SUPPLEMENTAL MATERIAL

Methods

siRNA and primers:

The siRNA duplex specific for human MiD49 mRNA was siMiD49 (sense 5'-GCGCUAUACAGUGGCCUACAGGAGC-3'; antisense 5'-GCUCCUGUAGGCCACUGUAUAGCGCAU-3'). The siRNA duplex specific for human MiD51 mRNA was siMiD51 (sense 5'- CGCAGAUACAUUUGAGAAGGUAGTG-3'; antisense 5'-CACUACCUUCUCAAAUGUAUCUGCGAC-3'). The siRNA duplex specific for human Fis1 mRNA was siFis1 (sense 5'- ACAGCGGGAUUACGUCUUCUACCTG-3'; antisense 5'-CAGGUAGAAGACGUAAUCCCGCUGUUC-3'). The siRNA duplex specific for human MFF mRNA was siMFF (sense 5'- ACCUGAAGAUGGAGCUAAUCUUUCC-3'; antisense 5'-GGAAAGAUUAGCUCCAUCUUCAGGUAA-3'). The siRNA duplex specific for rat MiD49 mRNA was RNC.RNAi.X001077609.12.1. The siRNA duplex specific for rat MiD51 mRNA was RNC.RNAi.N001007709.12.1. Control- siRNA (ctrl-siRNA) duplex, a sequence having no homology to any known mammalian genes, was used as a negative control (sense 5'-CGUUAAUCGCGUAUAAUACGCGUAT-3'; antisense 5'-AUACGCGUAUUAUACGCGAUUAACGAC-3').

The primers used were: MiD49 (sense): 5'-TGTGCTGGGCATTGCCACCCT-3', (antisense): 5'-TTGAGCAGGCTCAGTTCCTTCC-3', MiD51: (sense) 5'-ACTTGAGTGGCAGCCTCTACGA-3', (antisense): 5'-CAGGAAGAAGCCAGGGACATTC-3'.

All siRNA duplex and primers were purchased from Integrated DNA Technology, Coralville, IA, USA.

Adenovirus infection:

$Drp1^{Fl/Fl}$ MEFs were infected at a concentration of 100 pfu/cell for 48 h with Adv-Cre purchased from Robert E. Fitzhenry Vector Laboratory (McMaster University, ON, Canada). Adv-mNeon green was a kind gift from Dr. Mads Breum Larsen (University of Pittsburg, USA).

Immunoblotting and antibodies:

Whole-cell lysates were prepared in cell lysis buffer (Cell Signaling Technologies, Beverly MA, USA). For immunoblot analysis, cell lysates (40-60 μg) were analyzed on 4–12% NuPAGE gels (Life technologies, Carlsbad, CA, USA). The proteins were electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Life technologies, Carlsbad, CA, USA) and detection of specific proteins was carried out with indicated antibodies using the ECL-Plus Western Blotting Detection System (GE Healthcare, Piscataway, NJ, USA).

Polyclonal antibodies against MiD49 (16413-1-AP), MiD51 (20164-1-AP) and Total CDK4 (11026-1-AP) were obtained from Proteintech (Tucson, AZ, USA) and NovoPro Bio (115386) (Shanghai, China). Phospho-ERK1/2 (4370S), total ERK1/2 (9102S), phospho-Drp1 (ser616) (3455S), PDGFR-α (5241S) and β (3169S), p-Raf-1 (9421S), total Raf-1 (53745S), Mfn1 (14739S), OPA1 (80471S), phospho-Akt (ser473) (4060S), total Akt (4691S), Bax (5023S), Bak (6947S) antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Phospho-CDK2 (Tyr15) (ab76146) and Mfn2 (ab56889) were purchased from Abcam (Cambridge MA, USA) and phopho-CDK4 (Thr172) (PA5-64482) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Total Drp1 (611112) antibody was purchased from BD transduction Laboratories (San Jose, Ca, USA). β-actin antibodies (A5441) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Plasmid transfection to achieve overexpression of MiD49 and MiD51:

To overexpress the MiDs in PASMC we transfected cells with plasmids carrying the appropriate transgene. pCMV6-ENTRY (control vector), pCMV6-MiD49 and pCMV6-MiD51 plasmids were purchased from Origene (Rockville, MD, USA). Normal PASMC were seeded in 6 well plates and transfected with 2.5μg of either pCMV6-ENTRY, or pCMV6-MiD49 or pCMV6-MiD51 using lipofectamine 3000 (Life Technologies, CA, USA).

Rodent sugen5416/hypoxia PAH (Su/Hx PAH) model for the collection of whole blood and plasma:

The rat Su/Hx PAH model for whole blood and plasma RNA extraction was created at the University of Sheffield, UK. Briefly, it was created by a single subcutaneous injection of SU5416 at 20 mg/kg, followed by housing at 10% O_2 and 5% CO_2 as previously described[1]. The whole blood and plasma were collected for total RNA extraction.

Whole blood RNA extraction:

Total RNA from healthy volunteers, PAH patients (Supplementary Table 1), Monocrotaline (MCT) and Sugen5416/Hypoxia challenged rats was extracted from Tempus whole blood tubes (Applied Biosystems, UK) using Norgen Preserved Blood RNA Purification Kit (Norgen Biotek Corp, Canada). The entire content of a single Tempus whole blood tubes was emptied into a new 50 ml Falcon tube and mixed by vortexing for 30 seconds followed by centrifugation at 4000 rpm for 30 mins at 4°C. Supernatant was discarded. 600 μl of lysis solution and 300 μl of 100% ethanol were consecutively added and mixed by brief vortexing. 600 μl of lysate was added into the Norgen Preserved Blood RNA Purification Kit Spin Column and centrifuged at 10,000g for 1 minute at room temperature. The flow through was discarded and the process was repeated for the remaining lysates. 400 μl of wash solution was added into the spin column and centrifuged at 10,000 g for 1 min at room temperature. The flow through was discarded and this step was repeated. To elute column bound RNA, the spin column was removed and transferred into a new 1.5 ml elution tube. 50 μl of elution solution was added onto the spin column membrane and centrifuged at 200 g for 2 minutes followed by 14,000 g for 1 min. The quality and quantity of total extracted RNA were assessed by Spectrophotometry (Nanodrop 1000, Thermo Scientific, UK). Sample with 260/280 and 260/230 ratios of 2 to 2.2 were considered to be un-contaminated and pure. Extracted RNA sample was stored at -80°C.

Plasma RNA extraction:

Total RNA was extracted from plasma of healthy volunteers and IPAH patients (Supplementary Table 1). Plasma RNA was extracted using Norgen Plasma/Serum Circulating and Exosomal RNA Purification kit (Norgen Biotek Corp, Canada). Briefly, 500 µL of plasma sample was mixed with 0.2 mL of slurry C2

and 0.8 mL of Lysis Buffer A (10 µL of 2M DTT was added into each 1 mL-aliquots of Lysis Buffer A). The mixture was vortexed for 15 sec followed by 10 min incubation at 60°C. 1.5 mL of 100% ethanol was then added to the mixture, vortexed and centrifuged for 30 sec at 1,000 rpm. Supernatant was discarded and the pellet was resuspended in 0.3 mL of Lysis Buffer A followed by incubation at 60°C for 10 minutes. After incubation, 0.3 mL of 100% Ethanol was added and mixed by vortex. 650 µL of the mixture was repeatedly loaded into a Mini Filter Spin Column and centrifuged for 1 minute at 14,000 RPM with flow through discarded. 400 µL of Wash Solution A was added into the column and centrifuged for 1 minute at 14,000 rpm, flow through was discarded. The column was further washed twice with 400 µL Washing Solution A and centrifuged for 1 minute at 14,000 rpm with flow through discarded. The column was further centrifuged for 3 min at 14,000 rpm and transferred into an elution tube. 100 µL of elution solution A was added into the column and centrifuged for 2 min at 2,000 rpm followed by 3 min at 14,000 rpm. Extracted RNA sample was stored at -80°C.

Concentration of Plasma RNA:

The initial 100 µl elute of RNA was concentrated to 11.5µl with the RNA Clean and Concentrate-5 kit (Zymo Research Cop, U.S.A.). 200 µl of RNA Binding Buffer was added to the 100µl elute and mixed by vortexing. 300µl of 100% ethanol was added, mixed by pipetting and transferred to the spin column and centrifuged (10,000 rpm, 30 sec). 400µl of RNA Prep Buffer was added and centrifuged (10,000 rpm, 30 sec). The column was then washed twice by addition of 700µl and centrifugation (10,000 rpm, 30 sec). Flow-through was discarded and a final wash was performed with 400µl of wash buffer followed by centrifugation (10,000 rpm, 30 seconds). The column was dried by centrifugation (10,000 rpm, 1 minute). 11.5µl of DNase/RNase free water was then added directly to the membrane and eluted by centrifugation (10,000 rpm, 1 min). The column was discarded and RNA was stored at -80ºC.

Whole Cell Micropolarimetry

For all experiments, 25,000-50,000 of PAH PASMC transfected with either ctrl-siRNA or siRNA against MiD49 or MiD51. Twenty-four hours following transfection the cells were plated onto 24-well Seahorse XF^{e}24 Cell Culture Microplates and incubated at 37ºC for 24 hours to allow for adherence to plate. After substituting growth media with assay media (XF modified DMEM supplemented with 25mM glucose and 1mM sodium pyruvate for mitochondrial flux, XF minimal DMEM supplemented with 2mM glutamine for glycolytic flux), cells were incubated in a CO_2-free incubator for equilibration prior to micropolarimetry experiments. Oxygen consumption and extracellular acidification rates were measured using a Seahorse XFe24 Extracellular Flux Analyzer (Seahorse Bioscience/Agilent Technologies, North Billerca, MA). All measurements were obtained in 8-min cycles (3 min mixing, 2 min waiting, 3 min measuring). For mitochondrial flux, three cycles of baseline measurements were performed, followed by three 8 min cycles each after the addition of 2μM oligomycin (inhibitor of ATP synthase), 2μM FCCP (proton ionophore), and 2μM antimycin A + rotenone (mitochondrial complex III and complex I inhibitors, respectively). For glycolytic flux, 3 cycles of baseline measurements were performed in glucose-free media, followed by three 8 min cycles each after the addition of 10mM glucose (glycolysis stimulation), 2.5uM oligomycin, and 100mM 2-deoxyglucose (2-DG, glucose analog and inhibitor or glycolysis). All data were normalized to total protein per well, and are expressed as mean pmol O_2 or mpH/minute/μg protein ± SEM. (XF modified DMEM, XF minimal DMEM, oligomycin, FCCP, antimycin A, rotenone, glucose, 2-DG – Seahorse Bioscience/Agilent Technologies, North Billerca, MA; sodium pyruvate, glutamine – Gibco, Carlsbad, CA).

References:

1. Rothman AM, Arnold ND, Pickworth JA, Iremonger J, Ciuclan L, Allen RM, Guth-Gundel S, Southwood M, Morrell NW, Thomas M, Francis SE, Rowlands DJ, Lawrie A. Microrna-140-5p and smurf1 regulate pulmonary arterial hypertension. *The Journal of clinical investigation*. 2016;126:2495-2508.

Supplementary Table 1: Demographics of control and PAH patients

Variable	Tissue (Canada cohort)		BOEC (Canada cohort)		Plasma (UK cohort)		Whole blood (UK cohort)		Plasma (China cohort)		Whole blood (China cohort)	
	Ctrl	PAH	HV	PAH	HV	PAH	HV	PAH	HV	PAH	HV	PAH
	(n=6)	(n=6)	(n=5)	(n=6)	(n=29)	(n=27)	(n=11)	(n=14)	(n=19)	(n=36)	(n=20)	(n=39)
Age	51.3 ± 6.5	53.3 ± 5.1	23.6 ± 1.36	49.1 ± 2.5	49.5 ± 2.5	49.1 ± 2.5	48.1 (8.02)	48.9 (15.01)	33.2 (2.09)	31.9 (1.89)	31.3 (0.86)	29.5 (1.81)
Gender												
Female	4	5	4	4	18	18	7	10	9	22	10	29
Male	2	1	1	2	11	9	4	4	10	14	10	10
Race												
Caucasian	6	6	3	6	28	22	N/A	11	0	0	0	0
South Asian	0	0	0	0	0	4		2	0	0	0	0
Other Asian	0	0	0	0	1	1		1	19	36	20	39
Unknown	0	0	2	0	0	0		0	0	0	0	0

Supplementary Table 2: Twenty-five miRNAs are significantly downregulated in human PAH PASMC compared to normal PASMC.

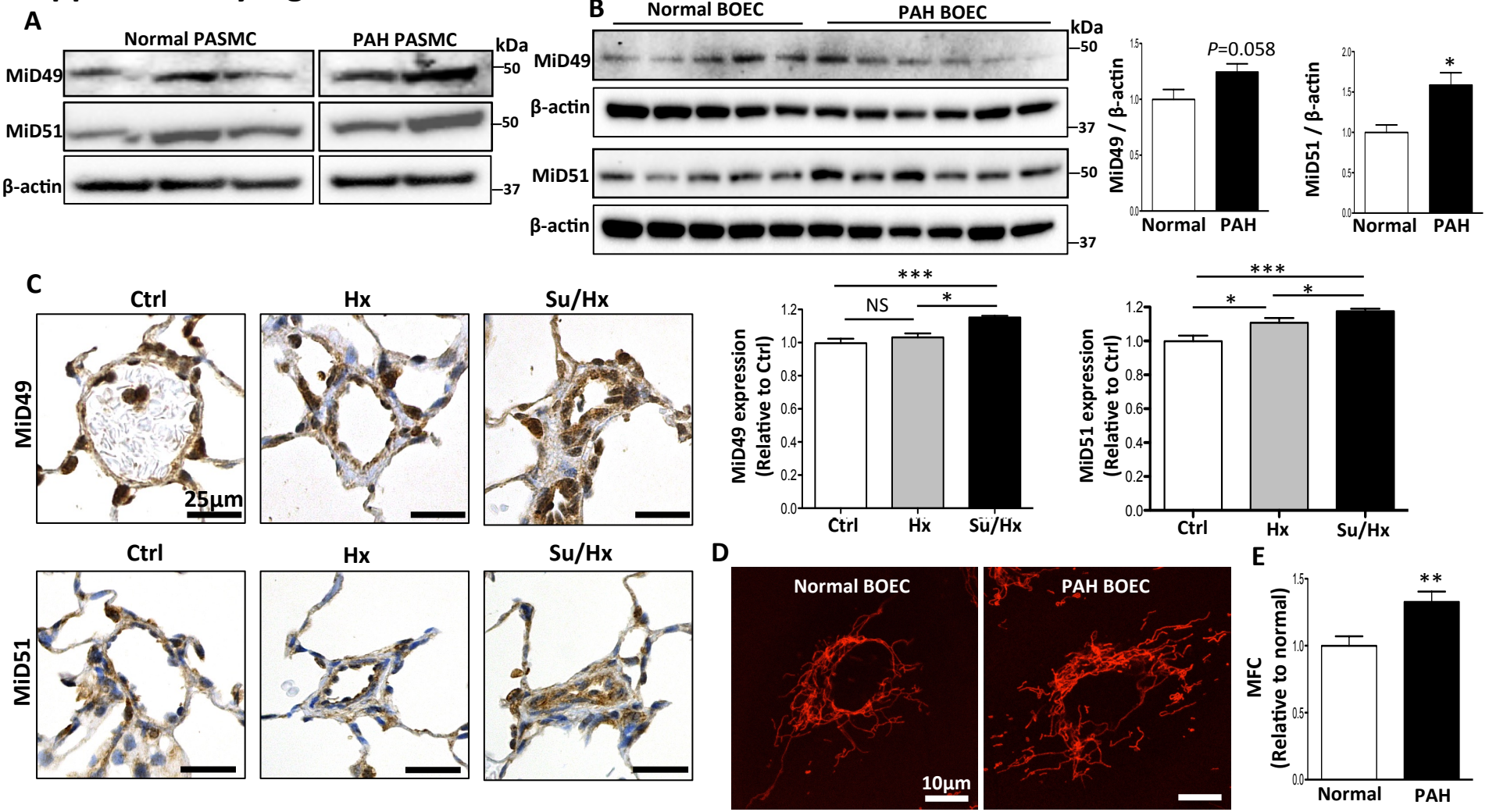
miRNAs	Accession ID	Fold Change (PAH PASMC vs. Normal PASMC)	ANOVA p-value
hsa-let-7i-5p	MIMAT0000415	-2.62	0.00089
hsa-miR-126-3p	MIMAT0000445	-3.82	0.043458
hsa-miR-143-5p	MIMAT0004599	-2.11	0.008419
hsa-miR-145-5p	MIMAT0000437	-2.33	0.027635
hsa-miR-146a-5p	MIMAT0000449	-248.37	0.000511
hsa-miR-148a-3p	MIMAT0000243	-2.29	0.048824
hsa-miR-148b-3p	MIMAT0000759	-3.38	0.031904
hsa-miR-154-5p	MIMAT0000452	-2.73	0.044662
hsa-miR-181c-5p	MIMAT0000258	-3.05	0.005317
hsa-miR-188-5p	MIMAT0000457	-2.76	0.009775
hsa-miR-193a-3p	MIMAT0000459	-2.27	0.047216
hsa-miR-21-3p	MIMAT0004494	-2.3	0.036548
hsa-miR-21-5p	MIMAT0000076	-2.51	0.024474
hsa-miR-224-3p	MIMAT0009198	-5	0.02764
hsa-miR-22-5p	MIMAT0004495	-4.59	0.037665
hsa-miR-29c-3p	MIMAT0000681	-9.33	0.017456
hsa-miR-30e-5p	MIMAT0000692	-2.95	0.024965
hsa-miR-34a-3p	**MIMAT0004557**	**-2.04**	**0.021964**
hsa-miR-34a-5p	MIMAT0000255	-2.52	0.023406
hsa-miR-425-3p	MIMAT0001343	-2.35	0.020163
hsa-miR-452-5p	MIMAT0001635	-4.6	0.003227
hsa-miR-6075	MIMAT0023700	-2.01	0.008258
hsa-miR-628-3p	MIMAT0003297	-2.52	0.000123
hsa-miR-660-5p	MIMAT0003338	-5.49	0.026855
hsa-miR-6782-5p	MIMAT0027464	-2.07	0.025352

This table contains the identity and fold-decrease in expression for 25 miRs that were downregulated in human PAH. miRs were isolated from cultured PASMC of six PAH patients and three healthy subjects and were extracted using the mirVana miRNA isolation kit (Ambion/Life Technologies) according to manufacturer's protocol. Microarray experiments were carried out at the Centre for Applied Genomics at the Hospital for Sick Children (Toronto, Canada) using the Affymetrix GeneChip miRNA 4.0 Array (Affymetrix).

Supplemental Movie legend:

Mitochondrial dynamics in PAH PASMC. Mitochondrial dynamics was imaged by transfecting PAH PASMC with (Movie 1) ctrl-siRNA, (Movie 2) siMiD49 and (Movie 3) siMiD51. Cells were also infected with Adv-mNeon Green and imaged after 48 h. Images were taken at real time by resonant scanning at 8000 Hz, 1024 X 256 pixels, line average of 2, 25.6 frames/sec and 6X zoom.

Supplementary Fig. 1



Supplementary Figure 1:

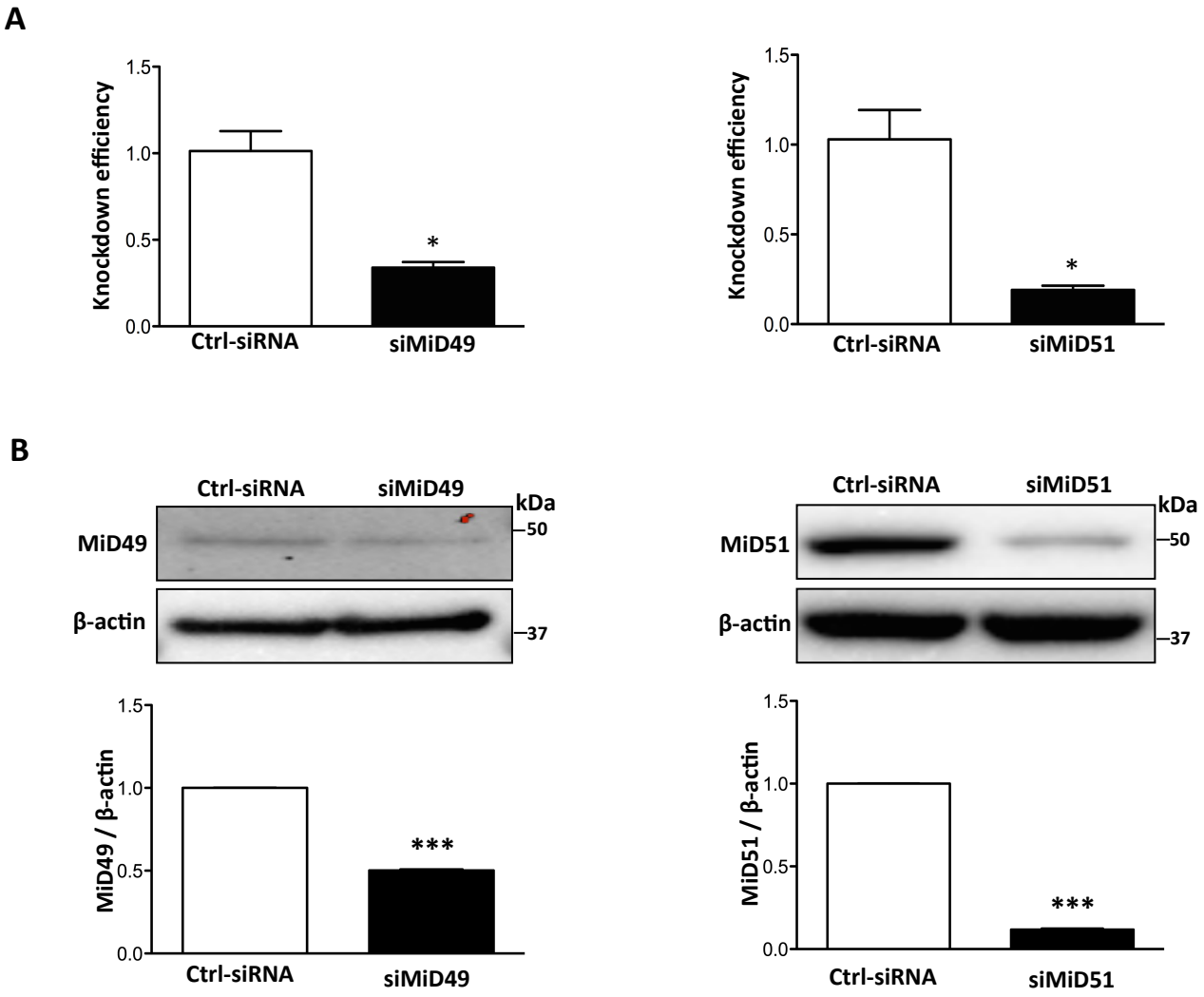
(A) Immunoblots showing expression of MiD49 and MiD51 in normal PASMC (n=3) vs PAH PASMC (n=2). β-actin was used as the loading control.

(B) Immunoblots and densitometries showing expression of MiD49 and MiD51 in BOEC isolated from normal individuals vs PAH patients. β-actin was used as the loading control (*$P < 0.05$; n=5-6/group).

(C) Representative images and quantifications of immunohistochemistry demonstrating increased expression of MiD49 and MiD51 (brown) in the media and intima of distal pulmonary arteries of Su/Hx PAH rats. 8-9 distal pulmonary arteries (<150 μm) from 3 animals per group (*$P < 0.05$, ***$P < 0.001$, NS: not significant). Scale bar: 25 μm.

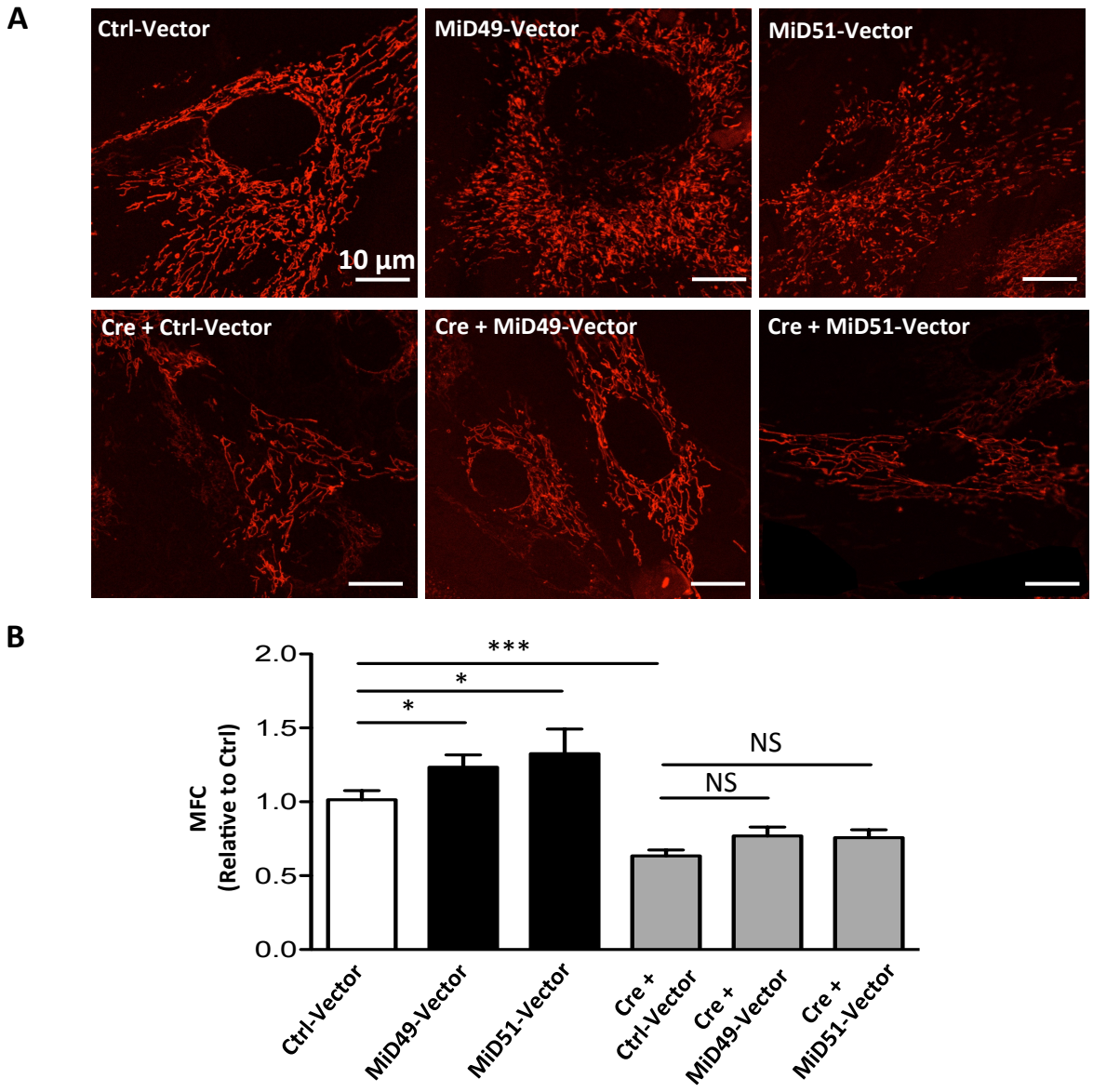
(D-E) BOEC from PAH patients have fragmented mitochondria. (D) Representative images of mitochondrial networks of BOEC isolated from normal individuals and PAH patients. Cells were loaded with the potentiometric dye TMRM (red). Scale bar: 10μm. (E) Mitochondrial fragmentation was quantified by mitochondrial fragmentation count (MFC) (**$P < 0.01$; n=15/group).

Supplementary Fig. 2



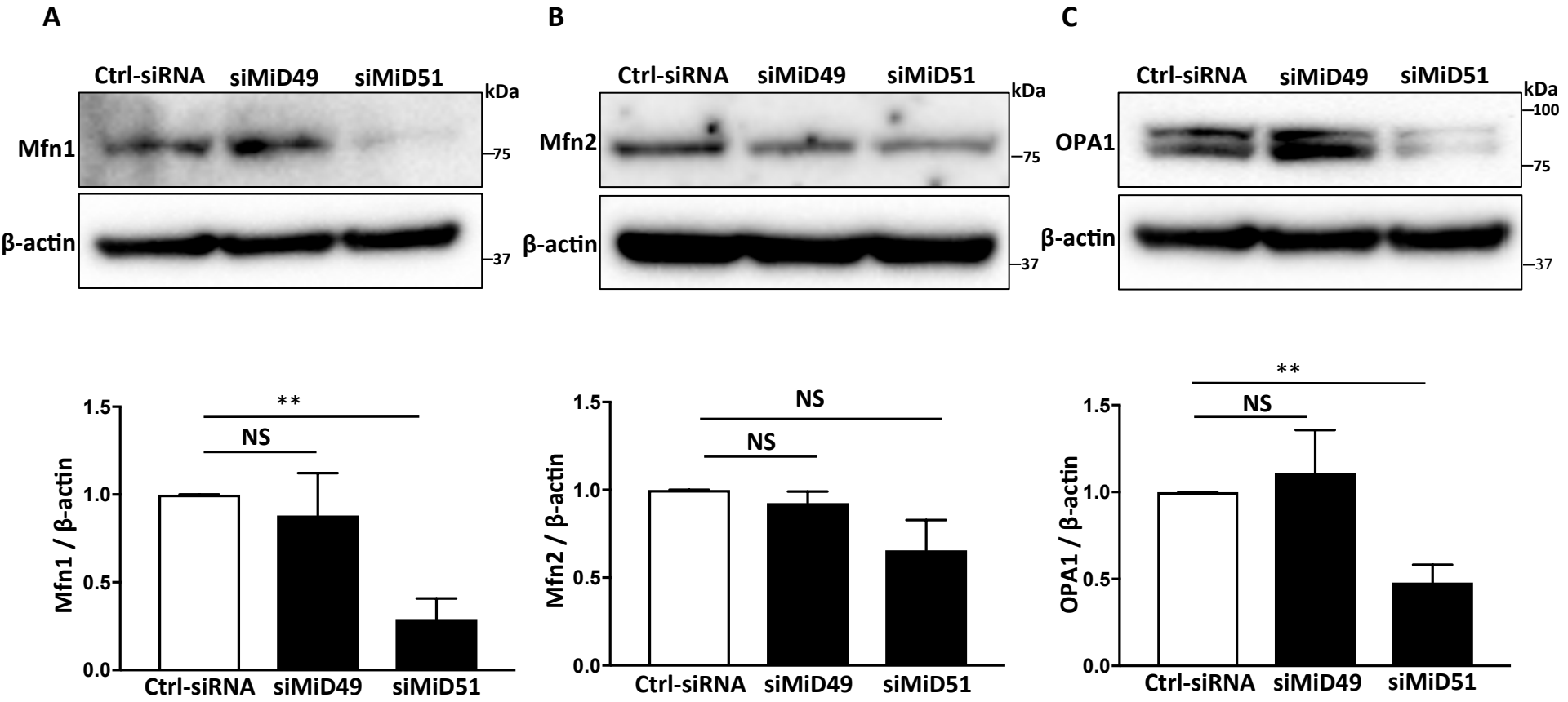
Supplementary Figure 2: Silencing of MiD49 and MiD51 at (A) RNA and (B) protein levels in human PAH PASMC by siRNA. RNA analyses were performed 48h after transfection with qRT-PCR; protein level was assayed 72h after transfection using immunoblot analysis (*$P < 0.05$, ***$P < 0.001$; n=3/group).

Supplementary Fig. 3



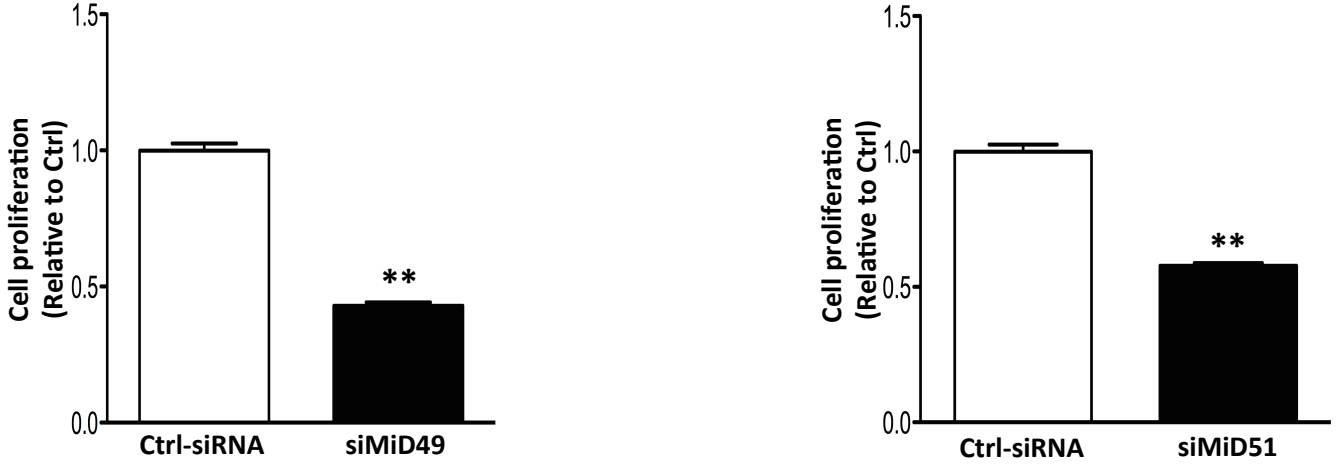
Supplementary Figure 3: Overexpression of MiD49 or MiD51 failed to mediate mitochondrial fission in Drp1 knockout MEFs.
(A) Representative images of mitochondrial networks of conditional Drp1 KO MEFs ($Drp1^{Fl/Fl}$ MEFs treated with Adv-Cre). Cells were loaded with the potentiometric dye TMRM (red). Scale bar: 10µm.
(B) Mitochondrial fragmentation was quantified by mitochondrial fragmentation count (MFC) (NS: not significant, $*P < 0.05$, $***P < 0.001$; n=15/group).

Supplementary Fig. 4



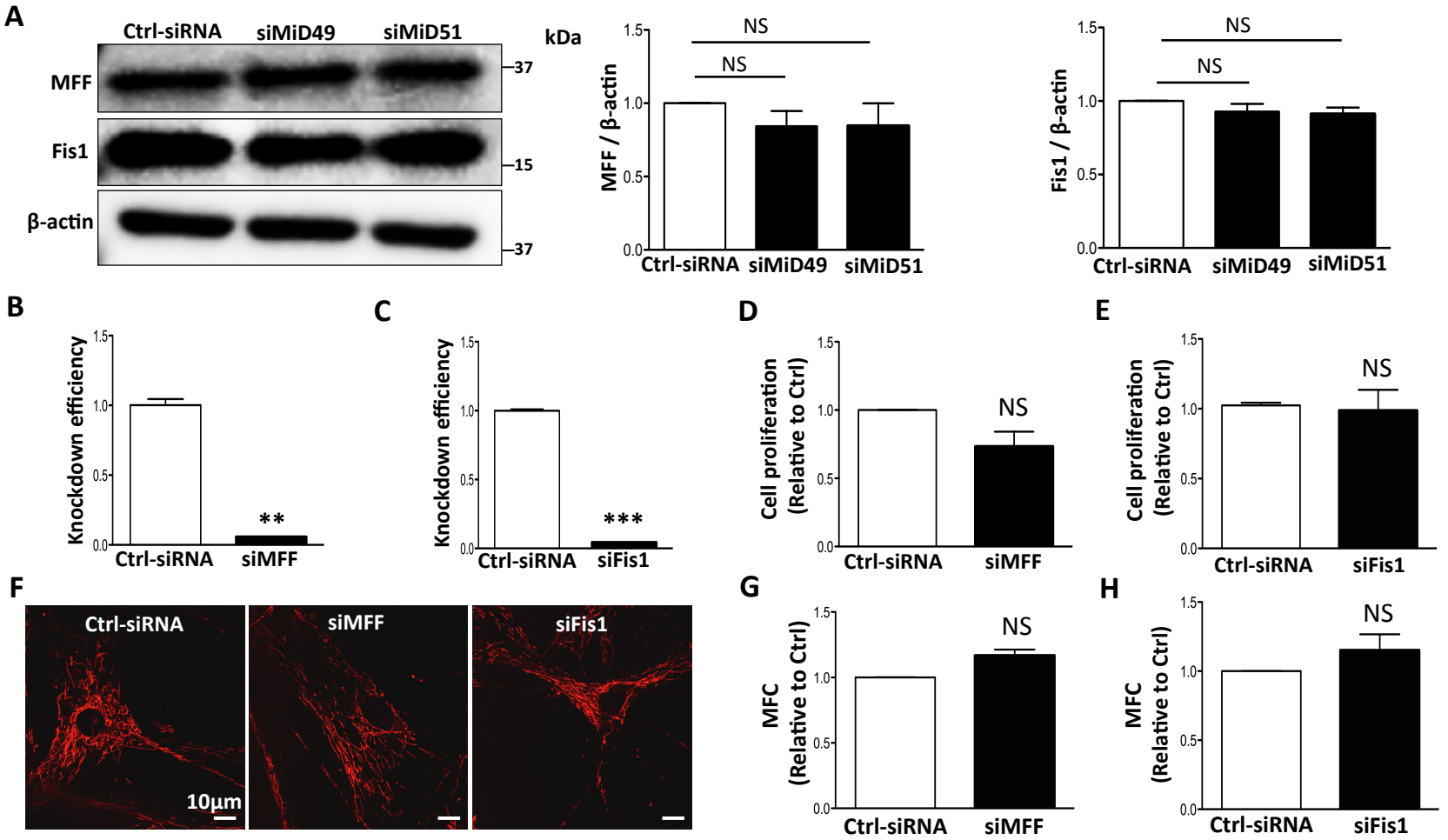
Supplementary Figure 4: MiD silencing did not upregulate the expression of mitochondrial fusion mediators. Representative immunoblots showing the expression of (A) Mfn1 (B) Mfn2 and (C) OPA1. β-actin was used as the loading control (NS: not significant, $^{**}P < 0.01$; n=3-4/group).

Supplementary Fig. 5



Supplementary Figure 5: Proliferation of normal PASMC was inhibited by silencing MiD49 or MiD51. Cell proliferation was analyzed 72h following transfection of siMiD49 or siMiD51 (**$P < 0.01$; n=3/group).

Supplementary Fig. 6



Supplementary Figure 6: MiD silencing did not alter the expression of other Drp1 binding partners (Fis1 and MFF) and silencing of Fis1 and MFF did not significantly alter cell proliferation and mitochondrial fission of PAH PASMC.

(A) Immunoblots and densitometries showing the expression of MFF and Fis1. Immunoblot analyses were performed 48h after knocking down MiD49 or MiD51 by siRNA. β-actin was used as the loading control (NS: not significant; n=3-4/group).

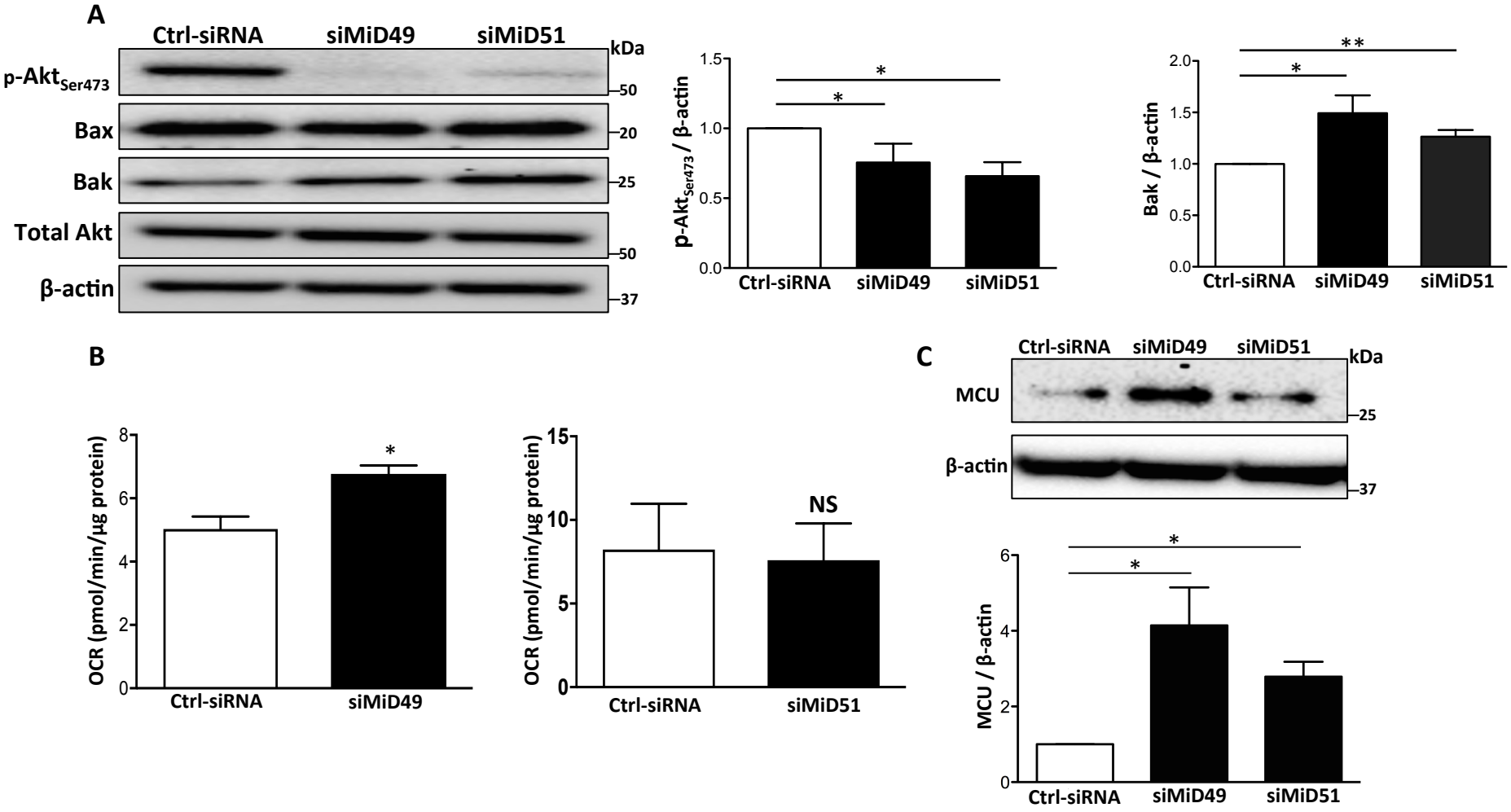
(B-C) Silencing of (B) MFF and (C) Fis1 at RNA level. RNA analyses were performed 48h after transfection with indicated siRNA by qRT-PCR (**$P < 0.01$, ***$P < 0.001$; n=3-4/group).

(D-E) Cell proliferation analysis performed after transfection with (D) siMFF or (E) siFis1. Cell proliferation was analyzed 72h following silencing of (D) MFF or (E) Fis1 by indicated siRNA (NS: not significant; n=3/group).

(F) Representative images of mitochondrial networks of PAH PASMC transfected with siMFF or siFis1. Cells were loaded with the potentiometric dye TMRM (red) and imaged with confocal microscope to assess the mitochondrial network structure. Scale bar: 10µm.

(G-H) Quantification of the mitochondrial fragmentation of (G) siMFF and (H) siFis1 (NS: not significant; n=6/group).

Supplementary Fig. 7



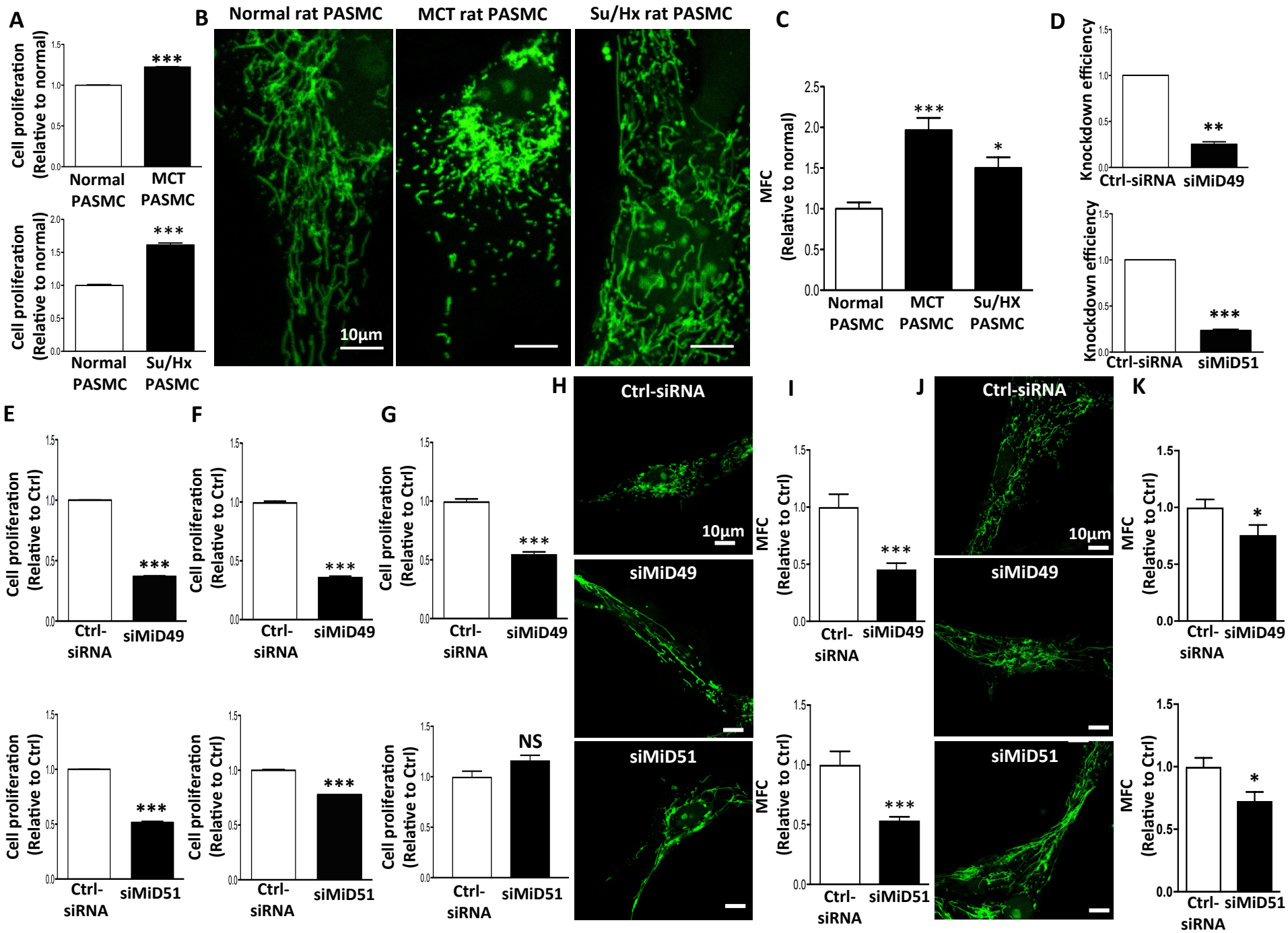
Supplementary Figure 7: Silencing MiDs modulates the expression of apoptotic mediators, increases basal oxygen consumption and restores MCU expression in PAH PASMC.

(A) Immunoblot analyses for the expressions of p-Akt$_{Ser473,}$ Bax, Bak and total Akt. Cells were transfected with siMiD49 or siMiD51 for 72h. β-actin was used as the loading control (*P < 0.05, **P < 0.01; n=3/group).

(B) Mitochondrial flux measured with Seahorse XFe24 in PAH PASMC transfected with siMiD49 or siMiD51 after 48h of transfection (NS: not significant, *P < 0.05; n=3/group).

(C) siMiDs restored MCU expression in PAH PASMC. PAH PASMCs were transfected with siMID49 or siMiD51. Cells were harvested for immunoblot analyses after 48h of transfection. β-actin was used as the loading control (*P < 0.05; n=3/group).

Supplementary Fig. 8



Supplementary Fig. 8 (continued)

Supplementary Figure 8: Silencing MiDs inhibits proliferation and mitochondrial fission in PASMC derived from MCT PAH or Su/Hx PAH rats.

(A-C) PASMC isolated from MCT PAH and Su/Hx PAH rats proliferate faster than normal rat PASMC and have fragmented mitochondrial network. (A) Cell proliferation was analyzed by Click-iT EdU flow cytometry assay kit (***$P < 0.001$; n=3/group). (B) Representative images of mitochondrial networks of PASMC isolated from normal or MCT PAH or Su/Hx PAH rats. Cells infected with Adv-mNeon Green and imaged after 48h with confocal microscopy to assess the mitochondrial network structure. Scale bar: 10µm. (C) Mitochondrial fragmentation was quantified by mitochondrial fragmentation count (MFC) (*$P < 0.05$, ***$P < 0.001$; n=8-15/group).

(D) Silencing of MiD49 or MiD51 at RNA level in normal rat PASMC. RNA analyses were performed 48h after transfection with indicated siRNA with qRT-PCR (**$P < 0.01$, ***$P < 0.001$; n=3/group).

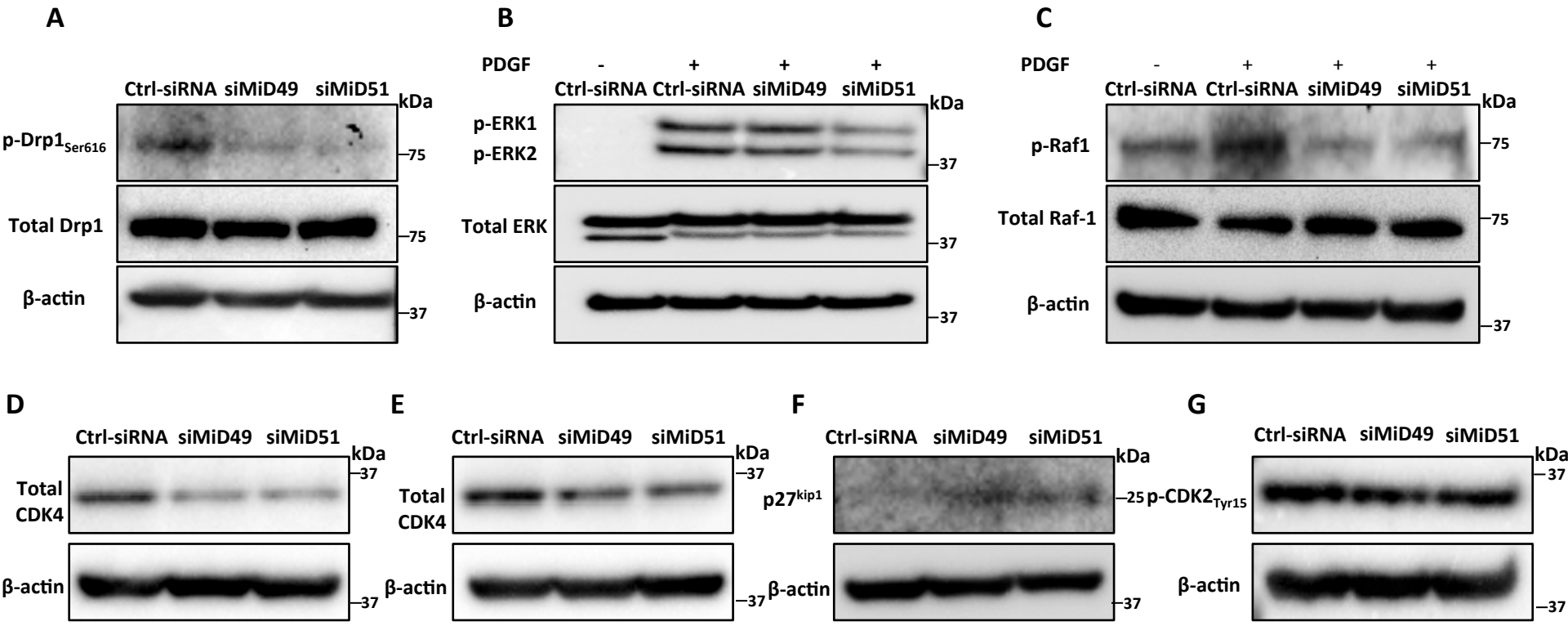
(E) Proliferation of PASMC isolated from normal rat was inhibited following silencing of MiD49 or MiD51 by indicated rat siRNAs. Cell proliferation was analyzed after 72h following transfection of indicated siRNA (***$P < 0.001$; n=3/group).

(F) Proliferation of PASMC isolated from MCT PAH rat was inhibited by silencing of MiD49 or MiD51. Cell proliferation was analyzed 72h following silencing of MiD49 or MiD51 (***$P < 0.001$; n=3/group).

(G) Proliferation of PASMC isolated from Su/Hx PAH rat was inhibited by silencing of MiD49. Cell proliferation was analyzed 72h following silencing of MiD49 or MiD51 (NS: not significant, ***$P < 0.001$; n=3/group).

(H-K) Mitochondrial fragmentation in PASMC isolated from MCT PAH and Su/Hx PAH rats was reversed by silencing of MiD49 or MiD51. (H) Representative images of mitochondrial networks of PASMC isolated from MCT PAH rat transfected with the specified siRNA, infected with Adv-mNeon Green and imaged after 48h following infection. Scale bar: 10µm. (I) Mitochondrial fragmentation was quantified by mitochondrial fragmentation count (MFC) (***$P < 0.001$; n=13-14/group). (J) Representative images of mitochondrial networks of PASMC isolated from Su/Hx PAH rat and transfected with the specified siRNA, and infected with Adv-mNeon Green and imaged 48h following infection. Scale bar: 10µm. (K) Mitochondrial fragmentation was quantified by mitochondrial fragmentation count (MFC) (*$P < 0.05$; n=15-16/group).

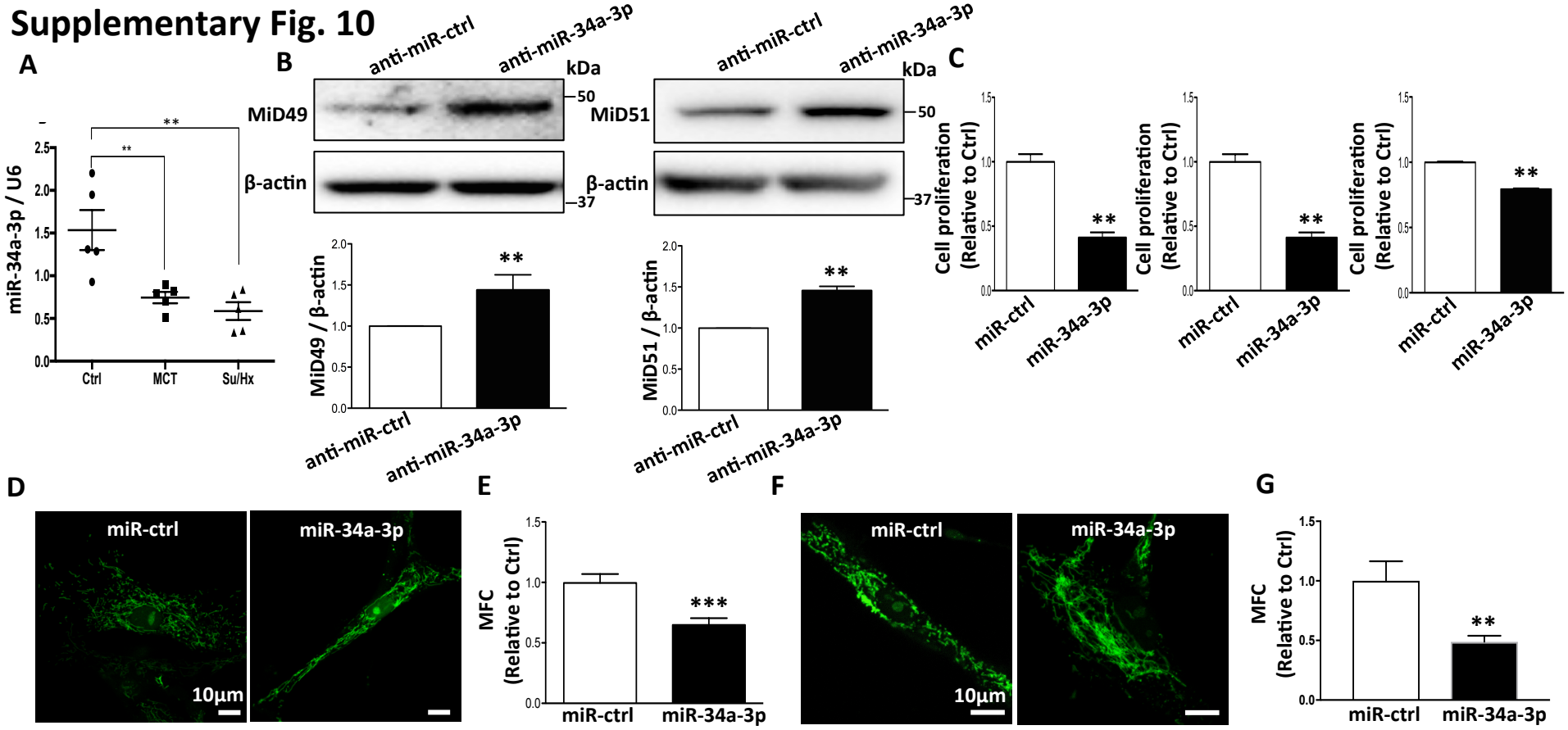
Supplementary Fig. 9



Supplementary Figure 9

(A-G) Knocking down MiDs inhibit mediators of mitochondrial fission and cell proliferation. Representative images of the immunoblots showing the expressions of (A) p-Drp1$_{Ser616}$ from MCT PAH PASMC, (B) p-ERK1/2 from MCT PAH PASMC, (C) p-Raf1 from human PAH PASMC, (D) Total CDK4 from human PAH PASMC, (E) Total CDK4 from MCT PAH PASMC, (F) p27^{Kip1} from MCT PAH PASMC and (G) p-CDK2$_{Tyr15}$ from human PAH PASMC**.** PAH PASMCs were transfected with siMiD49 or siMiD51. Cells were harvested for immunoblot analyses after 48h of transfection. β-actin was used as the loading control.

Supplementary Fig. 10



Supplementary Figure 10: miR-34a-3p is reduced in rodent models of PAH, regulates the expression of MiD49 and MiD51 and inhibits the proliferation of MCT PAH PASMC.

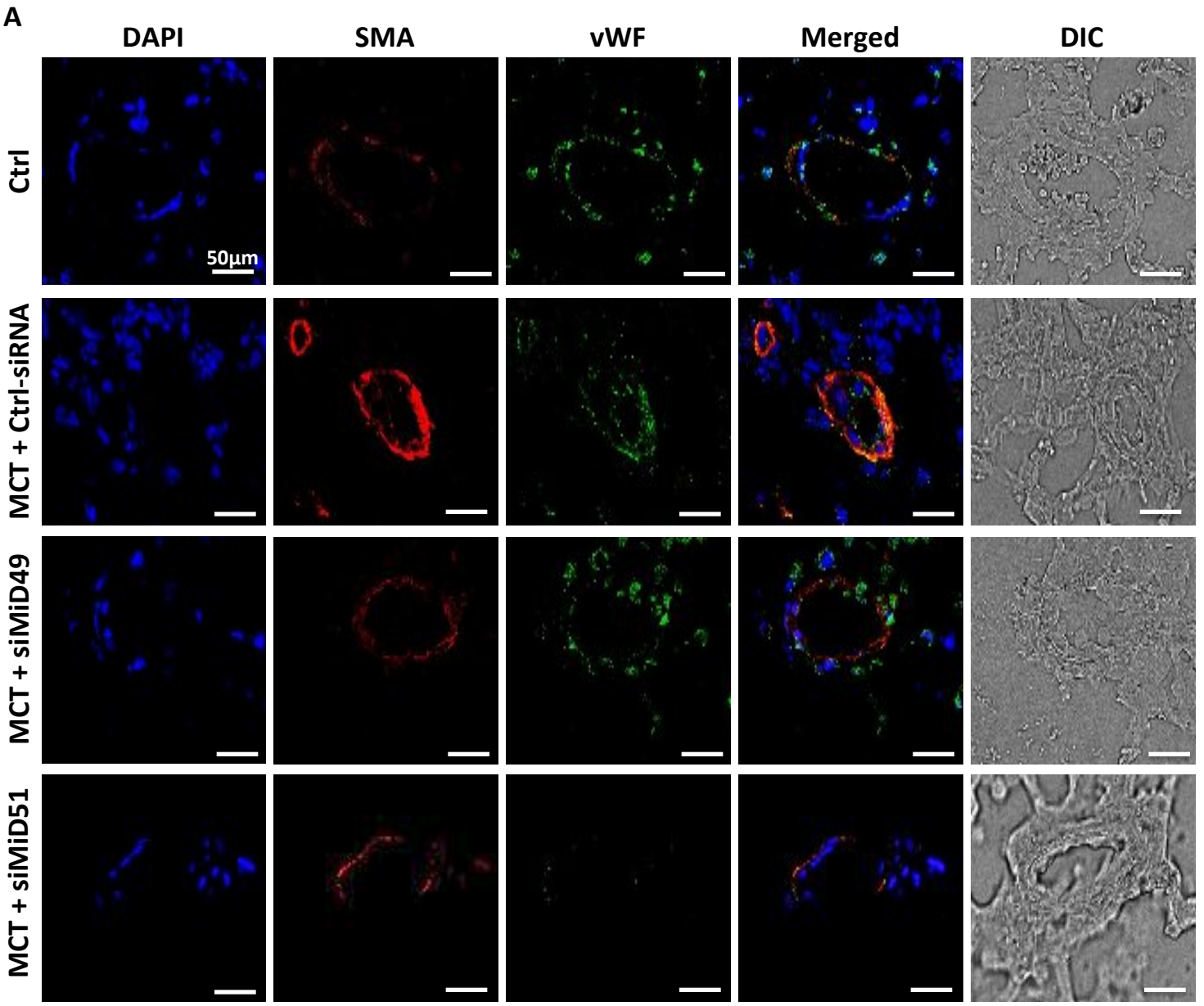
(A) Expression of miR-34a-3p isolated from the whole blood of MCT PAH or Su/Hx PAH rats. miR-34a-3p expression was assessed by qRT-PCR (***P* < 0.01; n=5/group).

(B) Anti-miR-34a-3p upregulates MiD49 and MiD51 expression in normal rat PASMC. Representative images of immunoblots and densitometries showing the expressions of MiD49 and MiD51 in normal rat PASMC. Normal rat PASMC were transfected with anti-miR-34a-3p for 72h. β-actin was used as the loading control (***P* < 0.01; n=3-4/group).

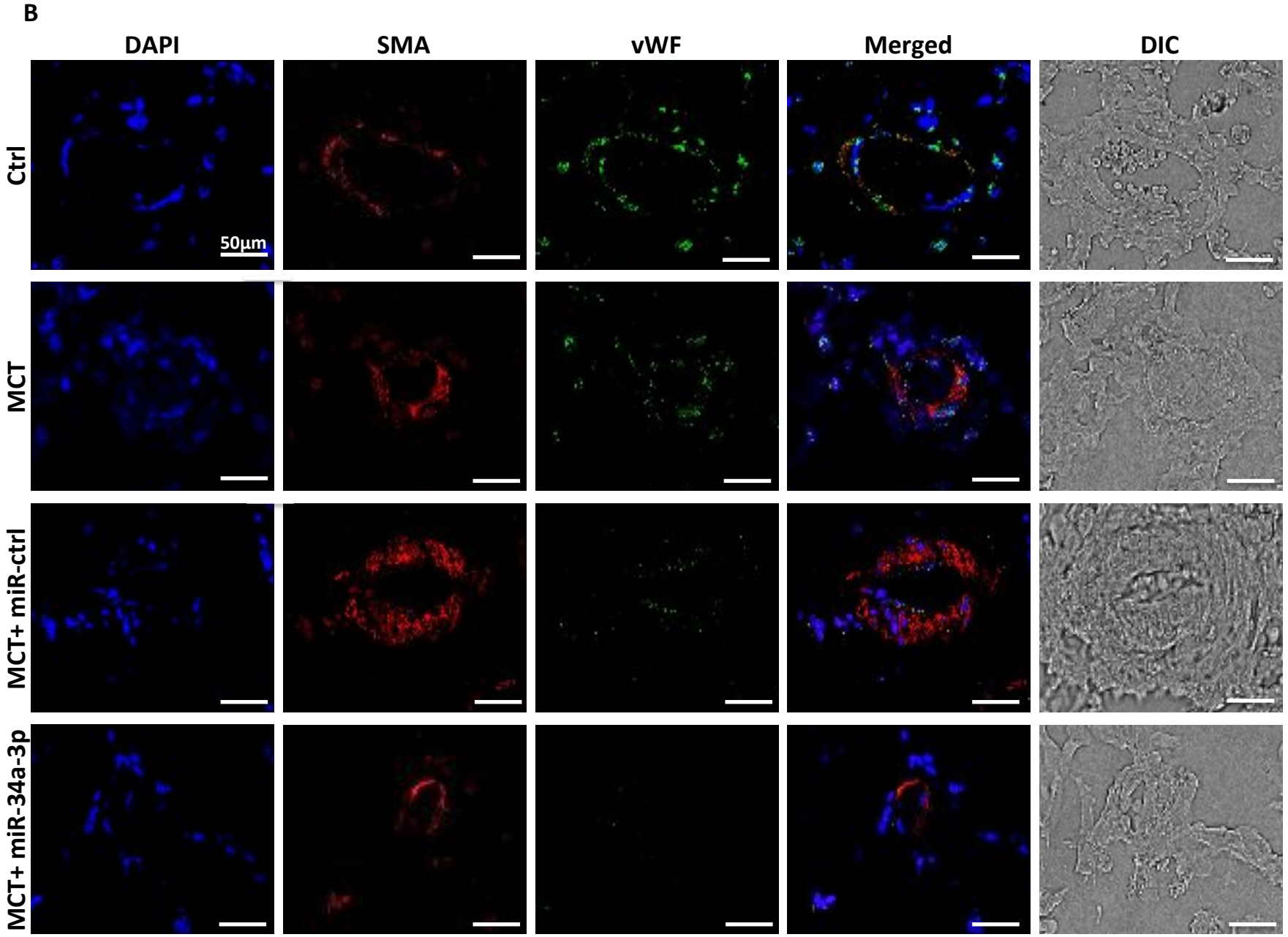
(C) Augmenting miR-34a-3p inhibits proliferation of PASMC isolated from normal, MCT PAH and Su/Hx PAH rats. Cell proliferation was analyzed 72h following miR-34a-3p mimic transfection (***P* < 0.01; n=3/group).

(D-G) Augmenting of miR-34a-3p inhibits mitochondrial fission. (D) Representative images of mitochondrial networks of PASMC isolated from MCT PAH rats and transfected with miR-34a-3p mimic. Cells infected with Adv-mNeon Green and imaged after 48h with confocal microscopy to assess the mitochondrial network structure. Scale bar: 10µm. (E) Mitochondrial fragmentation was quantified by mitochondrial fragmentation count (MFC) (****P* < 0.001; n=10-14/group). (F) Representative images of mitochondrial networks of PASMC isolated from Su/Hx PAH rats and transfected with miR-34a-3p mimic. Cells were infected with Adv-mNeon Green and imaged after 48h with confocal microscopy to assess the mitochondrial network structure. Scale bar: 10µm. (G) Mitochondrial fragmentation was quantified by mitochondrial fragmentation count (MFC) (***P* < 0.01; n=25/group).

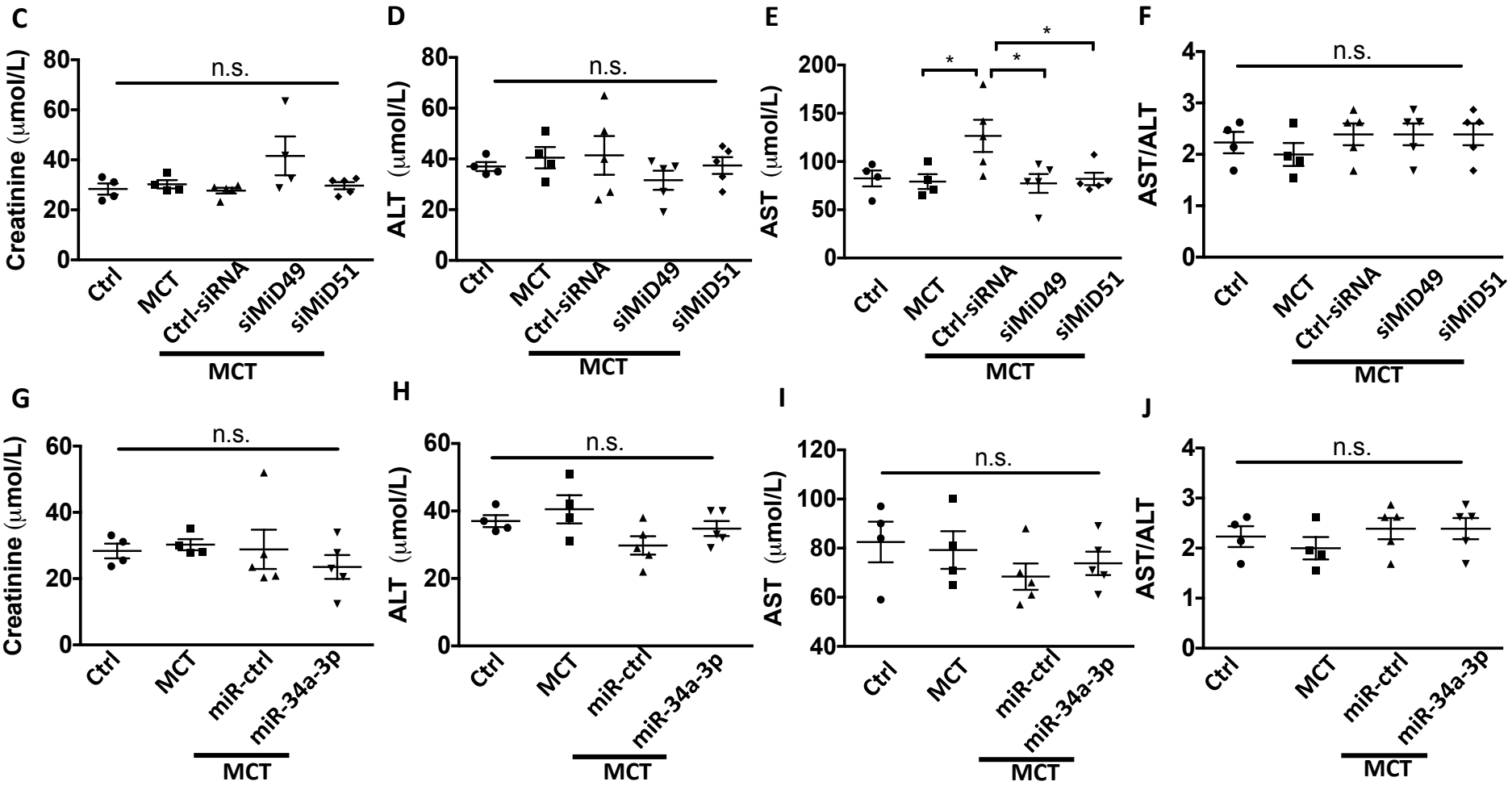
Supplementary Fig. 11



Supplementary Fig. 11 (continued)



Supplementary Fig. 11 (continued)



Supplementary Figure 11: Regression of PAH in MCT PAH rats treated with siMiD49 or siMiD51 or by miR-34a-3p mimic caused no hepatic or renal toxicity.

(A-B) Representative cross section images of small pulmonary arteries indicating the regression of PAH was associated with decrease in the wall thickness in MCT PAH rats treated with either (A) siMiD49 or siMiD51 or by (B) miR-34a-3p mimic. Scale bar: 50μm.

(C-F) Toxicity study carried out to confirm that neither siRNA against MiD49 or MiD51 caused hepatic or renal toxicity in rats (NS: not significant, **P* < 0.05; n=4-5/group).

(G-J) Toxicity study carried out to confirm that miR-34a-3p mimic caused no hepatic or renal toxicity in rats (NS: not significant; n=4-5/group).