Supplementary material

Mitochondrial dysfunction in human primary alveolar type II cells in emphysema

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Table E1. Primers used for qPCR. Mitochondrial DNA amount **(A)**, mitochondrial DNA damage **(B)** and common deletions **(C)** were determined in freshly isolated ATII cells and lung tissue obtained from non-smokers, smokers, and emphysema patients.

A

Short mtDNA fragment	F	GAATTAACTAGAAATAACTTTGCAAGG
	R	CCAGCTATCACCAGGCTC
Short nuclear DNA fragment	F	TGCTGTCTCCATGTTTGATGTATCT
	R	TCTCTGCTCCCCACCTCTAAGT

B

Long mtDNA fragment	F	ACAAAGAACCCTAACACCAGC
	R	TGTTGAGCTTGAACGCTTTC
Short mtDNA fragment	F	GAATTAACTAGAAATAACTTTGCAAGG
	R	CCAGCTATCACCAGGCTC

C

Common deletion	F	ACCCTATAGCACCCCCTCTAC
	R	CTTGTCAGGGAGGTAGCGATG
Short mtDNA fragment	F	GAATTAACTAGAAATAACTTTGCAAGG
	R	CCAGCTATCACCAGGCTC

Table E2. Primers used for RT-PCR. *TDP1*, *MFN1* and *DRP1* mRNA expression were evaluated in freshly isolated ATII cells and lung tissue obtained from non-smokers, smokers, and emphysema patients.

Gene		**Primer's sequence (5'-3')**
TDP1	F	TCTCCTTTATTTGGGACGCTTG
	R	GGATACTGTTTTACGAGCCAGTC
MFN1	F	GAGGTGCTATCTCGGAGACAC
	R	GCCAATCCCACTAGGGAGAAC
DRP1	F	TCAACCTCCGCGTCTACTC
	R	GATCTGGAACTCGATGTCGGG
GAPDH	F	GGAGCGAGATCCCTCCAAAAT
	R	GGCTGTTGTCATACTTCTCATGG
β-actin	F	GGCTGTATTCCCCTCCATCG
	R	CCAGTTGGTAACAATGCCAT

Table E3. Primers used for CHIP assay. CHIP assay was performed in lung tissue obtained from non-smokers, smokers and emphysema patients.

Short mtDNA fragment	F	GAATTAACTAGAAATAACTTTGCAAGG
	R	CCAGCTATCACCAGGCTC
GAPDH	F	GGCTCCCTAGGCCCCTCCTG
	R	TCCCAACTCGGCCCCCAACA

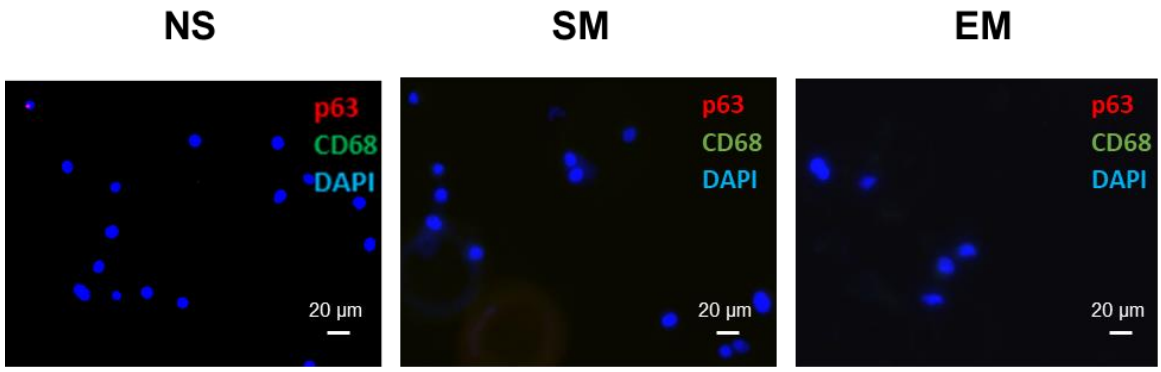


Figure E1. Purity of freshly isolated ATII cells. ATII cells were isolated from lung tissue obtained from non-smokers (NS), smokers (SM) and emphysema patients (EM). Cell purity was determined by p63 and CD68 expression by immunocytofluorescence (N=3 lungs per group).

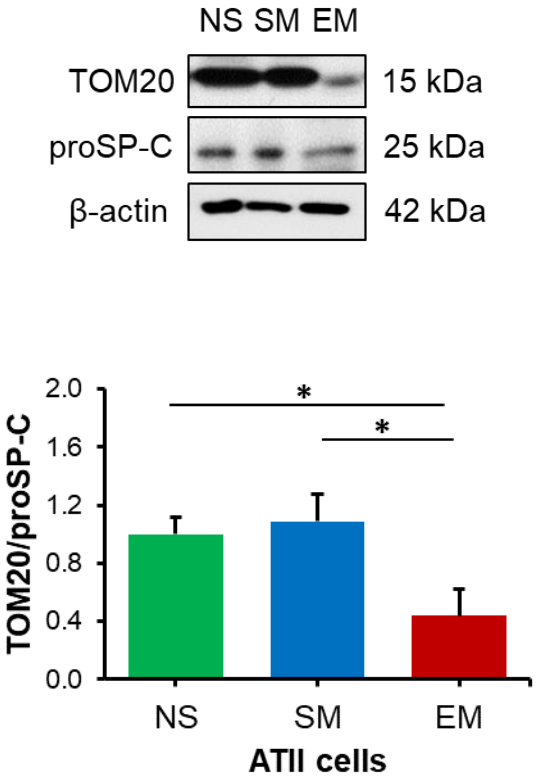


Figure E2. Protein expression in ATII cells. ATII cells were isolated from lung tissue obtained from non-smokers (NS), smokers (SM), and emphysema patients (EM). TOM20 and proSP-C expression in ATII cells was determined by Western blotting. Quantification is also shown. Data is expressed as means ± s.e.m, N=3 lungs per group, *P<0.05.

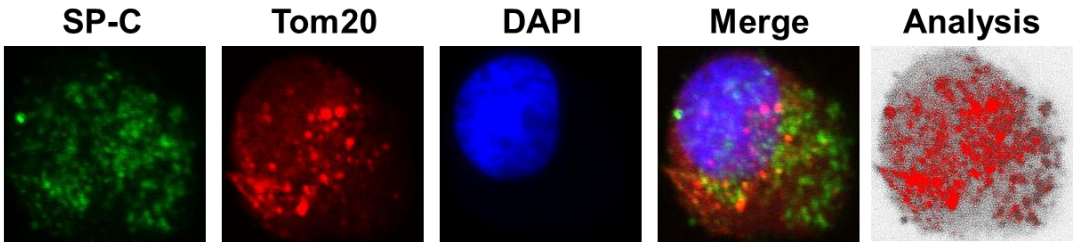


Figure E3. Mitochondrial swelling in ATII cells. Representative images of mitochondrial swelling analysis as described in the Materials and Methods section. SP-C and TOM20 were used to identify ATII cells and mitochondria, respectively (N=3 lungs per group).

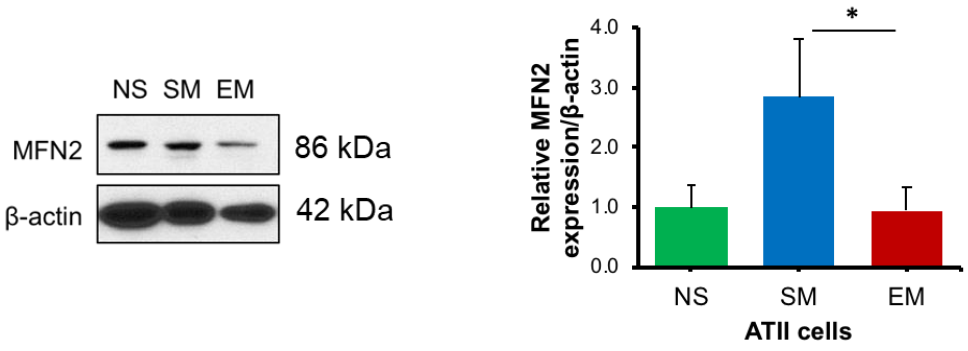


Figure E4. Decreased MFN2 expression in ATII cells in emphysema. ATII cells were isolated from lung tissue obtained from non-smokers (NS), smokers (SM) and emphysema patients (EM). MFN2 expression was determined by Western blotting. Quantification is also shown. Data is expressed as means ± s.e.m. *$P < 0.05$.

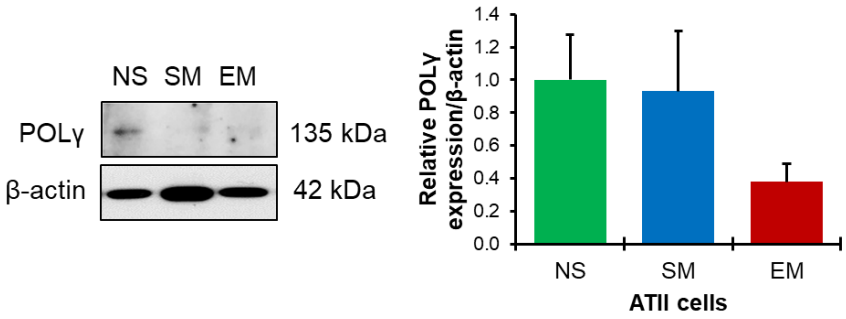


Figure E5. POLγ expression in ATII cells. ATII cells were isolated from lung tissue obtained from non-smokers (NS), smokers (SM) and emphysema patients (EM). POLγ expression was determined by Western blotting. Quantification is also shown. Data is expressed as means ± s.e.m.

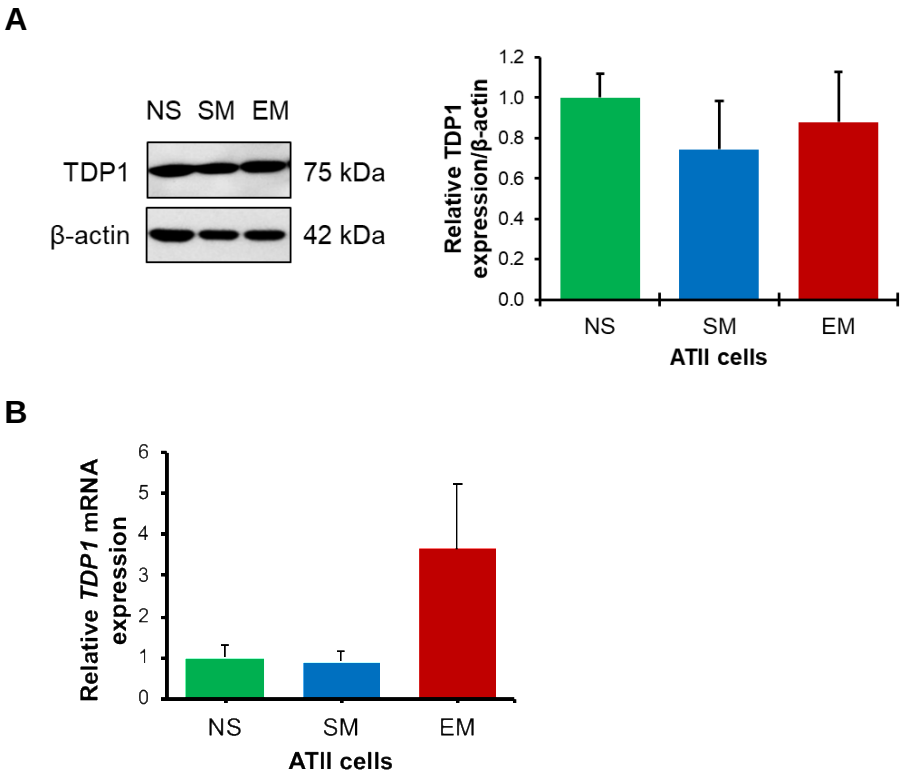


Figure E6. TDP1 expression in ATII cells. ATII cells were isolated from lung tissue obtained from non-smokers (NS), smokers (SM) and emphysema patients (EM). **A** – TDP1 expression was determined by Western blotting. Quantification is also shown. **B** – *TDP1* mRNA levels were analyzed by RT-PCR. Data is expressed as means ± s.e.m.

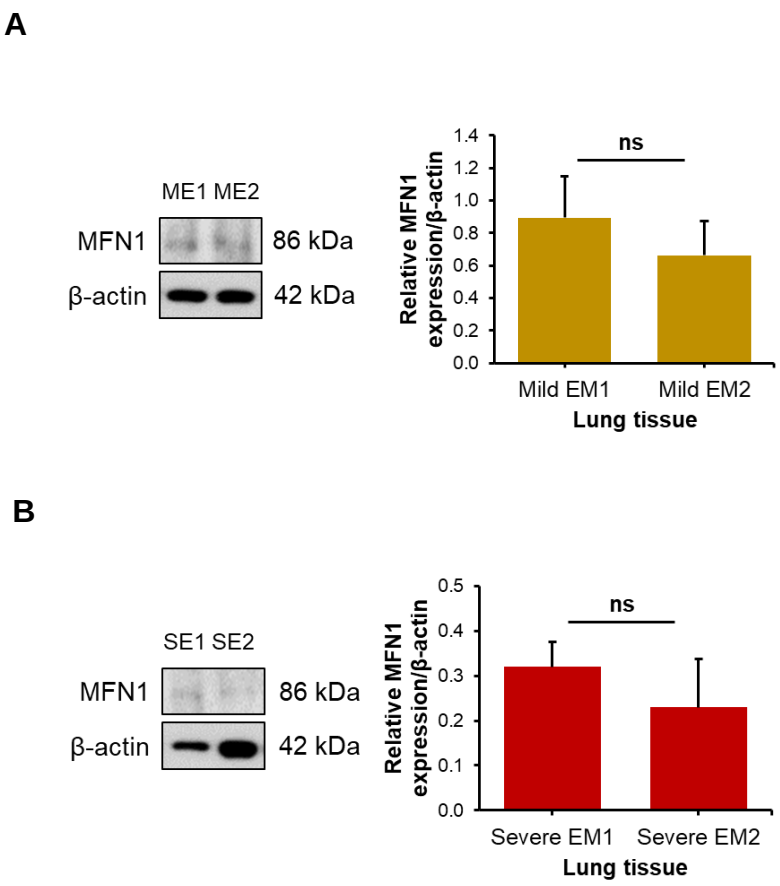


Figure E7. Variability in MFN1 expression in mild and severe emphysema. Lung tissue cores were obtained from 2 areas (EM1, EM2) with mild (**A**) and 2 areas with severe emphysema (**B**) from the same patient. MFN1 expression was determined by Western blotting. Quantification is also shown. ns – non-significant, N=3 lungs.

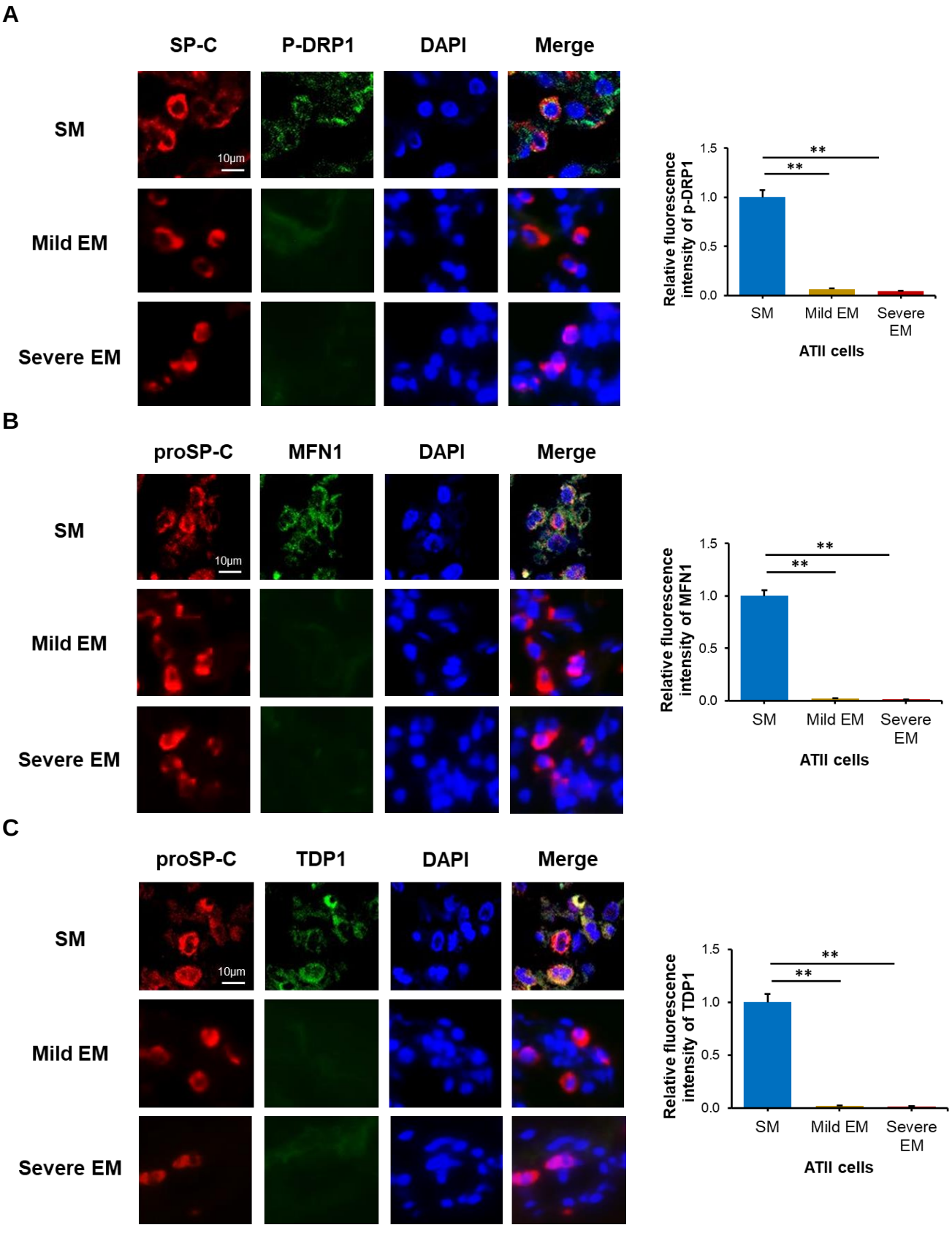

A
SP-C
P-DRP1
DAPI
Merge
SM
Mild EM
Severe EM
10μm
Relative fluorescence intensity of p-DRP1
1.5
1.0
0.5
0.0
**
**
SM
Mild EM
Severe EM
ATII cells
B
proSP-C
MFN1
DAPI
Merge
SM
Mild EM
Severe EM
10μm
Relative fluorescence intensity of MFN1
1.5
1.0
0.5
0.0
**
**
SM
Mild EM
Severe EM
ATII cells
C
proSP-C
TDP1
DAPI
Merge
SM
Mild EM
Severe EM
10μm
Relative fluorescence intensity of TDP1
1.5
1.0
0.5
0.0
**
**
SM
Mild EM
Severe EM
ATII cells

Figure E8. Low p-DRP1, MFN1, and TDP1 expression in ATII cells in areas with mild and severe emphysema. Lung sections were obtained from control smokers (SM) and emphysema patients (EM). p-DRP1, MFN1 or TDP1 expression (green) in ATII cells (red) identified using proSP-C or SP-C antibody in lung tissue sections by immunohistofluorescence. Quantification is also shown. Data is expressed as means ± s.e.m, N = 3 lungs per group, $**P < 0.001$.

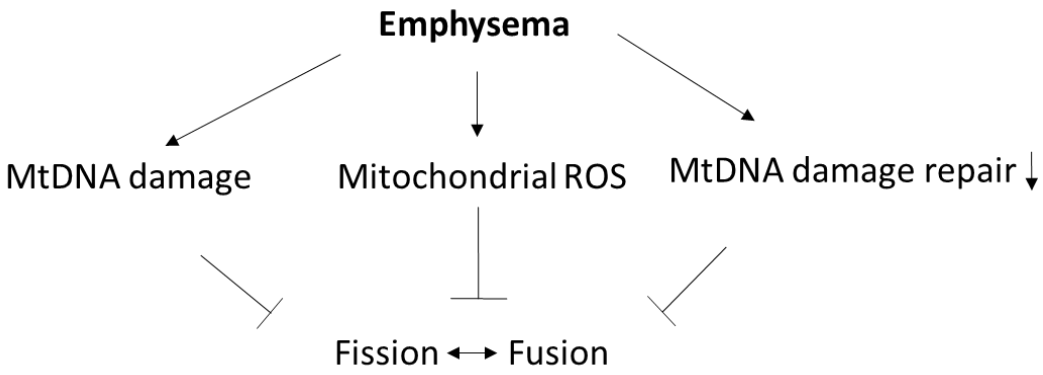
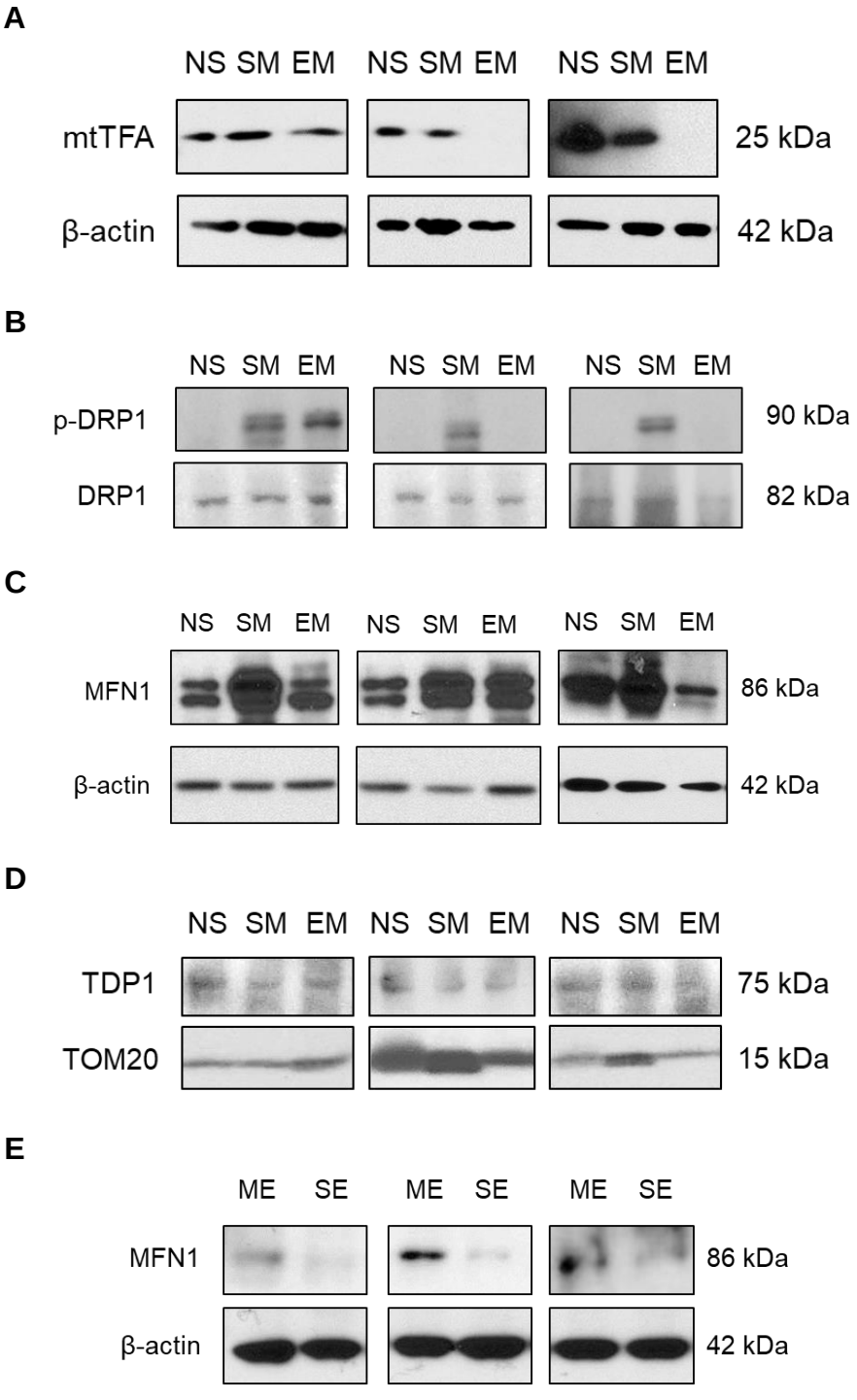


Figure E9. Mitochondrial dysfunction in ATII cells in emphysema.


A
NS SM EM NS SM EM NS SM EM
mtTFA
25 kDa
β-actin
42 kDa
B
NS SM EM NS SM EM NS SM EM
p-DRP1
90 kDa
DRP1
82 kDa
C
NS SM EM NS SM EM NS SM EM
MFN1
86 kDa
β-actin
42 kDa
D
NS SM EM NS SM EM NS SM EM
TDP1
75 kDa
TOM20
15 kDa
E
ME SE ME SE ME SE
MFN1
86 kDa
β-actin
42 kDa

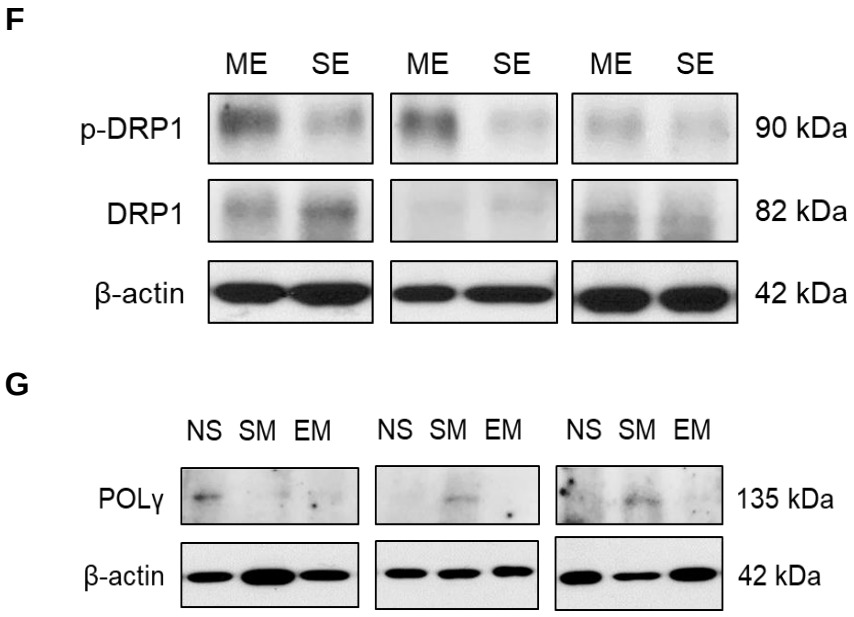


Figure E10. Representative images showing Western blots for figures 1E (A), 2A (B), 3A (C), 4C (D), 5F (E), 5E (F), and E5 (G).