Cellular Senescence Affects ECM Regulation in COPD Lung Tissue

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ABSTRACT

Rationale:
Higher levels of senescence have been demonstrated in COPD patients, including severe early onset (SEO)-COPD. Recently we demonstrated a link between senescence and extracellular matrix (ECM) dysregulation in lung fibroblasts. Whether this *in vitro* observation also translates *in vivo* has not been shown yet.

Objectives:
To determine whether senescence can contribute to COPD-associated ECM-related changes in lung tissue.

Methods:
Transcriptomics and proteomics analyses were performed on lung tissue from 60 COPD patients (including 18 SEO-COPD patients) and 32 controls. Expression levels of 471 ECM-related genes and proteins were compared between (SEO-)COPD and controls. Differentially expressed ECM-related genes and proteins were then correlated with six major senescence markers. ECM-senescence correlations were validated in primary human lung fibroblasts *in vitro*.

Results:
We identified 12 COPD- and 57 SEO-COPD-associated ECM-related genes and 4 COPD- and 9 SEO-COPD-associated ECM-related proteins. More than half (36 out of 68 unique genes) of the (SEO-)COPD-associated ECM-related proteins were significantly correlated with one or more senescence markers at transcript level, with the most and strongest correlations with p21 (26 genes). The correlation of 3 ECM-related genes with p21 was validated in primary lung fibroblasts cultured at baseline and ADAMTS1 was increased in senescence-induced lung fibroblasts.

Conclusions:
Many of the (SEO-)COPD-associated ECM-related changes in lung tissue were correlated with the senescence marker p21. As many of these ECM-related proteins are involved in ECM organization and include proteases, these results indicate a role for cellular senescence in disturbed ECM organization and protease-antiprotease imbalance in COPD.

Keywords: COPD, Cellular senescence, extracellular matrix, ECM regulation, proteases

INTRODUCTION

Accelerated ageing has been recognized to play a role in COPD pathogenesis (1, 2). Important features of ageing that are observed in COPD lungs compared to age-matched non-COPD (ex-)smokers are lung function decline, loss of elasticity, and increased cellular senescence (3). Cellular senescence is a major ageing hallmark demonstrated in COPD patients, both in lung tissue, and structural and immune cells (4-6). Cellular senescence is defined by irreversible cell cycle arrest. Senescent cells have an impaired cellular function and secrete a panel of pro-inflammatory chemokines, cytokines, growth factors and extracellular matrix (ECM) proteases, called 'senescence-associated secretory phenotype' (SASP). This SASP causes chronic inflammation in the surrounding tissue and contributes to tissue dysfunction and remodeling (7, 8).

A hallmark of lung ageing is ECM dysregulation (2). The ECM is essential for the function and structure of the lungs and is involved in healthy lung tissue repair. Lung fibroblasts are major producers of ECM and regulate ECM homeostasis. Age-related ECM changes can affect normal ECM homeostasis and healthy tissue function (9). In COPD, fibrosis of the large and small airways is present, characterized by increased protein levels of fibronectin and collagen (10, 11). In addition, reduced levels of proteoglycans involved in the organization of collagen crosslinking are found, including decorin and biglycan (12). In the parenchyma of COPD patients, a reduction and breakdown of elastic fibers and other ECM proteins is observed (10, 11). Furthermore, the protease-antiprotease balance is disturbed in COPD, which causes breakdown of the ECM mainly in the parenchyma eventually resulting in emphysema (13). With ageing, the general ECM changes include increased fibrosis and loss of elasticity (1, 11), which is similar to what is observed in COPD, indicating a role of accelerated ageing in the dysregulated ECM in COPD patients.

Recently, we demonstrated higher levels of cellular senescence in parenchymal lung fibroblasts derived from ex-smoking COPD patients compared to matched non-COPD controls, which was most pronounced in fibroblasts from SEO-COPD patients (14). These SEO-COPD patients are of particular interest as they have (very) severe airflow obstruction at relatively early age and therefore seem to be very susceptible to COPD development. In this previous study, we also found a link between higher levels of senescence and ECM dysregulation, including lower levels of decorin (14). In addition, we demonstrated that induction of senescence results in altered expression of ECM-related genes in fibroblasts, suggesting a direct link between senescence and ECM dysregulation.

Thus, we previously demonstrated that cellular senescence leads to ECM dysregulation in parenchymal lung fibroblasts *in vitro*. The next step is to investigate whether this association is also present *in vivo* and drives ECM dysregulation in COPD patients. Therefore, we investigated whether (SEO-)COPD-associated ECM-related changes in lung tissue are associated with markers of cellular senescence using comparative transcriptomics and proteomics analyses in lung tissue from (SEO-)COPD patients and non-COPD controls. Next, significant (SEO-)COPD-associated ECM and senescence correlations in lung tissue were validated in primary human lung fibroblasts at baseline culture and in our PQ-induced senescence model in lung fibroblasts for functional validation of the correlations.

MATERIALS & METHODS

The full description of the methods can be found in the online supplement.

Lung tissue collection for transcriptomic and proteomic analyses

Peripheral lung tissue from current-smoking and ex-smoking non-COPD controls (FEV_1/FVC>70% of predicted) and COPD (FEV_1/FVC<70% of predicted) patients (including SEO-COPD; FEV_1<40% of predicted and age≤55) was used from the PRoteogenomics Early onSeT cOpd (PRESTO) lung cohort (described in supplement).

Lung tissue transcriptomics

Transcriptomic data from 60 COPD patients and 32 controls was used (see table 1 for characteristics). Briefly, lung tissue frozen sections were used for RNA isolation and RNA sequencing was performed by GenomeScan B.V. A list of 471 ECM-related genes based on three Gene Ontology databases (GO:0085029, GO:0062023, GO:0005201) was used for differential expression analyses. ECM-related gene expression was compared between control current smokers (CS) and control ex-smokers (ES) first to exclude ECM-related genes affected by smoking status as smoking status is different between COPD and control. Next, ECM-related gene expression was compared between COPD and control, and between SEO-COPD and control using generalized linear models corrected for age and sex. Pearson's correlations were performed between six major senescence genes (*CDKN2A/p16, CDKN1A/p21, TP53, CDKN2B/p15, CDKN1B/p27,* and *LMNB1*) and the (SEO-)COPD-associated ECM-related genes. Benjamini-Hochberg False discovery rate (FDR) below 0.05 was considered statistically significant.

Lung tissue proteomics

Serial sections from the same lung tissue frozen section samples as used for transcriptomics, were used for proteomics. Briefly, discovery-based proteomics was done using a data independent acquisition method on an Orbitrap 480 LC-MS/MS platform. Protein levels of the 471 ECM-related proteins (as described above) were compared between control current smokers (CS) and control ex-smokers (ES) first to exclude ECM-related proteins affected by smoking status. Next, ECM-related gene expression was compared between COPD and control, and between SEO-COPD and control using generalized linear model corrected for age and sex. Pearson's correlations were performed between the six major senescence genes (as described above) and the (SEO-)COPD-associated ECM-related proteins. Benjamini-Hochberg False discovery rate (FDR) below 0.05 was considered statistically significant.

Primary human lung fibroblast culture

Primary human lung fibroblasts from SEO-COPD (n=10) and non-COPD patients (n=11) (see Table E1 for patient characteristics) were cultured at baseline conditions as described before and RNA sequencing was performed as described in the online supplement. In non-COPD-derived fibroblasts cellular senescence was induced using our previously described Paraquat-induced senescence model (Woldhuis et al. AJP 2020). Senescence induction was confirmed by SA-β-gal staining, reduced proliferation, and *CDKN1A* (p21) and *LMNB1* gene expression (Figure E1). RNA, secreted protein, and protein lysates were collected for gene expression analyses, secreted protein analyses, and Western Blot analysis.

RESULTS

Patient characteristics

The characteristics of the patients used from the PRESTO cohort for transcriptomics and proteomics analyses are shown in Table 1. In the analyses, all COPD patients (FEV_1/FVC <70%pred) and the subgroup of SEO-COPD patients (FEV_1/FVC <70%pred, FEV1 <40%pred, and age ≤55) are compared to (ex-)smoker controls (FEV_1/FVC >70%pred). Smoking status was significantly different between (SEO-)COPD and control, as all SEO-COPD patients were required to quit smoking for at least 6 months to be eligible for lung transplantation. In addition, age and pack-years of smoking were significantly different between SEO-COPD and control. Therefore, we corrected for age in our model.

Table 1: Patient characteristics of lung tissue subjects for transcriptomics and proteomics analyses

Variable	Non-COPD (ex-)smoker control	All COPD patients	Subgroup of SEO-COPD patients
Number	32	60	18
Age, median (range)	62 (45-76)	59 (39-79)	52 (39-55) #
Male/female, N	15/17	31/29	5/13
Smoking status (ES/CS)	13/19	55/5 *	18/0 #
Pack-years, median (range)	35 (10-75)	32 (6-130)	28 (6-54) #
non-COPD, N	32	-	-
COPD, N	-	60	18
GOLD 1	-	-	-
GOLD 2	-	14	-
GOLD 3	-	16	2
GOLD 4	-	30	16
FEV_1 %pred, median (range)	85.0 (59.8-125.0)	30.5 (10.6-77.0) *	17.3 (12.0-37.0) #
FEV_1/FVC, median (range)	76.9 (71.0-86.6)	41.6 (18.8-67.9) *	29.2 (20.5-50.0) #

ES = ex-smoker, CS = current smoker, FEV_1 = forced expiratory volume in the first second, FVC = Forced vital capacity, %pred = percentage of predicted. Mann–Whitney U tests or Chi-square tests were used to test differences in characteristics for COPD patients and SEO-COPD patients compared to non-COPD controls and indicated (for COPD and # for SEO-COPD) when significant (p<0.05). For transcriptomics analyses, 4 COPD samples did not pass QC and were therefore excluded for analyses, which did not affect the statistics.*

COPD-associated ECM-related genes and proteins

Of the 471 ECM(-associated) proteins selected from the Gene Ontology databases, 354 genes and 190 proteins were detected in the lung tissue derived transcriptomic and proteomic datasets, respectively. To avoid a possible smoking effect, we excluded differentially expressed (p<0.05) genes (n=16) and proteins (n=13) between current smoker controls and ex-smoker controls. On transcript level, 12 ECM-related genes were differentially expressed (FDR<0.05) between COPD-derived and control-derived lung tissue, with 7 genes higher expressed and 5 genes lower expressed in COPD (Figure 1A). On protein level, 4 ECM-related proteins were significantly different between COPD and control, with 3 proteins higher and 1 protein lower expressed in COPD (Figure 1B). *FBLN5* was significantly higher expressed in COPD on both transcript and protein level.

In the SEO-COPD subgroup, 57 ECM-related genes were differentially expressed compared to control (FDR<0.05), with 44 genes higher expressed and 13 genes lower expressed (Figure 1C). All the COPD-associated ECM-related genes were also significantly different in the same direction in SEO-

COPD. On protein level, 9 ECM-related proteins were significantly different between SEO-COPD and control, with 6 proteins higher and 3 proteins lower expressed in SEO-COPD (Figure 1D). Higher protein levels of FBLN5 and CTSH were observed for both COPD and SEO-COPD. All COPD- and SEO-COPD-associated ECM-related genes (57) and proteins (11) were used for further analyses.

COPD-associated ECM-related genes and proteins are correlated with markers of senescence

To assess the association between senescence and ECM, all significant (SEO-)COPD-associated ECM-related genes and proteins were correlated with six well-known senescence genes (*CDKN1A/p21, CDKN1B/p27, CDKN2A/p16, CDKN2B/p15, TP53*, (up with senescence) and *LMNB1* (down with senescence)). Of the 57 SEO-COPD associated ECM-related genes, 37 genes were significantly correlated with one of the senescence genes (Figure 2A). Most genes (26 genes) were correlated with p21, 18 with a positive and 8 with a negative correlation coefficient. All genes positively correlated with p21 were higher expressed in SEO-COPD and all negatively correlated genes were lower expressed in SEO-COPD. Most senescence-correlated ECM-related genes are regulators of ECM organization, including the three strongest correlated genes *ADAMTS4, THBS1,* and *ADAMTS1* (R = 0.84, 0.81 & 0.75 resp.) (Figure 3A-C). Some ECM-related genes were correlated with multiple senescence genes in the same direction, including *EMILIN1* and *SERPINE2*. In addition, 17 ECM-related genes were significantly correlated with *LMNB1*, with the majority (11 genes) being positively correlated. Of the 11 (SEO-)COPD associated ECM-related proteins, 5 were positively correlated with p21, which were all higher expressed in (SEO-)COPD (Figure 2B). FBLN5 (Figure 3D) and THBS1 protein levels showed the strongest correlations with p21 (both R = 0.41), while THBS1 was also strongly correlated with p21 on transcript level (R = 0.81) (Figure 3B). The significant correlations with p21 on transcript level were used for validation in primary human lung fibroblasts.

Validation of senescence-associated ECM-related genes in primary human lung fibroblasts

As the parenchymal lung tissue samples contain multiple (mostly structural) cell types, we aimed to validate our findings in a single cell type that best represented this tissue. First, we checked expression levels of our COPD- and p21-associated ECM-related genes in the single-cell dataset of the Human Lung Cell Atlas (HLCA)(15) and observed that the majority of these 26 genes are predominantly expressed by stromal cells (Figure E2B), including fibroblasts. The strongest p21 correlated (SEO-)COPD- associated ECM-related genes and protein (*ADAMTS4, THBS1, ADAMTS1*, and FBLN5) are highest expressed in fibroblasts, smooth muscle cells, and endothelial cells and *THBS1* was also highly expressed in myeloid cells (Figure E2C-F). For this reason and because fibroblasts are the main ECM producing and regulating cells, we decided to validate the 26 ECM-p21 correlations in RNA sequencing data obtained from primary human lung fibroblasts from COPD patients and controls. Twenty out of these 26 ECM-related genes were expressed in our lung fibroblast dataset. For *TGFB3*, we validated the positive correlation with p21 (Figure 4A+B), and similar trends ($P<0.1$) were observed for *ADAMTS1* and *SERPINE2* (Figure 4) at baseline culture. Opposite to the findings in lung tissue, a negative correlation was found between p21 and *SEMA7A* gene expression in fibroblasts (Figure 4A). No significant correlations were found for the other ECM-related genes, including *ADAMTS4* and *THBS1*. Similar to lung tissue, *TGFB3* was significantly higher expressed in SEO-COPD compared to control (Figure 4C).

Functional validation in senescence-induced parenchymal lung fibroblasts

To investigate a causal effect of senescence on the COPD-associated ECM changes, we assessed the effect of PQ-induced senescence induction on the strongest p21-ECM correlations found in lung tissue using our Paraquat-induced senescence model (14). Senescence induction was confirmed by strong induction of SA-β-gal positive cells and p21 expression and strong reduction in cell proliferation and *LMNB1* expression (Supplemental figure E2). After senescence induction the expression of *ADAMTS1* was significantly ($p<0.05$) increased as expected, while *ADAMTS4* expression was significantly decreased (Figure 5A+C). No significant differences were found on gene expression level for *THBS1* (Figure 5B). In line with gene expression, ADAMTS1 protein secretion was increased

after senescence induction as well (Figure 5E). Protein secretion of THBS1 was decreased after senescence induction (Figure 5D) and secreted ADAMTS4 protein levels were below detection limit.

(Cleaved) FBLN5 changes in senescence-induced parenchymal lung fibroblasts

As FBLN5 was positively correlated with p21 on protein level and not on transcript level, we assessed FBLN5 protein levels in our senescence induction model. It has previously been described that FBLN5 protein can be proteolytically cleaved by serin proteases resulting in a 10 kDa shorter and less functional cleaved protein variant (16). After senescence induction the protein levels of the full length FBLN5 protein (± 50 kDa) were significantly ($p<0.05$) decreased (Figure 6). However, the absolute levels of cleaved FBLN5 (± 40 kDa) and the ratio of cleaved FBLN5 over full length FBLN5 were significantly increased after senescence induction (Figure 6B), suggesting more cleavage and less functional FBLN5 protein with senescence.

DISCUSSION

The aim of this study was to assess whether COPD-associated ECM-related changes are associated with cellular senescence in lung tissue and investigate whether the observed ECM dysregulation in senescent lung fibroblasts also translates *in vivo* contributing to COPD and SEO-COPD pathology. Indeed, we confirmed that many COPD-, and in particular SEO-COPD-, associated ECM-related changes are correlated with the senescence marker p21 in lung tissue. Furthermore, in primary human lung fibroblasts, we validated the correlation with p21 for 3 of these ECM-related genes. For ADAMTS1 we also demonstrated a causal effect of senescence on *ADAMTS1* gene expression and protein secretion in senescence induced primary lung fibroblasts. Finally, we showed that cellular senescence may play a role in the cleavage of FBLN5, resulting in a non-functional protein.

One of the novel observations in our study was that among the correlations between senescence and (SEO-)COPD-associated ECM-related genes/proteins in lung tissue, there were many proteases and antiproteases, including MMP8, and several members of the ADAM (ADAM19, ADAMTS1, ADAMTS4, ADAMTS8, ADAMTS15 & ADAMTSL4) and SERPIN (SERPINB6, SERPINE2, SERPINF1 & SERPING1) protein families (13, 17). This indicates that senescence affects the regulators of ECM. Subsequently, we observed similar correlations with p21 in primary lung fibroblasts for *ADAMTS1* and *SERPINE2* and validated a causal effect on increased *ADAMTS1* expression and protein secretion in senescence-induced lung fibroblasts. While the protease-antiprotease imbalance has long been recognized to play a role in the pathogenesis of COPD (18), this was mainly attributed to immune cells, including neutrophils and macrophages, whereas our results indicate also involvement of fibroblast senescence, contributing to inadequate ECM homeostasis. Interestingly, in other tissues it has been demonstrated that senescent cells have higher expression and secretion of proteases, including MMP and ADAM proteins (8, 19, 20). In line with this, in a previous study we found higher secretion levels of multiple MMP proteins upon PQ-induced senescence in lung fibroblasts (21), including MMP9 (reported), and MMP-2, -3, and -10 (data not shown). Together, these data support the hypothesis that cellular senescence, including fibroblast senescence, may contribute to dysregulated ECM homeostasis in COPD through an imbalance in proteases and antiproteases.

ADAM/ADAMTS and SERPIN protein families are regulators of ECM homeostasis by their proteolytic and anti-proteolytic functions, respectively. Both families have important functions in inflammation, coagulation, and tissue remodeling. Dysregulation of both protein families has been demonstrated in multiple pathologic inflammatory processes (13, 17). In relation to COPD, higher gene expression of *ADAMTS4* was found in COPD lung tissue (22) and higher sputum levels of ADAMTS15 were found in severe COPD patients compared to mild COPD patients (23), suggesting a role in emphysema pathogenesis. The role of SERPINs in COPD has been widely described with the mutation in the *SERPINA1* gene as the most described genetic risk factor for COPD, called alpha-1 antitrypsin deficiency (A1AD) (24). In this study, we excluded this subgroup of patients. We found a

correlation between several other members of the SERPIN family and senescence markers, including *SERPINB6*, *SERPINE2*, and *SERPINF1*, but not *SERPINA1*. The role of SERPINB6 and SERPINF1 in COPD has not been described yet, but genetic variants of SERPINE2 have been associated with COPD before (13) and it is described as a susceptibility gene for COPD (25). So, our results indicate that senescence can contribute to the dysregulation of these protein families in COPD, likely contributing to ECM dysregulation.

Multiple of the (SEO-)COPD associated ECM-related genes and proteins that were correlated with senescence have been linked to COPD before with higher gene expression or protein levels of ADAMTS4, ADAMTSL4 ADAMTS15, CLU, COL18A1, ELN, EMILIN1, FBLN5, MFAP4, MMP8, TGM2, and THBS1 (22, 23, 26-29). Interestingly, these include glycoproteins involved in elastic fiber organization, including ELN, EMILIN1, FBLN5, MFAP4, and THBS1. We have described the role of elastogenesis genes in COPD before and proposed that these genes were higher expressed in COPD as a dysfunctional repair response (28). We now show the association with senescence, a key hallmark of ageing. With ageing of tissues, including lung tissue, there is stiffening of matrix, degradation of elastic fibers and loss of elasticity (30). In skin, higher levels of senescence in the epidermis resulted in altered elastic fiber morphology and structure (31), but what the exact role is of senescence in disturbed elastic fiber formation and structure in the lung is unclear. One of our hypotheses is that in regions with increased cellular senescence more proteases are produced (as described above) resulting in active breakdown of ECM and elastic fibers, which in turn leads to an increased need for repair and thus increased expression of elastogenesis genes. This combination of senescence-induced proteolysis and insufficient repair can contribute to emphysema in COPD patients.

To the best of our knowledge, higher *TGFB3* expression has not been linked to COPD before, but altered TGF-β signaling has been demonstrated in COPD (32, 33). In our current study, we found higher *TGFB3* expression in COPD-derived lung tissue and a positive correlation between p21 and *TGFB3* expression, which we both validated in primary lung fibroblasts. Indicating that lung fibroblast senescence can contribute to higher TGF-β3 levels in COPD patients. Till now, only lower TGF-β3 protein levels were observed in bronchial biopsies from COPD patients compared to control smokers (34), which is contrasting our results likely due to the difference in compartments, bronchial versus parenchymal tissue. Multiple studies have demonstrated higher TGF-β1 levels in COPD patients before, which contributes to the tissue remodeling in the airways of COPD patients (32, 33). In the peribronchial adventitia higher TGF-β leads to less decorin, weakening the peribronchial parenchymal attachments and likely leading to a vicious circle of persistent high TGF-β levels by reduced binding capacity to decorin (12, 35, 36). In addition, in the parenchyma TGF-β1 has been proposed to contribute to increased MMP levels and consequently resulting in emphysema (36-39). TGF-β3 is involved in tissue remodeling and wound healing and has been linked to lung fibrosis (40) and thus, like TGF-β1, may contribute to airway remodeling and perhaps also to emphysema in COPD patients. The higher *TGFB3* expression we observed in COPD lung tissue may be a sign of unsuccessful attempt for ECM production and tissue repair as no protein levels were detected. Whether TGF-β3 can activate MMP production and activity in the parenchyma, like TGF-β1, and thereby contribute to emphysema is unknown yet and thus should be investigated in future studies, including the role of senescence in here.

FBLN5 has been demonstrated to be a key player in elastic fiber formation and when FBLN5 is knocked out in mice it disturbs elastic fiber formation with these mice developing emphysema (41). Cleavage of FBLN5 leads to a non-functional protein and *in vitro* this leads to disturbed elastogenesis (41). While a previous study by our group demonstrated higher FBLN5 protein levels in COPD lung tissue, the level of non-functional cleaved FBLN5 seemed to be higher in COPD lung tissue as well (28). Here, we demonstrate that senescent lung fibroblasts can contribute to these higher levels non-functional cleaved FBLN5 in COPD lung tissue.

Multiple of the (SEO-)COPD-associated ECM-related genes and proteins that correlated with senescence markers have been linked to ageing and/or age-related disease before. In addition,

several have been linked to senescence before, including ADAM and SERPIN protein families, ANXA6, CLU, FBLN5, MMP8, PCOLCE, SRPX2, and THBS1 (19, 20, 42-45). Proteases and SERPINs have been shown to be SASP proteins in multiple studies and multiples cell types, including fibroblasts and epithelial cells (8, 19, 20). THBS1 is involved in mediating cellular senescence by promoting cell cycle arrest and is secreted by senescent human peritoneal mesothelial cells and thus part of the SASP (46). The role of THBS1 in COPD is not fully understood yet, but THBS1 has been demonstrated to activate TGF-β and thus may have a role in tissue remodeling in COPD (47), likely through senescence as our results suggest. The positive correlation between THBS1 and senescence was also validated in the stromal and myeloid cells at single-cell level, but we could not functionally validate this using our senescence model in lung fibroblasts, indicating that likely senescence in other stromal or myeloid cells may play a role.

Most observed correlations between (SEO-)COPD-associated ECM-related genes and senescence markers were with p21. As the senescence marker p21 is mainly involved in cellular senescence induced by oxidative stress and DNA damage (7), and oxidative stress has been proposed to be a main contributor to senescence in COPD (48, 49) the strong signal for p21 in COPD is not surprising.

The limitation of a cross-sectional study like ours is that changes in the proteins of the ECM itself in general concern longer lived proteins that in COPD take a long time to be remodeled with smaller, but likely persistent changes that may not be easily found significant different in a cross-sectional approach. Another limitation of our study is that the correlations between senescence and ECM-related genes/proteins are based on a mix of different cell types. To get more insight into these associations in a single cell type, we validated our findings in primary lung fibroblasts at baseline and after senescence induction. In primary lung fibroblasts, we could not validate the association with senescence for all selected genes *in vitro*, but for 3 genes we did, including a causal effect for *ADAMTS1*, which supports our *in vivo* observations. Of course, *in vitro* experiments do not completely reflect the *in vivo* situation as chronic effects cannot be studied *in vitro* and the patient-derived fibroblasts *in vitro* are not exposed anymore to the lung microenvironment, which includes inflammatory and oxidative effects in COPD lungs. Furthermore, it is known that different senescence inducers can lead to different senescent phenotypes, which may differ in disease context as well. We used our Paraquat model as this induces senescence by an increase in oxidative stress, likely the main contributor to senescence in COPD (as described above). In addition, the observed correlations *in vivo* may also reflect an indirect effect of accumulating senescent cells and their effect on the surrounding lung tissue and thus may be different from senescence induction in a single cell type, like our *in vitro* model. Another possibility is that some of the observed correlations are driven by senescence in other cell types. Finally, in our *in vitro* model we only assessed the effect of senescence on ECM-related changes, but it should be kept in mind that altered ECM composition, structure and organization may also influence cellular senescence, as reviewed by Blokland et al (50).

In conclusion, after demonstrating a link between senescence and ECM dysregulation in COPD-derived lung fibroblasts previously, we have now demonstrated that many (SEO-)COPD-associated ECM-regulating genes and proteins are correlated with markers of senescence *in vivo* in lung tissue. As these mainly include regulators of ECM organization, proteases, and elastogenesis genes, our findings suggest that senescence can contribute to ECM dysregulation in COPD by affecting the protease-antiprotease imbalance and the disturbance of elastic fibers. This effect may partly be caused by senescent fibroblasts. The exact mechanism of how senescent cells affect elastic fiber regulation in COPD should be investigated in more detail. In addition, the effects of senescence on long-term induced changes on structural ECM proteins needs investigation using longitudinal studies. Altogether, this will lead to a better understanding of the functional consequences of increased senescence on disease pathology in COPD patients and may open new opportunities to develop therapeutic strategies to restore ECM regulation in COPD by targeting senescence.

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Reference List

1. Ito K, Barnes PJ. Copd as a disease of accelerated lung aging. *Chest* 2009;135(1):173-180.
2. Meiners S, Eickelberg O, Königshoff M. Hallmarks of the ageing lung. *The European Respiratory Journal* 2015;45(3):807-827.
3. Brandsma CA, de Vries M, Costa R, Woldhuis RR, Konigshoff M, Timens W. Lung ageing and copd: Is there a role for ageing in abnormal tissue repair? *European respiratory review : an official journal of the European Respiratory Society* 2017;26(146):10.1183/16000617.16000073-16002017. Print 16002017 Dec 16000631.
4. Rutten EP, Gopal P, Wouters EF, Franssen FM, Hageman GJ, Vanfleteren LE, Spruit MA, Reynaert NL. Various mechanistic pathways representing the aging process are altered in copd. *Chest* 2016;149(1):53-61.
5. Tsuji T, Aoshiba K, Nagai A. Alveolar cell senescence in patients with pulmonary emphysema. *American journal of respiratory and critical care medicine* 2006;174(8):886-893.
6. Muller KC, Welker L, Paasch K, Feindt B, Erpenbeck VJ, Hohlfeld JM, Krug N, Nakashima M, Branscheid D, Magnussen H, et al. Lung fibroblasts from patients with emphysema show markers of senescence in vitro. *Respiratory research* 2006;7:32.
7. Munoz-Espin D, Serrano M. Cellular senescence: From physiology to pathology. *Nature reviewsMolecular cell biology* 2014;15(7):482-496.
8. Coppe JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: The dark side of tumor suppression. *Annual review of pathology* 2010;5:99-118.
9. Meschiari CA, Ero OK, Pan H, Finkel T, Lindsey ML. The impact of aging on cardiac extracellular matrix. *GeroScience* 2017;39(1):7-18.
10. Bidan CM, Veldsink AC, Meurs H, Gosens R. Airway and extracellular matrix mechanics in copd. *Frontiers in physiology* 2015;6:346.
11. Burgstaller G, Oehrle B, Gerckens M, White ES, Schiller HB, Eickelberg O. The instructive extracellular matrix of the lung: Basic composition and alterations in chronic lung disease. *The European respiratory journal* 2017;50(1):10.1183/13993003.13901805-13992016. Print 13992017 Jul.
12. van Straaten JF, Coers W, Noordhoek JA, Huitema S, Flipsen JT, Kauffman HF, Timens W, Postma DS. Proteoglycan changes in the extracellular matrix of lung tissue from patients with pulmonary emphysema. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 1999;12(0893-3952; 7):697-705.
13. Kelly-Robinson GA, Reihill JA, Lundy FT, McGarvey LP, Lockhart JC, Litherland GJ, Thornbury KD, Martin SL. The serpin superfamily and their role in the regulation and dysfunction of serine protease activity in copd and other chronic lung diseases. *International journal of molecular sciences* 2021;22(12):10.3390/ijms22126351.
14. Woldhuis RR, de Vries M, Timens W, van den Berge M, Demaria M, Oliver BGG, Heijink IH, Brandsma CA. Link between increased cellular senescence and extracellular matrix changes in copd. *American journal of physiologyLung cellular and molecular physiology* 2020;319(1):L48-L60.
15. Sikkema L, Ramirez-Suastegui C, Strobl DC, Gillett TE, Zappia L, Madissoon E, Markov NS, Zaragosi L-E, Ji Y, Ansari M, et al. An integrated cell atlas of the lung in health and disease. *Nature medicine* 2023;29(6):1563-1577.
16. Hirai M, Ohbayashi T, Horiguchi M, Okawa K, Hagiwara A, Chien KR, Kita T, Nakamura T. Fibulin-5/dance has an elastogenic organizer activity that is abrogated by proteolytic cleavage in vivo. *The Journal of cell biology* 2007;176(7):1061-1071.

17. Paulissen G, Rocks N, Gueders MM, Crahay C, Quesada-Calvo F, Bekaert S, Hacha J, El Hour M, Foidart JM, Noel A, et al. Role of adam and adamts metalloproteinases in airway diseases. *Respiratory research* 2009;10:127-127.
18. Gadek JE, Pacht ER, Gadek JE, Pacht ER. The protease-antiprotease balance within the human lung: Implications for the pathogenesis of emphysema. *Lung 1990 168:1* 1990/12;168(1).
19. Basisty N, Kale A, Jeon OH, Kuehnemann C, Payne T, Rao C, Holtz A, Shah S, Sharma V, Ferrucci L, et al. A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS biology* 2020;18(1):e3000599.
20. Ratushnyy A, Ezdakova M, Buravkova L. Secretome of senescent adipose-derived mesenchymal stem cells negatively regulates angiogenesis. *International Journal of Molecular Sciences* 2020 Mar 5;21(5).
21. Woldhuis RR, Heijink IH, van den Berge M, Timens W, Oliver BGG, de Vries M, Brandsma C-A. Copd-derived fibroblasts secrete higher levels of senescence-associated secretory phenotype proteins. *Thorax* 2021;76(5):508-511.
22. Ezzie ME, Crawford M, Cho JH, Orellana R, Zhang S, Gelinas R, Batte K, Yu L, Nuovo G, Galas D, et al. Gene expression networks in copd: Microrna and mrna regulation. *Thorax* 2012;67(2):122-131.
23. Singh D, Fox SM, Tal-Singer R, Plumb J, Bates S, Broad P, Riley JH, Celli B. Induced sputum genes associated with spirometric and radiological disease severity in copd ex-smokers. *Thorax* 2011-06-01;66(6).
24. Sandhaus RA, Turino G, Brantly ML, Campos M, Cross CE, Goodman K, Hogarth DK, Knight SL, Stocks JM, Stoller JK, et al. The diagnosis and management of alpha-1 antitrypsin deficiency in the adult. *Chronic Obstructive Pulmonary Diseases: Journal of the COPD Foundation* 2016 Jun 6;3(3).
25. DL D, TJ M, C L, S S, AA L, JC C, SL L, JJ R, HA C, BH M, et al. The serpine2 gene is associated with chronic obstructive pulmonary disease - pubmed. *American journal of human genetics* 2006 Feb;78(2).
26. JH V, JH L, JA J, R H, EF W. Increased activity of matrix metalloproteinase-8 and matrix metalloproteinase-9 in induced sputum from patients with copd - pubmed. *Chest* 2004 Dec;126(6).
27. T K, Y T, R N, T S, Y N, M I, M K, Y F, T T, T M, et al. Proteomics of serum extracellular vesicles identifies a novel copd biomarker, fibulin-3 from elastic fibres - pubmed. *ERJ open research* 03/22/2021;7(1).
28. Brandsma CA, van den Berge M, Postma DS, Jonker MR, Brouwer S, Pare PD, Sin DD, Bosse Y, Laviolette M, Karjalainen J, et al. A large lung gene expression study identifying fibulin-5 as a novel player in tissue repair in copd. *Thorax* 2015;70(1):21-32.
29. LH T, K B, CR L, N M, S B, GJ C, S K, M M, B K. The cytoprotective role of dj-1 and p45 nfe2 against human primary alveolar type ii cell injury and emphysema - pubmed. *Scientific reports* 02/23/2018;8(1).
30. A H. Elastic fibers during aging and disease - pubmed. *Ageing research reviews* 2021 Mar;66.
31. Waaijer MEC, Gunn DA, Adams PD, Pawlikowski JS, Griffiths CEM, van Heemst D, Slagboom PE, Westendorp RGJ, Maier AB. P16ink4a positive cells in human skin are indicative of local elastic fiber morphology, facial wrinkling, and perceived age. *The journals of gerontologySeries A, Biological sciences and medical sciences* 2016;71(8):1022-1028.
32. Königshoff M, Kneidinger N, Eickelberg O. Tgf-beta signaling in copd: Deciphering genetic and cellular susceptibilities for future therapeutic regimen. *Swiss Medical Weekly* 2009;139(39-40):554-563.

33. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda A. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (copd). *American journal of respiratory and critical care medicine* 2001;163(6):1476-1483.
34. A DS, C S, I G, P C, P B, FLM R, M C, A P, P M, P R, et al. Tgf-β signaling pathways in different compartments of the lower airways of patients with stable copd - pubmed. *Chest* 2018 Apr;153(4).
35. Postma DS, Timens W. Remodeling in asthma and chronic obstructive pulmonary disease. *ProcAmThoracSoc* 2006;3(1546-3222; 5):434-439.
36. Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. *AnnuRevPathol* 2009;4(1553-4014):435-459.
37. DG M, X H, N K, Y W, SD S, G D, A G, D S. Loss of integrin alpha(v)beta6-mediated tgf-beta activation causes mmp12-dependent emphysema - pubmed. *Nature* 03/13/2003;422(6928).
38. Kang H-R, Cho SJ, Lee CG, Homer RJ, Elias JA. Transforming growth factor (tgf)-beta1 stimulates pulmonary fibrosis and inflammation via a bax-dependent, bid-activated pathway that involves matrix metalloproteinase-12. *The Journal of Biological Chemistry* 2007;282(10):7723-7732.
39. Tajirian T, Dennis JW, Swallow CJ. Regulation of human monocyte prommp-9 production by fetuin, an endogenous tgf-beta antagonist. *Journal of Cellular Physiology* 2000;185(2):174-183.
40. T S, Z H, WC L, J Y, WY L, J W, JM V, J L, P C, S J, et al. Tgfβ2 and tgfβ3 isoforms drive fibrotic disease pathogenesis - pubmed. *Science translational medicine* 08/04/2021;13(605).
41. Nakamura T, Lozano PR, Ikeda Y, Iwanaga Y, Hinek A, Minamisawa S, Cheng C-F, Kobuke K, Dalton N, Takada Y, et al. Fibulin-5/dance is essential for elastogenesis in vivo. *Nature* 2002;415(6868):171-175.
42. AR P, JW G, A C, L H, A I, R D, L W, NA R. Age-related changes in tissue macrophages precede cardiac functional impairment - pubmed. *Aging* 2014 May;6(5).
43. Casella G, Munk R, Kim KM, Piao Y, De S, Abdelmohsen K, Gorospe M. Transcriptome signature of cellular senescence. *Nucleic acids research* 2019;47(21):11476.
44. Wiley CD, Flynn JM, Morrissey C, Lebofsky R, Shuga J, Dong X, Unger MA, Vijg J, Melov S, Campisi J. Analysis of individual cells identifies cell-to-cell variability following induction of cellular senescence. *Aging Cell* 2017;16(5):1043-1050.
45. AC F, D W, D W, SJ K. A signature of enhanced lipid metabolism, lipid peroxidation and aldehyde stress in therapy-induced senescence - pubmed. *Cell death discovery* 10/30/2017;3(1).
46. JS I, DD R. Thrombospondin-1 in maladaptive aging responses: A concept whose time has come - pubmed. *American journal of physiology Cell physiology* 07/01/2020;319(1).
47. JE M-U, MJ S. Thrombospondin-1 regulation of latent tgf-β activation: A therapeutic target for fibrotic disease - pubmed. *Matrix biology : journal of the International Society for Matrix Biology* 2018 Aug;68-69.
48. B E, S F-K, JM G, R M, J B, R F, M D. Heme oxygenase-1 induction attenuates senescence in chronic obstructive pulmonary disease lung fibroblasts by protecting against mitochondria dysfunction - pubmed. *Aging cell* 2018 Dec;17(6).
49. Barnes PJ. Oxidative stress in chronic obstructive pulmonary disease. *Antioxidants (Basel, Switzerland)* 2022;11(5):965.
50. Blokland KEC, Pouwels SD, Schuliga M, Knight DA, Burgess JK. Regulation of cellular senescence by extracellular matrix during chronic fibrotic diseases. *Clinical science (London, England : 1979)* 2020;134(20):2681-2706.

FIGURE LEGENDS

Figure 1: (SEO-)COPD-associated ECM-related genes and proteins in lung tissue. *ECM-related gene expression and protein levels were compared between COPD and controls (A+B) and SEO-COPD and controls (C+D) using transcriptomic (A+C) and proteomic (B+D) analyses. Volcano plots depict the LogFC (X-axis) and FDR (P adjust, Y-axis) of all detected ECM-related genes and proteins. Significant (P adjust < 0.05) differentially expressed ECM-related genes and proteins are depicted in color, with higher expression in COPD in red and lower expression in COPD in blue. Horizontal dotted lines represent significance cut-off (FDR = 0.05), and vertical dotted lines represent LogFC of 0.*

Figure 2: Correlations between senescence genes and (SEO-)COPD-associated ECM-related genes and proteins. *All (SEO-)COPD associated ECM-related genes (A) and proteins (B) were correlated with the six major senescence genes using Pearson's correlations. The heatmaps depict the significant correlations (FDR < 0.05) in color and the non-significant correlations in grey. The colors represent the strength (r coefficient) of the correlations, with positive correlations in red and negative correlations in blue. The ECM-related genes and proteins higher expressed in COPD are depicted on top and the ones lower expressed in COPD are depicted on the bottom of the y-axis in alphabetic order, the black line indicates the separation. The six senescence genes are depicted on the X-axis, in alphabetic order, with LMNB1 at last, as it is lower expressed in senescent cells.*

Figure 3: Strongest COPD-associated ECM and senescence correlations. *The strongest (SEO-)COPD-associated ECM and senescence correlations on transcript level, ADAMTS4, THBS1 and ADAMTS1, (A-C) and protein (FBLN5) level (D), in lung tissue are shown in scatter plots. Gene expression of p21 (CDKN1A) is depicted on x-axis and gene expression of ECM-related genes and protein is depicted on y-axis. The Pearson correlation coefficients (r) are depicted in the top left corner of the graphs.*

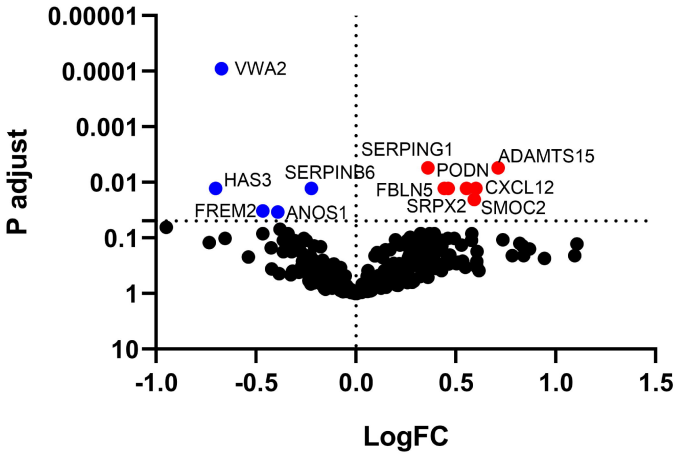
Figure 4: Validation of p21 and COPD-associated ECM correlations in primary human lung fibroblasts. *Gene expression of p21 (CDKN1A) and COPD-associated ECM was measured in primary human lung fibroblasts from COPD (n=10) and non-COPD patients (n=11) at baseline culture. COPD- & p21-associated ECM-related genes in lung tissue were correlated with p21 (CDKN1A) expression in lung fibroblasts (A) using Pearson's correlations and depicted in the heatmap (A). Colors indicate Pearson's correlation coefficients and significant correlations are indicated with a yellow star and correlations with a trend towards significance are indicated with a p-value. Examples of these correlations are depicted in the scatter plots (B). Gene expression of ECM of (trend towards) significant correlations are compared between COPD and non-COPD patients and depicted in scatter plot (C).*

Figure 5: Functional validation of ECM-senescence correlations in senescence-induced lung fibroblasts. *Senescence was induced in primary lung fibroblasts (n=9) with 250µM PQ and after 5 days cells were reseeded to reach the same confluency and gene expression and secreted protein levels were measured using RT-qPCR and ELISA, respectively, after 3 days of reseeding. Gene expression of ADAMTS4 (A), THBS1 (B), AND ADAMTS1 (C) of untreated (Basal, blue) and PQ-induced senescent (PQ, red) fibroblasts are shown in the dot plots. Relative gene expression was calculated using POLR2A as housekeeping gene and depicted. Secreted protein levels of THBS1 (D) and ADAMTS1 (E) are shown in the dot plots below. The lines represent matching donors. Statistical significance was tested using Wilcoxon signed-rank test, p-values are indicated and bold when < 0.05.*

Figure 6: FBLN5 protein level changes in senescence induced lung fibroblasts. *Senescence was induced in primary human lung fibroblasts (n=9) with 250µM PQ and after 5 days cells were reseeded to reach the same confluency. FBLN5 protein levels were measured using Western Blot after 3 days of reseeding. Examples of representative Western Blot membranes with visualization of full FBLN5, cleaved FBLN5 and Actin are shown in A. L = protein ladder, C = untreated fibroblasts & PQ = PQ-induced senescent fibroblasts. Quantification of total intensity of Full size FBLN5, Cleaved FBLN5 and the ratio of Cleaved FBLN5/Full size FBLN5 of untreated (Basal, blue) and PQ-induced senescent (PQ, red) fibroblasts are shown in the dot plots (B). The lines represent matching donors. Technical outliers were excluded. Statistical significance was tested using Wilcoxon signed-rank test, p-values are indicated and bold when < 0.05.*

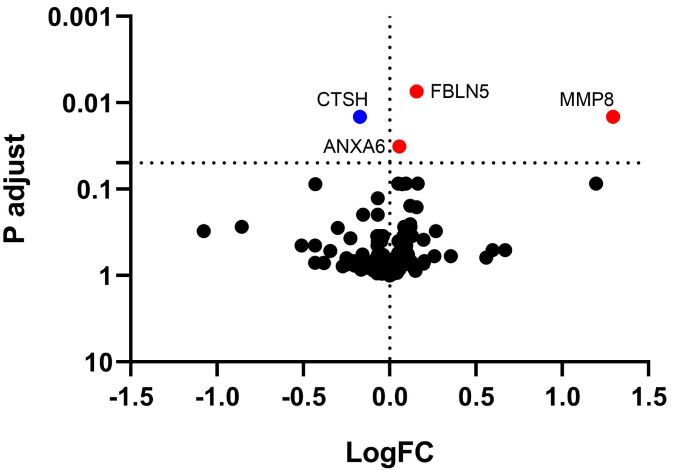
A

12 COPD-associated ECM genes



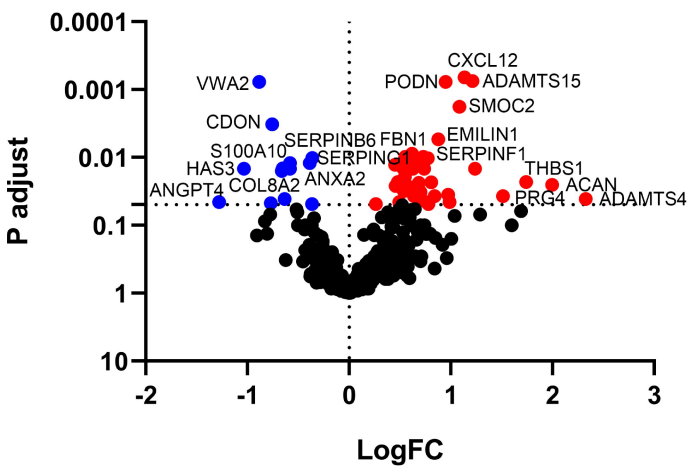
B

4 COPD-associated ECM proteins

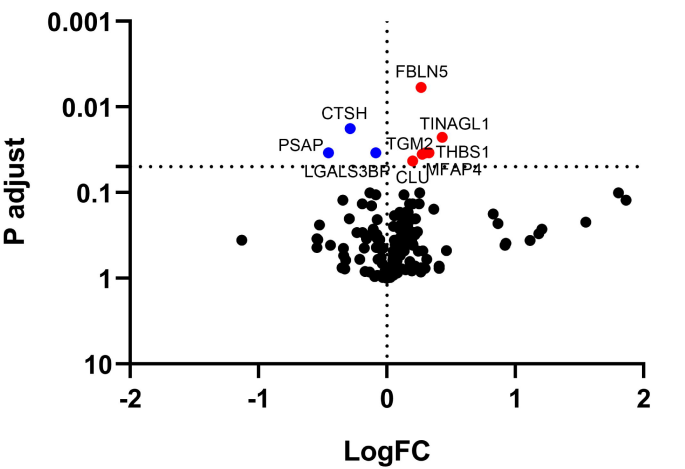


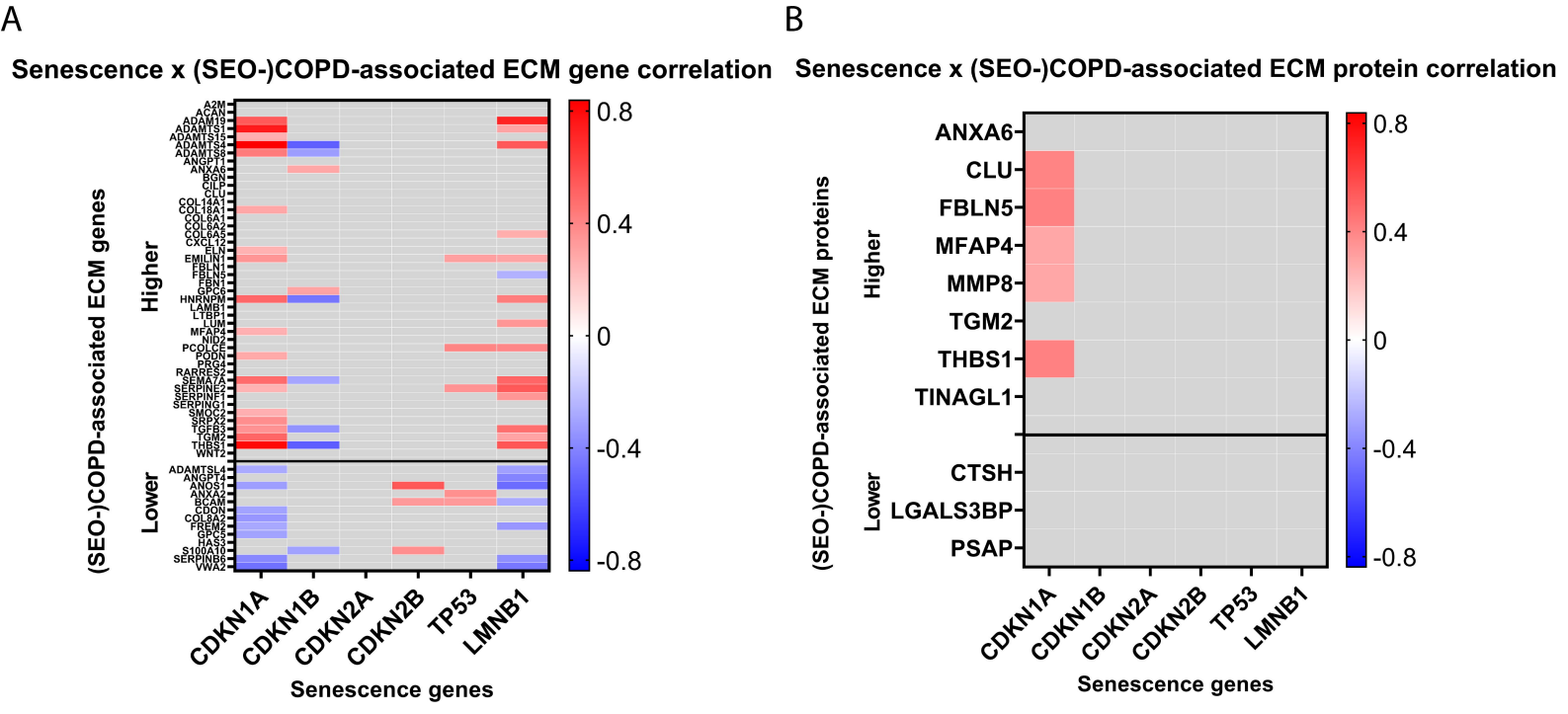
C

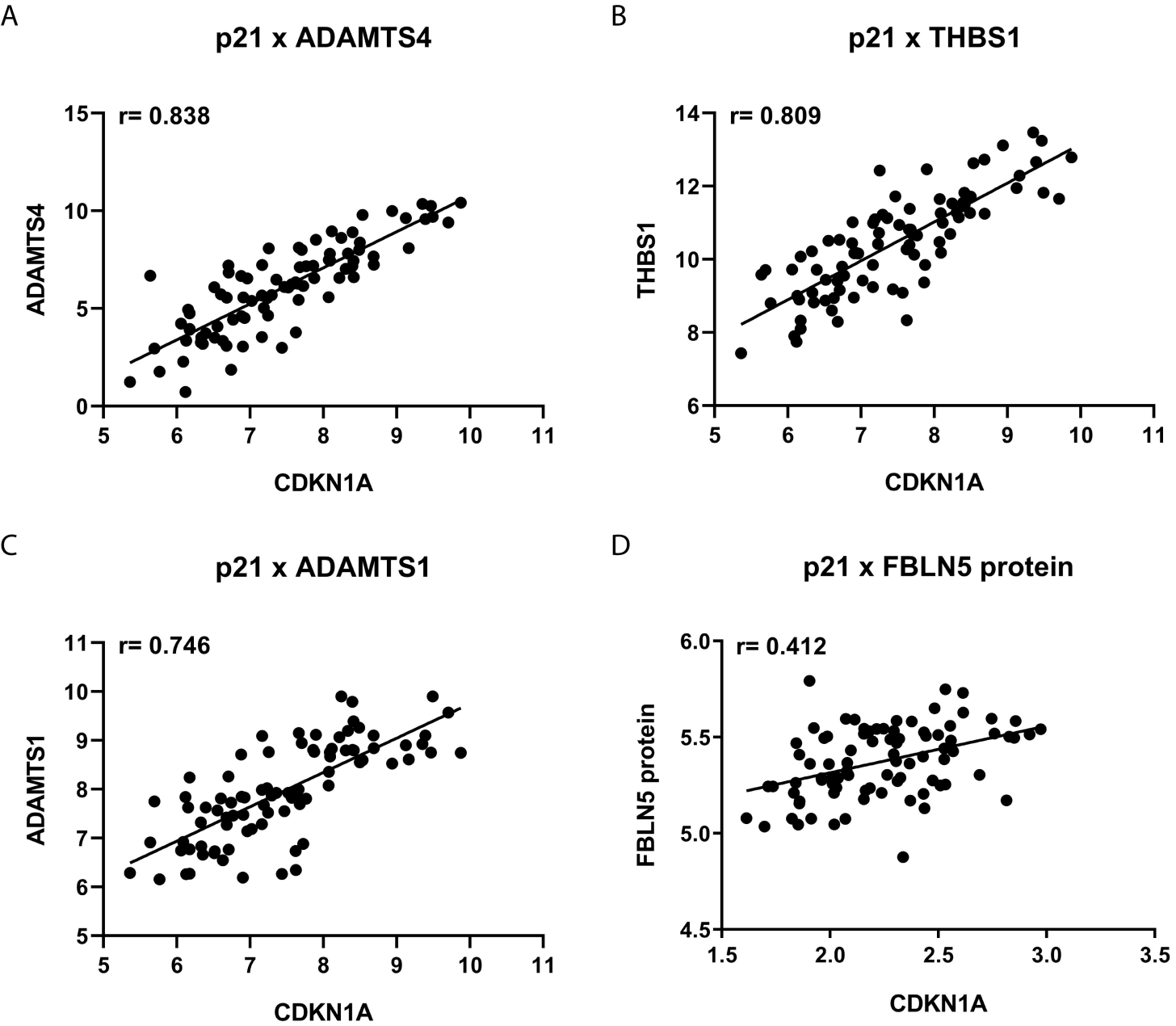
57 SEO-COPD-associated ECM genes

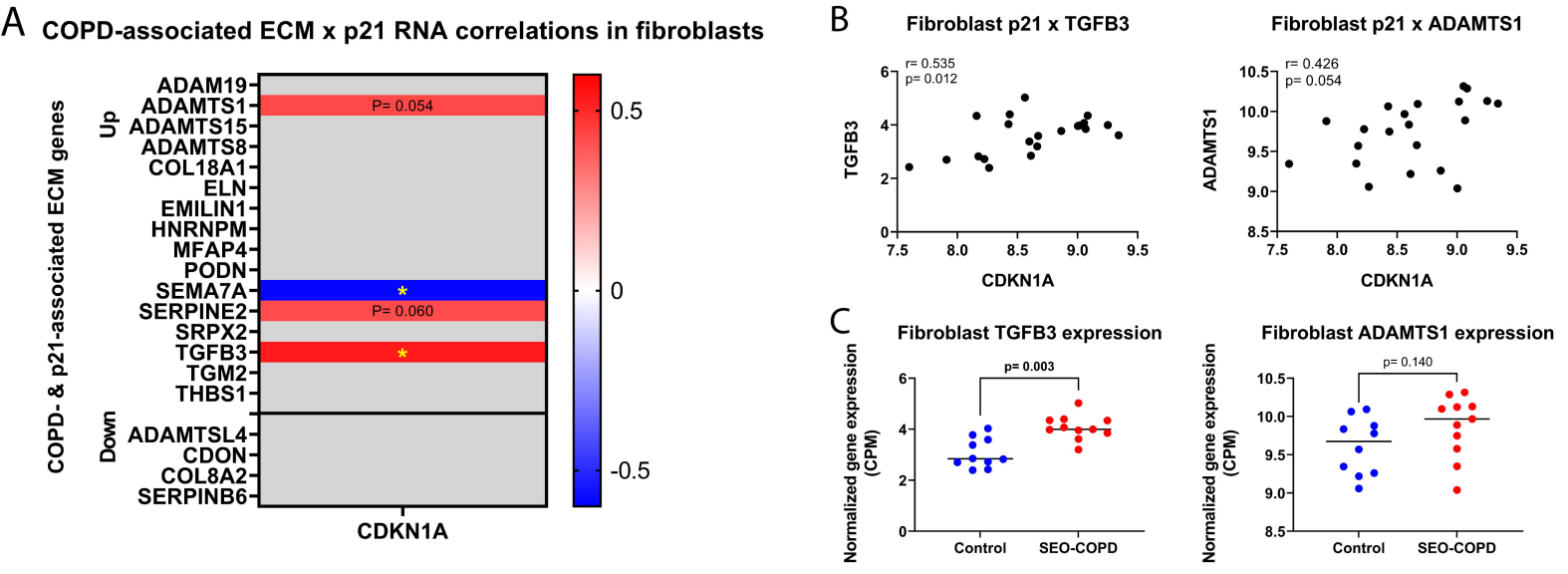


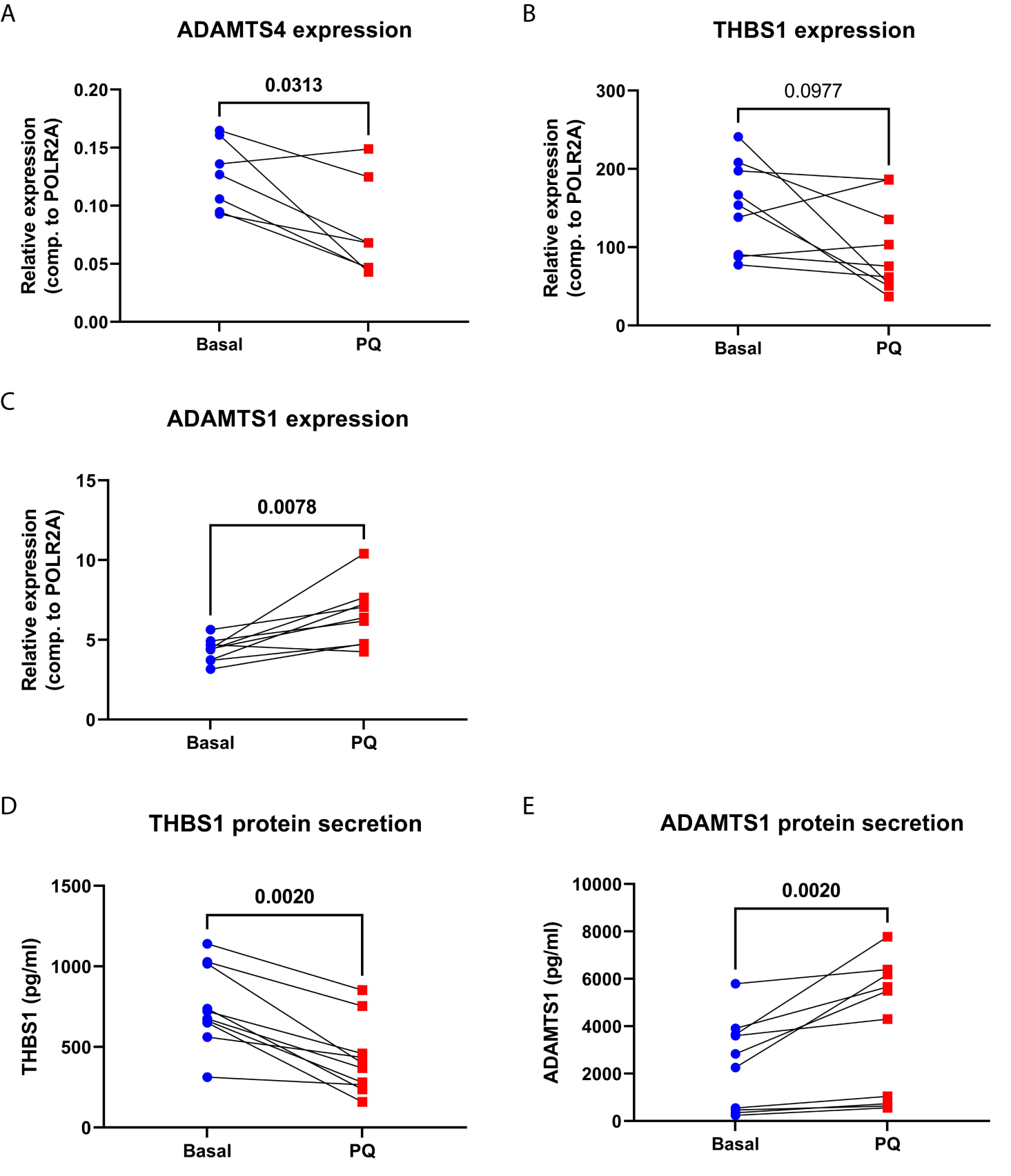
9 SEO-COPD-associated ECM proteins



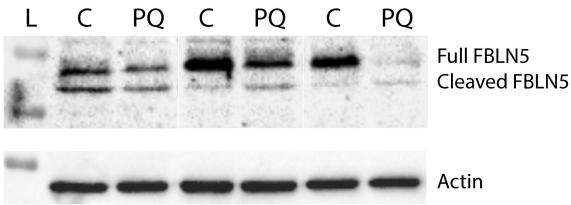

A
Senescence x (SEO-)COPD-associated ECM gene correlation
(SEO-)COPD-associated ECM genes
Higher
Lower
A2M
ACAN
ADAM19
ADAMTS1
ADAMTS15
ADAMTS4
ADAMTS8
ANGPT1
ANXA6
BGN
CILP
CLU
COL14A1
COL18A1
COL6A1
COL6A2
COL6A5
CXCL12
ELN
EMILIN1
FBLN1
FBLN5
FBN1
GPC6
HNRNPM
LAMB1
LTBP1
LUM
MFAP4
NID2
PCOLCE
PODN
PRG4
RARRES2
SEMA7A
SERPINE2
SERPINF1
SERPING1
SMOC2
SRPX2
TGFB3
TGM2
THBS1
WNT2
ADAMTSL4
ANGPT4
ANOS1
ANXA2
BCAM
CDON
COL8A2
FREM2
GPC5
HAS3
S100A10
SERPINB6
VWA2
0.8
0.4
0
-0.4
-0.8
CDKN1A
CDKN1B
CDKN2A
CDKN2B
TP53
LMNB1
Senescence genes
B
Senescence x (SEO-)COPD-associated ECM protein correlation
(SEO-)COPD-associated ECM proteins
Higher
Lower
ANXA6
CLU
FBLN5
MFAP4
MMP8
TGM2
THBS1
TINAGL1
CTSH
LGALS3BP
PSAP
0.8
0.4
0
-0.4
-0.8
CDKN1A
CDKN1B
CDKN2A
CDKN2B
TP53
LMNB1
Senescence genes

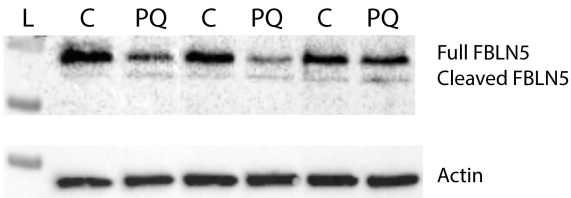

A
p21 x ADAMTS4
r= 0.838
ADAMTS4
CDKN1A
B
p21 x THBS1
r= 0.809
THBS1
CDKN1A
C
p21 x ADAMTS1
r= 0.746
ADAMTS1
CDKN1A
D
p21 x FBLN5 protein
r= 0.412
FBLN5 protein
CDKN1A


A
COPD-associated ECM x p21 RNA correlations in fibroblasts
COPD- & p21-associated ECM genes
Up
Down
ADAM19
ADAMTS1
ADAMTS15
ADAMTS8
COL18A1
ELN
EMILIN1
HNRNPM
MFAP4
PODN
SEMA7A
SERPINE2
SRPX2
TGFB3
TGM2
THBS1
ADAMTSL4
CDON
COL8A2
SERPINB6
P= 0.054
P= 0.060
CDKN1A
0.5
0
-0.5
B
Fibroblast p21 x TGFB3
r= 0.535
p= 0.012
TGFB3
CDKN1A
Fibroblast p21 x ADAMTS1
r= 0.426
p= 0.054
ADAMTS1
CDKN1A
C
Fibroblast TGFB3 expression
p= 0.003
Normalized gene expression (CPM)
Control
SEO-COPD
Fibroblast ADAMTS1 expression
p= 0.140
Normalized gene expression (CPM)
Control
SEO-COPD

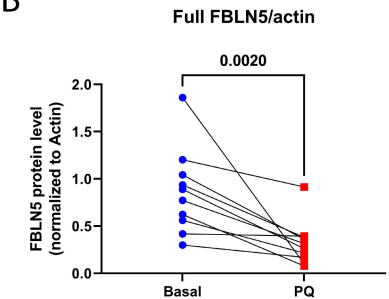

A
ADAMTS4 expression
0.0313
Relative expression (comp. to POLR2A)
0.20
0.15
0.10
0.05
0.00
Basal
PQ
B
THBS1 expression
0.0977
Relative expression (comp. to POLR2A)
300
200
100
0
Basal
PQ
C
ADAMTS1 expression
0.0078
Relative expression (comp. to POLR2A)
15
10
5
0
Basal
PQ
D
THBS1 protein secretion
0.0020
THBS1 (pg/ml)
1500
1000
500
0
Basal
PQ
E
ADAMTS1 protein secretion
0.0020
ADAMTS1 (pg/ml)
10000
8000
6000
4000
2000
0
Basal
PQ

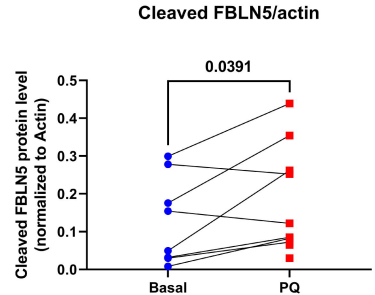
A

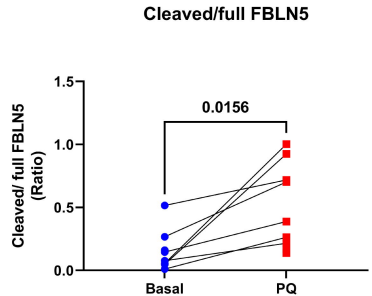

L C PQ C PQ C PQ
Full FBLN5
Cleaved FBLN5
Actin


L C PQ C PQ C PQ
Full FBLN5
Cleaved FBLN5
Actin

B


Full FBLN5/actin
0.0020
FBLN5 protein level (normalized to Actin)
2.0
1.5
1.0
0.5
0.0
Basal
PQ


Cleaved FBLN5/actin
0.0391
Cleaved FBLN5 protein level (normalized to Actin)
0.5
0.4
0.3
0.2
0.1
0.0
Basal
PQ


Cleaved/full FBLN5
0.0156
Cleaved/ full FBLN5 (Ratio)
1.5
1.0
0.5
0.0
Basal
PQ