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*Research Article*

# Inhibition of YAP1 Rescues Erectile Dysfunction by Inhibiting Phenotypic Modulation through Myocardin in Diabetic Rats

**Yangyan Lin**,[1] **Jialiang Hui**,[2] **Miaoxia Pan**,[3] **Li Wang**,[1] **Junqi Luo**,[1] **Zerong Chen**,[1] **Haibo Zhang**,[1] **and Anyang Wei**[1]

[1]*Department of Urology, Nanfang Hospital, Southern Medical University, Guangzhou, China*
[2]*Department of Transplantation, Nanfang Hospital, Southern Medical University, Guangzhou, China*
[3]*Department of Respiratory and Critical Care Medicine, Chronic Airways Diseases Laboratory, Nanfang Hospital, Southern Medical University, Guangzhou, China*

Correspondence should be addressed to Haibo Zhang; hai516@163.com and Anyang Wei; profwei@126.com

Yangyan Lin, Jialiang Hui, and Miaoxia Pan contributed equally to this work.

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*Background*. Phenotypic modulation was shown to be related to the pathological mechanism of diabetes mellitus erectile dysfunction (DMED), and excessive expression of YAP1 was measured in the cavernous tissues of rats with DMED. *Objectives*. This investigation was performed to explore the efficiency and mechanism of gene therapy through the knockdown of YAP1 in the field of ED treatment. *Materials and Methods*. Corpus cavernosum smooth muscle cells were obtained and cultivated in vitro employing the explant technique. EdU, CCK-8, and collagen gel lattice contraction assays were subsequently performed to assess cell proliferation and contractility, which indicate smooth muscle phenotypes. *Results*. Initially, overexpression of YAP1 enhanced the proliferation and expression of YAP1 and osteopontin (OPN) while reducing cell contractility and the expression of myocardin, $\alpha$-SMA, and calpontin, which is consistent with phenotypic modulation. Moreover, knocking down YAP1 resulted in the opposite effects, and the blockade of myocardin attenuated the reversal of phenotypic transformation. Then, 45 screened DMED rats were randomly divided into three equal groups, named streptozotocin (STZ)-induced DMED with intracavernosal injection of Ad-myocardin (DMED + myocardin), DMED with lenti-shYAP1 (DMED + shYAP1) and DMED with PBS (DMED + PBS), and 10 rats were in the negative control group. Staining with H&E and Masson's trichrome, immunochemistry, and determination of the ratio of the intracavernosal pressure/mean arterial pressure were performed to evaluate penile erection and histological changes. Western blotting was conducted to determine the molecular protein expression levels. In vivo, similar to myocardin overexpression, YAP1 inhibition rescued erectile function, repaired morphology, and maintained the contractile phenotype of smooth muscle tissues 14 days after treatment. *Discussion and Conclusion*. In conclusion, our study demonstrated the validity of gene therapy by downregulating excessive YAP1 expression to ameliorate ED in diabetic rats, and the ability of YAP1 inhibition to reverse phenotypic modulation was mediated through interaction with myocardin.

## 1. Introduction

Because patients with diabetes mellitus (DM) have a low response rate to phosphodiesterase inhibitor type 5 (PDE5i) and other nonoperative remedies, andrologists find it challenging to treat erectile dysfunction (ED) in these people [1]. The pathogenic causes of diabetes mellitus erectile dysfunction (DMED) may be manifold and are not fully understood [2]. In previous studies, we detected that in the corpus cavernosum of diabetic rats, phenotypic modulated and excessive collagen deposited in resident smooth muscle cells, which restricted contractility and relaxation, but the key regulators of these processes are still under exploration [3].

The ability to transition from the contractile (or differentiated) phenotype to the synthetic (or dedifferentiated) phenotype is referred to as phenotypic modulation, phenotypic transformation, or switching [4]. This switch occurs reversibly within one cell and has been shown to be a fundamental

pathophysiology during hypertension, myocardial hypertrophy, myocardial infarction, and other diseases linked with vascular smooth muscle cell (VSMC) dysfunction [5]. As corpus cavernosum smooth muscle cells (CCSMCs) and VSMCs are homologous, we demonstrated that CCSMCs have similar phenotypic modulation abilities and revealed their relationship with ED in rat models of DM [6] and bilateral cavernous nerve injury [7]. Furthermore, through gene therapy or gene-modified stem cell transfection, myocardin, a key regulator of phenotypic changes that directly encode contractile markers, such as $\alpha$-SMA, calponin, and smoothelin, was confirmed to ameliorate ED in diabetic rats [6, 8]. However, especially in individuals with DM, the molecular mechanism has not been fully elucidated.

The HIPPO signaling pathway central effector, Yes-associated protein 1 (YAP1), has been proven to be involved in promoting the extracellular matrix (ECM) in multiple organs [9]. HIPPO-YAP1 was reported to interact with the well-known molecule TGF-$\beta$-Smad 2/3 to induce collagen synthesis [10]. In addition, YAP1 is believed to promote phenotypic modulation in VSMCs [11], but its connection with CCSMCs and role in DMED have not been discussed. In this study, we demonstrated the function of YAP1 in the pathophysiological mechanism of diabetic ED and revealed the treatment potential of gene therapy by inhibiting excessive expression of YAP1.

## 2. Materials and Methods

*2.1. Animal Experimental Design.* Every experiment was conducted based on our institution's Institutional Animal Care and Use Committee approval criteria. Male Sprague–Dawley (SD) rats weighing between 250 and 300 g were acquired and kept at the Southern Medical University of China's Nanfang Hospital Experimental Animal Center. The apomorphine-induced erection testing was employed to screen the DMED rats after 8 weeks of STZ induction, as mentioned before [6]. DMED rats were used in the investigations that followed. Overall, three equal experimental groups of 15 rats were subjected to various treatments: streptozotocin (STZ)-induced DMED combined with intracavernosal injection of Ad-myocardin (DMED + myocardin; a total of 50 $\mu$L of PBS supplemented with $5 \times 10^8$ PFU of adenovirus [7]), lenti-shYAP1-mediated DMED (DMED + shYAP1, 50 $\mu$L of PBS with $5 \times 10^6$ TU lentivirus) or DMED with PBS (DMED + PBS) was used, and 10 rats in the negative control (NC) group received PBS (NC + PBS). After 14 days, penile erectile function evaluations were performed on all the rats before euthanasia.

*2.2. Penile Erectile Function Evaluation.* Mean arterial pressure (MAP) and intracavernosal pressure (ICP) were employed to evaluate every rat's erection; the resulting ratio, ICP/MAP, is reported. The rats were sedated, sterilized, and subjected to a lower abdomen incision to find the pair of cavernous nerves following our prior procedures. For erectile stimulation, a bipolar stainless steel electrode was employed at 2.5, 5, and 10 V. According to our previous experiments and the preexperimental results, 5 V was chosen as the best electrical stimulation value. The right penile crus was then punctured with a 25-G air-free needle that contained 100 U/mL heparin solution and was attached to the MP150 Biopac system's amplifier and transducer (Biopac Systems, Inc., California, USA). The results were analyzed using AcqKnowledge® V4.4 software.

*2.3. Histological Examinations.* Immunochemistry (IHC), Masson's trichrome staining, and H&E staining were employed for histological analysis after the recently dissected penile tissues were prepared. The penile strips were fixed with 4% paraformaldehyde, paraffinized, and subsequently subjected to staining with Masson's trichrome and H&E. Utilizing an Olympus microscope (Olympus, Japan), images were captured, and Image-Pro Plus 6.0 was employed to assess the SM-to-collagen ratio of Masson's trichrome staining. For the IHC test, segments were cut at 4 $\mu$m and underwent incubation with antibodies against YAP1 and COL1 (1 : 100; Abcam, UK) and $\alpha$-SMA and TAZ (or WWTR1; 1 : 100; Proteintech, USA). Leica microscope (Leica, Germany) was employed to obtain digital images, and image capture was performed using ImageScope V12.3.2.8013 software.

*2.4. Cell Culture and Processing.* Primary CCSMCs were isolated and underwent culturing as described above. In general, the tissue explants were removed after the cells climbed out and started to divide at days 3–5. The cells were passaged, and the cells of the third generation were used for subsequent experiments. Then, immunofluorescence (IF) was performed with an antibody of anticalponin (1 : 100; catalog # sc-58707; Santa Cruz, USA) to identify pure CCSMCs for subsequent experiments.

*2.5. Gene Transfection and Cytokine Stimulation.* In this study, the YAP1, shYAP1, and shMycoardin plasmids; the shYAP1 lentivirus (MOI = 20; Shanghai Genechem Co., Ltd., China); the myocardin adenovirus (MOI = 50; Hanbio Co., Ltd., China); and the platelet-derived growth factor (PDGF-BB; phenotypic regulation positive control; 20 ng/mL; PeproTech, USA) were used as stimulators. The cells were collected 48 hr later for additional investigation. The subsequent cell investigations were each conducted three times.

*2.6. EdU and CCK-8 Cell Proliferation Assays.* To quantify proliferative ability, assays of the Cell Counting Kit (CCK8; Dojindo, Japan) and 5-ethynyl-2′-deoxyuridine (EdU; RiboBio, China) were conducted based on the directions provided by the manufacturer. In the EdU incorporation reactions, quiescent cells underwent Hoechst 33342 staining alone, whereas growing cells were subjected to both EdU (red) and Hoechst (blue) stains. Utilizing a 400x microscope, the total and EdU+ cells in each cultivated well were counted in three separate fields, and the ratio of EdU+ to total cells was determined. However, in the CCK-8 test, cell proliferation was determined by determining the OD values using a microplate reader with multimode 1, 2, 4, 8, and 24 hr following PDGF-BB or gene stimulation. The GraphPad Prism 5 program was employed to generate the curves of time growth.

*2.7. Immunofluorescence Staining and Confocal Microscopy.* On glass coverslips, many CCSMC groups underwent seeding and growing. After the previous procedures, 0.25% Triton X-100/1% bovine serum albumin (BSA)/PBS was utilized to incubate

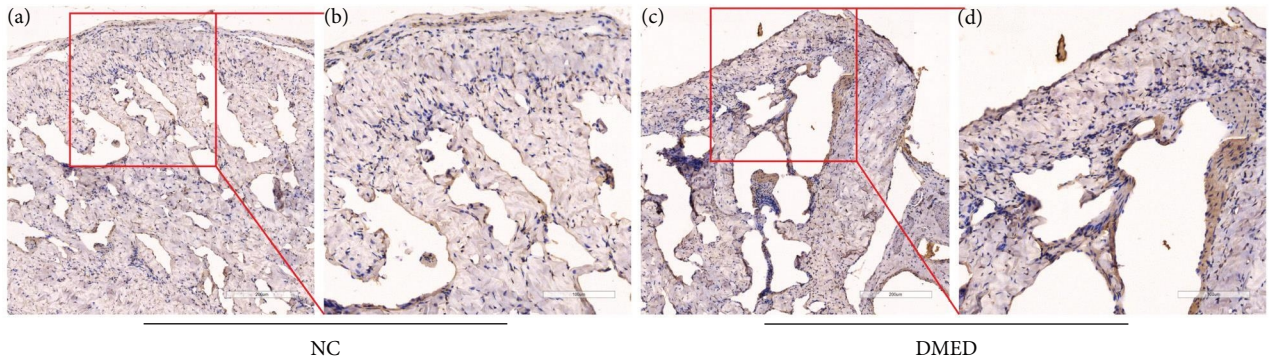



Figure 1: Expression of YAP1 was elevated in DMED rats. IHC revealed lower expression of YAP1 in the NC rats (a and b) in contrast with that in the DMED rats (c and d) at 100x and 200x magnification.

the cells before the fixation with 4% paraformaldehyde. The primary antibodies against YAP1 and myocardin (1 : 100; Abcam, UK) underwent overnight incubation at 4°C before they were double stained at room temperature for 1 hr with secondary antibodies (all 1 : 50; Bioworld, USA) conjugated with TRITC (red) or FITC (green) without light. After that, the cell nucleus was stained utilizing 4′,6-diamidino-2-phenylindole (DAPI; Abcam, UK). To confirm the colocalization of YAP1 with myocardin, an Olympus laser scanning confocal microscope was utilized at 1,200x magnification (Olympus, Shinjuku Monolith, Japan).

*2.8. Collagen Gel Lattice Contraction Assay.* As we earlier reported, an in vitro cell contractility experiment was conducted [7]. In summary, solubilized type I collagen (Sigma, USA) was combined with variously treated smooth muscle cells ($2 \times 10^5$ cells/mL) to create a cell-collagen solution that had 1 mg/mL as a final concentration. A 35-mm culture plate was loaded with the 200 $\mu$L of suspension instantly and underwent incubation in a growth medium for an additional 5 days. After that, the cell-collagen lattice was separated from the underlying plastic substratum. Next, the lattice underwent three different conditions: Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal bovine serum (FBS), DMEM plus 1 $\mu$M calcium ionophore (Ca-Ionophore, Sigma, USA), or DMEM with no serum. The diameters prior to lattice release and 10 min following different exposures were noted to determine the proportion of contraction that was relative. FBS was employed as the positive control, and DMEM with no serum as the NC.

*2.9. qRT-PCR.* RNAiso plus reagent (TaKaRa, Japan) was employed to extract the total RNA from CCSMCs, and the extracted RNA was subjected to real-time quantitative PCR analyses. A LightCycler® 480 II (Roche, Basel, Switzerland) was utilized with a SYBR Green PCR Kit (TaKaRa, Japan) to conduct qRT-PCR. The internal control $\beta$-actin was chosen, and the analysis of the melting curve was the method to determine the amplification product specificity. Every experiment has been investigated three times. The PCR primer sequences are illustrated in Table S1.

*2.10. Western Blotting.* Samples of proteins were made employing CCSMC or penile tissue lysates on ice. The following processes were conducted as we defined previously. The utilized primary antibodies and dilutions are listed below: antibodies against YAP1, OPN (1 : 1,000), myocardin (1 : 400) (Abcam, UK), calponin (1 : 400, Santa Cruz, USA), $\alpha$-SMA (1 : 100, Proteintech, USA), $\beta$-tubulin (1 : 8,000, ABclonal, Boston, MA, USA), and $\beta$-actin (1 : 8,000, Ray Antibody Biotech, Beijing, China), as well as a secondary antibody conjugated with horseradish peroxidase (Abcam, UK). Immunodetection was conducted utilizing an Enhanced chemiluminescence reagent (Bio-Rad, USA).

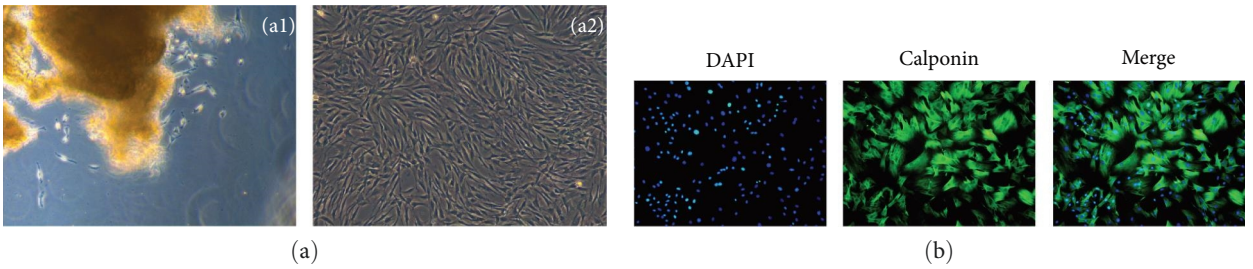
*2.11. Statistics.* For the statistical analysis, SPSS version 21.0 for Windows was utilized. The means ± SEMs are used to demonstrate the findings. One-way ANOVA or the Student's $t$-test was utilized to examine the statistical variations among the groups. The CCK-8 findings were tested employing a univariate general linear model with fixed factors of time and group. At the 5% confidence level, the statistical significance was established ($p < 0.05$).

## 3. Results

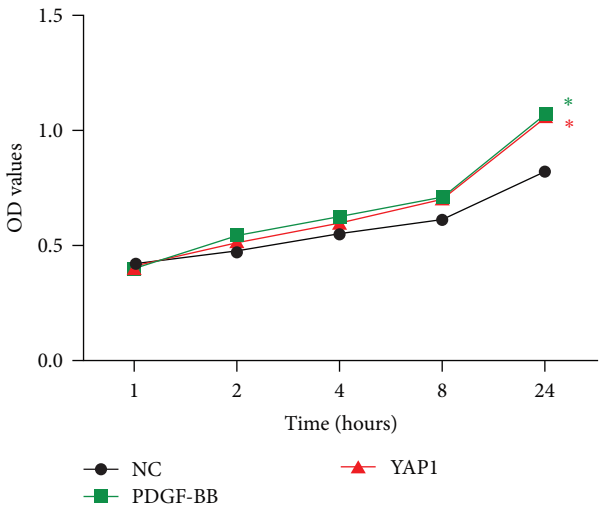
*3.1. Excessive Expression of YAP1 Was Detected in Rats with DMED.* In our previous studies, a rat model of DMED was established, and the phenotypic modulation features in CCSM cells were proven. We found that YAP1 was significantly upregulated in the DMED group by exploring the regulators of the CCSM cell phenotype (Figure 1).

*3.2. YAP1 Regulated CCSM Cell Proliferation.* Primary CCSM cells were cultured using the tissue block adherent culture method. Individual cells were isolated from cavernous tissues on days 3–5 (Figure 2(a)a1), after which they began to rapidly proliferate, and cells at passages 3–4 (Figure 2(a)a2) were used for our experiments. First, the CCSM cell purity was identified by IF staining with calponin (Figure 2(b)). Second, transmission of the YAP1 plasmid significantly increased the cell growth rate within 24 hr, as determined by CCK-8 assays (Figure 2(c)), and the number of proliferating cells within 48 hr, as determined by the EdU method (Figure 2d–2(e)), similar to the effects of the proven proliferation promoter PDGF-BB.

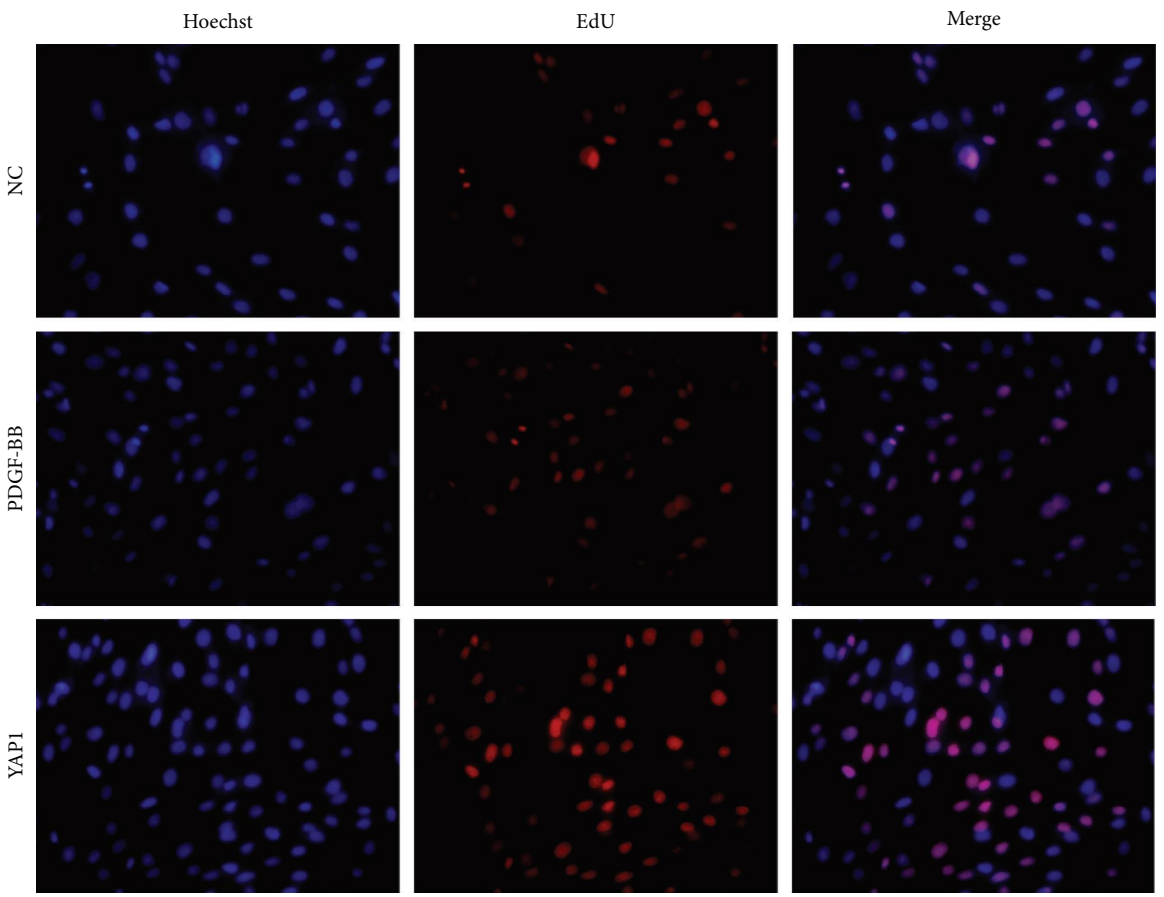
*3.3. Phenotypic Modulation Was Dominated by YAP1.* The qRT-PCR (Figure 3(a)) and western blotting (Figure 3(b))




(a) (b)




(c)




(d)

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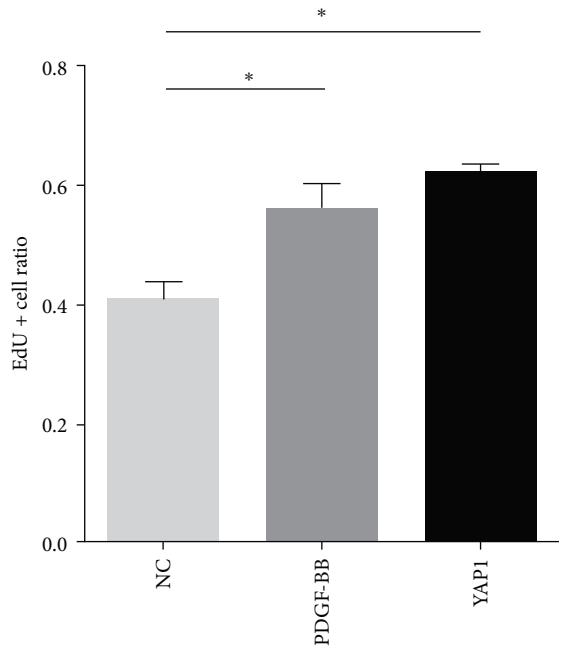



(e)

Figure 2: YAP1 enhanced the growth ability of CCSM cells in vitro. (a) Typical primary CCSM cells that migrated from the tissue block (a1) and growth image at passages 3 and 4 (a2) under 100x magnification. (b) CCSM cell identification via immunofluorescence staining with an anticalponin antibody under 100x amplification. (c) The proliferative capacity trends of the three groups were confirmed by CCK8 assays. Transmission of the YAP1 plasmid or stimulation with PDGF-BB significantly enhanced the CCSM cells' growth in contrast to that in the NC group. (d and e) At 400x magnification, the percentage of EdU+ cells (red) was counted, and an increase was detected in the YAP1-treated and PDGF-BB-stimulated groups. Cell investigations were conducted $n = 3$. $^*p < 0.05$.

were employed to assess the phenotypic markers' expression at the mRNA and protein levels, respectively. Compared with those in the NC group, $\alpha$-SMA and calponin as the contractile phenotype biomarkers and the key regulator myocardin were significantly downregulated, while the osteopontin (OPN), synthetic biomarker, was significantly upregulated in the YAP1 and PDGF-BB groups. Moreover, the type I collagen contraction test detected impaired contractility when YAP1 was overexpressed or affected by PDGF-BB (Figure 3(c)). Thus, similar to PDGF-BB, YAP1 promoted phenotypic modulation of CCSM cells in vitro.

*3.4. Blocking Myocardin Offset the Inhibitory Effect of YAP1 Knockdown on Reversing the Contractile Phenotype.* Myocardin is a vital regulator of the smooth muscle cell phenotypic switch, and it was reported to interact with YAP1. In the present study, colocalization of YAP1 and myocardin in both the nucleus and cytoplasm of CCSM cells was revealed via confocal microscopy at a magnification of 1,200x (Figure 4(a)), indicating interactions between YAP1 and myocardin. Subsequently, YAP1 knockdown resulted in impaired proliferation in CCSMCs (Figures 4(b) and 4(c)); increased mRNA and protein expression of myocardin, $\alpha$-SMA, and calponin; and diminished OPN expression (Figures 4(d) and 4(e)), which are similar to the findings of the myocardin overexpression group. However, further inhibition of myocardin by plasmid transfection inhibited the impacts of YAP1 knockdown on cell growth and marker alteration, suggesting that myocardin blockade offset the inhibitory effect of YAP1 knockdown of reversing the contractile phenotype.

*3.5. Inhibition of YAP1 Rescued Erectile Dysfunction in Diabetic Rats.* First, a total of 45 DMED rats were successfully established by a single injection of streptozotocin; the rats exhibited polydipsia, polyphagia, polyuria, and slowness of movement. The data shown in Table 1 indicate that the DMED rats underwent significant weight loss and hyperglycemia after 8 weeks compared with the NC rats ($n = 10$). Second, similar to the outcomes in the Ad-myocardin group, 14 days after the transfection of lenti-shYAP1, the ICP/MAP ratio was significantly greater (Figures 5(a) and 5(b)) in the lenti-shYAP1-treated group than in the DMED + PBS group but still did not reach the level observed in the NC group.

*3.6. CCSM Cell Function Was Improved by Gene Therapy.* Penile tissues were prepared for morphological and molecular detection. In all the rats with DM, disordered structures, such as deleted or ruptured endothelium and thinning smooth muscle layers, were detected by H&E staining, and a decreased smooth muscle (SM)/collagen (COL) ratio was found by Masson staining (Figures 5(c) and 5(d)). However, through the inhibition of YAP1 in penile tissue, gene therapy not only restored the morphology and SM/COL ratio but also improved CCSM cell function by reversing phenotypic transformation, as verified by the upregulation of the markers calponin and $\alpha$-SMA and the main regulator myocardin

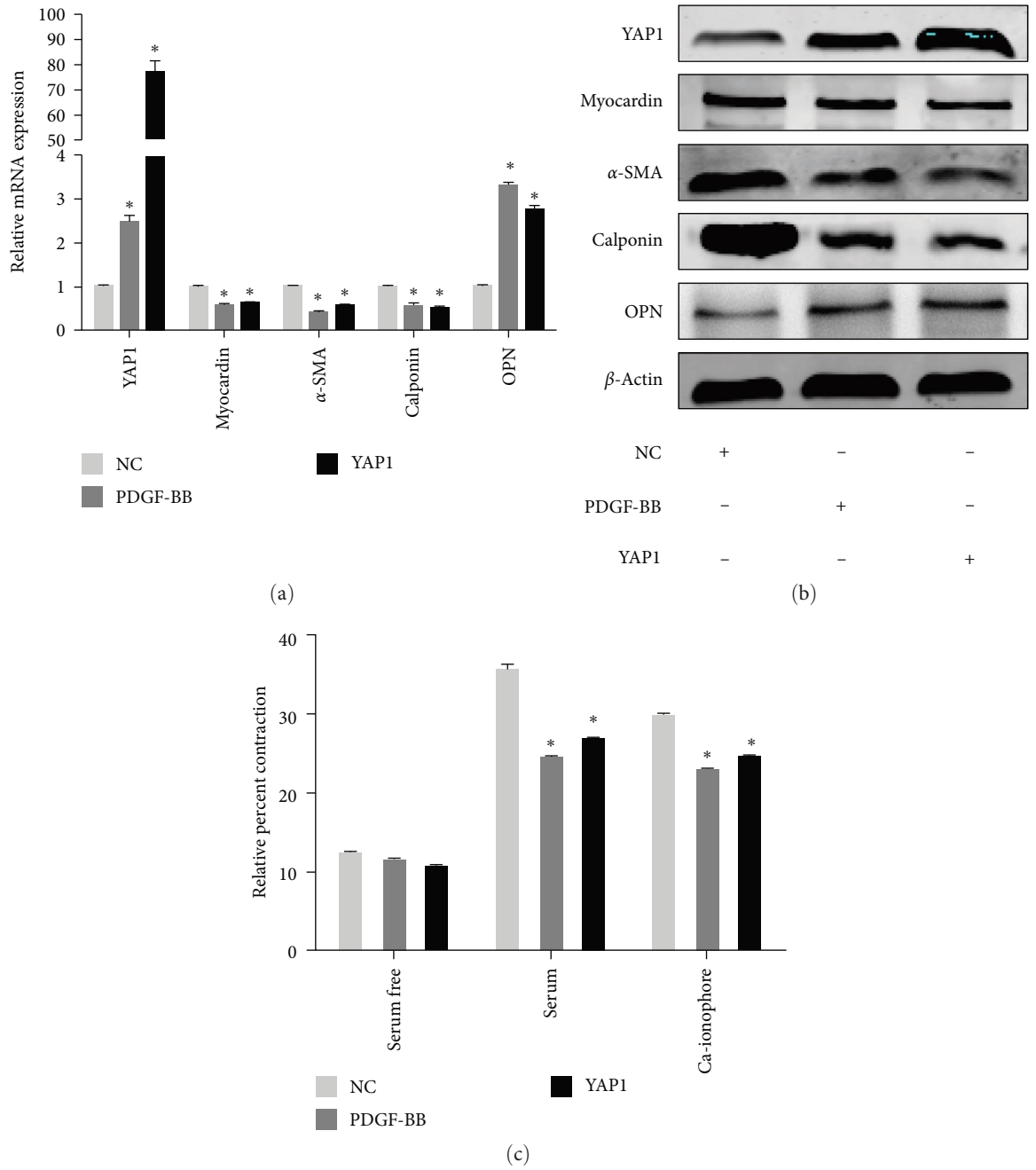
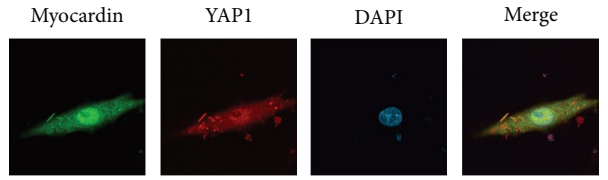



Figure 3: Phenotypic modulation was dominated by YAP1. (a) The cellular mRNA and protein (b) expression levels of YAP1, myocardin, $\alpha$-SMA, calponin, and OPN were measured utilizing qRT-PCR and western blotting. (c) Histogram of cell–collagen lattices stimulated with DMEM, DMEM plus 10% FBS or DMEM plus Ca-ionophore for 10 min. Cell contraction ratios were decreased in DMEM plus 10% FBS and in combination with Ca-ionophore-treated cells that underwent YAP1 and PDGF-BB treatment. There was no statistical variation among the groups with DMEM stimulation. Overall, these results suggest that similar to PDGF-BB, YAP1 promotes phenotypic modulation of CCSM cells in vitro. Cell experiments were conducted $n = 3$. $^{*}p < 0.05$.

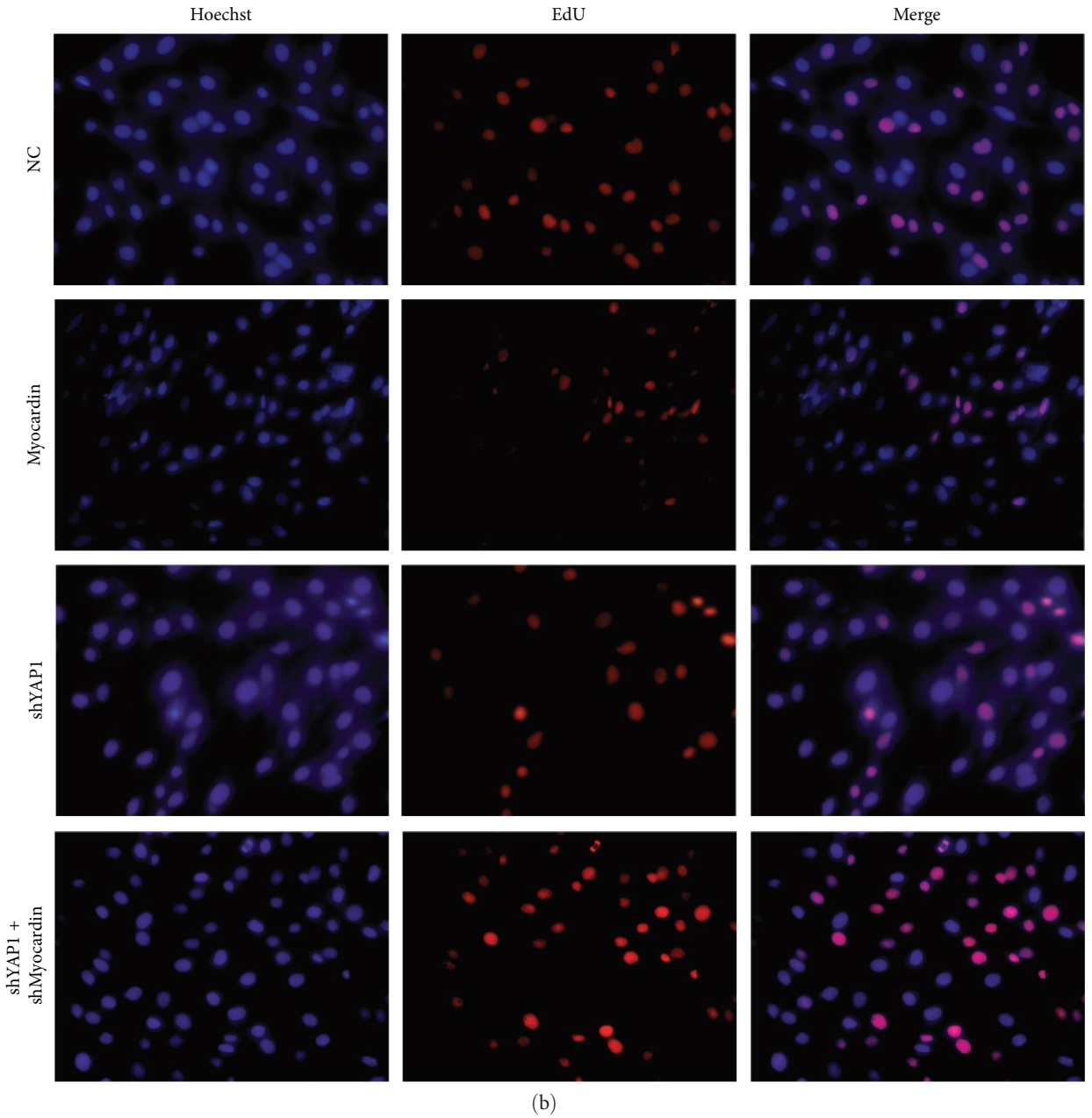
via immunochemistry and western blotting (Figures 6(a) and 6(b)).

## 4. Discussion

Patients with diabetes often complain of ED [12]. However, current treatments show relatively lower efficacy in these patients than treatments in individuals without diabetes [13]. It was reported that the efficacy of sildenafil for treating diabetic ED is only 53.3% [14], far lower than the efficacy of PDE5is, which is more than 80%. Moreover, the poor effects of PDE5is often indicate that patients have a poor response to other treatments and may require penile prosthesis transplantation, which refers to an irreversible invasive operation with




(a)




(b)

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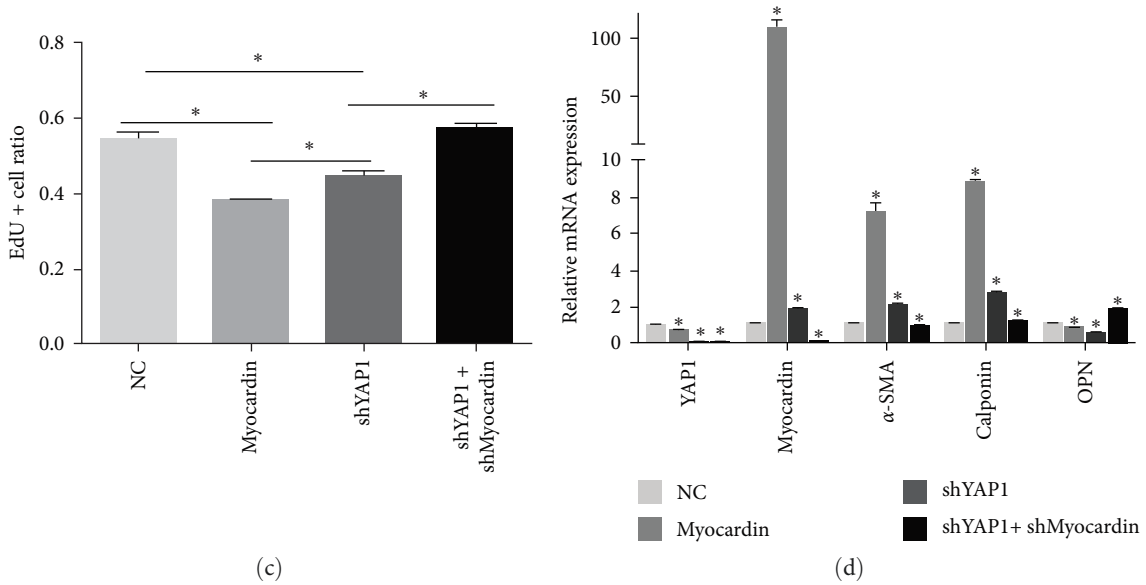
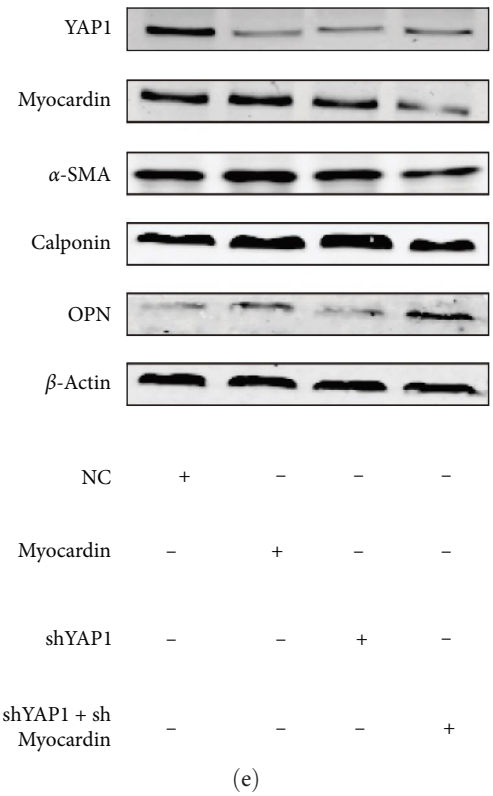



(c) (d)




(e)

Figure 4: Myocardin blockade offset the inhibitory effect of YAP1 knockdown on reversing the contractile phenotype. (a) Confocal microscopy images of NC cells at 1,200x magnification revealed that the fluorescence signals overlapped in the nucleus and cytoplasm, suggesting interactions between YAP1 and myocardin. (b and c) YAP1 knockdown caused a reduction in the EdU+ cells (in red) percentage, which was similar to that in the myocardin group, while further inhibition of myocardin inhibited the impacts of YAP1 knockdown on cell growth. (d and e) The mRNA and protein expression levels of YAP1, myocardin, $\alpha$-SMA, calponin, and OPN were measured employing qRT-PCR and western blotting. Transfection of shYAP1 lentivirus promoted the expression of myocardin, $\alpha$-SMA, and calponin and inhibited OPN, consistent with the effects of myocardin overexpression. However, further inhibition of myocardin expression in YAP1 knockdown cells significantly offset the reversal of the contractile phenotype. Cell investigations were conducted $n = 3$. $^{*}p < 0.05$.

Table 1: Weight of the body and levels of blood glucose in rats at 8 weeks.

| Groups | Body weight (g) | | Blood glucose (mmol/L) | |
|---|---|---|---|---|
| | 0 week | 8 weeks | 72 hr | 8 weeks |
| NC ($n=10$) | $251.90 \pm 7.52$ | $481.50 \pm 21.50$ | $5.44 \pm 0.54$ | $5.88 \pm 0.68$ |
| DMED ($n=45$) | $262.18 \pm 15.15^{**}$ | $228.87 \pm 18.11^{***}$ | $26.38 \pm 5.25^{***}$ | $28.26 \pm 3.47^{***}$ |
| $F$-value | 4.983 | 0.482 | 29.025 | 6.234 |
| $T$-value | −3.134 | 38.589 | −26.118 | −49.181 |
| $p$ | 0.004 | <0.001 | <0.001 | <0.001 |

*Note*: Uneven variances in homogeneity were detected for body weight at 0 weeks, blood glucose at 72 hr, and blood glucose at 8 weeks by the Levene test. $^{**}p<0.01$ vs. the NC group, $^{***}p<0.001$ vs. the NC group.

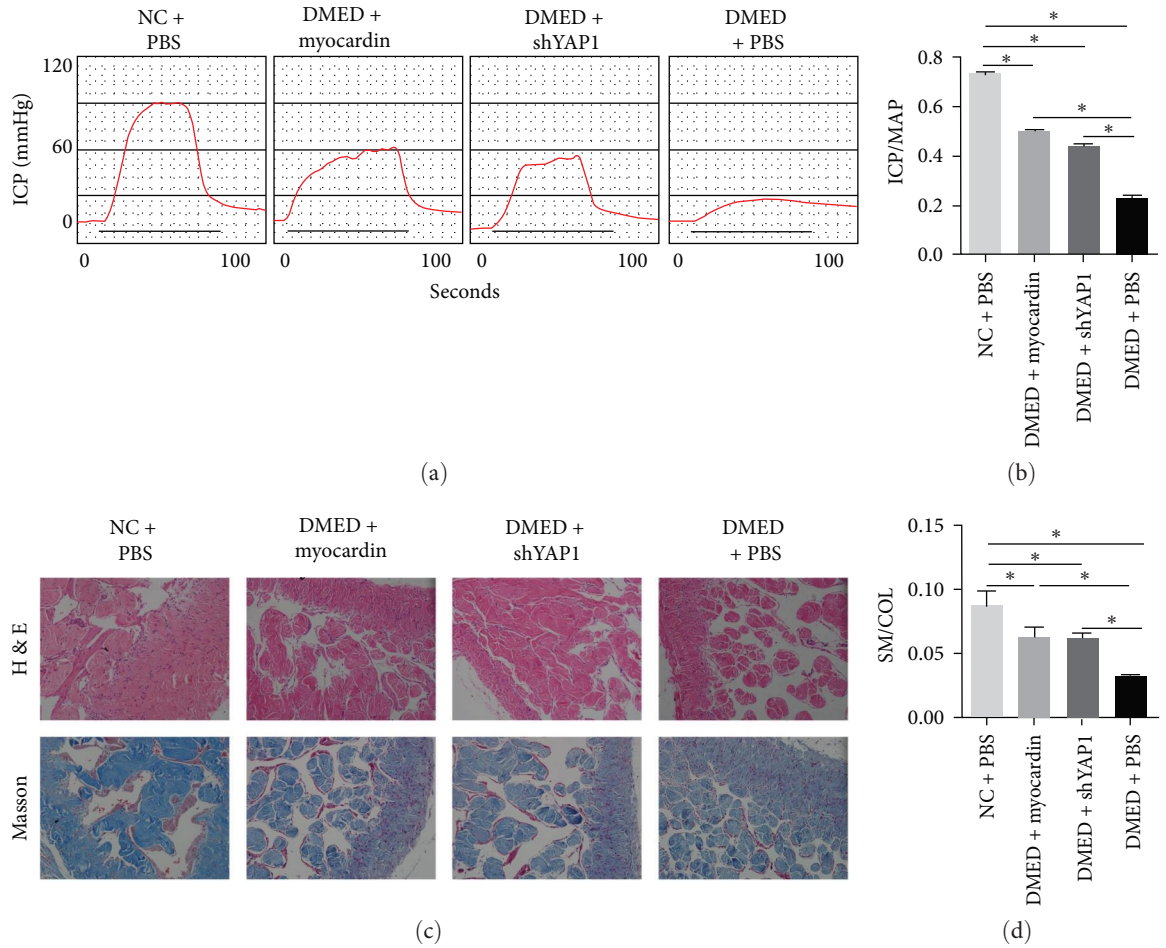
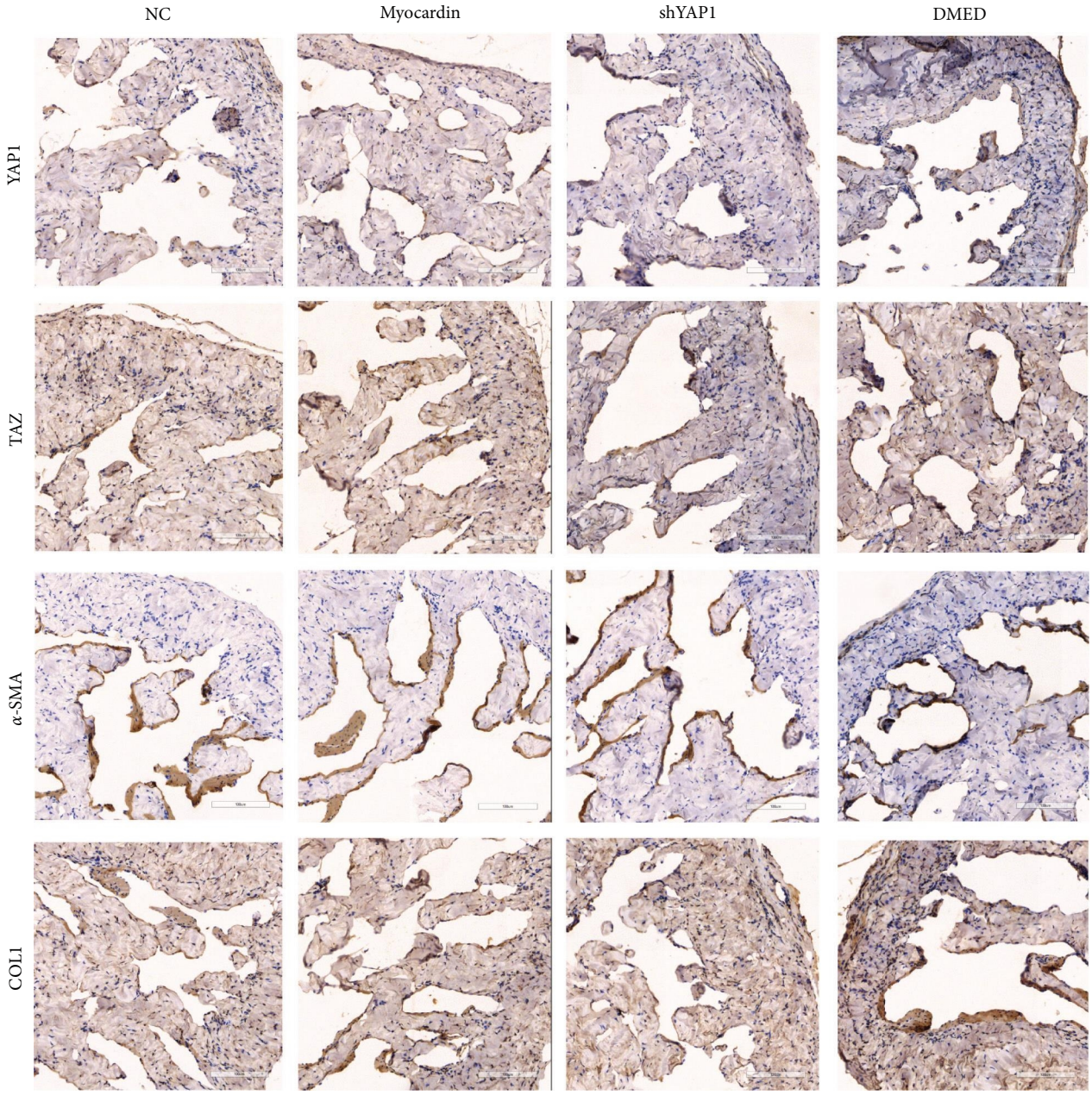



Figure 5: Inhibition of YAP1 rescued erectile dysfunction in diabetic rats. (a) Representative intracavernosal pressure (ICP) and (b) statistics of the ICP/mean arterial pressure (MAP) ratio. Transfection of lenti-shYAP1 or Ad-myocardin markedly enhanced the maximum ICP and the ICP/MAP ratio, but these values were still lower than those in the NC group. Moreover, no statistical variation between the shYAP1 and myocardin groups was detected. (c) H&E and Masson's trichrome staining showed a discontinuous endothelium and thinner smooth muscle layer in all diabetic rat groups, while lenti-shYAP1 and Ad-myocardin transfection significantly repaired the disordered structures. Moreover, compared with DMED + PBS, both the shYAP1 and myocardin treatments significantly increased the smooth muscle (SM)/collagen (COL) ratio (d). Original magnification: 200x $^{*}p<0.05$.

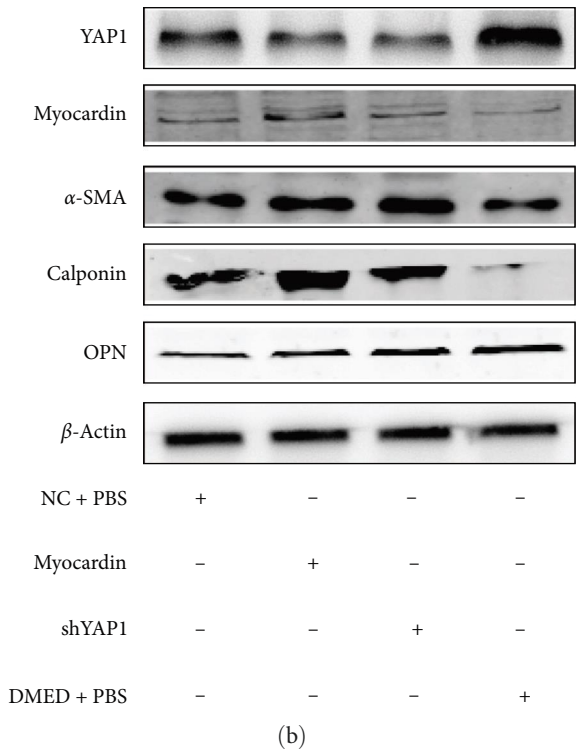
complications such as bleeding, infection, and pain; this treatment is not acceptable for many patients, especially in China [15–17]. Thus, novel therapeutic strategies are urgently needed.

Gene therapy has been introduced to the field of ED treatment in preclinical animal studies [18, 19]. Overexpression of Smad7 or inhibition of ROCK2 ameliorated erectile functions in animal models of bilateral cavernous nerve injury and spontaneously hypertensive rats [20, 21], providing evidence of the ability to correct the abnormal expression of specific genes during ED treatment. Moreover, as an important part of precision medicine, gene therapy has good application prospects [22]. In November 2017, the




(a)

Figure 6: Continued.




(b)

Figure 6: Gene therapy improved CCSM cell function. (a) Expressions of YAP1 and TAZ were confirmed in different treatments using immunochemistry, and lower $\alpha$-SMA expression and a higher collagen-1 level were observed in the penile tissues in the DMED groups compared with those in the NC rats. However, 2 weeks of treatment with lenti-shYAP1 or Ad-myocardin prominently reversed these changes. Original magnification: 200x. (b) The levels of the protein expression of contractile phenotype biomarkers $\alpha$-SMA and calponin, the synthetic biomarker OPN, myocardin, and YAP1 were measured via western blotting.

*New England Journal of Medicine* reported a phase I/II clinical trial of single-injection gene therapy. The 20-month survival time of 15 children with spinal muscular atrophy type I improved from the traditional 8%–100% [23]. In the same month, *Nature* reported a case of borderline bullous epidermolysis treated with epidermal tissue replacement differentiated from genetically modified stem cells [24]. Furthermore, clinical studies have shown promising curative effects in patients with hemophilia [25, 26] and Parkinson's disease [27]. These inspirational results have encouraged us to explore gene therapy for ED [28, 29]. In previous studies, we first described the characterization of the CCSMC phenotype in rats with DMED [3] and identified myocardin as the key regulator reversing phenotypic modulation to treat diabetic ED [6, 8]. However, as a terminal regulatory molecule [30], myocardin plays a simple role by directly activating the transcription of contractile markers such as $\alpha$-SMA, calponin, and smoothelin and is not a sufficient indicator of diabetic ED. However, additional unsolved issues related to ED in patients with DM remain to be elucidated.

The Hippo signaling pathway is a highly conserved signaling pathway that was identified in recent years. Its main function is to regulate cell proliferation, migration, and apoptosis [31]. Yes-associated protein 1 (YAP1) is the most critical effector molecule and acts as a transcriptional coactivator to induce subsequent effects by binding to a variety of transcription factors. Along with its paralog PDZ-binding motif (TAZ), YAP1 translocates from the cytoplasm to the nucleus upon dephosphorylation to activate TEA domain family member–binding domain (TEAD) transcription factors [32]. There are four members in the TEAD family, named TEAD 1-4, which share the same highly conserved TEA/ATTS DNA-binding domain in the N-terminus. In smooth muscle tissues [33], the family member that has been investigated most frequently is TEAD1, which has been revealed to consistently express and regulate smooth muscle cell-related markers, such as ACTA2, SM22$\alpha$, and calponin [34]. The 3D structure of the YAP1-TEAD1 complex has also been identified. It has been demonstrated that these molecules are crucial for the cardiovascular system development and vessel homeostasis maintenance [35]. However, aberrant activation of YAP1 is involved in multiple pathological conditions, such as pulmonary arterial hypertension [36]. Upregulated expression of YAP1 has been measured during the disease progression of vasoconstriction and abnormal remodeling of pulmonary vessels. YAP1 inhibition in pulmonary arterial smooth muscle cells (PASMCs) causes the depletion of cell proliferation and a reduction in ECM deposition [37]. Furthermore, interactions between HIPPO-YAP1 and cellular signaling pathways have been identified [38]. In this study, our data confirmed the correlation between YAP1 and myocardin, which regulates the CCSMC phenotype, in line with the findings of a prior investigation indicating that YAP1 interacts with myocardin to induce a phenotypic switch in VSMCs [39, 40]. Although they are homologous, CCSMCs and VSMCs have their own characteristics and regulatory systems. The effect of phenotypic modulation in ED has now been confirmed. We hypothesized that Hippo-YAP1 plays different

roles in disease processes, including in diabetes. In the early stage of DM, YAP1 is upregulated to help protect against damage from the disease, ensuring cell survival and continuity. However, in the late stage of diabetes, in patients with ED, for example, the excessive expression of YAP1 leads to abnormal cell function and the occurrence of complications. As a primary study, our results and those of previous studies suggest that YAP1 is a candidate therapeutic target. Due to the limitations of the study, the downstream signaling pathways of YAP1, including TEAD1, were not investigated, and future studies are needed.

## 5. Conclusions

Our study demonstrated the effectiveness of gene therapy by downregulating excessive YAP1 expression to ameliorate ED in diabetic rats, and the ability of YAP1 inhibition to reverse phenotypic modulation was mediated through the interaction myocardin.

## Data Availability

All data generated or analyzed during this study are included in this article.

## Ethical Approval

This study was approved by the Institutional Animal Care and Use Subcommittee of the Nanfang Hospital of Southern Medical University and every experiment was conducted in accordance with our institution's Institutional Animal Care and Use Committee approval criteria based on the Declaration of Helsinki.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Haibo Zhang and Anyang Wei designed the study. Yangyan Lin and Li Wang performed the animal modeling and the transfection of genes. Jialiang Hui and Miaoxia Pan performed the molecular biology experiments and histology. Junqi Luo and Zerong Chen analyzed the data. Yangyan Lin and Haibo Zhang wrote the manuscript. Miaoxia Pan and Anyang Wei revised the paper. All of the authors approved and submitted the final version of the manuscript.

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## Supplementary Materials

Table S1: primer sequences used in this study. (*Supplementary Materials*)

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