Phospholipase A2 receptor 1 promotes lung cell senescence and emphysema in obstructive lung disease

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PLA2R1 is a potent regulator of lung cell senescence in COPD, with JAK/STAT signalling as a major effector. Inhibition of JAK1/2 attenuates PLA2R1-induced lung alterations in murine models and so may represent a promising therapeutic approach for COPD. http://bit.ly/3i7yT3H

Cite this article as: Beaulieu D, Attwe A, Breau M, *et al*. Phospholipase A2 receptor 1 promotes lung cell senescence and emphysema in obstructive lung disease. *Eur Respir J* 2021; 58: 2000752 [DOI: 10.1183/13993003.00752-2020].

This article has supplementary material available from erj.ersjournals.com

Received: 18 March 2020
Accepted: 28 Dec 2020

Abstract

Background Cell senescence is a key process in age-associated dysfunction and diseases, notably chronic obstructive pulmonary disease (COPD). We previously identified phospholipase A2 receptor 1 (PLA2R1) as a positive regulator of cell senescence acting *via* Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling. Its role in pathology, however, remains unknown. Here, we assessed PLA2R1-induced senescence in COPD and lung emphysema pathogenesis.

Methods We assessed cell senescence in lungs and cultured lung cells from patients with COPD and controls subjected to *PLA2R1* knockdown, *PLA2R1* gene transduction and treatment with the JAK1/2 inhibitor ruxolitinib. To assess whether *PLA2R1* upregulation caused lung lesions, we developed transgenic mice overexpressing *PLA2R1* (*PLA2R1*-TG) and intratracheally injected wild-type mice with a lentiviral vector carrying the *Pla2r1* gene (LV-*PLA2R1* mice).

Results We found that *PLA2R1* was overexpressed in various cell types exhibiting senescence characteristics in COPD lungs. *PLA2R1* knockdown extended the population doubling capacity of these cells and inhibited their pro-inflammatory senescence-associated secretory phenotype (SASP). PLA2R1-mediated cell senescence in COPD was largely reversed by treatment with the potent JAK1/2 inhibitor ruxolitinib. Five-month-old *PLA2R1*-TG mice exhibited lung cell senescence, and developed lung emphysema and lung fibrosis together with pulmonary hypertension. Treatment with ruxolitinib induced reversal of lung emphysema and fibrosis. LV-*PLA2R1*-treated mice developed lung emphysema within 4 weeks and this was markedly attenuated by concomitant ruxolitinib treatment.

Conclusions Our data support a major role for PLA2R1 activation in driving lung cell senescence and lung alterations in COPD. Targeting JAK1/2 may represent a promising therapeutic approach for COPD.

Introduction

Exaggerated lung cell senescence is now emerging as a key pathogenic factor in several chronic respiratory diseases [1], notably chronic obstructive pulmonary disease (COPD), a major cause of morbidity and mortality in most countries for which no curative treatment is available [2–4]. COPD is characterised by slowly progressive airflow obstruction and emphysema due to lung parenchyma destruction developing in association with chronic lung inflammation [5]. We previously reported that a characteristic feature of COPD is the accumulation of senescent cells in the lung, which limits the potential for tissue renewal and

contributes to chronic inflammation through the senescence-associated secretory phenotype (SASP) [4, 6, 7]. In COPD, exaggerated lung cell senescence may result from a combination of replicative cell senescence due to telomere shortening and of premature cell senescence caused by various stress stimuli, such as oxidative stress and inflammatory mediators [8–10]. These processes engage a DNA damage response with p53-dependent upregulation of the cyclin-dependent kinase inhibitor p21 and expression of $p16^{INK4A}$ [10, 11].

A causal relationship between cell senescence and the lung manifestations of COPD is now supported by several studies. Some cases of familial lung emphysema are related to a loss-of-function mutation in the telomerase *TERT* gene and are therefore classified as telomere disorders [12]. Moreover, individuals with short telomeres experience an accelerated decline in lung function [13]. In mouse models, interventions that delay or inhibit cell senescence protect against cigarette smoke-induced lung emphysema, bronchial remodelling and inflammation [14, 15], whereas accelerated lung cell senescence promotes some of the lung alterations seen in COPD [7]. Consequently, there is an urgent need to identify molecular pathways that are relevant to the lung cell senescence programme in COPD and that could be targeted pharmacologically.

In previous studies, we identified phospholipase A2 receptor 1 (PLA2R1) as a crucial regulator of both replicative- and stress-induced cell senescence [16]. PLA2R1, a transmembrane protein belonging to the C-type lectin receptor superfamily and the mannose receptor family, binds a few secreted phospholipase A2s (sPLA2s), various collagen types and carbohydrate [17]. Recent studies by our groups indicate that an important function of PLA2R1 is related to its pro-senescent effect, which seems to be sPLA2 independent [18] and involves the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling pathway [19–23].

Here, we explored the potential importance of PLA2R1 and JAK/STAT signalling in lung cell senescence and COPD. We observed that PLA2R1 overexpression was a major mechanism leading to lung cell senescence in COPD, and that lung PLA2R1 overexpression in mice induced lung cell senescence and lung alterations, including lung emphysema, lung fibrosis and pulmonary hypertension. Treatment with the JAK1/2 inhibitor ruxolitinib delayed the senescence process in cells from patients with COPD, inhibited the SASP and attenuated the pathological lung alterations resulting from PLA2R1 overexpression in mice. Targeting PLA2R1 and/or the JAK/STAT pathway may represent a new therapeutic strategy for inhibiting lung cell senescence in COPD.

Methods

Patients and controls

Lung tissue samples were obtained from 46 patients who underwent lung resection surgery at the Institut Mutualiste Montsouris (Paris, France). Among them, 23 had COPD and 23 were control smokers matched to the COPD patients on age and sex (supplementary table S1). Inclusion criteria for the patients with COPD were ⩾10 pack-years smoking history and forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <70%. The controls had to have a smoking history >5 pack-years, FEV_1/FVC >70% and no evidence of chronic cardiovascular, hepatic or renal disease.

The study was approved by the Institutional Review Board of the Henri Mondor Teaching Hospital (Créteil, France). All patients and controls signed an informed consent document before study inclusion.

Animal studies

Adult male mice (C57BL/6J) were used according to institutional guidelines, which complied with national and international regulations (agreement 94-28245). Pulmonary arterial hypertension, lung emphysema and lung tissue analyses were performed according to the standard techniques detailed in the supplementary material.

The supplementary material provides details of the studies performed on lung samples and cultured cells from patients and controls, on animal studies, and on the statistical analysis.

Results

PLA2R1 *is overexpressed in lungs and derived cultured cells from patients with COPD*

To investigate *PLA2R1* expression and lung cell senescence in COPD, we studied lung tissues from 23 patients with COPD and 23 controls, as well as derived lung cells from a subset of eight patients and 10 controls (supplementary tables S1 and S2). Compared with control lungs, lungs from patients with COPD had higher protein levels of PLA2R1, p21 and p16 (figure 1a). Immunofluorescence studies of

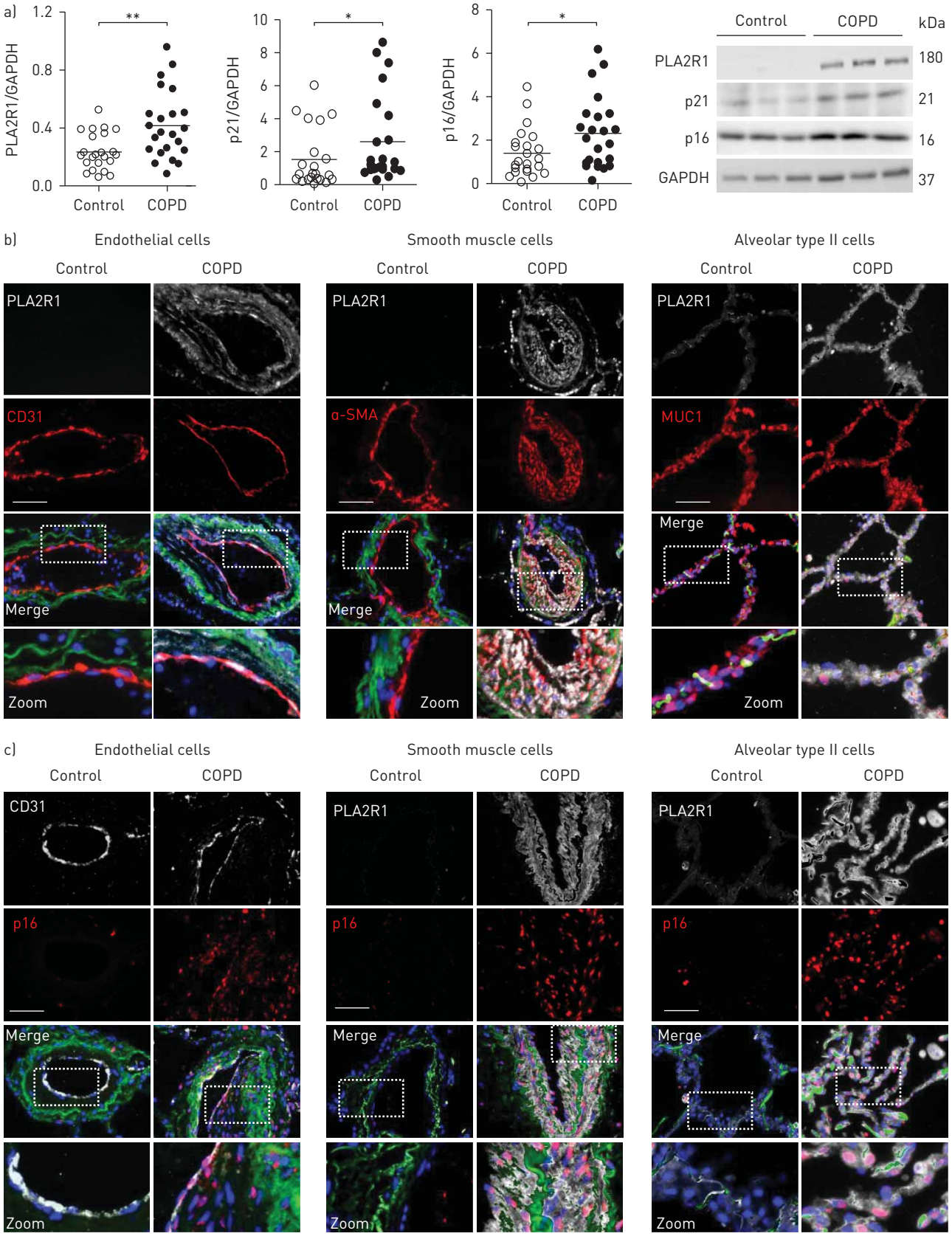

FIGURE 1 Analysis of lung samples from patients with chronic obstructive pulmonary disease (COPD) and controls. PLA2R1: phospholipase A2 receptor 1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; SMA: smooth muscle actin; MUC1: mucin 1. a) Protein levels of PLA2R1, p21 and p16 measured in the lungs of 23 patients with COPD and 23 controls. Representative immunoblots are shown on the right. Data are presented as

individual values with the mean. *: p<0.05; **: p<0.01 *versus* control. Two-tailed unpaired t-test. b) From left to right, representative micrographs of lung tissue from controls and patients with COPD showing immunofluorescence of PLA2R1 (white) in pulmonary vascular endothelial cells identified by CD31 (red), pulmonary artery smooth muscle cells identified by α-SMA (red) and alveolar epithelial type II cells identified by MUC1 (red). Elastin autofluorescence (green). Nuclei were labelled with 4′,6-diamidino-2-phenylindole (blue). c) Representative micrographs of PLA2R1-positive vascular cells and alveolar epithelial type II cells also stained for p16. The zoomed areas are indicated. Scale bar: 50 µm.

lungs from patients with COPD revealed prominent PLA2R1 staining in pulmonary vascular endothelial cells (P-ECs), pulmonary artery smooth muscle cells (PA-SMCs) and alveolar epithelial type II cells (AECs), all of which also stained for p16 (figure 1b and c, and supplementary figure S1) and for the DNA damage markers 53BP1 and phosphorylated (p)-γH2AX (figure 2a and b). In contrast, PLA2R1 immunofluorescence was weak in lungs from controls. *PLA2R1* mRNA levels were also higher in cultured PA-SMCs and P-ECs from patients with COPD, which exhibited early-onset cell senescence manifesting as fewer population doublings and higher β-galactosidase-positive cell counts compared with those from controls (figure 3a and b). *PLA2R1* mRNA levels increased in both PA-SMCs and P-ECs from patients and controls during repeated passages (figure 3a and b).

Downregulation of PLA2R1 *inhibits cell senescence in COPD*

To investigate the effect of *PLA2R1* knockdown on cellular senescence in COPD, we infected cultured PA-SMCs with a retroviral vector encoding a short hairpin RNA that targeted PLA2R1 (sh*PLA2R1*) [16]. *PLA2R1* mRNA levels were decreased by ∼80% in cells stably infected with the sh*PLA2R1*-encoding vector compared with cells infected with the control vector (supplementary figure S2). *PLA2R1* knockdown consistently decreased the number of β-galactosidase-positive cells and increased the population doubling to a greater extent in cells from patients with COPD (9-fold increase) than in those from controls (2-fold increase) (figure 4a and b). In addition, p16 and p21 protein levels were decreased in cells from both patients with COPD and controls (figure 4c). *PLA2R1* knockdown reduced the levels of the SASP components tested, notably in cells from patients with COPD, which released large amounts of interleukin (IL)-8, IL-6 and plasminogen activator inhibitor (PAI)-1 (figure 4d).

To better assess the effect of *PLA2R1* knockdown on cell senescence in COPD, we then compared the effects of *PLA2R1 versus p53* knockdown in PA-SMCs from a subset of five patients and five controls. Compared with cells infected with the vector encoding sh*PLA2R1*, cells infected with the sh*p53* vector exhibited a similar population doubling increase and a similar decrease in the percentage of β-galactosidase-positive cells (supplementary figure S3a). The levels of p16 and p21 proteins and of SASP components were reduced to a similar extent by both vectors (supplementary figure S3b and c).

JAK inhibition by ruxolitinib reverts PLA2R1*-induced cell senescence*

To explore the potential role for JAK1/2 on *PLA2R1*-induced cell senescence in primary lung cell cultures, we examined the effect of ectopic *PLA2R1* expression on cell senescence by using a retroviral vector encoding the *PLA2R1* gene [19]. PA-SMCs from controls with <55% β-galactosidase-positive cells were used. Immunofluorescence studies revealed that cells transduced with the retroviral vector and positive for PLA2R1 stained also for p-JAK2 and p-STAT3 (figure 5a). These cells became rapidly senescent, as shown by strong p16 immunofluorescence and β-galactosidase staining activity and an inability to divide, with a low population doubling and weak proliferating cell nuclear antigen (PCNA) staining (figure 5b and c). Cells infected with the control vector were not stained for p-JAK2 and p-STAT3 (figure 5a). We then investigated whether inhibition of JAK2 by the potent JAK1/2 inhibitor ruxolitinib altered *PLA2R1*-induced cell senescence. In cells undergoing cell senescence induced by *PLA2R1* gene transduction, ruxolitinib treatment almost fully inhibited the response, increasing the population doubling, decreasing the percentage of β-galactosidase-positive cells, and returning the levels of p16 and PCNA staining and of the SASP components IL-8, IL-6 and PAI-1 to basal values (figure 5b–d).

Treatment with ruxolitinib attenuates cell senescence and suppresses the SASP in cells from patients with COPD

We then investigated the link between *PLA2R1* expression, cell senescence and JAK/STAT in lungs and cells from patients with COPD. Immunofluorescence studies revealed strong PLA2R1 and JAK2 staining that co-localised in various cell types from COPD lungs, contrasting with weak JAK2 staining in control lungs (figure 6a). Strong immunostaining for p-STAT3 was also found in lungs from patients with COPD (figure 6b). JAK1 staining was undetectable. Ruxolitinib treatment of PA-SMCs from patients with COPD and controls increased the population doubling and decreased the percentage of β-galactosidase-positive cells, although these

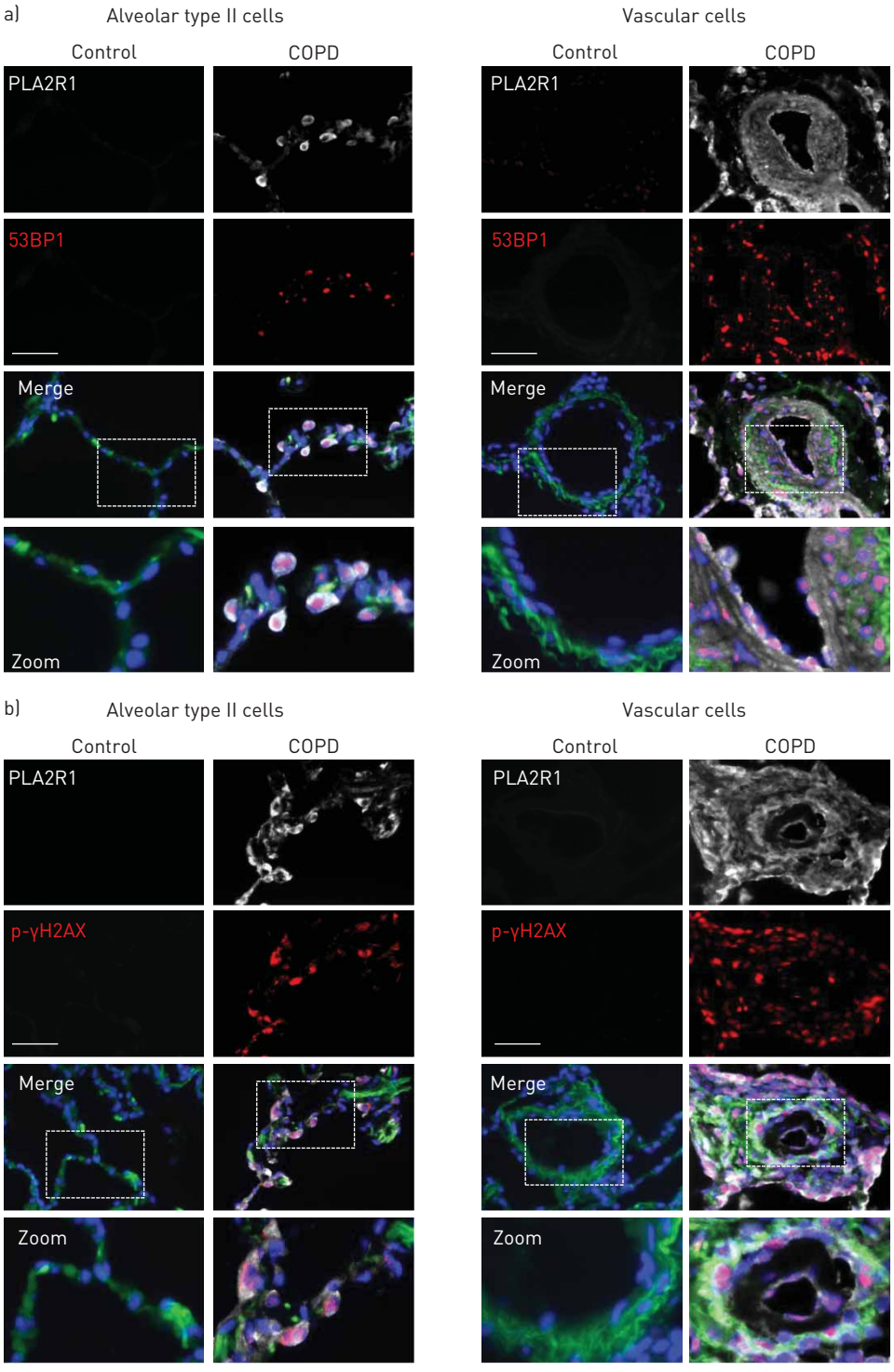

FIGURE 2 Co-localisation of phospholipase A2 receptor 1 (PLA2R1) and DNA damage markers (53BP1 and phosphorylated (p)-γH2AX) in lung samples from controls and patients with chronic obstructive pulmonary disease (COPD). a) Representative micrographs of PLA2R1-positive alveolar epithelial type II cells and vascular cells also stained for 53BP1 (red). b) Similar representation showing PLA2R1-positive cells also stained for p-γH2AX (red). Nuclei were labelled with 4′,6-diamidino-2-phenylindole (blue). The zoomed areas are indicated. Scale bar: 50 µm.

effects were weaker than those produced by *PLA2R1* knockdown (figure 6c). Strikingly, ruxolitinib potently suppressed the SASP of senescent cells from patients with COPD, as shown by decreases in the release of IL-8, IL-6 and PAI-1, which returned to the basal levels measured in cells from controls (figure 6d).

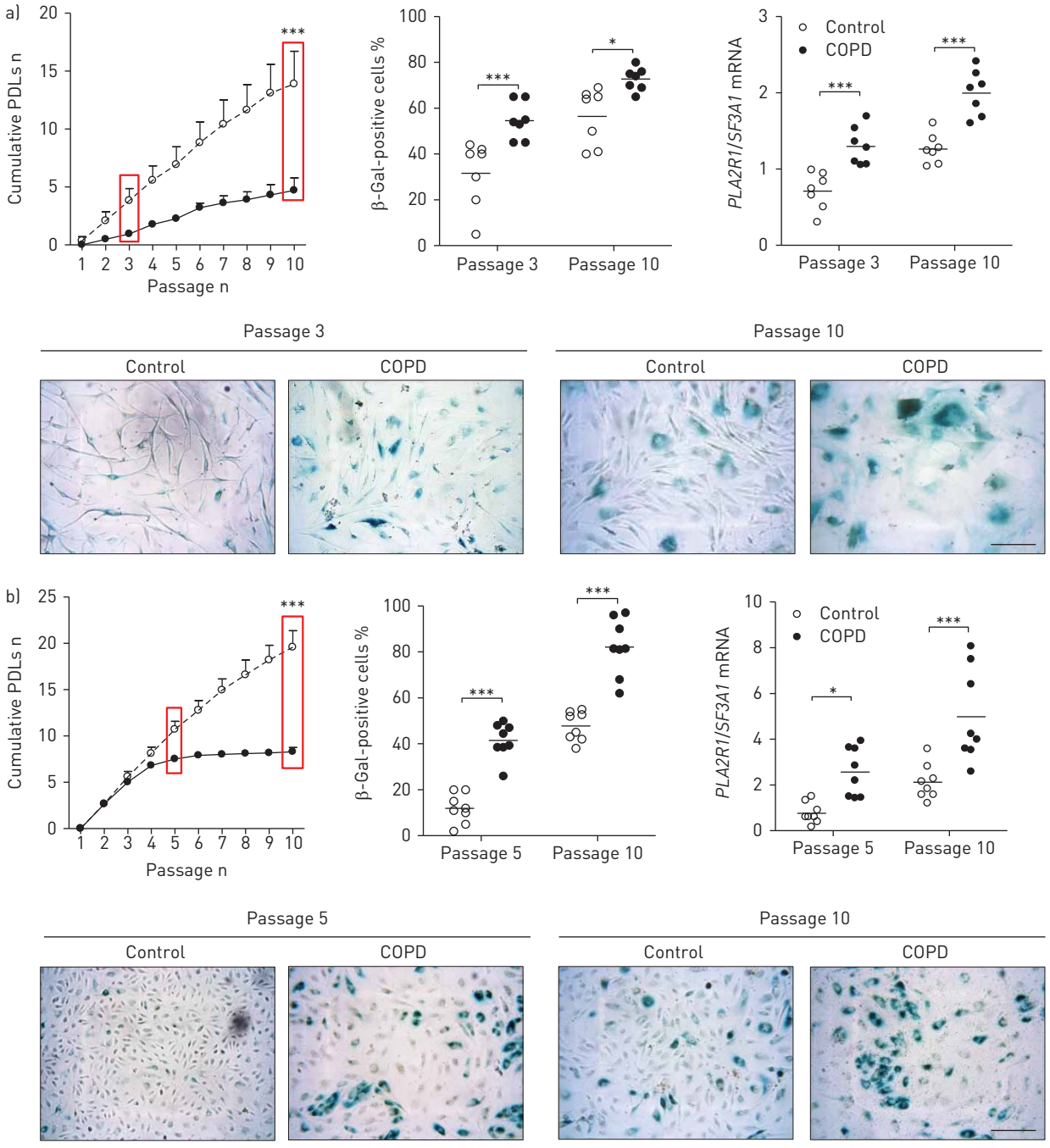

FIGURE 3 Analysis of primary pulmonary artery smooth muscle cells (PA-SMCs) and pulmonary vascular endothelial cells (P-ECs) isolated from pulmonary arteries of controls and patients with chronic obstructive pulmonary disease (COPD). PDL: population doubling; β-Gal: β-galactosidase. From left to right, graphs show cumulative PDLs, percentage of β-Gal-positive cells and *PLA2R1* mRNA levels shown at the corresponding cell passage in cultured a) PA-SMCs (n=7 in each group) and b) P-ECs (n=8 in each group) from patients with COPD and controls. Data are presented as mean±SEM or individual values with the mean. *: p<0.05; ***: p<0.001 *versus* control cells. Two-way ANOVA with Bonferroni's multiple comparisons test. Representative micrographs of cultured β-Gal-stained a) PA-SMCs and b) P-ECs at the indicated cell passages (red boxes) are shown below the graphs. Scale bar: 100 µm.

PLA2R1 *overexpression in transgenic mice induces lung cell senescence and structural lung alterations, which can be reversed by ruxolitinib*

To assess whether lung overexpression of *PLA2R1* replicated some of the lung alterations seen in COPD, we generated transgenic mice that constitutively expressed the human *PLA2R1* gene (*PLA2R1*-TG mice).

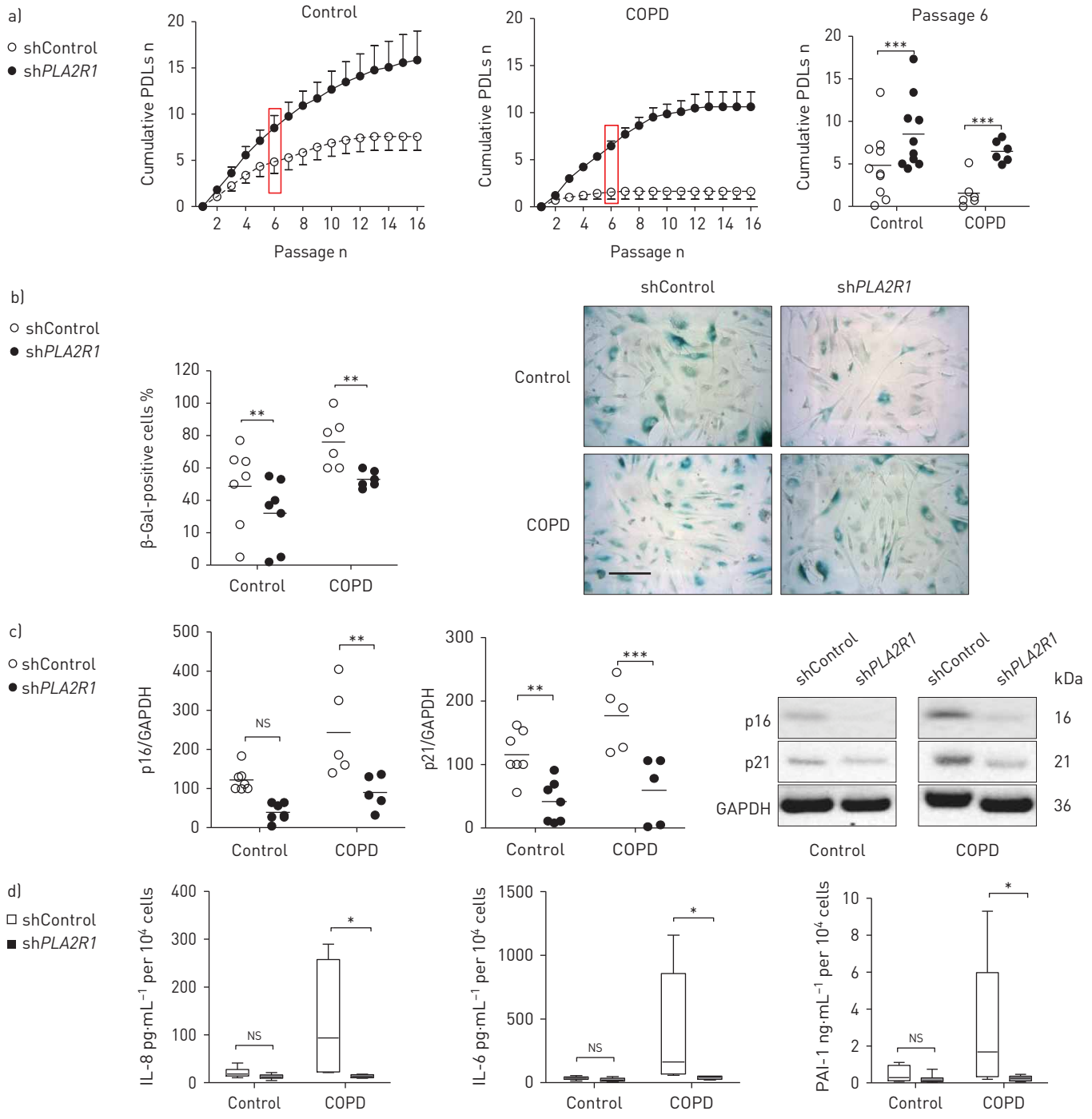

FIGURE 4 Effect of *PLA2R1* knockdown on senescence of pulmonary artery smooth muscle cells (PA-SMCs) from controls and patients with chronic obstructive pulmonary disease (COPD). sh: small hairpin; PDL: population doubling; β-Gal: β-galactosidase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; IL: interleukin; PAI: plasminogen activator inhibitor. Cells were infected with a retroviral vector encoding a shRNA that targeted *PLA2R1* (sh*PLA2R1*) or with a control vector (shControl) and then subjected to successive cell passages. a) Graphs showing mean±SEM of PDLs at successive cell passages of PA-SMCs from n=10 controls (left) and n=6 COPD patients (middle). PDLs at passage 6 are shown as individual values with the mean (right). b) Graph showing the percentage of β-Gal-positive cells at passage 6, with representative micrographs of cells stained for β-Gal. Scale bar: 100 µm. c) Graphs showing the cell protein levels of p16 and p21 with representative immunoblots in PA-SMCs from n=7 controls and n=5 COPD patients at passage 6. Data are presented as individual values with the mean. NS: nonsignificant; **: p<0.01; ***: p<0.001. Two-way ANOVA with Bonferroni's multiple comparisons test. d) Protein levels of IL-8, IL-6 and PAI-1 in PA-SMC-conditioned media at passage 6. Box plots show median (interquartile range) of n=5 (COPD patients) and n=10 (controls) values per group. Whiskers represent extreme values. NS: nonsignificant; *: p<0.05. Kruskal–Wallis and Dunn tests.

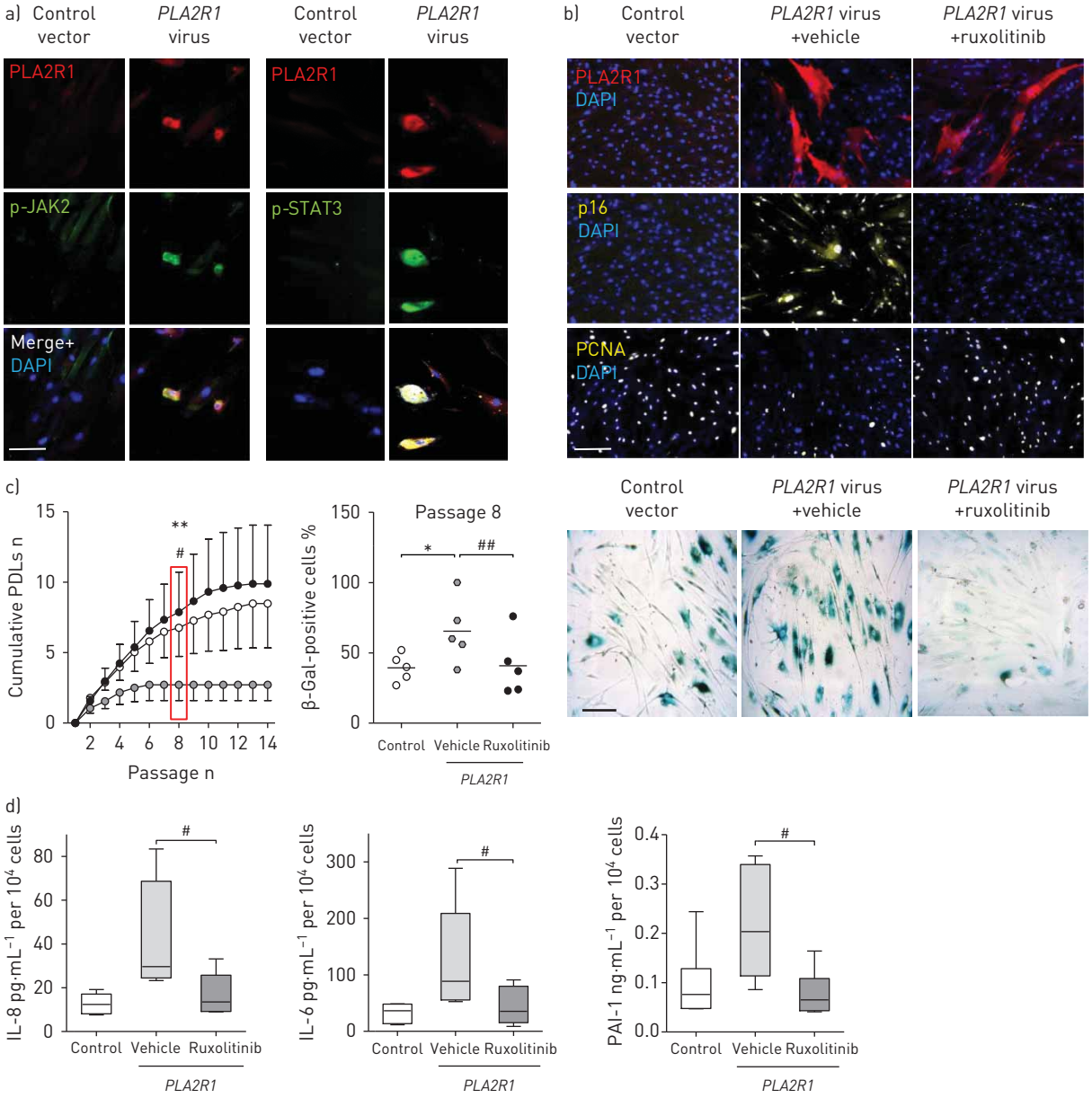

FIGURE 5 Effect of ectopic *PLA2R1* expression on senescence of pulmonary artery smooth muscle cells (PA-SMCs) treated with the Janus kinase (JAK) 1/2 inhibitor ruxolitinib or its vehicle. p: phosphorylated; STAT: signal transducer and activator of transcription; DAPI: 4′,6-diamidino-2-phenylindole; PCNA: proliferating cell nuclear antigen; PDL: population doubling; β-Gal: β-galactosidase; IL: interleukin; PAI: plasminogen activator inhibitor. PA-SMCs were infected with a retrovirus encoding the human *PLA2R1* gene (*PLA2R1* virus) or with a control vector and then subjected to successive passages in the presence of ruxolitinib (0.5 µM) or its vehicle. a) Representative micrographs of cells co-stained for PLA2R1 (red) and for p-JAK2 or p-STAT3 (green). Scale bar: 50 µm. b) Cells stained for PLA2R1 (red) were positive for p16 and negative for PCNA (yellow) when treated with the vehicle but not when treated with ruxolitinib. Scale bar: 200 µm. c) Graph showing mean±SEM of cumulative PDLs of cells infected with the control vector or with the *PLA2R1* virus and treated with vehicle or ruxolitinib (0.5 µM); n=5 in each group (left). Graph showing the percentage of β-Gal-positive cells at passage 8 (middle), with representative micrographs of cells stained for β-Gal (right). Data are presented as individual values with the mean. Scale bar: 100 µm. #: p<0.05; ##: p<0.01 comparison values from vehicle *versus* ruxolitinib; *: p<0.05; **: p<0.01 comparison values from control vector *versus* *PLA2R1* virus. One-tailed paired t-test. d) Protein levels of IL-8, IL-6 and PAI-1 in conditioned media from the indicated cultures at passage 8. Box plots show median (interquartile range) of n=5 values per group. Whiskers represent extreme values. #: p<0.05. Kruskal–Wallis followed by Wilcoxon's test.

Five-month-old *PLA2R1*-TG mice displayed an apparently normal phenotype but overexpressed lung human PLA2R1 and p16 protein compared with control mice (figure 7a). Lungs from *PLA2R1*-TG mice exhibited strong PLA2R1 and p16 immunofluorescence (figure 7a) with increased lung mRNA levels of the JAK/STAT target genes *Tap1*, *Socs1* and *Irf7* (figure 7b). These changes were associated with diffuse

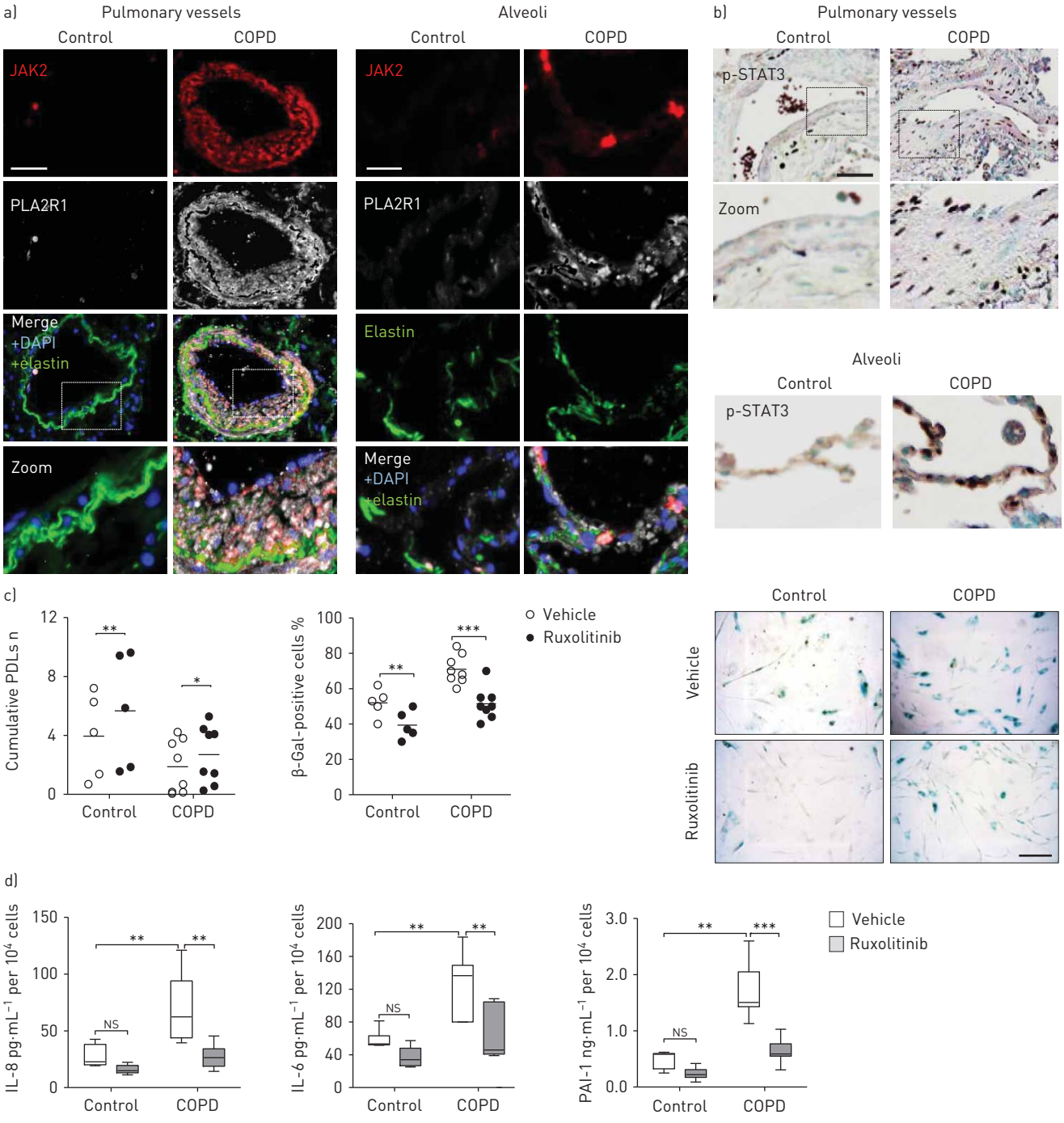

FIGURE 6 Effects of treatment with ruxolitinib on senescence of cultured pulmonary artery smooth muscle cells (PA-SMCs) from controls and patients with chronic obstructive pulmonary disease (COPD). JAK: Janus kinase; PLA2R1: phospholipase A2 receptor 1; DAPI: 4′,6-diamidino-2-phenylindole; p: phosphorylated; STAT: signal transducer and activator of transcription; PDL: population doubling; β-Gal: β-galactosidase; IL: interleukin; PAI: plasminogen activator inhibitor. a) Representative micrographs of lung tissue from controls and patients with COPD showing immunofluorescence of JAK2 (red) and PLA2R1 (white) in pulmonary vessels and alveoli. Elastin autofluorescence (green). Nuclei were labelled with DAPI (blue). The zoomed areas are indicated. Scale bars: 50 µm (left) and 25 µm (right). b) Representative micrographs showing p-STAT3-positive cells (brown) in vessels and alveoli. Scale bar: 50 µm. c) Effect of continuous ruxolitinib treatment on senescence of PA-SMCs from patients with COPD (n=8) and controls (n=5) as assessed by the number of PDLs and the percentage of β-Gal-positive cells at passage 6. Data are presented as individual values with the mean. Typical cells stained for β-Gal are shown on the right. Scale bar: 100 µm. *: p<0.05; **: p<0.01; ***: p<0.001. Two-way ANOVA with Bonferroni's multiple comparisons test. d) Protein levels of IL-8, IL-6, and PAI-1 in conditioned media of cells from controls and patients with COPD treated with ruxolitinib or its vehicle. Box plots show median (interquartile range) of n=5 values per group. Whiskers represent extreme values. NS: nonsignificant; **: p<0.01; ***: p<0.001 *versus* control. Two-tailed Mann–Whitney test.

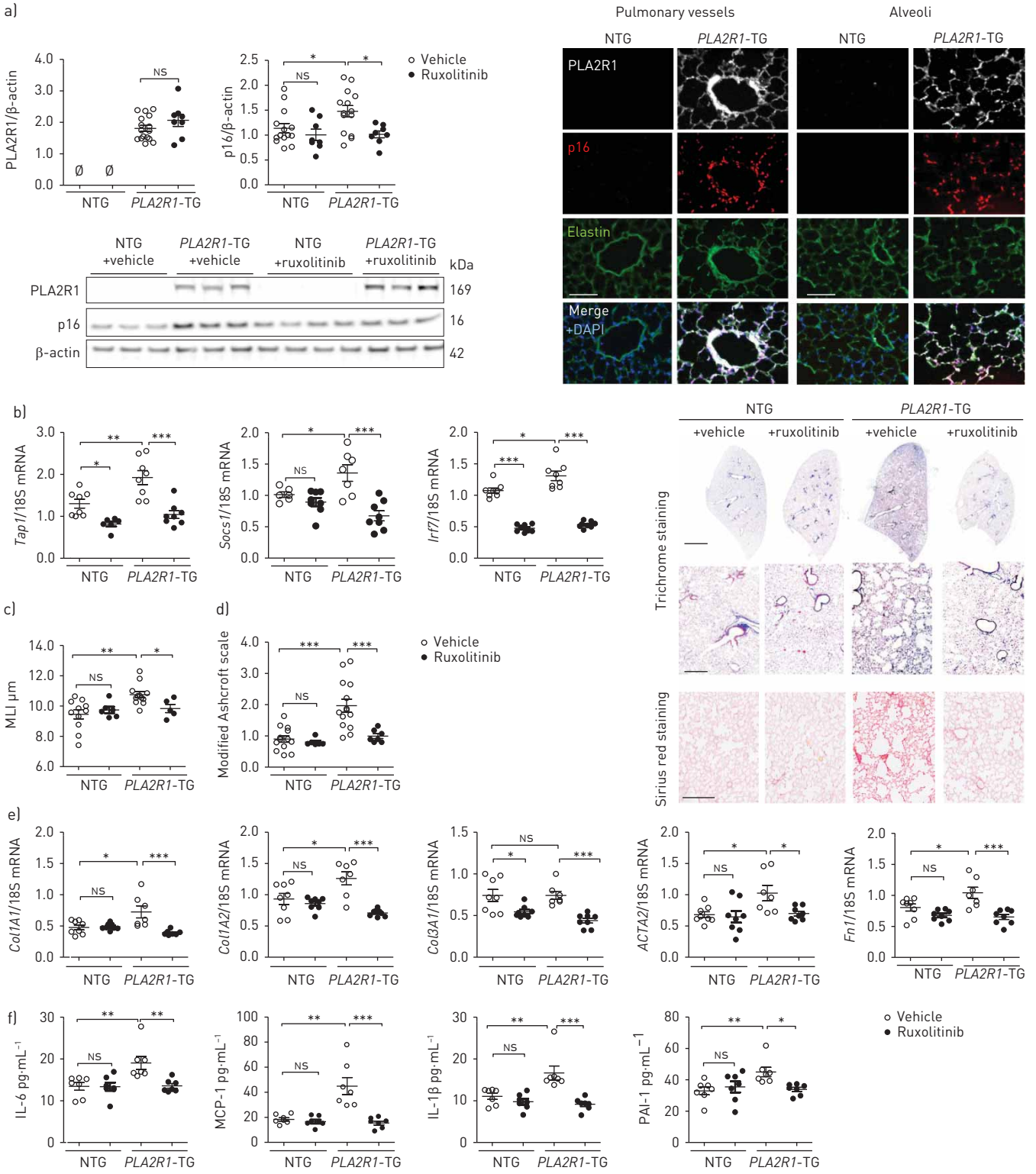

FIGURE 7 Lung characteristics of transgenic mice constitutively expressing the human phospholipase A2 receptor 1 gene (*PLA2R1*-TG) and their nontransgenic (NTG) littermates subjected to 15 days of ruxolitinib treatment. DAPI: 4′,6-diamidino-2-phenylindole; MLI: mean linear intercept; IL: interleukin; MCP: monocyte chemoattractant protein; PAI: plasminogen activator inhibitor. a) Lung protein levels of PLA2R1 and p16 in *PLA2R1*-TG (n=16) and NTG (n=15) mice treated with ruxolitinib (75 mg·kg^{-1}·day^{-1}) or its vehicle, with their representative immunoblots below. Representative micrographs of lung tissue from *PLA2R1*-TG and NTG showing co-localisation of PLA2R1 (white) and p16 (red) in pulmonary vessels and alveoli shown on the right. Scale bar: 50 µm. b) Lung mRNA levels of the Janus kinase 2 signalling target genes *Tap1*, *Socs1* and *Irf7* in each condition. c) Lung emphysema as assessed by measurement of the MLI and d) lung fibrosis as assessed by the modified Ashcroft score. Representative

images of lungs from the four groups of mice stained with Masson's trichrome (top and middle panels, in blue; scale bars: 2 and 0.5 mm, respectively) and Sirius red (bottom panel, in red; scale bar: 0.2 mm). e) Lung mRNA levels of collagen 1A1, collagen 1A2, collagen 3A1, actin α2 and fibronectin. f) Lung protein levels of IL-6, MCP-1, IL-1β and PAI-1. Data are presented as individual values with the mean. NS: nonsignificant; *: p<0.05; **: p<0.01; ***: p<0.001. Two-way ANOVA with Bonferroni's multiple comparisons test.

lung emphysema, as quantified by the mean distance separating alveolar septa (mean linear intercept) (figure 7c), and with fibrotic lung lesions, as quantified by the Ashcroft score of Sirius red-stained lung sections (figure 7d) and by lung mRNA levels of collagen 1A1, collagen 1A2, collagen 3A1, actin-α2 and

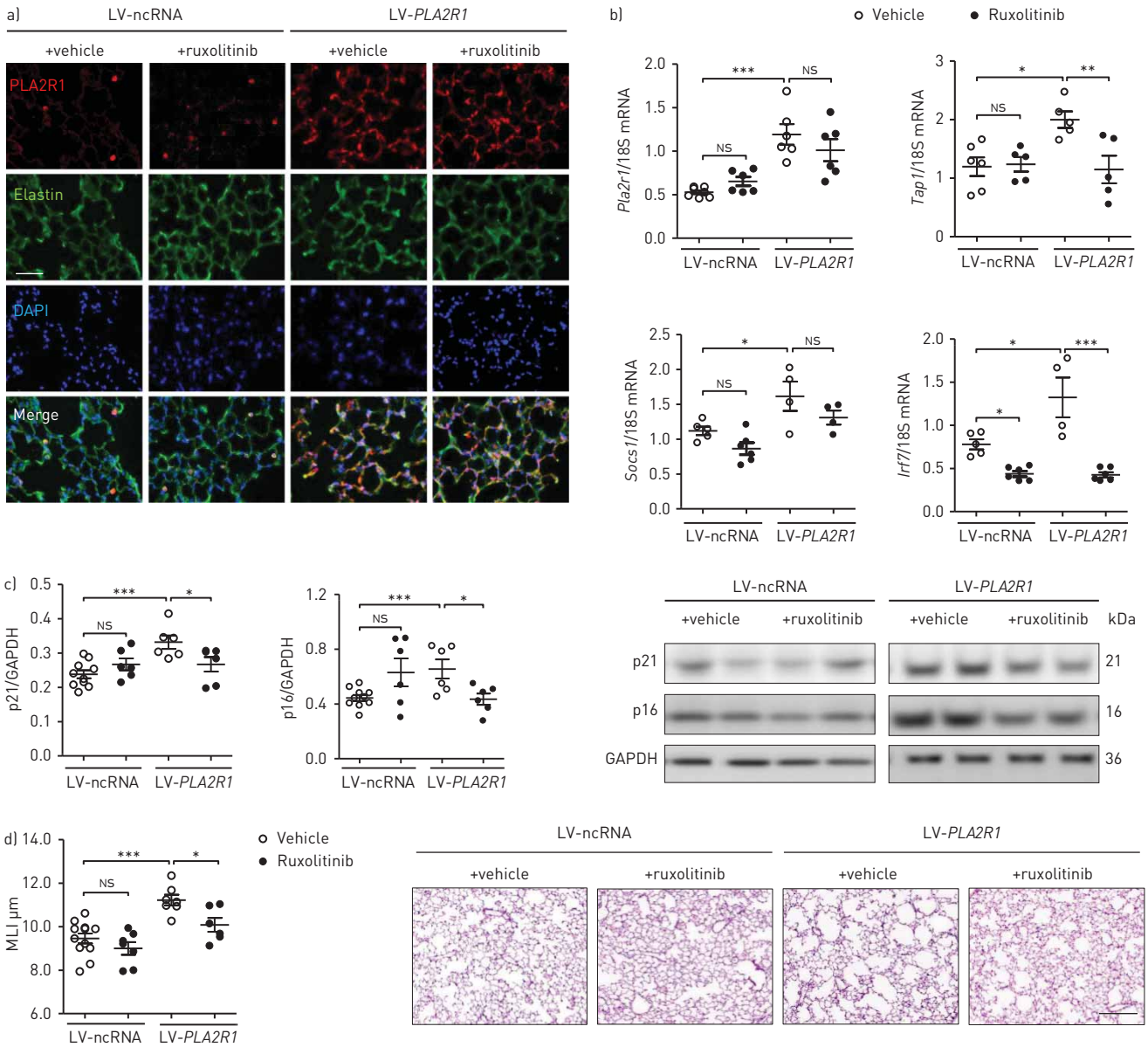

FIGURE 8 Lung characteristics of mice subjected to intratracheal injection of a lentivirus encoding the murine *Pla2r1* gene (LV-*PLA2R1*) and concomitantly treated with ruxolitinib. DAPI: 4′,6-diamidino-2-phenylindole; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; MLI: mean linear intercept. Mice treated with LV-*PLA2R1* or a noncoding control vector (LV-ncRNA) and simultaneously treated with ruxolitinib (75 mg·kg^{-1}·day^{-1}) or vehicle were studied 30 days after the injection. a) Representative micrographs showing PLA2R1 immunofluorescence (red) in mouse lung sections. Scale bar: 50 µm. b) Lung mRNA levels of *Pla2r1* and of the Janus kinas 2 signalling target genes *Tap1*, *Socs1* and *Irf7*. c) Lung protein levels of p21 and p16 with representative immunoblots in the indicated groups. d) Lung emphysema as assessed by measurement of the MLI in haematoxylin and eosin-stained lung sections. Scale bar: 200 µm. Data are presented as individual values with the mean. NS: nonsignificant; *: p<0.05; **: p<0.01; ***: p<0.001. t-test; two-way ANOVA with Bonferroni's multiple comparisons test.

fibronectin (figure 7e). Lung fibrosis in *PLA2R1*-TG mice was also illustrated by increases in Masson trichrome staining (figure 7d) and in vimentin and fibroblast-specific protein immunostaining (supplementary figure S4a and b). *PLA2R1*-TG mice also developed pulmonary hypertension, with increases in right ventricular systolic pressure, right heart hypertrophy and pulmonary vessel muscularisation (supplementary figure S5). Other abnormalities consisted of increased lung levels of IL-6, monocyte chemoattractant protein-1, IL-1β and PAI-1 in lungs from *PLA2R1*-TG mice but not in control mice (figure 7f). We then investigated the effect of ruxolitinib treatment at a dose of 75 $mg \cdot kg^{-1} \cdot day^{-1}$ for 15 consecutive days. This short-term ruxolitinib treatment reduced lung emphysema and fibrosis parameters, inducing marked reductions in lung p16 protein, JAK/STAT target genes and SASP members (figure 7a–f).

Ruxolitinib treatment prevents lung cell senescence and lung alterations induced by intratracheal delivery of the PLA2R1 *gene*

To further investigate the effect of ruxolitinib on lung alterations induced by *PLA2R1* overexpression, we studied mice injected intratracheally with a lentiviral vector encoding the murine *Pla2r1* gene (LV-*PLA2R1*) and given concomitant continuous ruxolitinib treatment (75 $mg \cdot kg^{-1} \cdot day^{-1}$). Preparatory experiments performed with a lentiviral vector encoding the reporter *mCherry* gene under the control of the cytomegalovirus promoter (LV-*mCherry*) revealed that ∼15% of lung cells were transduced by this procedure, including alveolar epithelial cells (supplementary figure S6). Gene transfer was efficient, as shown by diffuse PLA2R1 staining activity throughout the lung structures 4 weeks after LV-*PLA2R1* injection (figure 8a). At this time, the mice exhibited marked increases in lung mRNA levels of *Pla2r1*, *p21* and the JAK/STAT target genes *Tap1*, *Socs1* and *Irf7* (figure 8b and supplementary figure S7a), as well as in lung p21 and p16 protein levels, compared with mice injected with the noncoding control vector (LV-ncRNA) (figure 8c). Most importantly, LV-*PLA2R1* mice developed lung emphysema (figure 8d), increases in right ventricular systolic pressure and hypertrophy index, and pulmonary vessel remodelling (supplementary figure S7b–d). Ruxolitinib treatment started at the time of LV-*PLA2R1* administration markedly reduced the development of lung emphysema and pulmonary vascular remodelling (figure 8d and supplementary figure S7b–d), while also decreasing JAK/STAT downstream genes and p21 mRNA levels (figure 8b and supplementary figure S7a) and p21 and p16 protein levels (figure 8c). No consistent changes were seen in lung cytokine levels 4 weeks after LV-*PLA2R1* injection.

Discussion

Our data support a major role for *PLA2R1* activation in driving lung cell senescence and lung alterations in COPD. Here, we showed that *PLA2R1* overexpression was strongly involved in the exaggerated lung cell senescence seen in COPD, and that PLA2R1 overexpression in mice induced lung cell senescence and pathology. JAK2 activation emerged as an important downstream event to PLA2R1 signalling, notably as a SASP activator. The JAK1/2 inhibitor ruxolitinib delayed the senescence process, inhibiting the SASP in cells from patients with COPD, and also attenuated the pathological lung alterations resulting from *PLA2R1* overexpression in mice. Thus, targeting PLA2R1 and/or the JAK2 pathway may represent a new therapeutic strategy for counteracting lung cell senescence in COPD.

The potential role for PLA2R1 in pathology remains poorly understood. Until recently, efforts focused on the ability of PLA2R1 to bind soluble PLA2s, which catalyse phospholipid hydrolysis and generate inflammatory lipid mediators [17]. However, the physiological consequences of soluble PLA2s binding to PLA2R1 remain unclear [24] and accumulating evidence now supports an intrinsic role for PLA2R1 in several pathological conditions [20]. We have reported that *PLA2R1* can act as a tumour suppressor and that its decreased expression in various cancers promotes tumour progression [20, 21] or can promote some progeroid hallmarks [22]. Structure–function studies and chemical inhibition of shPLA2-related signalling pathways revealed that the ability of *PLA2R1* to exert its tumour-suppressive effect was independent of sPLA2 [18]. Here, we provide evidence for a new pathogenic role for *PLA2R1* due to *PLA2R1* overexpression in the lung in the context of COPD.

PLA2R1 was overexpressed in several cell types from lungs from COPD patients, but was only very weakly expressed in lungs from controls. PLA2R1-stained cells were senescent, as they also stained for p16, a major onco-suppressor expressed in senescent cells [25], and for 53BP1 and γ-H2AX, two well-established DNA damage markers. The prominent and intrinsic role for *PLA2R1* in the senescence process in COPD was further demonstrated by the ability of its knockdown to delay cell senescence in primary cultured cells from patients with COPD, independently of the pathological environment. We previously showed that cells from patients with COPD, compared with control cells, were characterised by a marked reduction in their population doubling and by the release of various inflammatory factors and

cytokines as part of the SASP [6, 7]. We show here that *PLA2R1* inactivation not only markedly improved the population doubling, but also completely suppressed the SASP.

An interesting point is that *PLA2R1* knockdown was almost as potent as *p53* neutralisation in inhibiting cell senescence and suppressing the SASP in cells from patients with COPD. That cell senescence was p53 dependent in COPD was not surprising, given that DNA damage and telomere dysfunction, both of which engage the p53 pathway, are among the main processes responsible for cell senescence in COPD [8, 26, 27]. Moreover, our previous studies suggest that p53 may be downstream of PLA2R1 [16]. Previous studies from our groups also showed that PLA2R1 activated the JAK2 pathway and that this activation mediated at least part of the pro-senescent effect of PLA2R1 [19]. Indeed, we showed that inhibition of JAK/STAT signalling by genetic or pharmacological tools reverted PLA2R1 effects [19], whereas inhibition by ruxolitinib phenocopied PLA2R1 loss, delaying senescence and improving premature ageing [22, 23]. Our present results are consistent with this finding since lungs from patients with COPD that stained for PLA2R1 also stained strongly for JAK2 and p-STAT3. A JAK/STAT increase was previously reported in lungs from patients with COPD, although its role was not investigated [28]. The JAK2 pathway has been associated with oncogenic effects, but its potential pathogenic role in COPD remained unclear. Here, we found that cells transduced with the *PLA2R1* gene and undergoing senescence became positive for p-JAK2 and p-STAT3. Moreover, pharmacological inhibition of JAK2 with ruxolitinib fully reversed the induction of cell senescence induced by *in vitro PLA2R1* transduction. These results indicate that one major mechanism responsible for JAK/STAT activation in COPD is linked to the senescence process driven by *PLA2R1*.

Previous studies established that JAK activation mediated the SASP in senescent cells from adipose tissue during ageing [29]. JAK1/2 inhibitors reduced inflammation and alleviated frailty in aged mice [29]. Similarly, our results support a major effect of the JAK/STAT pathway in promoting the SASP in senescent cells. Moreover, ruxolitinib treatment reversed *PLA2R1*-induced cell senescence and slightly but significantly delayed the onset of cell senescence in cells from patients with COPD. It is unlikely that the ability of ruxolitinib to decrease cell senescence was due to inhibition of the SASP. Indeed, in previous work by our groups, selectively suppressing the inflammatory arm of the SASP, *e.g. via* NF-κB inactivation, did not affect lung cell senescence in COPD [7]. Our results thus indicate that the JAK2 pathway mediates part of the pro-senescent effects of PLA2R1 in COPD, with prominent blunting of the SASP by inhibition of the pathway. However, it is likely that not all the effects of PLA2R1 are mediated *via* JAK/STAT and also that some of the effects of JAK/STAT inhibition on cell senescence may rely on mechanisms beyond PLA2R1 activation.

To assess whether *PLA2R1*-induced cell senescence played a causal role in COPD, we developed mice overexpressing *PLA2R1*. Although these mice had an apparently normal phenotype, they developed major lung alterations consisting of emphysema and fibrotic lesions at a young age, concomitantly with the accumulation of senescent lung cells stained for both p16 and PLA2R1, increased expression of lung JAK/STAT target genes, and increased expression of SASP members. These mice also developed pulmonary hypertension, a finding consistent with the reported association between pulmonary hypertension and the development of lung emphysema in mouse models [30]. Taken together, these results in mice show that, even in young mice, PLA2R1 pathway activation is sufficient to drive cell senescence with the concomitant development of lung emphysema and fibrosis. These findings are consistent with recent evidence of a pathogenic role for senescent cells in a murine lung fibrosis model [31] and with the accumulation of senescent cells in lungs from patients with idiopathic lung fibrosis [32, 33]. The pathogenic role for lung cell senescence in mediating either lung fibrosis or lung emphysema is further illustrated by reports of lung fibrosis, lung emphysema or both in individuals carrying a mutation in the telomerase *TERT* gene [14, 34]. The mechanisms that may favour development of lung emphysema, lung fibrosis or both in response to cell senescence still remain unclarified, although it is likely that different senescent cell types drive these different phenotypes. Consistent with this, we found that mice injected intratracheally with a LV-*PLA2R1* vector rapidly developed severe lung emphysema and some degree of pulmonary hypertension, but without significant lung fibrosis. It is therefore possible that the relatively small number of transduced cells, although sufficient to induce lung emphysema, was probably insufficient to induce lung fibrosis as seen with PLA2R1-TG mice.

In the *PLA2R1*-TG mouse model, short-term ruxolitinib treatment markedly diminished the lung fibrotic and emphysema lesions, lung p16 levels, SASP members, and JAK/STAT downstream gene expression. Moreover, in mice injected intratracheally with the LV-*PLA2R1* vector, ruxolitinib treatment started at the time of lentiviral administration prevented lung cell senescence and lung emphysema development. These results are consistent with an inhibitory effect of ruxolitinib on PLA2R1-induced lung cell senescence,

either by a direct effect or by an effect mediated by inhibition of the SASP. Inhibitors of the JAK/STAT pathways may have complex actions since they have also been shown to inhibit transforming growth factor-β signalling in lung cells. Our results therefore do not allow us to draw definitive conclusions about the mechanism of action of ruxolitinib and further studies are needed to assess the potential protective effects of this drug on lung alterations in other models.

Taken together, our findings support a causal role for *PLA2R1* activation-induced cell senescence in lung pathology and support the view that targeting the PLA2R1–JAK2 pathway represents a therapeutic option for inhibiting cell senescence or eliminating the SASP in patients with COPD. In contrast to interventions targeting major senescence pathways such as the p53 pathway, targeting PLA2R1 and/or the JAK pathway should not drastically impact tumour development. Anti-JAK agents are currently used to treat cancer and were recently found to reprogramme the SASP in senescent tumour cells, an effect that contributes to improve their antitumor effect. Further studies are needed to evaluate the potential of this approach as a new therapeutic option in COPD.

Acknowledgements: We are grateful to A. Lalot, D. Gelperowic, A. Ribeiro Da Silva, A. Da Silva and F. Weber from the Plateforme de Physiologie et Physiopathologie Expérimentale, as well as to X. Decrouy, and C. Micheli from the imaging platform facility at IMRB INSERM, Créteil, France; and to Thomas Lilin at the Centre de Recherche Biomédicale d'Alfort (CRBM), École Nationale Vétérinaire d'Alfort, Maisons-Alfort, France.

Data availability: The data that support the findings of this study are available from the corresponding author upon request.

Author contributions: D. Beaulieu, A. Attwe and S. Abid: conception and design, acquisition, analysis and interpretation of data, and drafting of the manuscript. M. Breau, E. Marcos, E. Born, J. Huang, A. Houssaini, N. Vienney, P. Bertolino, S. Jaber, D. Goehrig and D. Vindrieux: acquisition, analysis and interpretation of data. L. Lipskaia: conception and design, and acquisition, analysis and interpretation of data. G. Derumeaux, B. Maitre, M. Lefevre and H. Noureddine: analysis and interpretation of data, and revision of the manuscript. D. Bernard and S. Adnot: conception and design, analysis and interpretation of data, and drafting of the manuscript.

Conflict of interest: None declared.

Support statement: This study was supported by grants from the INSERM, Délégation à la Recherche Clinique de l'AP-HP–FHU SENEC, Fondation pour la Recherche Médicale (FRM), Legs Poix, Agence Nationale pour la Recherche (ANR) and Institut National Du Cancer (INCA). Funding information for this article has been deposited with the Crossref Funder Registry.

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