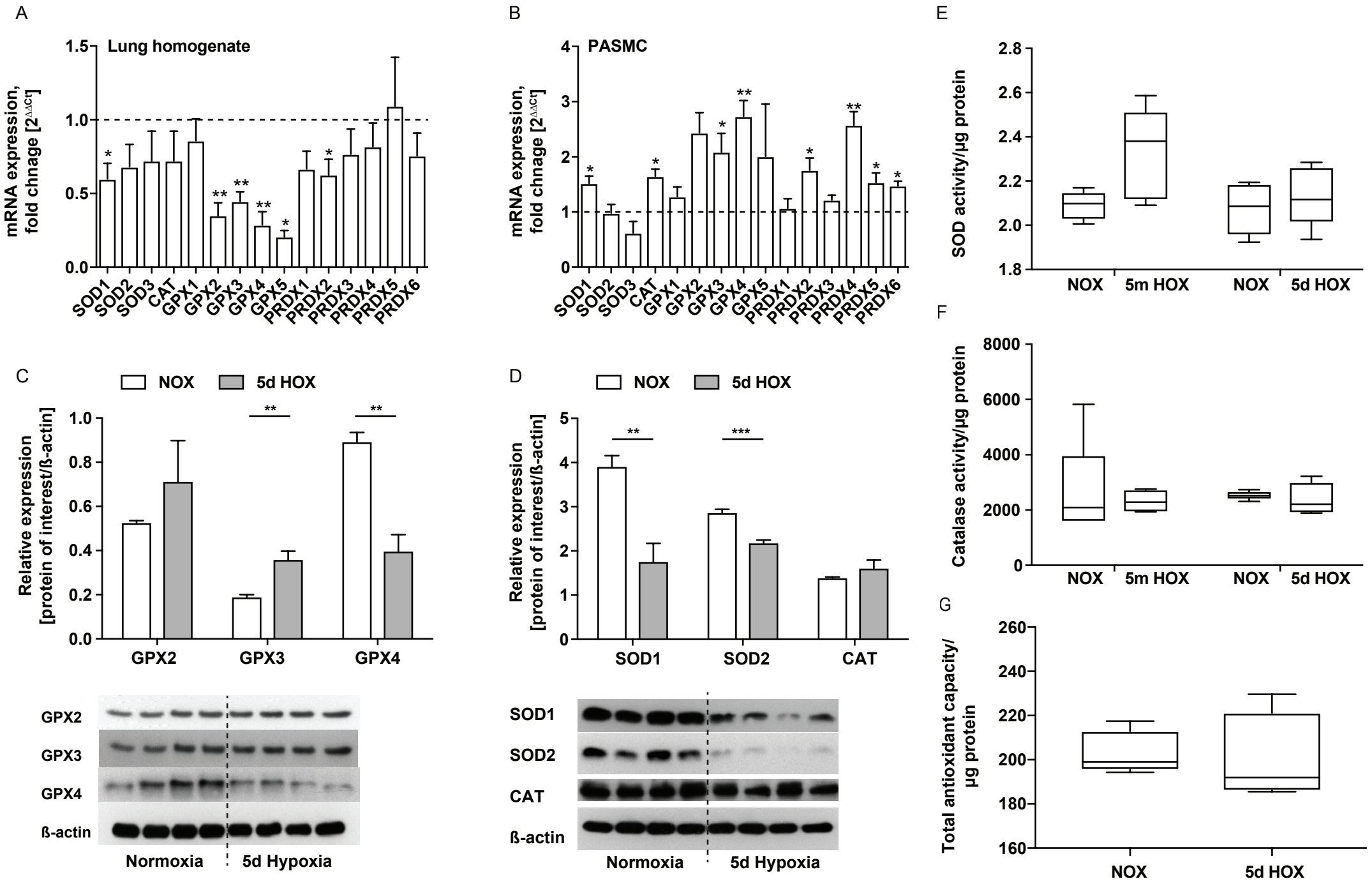

A
Lung homogenate
mRNA expression, fold chnage [2^ΔΔCt]
SOD1 SOD2 SOD3 CAT GPX1 GPX2 GPX3 GPX4 GPX5 PRDX1 PRDX2 PRDX3 PRDX4 PRDX5 PRDX6
B
PASMC
mRNA expression, fold chnage [2^ΔΔCt]
SOD1 SOD2 SOD3 CAT GPX1 GPX2 GPX3 GPX4 GPX5 PRDX1 PRDX2 PRDX3 PRDX4 PRDX5 PRDX6
C
NOX
5d HOX
Relative expression [protein of interest/ß-actin]
GPX2 GPX3 GPX4
GPX2
GPX3
GPX4
ß-actin
Normoxia
5d Hypoxia
D
NOX
5d HOX
Relative expression [protein of interest/ß-actin]
SOD1 SOD2 CAT
SOD1
SOD2
CAT
ß-actin
Normoxia
5d Hypoxia
E
SOD activity/µg protein
NOX 5m HOX NOX 5d HOX
F
Catalase activity/µg protein
NOX 5m HOX NOX 5d HOX
G
Total antioxidant capacity/ µg protein
NOX 5d HOX

Figure S6.

Effect of chronic hypoxia on mitochondrial DNA amount and cellular metabolism

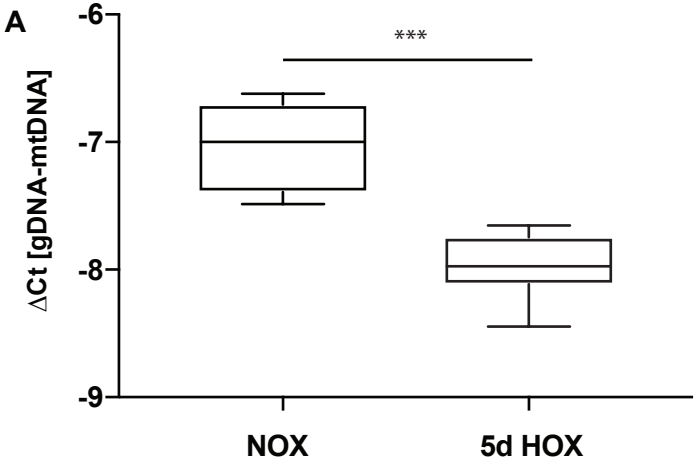
(A) The amount of mitochondrial DNA (mtDNA) normalized to genomic DNA (gDNA). (B) mRNA and (C) protein expression of lactate dehydrogenase A (LDHA) and pyruvate dehydrogenase kinase 1 (PDK1) in PASMC exposed to 1% O_2 for 5 days.

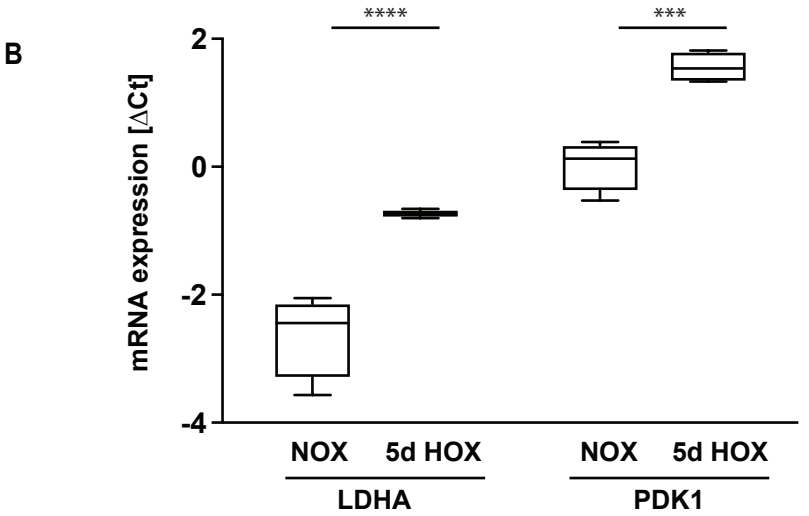
n=4 per group, *p<0.05, **p<0.01, *** p<0.001 and **** p<0.0001 compared to normoxic control by Student's *t* test with Welsh's correction.

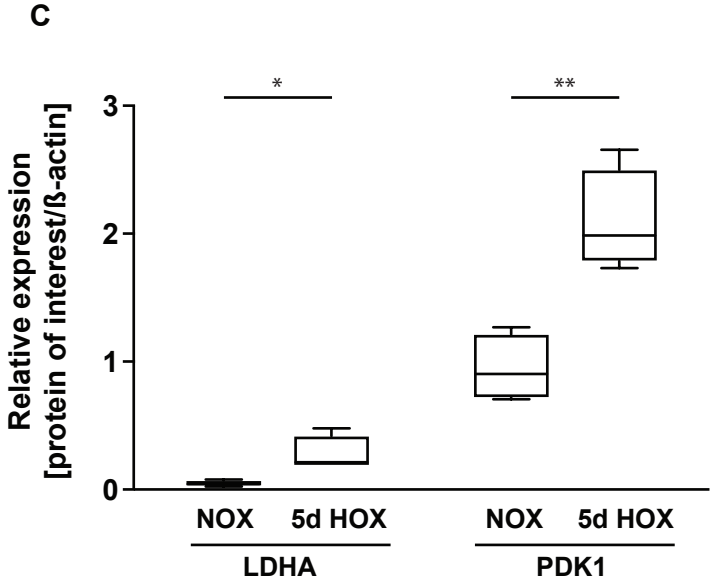
D) Superoxide concentration in PASMC exposed to chronic hypoxia (1% O_2) or normoxia (21% O_2) for 5 days in presence or absence of DCA.

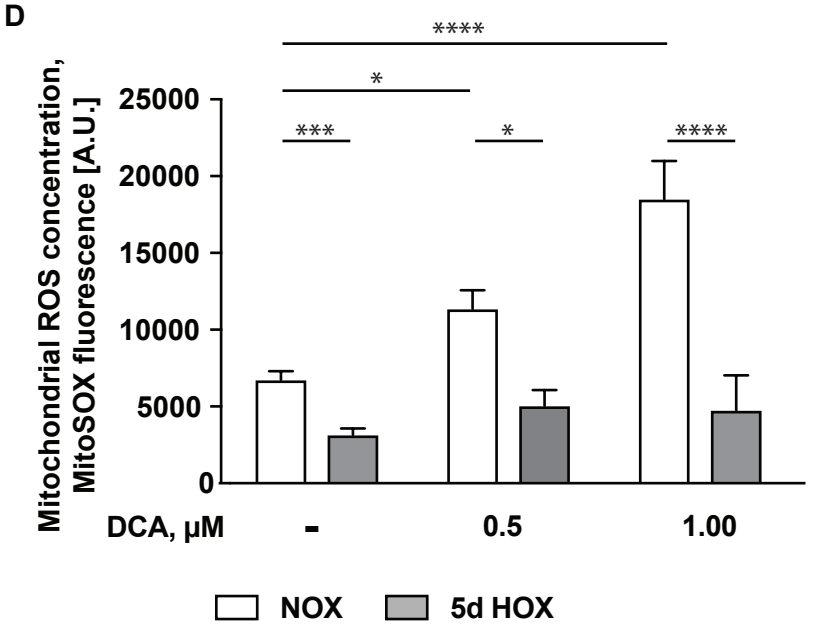
n=4 per group, *p<0.05 and **** p<0.0001 by two-way ANOVA.

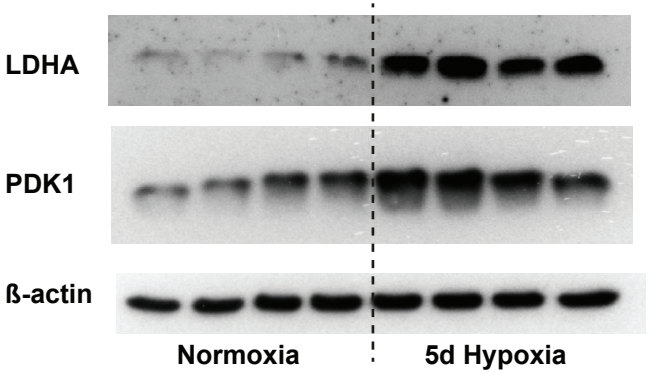
Figure S6


A
-6
-7
-8
-9
ΔCt [gDNA-mtDNA]
***
NOX
5d HOX


B
****
***
2
0
-2
-4
mRNA expression [ΔCt]
NOX
5d HOX
NOX
5d HOX
LDHA
PDK1


C
*
**
3
2
1
0
Relative expression
[protein of interest/ß-actin]
NOX
5d HOX
NOX
5d HOX
LDHA
PDK1


D
****
*
***
*
****
25000
20000
15000
10000
5000
0
Mitochondrial ROS concentration,
MitoSOX fluorescence [A.U.]
DCA, µM
-
0.5
1.00
NOX
5d HOX


LDHA
PDK1
ß-actin
Normoxia
5d Hypoxia

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