

ORIGINAL ARTICLE

Smooth Muscle *Ythdf2* Abrogation Ameliorates Pulmonary Vascular Remodeling by Regulating *Myadm* Transcript Stability

Jie Wang¹, Yueyao Shen, Yuhui Zhang, Donghai Lin, Qiang Wang, Xiaoxuan Sun, Dong Wei², Bin Shen³, Jingyu Chen, Yong Ji⁴, David Fulton⁵, Yanfang Yu, Feng Chen⁶, Li Hu⁷

BACKGROUND: The N6-methyladenosine (m⁶A) modification of RNA and its regulators have important roles in the pathogenesis of pulmonary hypertension (PH). *Ythdf2* (YTH N6-methyladenosine RNA binding protein 2) is best known for its role in degrading m⁶A-modified mRNAs such as *Hmox1* mRNA, which leads to alternative activation of macrophages in PH. Recent studies have also linked *Ythdf2* to the proliferation of pulmonary artery smooth muscle cells (PASMCs). However, its specific roles in PASMCs and downstream targets during the development of PH remain unclear.

METHODS: The expression and biological function of *Ythdf2* in PASMCs were investigated in human and experimental models of PH. Smooth muscle cell-specific *Ythdf2*-deficient mice were used to assess the roles of *Ythdf2* in PASMCs in vivo. Proteomic analysis, m⁶A sequencing, and RNA immunoprecipitation analysis were used to screen for potential downstream targets.

RESULTS: *Ythdf2* was significantly upregulated in human and rodent PH-PASMCs, and smooth muscle cell-specific *Ythdf2* deficiency ameliorated PASMC proliferation, right ventricular hypertrophy, pulmonary vascular remodeling, and PH development. Higher expression of *Ythdf2* promoted PASMC proliferation and PH by paradoxically stabilizing *Myadm* mRNA in an m⁶A-dependent manner. Loss of *Ythdf2* decreased the expression of *Myadm* in PASMCs and pulmonary arteries, both in vitro and in vivo. Additionally, silencing *Myadm* inhibited the *Ythdf2*-dependent hyperproliferation of PASMCs by upregulating the cell cycle kinase inhibitor p21.

CONCLUSIONS: We have identified a novel mechanism where the increased expression of *Ythdf2* stimulates PH-PASMC proliferation through an m⁶A/*Myadm*/p21 pathway. Strategies targeting *Ythdf2* in PASMCs might be useful additions to the therapeutic approach to PH. (*Hypertension*. 2024;81:1785–1798. DOI: 10.1161/HYPERTENSIONAHA.124.22801.)

• **Supplement Material.**

Key Words: hypertension, pulmonary ■ myocytes, smooth muscle ■ N-methyladenosine ■ pulmonary artery ■ RNA methylation

Pulmonary hypertension (PH) is a global health problem. Although the prevalence of PH varies based on the subgroups of PH, it is estimated that 1% of the world population and up to 10% of the elderly people aged 65 years and over are suffering from PH.^{1,2} Currently, PH is defined by a mean pulmonary artery pressure of over 20 mmHg measured by supine right heart

catheterization at rest. Meanwhile, the 6th World Symposium on PH also proposes that patients with mean pulmonary artery pressure of >20 mmHg are at increased risk of hospitalization and mortality compared with those with mean pulmonary artery pressure of 20 mmHg or lower.^{2,3} Because of the prevalence and severity of PH and the challenges of obtaining an early and accurate

Correspondence to: Li Hu, Department of Forensic Medicine, Nanjing Medical University, 101 Longmian Ave, Nanjing, Jiangsu 211166, China, Email huli@njmu.edu.cn; or Feng Chen, Key Laboratory of Targeted Intervention of Cardiovascular Disease, Collaborative Innovation Center for Cardiovascular Disease Translational Medicine, Nanjing Medical University, 101 Longmian Ave, Jiangning District, Nanjing, Jiangsu 210029, China, Email fchen@njmu.edu.cn; or Yanfang Yu, Department of Forensic Medicine, Nanjing Medical University, 101 Longmian Ave, Nanjing, Jiangsu 211166, China, Email yanfangyu223@njmu.edu.cn
Supplemental Material is available at <https://www.ahajournals.org/doi/suppl/10.1161/HYPERTENSIONAHA.124.22801>.

For Sources of Funding and Disclosures, see page 1797.

© 2024 American Heart Association, Inc.

Hypertension is available at www.ahajournals.org/journal/hyp

NOVELTY AND RELEVANCE

What Is New?

Herein, we provide evidence that increased protein expression of Ythdf2 (YTH N6-methyladenosine RNA binding protein 2) in pulmonary artery smooth muscle cells (PASMCs) of pulmonary hypertension (PH) animal models and patients with PH is associated with PH development. Loss of *Ythdf2* expression in smooth muscle cells ameliorates PASMC proliferation in vivo and in vitro, right ventricular hypertrophy, vascular remodeling, and PH development. In contrast to its conventional role in degrading N6-methyladenosine (m⁶A) mRNAs, we found that *Ythdf2* stabilized *Myadm* to repress *p21* expression and increase the proliferation of PASMCs.

What Is Relevant?

Our previous studies have identified roles for the m⁶A readers *Ythdf1* and *Ythdf2* in PASMCs and in alveolar macrophages, respectively, in the development of PH. However, the impact of *Ythdf2* on RNA degradation and translation is complex, and the mechanisms downstream of *Ythdf2* that drive functional changes in PASMCs to promote PH remain incompletely defined. This study represents a significant advancement in our understanding of the mechanisms by which m⁶A RNA methylation influences the pathogenesis of PH.

Clinical/Pathophysiological Implications?

This study suggests that *Ythdf2* promotes PASMC proliferation and vascular remodeling through the m⁶A/Myadm/p21 axis and that therapeutically targeting *Ythdf2* in PASMCs may have utility in the clinical approach for patients with PH.

Nonstandard Abbreviations and Acronyms

HPASMC	human pulmonary artery smooth muscle cell
m⁶A	N ⁶ -methyladenosine
mPASMC	mouse pulmonary artery smooth muscle cell
PASMC	pulmonary artery smooth muscle cell
PCNA	proliferating cell nuclear antigen
PDGF	platelet-derived growth factor
PH	pulmonary hypertension
PVR	pulmonary vascular remodeling
SMC	smooth muscle cell
Su/Hx	SU5416/hypoxia
Ythdf2	YTH N ⁶ -methyladenosine RNA binding protein 2

diagnosis, there is an unmet need for a greater number of effective targets for early diagnosis and precision therapy of PH.

PH is a chronic vascular disorder characterized by irreversible vascular remodeling of all layers of the pulmonary vascular wall. Past research has shown that multiple types of vascular cells exhibit a hyperproliferative phenotype in PH patients as well as in animal models.^{4,5} Among these, the excessive proliferation of vascular smooth muscle cells (SMCs) is one of the key underlying mechanisms driving pulmonary vascular remodeling (PVR).⁶ Pathological vascular remodeling in PH contributes to a sustained increase in pulmonary vascular resistance, primarily and

subsequently leading to increased pulmonary artery pressure. Ultimately, patients with PH are suffered from heart failure and even death. Despite significant progress in the therapeutic approach to PH, there remains a lack of effective/selective antiproliferative approaches to target the hyperproliferative phenotype of pulmonary artery smooth muscle cells (PASMCs).⁷ As a result, the prognosis of PH patients remains poor, and there remains an urgent need to improve our understanding of the underlying mechanisms and functionally significant pathways involved in PVR of PH.

RNA N⁶-methyladenosine (m⁶A) modification plays a significant role in various pathological and physiological processes. This modification, which is reversible and dynamic, is mediated by m⁶A writers and erasers. The fate of m⁶A-modified RNAs is predominantly determined by various m⁶A readers. Among these readers, *Ythdf1* (YTH N6-methyladenosine RNA binding protein 1) and *Ythdf3* have been shown to cooperatively enhance protein expression by promoting the translation of m⁶A-modified transcripts, while *Ythdf2* primarily facilitates the degradation of m⁶A-tagged mRNAs.⁸ Abnormal expression of m⁶A modulators (m⁶A writers, erasers, and readers) has also been confirmed to be tightly associated with the proliferation of PASMCs.^{4,9,10} Interestingly, we previously showed that *Ythdf1* is involved in regulating PASMC hyperproliferation.⁴ However, we found that only a small proportion of m⁶A-modified mRNAs in PH lungs was recognized by *Ythdf1*, suggesting other m⁶A readers might have important roles in PH.⁴ In our recent study, we have demonstrated that *Ythdf2* in alveolar macrophages contributes to the progression of PH and facilitates vascular

inflammation, oxidative stress, and alternative activation of macrophages through degrading *Hmox1* mRNA.⁹ In addition to alveolar macrophages, we also found higher levels of expression of *Ythdf2* in all 3 layers of pulmonary blood vessels in PH patients and PH animal models, but the highest levels were seen in the medial layer. Moreover, macrophage *Ythdf2* was found to be important in the early stage of PH development, while the elevated expression of *Ythdf2* in PH-PASMCs may play a crucial role throughout PH pathogenesis. Another study has found that the METTL14-driven m⁶A modification of *GRAP* mRNA promotes the proliferation of PSMCs and the development of PH, a process that is likely regulated by *Ythdf2*.¹⁰ Nevertheless, the functional role of *Ythdf2* in SMCs, in context with the excessive proliferation of PSMCs in PH, as well as the underlying mechanisms, remain undefined.

The dysregulation of the cell cycle has been established as a significant contributor to the development of PH. *Ythdf2* can bind and degrade multiple cell cycle-related mRNAs and consequently play a role in the pathophysiology of various diseases.^{11,12} However, it remains obscure whether *Ythdf2* can modulate PSMC hyperproliferation through the regulation of cell cycle genes. With advances in RNA research technology and an increased focus on m⁶A modification, the intricate mechanisms by which m⁶A effectors regulate various physiological and pathological processes have been gradually elucidated. On this basis, *Ythdf2* has been shown to play a role in facilitating the translation or stability of the m⁶A-modified mRNAs.^{13,14} Additionally, studies have indicated that the fate of *Ythdf2*-regulated m⁶A-modified transcripts may differ across various cell types.¹⁵ These findings indicate that greater investigation is necessary to better understand the regulatory mechanisms of *Ythdf2* that contribute to hyperproliferative PSMCs.

In the current study, we have comprehensively assessed the expression patterns and the underlying molecular mechanisms by which *Ythdf2* contributes to PSMC proliferation, vascular remodeling, and PH. Increased expression of *Ythdf2* protein was found in PSMCs of patients with PH and animal models of PH, as well as in platelet-derived growth factor (PDGF)-treated PSMCs. For the first time, SMC-specific *Ythdf2*-deficient mice were utilized for exploring the potential function and downstream mechanism of *Ythdf2* in PSMCs and in PH. Genetic ablation of *Ythdf2* in PSMCs improved cardiac dysfunction, alleviated PVR, and mitigated the proliferation of PSMCs both in vivo and in vitro. Mechanically, we elucidated that *Ythdf2* binds to and stabilizes *Myadm* mRNA in a noncanonical function of m⁶A methylation. Together, our results suggest that *Ythdf2* promotes PSMC proliferation and vascular remodeling through an m⁶A/*Myadm*/p21 axis, which may provide a potential and effective therapeutic target against the excessive proliferation of PSMCs in the pathogenesis of PH.

METHODS

All supporting data are available within the article and its online Supplementary Files.

Animals

The Animal Core Facility of Nanjing Medical University approved the present protocol for all animal experiments in this study (IACUC-2001008, 2004007). The *Ythdf2*^{flxed} mice used in this study have been described in detail in our previous study.⁹ To generate smooth muscle cell-specific *Ythdf2*-deficient mice, we crossed *Ythdf2*^{flxed} mice with *SM22α*^{Cre} mice to generate *Ythdf2*^{SM22α Cre} mice. Adult male Sprague-Dawley rats and C57BL/6 mice were purchased from the Animal Core Facility of Nanjing Medical University. All procedures and analyses were performed in a blinded manner.

Statistical Analyses

Data were presented as mean±SE, and the in vitro data are analyzed with at least 3 separate experimental repeats. Curve fitting was completed using GraphPad Prism 5.0 software. Statistical significance was assessed using an unpaired 2-tailed Student's *t* test and a 1-way ANOVA with a Tukey post hoc test wherever appropriate. A value of *P*<0.05 was defined as statistically significant. Randomization and blind analyses were used whenever possible.

RESULTS

Ythdf2 Is Significantly Upregulated in PSMCs of Patients With Pulmonary Arterial Hypertension (PAH) and Animal Models of PH

To investigate the role of *Ythdf2* in PH-PASMCs, we first examined YTHDF2 expression in lung slices of PAH patients and healthy controls. Immunohistochemical staining showed that YTHDF2 is widely distributed in all layers of small PAs in patients with PAH, especially in the vascular middle layer (Figure S1A). As shown in Figure 1A through 1D, immunofluorescence staining also revealed that *Ythdf2* is robustly enriched in PSMCs of lung sections from patients with PAH, monocrotaline or SU5416/hypoxia (Su/Hx)-induced PH rats, as well as Su/Hx-induced PH mice. Similarly, significant increases in *Ythdf2* protein levels were observed in PAs (Figure 1E) and PSMCs (Figure 1F) obtained from monocrotaline or Su/Hx-induced PH rats and Su/Hx-induced PH mice. These data indicate that the upregulation of *Ythdf2* in PSMCs is probably involved in the pathophysiological process of PH.

YTHDF2 Protein Expression Is Increased Through Changes in Translation in PDGF-Treated PSMCs

PASMC proliferation is a central event in the pathogenesis of vascular remodeling and PH. PDGF is a powerful

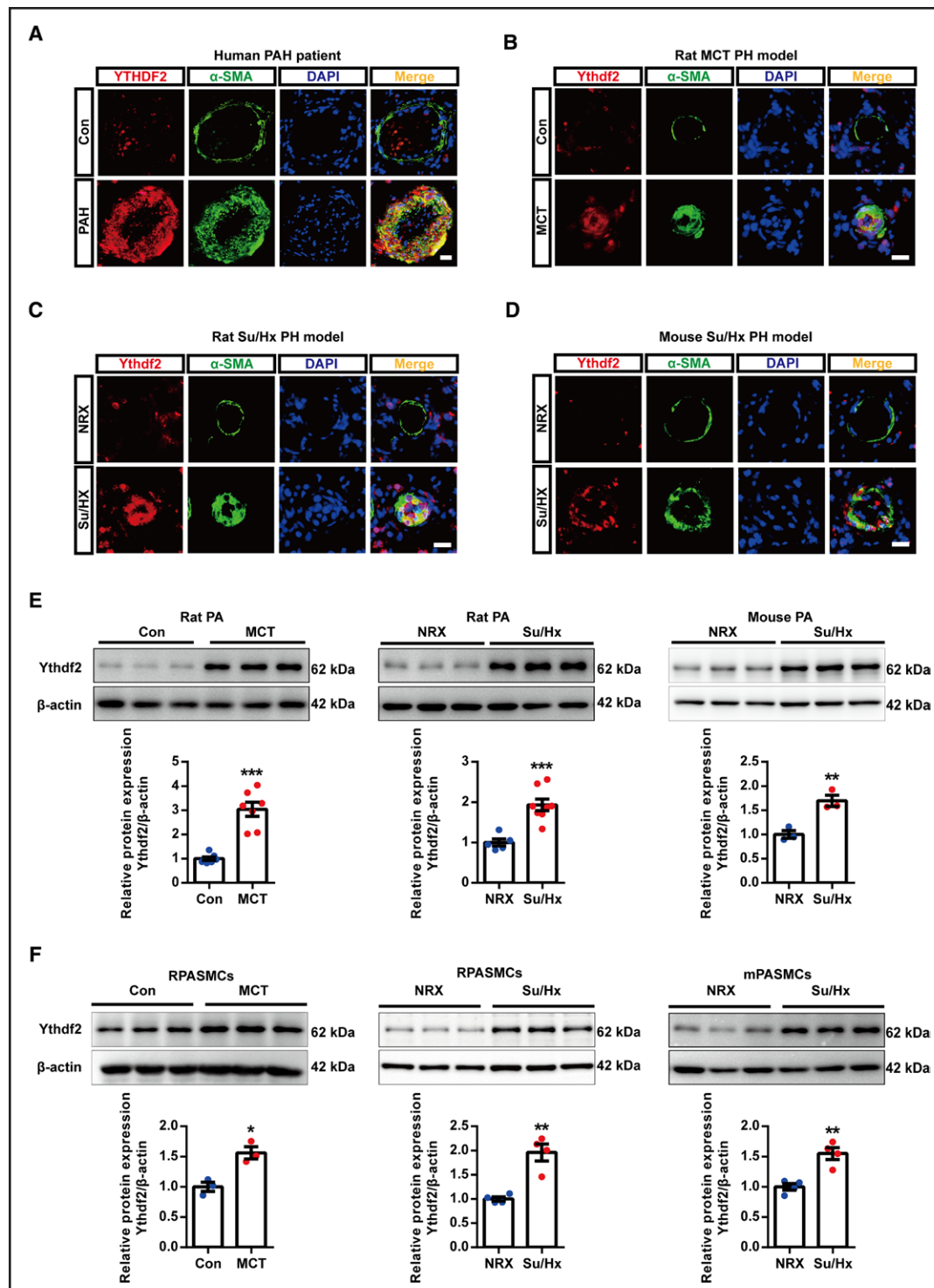


Figure 1. Increased Ythdf2 expression in PSMCs is associated with PH.

A through **D**, Representative immunofluorescence of Ythdf2 (red) and α -SMA (green) in pulmonary arteries (PAs) of human (**A**), rat (**B** and **C**), and mouse (**D**), nuclei were counterstained with DAPI (blue), scale bars=20 μ m. **E**, Representative immunoblots and relative densitometric analysis of Ythdf2 protein expression in PAs of rat PH (MCT and Su/Hx) models and mouse PH model (Su/Hx) normalized to β -actin, $n=3$ to 8 per group. **F**, Representative immunoblots and relative densitometric analysis of Ythdf2 protein expression in primary cultured PSMCs of rat PH (MCT and Su/Hx) models and mouse PH model (Su/Hx) normalized to β -actin; the experiments were done using primary cells isolated from 3 to 4 different male animals in each group. For bar graphs, data are shown as mean \pm SE; P values were determined by an unpaired 2-tailed Student's t test; * $P<0.05$, ** $P<0.01$, and *** $P<0.001$. MCT indicates monocrotaline; α -SMA, α -smooth muscle actin; NRX, normoxia; Su/Hx, SU5416/hypoxia; PAH, pulmonary arterial hypertension; PA, pulmonary arteries; PSMCs, pulmonary artery smooth muscle cells; RPASMCs, rat pulmonary artery smooth muscle cells; mPASMCs, mouse pulmonary artery smooth muscle cells; and Ythdf2, YTH N6-methyladenosine RNA binding protein 2.

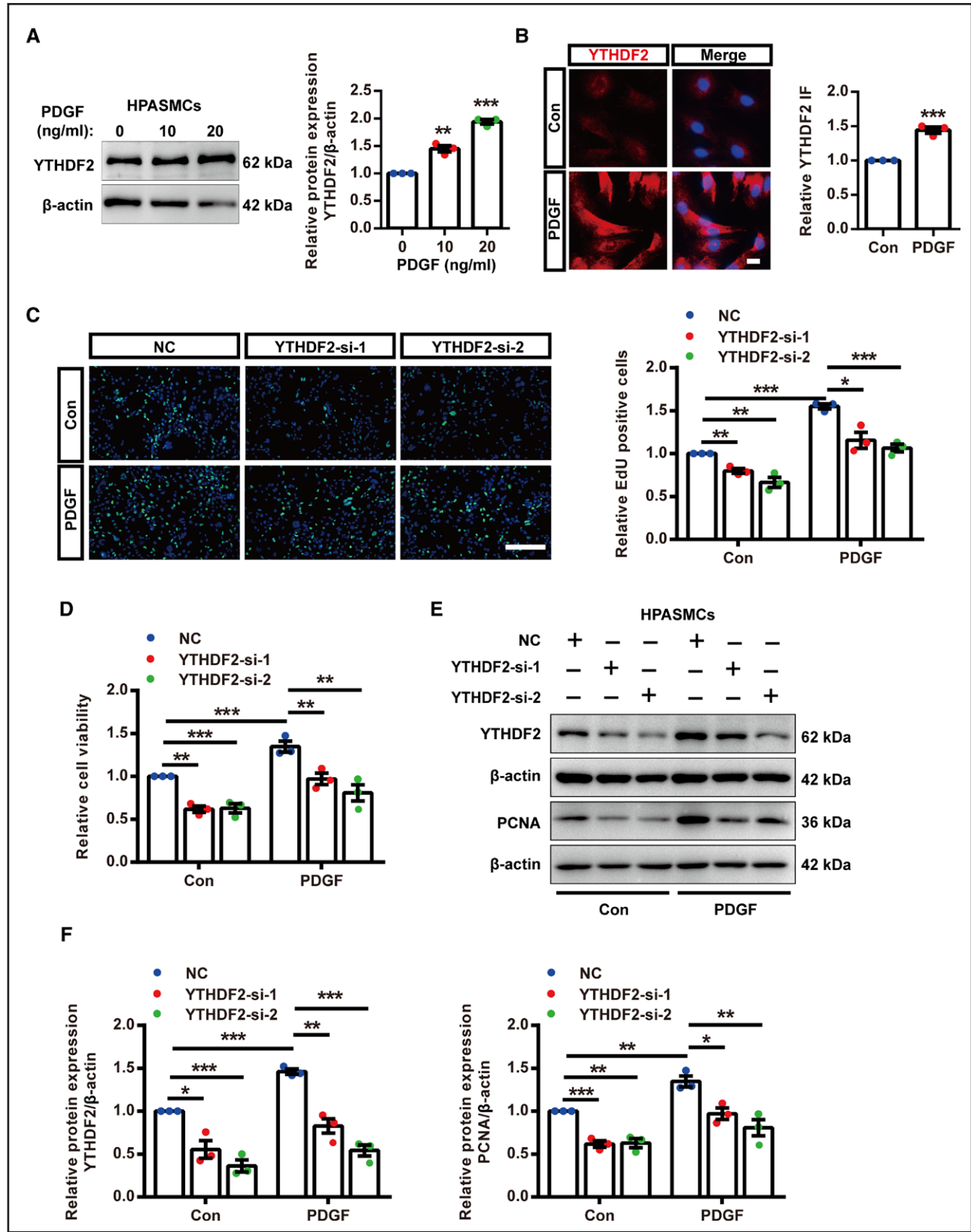


Figure 2. YTHDF2 deficiency inhibits PDGF-induced proliferation of HPASMCs. **A**, Representative immunoblots and relative densitometric analysis of YTHDF2 protein expression in HPASMCs with or without PDGF treatment. **B**, Representative immunofluorescence and quantitation of YTHDF2 (red) in HPASMCs (at least 15 randomly chosen fields were evaluated) exposed to PDGF treatment (20 ng/mL) for 24 h, nuclei were counterstained with DAPI (blue), scale bars=20 μ m. **C**, HPASMCs proliferation analysis was conducted by EdU assay. **Left**, Representative fluorescence microscopic images of EdU-positive cells (green) (Continued)

mitogen and is well known to stimulate a high level of proliferation in PASMCs.¹⁶ Western blot analysis and immunofluorescence staining analysis showed that elevated Ythdf2 protein expression is observed in rat pulmonary artery smooth muscle cells, mPASMCs (mouse pulmonary artery smooth muscle cells), and HPASMCs (human pulmonary artery smooth muscle cells) in response to PDGF treatment (Figure 2A and 2B; Figure S2A). Interestingly, the mRNA level of *Ythdf2* in PASMCs treated with PDGF was found to be comparable to those in the control group (Figure S3A). To further investigate the discord between the protein and mRNA levels of *Ythdf2*, an inhibitor of protein synthesis (cycloheximide) was used to assess the protein stability and translation efficiency of *Ythdf2* in PASMCs with or without PDGF treatment. The findings revealed that the rates of degradation of Ythdf2 protein were consistent between control PASMCs and those exposed to PDGF (Figure S3B), suggesting that PDGF-induced Ythdf2 expression was not influenced by protein stability. Nevertheless, PDGF treatment resulted in a further increase in Ythdf2 protein expression in PASMCs compared with the control group in the presence of MG-132 (Figure S3C). These findings collectively support the hypothesis that PDGF treatment enhances the translational efficiency of Ythdf2 protein in PASMCs.

YTHDF2 Deficiency Ameliorates the Proliferation of PDGF-Treated HPASMCs

Excessive proliferation of PASMCs and hypertrophic inward remodeling is a major driver of increased PVR in the pathogenesis of PH. Here, silencing *YTHDF2* in HPASMCs with siRNA significantly abolished PDGF-induced proliferation of HPASMCs, as determined by the 5-ethynyl-2-deoxyuridine assay (Figure 2C) and the Cell Counting Kit-8 assay (Figure 2D). Consistently, PDGF significantly increased PCNA (proliferating cell nuclear antigen) protein levels, whereas *YTHDF2* silencing significantly reduced the expression of PCNA in HPASMCs (Figure 2E and 2F).

Genetic Deletion of *Ythdf2* in SMCs Confers Protection in the Su/Hx Model of PH

To determine the function relevance of PASC *Ythdf2* in the development of PH, we next generated mice with

Ythdf2 deficiency in SMCs by breeding *Ythdf2*^{flxed} mice with *SM22α*^{Cre} transgenic mice (Figure S4A). Compared with PASCs in *Ythdf2*^{flxed} (wild-type) mice, decreased Ythdf2 expression was observed in PASCs of *Ythdf2*^{SM22α Cre} (cKO) mice both in vitro and in vivo (Figure S4B and S4C). To further clarify the function of *Ythdf2* in PASCs during PH pathogenesis, both *Ythdf2*^{flxed} and *Ythdf2*^{SM22α Cre} mice were subjected to Su/Hx treatment for 4 weeks to induce PH. As shown in Figure 3A and 3B, *Ythdf2* deficiency attenuated the increase in right ventricular systolic pressure and Fulton index (right ventricle/[left ventricle+septum]) in Su/Hx mice. Echocardiography also showed that *Ythdf2* deficiency significantly improved the pulmonary artery velocity time integral as well as the ratio of pulmonary artery acceleration time to ejection time caused by Su/Hx treatment (Figure 3C). H&E and immunofluorescence staining showed that *Ythdf2* deficiency in SMCs attenuates the occlusion and muscularization of PAs in Su/Hx-induced PH mice (Figure 3D). Furthermore, we observed fewer proliferating PASCs in the PAs of *Ythdf2*^{SM22α Cre} mice as compared with the PAs of *Ythdf2*^{flxed} mice in Su/Hx-treated groups (Figure 3E). In parallel, the expression of Pcn protein was decreased in the lungs of *Ythdf2*^{SM22α Cre} mice relative to *Ythdf2*^{flxed} mice in Su/Hx-treated groups (Figure S5A). In summary, these results support the hypothesis that SMC *Ythdf2* contributes to the development of PH in mice.

Ythdf2 Promotes the Proliferation of Primary mPASMCs

Next, we investigated the effect of *Ythdf2* on cell proliferation in mPASMCs isolated from *Ythdf2*^{flxed} and *Ythdf2*^{SM22α Cre} mice. Both Cell Counting Kit-8 and 5-ethynyl-2-deoxyuridine assays showed that loss of *Ythdf2* in mPASMCs significantly reduced cell proliferation in the absence or presence of PDGF (Figure 4A and 4B). Western blot analysis showed that the expression level of Pcn protein was lower in PASCs isolated from *Ythdf2*^{SM22α Cre} mice as compared with *Ythdf2*^{flxed} mice (Figure 4C). Moreover, our data also demonstrated that overexpression of *Ythdf2* enhanced cell viability (Figure 4D) and cell proliferation (Figure 4E) in primary mPASMCs. Meanwhile, Pcn protein expression level was also significantly increased in *Ythdf2*-overexpressing mPASMCs as compared with control cells (Figure 4F).

Figure 2 Continued. and nuclei were counterstained with DAPI (blue), scale bars=200 μm. **Right,** Quantitative measurement of EdU incorporation into HPASMCs, at least 15 randomly chosen fields were evaluated. **D,** Cell viability in HPASMCs was measured by the cell counting kit-8 assay. **E and F,** YTHDF2 and PCNA protein levels were measured by Western blot, β-actin was used as a loading control. For **C** through **F,** HPASMCs were transfected with YTHDF2 siRNA (50 nmol/L) using RNAiMax for 48 h before PDGF treated for 24 h. For **A** through **F,** the experiments were done using cell lines isolated from 3 different males, and the results are representative of 3 separate experiments. For bar graphs, data are shown 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. EdU indicates 5-ethynyl-2-deoxyuridine; HPASMCs, human pulmonary artery smooth muscle cells; NC, negative control; PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor; si-1, siRNA-1; si-2, siRNA-2; and YTHDF2, YTH N6-methyladenosine RNA binding protein 2.

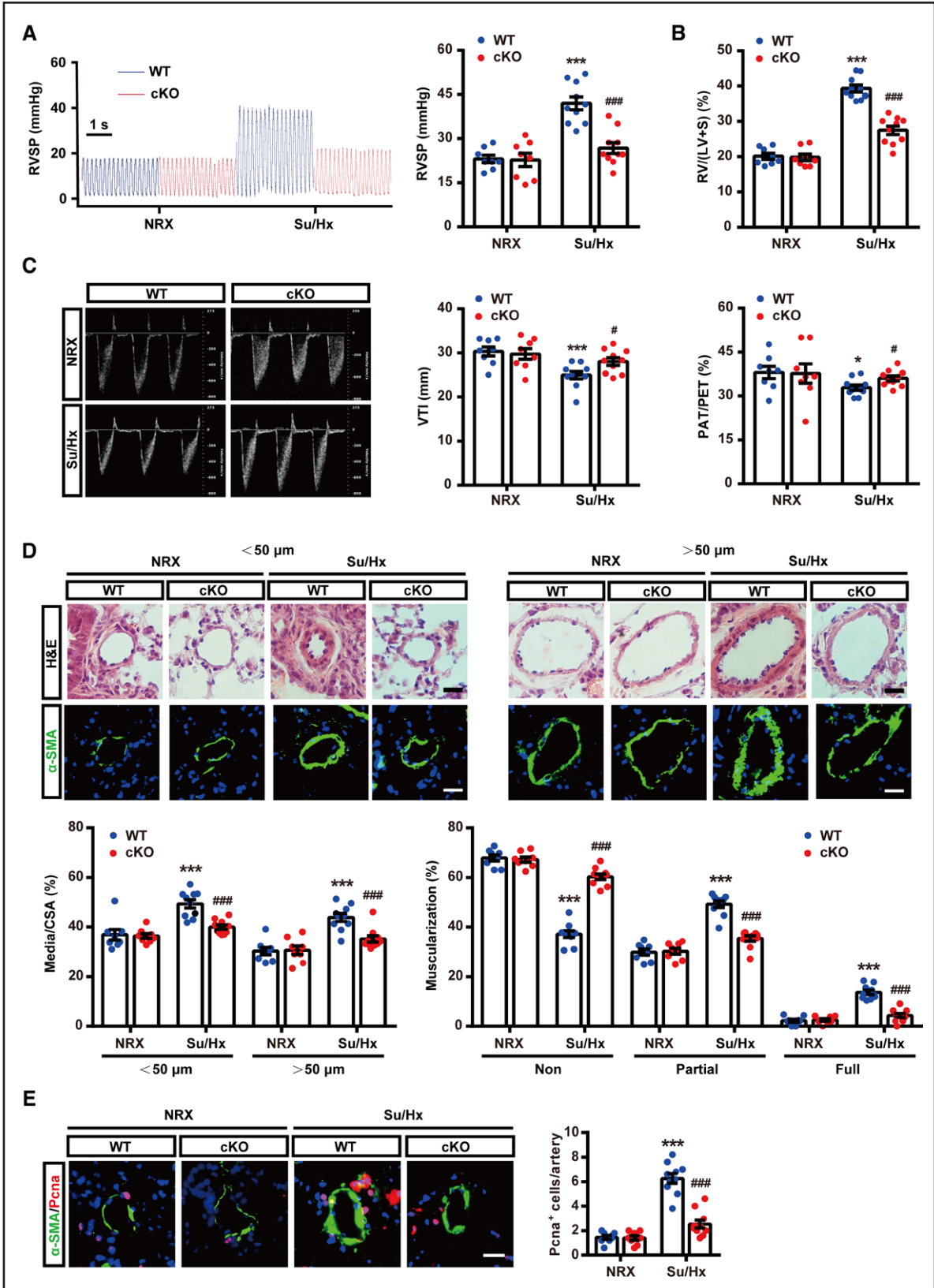


Figure 3. SMC-specific *Ythdf2* deletion confers protection against experimental PH in Su/Hx-induced PH mouse model. SMC-specific *Ythdf2* knockout mice were generated by crossing noninducible *SM22 α ^{Cre}* transgenic mice (C57BL/6 background) with *Ythdf2^{flxed}* mice. **A** through **C**, RVSP (**A**), the ratio of RV to LV wall plus septum (S; RV/[LV+S]; **B**), the velocity time integral (VTI), and the ratio of pulmonary artery accelerate time to ejection time (PAT/PET; **C**) in *Ythdf2^{flxed}* (WT) mice and *Ythdf2^{SM22 α Cre}* (cKO) mice after 4 weeks of normoxia or Su/Hx treatment. **D**, H&E staining and α -SMA (green) immunofluorescent staining of the pulmonary vascular vessels, (Continued)

Myadm Is a Target of m⁶A Modification and Ythdf2 in PSMCs in PH

To determine the mechanisms by which *Ythdf2* functions in PSMCs in the PH pathogenesis, we interrogated the downstream targets of *Ythdf2* using proteomic analysis. The differentially expressed proteins in mPSMCs from *Ythdf2*^{flxed} versus *Ythdf2*^{SM22α Cre} mice were visualized as a volcano plot (Figure 5A). Previous studies have shown that *Ythdf2* induces target mRNA degradation by recruiting the mRNA degradation system and thus should universally inhibit the protein expression of the corresponding targets.^{10,17} Interestingly, our results showed that 29 proteins were significantly upregulated, whereas 98 proteins were significantly downregulated in mPSMCs from *Ythdf2*^{SM22α Cre} mice as compared with *Ythdf2*^{flxed} mice. Gene set enrichment analysis was conducted on these differentially expressed proteins, revealing a noteworthy enrichment in the pathway associated with negative regulation of smooth muscle cell proliferation (Figure S6A). This finding suggests a negative association between the loss of *Ythdf2* expression and PSMC proliferation. Among the identified proteins, *Myadm* was chosen as a focal point for this study due to its significant role in promoting PSMC proliferation in PH.^{18–20} Previous research has demonstrated that *Myadm* facilitates PSMC proliferation by suppressing the expression of the cell cycle kinase inhibitor p21.¹⁹ Here, as shown in Figure 5B, the protein expression level of *Myadm* was lower in PSMCs from *Ythdf2*^{SM22α Cre} mice versus PSMCs from *Ythdf2*^{flxed} mice in the absence or presence of PDGF, followed by a significant increase in the expression level of p21 (Figure 5C). Correspondingly, upregulation of *Ythdf2* increased the protein level of *Myadm* and decreased the protein level of p21 in mPSMCs (Figure S7A). In addition, downregulation of *Myadm* expression and upregulation of p21 expression were also detected in PAs of *Ythdf2*^{SM22α Cre} mice compared with *Ythdf2*^{flxed} mice under PH conditions in vivo (Figure S8A and S8B).

Notably, analysis of m⁶A RNA immunoprecipitation sequencing and *Ythdf2* RNA immunoprecipitation sequencing of mPSMCs data sets revealed that there are 2 good fits between the m⁶A peaks and the *Ythdf2*-binding peaks in the 5′ untranslated region (peak 1) and coding sequence region (peak 2) of *Myadm* mRNA (Figure 5D). Further study confirmed that m⁶A-modified

Myadm mRNA in the coding sequence region (peak 2) rather than in the 5′ untranslated region (peak 1) was significantly increased in PDGF-treated mPSMCs as compared with controls (Figure 5E). *Ythdf2* RNA immunoprecipitation analysis also revealed significantly higher enrichment of *Myadm* in the *Ythdf2* immunoprecipitated fragment in mPSMCs with PDGF treatment (Figure 5E). Moreover, reduced MYADM protein levels and increased P21 protein levels were observed in YTHDF2-silenced HPASMCs with or without PDGF treatment as compared with controls (Figure 5F). Collectively, these data support the hypothesis that *Myadm* expression is regulated by *Ythdf2* in an m⁶A-dependent manner in PSMCs, which influences p21 expression.

Ythdf2 Promotes PSMCs Proliferation in PH by Stabilizing Myadm Transcripts

Recently, *Ythdf2* was reported to stabilize mRNA transcripts in an m⁶A-dependent manner that is distinct from its typical role in degrading mRNAs.²¹ To further explore the mechanisms underlying the ability of *Ythdf2* to regulate *Myadm* protein expression, we next examined the mRNA expression level of *Myadm* in PSMCs with or without *Ythdf2*. Results showed that *Myadm* mRNA was significantly downregulated in mPSMCs from *Ythdf2*^{SM22α Cre} mice compared with those from *Ythdf2*^{flxed} mice and that *Ythdf2*-overexpression enhanced the mRNA expression level of *Myadm* in mPSMCs (Figure 6A). These results indicate that *Ythdf2* regulates *Myadm* expression at the RNA level. RNA stability in mPSMCs was investigated by suppressing new RNA synthesis with actinomycin D. Real-time quantitative polymerase chain reaction analysis revealed that the stability of *Myadm* mRNA was markedly reduced in mPSMCs lacking *Ythdf2* (Figure 6B), which further supports the positive role of *Ythdf2* in regulating *Myadm* mRNA stability. To confirm the role of m⁶A modification in this process, *Mettl3*-overexpression was used to enhance the m⁶A levels. We found that *Mettl3*-overexpression in mPSMCs increased both the mRNA and protein levels of *Myadm* and, correspondingly, decreased the expression of p21, while *Ythdf2* deficiency diminished these effects (Figure 6C and 6D; Figure S9A). In addition, HPASMCs with YTHDF2 inhibition also displayed similar results (Figure S10A through S10D). To determine whether *Ythdf2* regulates PSMC proliferation

Figure 3 Continued. quantification of medial wall thickness index (each point represents the area ratios of >20 vessels per animal), and proportion of non, partially, or fully muscularized pulmonary arteries are shown (40–60 intra-acinar vessels were evaluated for each animal), scale bars=20 μm. **E**, Immunofluorescence staining of lung samples from WT and cKO mice for α-SMA (green) and PCNA (red) after 4 weeks of normoxia or Su/Hx treatment, and Pcn⁺ cells were quantified in each pulmonary artery (>20 vessels per animal were evaluated), scale bars=20 μm. For **A** through **E**, n=8 to 10 per group. For bar graphs, data are shown as mean±SE; *P* values were determined by 1-way ANOVA with Tukey post hoc test; **P*<0.05, ****P*<0.001 vs WT (NRX) group; #*P*<0.05, ###*P*<0.001 vs WT (Su/Hx) group. cKO indicates *Ythdf2*^{SM22α Cre}; CSA, cross-sectional area; H&E, hematoxylin and eosin; LV, left ventricle; NRX, normoxia; Pcn⁺, proliferating cell nuclear antigen; PH, pulmonary hypertension; RVSP, right ventricular systolic pressure; α-SMA, α-smooth muscle actin; Su/Hx, SU5416/hypoxia; SMC, smooth muscle cell; WT, *Ythdf2*^{flxed}; and Ythdf2, YTH N6-methyladenosine RNA binding protein 2.

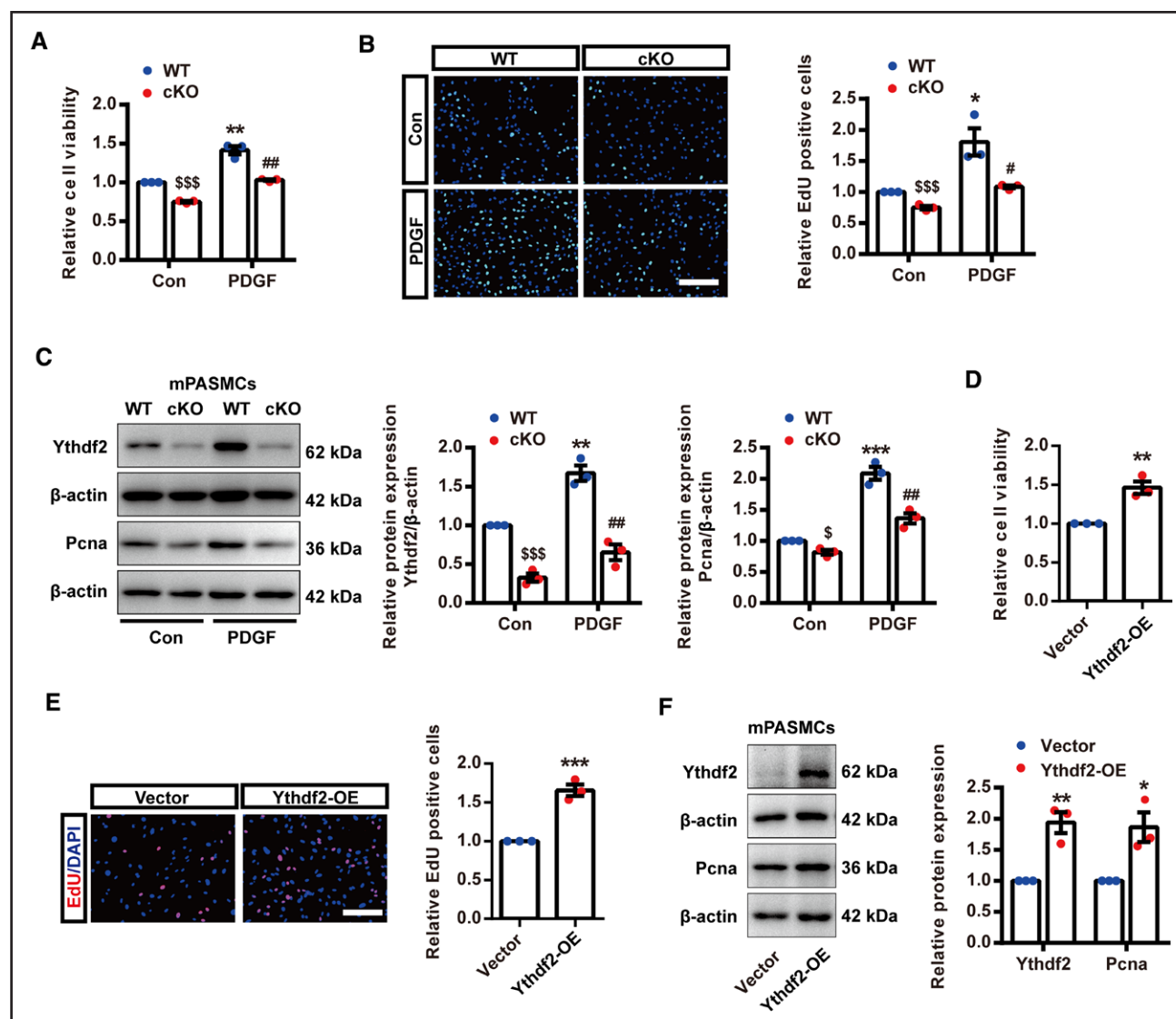


Figure 4. *Ythdf2* regulates the proliferation of primary mPASCs.

Primary mPASCs were generated from *Ythdf2*^{flxed} (WT) or *Ythdf2*^{SM22α Cre} (cKO) mice. **A** and **B**, The cell viability and proliferation were determined by cell counting kit-8 (**A**) and EdU assays (**B**) in mPASCs from WT and cKO mice with or without PDGF treatment. **C**, *Ythdf2* and *Pcn* protein levels in mPASCs from WT and cKO mice with or without PDGF treatment were measured by Western blot, β -actin was used as a loading control. **D** and **E**, Cell counting kit-8 (**D**) and EdU assays (**E**) were conducted in *Ythdf2*-overexpression adenovirus (*Ythdf2*-OE) or control virus (Vector)-infected mPASCs. **F**, *Ythdf2* and *Pcn* protein levels in mPASCs transduced with *Ythdf2*-overexpression adenovirus or control virus were measured by Western blot, β -actin was used as a loading control. For **A** through **F**, the experiments were done using primary cells isolated from 3 different male animals in each group, and the results are representative of 3 separate experiments. For the quantitation of EdU-positive cells in **B** and **E**, at least 15 randomly chosen fields were evaluated. For bar graphs, data are shown as mean \pm SE; *P* values were determined by unpaired 2-tailed Student's *t* test or 1-way ANOVA with Tukey post hoc test; **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 vs WT (Con) group or Vector group. #*P* < 0.05, ##*P* < 0.01 vs WT (PDGF) group. cKO indicates *Ythdf2*^{SM22α Cre}; EdU, 5-ethynyl-2-deoxyuridine; mPASCs, mouse pulmonary artery smooth muscle cells; *Pcn*, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor; WT, *Ythdf2*^{flxed}; and *Ythdf2*, YTH N6-methyladenosine RNA binding protein 2.

through the *Ythdf2*/*Myadm*/*p21* pathway, we next evaluated whether *Myadm* silencing could diminish the excessive proliferation of PASCs triggered by *Ythdf2*-overexpression. In *Ythdf2*-overexpressing mPASCs, the increased cell viability, cell proliferation, and *Pcn* protein expression, as well as the decreased *p21* protein expression, were reversed by *Myadm* silencing (Figure 6E and 6F; Figure S9B). Together, these findings indicate that

Ythdf2 promotes PASC proliferation, at least in part, through the *Ythdf2*/*Myadm*/*p21* pathway (Figure S11).

DISCUSSION

The m⁶A modification of RNA has been intricately linked to PASC proliferation and the development of PH. This has been demonstrated in global *Ythdf1* knockout mice,

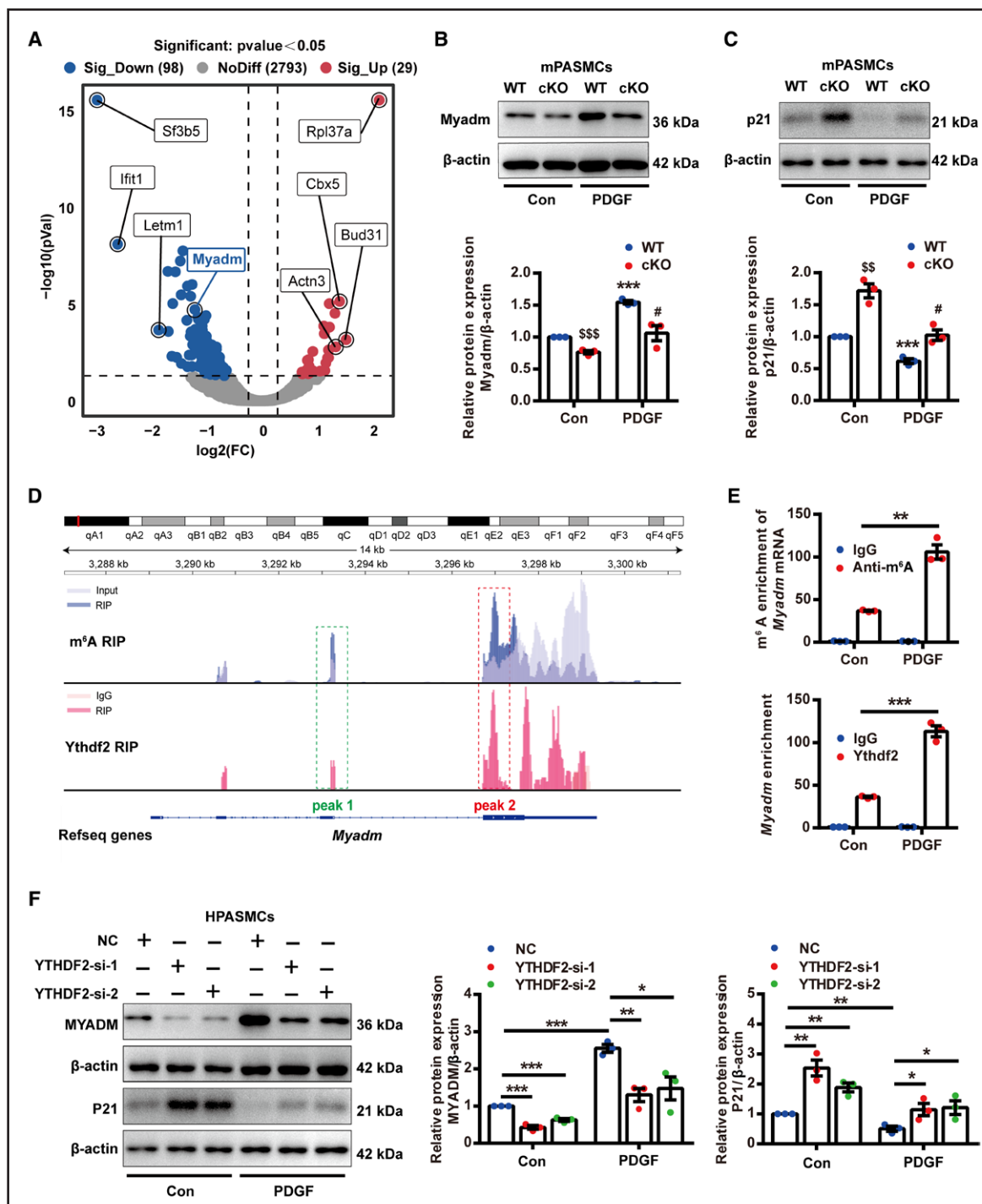


Figure 5. Myadm mRNA is modified by m⁶A and mediated by the m⁶A reader Ythdf2 in primary mPAMSCs.

A, The volcano plot showing the differentially expressed proteins in mPAMSCs of WT and cKO mice after 24 h of PDGF treatment, $n=4$ per group (significance cutoff $P<0.05$). **B** and **C**, Immunoblotting of Myadm and p21 in mPAMSCs of WT and cKO mice with or without PDGF treatment. **D**, IGV analysis for potential m⁶A-modified peaks and Ythdf2 binding sites of *Myadm* mRNA in PDGF-treated mPAMSCs. **E**, **Top**, MeRIP-qPCR was applied to detect the m⁶A enrichment of *Myadm* mRNA in mPAMSCs with or without PDGF treatment. **Bottom**, RIP analysis of Ythdf2 protein binding to *Myadm* mRNA in mPAMSCs with or without PDGF treatment. **F**, Protein levels of MYADM and P21 in HPAMSCs were measured by Western blot, and β-actin was used as a loading control. HPAMSCs were transfected with YTHDF2 siRNA (50 nmol/L) using RNAiMax for 48 h before being treated with PDGF for 24 h. For **B** through **F**, the experiments were done using cells isolated from 3 different individuals in each group, and the results are representative of 3 separate experiments. For bar graphs, data are shown as mean±SE; P values were determined by 1-way ANOVA with Tukey post hoc test; $^{\#}P<0.05$ vs WT (Con) group. $^{\#}P<0.05$ vs WT (PDGF) group. cKO indicates *Ythdf2*^{SM22α Cre}; HPAMSCs, human pulmonary artery smooth muscle cells; m⁶A, N⁶-methyladenosine; mPAMSCs, mouse pulmonary artery smooth muscle cells; NC, negative control; PDGF, platelet-derived growth factor; si-1, siRNA-1; si-2, siRNA-2; WT, *Ythdf2*^{flxed}; and Ythdf2, YTH N⁶-methyladenosine RNA binding protein 2.

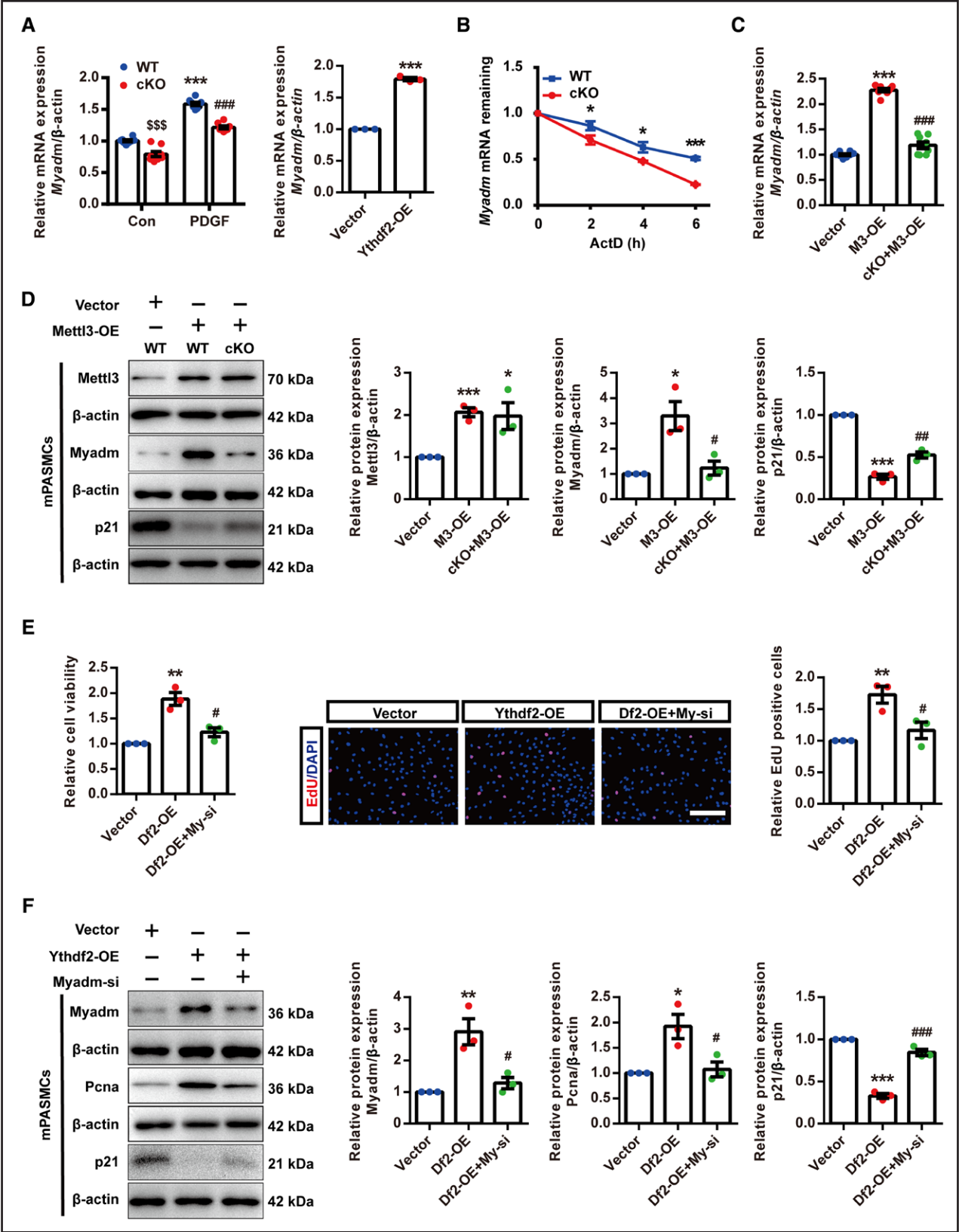


Figure 6. Ythdf2 promotes the proliferation of primary mPASCs by stabilizing *Myadm* mRNA in an m⁶A-dependent manner. **A, Left**, The mRNA levels of *Myadm* in mPASCs from WT and cKO mice with or without PDGF treatment were detected by RT-qPCR analysis. **Right**, The mRNA levels of *Myadm* in *Ythdf2*-overexpression adenovirus-infected mPASCs were detected by RT-qPCR analysis. **B**, RT-qPCR analysis of the decay rate of *Myadm* mRNA at the indicated times after Actinomycin D treatment in mPASCs from WT and cKO mice under PDGF exposure. **C and D**, The mRNA level of *Myadm* (**C**) as well as the protein levels of Mettl3, Myadm, (Continued)

which have reduced PSMC proliferation and PVR following Su/Hx.⁴ However, given the multifaceted roles of *Ythdf1* in inflammation, epithelial-mesenchymal transition, and stem cell self-renewal, it is important to acknowledge the limitations of using global *Ythdf1* knockout mice in our previous study.²² Therefore, in this investigation, SMCs-specific *Ythdf2* knockout mice were used to assess the distinct functions of *Ythdf2* in the proliferation of PSMCs and the pathogenesis of PH, thereby eliminating the potential influences of *Ythdf2* in other cell types during the progression of PH. The findings of our study provide novel perspectives on the roles of m⁶A modification in the cellular and molecular regulatory processes of PVR in PH and will aid in the identification of novel therapeutic targets to improve pharmaceutical interventions for PH.

Increased PVR is one of the major pathological changes during the progression of PH, and the excessive proliferation of PSMCs is a key mechanism of pathological PVR. Irreversible vascular remodeling, accompanied by chronic vasoconstriction, ultimately leads to heart failure and death in PH patients.²³ Although both clinical and basic science studies have recognized that PSMC proliferation is the most significant pathological feature in the pathogenesis of PH, a more detailed understanding of the underlying regulatory mechanisms and signaling pathways is needed. Indeed, the prognosis of PH patients remains poor due to the unmet clinical needs for targeting hyperproliferative PSMCs.

Impaired regulation of the cell cycle plays a major role in the development of various proliferative diseases. Cell cycle inhibition has been demonstrated to be therapeutically beneficial in PH animals.^{24,25} m⁶A RNA methylation can modulate multiple cell cycle-related proteins via different m⁶A readers. *Ythdf2* has been reported to promote mitotic entry and be regulated by cell cycle mediators.²⁶ Moreover, *Ythdf2* can enhance the degradation of *CDKN1B* and *KDM1A* mRNA and subsequently contribute to the progression of several cancers.^{11,12} PDGF promotes PSMC proliferation by driving mitogenic signaling and has been frequently used as a PH stimulus. In our study, PDGF treatment increased the expression of Ythdf2 protein in PSMCs from humans, mice, and rats. Inhibition of *Ythdf2* in PSMCs by knockdown or knock-out approaches significantly alleviated the proliferation

of PSMCs induced by PDGF treatment and decreased the protein expression of PcnA while increasing that of p21. Our findings are supported by other studies showing increased protein levels of Ythdf2 in hypoxia-treated PSMCs.¹⁰ Accordingly, these results indicate that upregulation of *Ythdf2* is critically associated with the proliferation of PSMCs in PH.

In its most widely appreciated role as an m⁶A reader, *Ythdf2* transports m⁶A-modified transcripts to the processing body in the cytoplasm where they are degraded.^{27,28} However, a growing number of studies have revealed contradictory functions of *Ythdf2*, such as the ability to promote m⁶A mRNA translation and stabilize m⁶A-modified transcripts.^{13,14,21,29} Because of these seemingly contradictory effects on RNA expression, we utilized proteomic analysis to screen potential targets of *Ythdf2* in the proliferating PSMCs instead of transcriptomic analysis. Interestingly, the loss of *Ythdf2* expression in mPSMCs resulted in the upregulation of 29 proteins and the downregulation of 98 proteins, indicating that the biology of *Ythdf2* is considerably more complex than just the degradation of m⁶A-tagged mRNAs. Further studies also indicate that *Ythdf2* has a positive effect on m⁶A RNA preservation by stabilizing the *Myadm* transcript, supporting a similarly noncanonical function of *Ythdf2*. These data suggest that the regulatory mechanisms of *Ythdf2* in hyperproliferative PSMCs differ from those in hypoxia-treated alveolar macrophages in PH, indicating that m⁶A modification and the fate of m⁶A-modified transcripts are modulated in a cell-type-specific manner.

More recently, integrative network analysis has been used to identify cell-specific trans-regulators of the m⁶A modification.¹⁵ The temporal and spatial dynamics of the m⁶A modification may account for the diverse functions of Ythdf2 in various cells, tissues, and diseases. In the future, large-scale m⁶A sequencing or single-cell imaging of m⁶A-modified RNAs is expected to provide novel insights into the different mechanisms through which m⁶A selectively functions in certain physiological and pathological processes.

Although used as a myeloid-differentiated marker, *Myadm* is not confined to roles in the hematopoietic system. It has a close association with various cardiopulmonary diseases, including asthma, hypertension,

Figure 6 Continued. and p21 (**D**) were detected in mPSMCs from WT and cKO mice infected with a Mettl3-overexpression lentivirus or control virus (Vector). **E** and **F**, mPSMCs were transduced with a *Ythdf2*-overexpression adenovirus (*Ythdf2*-OE) for 24 h with or without *Myadm* siRNA transfection for another 48 h before cell viability and proliferation were determined by cell counting kit-8 assay, EdU assay (**E**), and the Western blot analysis of *Myadm*, PcnA, and p21 (**F**). For **E**, scale bars=200 μ m. For **A** through **F**, the experiments were done using primary cells isolated from 3 different male animals in each group, and the results are representative of 3 separate experiments. For the quantitation of EdU-positive cells in **E**, at least 15 randomly chosen fields were evaluated. For bar graphs, data are shown as mean \pm SE; *P* values were determined by unpaired 2-tailed Student's *t* test or 1-way ANOVA with Tukey post hoc test; \$\$\$*P*<0.001, **P*<0.05, ***P*<0.01 and ****P*<0.001 vs WT (Con) group or Vector group. **P*<0.05, ***P*<0.01 and ****P*<0.001 vs M3-OE group or Df2-OE group. ActD indicates actinomycin D; cKO, *Ythdf2*^{SM22 α Cre}; Df2-OE, *Ythdf2*-overexpression; EdU, 5-ethynyl-2-deoxyuridine; M3-OE, Mettl3-overexpression; My-si, *Myadm* siRNA; mPSMCs, mouse pulmonary artery smooth muscle cells; PDGF, platelet-derived growth factor; RT-qPCR, real-time quantitative polymerase chain reaction; WT, *Ythdf2*^{flxed}; and Ythdf2, YTH N6-methyladenosine RNA binding protein 2.

and PH.^{20,30,31} Targeting *Myadm* has been confirmed to improve hypoxia-induced PH and mitigate the detrimental effects on the cardiac-cerebral development of offspring from maternal PH in rats.^{18,20} Furthermore, overexpression of *Myadm* in PSMCs can promote the expression of cell cycle-related proteins, such as PCNA, Cyclin D1, and CDK2, while concurrently inhibiting the cell cycle kinase inhibitor p21 by promoting Klf4 nuclear export, all of which provides a basis for the excessive proliferation of PSMCs in PH.¹⁹ In this study, *Myadm* was identified as a target gene of *Ythdf2* based on multiomics sequencing analysis and its biological functions in PSMC proliferation. *Ythdf2* deficiency in PSMCs significantly inhibited the expression of *Myadm* and reciprocally upregulated the expression of *p21*. Overexpression of *Ythdf2* increased *Myadm* mRNA stability, whereas *Ythdf2* inhibition failed to extend the lifespan of *Myadm* mRNA. These data provide the first evidence that *Myadm* mRNA is modified by m⁶A and stabilized by *Ythdf2* in an m⁶A-dependent manner and that *Myadm* silencing blocks the pro-proliferative effects of *Ythdf2* overexpression in PSMCs. However, knockout of *Ythdf2* in *Mettl3*-overexpressing PSMCs could not entirely eliminate the expression of *Myadm*, suggesting that *Myadm* may also be regulated by other m⁶A readers in PSMCs. These results suggest that during the development of PH, increases in m⁶A labeling facilitate the recruitment of *Ythdf2* to *Myadm* mRNA, thereby enhancing *Myadm* mRNA stability and protein expression and driving the m⁶A/*Myadm*/p21 signaling pathway, which increases PSMCs proliferation and subsequently vascular remodeling.

In our prior investigation, we confirmed that molecular triggers of PH can bolster the stability of the *Ythdf1* protein, leading to elevated expression in PSMCs.⁴ Notably, the mRNA and protein levels of *Ythdf2* in PSMCs treated with PDGF also exhibited a divergent correlation. Subsequent analysis revealed that PDGF enhanced the translational efficacy of *Ythdf2* protein in PSMCs, a mechanism distinct from that governing *Ythdf1* regulation. Several studies have suggested that m⁶A modulators may be influenced by different posttranslational modifications, impacting their expression, function, and activity.^{32,33} However, the precise mechanisms governing the posttranslational modifications of m⁶A readers, such as *Ythdf1* and *Ythdf2*, in the context of PH, remain to be fully elucidated.

To date, a few studies have reported on the association between PSMCs, *Ythdf2*, and PH. However, the specific mechanisms and clinical significance of *Ythdf2* in hyperproliferative PSMCs remain unclear. Our study demonstrates that *Ythdf2* promotes PSMC proliferation and vascular remodeling through stabilizing *Myadm* mRNA in an m⁶A-dependent manner both in vivo and in vitro. Meanwhile, increased expression of *Ythdf2* is also observed in PAH patients, PH animal models, and

PDGF-treated PSMCs. Additionally, our study is the first to show a functional role for SMC *Ythdf2* in PH pathogenesis using a novel SMC-specific knockout mouse. Genetic ablation of *Ythdf2* alleviated the hemodynamic parameters and vascular remodeling in Su/Hx-induced PH mice. Moreover, the increased proliferation of PSMC resulting from *Ythdf2* overexpression was significantly reduced by subsequent *Myadm* knockdown. Overall, this study identifies a novel, PSMC-specific role for *Ythdf2* in the development of PH, and interventions targeting this pathway might be of value in the therapeutic approach to PH.

PERSPECTIVES

Our previous studies have demonstrated that m⁶A readers have important but poorly defined roles in the development of PH. This study focused on the role of *Ythdf2* in PSMCs, and our results showed that *Ythdf2* critically contributes to PSMC proliferation and PH development by stabilizing *Myadm* mRNA in an m⁶A-dependent manner. These novel results shed light on a deeper understanding of m⁶A RNA methylation and its role in PH pathogenesis and provide a promising therapeutic target to improve the treatment of PH.

ARTICLE INFORMATION

Received January 25, 2024; accepted May 10, 2024.

Affiliations

Department of Forensic Medicine (J.W., Y.S., Y.Z., D.L., Y.Y., F.C., L.H.), Key Laboratory of Targeted Intervention of Cardiovascular Disease, Collaborative Innovation Center for Cardiovascular Disease Translational Medicine (J.W., F.C., L.H.), State Key Laboratory of Reproductive Medicine (B.S.), and Key Laboratory of Cardiovascular and Cerebrovascular Medicine, Key Laboratory of Targeted Intervention of Cardiovascular Disease, Collaborative Innovation Center for Cardiovascular Disease Translational Medicine (Y.J.), Nanjing Medical University, China. Department of Rheumatology, the First Affiliated Hospital of Nanjing Medical University, China (Q.W., X.S.). Wuxi Lung Transplantation Center, Wuxi People's Hospital Affiliated with Nanjing Medical University, China (D.W., J.C., F.C.). Vascular Biology Center, Medical College of Georgia at Augusta University (D.F., F.C.).

Author Contributions

All authors listed contributed to this article; L. Hu, F. Chen, Y. Yu, and J. Wang contributed to conceptualization. J. Wang, L. Hu, Y. Shen, Y. Zhang, D. Lin, Q. Wang, X. Sun, D. Wei, B. Shen, J. Chen, Y. Ji, and D. Fulton contributed to methodology, human patient samples, animal models, and writing-original draft preparation, major editing, and formalizing. L. Hu, F. Chen, and J. Wang contributed to funding acquisition. All authors have read and agreed to the published version of the article.

Sources of Funding

This study was supported by the National Natural Science Foundation of China grants (82200057, 82225023, 82300069, and 82121001), the Natural Science Foundation of Jiangsu Province (BK20220321 and BK20230304), and the Natural Science Foundation of Jiangsu Higher Education Institutions of China (23KJB320005).

Disclosures

None.

Supplemental Material

Expanded Methods
Figures S1–S11
Tables S1–S3

REFERENCES

- Hassoun PM. Pulmonary arterial hypertension. *N Engl J Med*. 2021;385:2361–2376. doi: 10.1056/NEJMra2000348
- Ruopp NF, Cockrill BA. Diagnosis and treatment of pulmonary arterial hypertension: a review. *JAMA*. 2022;327:1379–1391. doi: 10.1001/jama.2022.4402
- Farishta M, Sankari A. Pulmonary hypertension due to lung disease or hypoxia. In: *StatPearls*. StatPearls Publishing; 2024.
- 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
- Zhang H, Wang D, Li M, Plecita-Hlavata L, D'Alessandro A, Tauber J, Riddle S, Kumar S, Flockton A, McKeon BA, et al. Metabolic and proliferative state of vascular adventitial fibroblasts in pulmonary hypertension is regulated through a microRNA-124/PTBP1 (polypyrimidine tract binding protein 1)/pyruvate kinase muscle axis. *Circulation*. 2017;136:2468–2485. doi: 10.1161/CIRCULATIONAHA.117.028069
- Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, Olschewski AJ, Pullamsetti SS, Schermuly RT, Stenmark KR, et al. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J*. 2019;53:1801887. doi: 10.1183/13993003.01887-2018
- Hoeper MM, Badesch DB, Ghofrani HA, Gibbs JSR, Gombert-Maitland M, McLaughlin VV, Preston IR, Souza R, Waxman AB, Grunig E, et al; STELLAR Trial Investigators. Phase 3 trial of sotatercept for treatment of pulmonary arterial hypertension. *N Engl J Med*. 2023;388:1478–1490. doi: 10.1056/NEJMoa2213558
- Zou Z, Sepich-Poore C, Zhou X, Wei J, He C. The mechanism underlying redundant functions of the YTHDF proteins. *Genome Biol*. 2023;24:17. doi: 10.1186/s13059-023-02862-8
- 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
- Liu P, Zhang A, Ding Y, Dai D, Li B, Liu SF, Xu J, Cheng Z, Zhao S, Zhao X, et al. m(6)A modification-mediated GRAP regulates vascular remodeling in hypoxic pulmonary hypertension. *Am J Respir Cell Mol Biol*. 2022;67:574–588. doi: 10.1165/rccb.2021-0429OC
- Huang CS, Zhu YQ, Xu QC, Chen S, Huang Y, Zhao G, Ni X, Liu B, Zhao W, Yin XY. YTHDF2 promotes intrahepatic cholangiocarcinoma progression and desensitizes cisplatin treatment by increasing CDKN1B mRNA degradation. *Clin Transl Med*. 2022;12:e848. doi: 10.1002/ctm.2848
- Li X, Zhang K, Hu Y, Luo N. YTHDF2 regulates cell growth and cycle by facilitating KDM1A mRNA stability. *Am J Pathol*. 2023;193:442–455. doi: 10.1016/j.ajpath.2022.12.010
- Yang Y, Yan Y, Yin J, Tang N, Wang K, Huang L, Hu J, Feng Z, Gao Q, Huang A. O-GlcNAcylation of YTHDF2 promotes HBV-related hepatocellular carcinoma progression in an N(6)-methyladenosine-dependent manner. *Signal Transduct Target Ther*. 2023;8:63. doi: 10.1038/s41392-023-01316-8
- Zhang C, Huang S, Zhuang H, Ruan S, Zhou Z, Huang K, Ji F, Ma Z, Hou B, He X. YTHDF2 promotes the liver cancer stem cell phenotype and cancer metastasis by regulating OCT4 expression via m6A RNA methylation. *Oncogene*. 2020;39:4507–4518. doi: 10.1038/s41388-020-1303-7
- An S, Huang W, Huang X, Cun Y, Cheng W, Sun X, Ren Z, Chen Y, Chen W, Wang J. Integrative network analysis identifies cell-specific trans regulators of m6A. *Nucleic Acids Res*. 2020;48:1715–1729. doi: 10.1093/nar/gkz1206
- Deng L, Chen J, Chen B, Wang T, Yang L, Liao J, Yi J, Chen Y, Wang J, Linneman J, et al. LncPTSR triggers vascular remodeling in pulmonary hypertension by regulating [Ca(2+)](i) in pulmonary arterial smooth muscle cells. *Am J Respir Cell Mol Biol*. 2022;66:524–538. doi: 10.1165/rccb.2020-0480OC
- Zhong L, Liao D, Zhang M, Zeng C, Li X, Zhang R, Ma H, Kang T. YTHDF2 suppresses cell proliferation and growth via destabilizing the EGFR mRNA in hepatocellular carcinoma. *Cancer Lett*. 2019;442:252–261. doi: 10.1016/j.canlet.2018.11.006
- Bai Y, Wang J, Chen Y, Lv T, Wang X, Liu C, Xue H, He K, Sun L. The miR-182/Myadm axis regulates hypoxia-induced pulmonary hypertension by balancing the BMP- and TGF-beta-signalling pathways in an SMC/EC-crosstalk-associated manner. *Basic Res Cardiol*. 2021;116:53. doi: 10.1007/s00395-021-00892-6
- Sun L, Lin P, Chen Y, Yu H, Ren S, Wang J, Zhao L, Du G. miR-182-3p/Myadm contribute to pulmonary artery hypertension vascular remodeling via a KLF4/p21-dependent mechanism. *Theranostics*. 2020;10:5581–5599. doi: 10.1071/tno.44687
- Wang J, Zhang Z, Liang C, Lv T, Yu H, Ren S, Lin P, Du G, Sun L. Targeting myadm to intervene pulmonary hypertension on rats before pregnancy alleviates the effect on their offspring's cardiac-cerebral systems. *Front Pharmacol*. 2021;12:791370. doi: 10.3389/fphar.2021.791370
- Dixit D, Prager BC, Gimple RC, Poh HX, Wang Y, Wu Q, Qiu Z, Kidwell RL, Kim LJ, Xie Q, et al. The RNA m6A reader YTHDF2 maintains oncogene expression and is a targetable dependency in glioblastoma stem cells. *Cancer Discov*. 2021;11:480–499. doi: 10.1158/2159-8290.CD-20-0331
- Chen Z, Zhong X, Xia M, Zhong J. The roles and mechanisms of the m6A reader protein YTHDF1 in tumor biology and human diseases. *Mol Ther Nucleic Acids*. 2021;26:1270–1279. doi: 10.1016/j.omtn.2021.10.023
- Naeije R, Richter MJ, Rubin LJ. The physiological basis of pulmonary arterial hypertension. *Eur Respir J*. 2022;59:2102334. doi: 10.1183/13993003.02334-2021
- Knight H, Abis G, Kaur M, Green HLH, Krasemann S, Hartmann K, Lynham S, Clark J, Zhao L, Ruppert C, et al. Cyclin D-CDK4 disulfide bond attenuates pulmonary vascular cell proliferation. *Circ Res*. 2023;133:966–988. doi: 10.1161/CIRCRESAHA.122.321836
- Weiss A, Neubauer MC, Yerabolu D, Kojonazarov B, Schlueter BC, Neubert L, Jonigk D, Baal N, Ruppert C, Dorfmüller P, et al. Targeting cyclin-dependent kinases for the treatment of pulmonary arterial hypertension. *Nat Commun*. 2019;10:2204. doi: 10.1038/s41467-019-10135-x
- Fei Q, Zou Z, Roundtree IA, Sun HL, He C. YTHDF2 promotes mitotic entry and is regulated by cell cycle mediators. *PLoS Biol*. 2020;18:e3000664. doi: 10.1371/journal.pbio.3000664
- Chai RC, Chang YZ, Chang X, Pang B, An SY, Zhang KN, Chang YH, Jiang T, Wang YZ. YTHDF2 facilitates UBXN1 mRNA decay by recognizing METTL3-mediated m(6)A modification to activate NF-kappaB and promote the malignant progression of glioma. *J Hematol Oncol*. 2021;14:109. doi: 10.1186/s13045-021-01124-z
- Lv D, Zhong C, Dixit D, Yang K, Wu Q, Godugu B, Prager BC, Zhao G, Wang X, Xie Q, et al. EGFR promotes ALKBH5 nuclear retention to attenuate N6-methyladenosine and protect against ferroptosis in glioblastoma. *Mol Cell*. 2023;83:4334–4351.e7. doi: 10.1016/j.molcel.2023.10.025
- Fu D, Si Q, Yu C, Han Z, Zang L. USF1-mediated ALKBH5 stabilizes FLII mRNA in an m6A-YTHDF2-dependent manner to repress glycolytic activity in prostate adenocarcinoma. *Mol Carcinog*. 2023;62:1700–1716. doi: 10.1002/mc.23609
- Tanyaratrisakul S, Dy ABC, Poverino F, Numata M, Ledford JG. Myeloid-associated differentiation marker is associated with type 2 asthma and is upregulated by human rhinovirus infection. *Front Immunol*. 2023;14:1237683. doi: 10.3389/fimmu.2023.1237683
- Zeller T, Schürmann C, Schramm K, Müller C, Kwon S, Wild PS, Teumer A, Herrington D, Schillert A, Iacoviello L, et al. Transcriptome-wide analysis identifies novel associations with blood pressure. *Hypertension*. 2017;70:743–750. doi: 10.1161/HYPERTENSIONAHA.117.09458
- Chen Y, Jiang Z, Yang Y, Zhang C, Liu H, Wan J. The functions and mechanisms of post-translational modification in protein regulators of RNA methylation: current status and future perspectives. *Int J Biol Macromol*. 2023;253:126773. doi: 10.1016/j.ijbiomac.2023.126773
- Wang J, Wang Z, Inuzuka H, Wei W, Liu J. PRMT1 methylates METTL14 to modulate its oncogenic function. *Neoplasia*. 2023;42:100912. doi: 10.1016/j.neo.2023.100912