



PFN1 Knockdown Aggravates Mitophagy to Retard Lung Adenocarcinoma Initiation and M2 Macrophage Polarization

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Abstract

Tumor-associated macrophages (TAM) are considered as crucial influencing factors of lung adenocarcinoma (LUAD) carcinogenesis and metastasis. Profilin 1 (PFN1) has been proposed as a potent driver of migration and drug resistance in LUAD. The focus of this work was to figure out the functional mechanism of PFN1 in macrophage polarization in LUAD. PFN1 expression and its significance in patients' survival were detected by ENCORI and Kaplan–Meier Plotter. RT-qPCR and western blotting examined PFN1 expression in LUAD cells. CCK-8 assay and colony formation assay detected cell proliferation. Flow cytometry detected cell apoptosis. Relevant assay kit tested caspase3 concentration. Western blotting analyzed the expression of proliferation- and apoptosis-related proteins. RT-qPCR and immunofluorescence staining measured M1 and M2 macrophages markers. Mitophagy was assessed by MitoTracker Red staining, immunofluorescence staining, and western blotting. PFN1 expression was increased in LUAD tissues and cells and correlated with the poor survival rate of LUAD patients. Deficiency of PFN1 hindered the proliferation, whereas facilitated the apoptosis of LUAD cells. Additionally, PFN1 interference impaired M2 macrophage polarization. Moreover, PFN1 knockdown exacerbated the mitophagy in LUAD cells and mitophagy inhibitor mitochondrial division inhibitor 1 (Mdivi-1) notably reversed the effects of PFN1 down-regulation on the proliferation, apoptosis as well as macrophage polarization in LUAD cells. To sum up, activation of mitophagy initiated by PFN1 depletion might obstruct the occurrence and M2 macrophage polarization in LUAD.

Keywords Apoptosis · Lung adenocarcinoma · Macrophage polarization · Mitophagy · PFN1

Introduction

Lung cancer represents a prevalent form of neoplasia of the respiratory system worldwide because of its highest incidence and fatality rate among all human malignancies [1]. Non-small cell lung cancer is a predominant class of lung cancer, accounting for approximately 85% of all lung cancer cases [2], with lung adenocarcinoma (LUAD) being the most frequently occurring histologic type [3]. Admittedly, in addition to the conventional treatment approaches including

surgery, radiation, and chemotherapy [4], the emerging targeted, individualized therapy or immunotherapy have achieved certain efficiency in treating patients with LUAD [5, 6]. The outcome of LUAD patients at advanced stages is suboptimal and the life expectancy of LUAD patients is severely impaired, ascribed to high propensity for relapse and metastasis [7]. In terms of epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer, exceptional progresses that achieved have significantly impacted patients' survival [8, 9]. However, the unavoidable side effects should be taken into account [10]. Thence, exploring the underlying action mechanism of LUAD and developing novel therapeutic targets are of utmost urgency.

Profilin (PFN) family of actin-binding proteins are key regulators in actin assembly at the leading edge of migrating cells [11]. Profilin 1 (PFN1) is a small evolutionarily conserved ubiquitous actin-binding protein present in all eukaryotes, which is engaged in various cellular biological behaviors, such as proliferation, survival, motility, endocytosis, membrane trafficking, via interaction with actin and

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proline-rich-motif (PRM) proteins [12]. Abundant evidence has supported that PFN1 is abnormally expressed in various cancers and diseases and modulates disease development [11–13]. Importantly, PFN1 has been proposed to be overexpressed in lung cancer and drive tumor metastasis and drug resistance [14, 15].

As a well-studied type of cargo-specific autophagy, mitophagy is responsible for the elimination of senescent and dysfunctional mitochondria to maintain cellular energy homeostasis and functions in response to multiple stress conditions including nutritional deficiency, hypoxia, DNA damage, and inflammation [16]. It has been demonstrated that mitophagy is implicated in a wide range of human disorders and malignancies via sensing different extracellular signals [17–19]. Moreover, it is worth noting that inactivation of mitophagy may protect against LUAD tumorigenesis [20].

Accordingly, the aim of this work was to figure out the functional mechanism of PFN1 associated with mitophagy in LUAD.

Materials and Methods

Bioinformatics Tools

PFN1 expression and its significance in LUAD patients' survival were detected by ENCORI (<https://rnasysu.com/encori/>) and Kaplan–Meier Plotter (<https://kmplot.com/analysis/>).

Cell Culture, Treatment, and Transfection

Human bronchial epithelial cell line BEAS-2B, LUAD cell lines (HCC827, PC-9, A549, and NCI-H1975) and human peripheral blood monocytes THP-1 were all sourced from Typical Culture Preservation Commission Cell Bank, Chinese Academy of Sciences (Shanghai, China). Bronchial epithelial growth medium (BEGM; Lonza, Walkersville, MD, USA) was to cultivate BEAS-2B cells. All LUAD cells and THP-1 cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (Biosera, France) containing 10% fetal bovine serum (FBS; NQBB, Australia) while A549 cell line was fostered in F-12 K medium (Biosera, France) at 37 °C in a 5% (v/v) CO₂ atmosphere. Besides, A549 cells were pretreated by 10 μM mitochondrial division inhibitor 1 (Mdivi-1) [21].

The short hairpin RNA (shRNA) lentiviral particles against PFN1 constructed by Biosettia Inc (San Diego, CA, USA) were transfected into A549 cells using Lipofectamine 3000 Transfection Reagent (Thermo Fisher Scientific, USA).

In addition, THP-1 cells that treated with 100 ng/ml phorbol 12-myristate 13-acetate (PMA) for 24 h differentiated into M0 macrophages. Subsequently, THP-1 cells were

exposed to 10 pg/ml lipopolysaccharide (LPS; Sigma, USA) and 20 ng/ml INF-γ (PeproTech, Rocky Hill, NJ, USA) or 20 ng/ml IL-4 (PeproTech, Rocky Hill, NJ, USA) and 20 ng/ml IL-13 (PeproTech, Rocky Hill, NJ, USA) to polarize into M1 macrophages or M2 macrophages [22]. Then M0 macrophages were also treated by the conditioned medium of transfected A549 cells.

Cell Counting Kit-8 (CCK-8)

In a brief, A549 cells were inoculated into a 96-well plate at a density of 5×10^3 cells/well. Subsequently, the cells were incubated with CCK8 reagent (Peptide Institute) for 2 h. With the employment of a microplate reader (SLT Lab Instruments GmbH, Salzburg, Austria), the absorbance at 450 nm was detected.

Colony Formation Assay

A549 cells (1×10^4) were injected into 6-well plates for 14 d. Then, the cells were fixed with 4% paraformaldehyde and stained by 0.1% crystal violet. The number of colonies was eventually counted under a light microscope (Leica, Wetzlar, Germany).

Flow Cytometry Analysis

Cell apoptosis was measured with the employment of Annexin V-FITC/PI Apoptosis Detection Kit (Biovision, Milpitas, CA, USA) in the light of the recommended protocol. Before detecting the apoptotic rate by a flow cytometer (Ex, 488 nm; Em, 530 nm) (Sysmex Co., Kobe, Japan), A549 (1×10^6 cells/well) injected in 6-well plates were subjected to resuspension in 500 μL of $1 \times$ binding buffer, following which was the treatment with 5 μL Annexin V-FITC/propidium iodide (PI) for 5 min at room temperature in the dark. The total apoptotic cells was calculated as the sum of the right upper quadrant which indicated early apoptotic cells and right lower quadrant which indicated late apoptotic cells as previously reported [23].

Caspase3 Activity Assay

Caspase3 activity was examined with Caspase 3 Activity Assay Kit (Bestbio, Shanghai, China), according to the manufacturer's instructions. The absorbance at 405 nm was determined using. The activity of caspase 3 was calculated as the OD405 relative to the control.

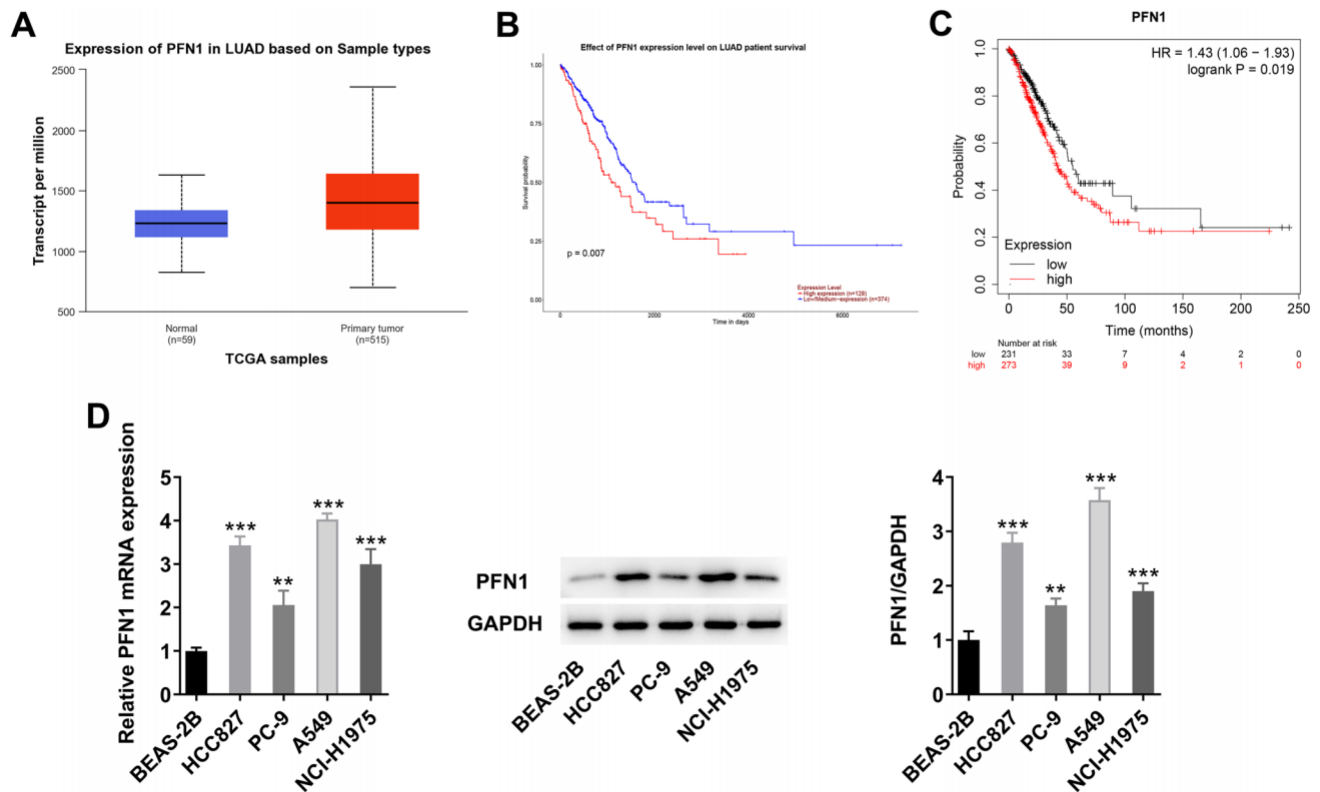


Fig. 1 Up-regulated PFN1 expression predicts unfavorable survival of LUAD patients. **A–B** ENCORI and **C** Kaplan–Meier Plotter detected PFN1 expression and its significance in survival of LUAD patients.

D RT-qPCR and western blotting tested PFN1 expression in LUAD cells. n=3. **P<0.01, ***P<0.001 vs. BEAS-2B

Reverse Transcription-Quantitative PCR (RT-qPCR)

The total RNA that extracted from A549 cells with RNA isolation kit (Gentra, Minneapolis, MN, USA) was reverse transcribed into cDNA using ProtoScript® II First Strand cDNA Synthesis Kit (NEB). PFN1 transcription was quantified employing Luna® Universal qPCR Master Mix (LMAI Bio, Shanghai, China) and relative gene expression was calculated using $2^{-\Delta\Delta C_t}$ equation.

Western Blot

Total proteins were extracted from A549 cells using RIPA lysis buffer (Bio Basic, Canada) and the protein concentration was quantified with BCA method (Bio Basic, Canada). Separated by 12% SDS-PAGE, equal amounts of sample proteins were subsequently transferred onto the PVDF membranes sealed by 5% BSA. Then, the membranes were incubated with primary antibodies. On the next day, the membranes were incubated with HRP-linked secondary antibody. The binding signals were scanned by the ECL reagent (Proanti Biotechnology Development Co., Ltd., Shanxi, China). Images were captured using Bio-Rad ChemiDOC

XRS + system (Bio-Rad Laboratories, Inc.) and analyzed by Image Lab Software (version 5.2.1; Bio-Rad Laboratories, Inc.) [24].

Immunofluorescence (IF) Staining

Following the treatment with 4% paraformaldehyde, 0.5% Triton X-100, and the inhibition by 5% BSA, A549 cells were incubated with primary antibodies targeting CD68, iNOS, and CD206 overnight at 4 °C. On the next day, the cells were incubated with goat anti-rabbit IgG (Alexa Fluor®488) secondary antibody at room temperature for 1 h.

To detect mitochondria and LC3 co-localization, cells were dyed by the MitoTracker Red kit (Invitrogen, Thermo Fisher, USA). Subsequently, the cells were incubated with primary antibody against LC3 and secondary antibody. DAPI was used for nuclear staining. Images were observed under a fluorescence microscope.

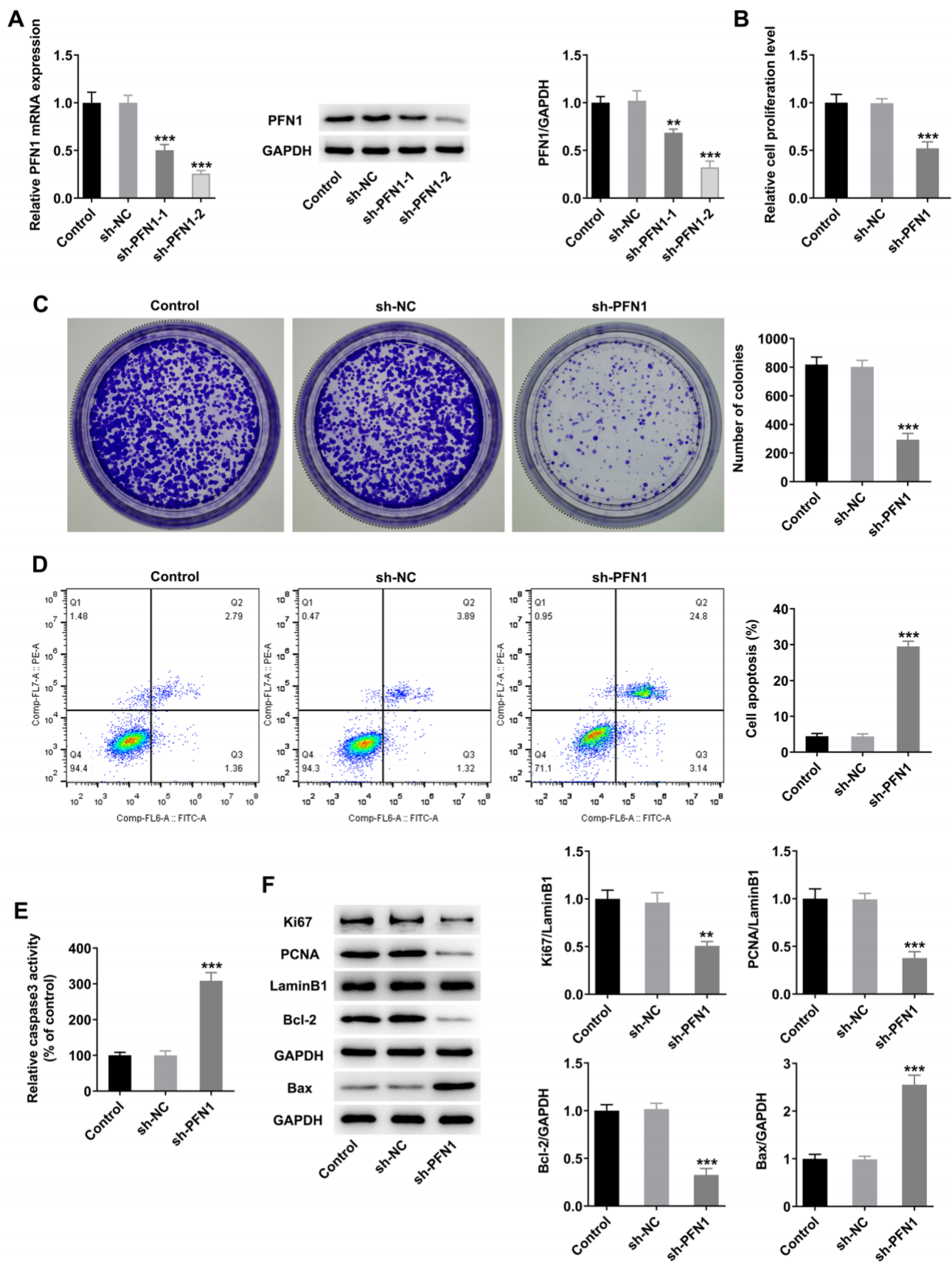


Fig. 2 Knockdown of PFN1 represses the proliferation and facilitates the apoptosis of LUAD cells. **A** RT-qPCR and western blotting measured the transduction efficacy of PFN1 interference plasmids. **B** CCK-8 assay detected cell viability. **C** Colony formation assay evaluated the colony-forming capacity of cells. **D** Flow cytometry analysis estimated cell apoptosis. **E** Relevant assay kit examined caspase3 concentration. **F** Western blotting tested the expression of proliferation- and apoptosis-related proteins. $n=3$. ** $P<0.01$, *** $P<0.001$ vs. sh-NC

Statistics

All statistics were analyzed using SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA) and then presented as mean \pm standard deviation. By means of one-way ANOVA as well as Tukey's post hoc test, statistical significance was ascertained. P value less than 0.05 indicated statistical significance. All experiments were repeated ≥ 3 times.

Results

Up-Regulated PFN1 Expression Predicts Unfavorable Survival of LUAD Patients

Firstly, it was noted from the data of ENCORI database that PFN1 expression was significantly increased in LUAD tissues and PFN1 upregulation predicted worse survival of LUAD patients (Fig. 1A–B). The similar trend can also be observed in Kaplan–Meier Plotter database, suggesting that PFN1 might act as a negative prognostic marker for LUAD (Fig. 1C). Through RT-qPCR and western blotting, PFN1 expression was also significantly increased in LUAD cell lines (HCC827, PC-9, A549, and NCI-H1975) by contrast with human bronchial epithelial cell line BEAS-2B (Fig. 1D). A549 cells displayed the highest PFN1 expression, hence being chosen for following experiments.

Knockdown of PFN1 Represses the Proliferation and Facilitates the Apoptosis of LUAD Cells

With the aim of exploring the effects of PFN1 on LUAD cellular behaviors, PFN1 was initially transfected with PFN1 interference plasmids (sh-PFN1-1/2) to reduce PFN1 expression (Fig. 2A). Moreover, sh-PFN1-2 was selected for the follow-up studies because of its better transfection efficiency. Through CCK-8 and colony formation assays, it was observed that the proliferation and colony forming abilities of A549 cells were both inhibited when PFN1 was down-regulated (Fig. 2B–C). Results obtained from flow cytometry analysis showed that interference with PFN1 significantly increased the number of apoptotic A549 cells (Fig. 2D). Also, caspase3 activity was also discovered to be increased

after PFN1 was knocked down (Fig. 2E). Further, the expressions of proliferation-associated proteins Ki67, PCNA, and anti-apoptotic protein Bcl-2 were all decreased while the expression of pro-apoptotic protein Bax was increased in A549 cells transfected with sh-PFN1 (Fig. 2F).

Knockdown of PFN1 Attenuates Macrophage Polarization Toward M2 in LUAD Cells

Dysregulated macrophage polarization has emerged as a critical event in the progression of LUAD. To investigate the role of PFN1 in macrophage polarization in LUAD cells, the expression of M1 and M2 macrophage markers were analyzed. RT-qPCR and immunofluorescence staining presented that the differentiation of M1 and M2 macrophages were identified, as evidenced by the up-regulated expression of M1 macrophage surface markers including CD80, IL-6, IL-1 β , CD68/iNOS in the M1 group and the raised expression of M2 macrophage surface markers including CD163, Arg-1, CD68/CD206 in the M2 group (Fig. 3A–B). Intriguingly, as illustrated in Fig. 3C–D, after M0 macrophages were cultivated with the conditioned medium of PFN1-silencing A549 cells, CD80, IL-6, IL-1 β , and CD68/iNOS expression were increased, whereas CD163, Arg-1, and CD68/CD206 expression were decreased (Fig. 3C–D), implying that reduction of PFN1 aggravated proinflammatory M1 macrophage polarization and suppressed anti-inflammatory M2 macrophage polarization in LUAD cells.

Knockdown of PFN1 Triggers Mitophagy in LUAD Cells

As depicted in Fig. 4A, the fluorescence intensity of LC3-FITC and MitoTracker Red were significantly increased in PFN1-silencing A549 cells, suggesting the existence of mitophagy by PFN1 deficiency. In addition, knockdown of PFN1 increased mitochondrial proteins PINK1, Parkin expression in the mitochondria, PINK1, Parkin, and autophagy-associated LC3II/I, Beclin-1 expression in the cytoplasm of cells while reduced p62 expression (Fig. 4B).

PFN1 Reduction Retards the Initiation of LUAD Dependent on Activation of Mitophagy

To determine the mechanism of PFN1 associated with mitophagy in LUAD, mitophagy inhibitor Mdivi-1 was applied. From CCK-8 assay, it turned out that the reduced viability of A549 cells due to PFN1 interference was revived by Mdivi-1 treatment (Fig. 5A). As expected,

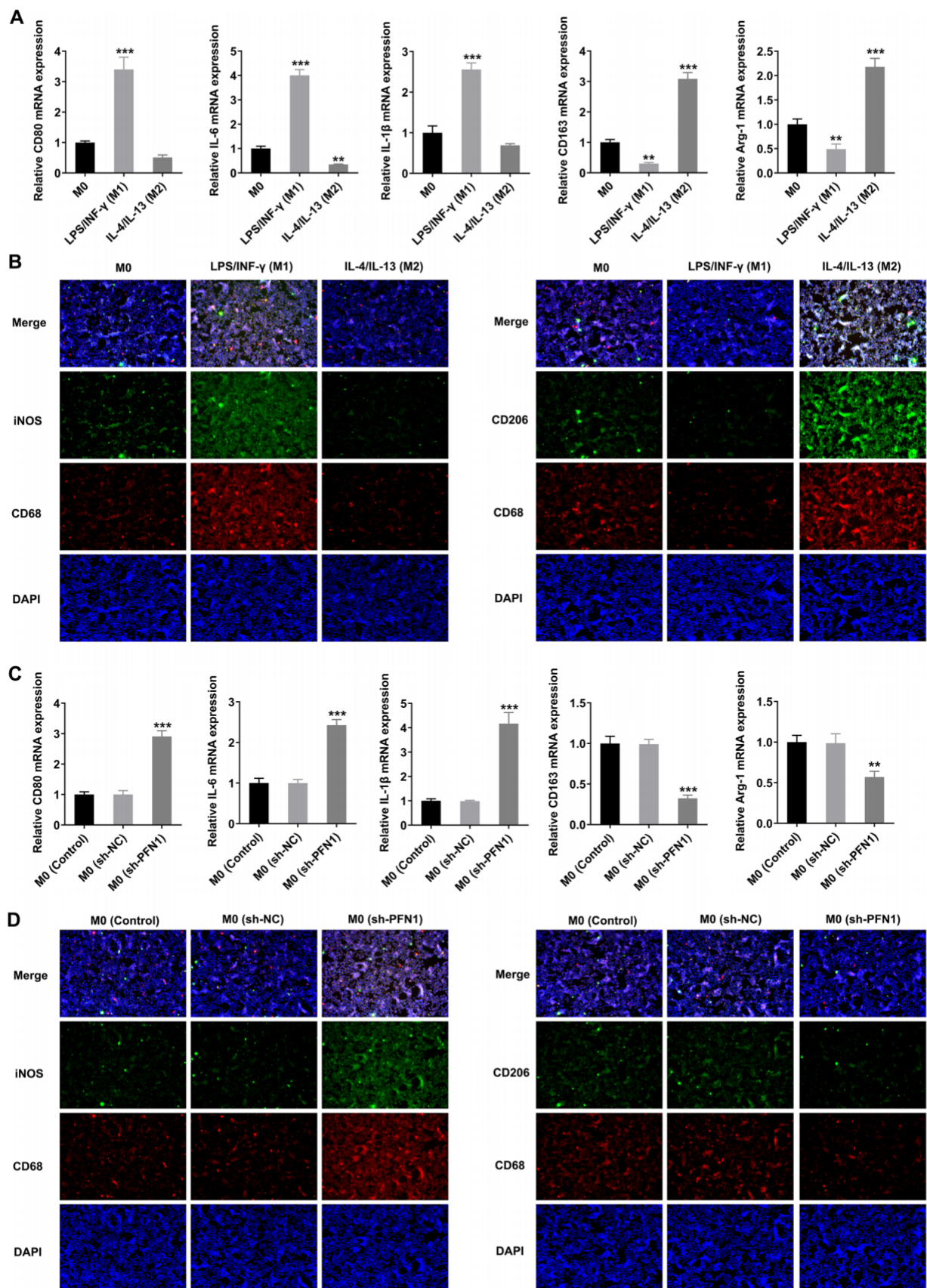


Fig. 3 Knockdown of PFN1 attenuates macrophage polarization toward M2 in LUAD cells. **A** RT-qPCR and **B** immunofluorescence staining estimated M1 and M2 macrophages markers. ** $P < 0.01$, *** $P < 0.001$ vs. M0. **C** RT-qPCR and **D** immunofluorescence staining estimated M1 and M2 macrophages markers after silencing of PFN1. $n = 3$. ** $P < 0.01$, *** $P < 0.001$ vs. M0 (sh-NC)

PFN1 interference depleted the number of formed colonies, which was then increased by Mdivi-1 (Fig. 5B). Conversely, Mdivi-1 markedly decreased the apoptotic rate of PFN1-silencing A549 cells (Fig. 5C), accompanied by decreased caspase3 content (Fig. 5D). Also, the decreased Ki67, PCNA, and Bcl-2 expression and the increased Bax expression caused by PFN1 knockdown in A549 cells were all partially restored again by Mdivi-1 (Fig. 5E).

PFN1 Reduction Polarizes Macrophages to M1 Phenotype in LUAD Cells Dependent on Activation of Mitophagy

At the same time, to determine the association between PFN1 and mitophagy in macrophage polarization during the process of LUAD, the expression of M1 and M2 macrophage markers were evaluated again in macrophages stimulated with the conditioned medium of PFN1-silencing A549 cells treated by Mdivi-1. From RT-qPCR and immunofluorescence staining, the expression of M1 macrophage markers CD80, IL-6, IL-1 β , and CD68/iNOS were up-regulated and the expression of M2 macrophage markers CD163, Arg-1, and CD68/CD206 expression were down-regulated in M0 (sh-PFN1) group compared with the M0 (Control) group. However, CD80, IL-6, IL-1 β , and CD68/iNOS expression were inhibited while CD163, Arg-1, and CD68/CD206 expression were enhanced again in the M0 (Mdiv-1 + sh-PFN1) group by contrast with the M0 (sh-PFN1) group (Fig. 6A–B).

Discussion

LUAD remains a highly heterogenous disease from the clinical and cytogenetic aspect, the carcinogenesis of which may be driven by the activation of oncogenes and the inactivation of suppressor genes [25]. As an extensively expressed protein, PFN1 is related with cellular motility, survival, and actin remodeling, and has been reported to be overexpressed in metastatic non-small cell lung cancer tissues [14]. The major findings of the current research expounded that PFN1 expression was higher in LUAD tissues and cells, and was correlated with the worse survival of LUAD patients. The onset of LUAD is characterized by uncontrolled proliferation and restrained apoptosis. However, the existing studies

have presented the controversial role of PFN1 in cancer cell proliferation. For instance, PFN1 elevation suppresses cell proliferation in pancreatic cancer [26] while promotes cell proliferation in colorectal cancer [27]. In this study, PFN1 interference inhibited the proliferation and colony forming abilities of A549 cells, accompanied by down-regulated expression of proliferation markers Ki67 and PCNA. Besides, Bcl-2 is a well-recognized anti-apoptotic molecule in LUAD [28, 29] and Bax and caspase3 are considered as common pro-apoptotic proteins in LUAD conversely [30, 31]. MiR-330-3p or miR-299-3p has been reported to promote the apoptosis of colorectal cancer cells via targeting PFN1 [27, 32]. Intriguingly, deficiency of PFN1 has been clarified to decrease the percentage of early apoptotic cells in A549 cells [15]. However, the specific effects of PFN1 on apoptotic proteins in LUAD remain unclear. The experimental results in this study confirmed that PFN1 knockdown noticeably increased the apoptotic rate of A549 cells. Further analysis presented that PFN1 knockdown significantly increased pro-apoptotic caspase3 activity, reduced anti-apoptotic Bcl-2 expression and increased pro-apoptotic Bax expression. All these findings underlined the inhibitory role of PFN1 knockdown in the occurrence of LUAD.

As reported, multiple cytokines produced by different cell types are implicated in the tumor microenvironment (TME) [33]. TME is commonly considered to cause pro-tumorigenic outcomes by impacting macrophage recruitment and polarization [34]. Tumor-associated macrophages (TAMs) are abundant immunosuppressive cells infiltrating the TME [34]. TAMs greatly affect LUAD progression [35], and targeting TAMs has become one of the most favored immunotherapy strategies [36]. In tumor microenvironment, macrophages under stress can be generally converted into classical activated M1 macrophages and alternatively activated M2 macrophages [34]. Specifically, M1 macrophages (CD86, CD80, and iNOS) stimulated by LPS and INF- γ release pro-inflammatory cytokines (e.g., IL-6, IL-1 β) to play an anti-tumor role, while M2 macrophages (CD163, CD206, and Arg-1) stimulated by IL-4 and IL-13 release pro-tumor cytokines (e.g., IL-4, IL-10, IL-13) to promote tumor progression and metastasis [37]. CD68 is considered as a pan-macrophage marker [38]. Moreover, Zhang et al. have introduced that PFN1 may modulate macrophage polarization in colorectal cancer [39]. Consistently, in unpolarized macrophages cultured with the conditioned medium of PFN1-silencing LUAD cells, the expression of M1 macrophage markers including CD80, IL-6, IL-1 β , CD68/iNOS were up-regulated and the expression of M2 macrophage markers including CD163, Arg-1, CD68/CD206 were down-regulated here, suggesting the inhibitory role of PFN1 reduction in M2 macrophage polarization in LUAD cells, which further testified the tumor-suppressing activities of PFN1 depletion in LUAD.

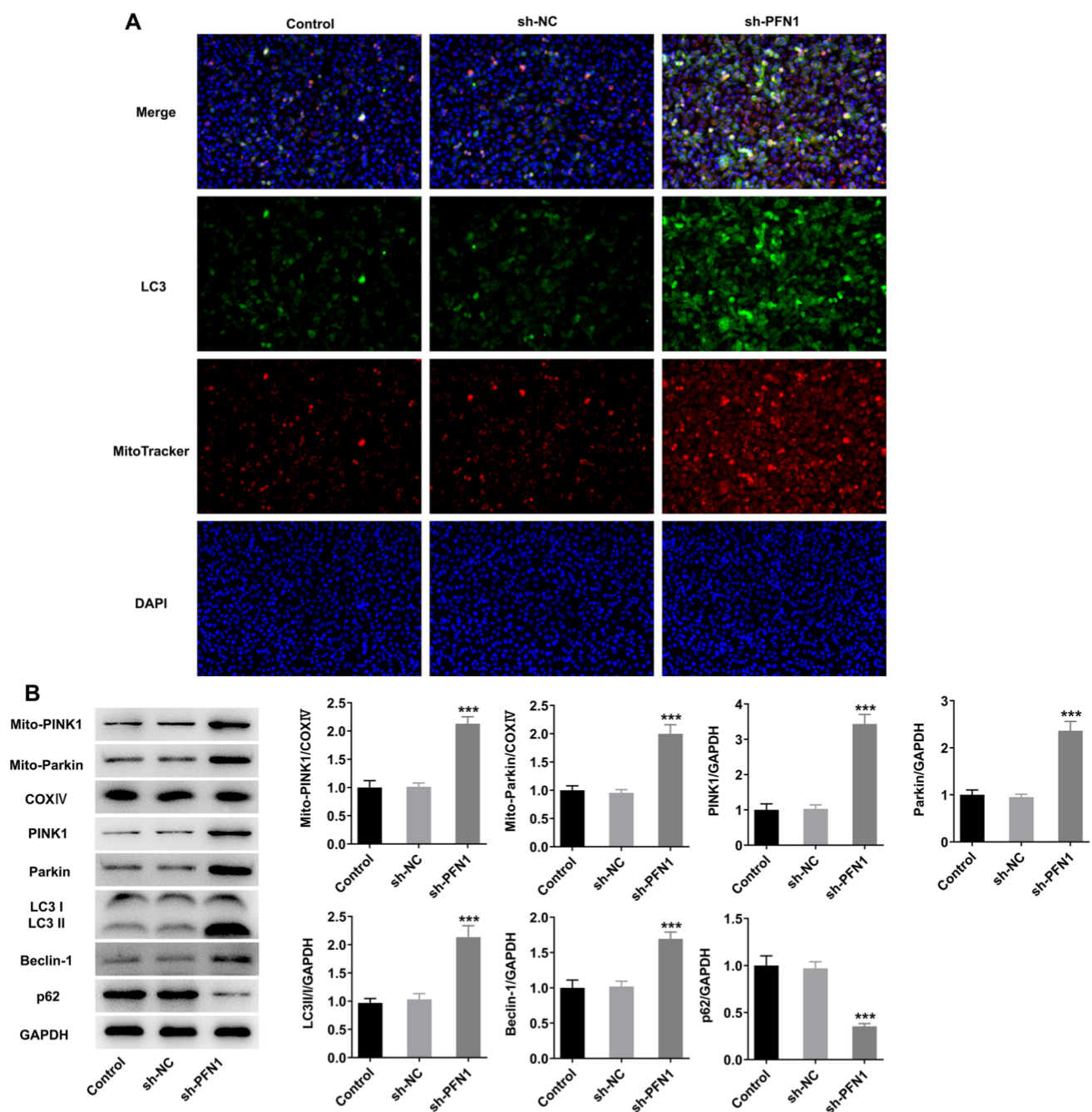


Fig. 4 Knockdown of PFN1 triggers mitophagy in LUAD cells. **A** MitoTracker Red and immunofluorescence staining measured mitochondria and LC3 colocalization. **B** Western blotting tested the

expression of mitochondrial proteins and autophagy-associated proteins. $n=3$. *** $P<0.001$ vs. sh-NC

Accumulated studies have revealed that mitochondria dysregulation plays a vital role in cancer progression and chemoresistance [40–42]. It has also been showed that mitophagy is often defective in various cancer cells [19] and targeting key mitophagy-related genes may influence the development and prognosis of LUAD [43]. Notably, recent investigation has mentioned that mitophagy is activated upon PFN1 deficiency [44]. During the process of

mitophagy, autophagosomes are formed following the wrapping of damaged mitochondria with endoplasmic reticulum membrane, and the subsequent modification of the LC3-I protein to LC3-II protein accelerates their fusion with lysosomes [45]. The ubiquitin-binding protein p62 degradation also contributes to the autophagic flux [46]. Beclin-1 cleavage by caspases inactivates autophagy and triggers the apoptotic cascade by expediting cytochrome C release

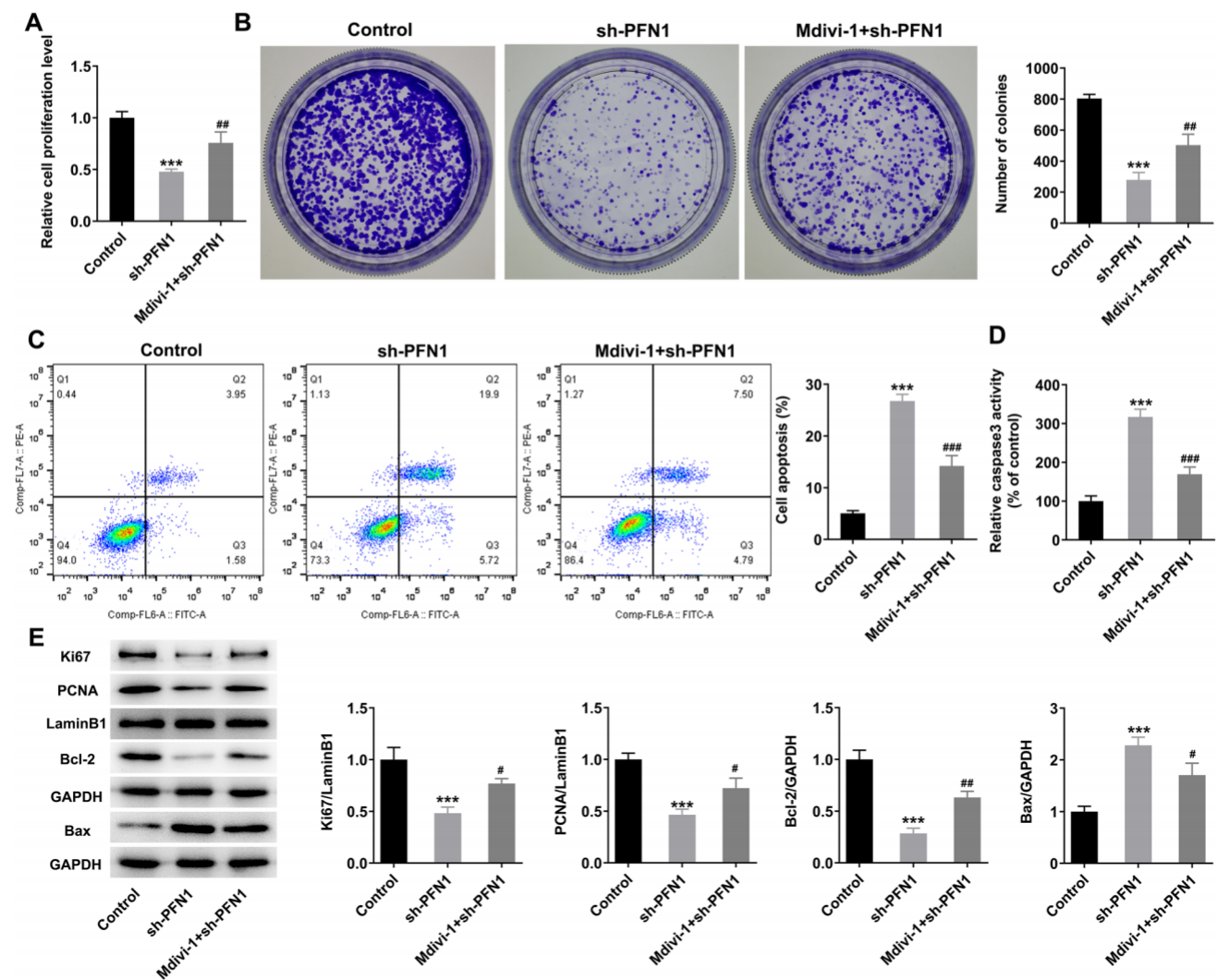


Fig. 5 PFN1 reduction retards the initiation of LUAD dependent on activation of mitophagy. **A** CCK-8 assay detected cell viability. **B** Colony formation assay evaluated the colony-forming capacity of cells. **C** Flow cytometry analysis estimated cell apoptosis. **D** Relevant

assay kit examined caspase3 concentration. **E** Western blotting tested the expression of proliferation and apoptosis-related proteins. $n=3$. *** $P<0.001$ vs. Control; # $P<0.05$, ## $P<0.01$, ### $P<0.001$ vs. sh-PFN1

from mitochondria [47]. In addition, PINK1, a serine/threonine kinase, interacts with Parkin, an E3 ubiquitin ligase, to target damaged mitochondria to the lysosome for degradation [48]. Our current study demonstrated that PFN1 silencing increased the fluorescence intensity of LC3-FITC and MitoTracker Red, elevated PINK1, Parkin expression in the mitochondria, PINK1, Parkin, and LC3II/I, Beclin-1 expression in the cytoplasm of A549 cells while reduced p62 expression, implying that mitophagy was activated by interference with PFN1 in LUAD cells. Numerous literatures have revealed that repressive mitophagy drives macrophage activation in multiple human diseases, such as sepsis [49], chronic kidney disease [50], and osteoarthritis [51]. The current work also proved that the addition of mitophagy inhibitor Mdivi-1 partially counteracted the

effects of PFN1 deletion on the proliferation and apoptosis in LUAD cells, also evidenced by the increased Ki67, PCNA, and anti-apoptotic Bcl-2 expression and the decreased pro-apoptotic Bax expression in PFN1-silencing LUAD cells. Also, Mdivi-1 pretreatment decreased the expression of M1 macrophage markers including CD80, IL-6, IL-1 β , CD68/iNOS and increased the expression of M2 macrophage markers including CD163, Arg-1, CD68/CD206 in macrophages cultured with the conditioned medium of PFN1-silencing LUAD cells.

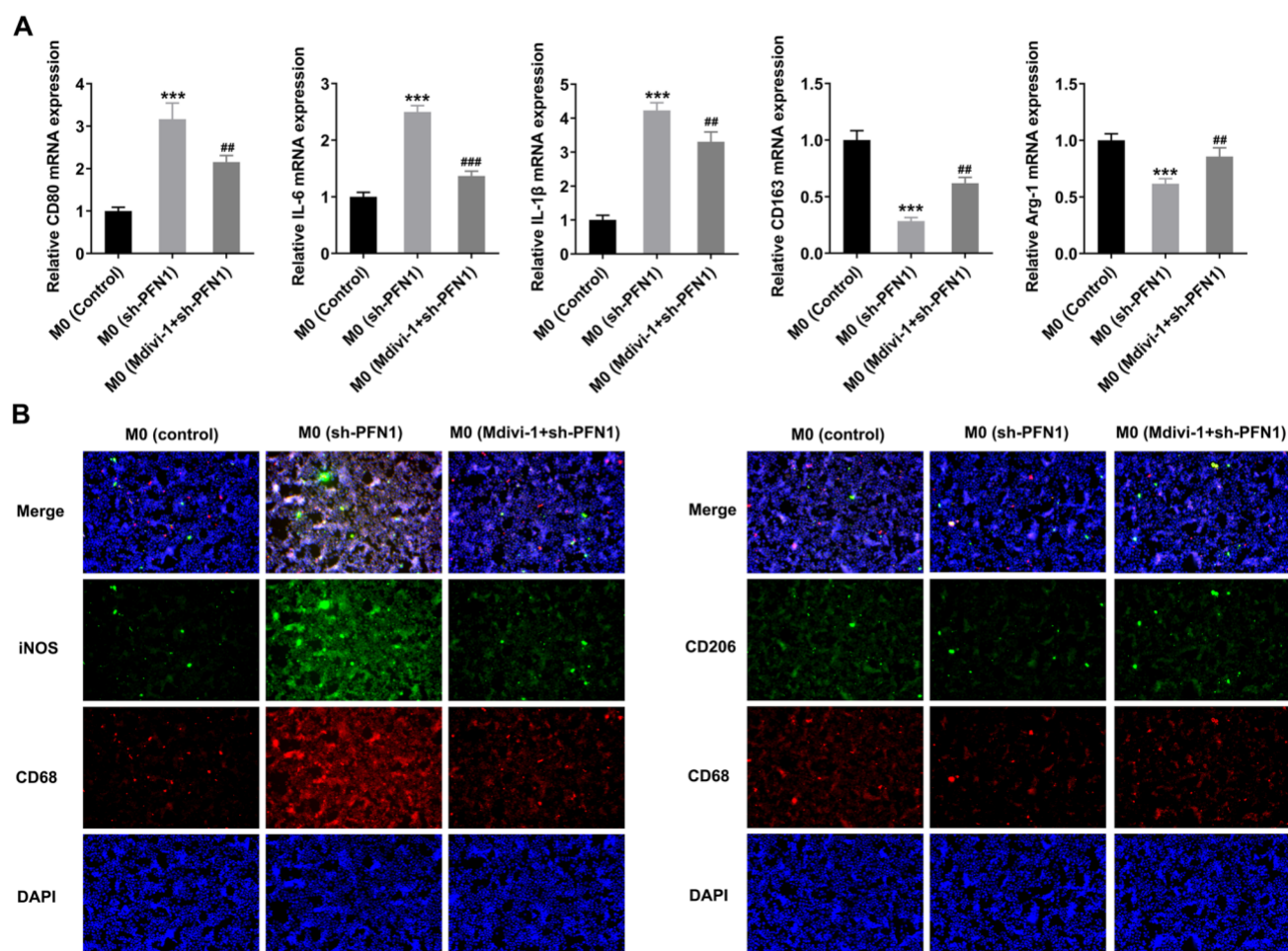


Fig. 6 PFN1 reduction polarizes macrophages to M1 phenotype in LUAD cells dependent on activation of mitophagy. **A** RT-qPCR and **B** immunofluorescence staining estimated M1 and M2 mac-

rophages markers. $n=3$. *** $P<0.001$ vs. M0 (Control); ## $P<0.01$, ### $P<0.001$ vs. M0 (sh-PFN1)

Conclusion

To be concluded, the activation of mitophagy by PFN1 inhibition may inhibit the occurrence of LUAD via polarizing macrophages to anti-tumor M1 phenotype. Collectively, PFN1 may be a therapeutic target in LUAD.

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Data Availability The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

References

1. Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, 70(1), 7–30.
2. Relli, V., Trerotola, M., Guerra, E., & Alberti, S. (2019). Abandoning the notion of non-small cell lung cancer. *Trends in Molecular Medicine*, 25(7), 585–594.
3. Travis, W. D. (2020). Lung cancer pathology: Current concepts. *Clinics in Chest Medicine*, 41(1), 67–85.
4. Mott, T. F. (2018). Lung cancer: Management. *FP Essent*, 464, 27–30.
5. Wang, M., Herbst, R. S., & Boshoff, C. (2021). Toward personalized treatment approaches for non-small-cell lung cancer. *Nature Medicine*, 27(8), 1345–1356.
6. Alduais, Y., Zhang, H., Fan, F., Chen, J., & Chen, B. (2023). Non-small cell lung cancer (NSCLC): A review of risk factors, diagnosis, and treatment. *Medicine (Baltimore)*, 102(8), e32899.
7. Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer Statistics, 2021. *CA: A Cancer Journal for Clinicians*, 71(1), 7–33.
8. Jurišić, V., Obradovic, J., Pavlović, S., & Djordjevic, N. (2018). Epidermal growth factor receptor gene in non-small-cell lung

- cancer: the importance of promoter polymorphism investigation. *Analytical Cellular Pathology (Amsterdam)*, 2018, 6192187.
9. Jurisic, V., Vukovic, V., Obradovic, J., Gulyaeva, L. F., Kushlinskii, N. E., & Djordjević, N. (2020). EGFR polymorphism and survival of NSCLC patients treated with TKIs: A systematic review and meta-analysis. *J Oncology*, 2020, 1973241.
 10. Obradovic, J., Todosijevic, J., & Jurisic, V. (2023). Side effects of tyrosine kinase inhibitors therapy in patients with non-small cell lung cancer and associations with EGFR polymorphisms: A systematic review and meta-analysis. *Oncology Letters*, 25(2), 62.
 11. Murk, K., Ornaghi, M., & Schiweck, J. (2021). Profilin isoforms in health and disease—All the same but different. *Front Cell Dev Biol.*, 9, 681122.
 12. Wang, Y., Wang, Y., Wan, R., Hu, C., & Lu, Y. (2021). Profilin 1 protein and its implications for cancers. *Oncology (Williston Park)*, 35(7), 402–409.
 13. Alkam, D., Feldman, E. Z., Singh, A., & Kiaei, M. (2017). Profilin1 biology and its mutation, actin(g) in disease. *Cellular and Molecular Life Sciences*, 74(6), 967–981.
 14. Wang, Y., Lu, Y., Wan, R., Wang, Y., Zhang, C., Li, M., et al. (2022). Profilin 1 induces tumor metastasis by promoting microvesicle secretion through the ROCK 1/p-MLC pathway in non-small cell lung cancer. *Frontiers in Pharmacology*, 13, 890891.
 15. Gagat, M., Hałas-Wisniewska, M., Zielińska, W., Izdebska, M., Grzanka, D., & Grzanka, A. (2018). The effect of piperlongumine on endothelial and lung adenocarcinoma cells with regulated expression of profilin-1. *Oncotargets and Therapy*, 11, 8275–8292.
 16. Lu, Y., Li, Z., Zhang, S., Zhang, T., Liu, Y., & Zhang, L. (2023). Cellular mitophagy: Mechanism, roles in diseases and small molecule pharmacological regulation. *Theranostics*, 13(2), 736–766.
 17. Picca, A., Faitg, J., Auwerx, J., Ferrucci, L., & D'Amico, D. (2023). Mitophagy in human health, ageing and disease. *Nature Metabolism*, 5(12), 2047–2061.
 18. Wang, S., Long, H., Hou, L., Feng, B., Ma, Z., Wu, Y., et al. (2023). The mitophagy pathway and its implications in human diseases. *Signal Transduction and Targeted Therapy*, 8(1), 304.
 19. Panigrahi, D. P., Prahara, P. P., Bhol, C. S., Mahapatra, K. K., Patra, S., Behera, B. P., et al. (2020). The emerging, multifaceted role of mitophagy in cancer and cancer therapeutics. *Seminars in Cancer Biology*, 66, 45–58.
 20. Ji, J., Wang, K., Meng, X., Zhong, H., Li, X., Zhao, H., et al. (2022). Elaiophyllin inhibits tumorigenesis of human lung adenocarcinoma by inhibiting mitophagy via suppression of SIRT1/Nrf2 signaling. *Cancers (Basel)*, 14(23), 5812.
 21. Cao, S., Xiao, H., Li, X., Zhu, J., Gao, J., Wang, L., et al. (2021). AMPK-PINK1/parkin mediated mitophagy is necessary for alleviating oxidative stress-induced intestinal epithelial barrier damage and mitochondrial energy metabolism dysfunction in IPEC-J2. *Antioxidants (Basel)*, 10(12), 2010.
 22. Liu, R., Sun, X., Hu, Z., Peng, C., & Wu, T. (2022). Knockdown of long non-coding RNA MIR155HG suppresses melanoma cell proliferation, and deregulated MIR155HG in melanoma is associated with M1/M2 balance and macrophage infiltration. *Cells Development*, 170, 203768.
 23. Jurisic, V., Srdic-Rajic, T., Konjevic, G., Bogdanovic, G., & Colic, M. (2011). TNF- α induced apoptosis is accompanied with rapid CD30 and slower CD45 shedding from K-562 cells. *Journal of Membrane Biology*, 239(3), 115–122.
 24. Lovett, J. V., Manalo, P. B., Barcia, T. C., Bomberger, R. A., & McGregor, D. B. (1988). Diagnosis of pulmonary masses by fine-needle aspiration. *American Journal of Surgery*, 156(6), 441–445.
 25. Inamura, K. (2017). Major tumor suppressor and oncogenic non-coding RNAs: Clinical relevance in lung cancer. *Cells*, 6(2), 12.
 26. Yao, W., Ji, S., Qin, Y., Yang, J., Xu, J., Zhang, B., et al. (2014). Profilin-1 suppresses tumorigenicity in pancreatic cancer through regulation of the SIRT3-HIF1 α axis. *Molecular Cancer*, 13, 187.
 27. Bai, N., Ma, Y., Zhao, J., & Li, B. (2020). Knockdown of lncRNA HCP5 suppresses the progression of colorectal cancer by miR-299-3p/PFN1/AKT axis. *Cancer Management Research*, 12, 4747–4758.
 28. Vuletic, A., Konjevic, G., Milanovic, D., Ruzdijic, S., & Jurisic, V. (2010). Antiproliferative effect of 13-cis-retinoic acid is associated with granulocyte differentiation and decrease in cyclin B1 and Bcl-2 protein levels in G0/G1 arrested HL-60 cells. *Pathology Oncology Research*, 16(3), 393–401.
 29. Nalluri, S., Ghoshal-Gupta, S., Kutiyanaawalla, A., Gayatri, S., Lee, B. R., Jiواني, S., et al. (2015). TIMP-1 inhibits apoptosis in lung adenocarcinoma cells via interaction with Bcl-2. *PLoS ONE*, 10(9), e0137673.
 30. Lu, W. L., Yu, C. R., Lien, H. M., Sheu, G. T., & Cherng, S. H. (2020). Cytotoxicity of naringenin induces bax-mediated mitochondrial apoptosis in human lung adenocarcinoma A549 cells. *Environmental Toxicology*, 35(12), 1386–1394.
 31. Wang, X., Wang, D., & Zhao, Y. (2015). Effect and mechanism of resveratrol on the apoptosis of lung adenocarcinoma cell line A549. *Cell Biochemistry and Biophysics*, 73(2), 527–531.
 32. Huang, Y., Sun, H., Ma, X., Zeng, Y., Pan, Y., Yu, D., et al. (2020). HLA-F-AS1/miR-330-3p/PFN1 axis promotes colorectal cancer progression. *Life Sciences*, 254, 117180.
 33. Jurisic, V. (2020). Multiomic analysis of cytokines in immunoncology. *Expert Review of Proteomics*, 17(9), 663–674.
 34. Boutilier, A. J., & Elswa, S. F. (2021). Macrophage polarization states in the tumor microenvironment. *International Journal of Molecular Sciences*, 22(13), 6995.
 35. Yuan, S., Dong, Y., Peng, L., Yang, M., Niu, L., Liu, Z., et al. (2019). Tumor-associated macrophages affect the biological behavior of lung adenocarcinoma A549 cells through the PI3K/AKT signaling pathway. *Oncology Letters*, 18(2), 1840–1846.
 36. Shikanai, S., Yamada, N., Yanagawa, N., Sugai, M., Osakabe, M., Saito, H., et al. (2023). Prognostic impact of tumor-associated macrophage-related markers in patients with adenocarcinoma of the lung. *Annals of Surgical Oncology*, 30(12), 7527–7537.
 37. Gunassekaran, G. R., Poongkavithai Vadevoo, S. M., Baek, M. C., & Lee, B. (2021). M1 macrophage exosomes engineered to foster M1 polarization and target the IL-4 receptor inhibit tumor growth by reprogramming tumor-associated macrophages into M1-like macrophages. *Biomaterials*, 278, 121137.
 38. Jeong, H., Hwang, I., Kang, S. H., Shin, H. C., & Kwon, S. Y. (2019). Tumor-associated macrophages as potential prognostic biomarkers of invasive breast cancer. *Journal of Breast Cancer*, 22(1), 38–51.
 39. Zhang, J., Li, S., Zhang, X., Li, C., Zhang, J., & Zhou, W. (2021). LncRNA HLA-F-AS1 promotes colorectal cancer metastasis by inducing PFN1 in colorectal cancer-derived extracellular vesicles and mediating macrophage polarization. *Cancer Gene Therapy*, 28(12), 1269–1284.
 40. Zong, W. X., Rabinowitz, J. D., & White, E. (2016). Mitochondria and cancer. *Molecular Cell*, 61(5), 667–676.
 41. Mukherjee, S., Bhatti, G. K., Chhabra, R., Reddy, P. H., & Bhatti, J. S. (2023). Targeting mitochondria as a potential therapeutic strategy against chemoresistance in cancer. *Biomedicine & Pharmacotherapy*, 160, 114398.
 42. Musicco, C., Signorile, A., Pesce, V., Loguercio Polosa, P., & Cormio, A. (2023). Mitochondria deregulations in cancer offer

- several potential targets of therapeutic interventions. *International Journal of Molecular Sciences.*, 24(13), 10420.
43. Dai, D., Liu, L., Guo, Y., Shui, Y., & Wei, Q. (2022). A comprehensive analysis of the effects of key mitophagy genes on the progression and prognosis of lung adenocarcinoma. *Cancers (Basel)*, 15(1), 57.
 44. Read, T. A., Cisterna, B. A., Skrubber, K., Ahmadi, S., Lindamood, H. L., Vitriol, J. A., et al. (2023). The actin binding protein profilin 1 is critical for mitochondria function. *bioRxiv*, 29, 969.
 45. Towers, C. G., Wodetzki, D. K., Thorburn, J., Smith, K. R., Caino, M. C., & Thorburn, A. (2021). Mitochondrial-derived vesicles compensate for loss of LC3-mediated mitophagy. *Developmental Cell*, 56(14), 2029–42.e5.
 46. Yamada, T., Dawson, T. M., Yanagawa, T., Iijima, M., & Sesaki, H. (2019). SQSTM1/p62 promotes mitochondrial ubiquitination independently of PINK1 and PRKN/parkin in mitophagy. *Autophagy*, 15(11), 2012–2018.
 47. Sun, Y., Yao, X., Zhang, Q. J., Zhu, M., Liu, Z. P., Ci, B., et al. (2018). Beclin-1-dependent autophagy protects the heart during sepsis. *Circulation*, 138(20), 2247–2262.
 48. Eiyama, A., & Okamoto, K. (2015). PINK1/Parkin-mediated mitophagy in mammalian cells. *Current Opinion in Cell Biology*, 33, 95–101.
 49. Patoli, D., Mignotte, F., Deckert, V., Dusuel, A., Dumont, A., Rieu, A., et al. (2020). Inhibition of mitophagy drives macrophage activation and antibacterial defense during sepsis. *The Journal of Clinical Investigation*, 130(11), 5858–5874.
 50. Cao, Y., Xiong, J., Guan, X., Yin, S., Chen, J., Yuan, S., et al. (2023). Paeoniflorin suppresses kidney inflammation by regulating macrophage polarization via KLF4-mediated mitophagy. *Phytomedicine*, 116, 154901.
 51. Gu, C., Chen, M., Li, X., Geng, D., & Wang, C. (2023). MAGL regulates synovial macrophage polarization via inhibition of mitophagy in osteoarthritic pain. *Molecular Medicine Reports*. <https://doi.org/10.3892/mmr.2023.13004>

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