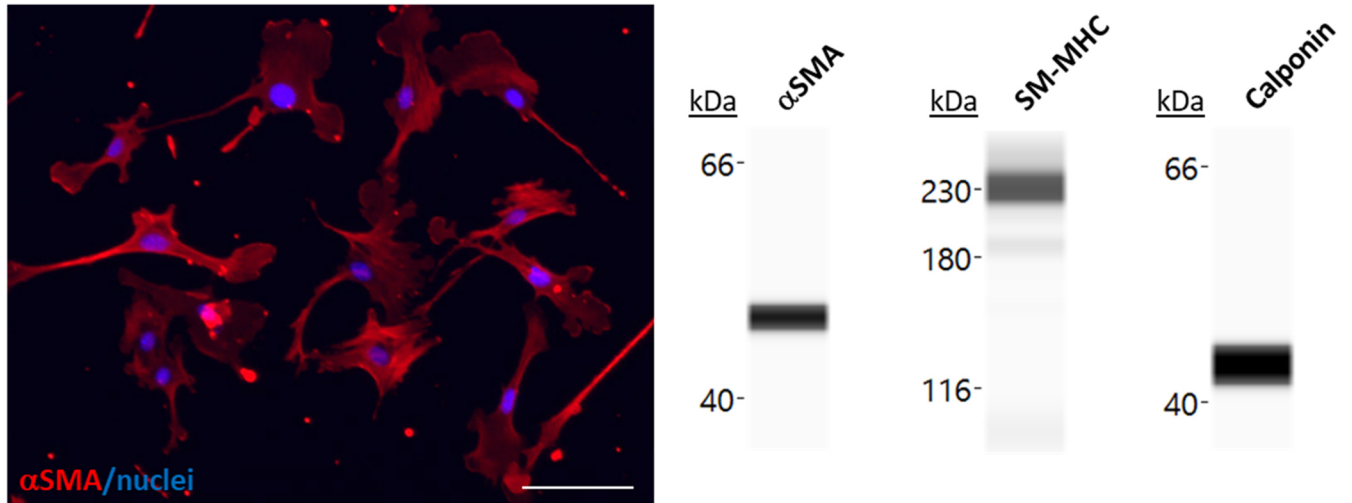
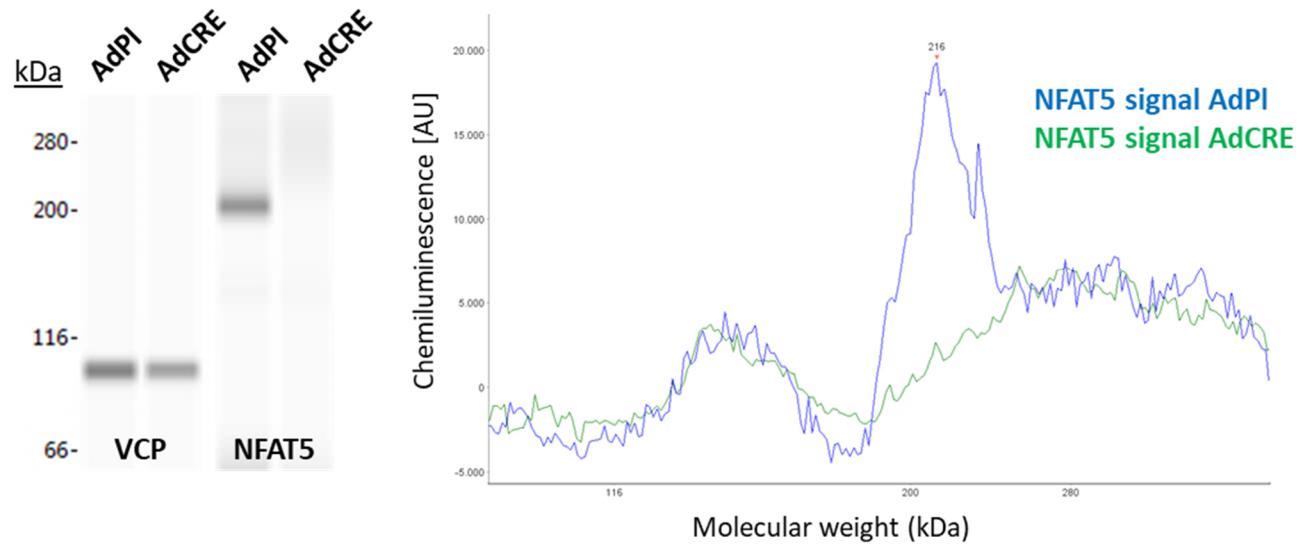


## Supplement 1



Murine pulmonary artery smooth muscle cells (mpaSMCs) were cultured and SMC markers ( $\alpha$ SMA, SM-MHC, calponin) were detected by immunofluorescence-based techniques ( $\alpha$ SMA - red fluorescence, top image, scale bar: 100  $\mu$ m) or immunodetection in lysates after capillary electrophoresis (lower panel).

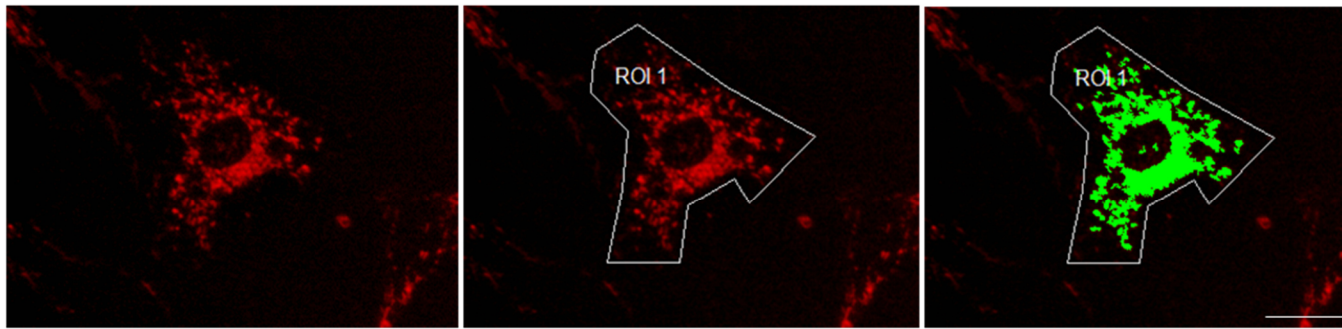
## Supplement 2



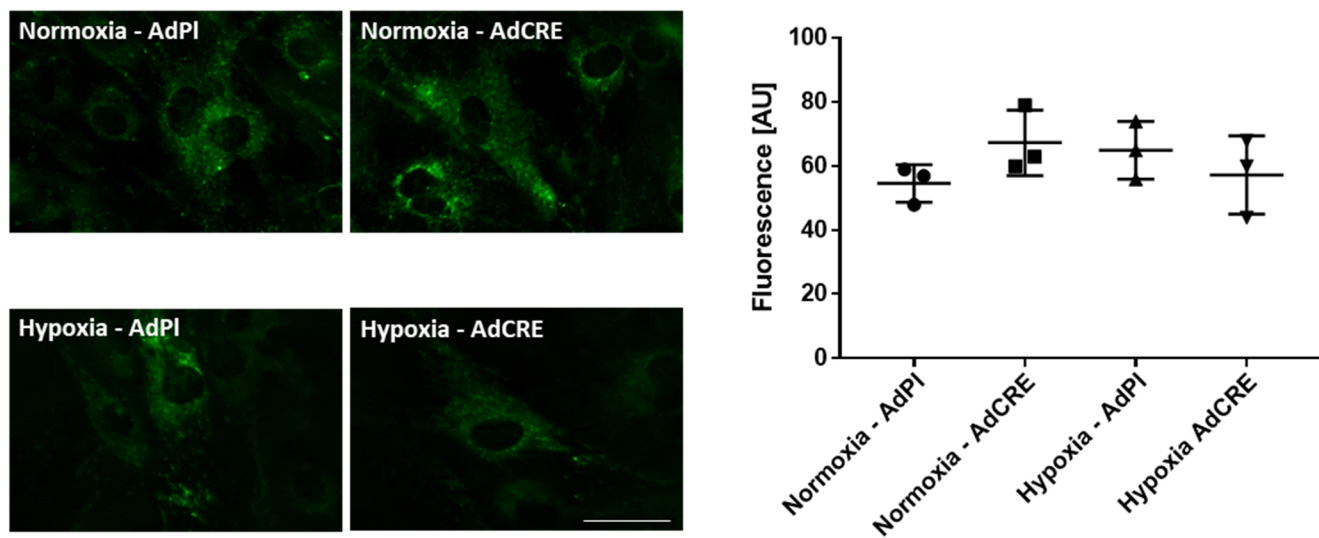
Capillary electrophoresis-based immunodetection of NFAT5 in lysates of maoSMCs treated with AdCRE adenovirus to genetically ablate *Nfat5* (*Nfat5*<sup>-/-</sup>) or AdPI adenovirus as a control (*Nfat5*<sup>fl/fl</sup>). VCP (valosin containing protein) served as loading reference. The right panel shows the corresponding signal peaks detected by the analysis software.

## Supplement 3

**A**



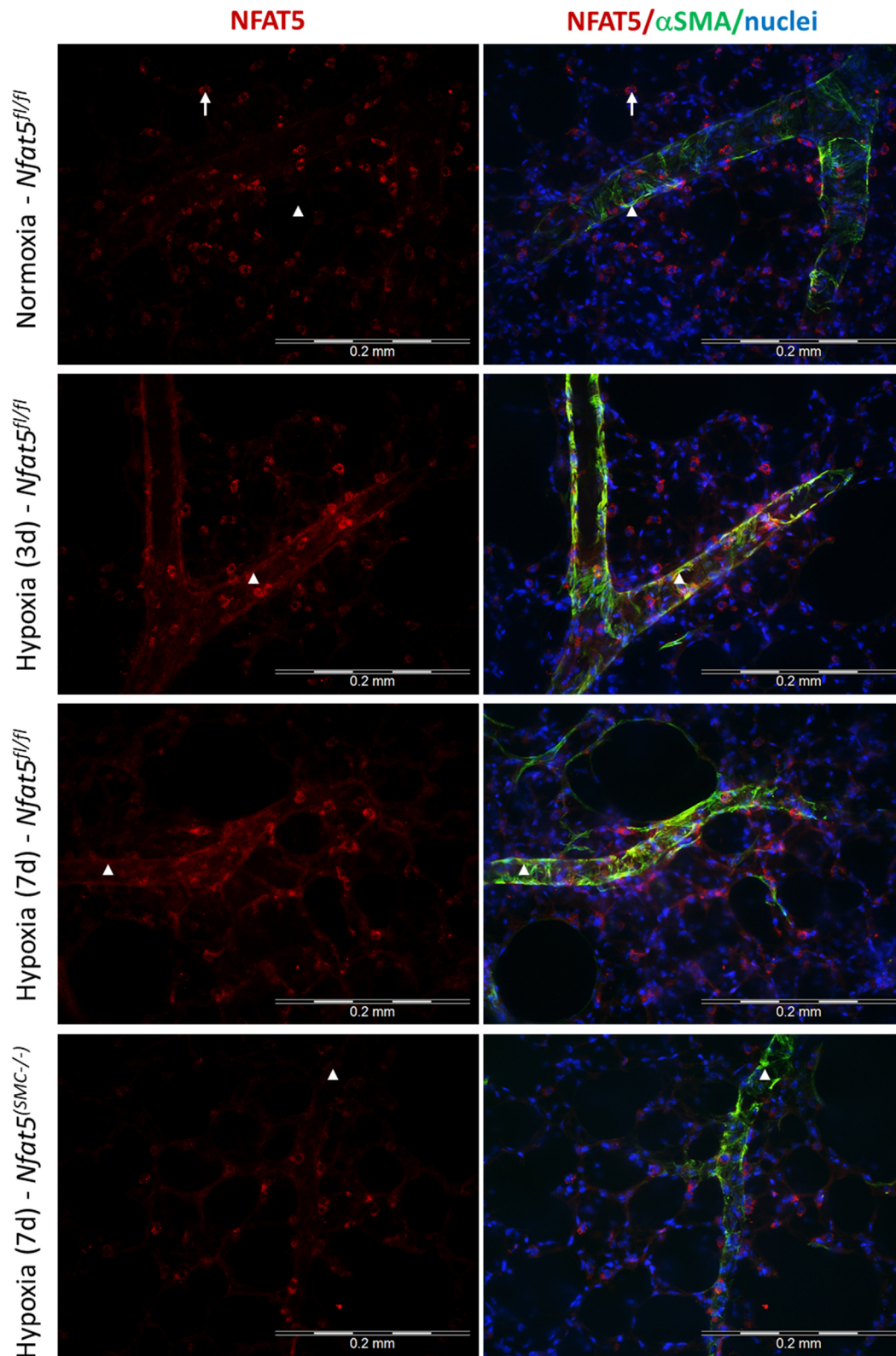
**B**



**(A)** Immunofluorescence-based mitochondrial ROS detection in maoSMCs (red fluorescence) and its software-supported evaluation in a region of interest (ROI, green color, right image, scale bar: 50  $\mu$ m).

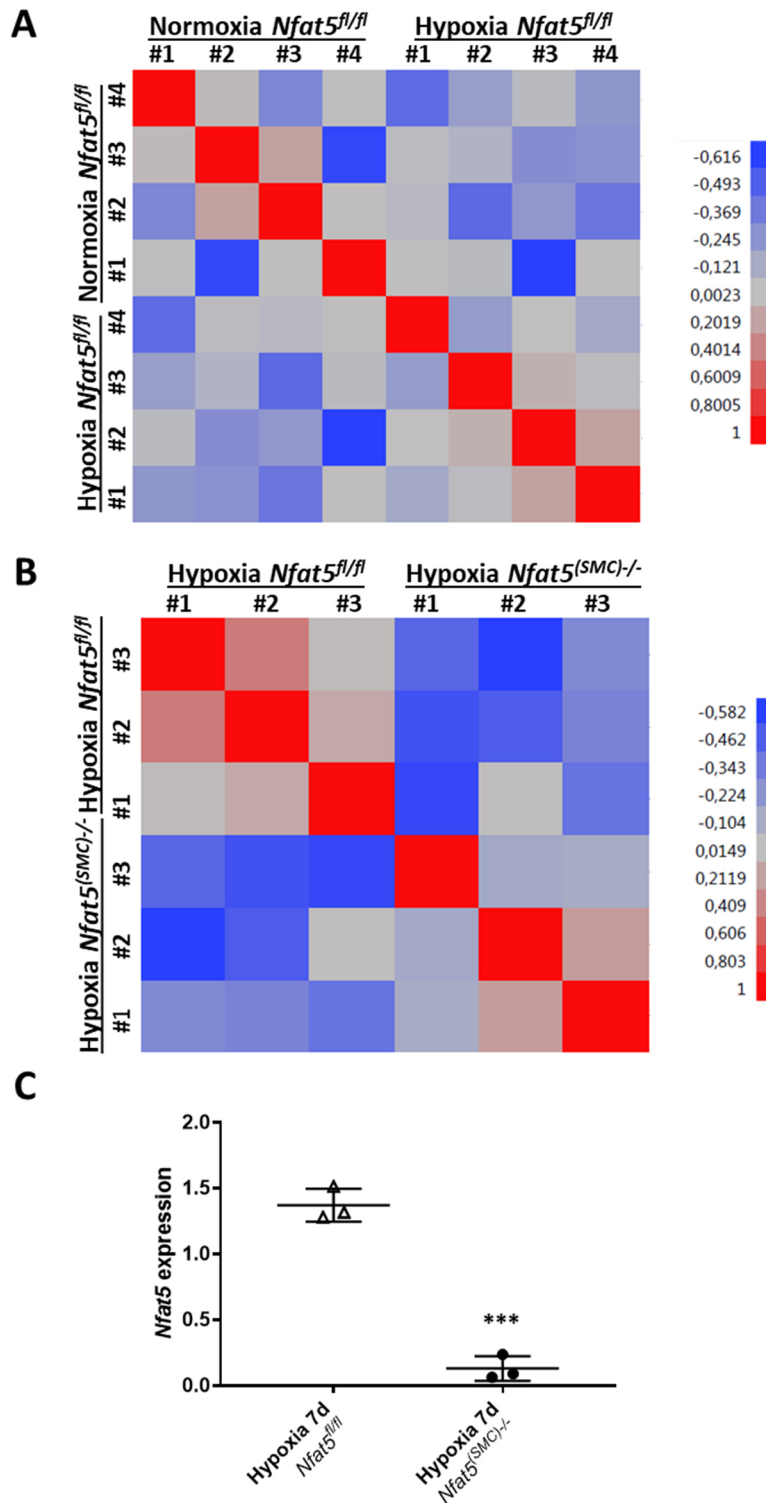
**(B)** Detection of mitochondria by the fluorophore MitoTracker™ Green FM in maoSMCs treated with AdCRE adenovirus to knockout *Nfat5* or AdPI adenovirus as a control and exposed to normoxia/hypoxia for 24 h. The graph summarizes the data of one experiment performed in triplicate. No significant changes were observed (scale bar: 50  $\mu$ m).

## Supplement 4



Vibratome sections of lungs from hypoxia/normoxia-exposed *Nfat5<sup>fl/fl</sup>* and *Nfat5<sup>(SMC)-/-</sup>* mice were processed to detect  $\alpha$ SMA (green) and NFAT5 (red) by immunofluorescence-based techniques (directly labelled antibodies were utilized only) and image stacks were generated by confocal microscopy (scale bars: 200  $\mu$ m, note that NFAT5 localized in the nuclei cannot be detected in formalin-fixed vibratome sections). In normoxic lungs, NFAT5 was preferentially detectable in immune cells (arrows) but not in vascular smooth muscle cells (arrowheads). NFAT5-associated IF was detectable in  $\alpha$ SMA-positive cells in hypoxia-exposed *Nfat5<sup>fl/fl</sup>* but not *Nfat5<sup>(SMC)-/-</sup>* lungs.

## Supplement 5



**(A,B)** Principal component analyses of RNA samples of lungs from *Nfat5<sup>fl/fl</sup>* mice exposed to normoxia/hypoxia for 7 d (A, n=4) and from *Nfat5<sup>(SMC)-/-</sup>* and *Nfat5<sup>fl/fl</sup>* mice exposed to hypoxia for 7 d (B, n=3). The hierarchical clustering analysis indicated a homogenous correlation of the clustering of samples from individual experimental groups. **(C)** qPCR-based comparison of the expression of *Nfat5* (transcriptional target of NFAT5) in lungs from *Nfat5<sup>(SMC)-/-</sup>* and *Nfat5<sup>fl/fl</sup>* mice exposed to hypoxia for 7 d (\*\*\*)  $p < 0.001$  vs. *Nfat5<sup>fl/fl</sup>* mice, n=3).

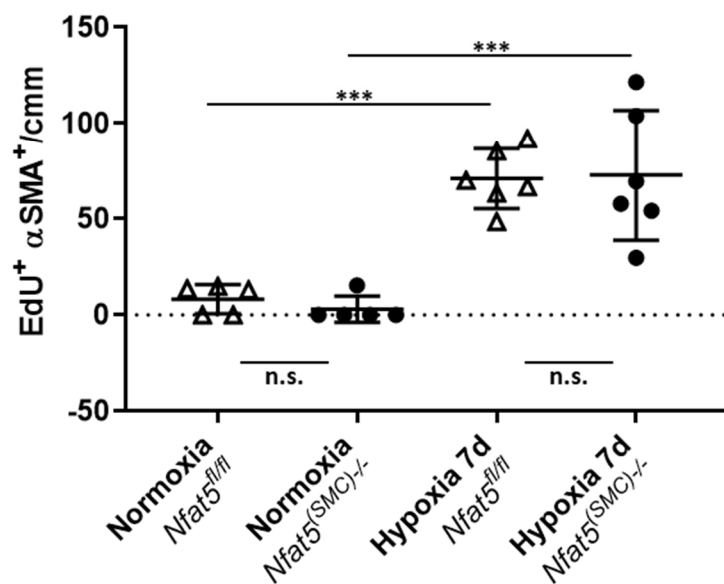
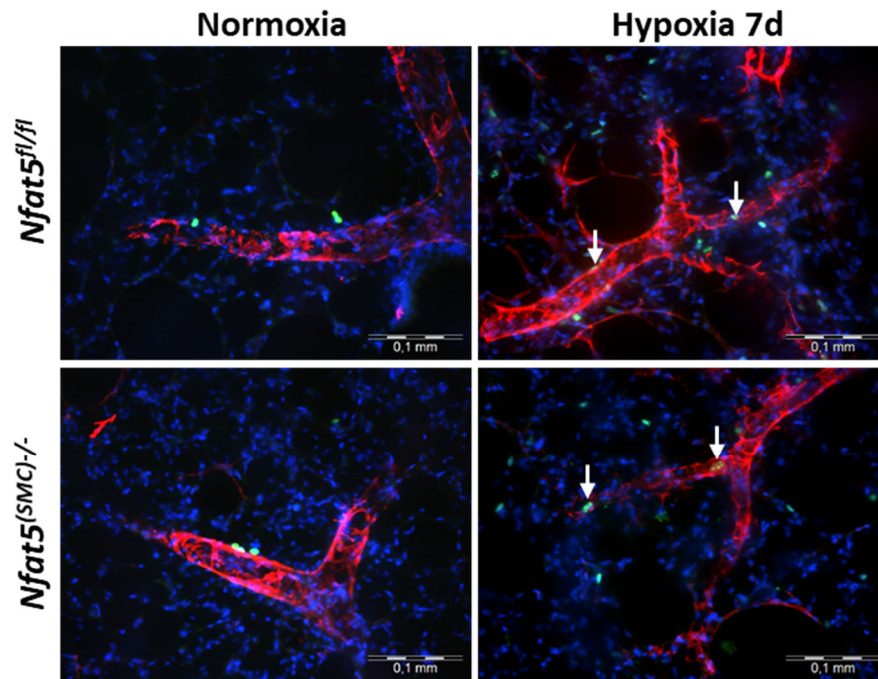
## Supplement 6

Gene name	Category	FR (log2) - <i>Nfat5</i> <sup>fl/fl</sup> : Hyp. vs. Norm.	p-value	FR (log2) - Hyp.: <i>Nfat5</i> <sup>(SMC)-/-</sup> vs. <i>Nfat5</i> <sup>fl/fl</sup>	p-value
<i>Nfat5</i>	Transcription factor	n.s.	>0.05	-1.06	<0.01
<i>Pdgfb</i>	SMC biology	0.71	<0.01	n.s.	>0.05
<i>Pdgfrb</i>	SMC biology	n.s.	>0.05	n.s.	>0.05
<i>Myh2</i>	SMC biology	n.s.	>0.05	n.s.	>0.05
<i>Myh11 (SMMHC)</i>	SMC biology	n.s.	>0.05	n.s.	>0.05
<i>Myl4</i>	SMC biology	-0.64	<0.01	n.s.	>0.05
<i>Myl7</i>	SMC biology	-1.05	<0.001	n.s.	>0.05
<i>Vim</i>	SMC biology	0.45	<0.05	n.s.	>0.05
<i>Tagln (SM22)</i>	SMC biology	0.62	<0.05	n.s.	>0.05
<i>Acta2 (αSMA)</i>	SMC biology	0.71	<0.05	n.s.	>0.05
<i>Cacna1c</i>	SMC biology	0.84	<0.01	-0.77	<0.01
<i>Ednra</i>	SMC biology	n.s.	>0.05	-0.44	<0.01
<i>Plcb1</i>	SMC biology	n.s.	>0.05	-0.70	<0.01
<i>Prkg1</i>	SMC biology	-0.71	<0.05	-0.71	<0.05
<i>Rock1</i>	SMC biology	n.s.	>0.05	-0.50	<0.05
<i>Rock2</i>	SMC biology	n.s.	>0.05	-0.69	<0.05

Expression of individual genes associated with the SMC phenotype in lungs of *Nfat5*<sup>fl/fl</sup> and *Nfat5*<sup>(SMC)-/-</sup> mice exposed to normoxia/hypoxia for 7 days. The left panel lists significantly regulated genes (downregulated: green, upregulated: red, FR – fold regulation (log2-values)) when comparing hypoxia (7d)/*Nfat5*<sup>fl/fl</sup> and the corresponding normoxia/*Nfat5*<sup>fl/fl</sup> group (left panel, n=4, n.s. – not significant). The right panel shows the change in gene expression comparing hypoxia-exposed (7 d) *Nfat5*<sup>(SMC)-/-</sup> and *Nfat5*<sup>fl/fl</sup> mice (n=3).

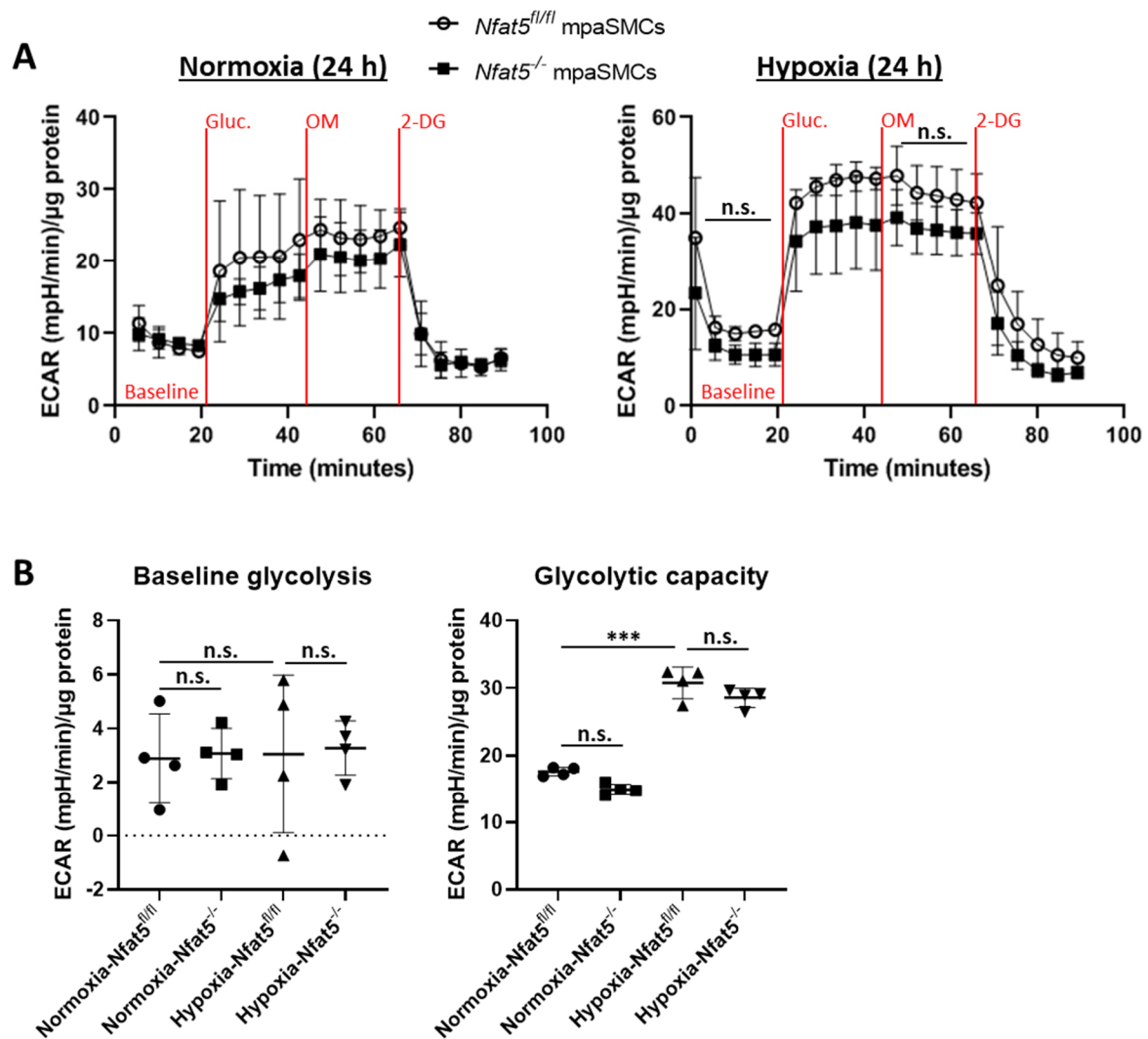


## Supplement 7



Vibratome sections of lungs from hypoxia/normoxia-exposed *Nfat5<sup>(SMC)-/-</sup>* and *Nfat5<sup>fl/fl</sup>* mice were processed to detect EdU (green fluorescence, labels cells with DNA synthesis during the last 2 hours of the experiment) and the SMC marker  $\alpha$ SMA (red fluorescence) by immunofluorescence-based techniques (scale bars: 100  $\mu\text{m}$ ). Images stacks of confocal images were morphometrically evaluated to determine the number of  $\text{EdU}^+/\alpha\text{SMA}^+$  cells (arrows) per cubic mm in arterial segments with a diameter  $<30 \mu\text{m}$  (n.s.- not significant, \*\*\* $p < 0.001$  as indicated,  $n=5-6$ ).

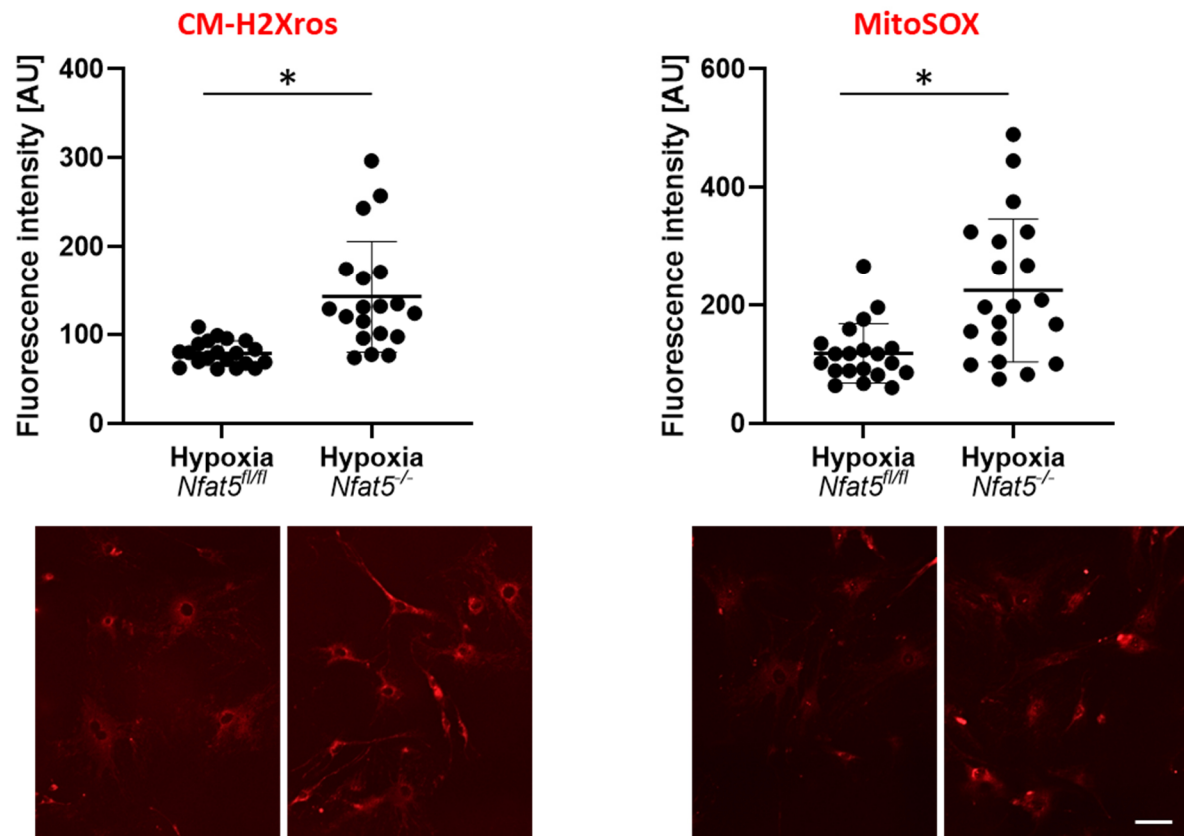
## Supplement 8



Glycolysis was assessed by analysis of the extracellular acidification rate (ECAR) using a Seahorse XF analyzer. mpaSMCs were treated with adeno-associated virus to overexpress CRE recombinase (*Nfat5<sup>-/-</sup>*) or GFP (*Nfat5<sup>fl/fl</sup>*) and exposed to normoxia/hypoxia for 24 h prior to analysis. **(A)** ECAR was determined while treating the cells with glucose (Gluc.), oligomycin (OM) and 2-deoxy-D-glucose (2-DG) to analyze **(B)** baseline glycolysis and glycolytic capacity. Three replicates were performed for each sample (\*\*\*)  $p < 0.001$  as indicated). ECAR was normalized to the total cellular protein per sample.

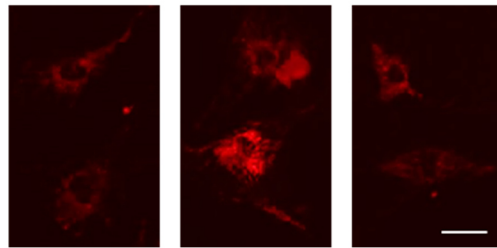
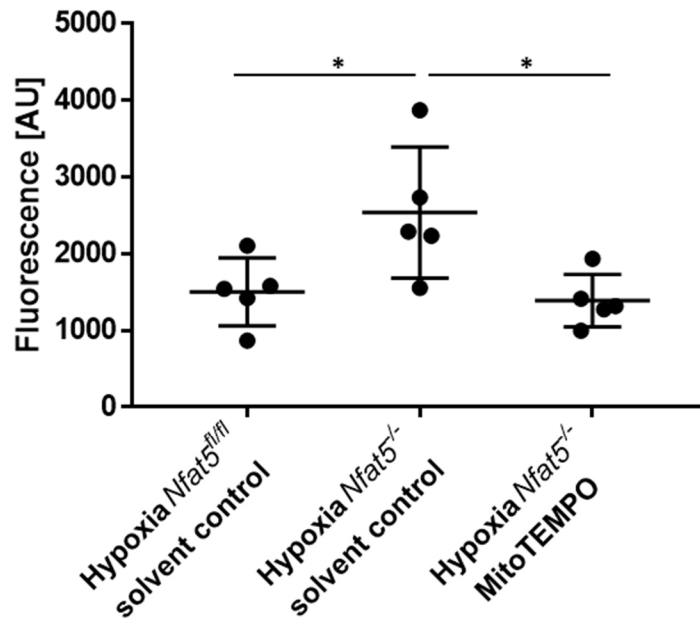


## Supplement 9

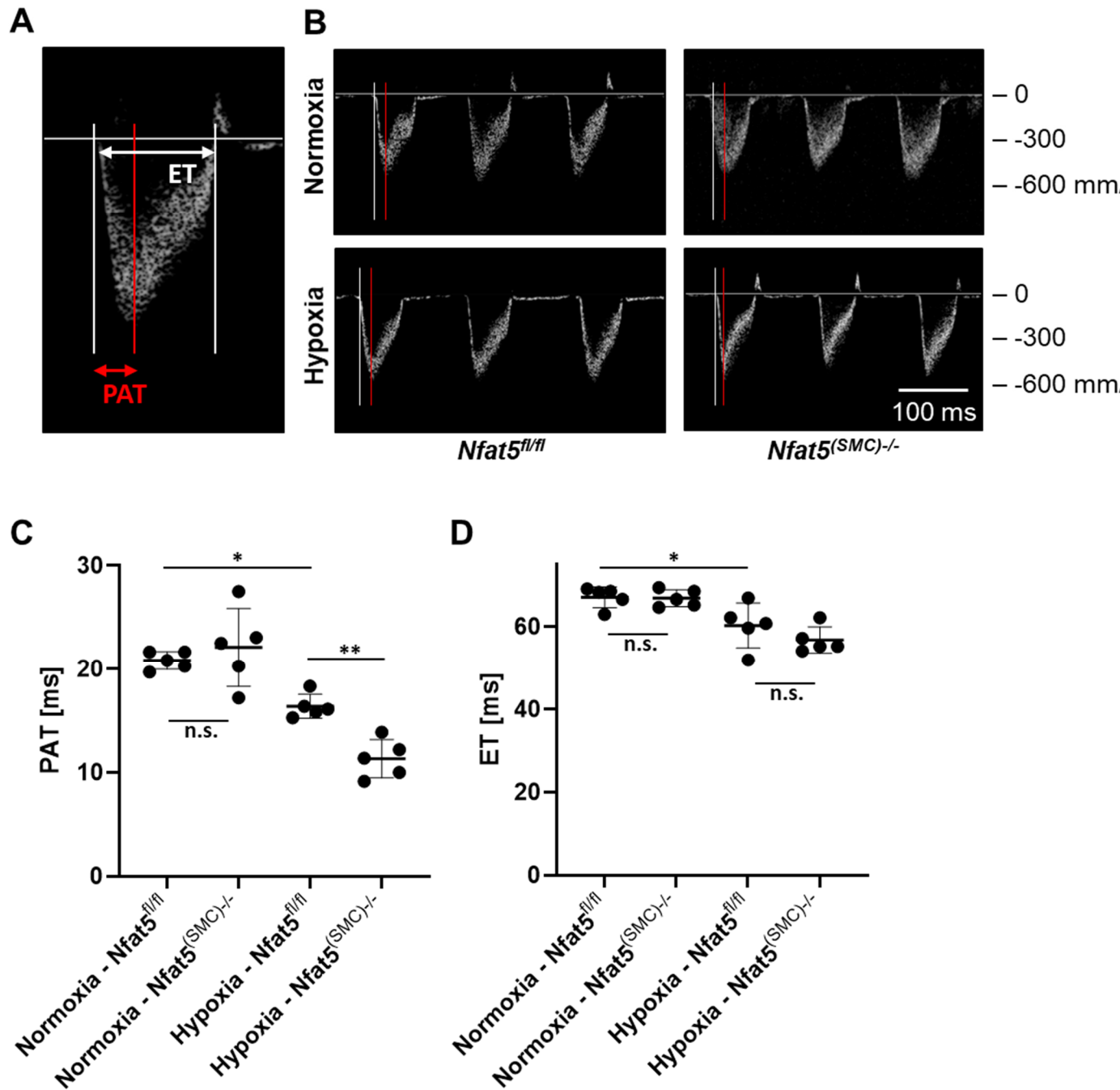


Mitochondrial ROS production was assessed by determining the fluorescence intensity of ROS-sensitive mitochondrion-selective probes (MitoTracker™ Red CM-H2Xros and MitoSOX Red) in mpaSMCs from *Nfat5<sup>fl/fl</sup>* mice, treated with control (*Nfat5<sup>fl/fl</sup>*) and Cre recombinase-transducing (*Nfat5<sup>-/-</sup>*) adenoviral vectors and exposed to hypoxia for 24 h (\*p<0.05 as indicated, the results of 19-20 randomly selected regions of interest (ROI) from one experiment are shown, scale bar: 50  $\mu$ m).

## Supplement 10

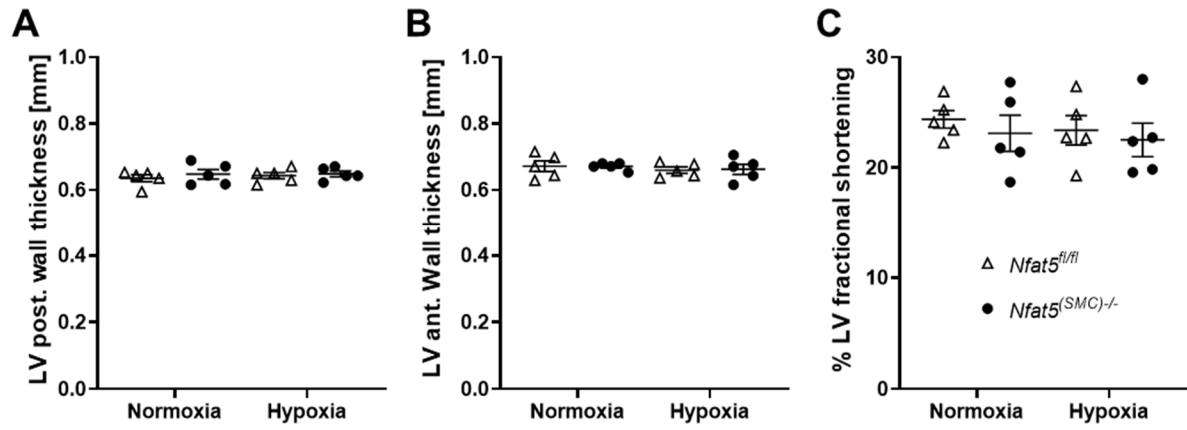


Mitochondrial ROS production was assessed by recording the fluorescence intensity of a ROS-sensitive mitochondrion-selective probe (MitoTracker™ Red CM-H2Xros) in mpaSMCs, treated with adenoviral vectors (AdCRE) to genetically ablate *Nfat5* (*Nfat5*<sup>-/-</sup>) or AdPI vectors as control (*Nfat5*<sup>fl/fl</sup>) and exposed to hypoxia for 24 h. Hypoxia-exposed (24 h) *Nfat5*<sup>-/-</sup> mpaSMCs were treated with the mitochondrion-specific ROS scavenger MitoTEMPO (20 μM) for 1 h before fluorescence recording (\*p<0.05 as indicated, the results of one experiment performed in pentaplicate are shown, scale bar: 50 μm).



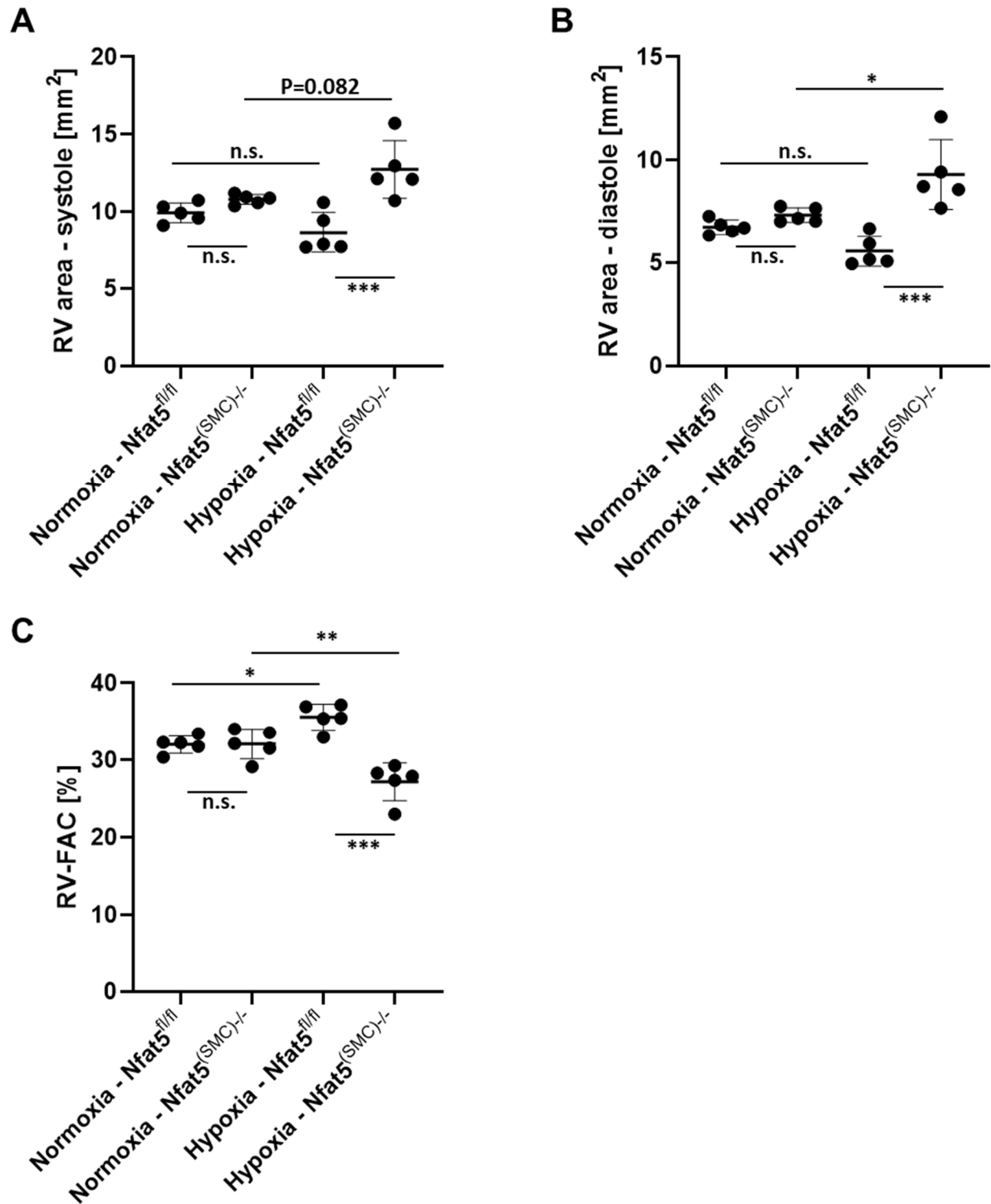
**(A)** Echocardiographic analysis of right ventricular pulmonary acceleration time (PAT) and ejection time (ET). **(B)** Representative (Doppler) echocardiographic recordings of the pulmonary artery ejection in *Nfat5<sup>fl/fl</sup>* and *Nfat5<sup>(SMC)-/-</sup>* mice exposed to normoxia/hypoxia for 21 d. **(C and D)** Graphs summarizing individual PAT and ET values from mice (\* $p < 0.05$ , \*\* $p < 0.01$  as indicated, n.s. – not significant,  $n = 5$ ). Data is shown as mean  $\pm$  SD.

## Supplement 12



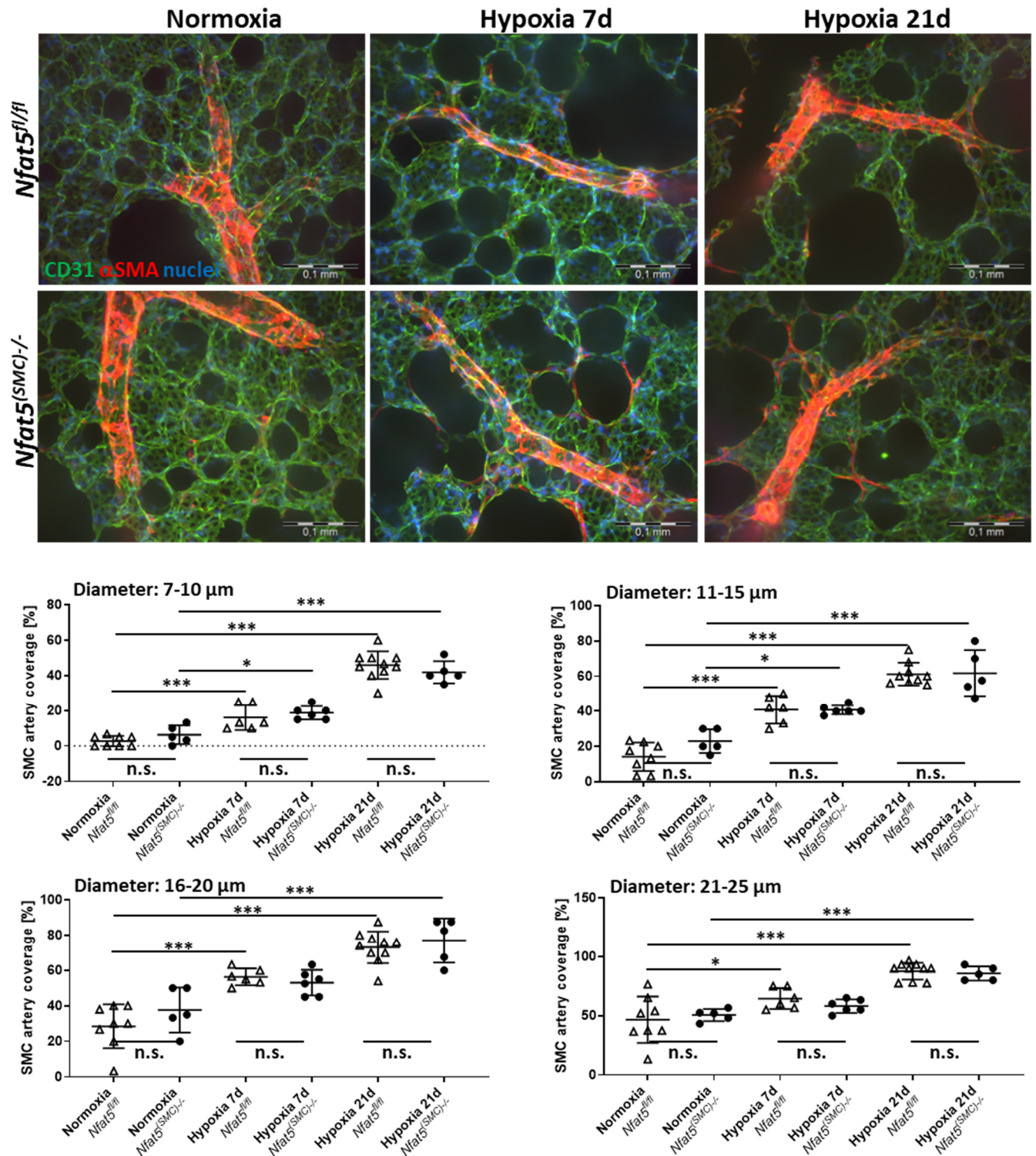
**(A-C)** Echocardiographic analysis of left ventricular (LV) structural and functional parameters of *Nfat5<sup>fl/fl</sup>* and *Nfat5<sup>(SMC)-/-</sup>* mice exposed to normoxia/hypoxia for 21 d. No significant differences were observed (n=5). Data is shown as mean  $\pm$  SEM.

# Supplement 13



(A-C) Echocardiographic data of right ventricular (RV) area during systole (A) and diastole (B) as well as (C) RV fractional area change (FAC) in *Nfat5*<sup>fl/fl</sup> and *Nfat5*<sup>(SMC)-/-</sup> mice exposed to normoxia/hypoxia for 21 d (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 as indicated, n.s. – not significant, n=5). Data is shown as mean ±SD.

## Supplement 14



SMC coverage of arterial blood vessels with different caliber (extension of Figure 4). Vibratome sections of lungs from hypoxia/normoxia-exposed *Nfat5<sup>(SMC)-/-</sup>* and *Nfat5<sup>fl/fl</sup>* mice were processed to detect CD31 (green) and  $\alpha$ SMA (red) by immunofluorescence-based techniques (scale bars: 100  $\mu$ m). Image stacks of confocal images were morphometrically evaluated to determine the coverage of arterial segments with different caliber comprising the following groups: 7-10  $\mu$ m, 11-15  $\mu$ m, 16-20  $\mu$ m and 21-25  $\mu$ m (n.s. – not significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 as indicated, n=5-10).