

SUPPLEMENTARY INFORMATION for

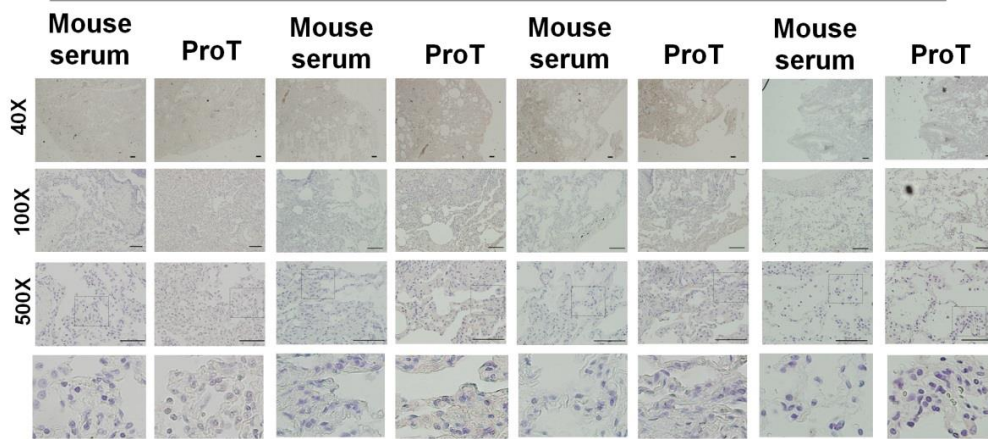
**Prothymosin α overexpression contributes to the development of pulmonary
emphysema**

Bing-Hua Su, Yau-Lin Tseng, Gia-Shing Shieh, Yi-Cheng Chen, Ya-Chieh Shiang,

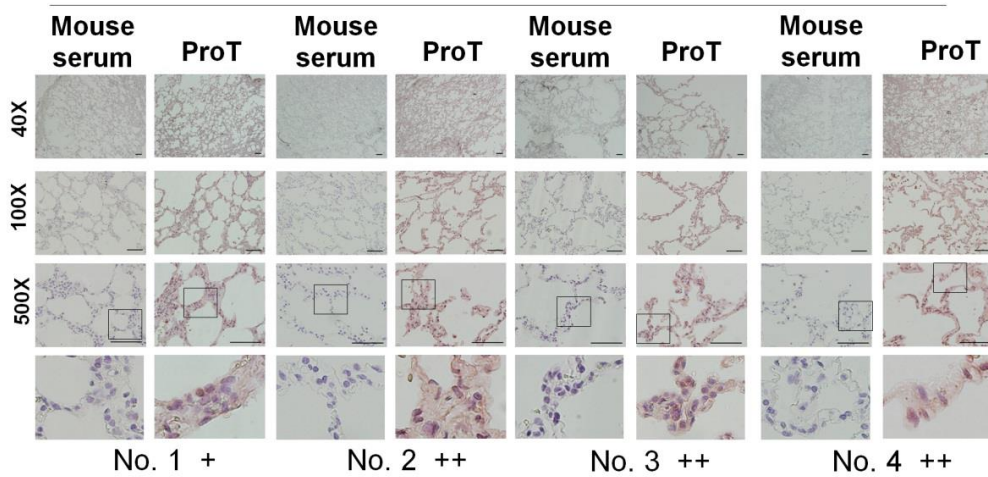
Pensee Wu, Kuo-Jung Li, Te-Hsin Yen, Ai-Li Shiau & Chao-Liang Wu

Supplementary Figure S1

Normal

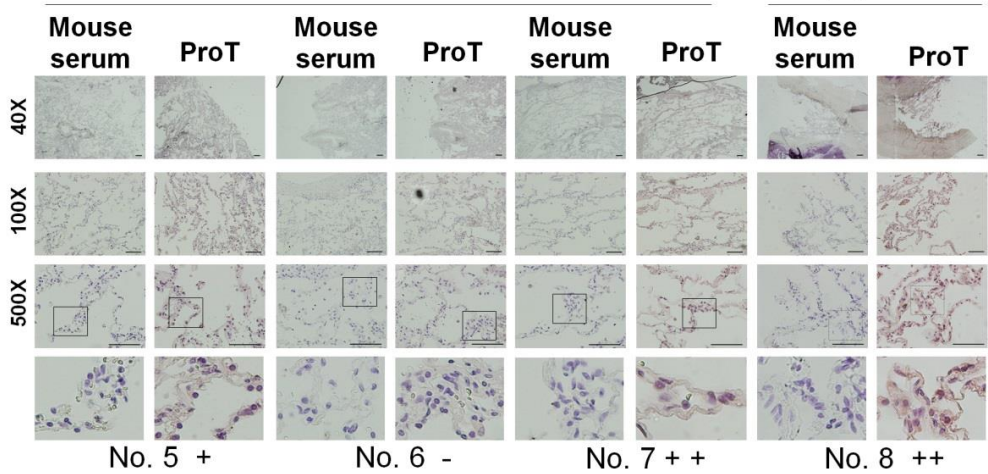


Mild emphysema



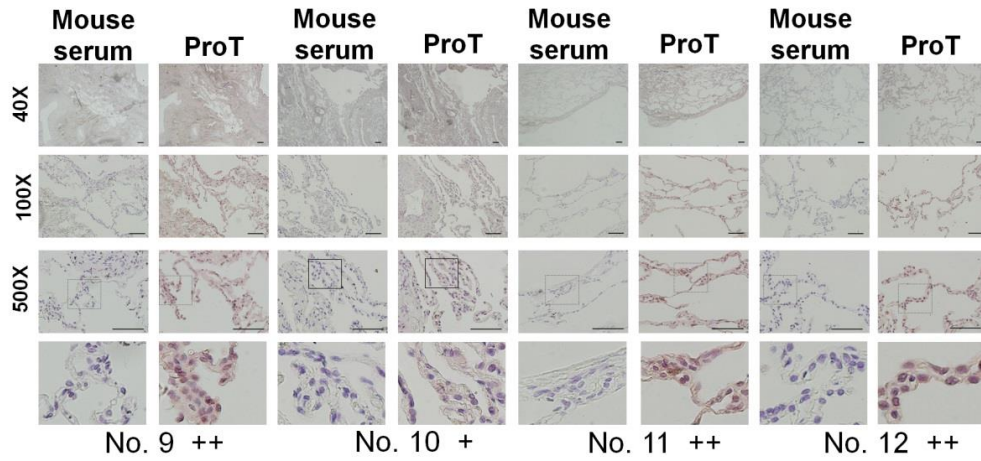
Mild emphysema

Moderate emphysema

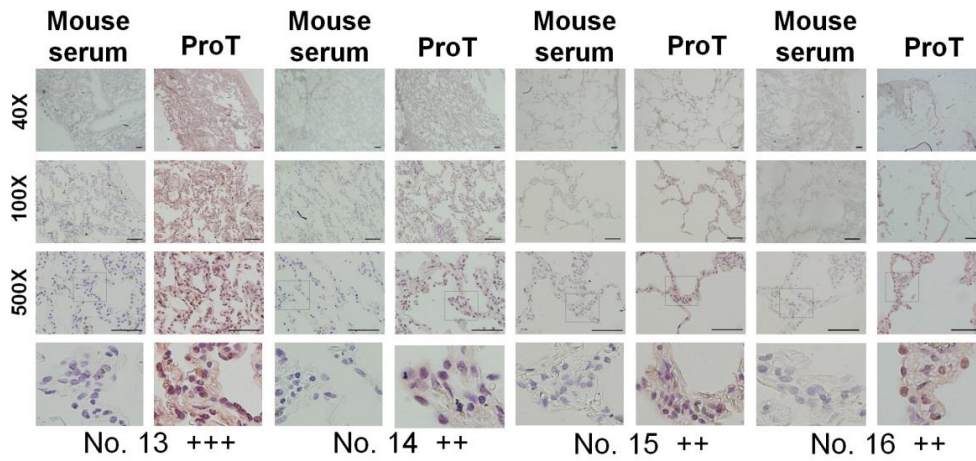


Supplementary Figure S1 continued

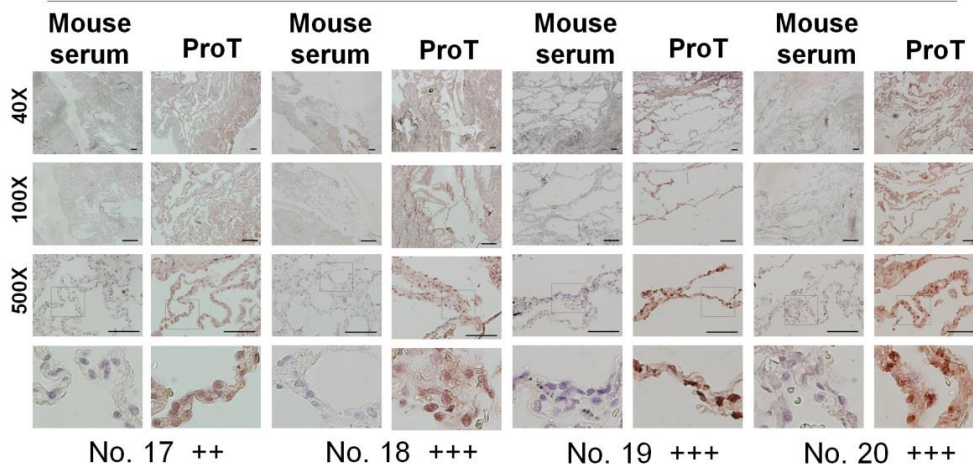
Moderate emphysema



Severe emphysema

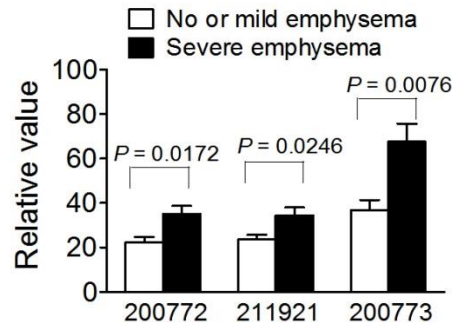


Severe emphysema



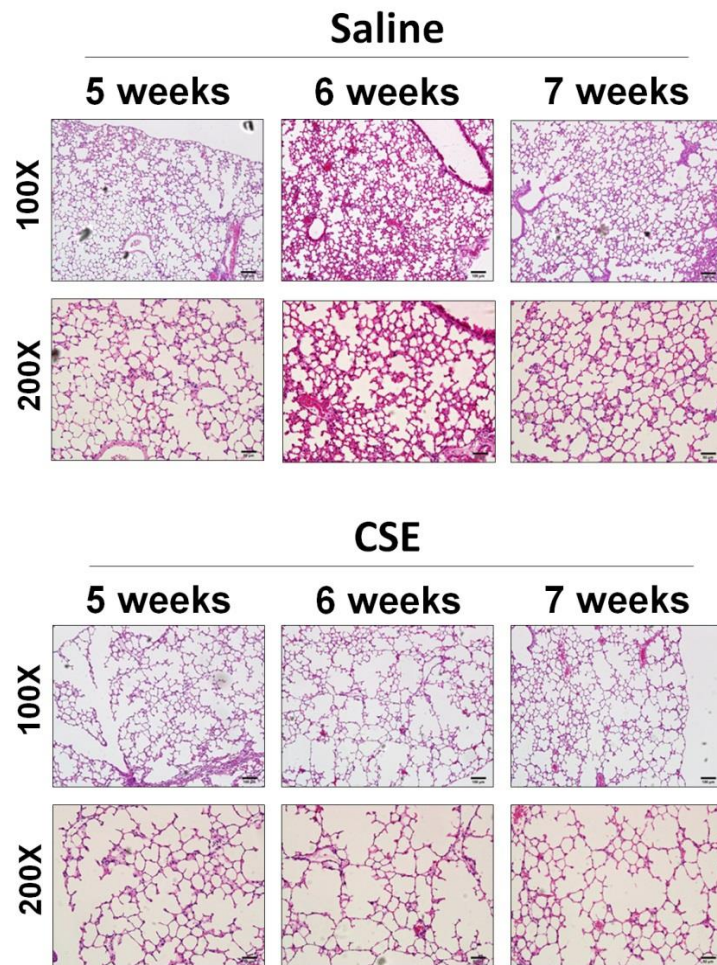
Supplementary Figure S1. The expression levels of ProT in four non-emphysema individuals and 20 patients with mild, moderate or severe emphysema. The immunohistochemical detection of ProT with anti-ProT monoclonal antibody or normal mouse serum for negative control staining in human emphysematous lung tissues of varying degrees of severity and in non-tumour (grossly normal) lung tissues obtained from patients with lung cancer. The tissue sections from the emphysema patients revealed airspace enlargement. Immunohistochemistry for ProT was performed and the immunoreactive intensity was quantitatively analysed by MetaMorph software. In each analysis, three randomly selected fields (500×) within the same tissue section were examined. Scale bars shown on 40×, 100× and 500× images correspond to 100 μm, 200 μm and 50 μm, respectively, and the boxed areas on 500× images are magnified below each panel. The cells stained positively for ProT in the emphysematous specimens are defined as those with immunoreactive signals equal to or greater than 2 times the mean signal from the normal specimens. The expression of ProT is classified as follows: positive staining in <10% of cells, (-); 10% to <30%, (+); 30% to <60%, (++); \geq 60%, (+++).

Supplementary Figure S2



Supplementary Figure S2. The levels of ProT transcripts are higher in the lung tissues of smokers with severe emphysema than in those with mild emphysema or without disease. The microarray data (GEO accession number GPL96, DataSet Record GDS737, Affymetrix Human Genome U133A Array) from the Gene Expression Omnibus (GEO, NCBI) were analysed. The record includes data for comparison of the lung tissues from smokers with severe emphysema (removed at the time of lung volume reduction surgery) and smokers with mild or no emphysema. Accession numbers 200772 (BF686442), 211921(AF348514) and 200773 (NM_002823) correspond to the ProT gene. Because the level of lung-specific surfactant protein C (SP-C, pulmonary-associated protein C) is not altered in COPD⁶¹, the expression level of ProT mRNA was normalised with that of surfactant protein C (SFTPC, accession number 38691). Note that the levels of ProT mRNA were significantly increased in severely emphysematous lung tissues ($n=18$) as compared with those in normal or mildly emphysematous lung tissues ($n=12$) from smokers. Values shown are the mean \pm s.e.m. (Student's *t*-test).

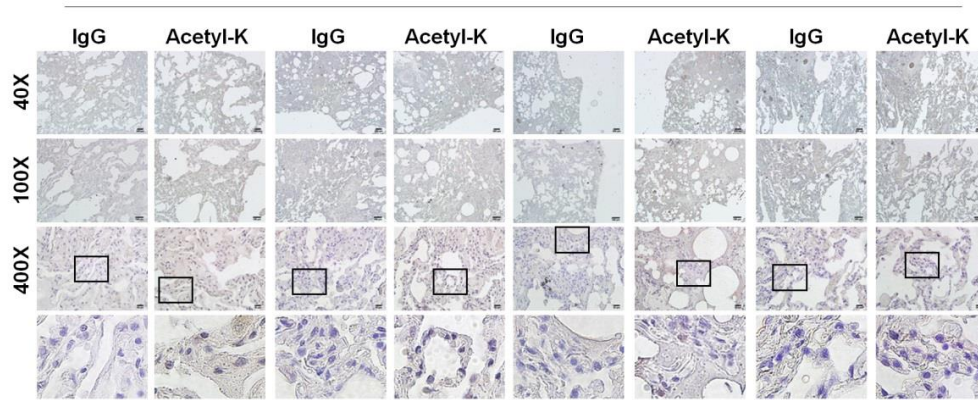
Supplementary Figure S3



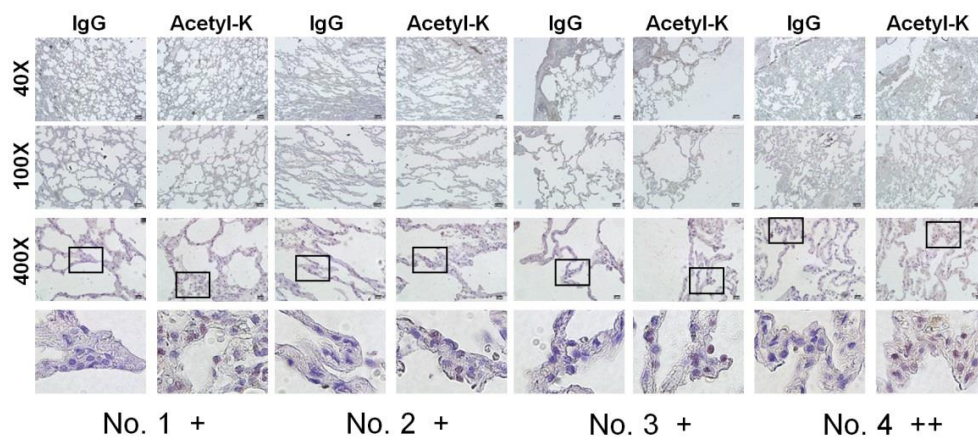
Supplementary Figure S3. Establishment of a cigarette smoke extract (CSE)-induced emphysema model in FVB mice. Six-week-old FVB male mice were intraperitoneally injected with CSE or PBS saline twice a week for 5, 6 or 7 weeks. Subsequently, the mice were sacrificed and their lung tissue sections were subjected to H&E staining for evaluating the severity of emphysema. Note that mice treated with CSE displayed alveolar airspace enlargement compared to those treated with PBS saline. Scale bars on 100 \times and 200 \times images correspond to 100 μ m and 50 μ m, respectively

Supplementary Figure S4

Normal

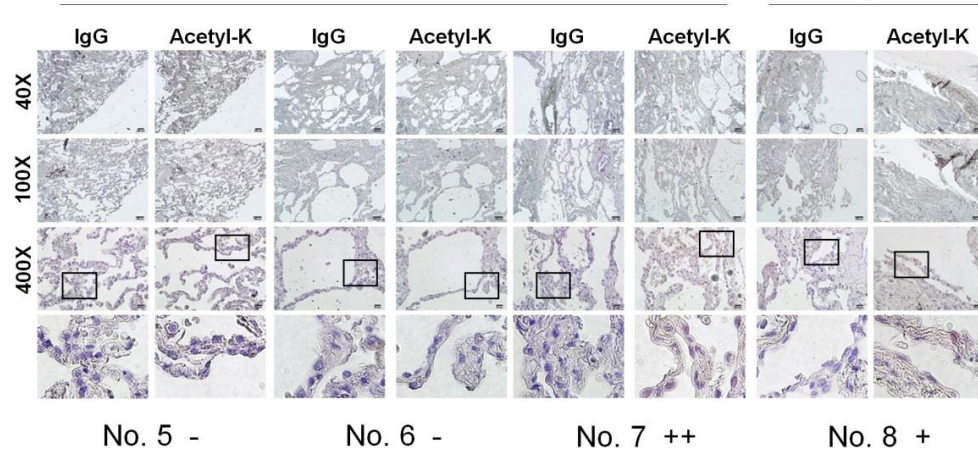


Mild emphysema



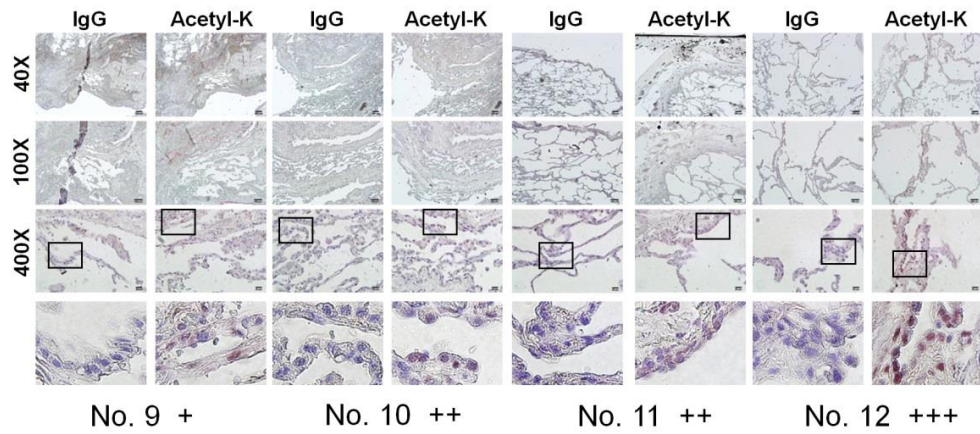
Mild emphysema

Moderate emphysema

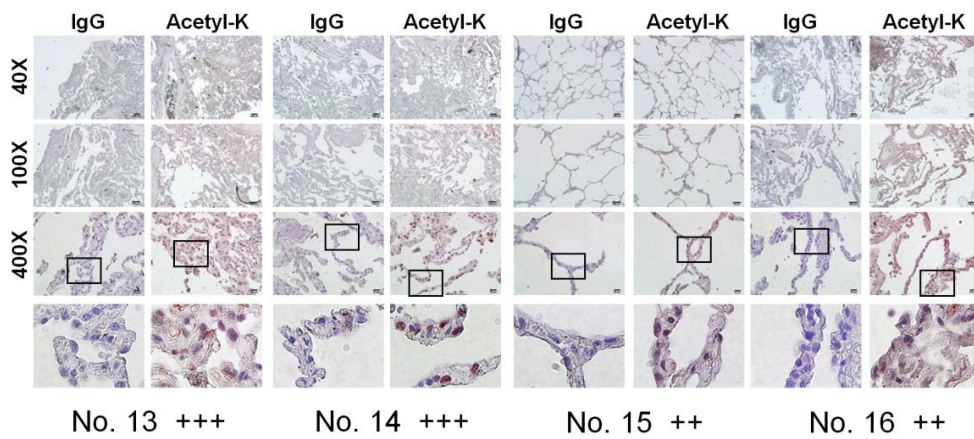


Supplementary Figure S4 continued

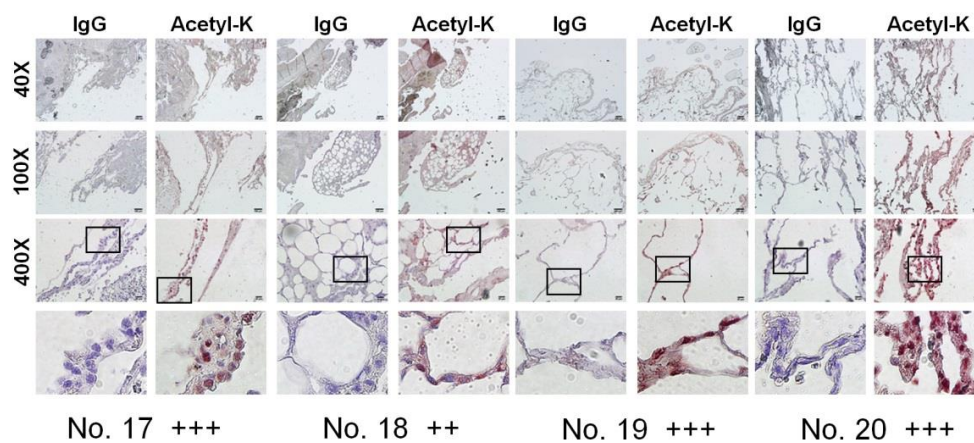
Moderate emphysema



Severe emphysema

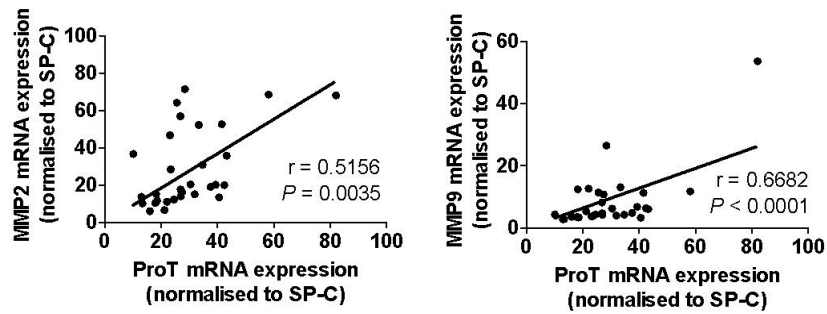


Severe emphysema



Supplementary Figure S4. The levels of acetyl-K in four non-emphysema individuals and 20 patients with mild, moderate or severe emphysema. The immunohistochemical detection of acetyl-K with anti-acetyl-K polyclonal antibody or control IgG for negative control staining in human emphysematous lung tissues of varying severity and in non-tumour (grossly normal) lung tissues obtained from patients with lung cancer. Immunohistochemistry for acetyl-K was performed and the immunoreactive intensity was quantitatively analysed by MetaMorph software. In each analysis, three randomly selected fields (400×) within the same tissue section were examined. The cells stained positively for acetyl-K in the emphysematous specimens are defined as those with immunoreactive signals equal to or greater than 2 times the mean signal from the normal specimens. The expression of acetyl-K is classified as follows: positive staining in <10% of cells, (-); 10% to <30%, (+); 30% to <60%, (++); $\geq 60\%$, (+++). Note that tissue sections from emphysema patients revealed acetyl-K accumulation. Scale bars shown on 40×, 100× and 400× images correspond to 200 μm , 100 μm and 20 μm , respectively, and the boxed areas on 400× images are magnified below each panel.

Supplementary Figure S5



Supplementary Figure S5. Positive correlations between ProT and MMP2 expressions as well as between ProT and MMP9 expressions in the lung tissues from smokers with varying degrees of emphysema. The microarray data used for analysis were described in Fig. S2. Accession numbers 200772 (BF686442), 211921(AF348514) and 200773 (NM_002823) correspond to the ProT gene. Accession numbers 201069 and 203936 correspond to the *MMP2* and *MMP9* gene, respectively. Correlations were measured using Pearson's correlation coefficient.

Supplementary Figure S6

Fig. 3c

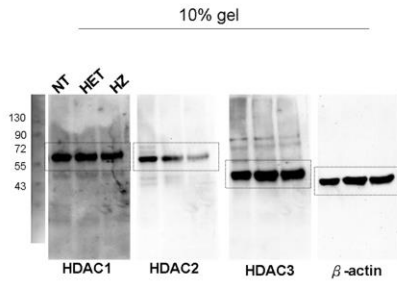


Fig. 3d

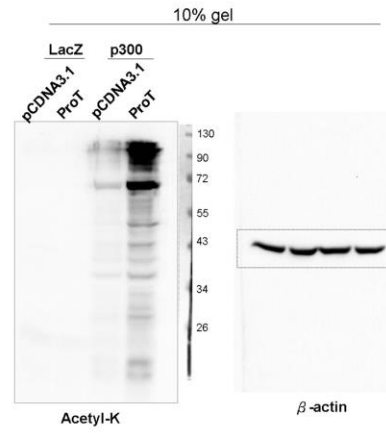


Fig. 3d

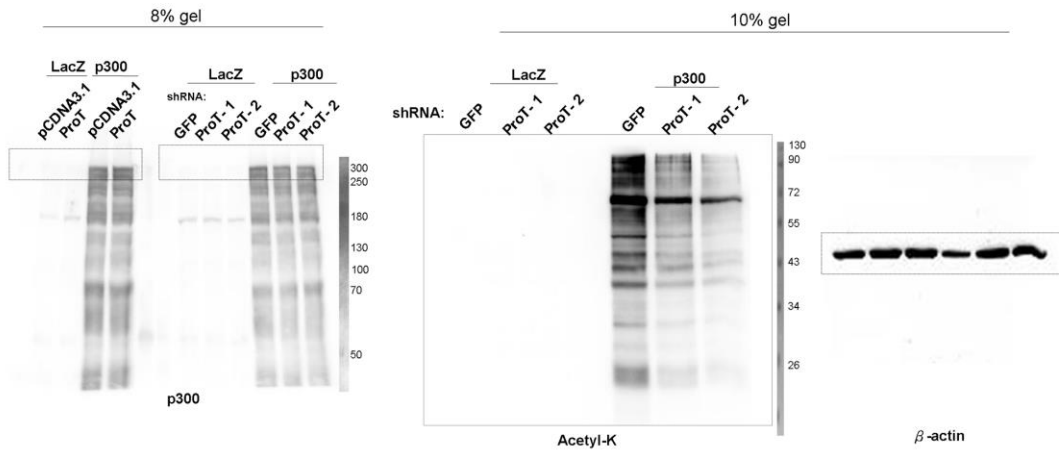


Fig. 3e

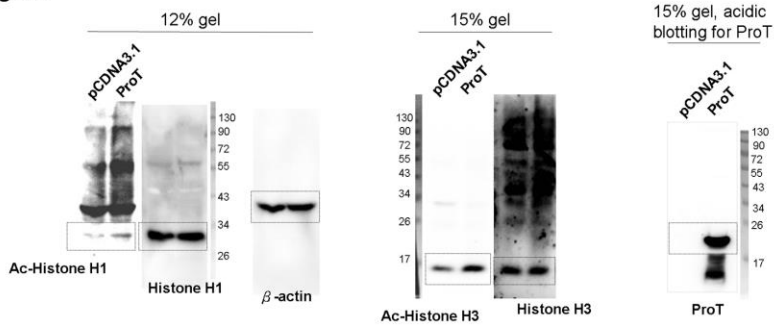


Fig. 3f (left)

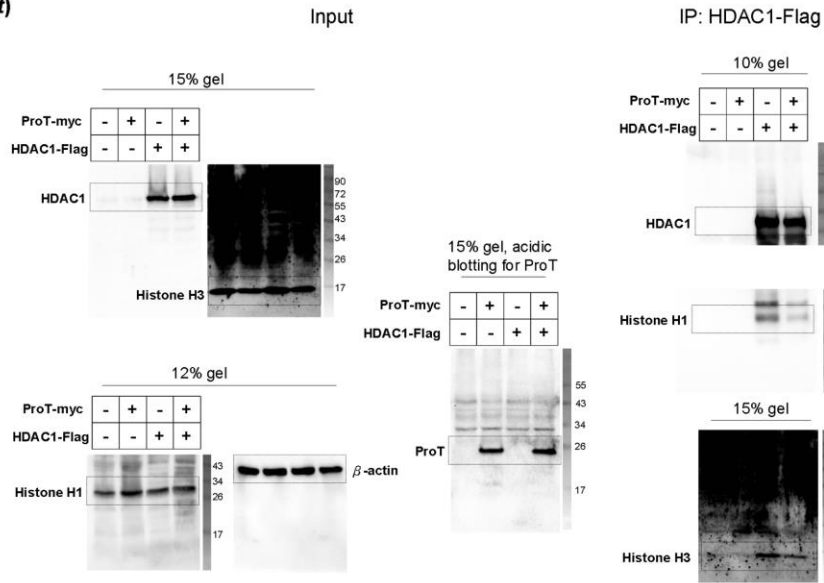


Fig. 3f (right)

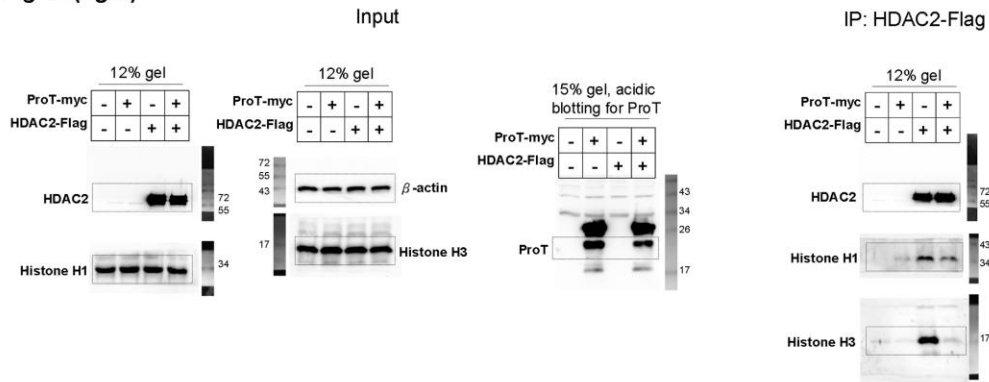
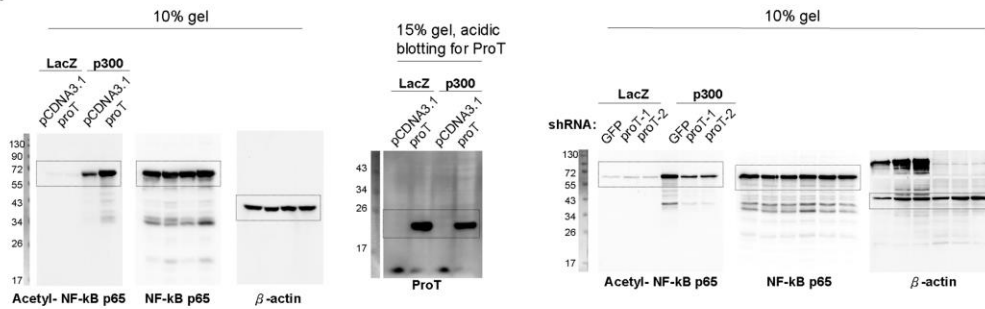


Fig. 5a



Full-length images of immunblots.#

Fig. 5c

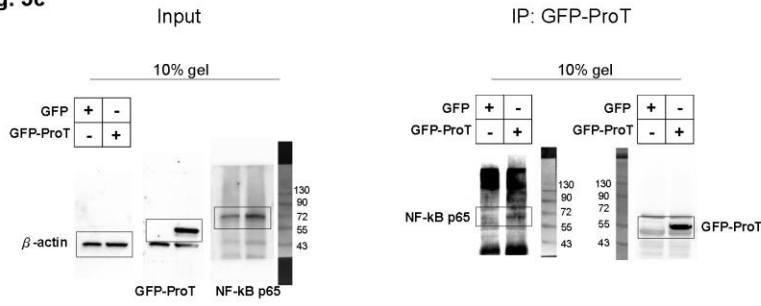


Fig. 5d (left)

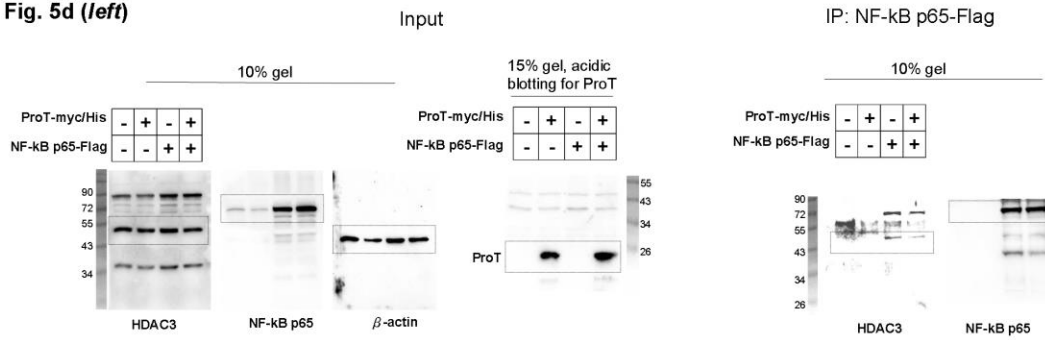


Fig. 5d (right)

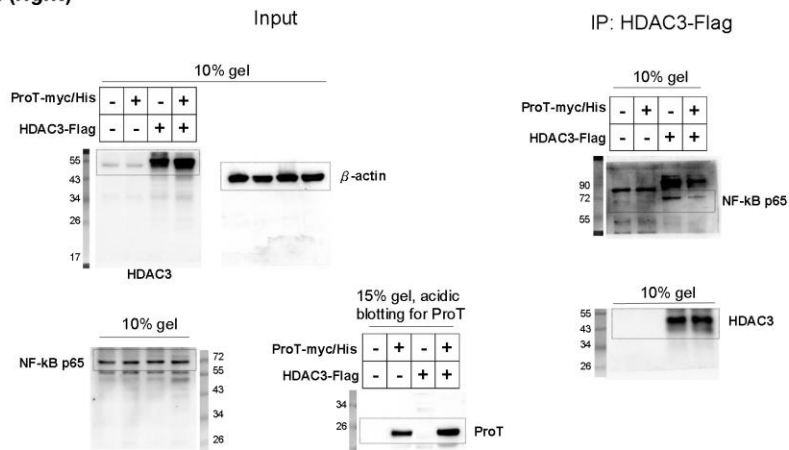
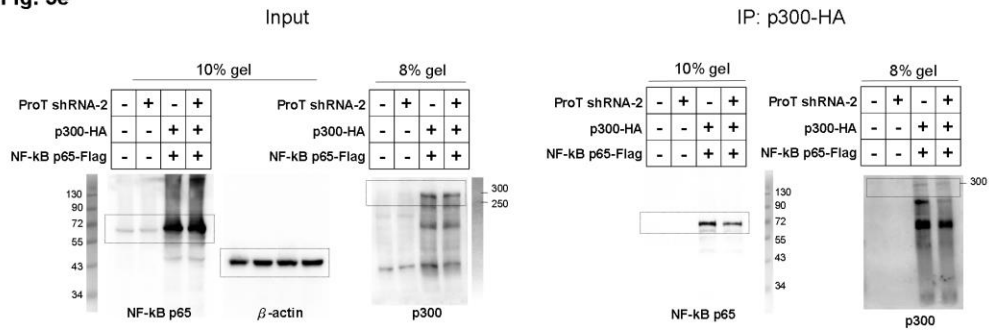


Fig. 5e



Supplementary Table S1. The clinical characteristics of COPD patients.

Patient number	Age/Sex	Smoking	Severity (by PFT)	CT findings (type of emphysema)	Presentation for surgery	Surgical procedures	Treatment before surgery	IHC for ProT
1	32/M	No	Mild FEV1: 88%	Paraseptal	Chest pain and pneumothorax	Wedge resection (V)	None	+
2	34/M	No	Mild FEV1: 89%	Paraseptal	Chest pain and pneumothorax	Wedge resection (V)	None	++
3	61/M	Yes	Mild FEV1: 86%	Paraseptal	Chest pain and pneumothorax	Wedge resection (V)	None	++
4	48/M	No	Mild FEV1: 85%	Paraseptal	Chest pain and pneumothorax	Wedge resection (V)	None	++
5	66/M	Yes	Mild FEV1: 80%	Paraseptal	Chest pain and pneumothorax	Wedge resection (T)	None	+
6	55/M	Yes	Mild FEV1: 83%	Paraseptal & centrilobular	Chest pain and pneumothorax	Wedge resection (T)	None	-
7	40/M	Yes	Mild FEV1: 80%	Paraseptal	Chest pain and pneumothorax	Wedge resection (V)	None	++
8	49/M	Yes	Moderate FEV1: 69%	Paraseptal & centrilobular	Dyspnea and pneumothorax	Wedge resection (V)	Bronchodilators	++
9	65/M	Yes	Moderate FEV1: 55%	Paraseptal & centrilobular	Dyspnea and pneumothorax	Wedge resection (T)	Bronchodilators	++
10	80/M	Yes	Moderate FEV1: 54%	Paraseptal & centrilobular	Dyspnea and pneumothorax	Wedge resection (T)	Bronchodilators	+
11	41/F	No	Moderate FEV1: 72%	Paraseptal & centrilobular	Chest pain and pneumothorax	Wedge resection (V)	None	++
12	39/M	Yes	Moderate FEV1: 74%	Paraseptal & centrilobular	Chest pain and pneumothorax	Wedge resection (V)	None	++
13	50/M	Yes	Severe FEV1: 32%	Panlobular	Dyspnea	Bilateral LVRS	Inhaled steroids and bronchodilators	+++
14	59/M	Yes	Very severe FEV1: 18%	Panlobular	Dyspnea and pneumothorax	Wedge resection (V)	Inhaled steroids and bronchodilators	++
15	77/M	Yes	Severe FEV1: 45%	Panlobular	Dyspnea and pneumothorax	Wedge resection (V)	Inhaled steroids and bronchodilators	++
16	56/M	Yes	Very severe FEV1: 13%	Panlobular	Dyspnea	Bilateral LVRS	Inhaled steroids and bronchodilators	++

17	82/M	Yes	Very severe FEV1: 28%	Panlobular	Dyspnea	Bilateral LVRS	Inhaled steroids and bronchodilators	++
18	55/M	Yes	Very severe FEV1: 25%	Panlobular	Dyspnea	Lung transplantation	Inhaled steroids and bronchodilators	+++
19	54/M	No	Very severe FEV1: 16%	Panlobular	Dyspnea	Lung transplantation	Inhaled steroids and bronchodilators	+++
20	66/M	Yes	Very severe FEV1: 11%	Panlobular	Dyspnea	Lung transplantation	Inhaled steroids and bronchodilators	+++

All patients were diagnosed with emphysema by pulmonary function test results, CT scans and pathological reports from resected lung specimens. The severity was classified according to the Global Initiative on Obstructive Lung Disease (GOLD) emphysema staging system. All patients had FEV1/FEV (percent forced expiratory volume in one second) less than 70%. Immunohistochemistry (IHC) for ProT was performed and the immunoreactive intensity was quantitatively analysed by MetaMorph software. In each analysis, three randomly selected fields (500×) within the same tissue section were examined. The cells stained positively for ProT in the emphysematous specimens are defined as those with immunoreactive signals equal to or greater than 2 times the mean signal from the normal specimens. The expression of ProT is classified as follows: positive staining in <10% of cells, (-); 10% to <30%, (+); 30% to <60%, (++); \geq 60%, (+++). PFT, pulmonary function test; V, video-assisted thoracoscopic surgery; T, thoracotomy; LVRS, lung volume reduction surgery.

Supplementary Reference

61. Ohlmeier, S. *et al.* Proteomics of human lung tissue identifies surfactant protein A as a marker of chronic obstructive pulmonary disease. *J Proteome Res* **7**, 5125-5132 (2008).