

ORIGINAL ARTICLE

Peli1 Deficiency in Macrophages Attenuates Pulmonary Hypertension by Enhancing Foxp1-Mediated Transcriptional Inhibition of IL-6

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BACKGROUND: The infiltration of macrophages into the lungs is a common characteristic of perivascular inflammation, contributing to vascular remodeling in pulmonary hypertension (PH). Peli1 (pellino E3 ubiquitin-protein ligase 1) plays a critical role in regulating the production of proinflammatory cytokines and the polarization of macrophages in various diseases. However, the role of Peli1 in PH remains to be investigated.

METHODS: The expression and biological function of Peli1 were investigated in both human and experimental models of PH. Peli1-deficient mice and bone marrow transplant mice were utilized to explore the roles of Peli1 in macrophages in vivo. Proteomic analysis and molecular biology techniques were used to uncover the underlying mechanisms.

RESULTS: The upregulation of Peli1 in the lungs and alveolar macrophages was observed in hypoxia-treated mice. Peli1 knockout mice and myeloid Peli1-deficient mice significantly ameliorated hypoxia-induced right ventricular systolic pressure, right ventricular hypertrophy, and pulmonary vascular remodeling. Mechanistically, Peli1 facilitated the ubiquitination and subsequent proteasomal degradation of Foxp1 (forkhead box p1), thereby alleviating its suppression of *IL (interleukin)-6* transcription and contributing to macrophage activation. Furthermore, myeloid Foxp1 deficiency partially eliminates the protective effect of myeloid Peli1 deficiency in hypoxia-induced PH mice.

CONCLUSIONS: Our findings demonstrate that the Peli1-Foxp1-IL-6 pathway plays a crucial role in macrophage activation and recruitment during the development of PH, underscoring the potential of Peli1 as a therapeutic target for PH. (*Hypertension*. 2025;82:445–459. DOI: 10.1161/HYPERTENSIONAHA.124.23542.) • **Supplement Material.**

Key Words: hypertension, pulmonary ■ inflammation ■ interleukin ■ macrophage ■ mice

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Pulmonary hypertension (PH) is a chronic and progressive disease characterized by sustained perivascular inflammation and progressive pulmonary vascular remodeling, ultimately leading to right ventricular (RV) hypertrophy and death.^{1,2} Previous research has indicated that PH is, at least partially, an inflammatory disorder.^{1,3} Pulmonary inflammation is observed before the onset of vascular remodeling, with macrophages serving as a

significant source of growth factors and proinflammatory cytokines.⁴ In the monocrotaline-induced PH rat model, there is a notable increase in the number of cluster of differentiation (CD)68⁺ NOS2 (nitric oxide synthase 2)⁺ M1-like macrophages 3 days postadministration, whereas the number of CD68⁺ CD206⁺ M2 macrophages progressively accumulates at later stages.⁵ Depending on their location and roles in the defense response to

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NOVELTY AND RELEVANCE

What Is New?

Here, we provide evidence that increased expression of Peli1 (pellino E3 ubiquitin-protein ligase 1) in macrophages of pulmonary hypertension (PH) animal models and in patients with idiopathic pulmonary artery hypertension is associated with the development of PH.

Peli1 deficiency in macrophages ameliorates perivascular inflammation in vivo and in vitro, right ventricular hypertrophy, vascular remodeling, and PH development.

Peli1 facilitated the ubiquitination and subsequent proteasomal degradation of Foxp1 (forkhead box p1), thereby alleviating its suppression of *IL* (interleukin) -6 transcription and contributing to macrophage activation.

What Is Relevant?

Previous research has elucidated various functions of *Peli1* in modulating the expression of proinflammatory cytokines and the activities of macrophages within immune responses. Nonetheless, the precise role of *Peli1* in the recruitment and activation of macrophages during the progression of PH, as well as its contribution to pulmonary vascular remodeling, remains to be fully characterized. This study constitutes a substantial advancement in our comprehension of the mechanisms through which *Peli1* impacts the pathogenesis of PH.

Clinical/Pathophysiological Implications?

This study demonstrated that Peli1-Foxp1-IL-6 pathway plays a crucial role in macrophage activation and recruitment in the development of PH, underlining Peli1's potential as a therapeutic target for PH.

Nonstandard Abbreviations and Acronyms

AMs	alveolar macrophages
ARG1	arginase 1
BMDMs	bone marrow–derived macrophages
F13A1	coagulation factor XIII A chain
Foxp1	forkhead box P1
HNF4α	hepatocyte nuclear factor 4 alpha
IFN-γ	interferon γ
IL	interleukin
IMs	interstitial macrophages
iNOS	inducible NO synthase
IPAH	idiopathic pulmonary artery hypertension
IRF5	interferon regulatory factor 5
mPASCs	mouse pulmonary artery smooth muscle cells
Peli1	pellino E3 ubiquitin-protein ligase 1
PH	pulmonary hypertension
RVSP	RV systolic pressure
TGF	transforming growth factor
TNF	tumor necrosis factor

damage, pulmonary macrophages can be categorized as alveolar macrophages (AMs) and interstitial macrophages (IMs).⁶ The early recruitment and activation of macrophages (M1-like or M2-like) contribute to the injury and apoptosis resistance of pulmonary artery endothelial cells,⁷ as well as promote the proliferation and hypertrophy of pulmonary artery smooth muscle cells (PASCs), resulting in pulmonary vascular remodeling.^{4,8,9} Therefore,

inhibiting the aberrant activation of macrophages may reverse pulmonary vascular remodeling and provide therapeutic benefits for patients with PH. However, the precise mechanisms underlying macrophage recruitment and activation in PH remain incompletely understood.

Peli1 (pellino E3 ubiquitin-protein ligase 1) is an evolutionarily conserved immunomodulator that regulates the expression of proinflammatory cytokines and the activation of macrophages during immune responses.^{10,11} Silencing Peli1 in mice significantly protects against myocardial infarction–induced cardiac dysfunction and remodeling by inhibiting inflammatory cell infiltration into the myocardium and reducing proinflammatory cytokine production.¹² Kim et al¹³ demonstrated that Peli1 facilitates the nuclear translocation of IRF5 (interferon regulatory factor 5) through binding and K63-linked ubiquitination, which promotes M1 macrophage polarization and exacerbates glucose intolerance in obesity. In the context of acute lung injury, TGF (transforming growth factor)-β1 induces the upregulation of DNA methyltransferase 1, leading to the activation of Peli1 and the nuclear translocation of IRF5, ultimately driving M1 polarization of AMs and worsening acute lung injury in mice.¹¹ However, the specific role of Peli1 in the recruitment and activation of macrophages during PH development and its contribution to pulmonary vascular remodeling remains undefined.

In this study, we found that Peli1 plays a critical role in the M1 polarization of AMs during the early stages of PH. Peli1 promotes the degradation of Foxp1 (forkhead box p1), relieving its suppression of *IL*-6 transcription. This process facilitates the M1 polarization of macrophage and subsequently enhances the proliferation and migration of PASCs. In hypoxia-induced PH mice, Peli1

knockout attenuates pulmonary vascular remodeling and RV hypertrophy by mitigating perivascular macrophage infiltration and polarization. These findings suggest that Peli1 may serve as a promising therapeutic target for PH.

METHODS

Data Availability

All data related to this study's findings are available from the corresponding author on reasonable request. See the [Supplemental Methods](#) for detailed materials and methods.

Human Samples

The human lung sections and peripheral blood mononuclear cells utilized in this study were obtained from Wuxi People's Hospital and the First Affiliated Hospital of Nanjing Medical University, respectively. Clinical information is available in [Tables S2 and S3](#). Human research protocols for this study were approved by the Ethics committee of Nanjing Medical University.

Animal Studies

All animal experiments complied with the guidelines for the care and use of laboratory animals and received approval from the Committee on the Ethics of Animal Experiments at Nanjing Medical University (IACUC-1705029 and 2005016). *Peli1*^{+/-} mice (C57BL/6 background) were generated as previously described and backcrossed onto the C57BL/6 background for at least seven generations to generate the *Peli1*^{-/-} mice.¹⁴ All the operations and analyses were performed in a blinded manner. For PH parameter assessment, right cardiac function, RV systolic pressure (RVSP), RV hypertrophy, vascular wall thickness, and vessel muscularization were used to evaluate PH parameters as in our previous studies.¹⁵⁻¹⁷

Quantification and Statistical Analysis

Graphs and statistical analyses were conducted using GraphPad Software. Unless otherwise specified, all data represent the results of at least 5 independent replicates. Before conducting statistical analyses, the normality of the data was thoroughly assessed using the Shapiro-Wilk test, whereas the homogeneity of group variances was evaluated via the Brown-Forsythe test. For comparisons between 2 groups with equal variances, Student *t* test was utilized, whereas the Welch correction was applied for analyses involving 2 groups with unequal variances. For comparisons among multiple groups, a 1-way ANOVA followed by Tukey post hoc test was used. Data are expressed as the mean±SEM, and a *P* value of <0.05 was considered statistically significant.

RESULTS

Peli1 Is Significantly Upregulated in Pulmonary Macrophages of PH

To investigate the role of Peli1 in the development of PH, we assessed the expression of Peli1 protein in the lungs of hypoxia-treated mice during the progression of PH ([Figure S1A and S1B](#); [Figure 1A](#)). The results showed

that Peli1 protein expression was significantly increased in the earlier period of PH, suggesting Peli1 may be related to pulmonary inflammation in PH. Similarly, the expression of Peli1 was also elevated in the lungs of 5-day monocrotaline-treated rats ([Figure S1C](#)). Immunofluorescence staining demonstrated a significant enrichment of Peli1 in macrophages within lung sections from both 4-day and 28-day hypoxia-treated mice, as well as in 5-day monocrotaline-treated rats ([Figure 1B](#); [Figure S1D](#)). In addition, Western blot analysis and immunofluorescence staining indicated that Peli1 was predominantly localized in pulmonary macrophages from patients with idiopathic pulmonary artery hypertension and connective tissue disease-pulmonary artery hypertension, with an observed increase of Peli1 protein expression in peripheral blood mononuclear cells from patients with idiopathic pulmonary artery hypertension ([Figure 1C and 1D](#)). Furthermore, AMs and IMs were isolated from hypoxia-treated mice, and the results of real-time polymerase chain reaction demonstrated that hypoxia induces significantly higher levels of *Peli1* mRNA in AMs compared with IMs ([Figure S1E](#)). These data support the hypothesis that upregulated Peli1 expression in pulmonary macrophages is likely involved in the pathophysiological processes associated with PH.

Peli1 Deficiency Attenuated PH Parameters in Mice With Hypoxia or SU5416/Hypoxia-Induced PH

To investigate the role of Peli1 in the pathogenesis of PH, *Peli1*^{+/-} mice, and *Peli1*^{-/-} mice were generated ([Figure S2A](#)) and subjected to hypoxia for 28 days to induce PH ([Figure 2A](#)). Tail-cuff measurements revealed no significant differences in blood pressure between *Peli1*^{+/-} and *Peli1*^{-/-} mice under both normoxic and hypoxic conditions ([Figure S3A](#)). As illustrated in [Figure 2B and 2D](#), the hypoxia-induced *Peli1*^{-/-} mice exhibited a 49.81% reduction in RVSP and a 34.75% reduction in the ratio of RV to left ventricle plus septum, as well as a decrease in the occlusion and muscularization of pulmonary arterioles compared with *Peli1*^{+/-} mice. Similarly, in the SU5416 (Semaxinib)/hypoxia-induced PH model, *Peli1*^{-/-} mice exhibited attenuated PH parameters relative to *Peli1*^{+/-} mice ([Figure S4A through S4C](#)). Double-staining for PCNA (proliferating cell nuclear antigen) and α -SMA (smooth muscle actin) indicated a significant reduction in the number of PCNA-positive vascular cells in the lungs of hypoxia-induced *Peli1*^{-/-} mice compared with *Peli1*^{+/-} mice ([Figure 2E](#)). Furthermore, immunofluorescence staining for F4/80 and α -SMA indicated a significant decrease in perivascular macrophages in the lungs of 4-day hypoxia-treated *Peli1*^{-/-} mice ([Figure 2F](#)). These findings support the theory that Peli1 deficiency in mice leads to decreased macrophage recruitment and improved pulmonary vascular remodeling in hypoxia-induced PH mice.

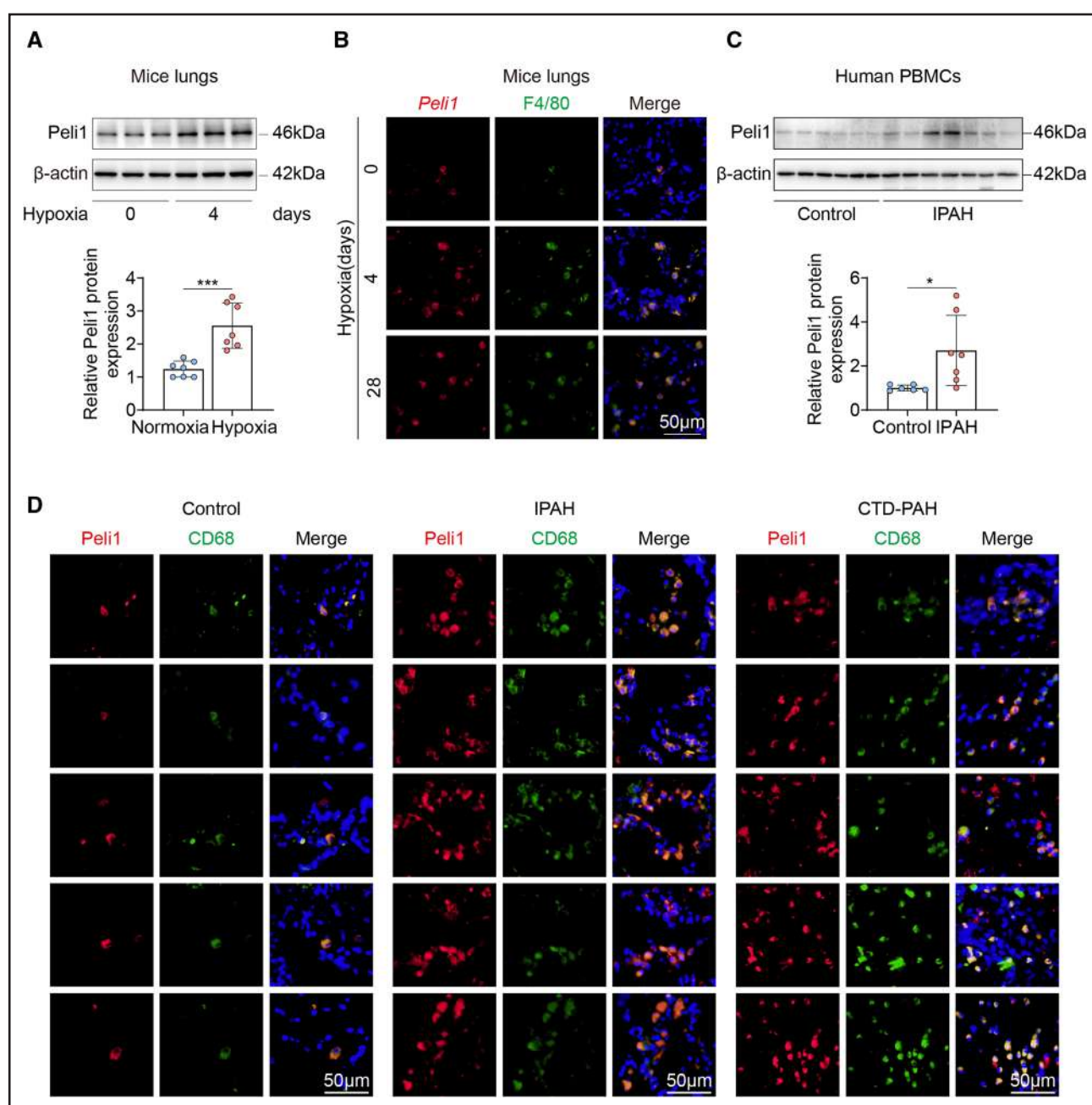


Figure 1. Increased expression of Peli1 (pellino E3 ubiquitin-protein ligase 1) in pulmonary macrophages is associated with pulmonary hypertension (PH).

A and **C**, Representative immunoblots and relative quantitative densitometric analysis of Peli1 protein expression in the lungs of 4-day hypoxia-treated mice ($n=7$ mice per group) and peripheral blood mononuclear cells (PBMCs) of control individuals ($n=6$) and patients with idiopathic pulmonary artery hypertension (IPAH; $n=7$), normalized to β -actin. **B** and **D**, Immunofluorescence staining for Peli1 in the lungs of normoxia, 4-day, and 28-day hypoxia-treated mice (**B**), and control individuals, patients with IPAH, and connective tissue disease (CTD)-PAH (**D**). Pulmonary macrophages were visualized using F4-80 (green), and cluster of differentiation 68 (CD68; green), respectively. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; blue color), scale bar=50 μ m. The data are presented as mean \pm SE; P values were determined by Student t test; * $P<0.05$, and *** $P<0.001$.

Myeloid Peli1 Deficiency Attenuated PH Progression in Mice With Hypoxia-Induced PH

To investigate the impact of Peli1 deletion in macrophages on the progression of PH, bone marrow transplantation was performed to establish mice with Peli1

deficiency in myeloid cells. As illustrated in Figure 3A, Mac (macrophage reconstruction) ($Peli1^{+/+}$) and Mac ($Peli1^{-/-}$) mice were subjected to hypoxia treatment for 28 days to induce PH. Immunofluorescence staining for Peli1 and F4/80 was conducted to confirm the reduction in the number of $Peli1^{+}$ macrophages in the lungs

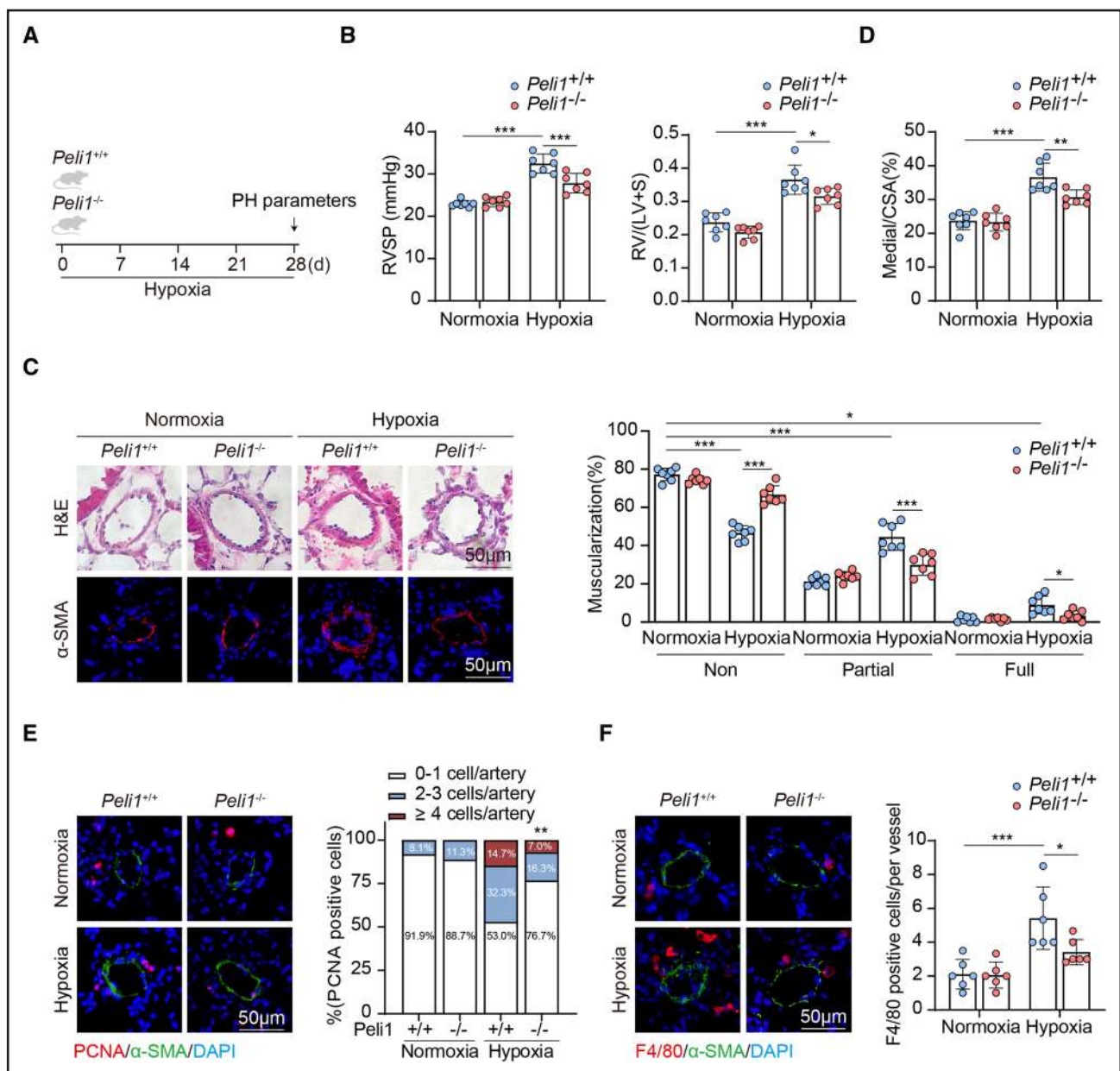


Figure 2. Genetic ablation of *Peli1* (pellino E3 ubiquitin-protein ligase 1) attenuates hypoxia-induced pulmonary hypertension (PH) in mice.

A, Schematic presentation of experimental protocol for *Peli1*^{+/+} and *Peli1*^{-/-} mice in the hypoxia-induced PH model. **B**, **left**, Quantification of the right ventricular systolic pressure (RVSP), **(right)** Fulton index: the ratio of right ventricular (RV) to left ventricular wall plus septum (S; RV/[LV+S]) in *Peli1*^{+/+} and *Peli1*^{-/-} mice after 4 weeks of normoxia or hypoxia treatment. **C**, **left**, Representative images of hematoxylin and eosin (H&E) and immunofluorescence (IF) staining for α-SMA (smooth muscle actin; red) in the lungs of normoxia- or hypoxia-treated mice; nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; blue), scale bar=50 μm; **(right)** The proportion of non, partially, or fully muscularized pulmonary arterioles (20–50 μm in diameter) from hypoxia-induced PH mice. **D**, Quantifying medial/cross-sectional area (CSA, %) for image **C** (left). **E**, Representative IF staining for PCNA (proliferating cell nuclear antigen) (red) and α-SMA (green) staining in the lungs of normoxia- or hypoxia-treated mice; nuclei were counterstained with DAPI (blue), scale bar=50 μm. The frequency bar chart represents the percentage of pulmonary arteries with PCNA-positive cells categorized into 3 groups (0–1 positive cell/artery, 2–3 positive cells/artery, and ≥4 positive cells per artery, 6–8 pulmonary arteries per mouse). For **A** through **E**, n=7 mice per group. **F**, Representative IF staining and quantification of perivascular macrophages in normoxia- or 4-day hypoxia-treated *Peli1*^{+/+} and *Peli1*^{-/-} mice lung sections, nuclei were counterstained with DAPI (blue), scale bar=50 μm. n=6 mice per group. The data are presented as mean±SE; P values were determined by 1-way ANOVA with Tukey post hoc test; *P<0.05, **P<0.01, and ***P<0.001.

of Mac (*Peli1*^{-/-}) mice compared with Mac (*Peli1*^{+/+}) mice (Figure S5A). As shown in Figure 3B and 3E, Mac (*Peli1*^{-/-}) mice exhibited an attenuated hypoxia-induced increase in RVSP and the ratio of RV to left

ventricle plus septum, as well as a reduction in occlusion and muscularization of distal pulmonary arterioles as compared with Mac (*Peli1*^{+/+}) mice. Furthermore, immunofluorescence staining for PCNA and α-SMA

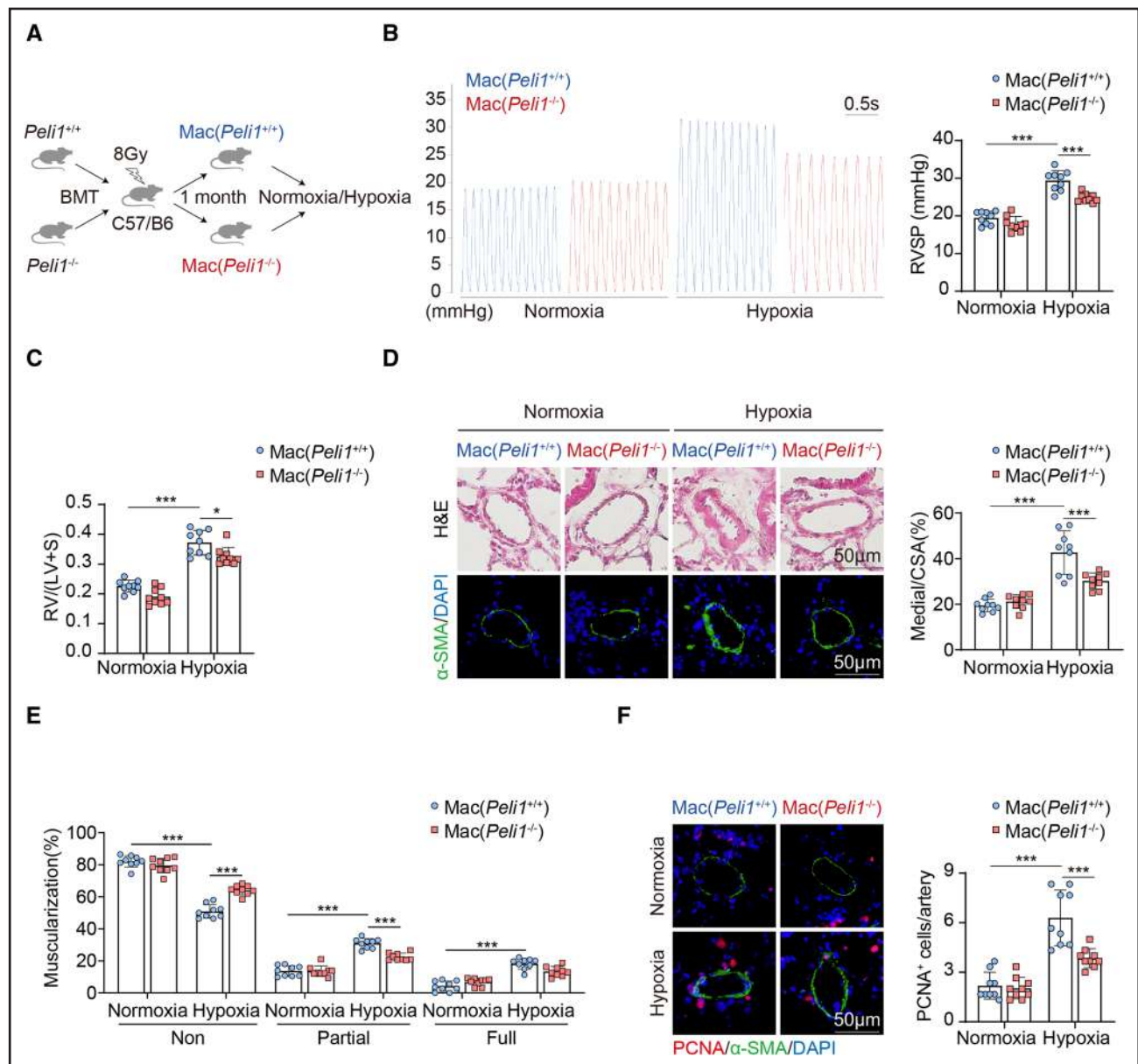


Figure 3. Genetic ablation of *Peli1* (pellino E3 ubiquitin-protein ligase 1) in myeloid cells attenuates hypoxia-induced pulmonary hypertension (PH) in mice.

A, Schematic presentation of the experimental protocol for Mac (macrophage reconstruction) (*Peli1*^{+/+}) and Mac (*Peli1*^{-/-}) bone marrow transplant (BMT) mice in the hypoxia-induced PH model. The bone marrow cells of *Peli1*^{+/+} mice and *Peli1*^{-/-} mice were transplanted into recipient mice and housed in normoxia for 1 month before exposure to hypoxia (10% O₂) for 4 weeks. **B**, Representative images and quantification of the right ventricular (RV) systolic pressure (RVSP), **(C)** the ratio of RV to left ventricular (LV) wall plus septum (RV/[LV+S]) in Mac (*Peli1*^{+/+}) and Mac (*Peli1*^{-/-}) BMT mice after 4 weeks of normoxia or hypoxia treatment. **D**, **left**, Representative hematoxylin and eosin (H&E) and immunofluorescence (IF) staining for α-SMA (smooth muscle actin; green) in the lung sections and nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; blue), scale bar=50 μm; **(right)** Quantification of vascular media/cross-sectional area (CSA, %) for images in **D**. **E**, The proportion of non, partially, or fully muscularized pulmonary arterioles (20–50 μm in diameter) from hypoxia-induced PH mice. **F**, Representative IF staining and quantification of PCNA (proliferating cell nuclear antigen)* (red) cells in pulmonary arteries within the lungs of normoxia- or hypoxia-treated mice; nuclei were counterstained with DAPI (blue), scale bar=50 μm. For **A** through **F**, n=9 mice per group. The data are presented as mean±SE; *P* values were determined by 1-way ANOVA with Tukey post hoc test; **P*<0.05, and ****P*<0.001.

revealed a significant decrease in PCNA-positive vascular cells in the hypoxia-induced pulmonary arteries of Mac (*Peli1*^{-/-}) mice (Figure 3F). These findings support the hypothesis that *Peli1* deficiency in myeloid cells mitigates pulmonary vascular remodeling in hypoxia-induced PH mice.

Peli1 Deficiency Diminished Macrophage Activation and Attenuated the Proliferation and Migration of Mouse PSMCs

To investigate the impact of *Peli1* on macrophages in the lungs of PH, AMs were isolated from *Peli1*^{+/+} and

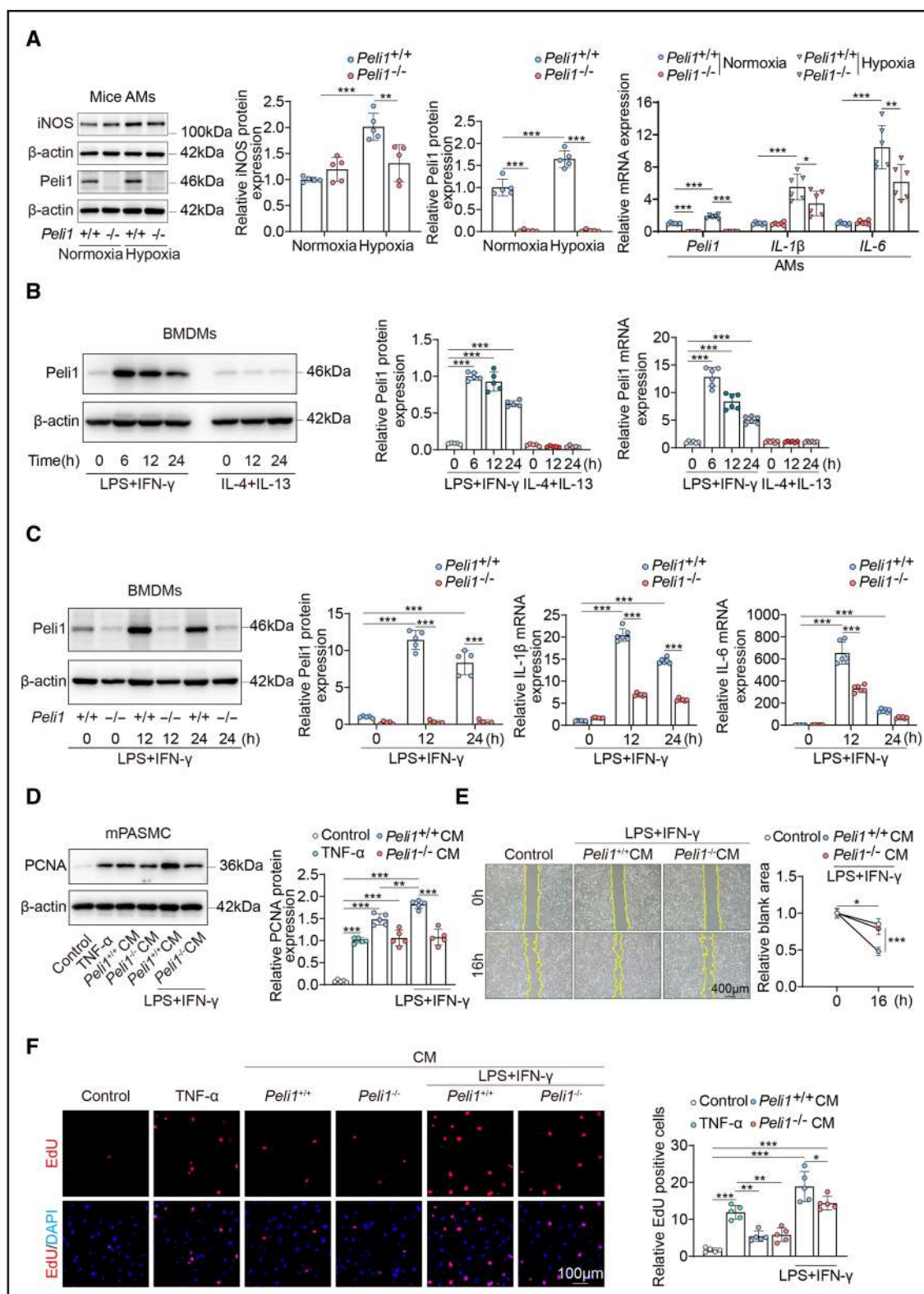


Figure 4. Macrophages with Peli1 (pellino E3 ubiquitin-protein ligase 1) deficiency attenuate the proliferative and migratory effects on mouse pulmonary artery smooth muscle cells (mPASMCs).

A, left, Representative immunoblots and relative densitometric analysis of iNOS (inducible NO synthase) and Peli1 protein expression, **(right)** real-time PCR (RT-PCR) analysis of *Peli1*, *IL* (*interleukin*)-1β, and *IL*-6 mRNA levels in alveolar macrophages (AMs) isolated from normoxia-treated or 4-day hypoxia-treated *Peli1*^{+/+} and *Peli1*^{-/-} mice. **B, left**, Representative immunoblots and relative densitometric analysis of Peli1 protein expression, and **(right)** RT-PCR analysis of *Peli1* mRNA levels in M1 (lipopolysaccharides [LPS] and IFN-γ [interferon γ]) and M2 (IL-4 and IL-13) bone marrow-derived macrophages (BMDMs). **C, left**, Representative immunoblots (*Continued*)

Peli1^{-/-} mice subjected to 4-day hypoxia. The results in Figure 4A demonstrated an upregulation of Peli1 at both the protein and mRNA levels in AMs after 4-day hypoxia exposure. In contrast, Peli1 deficiency reduced protein levels of iNOS (inducible NO synthase) and decreased mRNA levels of *IL-1β* and *IL-6* in AMs. Immunofluorescence staining for mannose receptor c-type 1 (CD206) and F4/80 further indicated that Peli1 deficiency diminished the proportion of M2 macrophages in the lungs of hypoxia-induced mice (Figure S6A). In addition, we assessed the expression of Peli1 protein in M1 (lipopolysaccharides and IFN-γ [interferon γ]) and M2 (IL-4 and IL-13) polarized bone marrow-derived macrophages (BMDMs). As shown in Figure 4B, the protein and mRNA levels of Peli1 were significantly elevated in M1 but not in M2-polarized BMDMs. Furthermore, the mRNA levels of *IL-1β* and *IL-6* were significantly reduced in M1-polarized *Peli1*^{-/-} BMDMs (Figure 4C).

Previous studies have indicated that M1 macrophages play a role in the proliferation of PASMCs in PH.⁸ Given the reduction in pulmonary vascular resistance observed in hypoxia-induced myeloid Peli1-deficiency PH mice, we proceeded to investigate the impact of macrophage Peli1 on the proliferation and migration of mouse PASMCs (mPASMCs). Conditioned medium from *Peli1*^{+/+} and *Peli1*^{-/-} BMDMs was collected and incubated with mPASMCs for 24 hours. Results from Western blot analysis (Figure 4D), wound-healing assay (Figure 4E), and EdU (5-ethynyl-2-deoxyuridine) assay (Figure 4F) showed that conditioned media from *Peli1*^{-/-} M1 polarized BMDMs significantly attenuated the proliferation and migration of mPASMCs, which was consistent with our in vivo findings. Furthermore, adding IL-6 to the conditioned medium from *Peli1*^{-/-} BMDMs mitigated the observed reduction in the proliferation and migration of mPASMCs (Figure S7A and S7B). These data support the hypothesis that Peli1 deficiency reduces the presence of classically activated macrophages and mitigates their proliferative and migratory effects on mPASMCs.

Foxp1 Is Identified as a Target of Peli1 in Macrophages

To further investigate the molecular mechanisms underlying the impact of Peli1 on the progression of PH, quantitative proteomics analysis was conducted on M1

polarized *Peli1*^{+/+} and *Peli1*^{-/-} BMDMs. Significant differences were defined as absolute values of log2-fold change ≥1 in intergroup comparisons. Mass spectrometry identified 2917 proteins, with 13 proteins exhibiting significant upregulation and 8 proteins demonstrating considerable downregulation in *Peli1*^{-/-} BMDMs compared with *Peli1*^{+/+} BMDMs (Figure 5A). Consistent with the quantitative proteomics analysis, the mRNA expression levels of *ARG1* (arginase 1) and *F13A1* (coagulation factor XIII A chain) were significantly decreased in M1 polarized *Peli1*^{-/-} BMDMs relative to *Peli1*^{+/+} BMDMs (Figure S8A). Previous studies have shown that inhibiting M2 macrophages can effectively reduce the proliferation of PASMCs and improve pulmonary vascular remodeling. Given the critical roles of ARG1 and F13A1 in M2 macrophages, our study further explored the impact of Peli1 on M2 macrophages. However, our findings revealed no significant difference in CD206 protein expression between *Peli1*^{+/+} and *Peli1*^{-/-} BMDMs (Figure S8B), suggesting that Peli1 deficiency does not promote M2 polarization in BMDMs.

Foxp1, a well-known transcriptional regulator, plays a crucial role in monocyte differentiation and macrophage function. Western blot analysis demonstrated a significant increase in Foxp1 protein levels in M1-polarized *Peli1*^{-/-} BMDMs compared with *Peli1*^{+/+} BMDMs (Figure 5B). In contrast, the mRNA levels of *Foxp1* were decreased in M1-polarized BMDMs (Figure S9A). Overexpression of Peli1 using an adenoviral vector in BMDMs resulted in a significant reduction in Foxp1 protein expression without affecting *Foxp1* mRNA levels (Figure 5C; Figure S9B). Immunofluorescence staining revealed that Foxp1 predominantly localizes to the nucleus, with no observable differences in subcellular localization between *Peli1*^{+/+} and *Peli1*^{-/-} BMDMs (Figure S9C). Peli1 functions as an E3 ubiquitin ligase that facilitates K48 or K63 ubiquitination of target proteins through substrate recognition via a noncanonical forkhead-associated domain, which is a well-defined phosphothreonine-binding module with significant sequence homology.¹⁸ The amino acid sequence analysis suggests that Foxp1 possesses numerous ubiquitination sites (Table S5) and multiple peptide-binding motifs for forkhead-associated domains (Tables S6 and S7).

To further investigate the role of Peli1 in regulating Foxp1 expression, experiments were conducted utilizing

Figure 4 Continued. and relative densitometric analysis of Peli1 protein expression in *Peli1*^{+/+} and *Peli1*^{-/-} M1 BMDMs; (right) RT-PCR analysis of *IL-1β* and *IL-6* mRNA levels in *Peli1*^{+/+} and *Peli1*^{-/-} M1 BMDMs. **D**, Representative immunoblots and relative densitometric analysis of PCNA (proliferating cell nuclear antigen) protein expression in mPASMCs treated with conditioned medium (CM) from *Peli1*^{+/+} and *Peli1*^{-/-} M1 BMDMs for 24 hours. For **A** through **D**, normalized to β-actin or RPLP0 (ribosomal protein lateral stalk subunit P0), n=5–6 biologically independent samples per group. **E**, mPASMCs were treated with CM from *Peli1*^{+/+} and *Peli1*^{-/-} M1 BMDMs for 16 hours, and images were captured at the indicated time to quantify the relative blank area, scale bar=400 μm. **F**, Representative images and quantification of EdU (5-ethynyl-2-deoxyuridine)⁺ (red) in mPASMCs, mPASMCs were treated with CM from *Peli1*^{+/+} and *Peli1*^{-/-} M1 BMDMs for 24h, and nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; blue), scale bar=100 μm. For **E** and **F**, n=5 biologically independent samples per group. The data are presented as mean±SE; P values were determined by 1-way ANOVA with Tukey post hoc test. TNF indicates tumor necrosis factor. *P<0.05, **P<0.01, and ***P<0.001.

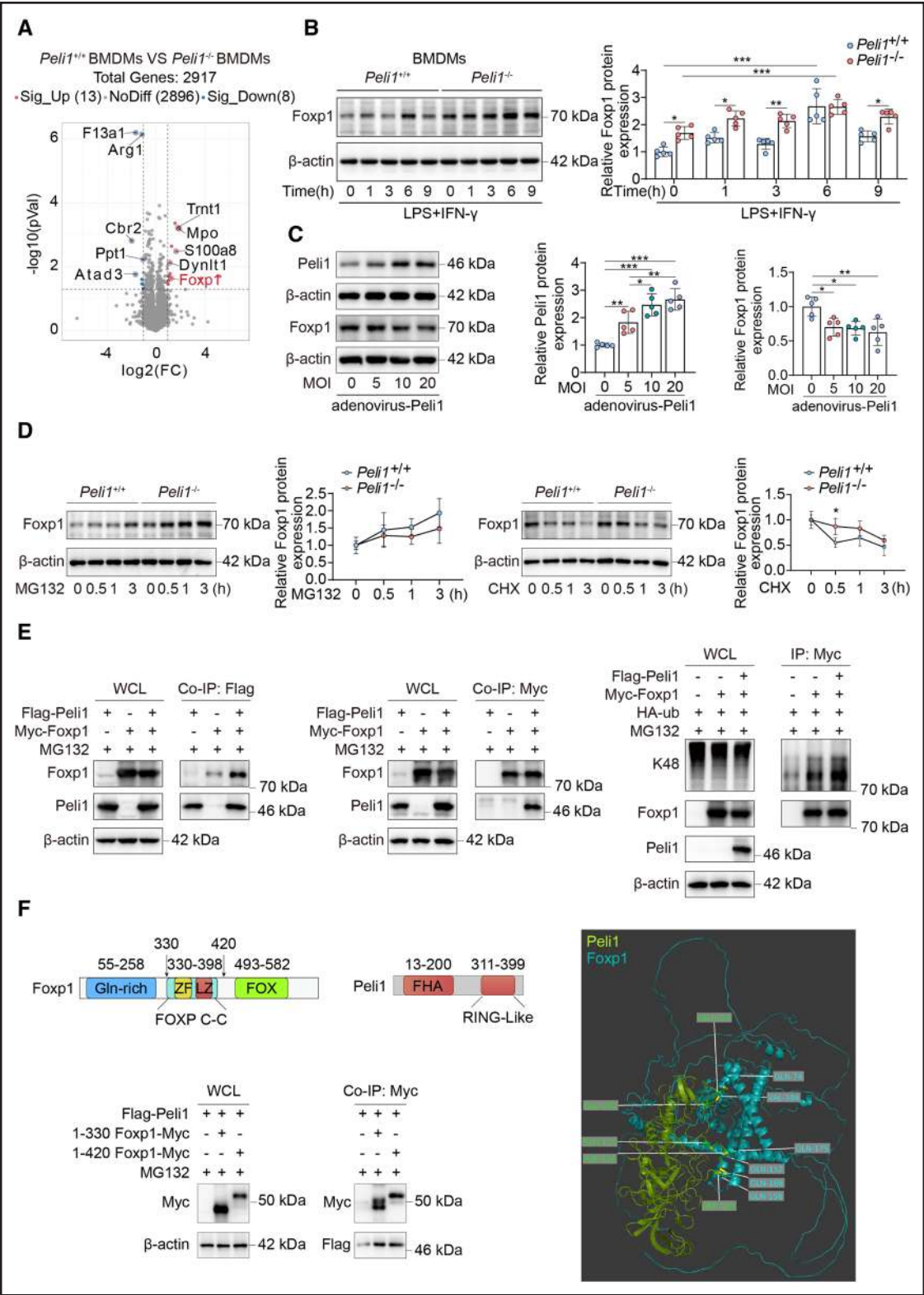


Figure 5. Peli1 (pellino E3 ubiquitin-protein ligase 1) regulates Foxp1 (forkhead box p1) protein expression in macrophages. **A**, The volcano plot depicting the differentially expressed proteins in the *Peli1*^{+/+} and *Peli1*^{-/-} bone marrow–derived macrophages (BMDMs) after lipopolysaccharides (LPS) and IFN (interferon)-γ treated for 6 hours, n=6 mice per group (significance cutoff *P*<0.05). **B**, Representative immunoblots and relative densitometric analysis of Foxp1 expression in *Peli1*^{+/+} and *Peli1*^{-/-} M1 BMDMs. **C**, Representative immunoblots and relative densitometric analysis of Peli1 and Foxp1 expression in BMDMs transduced with increasing amounts of adenovirus-*Peli1* for 24 hours. **D**, Representative immunoblots and relative densitometric analysis of Foxp1 expression in MG132 (Z-Leu-Leu-Leu-al; 5 μM) or cycloheximide (CHX; 5 μM) treated *Peli1*^{+/+} and *Peli1*^{-/-} BMDMs. For **B** through **D**, normalized to β-actin, n=5 biologically independent (Continued)

the proteasome inhibitor MG132 (Z-Leu-Leu-Leu-al) and the protein synthesis inhibitor cycloheximide in both *Peli1*^{+/+} and *Peli1*^{-/-} BMDMs. As shown in Figure 5D, the degradation of Foxp1 probably involves the ubiquitination-mediated proteasomal degradation system, with a more pronounced attenuation observed in *Peli1*^{-/-} BMDMs compared with *Peli1*^{+/+} BMDMs. Furthermore, coimmunoprecipitation and immunoprecipitation assays were performed to confirm the interaction between Peli1 and Foxp1, which promotes the K48 ubiquitination of Foxp1 (Figure 5E). The results from molecular docking and coimmunoprecipitation using the Foxp1 truncated mutant plasmid indicated a potential binding site for Peli1 within the glutamine-rich domain of Foxp1 (Figure 5F). These findings support the hypothesis that Peli1 may facilitate the degradation of Foxp1 in macrophages.

Myeloid Foxp1 Knockdown Partially Eliminates the Protective Effect of Myeloid Peli1 Deficiency in Hypoxia-Induced PH Mice

Chokas et al¹⁹ have provided evidence that Foxp1 functions as a transcriptional inhibitor of *IL-6* in the lungs of embryonic mice by directly binding to the promoter region of *IL-6*. To investigate the role of Foxp1 in mediating *IL-6* transcription in BMDMs, Foxp1 was knocked down using siRNA (small interfering RNA), which was confirmed by Western blot analysis (Figure 6A, left). As anticipated, the knockdown of Foxp1 led to elevated mRNA levels of *IL-6* and *IL-1β* in M1 BMDMs (Figure 6A, middle). In addition, Foxp1 silencing reinstated the previously reduced *IL-6* mRNA levels, whereas *IL-1β* levels remained unchanged in *Peli1*^{-/-} BMDMs (Figure 6A, right).

To elucidate the role of Foxp1 in the lungs of PH models, we assessed the expression of Foxp1 protein in the lungs of 4-day hypoxia-treated mice. Our results indicated no significant alteration in Foxp1 protein expression in the lungs of PH mice (Figure S10A). Immunofluorescence staining revealed extensive expression of Foxp1, with partial colocalization in pulmonary macrophages from hypoxia-induced mice, monocrotaline-induced rats, and patients with idiopathic pulmonary artery hypertension (Figure 6B). Moreover, *Peli1*^{-/-} mice exhibited an increased proportion of Foxp1⁺ pulmonary macrophages compared with *Peli1*^{+/+} mice (Figure S10B). Notably,

Foxp1 was predominantly localized in the nuclei of pulmonary macrophages from both 4-day hypoxia-treated *Peli1*^{+/+} and *Peli1*^{-/-} mice (Figure 6B, left).

To further investigate the impact of Foxp1 deletion in macrophages on PH progression, lentiviral vectors were engineered for Foxp1 knockdown, and the knockdown efficiency was confirmed in H293T cells and BMDMs (Figure S11A and S11B). As shown in Figure 6C, bone marrow transplant was performed to generate Mac (*Peli1*^{+/+}) and Mac (*Peli1*^{-/-}) mice with or without Foxp1 deficiency in myeloid cells. The mice were subjected to hypoxia treatment for 28 days to induce PH. Immunofluorescence staining for EGFP (enhanced green fluorescent protein) and Foxp1 was conducted to validate the successful transduction of the lentivirus and to confirm the reduced expression of Foxp1 in the pulmonary macrophages of hypoxia-induced bone marrow transplant mice (Figure S12A through S12C). As shown in Figure 6D, RVSP and the ratio of RV to left ventricle plus septum were significantly lower in Mac (*Peli1*^{-/-}) mice compared with Mac (*Peli1*^{+/+}) mice, which is consistent with previous findings. Furthermore, the deficiency of myeloid Foxp1 further elevated RVSP and reversed the ratio of RV to left ventricle plus septum in Mac (*Peli1*^{-/-}) mice. However, histological analysis indicated that myeloid Foxp1 deficiency did not reverse the reduced occlusion and muscularization of distal pulmonary arterioles in hypoxia-induced Mac (*Peli1*^{-/-}) mice (Figure 6E). These findings suggest that Peli1 may mediate macrophage activation, partially through the Foxp1/*IL-6* pathway, in the development of PH (Figure 6F).

DISCUSSION

The infiltration and activation of perivascular macrophages play a crucial role in driving pulmonary artery remodeling.^{20,21} When exposed to different stimuli, monocytes/macrophages can be recruited and retained in the local tissue microenvironment, activating to exhibit either proinflammatory or proremodeling phenotypes.^{2,22,23} The heightened expression of cytokines (*IL-1β*, *IL-6*, leukotriene B₄), chemokines (CCL [chemokine (C-C motif) ligand] 2, CCL5), and enzymes (iNOS, matrix metalloproteinases-10) by M1 macrophages have been shown to induce damage and confer apoptosis resistance

Figure 5 Continued. samples per group. **E, left and middle,** The interaction between Peli1 and Foxp1 was detected by Co-IP (co-immunoprecipitation) assay. H293T cells were cotransfected with flag-tagged Peli1 and myc-tagged Foxp1 plasmids. The cells were harvested and subjected to immunoprecipitation with anti-Flag and anti-Myc antibodies, respectively. **Right,** Western blot analysis of K48-linked polyubiquitination of Foxp1 immunoprecipitated from H293T cells co-overexpressing Peli1 and Foxp1. **F, top,** Schematic representation of the structural domains of Foxp1 and Peli1 proteins: glutamine-rich (Gln-rich) domain, zinc finger (ZF) domain, leucine zipper (LZ) domain, forkhead box DNA-binding domain (FOX), FOXP coiled-coil (FOXP C-C) domain, forkhead-associated (FHA) domains. **Right,** The docking simulation explored the potential interaction between Peli1 (green) and Foxp1 (blue); the docking results suggest a potential interaction between Peli1 and Foxp1, with specific residues implicated in the binding interface. **Bottom,** Co-IP analysis of Foxp1 mutants binding to Peli1. H293T cells were cotransfected with Foxp1 deletion mutants (myc-tagged 1-330 Foxp1, or myc-tagged 1-420 Foxp1) and flag-tagged Peli1 plasmids, and Co-IP analysis was performed with anti-Myc antibody. The data are presented as mean±SE; *P* values were determined by 1-way ANOVA with Tukey post hoc test; **P*<0.05, ***P*<0.01, and ****P*<0.001. HA-ub indicates HA tagged ubiquitin plasmids; MOI, multiplicity of infection; Myc, MYC tag; RING, really interesting new gene; and WCL, whole cell lysate.

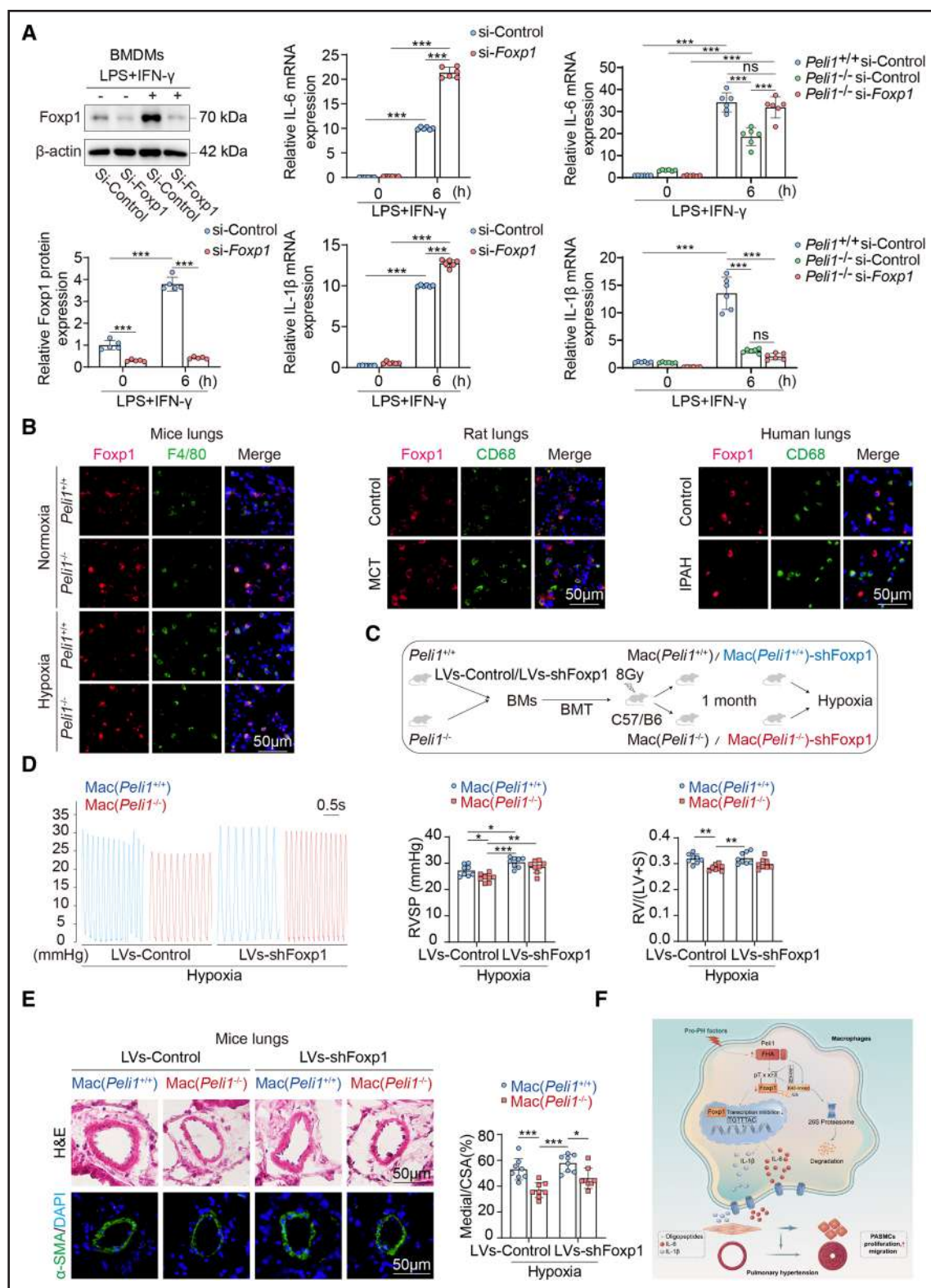


Figure 6. The protective effect of myeloid *Peli1* (pellino E3 ubiquitin-protein ligase 1) knockdown on hypoxia-induced pulmonary hypertension (PH) in mice is partially attenuated by myeloid *Foxp1* (forkhead box p1) knockdown.

A, left, Representative immunoblots and relative densitometric analysis of *Foxp1* expression, (**middle and right**) relative mRNA levels of *IL-6* (interleukin) 6, and *IL-1β* in *Peli1*^{+/+} and *Peli1*^{-/-} M1 bone marrow (BM)-derived macrophages (BMDMs) transfected with or without *Foxp1* small interfering RNA (siRNA), normalized to β-actin, n=5 or 6 biologically independent samples per group. **B**, Immunofluorescence (IF) staining for *Foxp1* (red) in the lungs of normoxia- or hypoxia-induced *Peli1*^{+/+} and *Peli1*^{-/-} mice (**left**), in control and MCT (monocrotaline) rats (**middle**), and in non-idiopathic pulmonary artery hypertension (IPAH) individuals and patients with IPAH (**right**). Pulmonary macrophages (Continued)

in PAECs, as well as stimulate the proliferation, migration, and dedifferentiation of PSMCs. These processes ultimately lead to vasomotor dysfunction, vascular remodeling, and RV remodeling.^{7,24–26} Inhibition of macrophage recruitment and activation, as well as the blockade of macrophage products, has been demonstrated to mitigate or reverse the progression of PH.^{4,9,27} Nonetheless, macrophages play a pivotal role in innate immunity, and broad-spectrum interventions targeting these cells may pose inherent risks. Moreover, AMs and IMs exhibit notable heterogeneity across various stages of PH. Therefore, a comprehensive examination of the distinct phenotypic characteristics and regulatory mechanisms of AMs and IMs throughout the progression of PH is imperative for developing innovative, targeted therapeutic modalities. In this study, our results demonstrated that a 4-day hypoxia exposure significantly increased the infiltration of perivascular macrophages in the lungs of mice and upregulated the expression of M1 polarization-related genes (iNOS, IL-1 β , IL-6) in AMs, consistent with previous studies.^{9,28}

Peli1 plays a crucial role in facilitating the polyubiquitination of various substrate proteins. In myocardial ischemia/reperfusion, Peli1 interacts with P62 via its RING (really interesting new gene)-like domain to promote K63-linked ubiquitination of P62 at lysine (K) 7. This modification inhibits P62 dimerization and autophagic degradation, thereby disrupting autophagic flux and ultimately exacerbating cardiomyocyte death.²⁹ In addition, Peli1 is involved in the degradation of HNF4 α (hepatocyte nuclear factor 4 α) through ubiquitination at residues K307 and K309, which impairs fatty acid oxidation in cardiomyocytes and accelerates myocardial hypertrophy induced by pressure overload.³⁰ Silencing Peli1 in macrophages has been shown to suppress M1 polarization of macrophages and attenuate myocardial ischemia/reperfusion injury.³¹ Meanwhile, the nuclear translocation of Peli1 and IRF5 promotes the M1 polarization of AMs, thereby aggravating acute lung injury in mice.¹¹ Our results demonstrate that Peli1 expression is increased in the lungs of 4-day hypoxia-induced PH mice, in 5-day monocrotaline-treated rats, and the peripheral blood mononuclear cells of patients with idiopathic pulmonary artery hypertension. Immunofluorescence staining demonstrated significant enrichment of Peli1 in

pulmonary macrophages. The expression pattern of Peli1 may correlate with the time course of macrophage infiltration in the disease progress of PH. Genetic depletion of Peli1 deactivates pulmonary macrophages, reduces the number of perivascular macrophages, weakens distal pulmonary vascular muscularization, and alleviates hypoxia-induced PH in mice. These suggest that Peli1 may serve as a valuable biomarker for the early detection of PH and remains elevated throughout the progression of the disease. The early application of Peli1-targeted therapies could reduce ongoing inflammation and limit further vascular remodeling. Because the activation of inflammatory macrophages is involved during the progression of PH, it is speculated that the inhibition of Peli1 could also benefit patients with established PH.

This research indicates that Peli1 facilitates M1 polarization of macrophages, partially dependent on the Foxp1/IL-6 axis. Foxp1, a forkhead box P transcription factor family member, contains forkhead DNA-binding and protein-protein interaction domains. It is predominantly localized in the nucleus and exhibits high expression levels in the spleen, thymus, lung, and brain.^{32,33} By interacting with the forkhead-binding motif (TGTTTAC), Foxp1 can specifically inhibit the transcription of target genes.^{34,35} Numerous studies have demonstrated that the downregulation of Foxp1 modulates the differentiation of monocytes into macrophages and influences macrophage function.^{36–38} Increasing Foxp1 expression in macrophages may represent a novel strategy for anti-inflammatory intervention.^{39–41} Our research demonstrates that Peli1 can modulate the protein stability of Foxp1 in macrophages without affecting its mRNA levels or subcellular localization. Depletion of Peli1 in BMDMs resulted in elevated Foxp1 expression, whereas overexpression of Peli1 led to decreased Foxp1 levels. The regulatory mechanism by which Peli1 influences Foxp1 expression may involve K48-linked ubiquitination and proteasome-mediated degradation.

Patients with PH exhibit elevated levels of the pro-inflammatory cytokine IL-6 in their serum and lung tissues.^{42,43} As a nonclassical activator of M2 polarization, IL-6 can induce the expression of CD206, a well-established marker of M2 macrophages. Elevated IL-6 levels have been shown to promote various physiological

Figure 6 Continued. were identified using F4-80 (green) or CD (cluster of differentiation) 68 (green) staining. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI), scale bar=50 μ m. **C**, Schematic presentation of the experimental protocol for BM transplant (BMT) mice in the hypoxia-induced PH model. BM cells from *Peli1*^{+/+} and *Peli1*^{-/-} mice were transfected with lentiviruses control (LVs-Con) or LVs-sh (short hairpin) Foxp1, respectively. These transfected BM cells were then transplanted into recipient mice, which were subsequently exposed to normoxic conditions for 1 month before being subjected to hypoxia (10% O₂) for 4 weeks. **D, left and middle**, Representative images and quantification of the right ventricular systolic pressure (RVSP; **right**) ratio RV to left ventricular (LV) wall plus septum (RV/[LV+S]) ratio in BMT mice after 4 weeks of hypoxia treatment. **E**, Representative hematoxylin and eosin (H&E) and IF staining for α -SMA (smooth muscle actin; green) in the lung sections and nuclei were counterstained with DAPI (blue), scale bar=50 μ m. The media/cross-sectional area (CSA; %) was quantified in each pulmonary artery. For **C** through **E**, n=8 mice per group. **F**, Schematic diagram of Peli1 in macrophages promoting pulmonary vascular remodeling during PH pathogenesis. Forkhead-associated (FHA) domain-binding motifs=pTx+3 (pT, phosphorylated threonine residue; x, non-specific amino acids; +3, the third amino acid after pT.); Forkhead-binding motif=TGTTTAC. The data are presented as mean \pm SE; *P* values were determined by 1-way ANOVA with Tukey post hoc test.**P*<0.05, ***P*<0.01, and ****P*<0.001. A indicates alanine; L, leucine; M, methionine; Mac, macrophage reconstruction; PSMC, pulmonary artery smooth muscle cells; S, serine; and T, threonine.

responses, including neutrophil egress from the bone marrow, proliferation of endothelial cells,⁴⁴ chemokine production,⁴⁵ and proliferation of PSMCs.²⁵ Transgenic mice that overexpress IL-6 develop spontaneous PH and exhibit exacerbated hypoxia-induced PH. Inhibition of IL-6 through genetic depletion or the use of neutralizing antibodies in mice has been found to suppress the expression of genes associated with M2 polarization in pulmonary macrophages, such as hypoxia-induced mitogenic factor, ARG1, chitinase 3-like 3, CD206, and C-X-C motif chemokine ligand 12, thereby protecting mice from hypoxia-induced PH. However, Toshner et al⁴⁶ reported that tocilizumab, an IL-6-specific antibody, did not demonstrate a consistent therapeutic effect in patients with PH. In contrast, Yaku et al⁴⁷ found that regnase-1 plays a role in maintaining lung innate immune homeostasis by degrading *IL-6* and *platelet-derived growth factor* mRNA in AMs, thereby suppressing the development of hypoxia-induced PH in mice. Consequently, future research may prioritize cell-specific targeted interventions for IL-6 expression. In addition, Foxp1 has been shown to inhibit the transcription of IL-6 in embryonic mouse lungs.¹⁹ The present study provides further evidence supporting the inhibitory effect of Foxp1 on *IL-6* transcription in macrophages, leading to reduced proliferation and migration of PSMCs.

There are several limitations to this study. First, it remains to be determined whether Peli1 interacts with Foxp1 through the RING-like domain and which specific lysine residues of Foxp1 are targeted for ubiquitination by Peli1. Second, silencing of Foxp1 did not restore the reduced *IL-1 β* mRNA levels in *Peli1*^{-/-} BMDMs, and the knockdown of myeloid Foxp1 was unable to reverse the improved pulmonary vascular remodeling observed in hypoxia-induced Mac (*Peli1*^{-/-}) mice. These findings suggest that Peli1 may regulate PH progression through additional signaling pathways, which require further investigation.

In conclusion, our study indicates that Peli1 plays a role in the development of PH by facilitating the recruitment and activation of macrophages through the attenuation of Foxp1-mediated transcriptional inhibition of *IL-6*. This finding suggests that targeting Peli1 in macrophages may represent a promising therapeutic strategy for PH.

PERSPECTIVES

The findings in this study are an exciting advance in our understanding of the mechanisms underlying E3 ubiquitin ligase Peli1 in macrophages and PH pathogenesis and provide new insight into Peli1 on the cellular and molecular regulatory mechanisms of pulmonary vascular remodeling in PH. Our results showed that the Peli1-Foxp1-IL-6 pathway plays a crucial role in macrophage

activation and recruitment in the development of PH, and targeting this pathway may be an effective therapeutic approach to improve macrophage polarization and vascular remodeling.

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Disclosures

None.

Supplemental Material

Supplemental Methods
Tables S1–S7
Figures S1–S13

REFERENCES

- Wang L, Zhang X, Cao Y, Ma Q, Mao X, Xu J, Yang Q, Zhou Y, Lucas R, Fulton DJ, et al. Mice with a specific deficiency of Pfkfb3 in myeloid cells are protected from hypoxia-induced pulmonary hypertension. *Br J Pharmacol*. 2021;178:1055–1072. doi: 10.1111/bph.15339
- Kumar S, Frid MG, Zhang H, Li M, Riddle S, Brown RD, Yadav SC, Roy MK, Dzieciatkowska ME, D'Alessandro A, et al. Complement-containing small extracellular vesicles from adventitial fibroblasts induce proinflammatory and metabolic reprogramming in macrophages. *JCI Insight*. 2021;6:1–17. doi: 10.1172/jci.insight.148382
- Amsellem V, Lipskaia L, Abid S, Poupel L, Houssaini A, Quarck R, Marcos E, Mouraret N, Parpaleix A, Bobe R, et al. CCR5 as a treatment target in pulmonary arterial hypertension. *Circulation*. 2014;130:880–891. doi: 10.1161/CIRCULATIONAHA.114.010757
- Vergadi E, Chang MS, Lee C, Liang OD, Liu X, Fernandez-Gonzalez A, Mitsialis SA, Kourembanas S. Early macrophage recruitment and alternative activation are critical for the later development of hypoxia-induced pulmonary hypertension. *Circulation*. 2011;123:1986–1995. doi: 10.1161/CIRCULATIONAHA.110.978627
- Fan Y, Hao Y, Gao D, Li G, Zhang Z. Phenotype and function of macrophage polarization in monocrotaline-induced pulmonary arterial hypertension rat model. *Physiol Res*. 2021;70:213–226. doi: 10.33549/physiolres.934456
- Liu G, Zhai H, Zhang T, Li S, Li N, Chen J, Gu M, Qin Z, Liu X. New therapeutic strategies for IPF: Based on the "phagocytosis-secretion-immunization"

network regulation mechanism of pulmonary macrophages. *Biomed Pharmacother*. 2019;118:109230. doi: 10.1016/j.biopha.2019.109230

7. Tian W, Jiang X, Tamosiuniene R, Sung YK, Qian J, Dhillion G, Gera L, Farkas L, Rabinovitch M, Zamanian RT, et al. Blocking macrophage leukotriene b4 prevents endothelial injury and reverses pulmonary hypertension. *Sci Transl Med*. 2013;5:200ra117. doi: 10.1126/scitranslmed.3006674
8. Abid S, Marcos E, Parpaleix A, Amsellem V, Breau M, Houssaini A, Vienney N, Lefevre M, Derumeaux G, Evans S, et al. CCR2/CCR5-mediated macrophage-smooth muscle cell crosstalk in pulmonary hypertension. *Eur Respir J*. 2019;54:1802308. doi: 10.1183/13993003.02308-2018
9. Hudalla H, Michael Z, Christodoulou N, Willis GR, Fernandez-Gonzalez A, Filatava EJ, Dieffenbach P, Fredenburgh LE, Stearman RS, Geraci MW, et al. Carbonic anhydrase inhibition ameliorates inflammation and experimental pulmonary hypertension. *Am J Respir Cell Mol Biol*. 2019;61:512–524. doi: 10.1165/rcmb.2018-0232OC
10. Xiao Y, Jin J, Chang M, Chang JH, Hu H, Zhou X, Brittain GC, Stansberg C, Torkildsen O, Wang X, et al. Peli1 promotes microglia-mediated CNS inflammation by regulating Traf3 degradation. *Nat Med*. 2013;19:595–602. doi: 10.1038/nm.3111
11. Wang Y, Wang X, Zhang H, Han B, Ye Y, Zhang M, Wang Y, Xue J, Wang C. Transforming growth factor-beta1 promotes M1 alveolar macrophage polarization in acute lung injury by up-regulating DNMT1 to mediate the microRNA-124/Peli1/IRF5 axis. *Front Cell Infect Microbiol*. 2021;11:693981. doi: 10.3389/fcimb.2021.693981
12. Wu W, Hu Y, Li J, Zhu W, Ha T, Que L, Liu L, Zhu Q, Chen Q, Xu Y, et al. Silencing of pellino1 improves post-infarct cardiac dysfunction and attenuates left ventricular remodeling in mice. *Cardiovasc Res*. 2014;102:46–55. doi: 10.1093/cvr/cvu007
13. Kim D, Lee H, Koh J, Ko JS, Yoon BR, Jeon YK, Cho YM, Kim TH, Suh YS, Lee HJ, et al. Cytosolic pellino-1-mediated K63-linked ubiquitination of IRF5 in M1 macrophages regulates glucose intolerance in obesity. *Cell Rep*. 2017;20:832–845. doi: 10.1016/j.celrep.2017.06.088
14. Zhao Q, Yang J, Chen H, Li J, Que L, Zhu G, Liu L, Ha T, Chen Q, Li C, et al. Peli1 induction impairs cardiac microvascular endothelium through Hsp90 dissociation from IRE1alpha. *Biochim Biophys Acta Mol Basis Dis*. 2019;1865:2606–2617. doi: 10.1016/j.bbadis.2019.06.017
15. Hu L, Wang J, Huang H, Yu Y, Ding J, Yu Y, Li K, Wei D, Ye Q, Wang F, et al. YTHDF1 regulates pulmonary hypertension through translational control of MAGED1. *Am J Respir Crit Care Med*. 2021;203:1158–1172. doi: 10.1164/rccm.202009-3419OC
16. Hu L, Yu Y, Shen Y, Huang H, Lin D, Wang K, Yu Y, Li K, Cao Y, Wang Q, et al. Ythdf2 promotes pulmonary hypertension by suppressing Hmox1-dependent anti-inflammatory and antioxidant function in alveolar macrophages. *Redox Biol*. 2023;61:102638. doi: 10.1016/j.redox.2023.102638
17. Wang J, Shen Y, Zhang Y, Lin D, Wang Q, Sun X, Wei D, Shen B, Chen J, Ji Y, et al. Smooth muscle Ythdf2 abrogation ameliorates pulmonary vascular remodeling by regulating myadm transcript stability. *Hypertension*. 2024;81:1785–1798. doi: 10.1161/HYPERTENSIONAHA.124.22801
18. Huoh YS, Ferguson KM. The pellino e3 ubiquitin ligases recognize specific phosphothreonine motifs and have distinct substrate specificities. *Biochemistry*. 2014;53:4946–4955. doi: 10.1021/bi5005156
19. Chokas AL, Bickford JS, Barilovits SJ, Rogers RJ, Qiu X, Newsom KJ, Beachy DE, Nick HS. A TEAD1/p65 complex regulates the eutherian-conserved MnSOD intronic enhancer, eRNA transcription and the innate immune response. *Biochim Biophys Acta*. 2014;1839:1205–1216. doi: 10.1016/j.bbaggm.2014.06.012
20. Florentin J, Coppin E, Vasamsetti SB, Zhao J, Tai YY, Tang Y, Zhang Y, Watson A, Sembrat J, Rojas M, et al. Inflammatory macrophage expansion in pulmonary hypertension depends upon mobilization of blood-borne monocytes. *J Immunol*. 2018;200:3612–3625. doi: 10.4049/jimmunol.1701287
21. Hu Y, Chi L, Kuebler WM, Goldenberg NM. Perivascular inflammation in pulmonary arterial hypertension. *Cells*. 2020;9:2338. doi: 10.3390/cells9112338
22. El Kasbi KC, Pugliese SC, Riddle SR, Poth JM, Anderson AL, Frid MG, Li M, Pullamsetti SS, Savai R, Nagel MA, et al. Adventitial fibroblasts induce a distinct proinflammatory/profibrotic macrophage phenotype in pulmonary hypertension. *J Immunol*. 2014;193:597–609. doi: 10.4049/jimmunol.1303048
23. Li M, Riddle S, Kumar S, Pocobutt J, McKeon BA, Frid MG, Ostaff M, Reisz JA, Nemkov T, Fini MA, et al. Microenvironmental regulation of macrophage transcriptomic and metabolomic profiles in pulmonary hypertension. *Front Immunol*. 2021;12:640718. doi: 10.3389/fimmu.2021.640718
24. Ou M, Zhang C, Chen J, Zhao S, Cui S, Tu J. Overexpression of microRNA-340-5p inhibits pulmonary arterial hypertension induced by APE by downregulating IL-1beta and IL-6. *Mol Ther Nucleic Acids*. 2020;21:542–554. doi: 10.1016/j.omtn.2020.05.022
25. Batoon M, Berghausen EM, Zierden M, Vantler M, Schermuly RT, Baldus S, Rosenkranz S, Ten Freyhaus H. The six-transmembrane protein Stamp2 ameliorates pulmonary vascular remodeling and pulmonary hypertension in mice. *Basic Res Cardiol*. 2020;115:68. doi: 10.1007/s00395-020-00826-8
26. Chi PL, Cheng CC, Hung CC, Wang MT, Liu HY, Ke MW, Shen MC, Lin KC, Kuo SH, Hsieh PP, et al. MMP-10 from M1 macrophages promotes pulmonary vascular remodeling and pulmonary arterial hypertension. *Int J Biol Sci*. 2022;18:331–348. doi: 10.7150/ijbs.66472
27. Ntokou A, Dave JM, Kauffman AC, Sauler M, Ryu C, Hwa J, Herzog EL, Singh I, Saltzman WM, Greif DM. Macrophage-derived PDGF-B induces muscularization in murine and human pulmonary hypertension. *JCI Insight*. 2021;6:e139067. doi: 10.1172/jci.insight.139067
28. Pugliese SC, Kumar S, Janssen WJ, Graham BB, Frid MG, Riddle SR, El Kasbi KC, Stenmark KR. A time- and compartment-specific activation of lung macrophages in hypoxic pulmonary hypertension. *J Immunol*. 2017;198:4802–4812. doi: 10.4049/jimmunol.1601692
29. Yang J, Tong T, Zhu C, Zhou M, Jiang Y, Chen H, Que L, Liu L, Zhu G, Ha T, et al. Peli1 contributes to myocardial ischemia/reperfusion injury by impairing autophagy flux via its E3 ligase mediated ubiquitination of P62. *J Mol Cell Cardiol*. 2022;173:30–46. doi: 10.1016/j.jmcc.2022.09.004
30. Hou Y, Shi P, Du H, Zhu C, Tang C, Tang C, Qye J, Qye J, Liu L, Chen Q, Li C, et al. HNF4alpha ubiquitination mediated by Peli1 impairs FAO and accelerates pressure overload-induced myocardial hypertrophy. *Cell Death Dis*. 2024;15:135. doi: 10.1038/s41419-024-06470-7
31. Chen H, Hou Y, Zhai Y, Yang J, Que L, Liu J, Lu L, Ha T, Li C, Xu Y, et al. Peli1 deletion in macrophages attenuates myocardial ischemia/reperfusion injury by suppressing M1 polarization. *J Leukoc Biol*. 2023;113:95–108. doi: 10.1093/leuko/qjac012
32. Santos ME, Athanasiadis A, Leitao AB, DuPasquier L, Sucena E. Alternative splicing and gene duplication in the evolution of the FoxP gene subfamily. *Mol Biol Evol*. 2011;28:237–247. doi: 10.1093/molbev/msq182
33. Sollis E, Graham SA, Vino A, Froehlich H, Vreeburg M, Dimitropoulou D, Glissen C, Pfundt R, Rappold GA, Brunner HG, et al. Identification and functional characterization of de novo FOXP1 variants provides novel insights into the etiology of neurodevelopmental disorder. *Hum Mol Genet*. 2016;25:546–557. doi: 10.1093/hmg/ddv495
34. Shu W, Yang H, Zhang L, Lu MM, Morrissey EE. Characterization of a new subfamily of winged-helix/forkhead (Fox) genes that are expressed in the lung and act as transcriptional repressors. *J Biol Chem*. 2001;276:27488–27497. doi: 10.1074/jbc.M100636200
35. Chokas AL, Trivedi CM, Lu MM, Tucker PW, Li S, Epstein JA, Morrissey EE. Foxp1/2/4-NuRD interactions regulate gene expression and epithelial injury response in the lung via regulation of interleukin-6. *J Biol Chem*. 2010;285:13304–13313. doi: 10.1074/jbc.M109.088468
36. Shi C, Sakuma M, Mooroka T, Liscio A, Gao H, Croce KJ, Sharma A, Kaplan D, Greaves DR, Wang Y, et al. Down-regulation of the forkhead transcription factor Foxp1 is required for monocyte differentiation and macrophage function. *Blood*. 2008;112:4699–4711. doi: 10.1182/blood-2008-01-137018
37. Song Y, Li X, Zeng Z, Li Q, Gong Z, Liao Q, Li X, Chen P, Xiang B, Zhang W, et al. Epstein-Barr virus encoded miR-BART11 promotes inflammation-induced carcinogenesis by targeting FOXP1. *Oncotarget*. 2016;7:36783–36799. doi: 10.18632/oncotarget.9170
38. Mulvaney EP, O'Sullivan AG, Eivers SB, Reid HM, Kinsella BT. Differential expression of the TPalpha and TPbeta isoforms of the human T Prostanoid receptor during chronic inflammation of the prostate: Role for FOXP1 in the transcriptional regulation of TPbeta during monocyte-macrophage differentiation. *Exp Mol Pathol*. 2019;110:104277. doi: 10.1016/j.yexmp.2019.104277
39. Shi C, Miley J, Nottingham A, Morooka T, Prosdocimo DA, Simon DI. Leukocyte integrin signaling regulates FOXP1 gene expression via FOXP1-IT1 long non-coding RNA-mediated IRAK1 pathway. *Biochim Biophys Acta Gene Regul Mech*. 2019;1862:493–508. doi: 10.1016/j.bbaggm.2019.02.008
40. Gao F, Zhao Y, Zhang B, Xiao C, Sun Z, Gao Y, Dou X. Forkhead box protein 1 transcriptionally activates sestrin1 to alleviate oxidized low-density lipoprotein-induced inflammation and lipid accumulation in macrophages. *Bioengineered*. 2022;13:2917–2926. doi: 10.1080/21655979.2021.2000228
41. Hu J, Liu X, Tang Y. HMGB1/Foxp1 regulates hypoxia-induced inflammatory response in macrophages. *Cell Biol Int*. 2022;46:265–277. doi: 10.1002/cbin.11728

42. Steiner MK, Syrkina OL, Kolliputi N, Mark EJ, Hales CA, Waxman AB. Interleukin-6 overexpression induces pulmonary hypertension. *Circ Res*. 2009;104:236–44, 28p following 244. doi: 10.1161/CIRCRESAHA.108.182014
43. Simpson CE, Chen JY, Damico RL, Hassoun PM, Martin LJ, Yang J, Nies M, Griffiths M, Vaidya RD, Brandal S, et al. Cellular sources of interleukin-6 and associations with clinical phenotypes and outcomes in pulmonary arterial hypertension. *Eur Respir J*. 2020;55:1901761. doi: 10.1183/13993003.01761-2019
44. Florentin J, Zhao J, Tai YY, Vasamsetti SB, O'Neil SP, Kumar R, Arunkumar A, Watson A, Sembrat J, Bullock GC, et al. Interleukin-6 mediates neutrophil mobilization from bone marrow in pulmonary hypertension. *Cell Mol Immunol*. 2021;18:374–384. doi: 10.1038/s41423-020-00608-1
45. Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ Res*. 2014;115:165–175. doi: 10.1161/CIRCRESAHA.113.301141
46. Toshner M, Church C, Harbaum L, Rhodes C, Villar Moreschi SS, Liley J, Jones R, Arora A, Batai K, Desai AA, et al; Uniphy Clinical Trials Network. Mendelian randomisation and experimental medicine approaches to interleukin-6 as a drug target in pulmonary arterial hypertension. *Eur Respir J*. 2022;59:2002463. doi: 10.1183/13993003.02463-2020
47. Yaku A, Inagaki T, Asano R, Okazawa M, Mori H, Sato A, Hia F, Masaki T, Manabe Y, Ishibashi T, et al. Regnase-1 prevents pulmonary arterial hypertension through mRNA degradation of interleukin-6 and platelet-derived growth factor in alveolar macrophages. *Circulation*. 2022;146:1006–1022. doi: 10.1161/CIRCULATIONAHA.122.059435