Supplementary Figures

Fig. S1

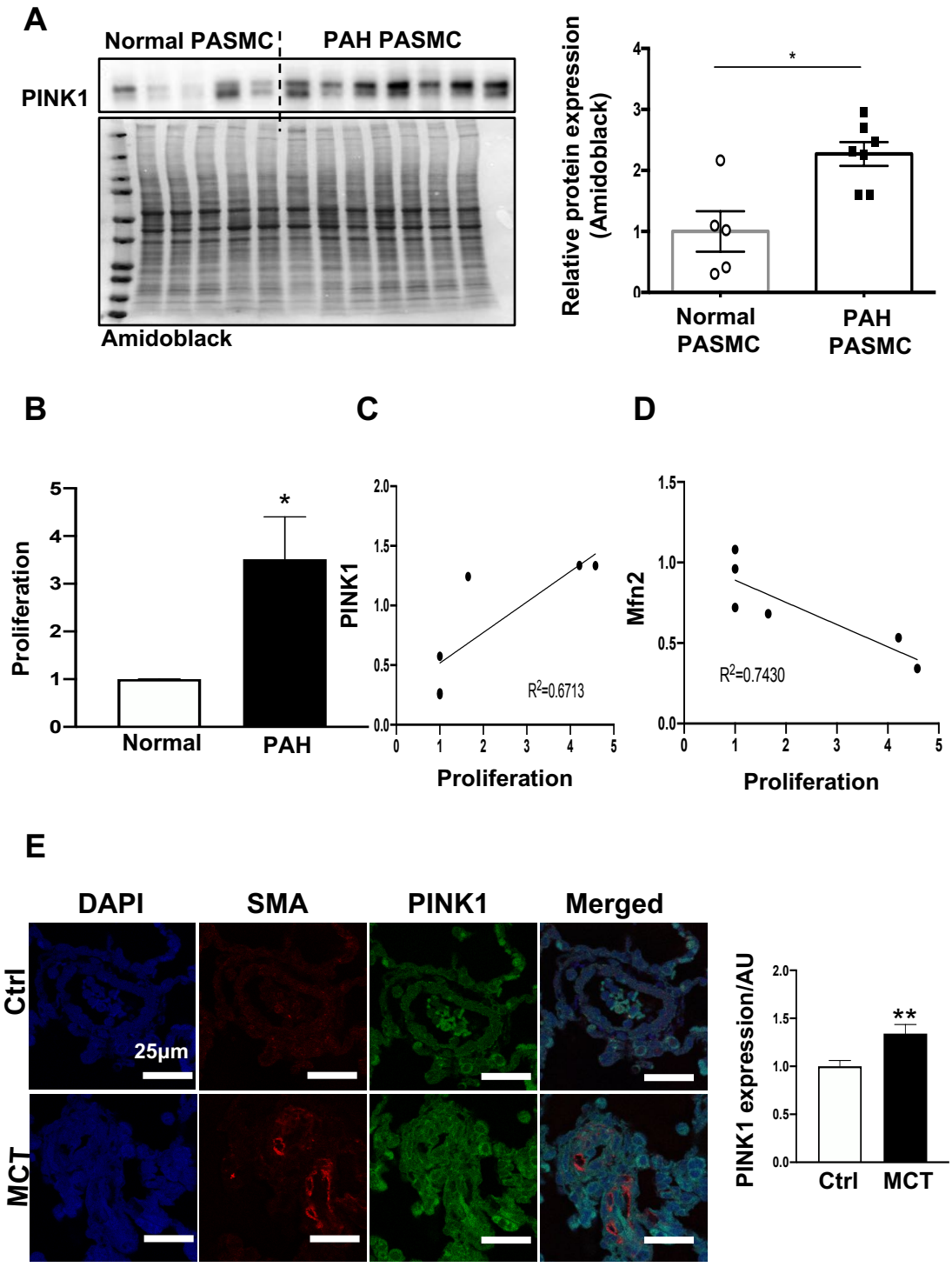
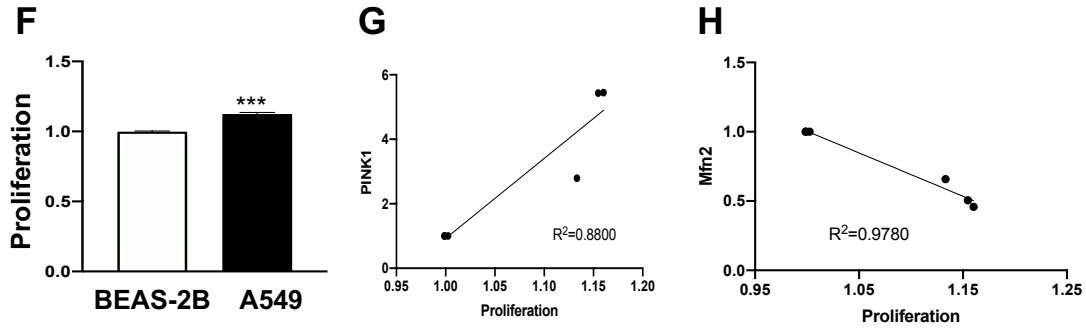

A
Normal PASMC
PAH PASMC
PINK1
Amidoblack
Relative protein expression (Amidoblack)
Normal PASMC
PAH PASMC
B
Proliferation
Normal
PAH
C
PINK1
R2=0.6713
Proliferation
D
Mfn2
R2=0.7430
Proliferation
E
DAPI
SMA
PINK1
Merged
Ctrl
MCT
25μm
PINK1 expression/AU
Ctrl
MCT

Fig. S1 (continued)



S1: PINK1 is upregulated in human and MCT PAH and proliferation of human PASMC is positively correlated with PINK1 expression and inversely correlated with Mfn2 expression.

A) Representative immunoblots and densitometry demonstrating increased protein expression of PINK1 in human PAH PASMC vs normal human PASMC (n=5 for normal PASMC and n=7 for PAH PASMC). The protein expression was normalized to amidoblack. **P* < 0.05.

B) PAH PASMC proliferates faster than normal human PASMC (n=3 for normal PASMC and n=3 for PAH PASMC). **P* < 0.05.

C) PINK1 expression is positively correlated to the proliferation of PASMC. Correlation analysis showing that expression level of PINK and proliferation of PASMC are positively correlated (n=6). R^2=0.6713.

D) Mfn2 expression is inversely correlated to the proliferation of PASMC. Correlation analysis showing that expression level of Mfn2 and proliferation of PASMC are inversely correlated (n=6). R^2=0.7430.

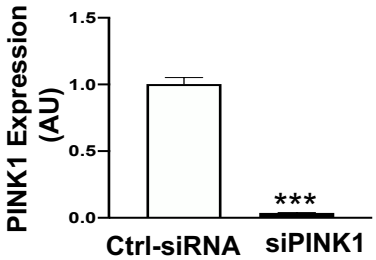
E) PINK1 is upregulated in MCT PAH. Representative cross section images of small pulmonary arteries indicating increased PINK1 expression in MCT PAH rats. (n=14-15/group). ***P* < 0.01. Scale bar: 25μm.

F) NSCLC cells, A549, proliferates faster than the non-neoplastic cells, BEAS-2B (n=3). ****P* < 0.001.

G) PINK1 expression is positively correlated to the proliferation of A549 and BEAS-2B cells. Correlation analysis showing that expression level of PINK and proliferation of A549 and BEAS2B cells are positively correlated (n=6). R^2=0.8800.

H) Mfn2 expression is inversely correlated to the proliferation of A549 and BEAS-2B cells. Correlation analysis showing that expression level of Mfn2 and proliferation of A549 and BEAS2B cells are inversely correlated (n=6). R^2=0.09780.

Fig. S2



S2: Knockdown efficiency of siPINK1 in A549 cells. RNA analyses were performed with qRT-PCR 48 hours after transfection (n=3/group). ****P* < 0.001.

Fig. S3

A

Program	Site	Sequence	Score	Prediction
NetPhos 2.0	S442	IRRL**S**VLVD	0.993	*S*

Program	Position	Sequence	Corresponding motif	Features of motifs
PhosphoMotif	440-442	R**S**L	RXpS	Protein kinase A substrate motif

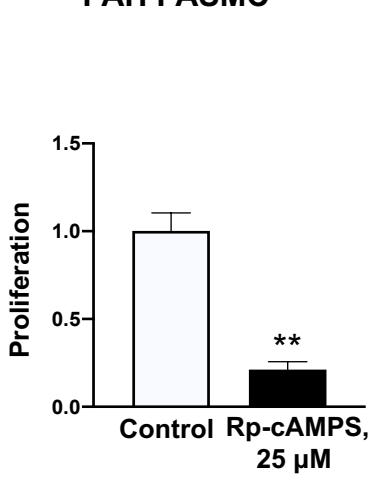
http://www.cbs.dtu.dk/services/NetPhos/

B

```
# Sequence          442 S   IRRLSVLVD   0.993   unsp
# Sequence          442 S   IRRLSVLVD   0.859   PKA
# Sequence          442 S   IRRLSVLVD   0.545   RSK
# Sequence          442 S   IRRLSVLVD   0.459   GSK3
# Sequence          442 S   IRRLSVLVD   0.458   CaM-II
# Sequence          442 S   IRRLSVLVD   0.396   cdc2
# Sequence          442 S   IRRLSVLVD   0.390   ATM
# Sequence          442 S   IRRLSVLVD   0.368   CKI
# Sequence          442 S   IRRLSVLVD   0.362   PKG
# Sequence          442 S   IRRLSVLVD   0.358   PKC
# Sequence          442 S   IRRLSVLVD   0.347   DNAPK
# Sequence          442 S   IRRLSVLVD   0.326   CKII
# Sequence          442 S   IRRLSVLVD   0.313   p38MAPK
# Sequence          442 S   IRRLSVLVD   0.210   PKB
# Sequence          442 S   IRRLSVLVD   0.173   cdk5
```

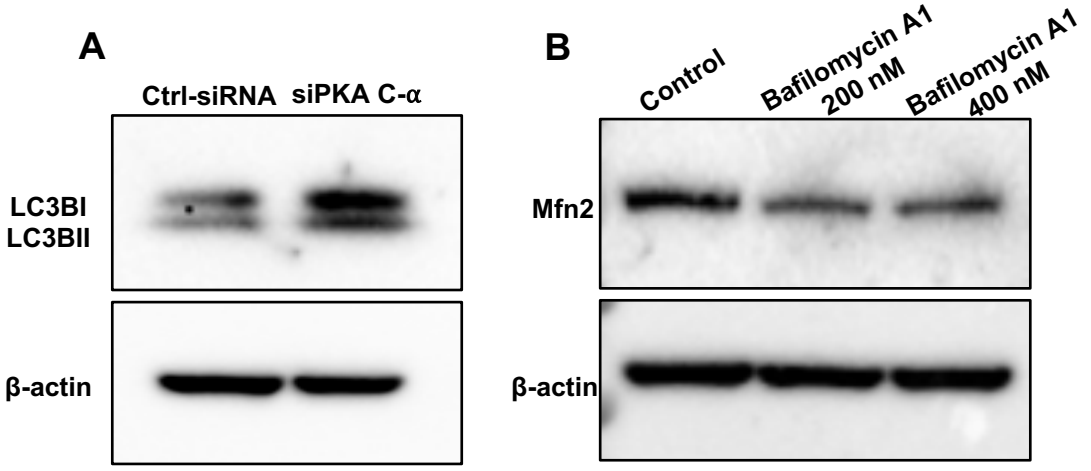
S3: A) Serine 442 of mitofusin2 amino acid sequence is a candidate phosphorylation site of protein kinase A predicted by NetPhos 2.0 and PhosphoMotif programs with high possibility. B) FASTA from http://www.cbs.dtu.dk/services/NetPhos/ showing PKA is the predicted kinase with highest score that can phosphorylate serine 442 site of Mfn2.

Fig. S4



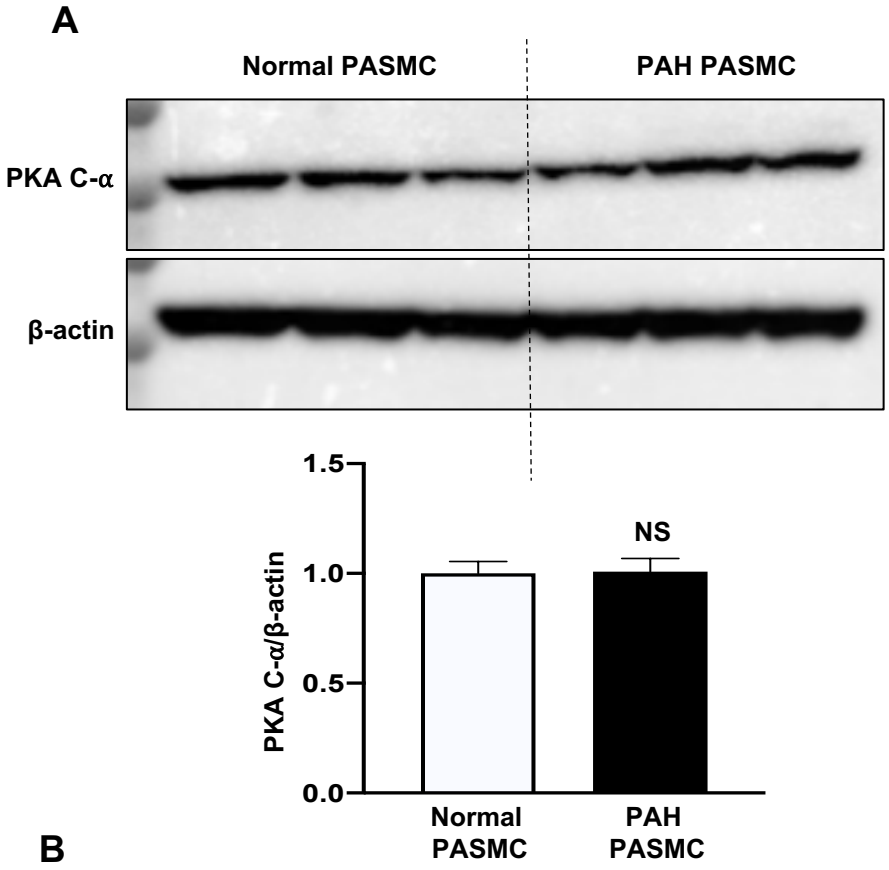
S4: Pharmacological inhibition of PKA inhibits cell proliferation in PAH PASMC. PAH PASMCs were treated with or without the PKA inhibitor, Rp-cAMPS (25 μM). Cell proliferation was analyzed by Click-iT EdU flow cytometry assay kit 72 hours following treatment (n=3/group). $^{**}P < 0.01$.

Fig. S5



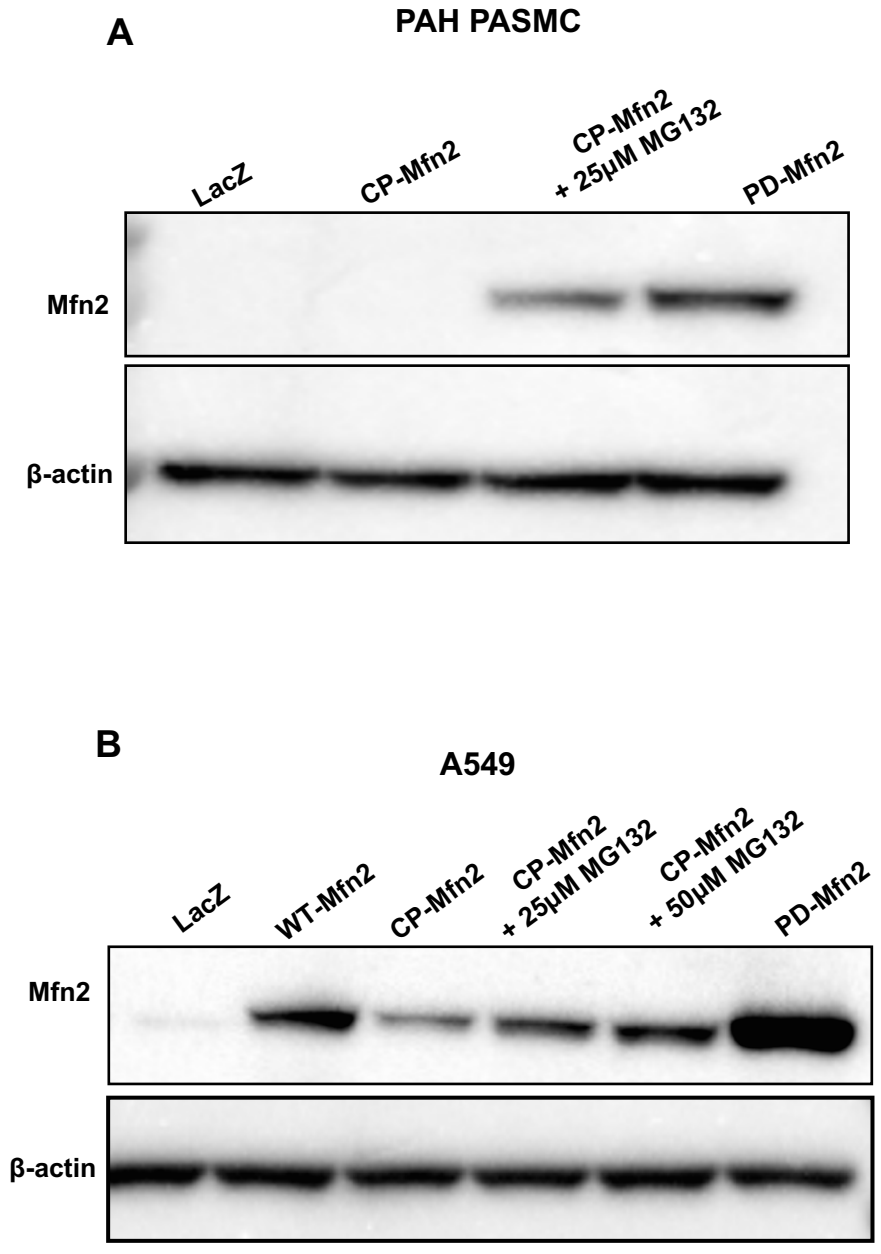
S5: Upregulation of Mfn2 following PKA silencing is not due to inhibition of mitophagy or lysosomal inhibition. Representative immunoblots showing A) expression of LC3BII 48 hours following siPKA C-α silencing; B) expression of Mfn2 following 24 hours of treatment with a lysosomal inhibitor bafilomycin A1. β-actin was used as the loading control.

Fig. S6



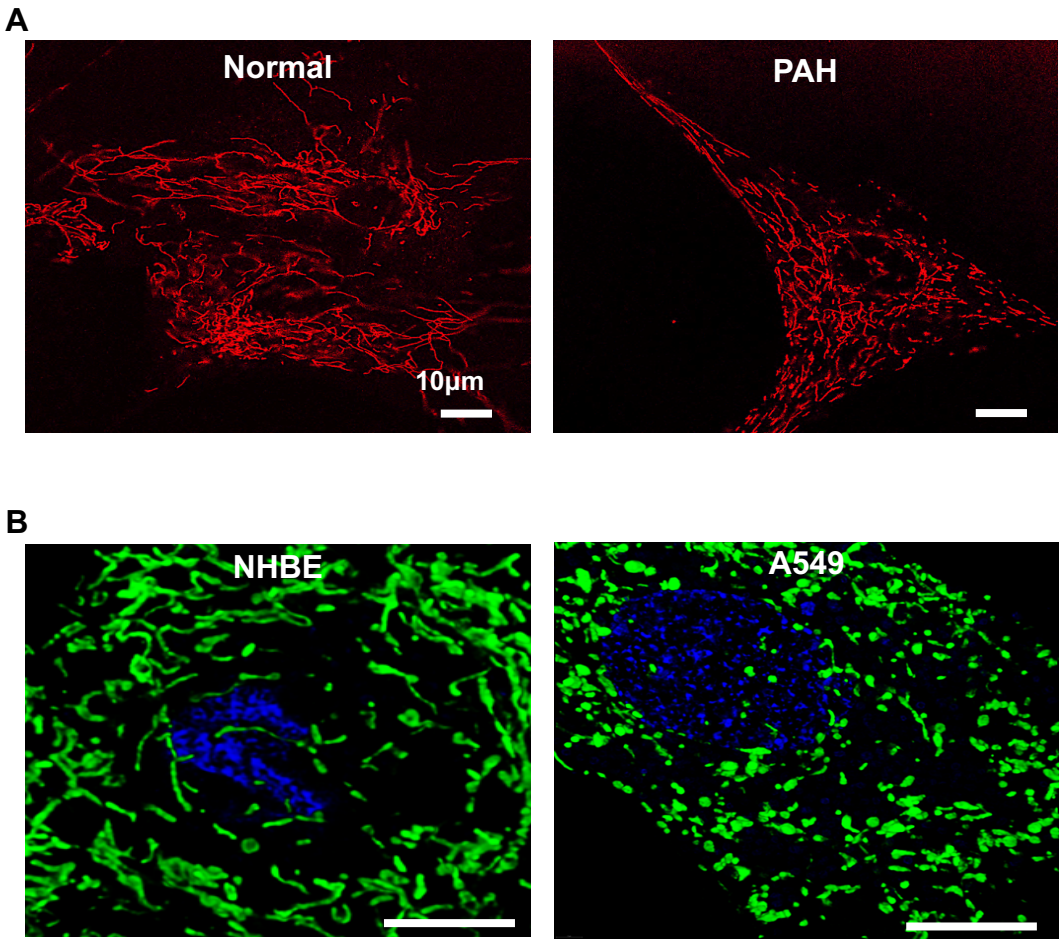
S6: A) Representative immunoblots and densitometry showing the expression of PKA-C-α in normal vs PAH PASMC (n=3). NS: not significant. B) Representative immunoblot showing the expression of PKA-C-α in NHBE vs A549 cells.

Fig. S7



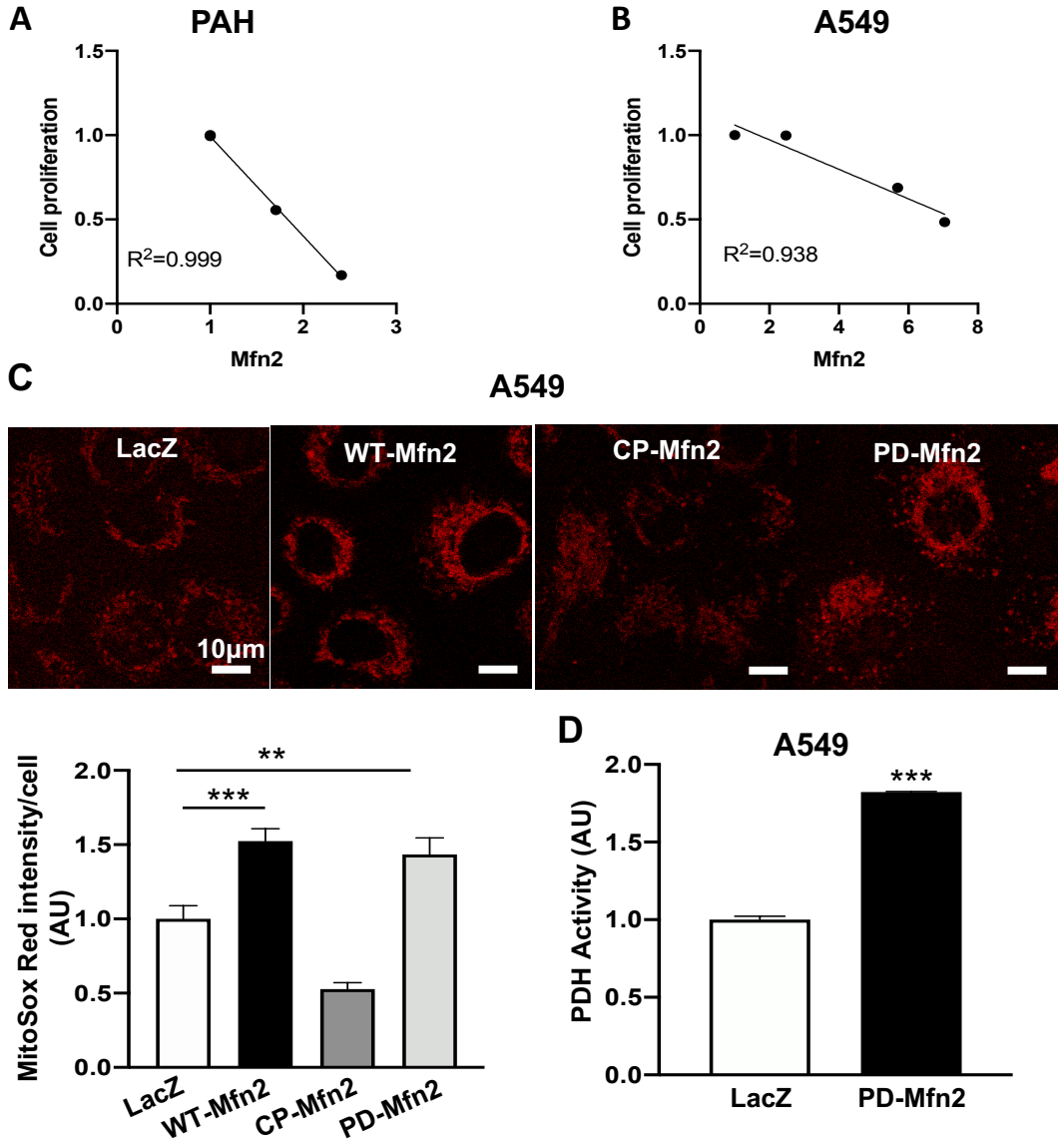
S7: Representative immunoblots showing increased expression of CP-Mfn2 following proteasomal blockade. A) PAH PASMC and B) A549 cells were infected by adenovirus carrying indicated Mfn2 constructs for 40 hours. CP-Mfn2 overexpressed cells were treated with or without the indicated dose of a proteasome inhibitor, MG132 for 8 hours before harvesting. β-actin was used as the loading control.

Fig. S8



S8: PAH PASMC and NSCLC cells have fragmented mitochondrial network. Representative images of mitochondrial networks of A) Normal PASMC and PAH PASMC and B) NHBE and A549. Cells were stained with either TMRM (red) or Mitotracker Green (green). Scale bar: 10μm.

Fig. S9

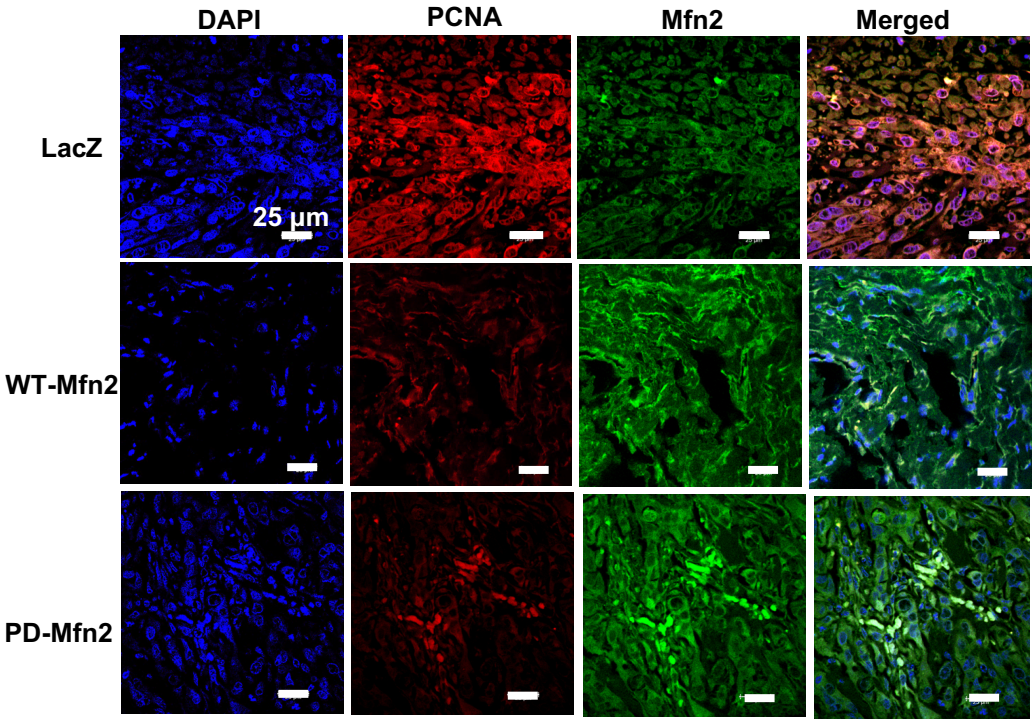


S9 A-B) Correlation analyses showing cell proliferation is inversely correlated with Mfn2 expression in A) PAH PASMC and B) A549 cells. Mfn2 constructs (LacZ, WT-Mfn2, CP-Mfn2 and PD-Mfn2) were overexpressed by adenoviral infection (n=3-4). R^2=0.999 for PAH PASMC and R^2=0.938 for A549 cells.

C) **Mfn2 overexpression increases mitochondrial ROS generation.** The A549 cells were infected with adenovirus carrying PD-Mfn2 constructs. Adenovirus carrying LacZ gene was used as a control. Mitochondrial ROS generation was assessed by staining the cells with MitoSOX™ Red Mitochondrial Superoxide Indicator 48 hours post infection. (n=19-20 cells/group). $^{**}P < 0.01$; $^{***}P < 0.001$.

D) **PD-Mfn2 increased PDH activity.** The A549 cells were infected with adenovirus carrying PD-Mfn2 constructs. Adenovirus carrying LacZ gene was used as a control. PDH activity assay was conducted 48 hours post infection. (n=3/group). $^{***}P < 0.001$.

Fig. S10



S10: Representative images of tumor sections indicating a superior regression of tumor growth by PD-Mfn2 treatment. Scale bar: 25µm.