



## Research Article

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# MiR-4465-modified mesenchymal stem cell-derived small extracellular vesicles inhibit liver fibrosis development via targeting LOXL2 expression

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**Abstract:** Liver fibrosis is a significant health burden, marked by the consistent deposition of collagen. Unfortunately, the currently available treatment approaches for this condition are far from optimal. Lysyl oxidase-like protein 2 (LOXL2) secreted by hepatic stellate cells (HSCs) is a crucial player in the cross-linking of matrix collagen and is a significant target for treating liver fibrosis. Mesenchymal stem cell-derived small extracellular vesicles (MSC-sEVs) have been proposed as a potential treatment option for chronic liver disorders. Previous studies have found that MSC-sEV can be used for microRNA delivery into target cells or tissues. It is currently unclear whether microRNA-4465 (miR-4465) can target LOXL2 and inhibit HSC activation. Additionally, it is uncertain whether MSC-sEV can be utilized as a gene therapy vector to carry miR-4465 and effectively inhibit the progression of liver fibrosis. This study explored the effect of miR-4465-modified MSC-sEV (MSC-sEV<sup>miR-4465</sup>) on LOXL2 expression and liver fibrosis development. The results showed that miR-4465 can bind specifically to the promoter of the *LOXL2* gene in HSC. Moreover, MSC-sEV<sup>miR-4465</sup> inhibited HSC activation and collagen expression by downregulating LOXL2 expression in vitro. MSC-sEV<sup>miR-4465</sup> injection could reduce HSC activation and collagen deposition in the CCl<sub>4</sub>-induced mouse model. MSC-sEV<sup>miR-4465</sup> mediating via LOXL2 also hindered the migration and invasion of HepG2 cells. In conclusion, we found that MSC-sEV can deliver miR-4465 into HSC to alleviate liver fibrosis via altering LOXL2, which might provide a promising therapeutic strategy for liver diseases.

**Key words:** Mesenchymal stem cell (MSC); Small extracellular vesicle (sEV); MicroRNA-4465 (miR-4465); Hepatic stellate cell (HSC); Liver fibrosis

## 1 Introduction

Liver fibrosis severely threatens human health, with approximately two million people dying from it each year (Asrani et al., 2019). It might develop into cirrhosis or even liver cancer (Paik et al., 2020; Jepsen

and Younossi, 2021), which may be due to a variety of factors, including obesity, alcohol consumption, hepatitis B or C infection (Ginès et al., 2021), and excessive medication (Cai et al., 2022). However, no effective clinical treatment has been developed for this illness except liver transplantation (Friedman and Pinzani, 2022). Liver fibrosis is a pathophysiological process involving the inflammatory cascade, excessive reactive oxygen species, and the deposition of extracellular matrix (ECM). The activation of hepatic stellate cell (HSC) is a crucial step in the development of liver fibrosis, which is triggered by the inflammatory and oxidative stress response, leading to consistent collagen deposition and worsening of the condition. Therefore, it is essential to devise a strategy that explicitly targets HSC activation and collagen deposition to prevent the progression of

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liver fibrosis (Kisseleva and Brenner, 2021; Liu et al., 2021).

Lysyl oxidase-like protein 2 (LOXL2), a member of the lysyl oxidase family, has been shown to play a significant role in producing cross-linked matrix collagen and elastin components outside of cells (Chen et al., 2020). Recent research has suggested the obvious therapeutic benefits of inhibiting LOXL2 in liver fibrosis mice models, illuminating the possibility that LOXL2 can serve as a new therapeutic target for hepatic fibrosis (Ikenaga et al., 2017; Magdaleno and Trebicka, 2017). Therefore, in this study, we attempted to find a new therapy to alleviate liver fibrosis via downregulating LOXL2 expression. MicroRNAs (miRNAs) are small non-coding RNAs that regulate messenger RNA (mRNA) expression and translational efficiency in most cell types (Li and Rana, 2014; Zhang et al., 2022). However, one of the significant challenges is to effectively deliver miRNA to target cells without side effects.

Mesenchymal stem cell (MSC) treatment in healing tissue injury faced low efficiency for ferroptosis in transplanted MSC (Xu et al., 2023). Small extracellular vesicles (sEVs) are nanoscale vesicles that can deliver a variety of biologically active molecules into various cells (Dixson et al., 2023). Numerous studies have supported that MSC-derived sEVs (MSC-sEVs) could potentially treat liver diseases and regeneration (Kostallari et al., 2021; Psaraki et al., 2022). Our research team previously reported that MSC-sEV could ameliorate CCl<sub>4</sub>-induced mice liver fibrosis (Li et al., 2013; Jiang et al., 2018; Tan et al., 2022). Moreover, MSC-sEVs achieved significant advances in therapy for several diseases as a novel drug or miRNA delivery vehicle for their low immunogenicity and lack of moral and ethical constraints (Rao et al., 2022; Sohrabi et al., 2022). For example, Shao et al. (2020) proposed that MSC-sEV may improve interleukin-6 (IL-6)-induced acute liver injury by transporting miR-455-3p. Yuan et al. (2021) found that MSC-sEV could deliver exogenous miR-26a-5p to inhibit nucleus pulposus cell pyroptosis through methyltransferase 14 (METTL14)/nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3). Thus, MSC-sEV may be an ideal therapeutic tool for treating liver fibrosis by transporting miRNA. However, the role of miR-4465-modified MSC-sEV (MSC-sEV<sup>miR-4465</sup>) in inhibiting *LOXL2* expression and fibrotic progression has not been reported.

The present study aimed to identify MSC-sEV<sup>miR-4465</sup> in LOXL2 inhibition and liver fibrosis development. We demonstrated that miR-4465 could downregulate *LOXL2* mRNA expression and decrease collagen deposition in HSC. Besides, we attempted to explore MSC-sEV as a more efficient vehicle to deliver miR-4465 into HSC by targeting LOXL2, thereby providing a novel therapeutic therapy for liver fibrosis development.

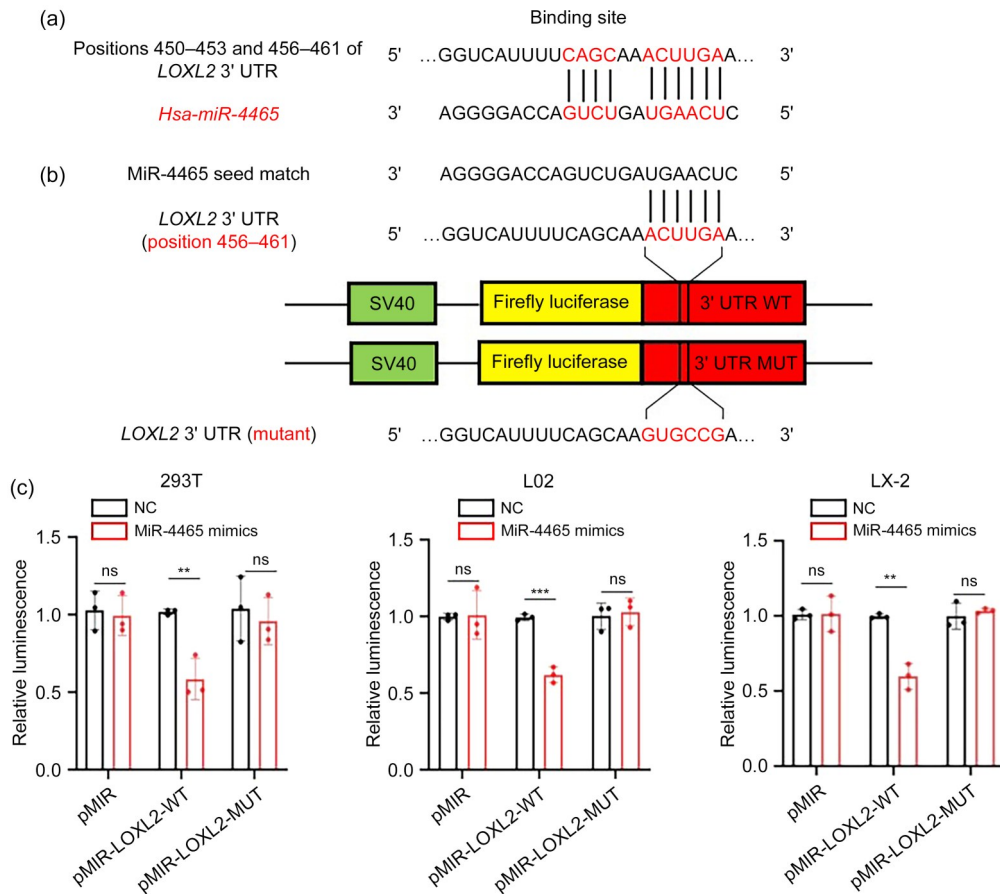
## 2 Results

### 2.1 MiR-4465 targeting human *LOXL2* in LX-2

MiRNAs regulate gene expression at the post-transcriptional level. TargetScan (<https://www.targetscan.org>), a bioinformatics database, suggests that miR-4465 may target *LOXL2* (Fig. 1a). To confirm this hypothesis, mutant and wild-type plasmids were designed with a similar binding sequence to miR-4465 and *LOXL2* (Fig. 1b). We performed the co-transfection of plasmid with wild-type *LOXL2* possessing miR-4465-binding sites (pMIR-*LOXL2*-WT) and miR-4465 mimics into 293T, L02, and LX-2 cells. Luciferase activity was measured 48 h later. The results from the dual luciferase reporter gene experiment showed the significant inhibition of luciferase activity of *LOXL2* by miR-4465 mimics in comparison with the NC group (Fig. 1c). However, transfection by plasmid with mutated *LOXL2* possessing miR-4465-binding sites (pMIR-*LOXL2*-MUT) and miR-4465 mimics did not induce a reduction in luciferase activity in 293T, L02, or LX-2 cells (Fig. 1c), suggesting that miR-4465 is capable of binding specifically to *LOXL2* and inhibiting its expression.

### 2.2 Inhibition of collagen deposition by miR-4465 in LX-2 via downregulating *LOXL2* expression

In order to investigate the role of miR-4465 in LX-2, we transfected the miR-4465 mimic at concentrations of 5 and 10 nmol/L. The efficiency of miR-4465 transfection was determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) (Fig. 2a). The results showed that the protein levels of *LOXL2*, collagen type III  $\alpha 1$  (COL3A1), and COL4A2 were downregulated (Fig. 2b). The mRNA levels of *LOXL2* were significantly reduced in LX-2 cells transfected with miR-4465 mimics. *COL3A1*, *COL4A2*,



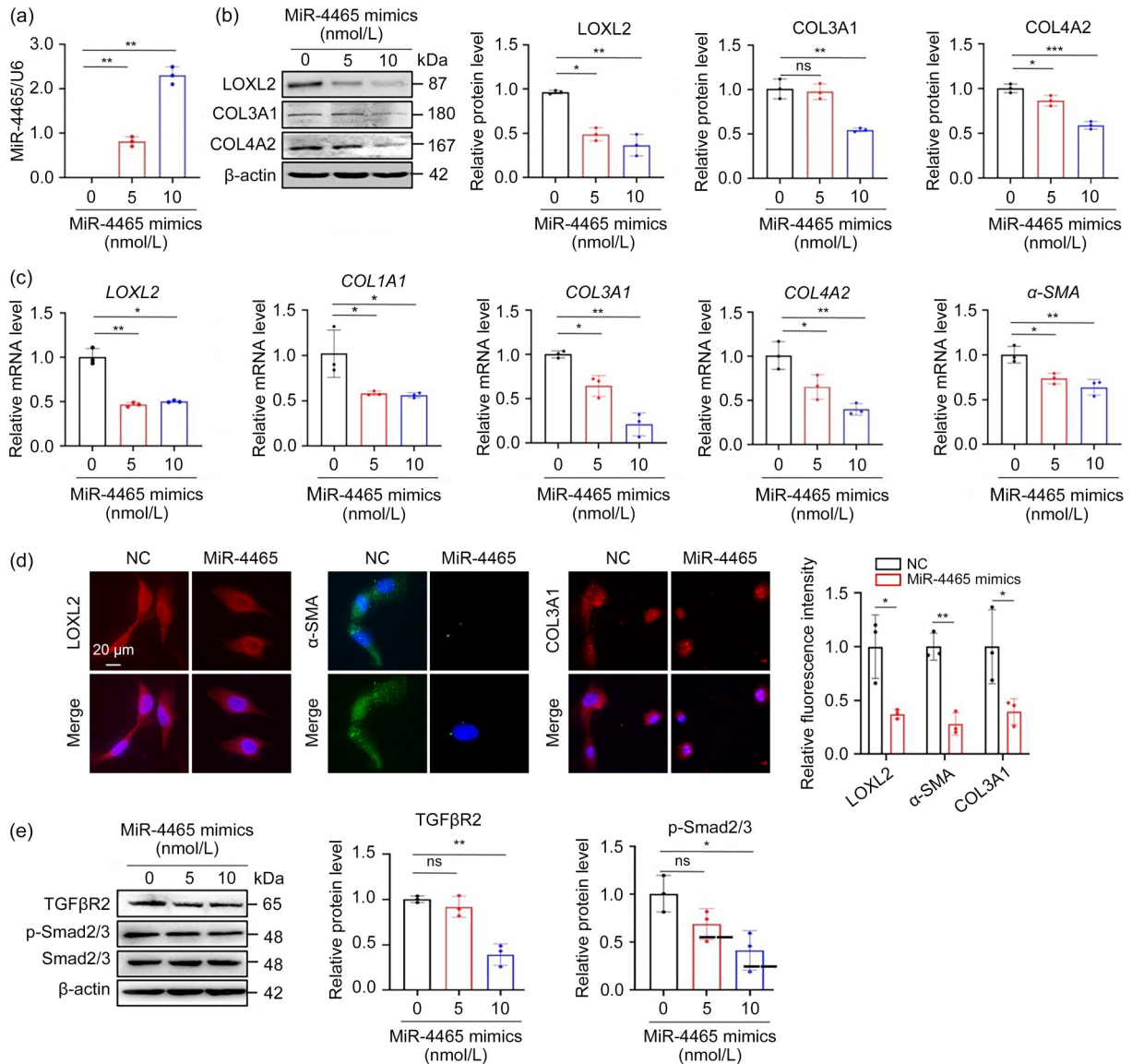
**Fig. 1** MiR-4465 targeting human *LOXL2* in LX-2. (a) The miR-4465-binding sites in *LOXL2* 3' UTR as predicted by TargetScan (<https://www.targetscan.org>); (b) The wild-type (WT) and mutated (MUT) reporter constructs of the *LOXL2* 3' UTR sequence are shown in the schematic diagram. (c) The relative luciferase activity was normalized to the Renilla luciferase activity and calculated compared with negative control (NC) in transfected 293T, L02, and LX-2. Data are expressed as mean±standard deviation (SD) ( $n=3$ ). \*\*  $P<0.01$ ; \*\*\*  $P<0.001$ ; ns, no significant difference. MiR: microRNA; *LOXL2*: lysyl oxidase-like protein 2; UTR: untranslated region; pMIR-*LOXL2*-WT: plasmid with wild-type *LOXL2* possessing miR-4465-binding sites; pMIR-*LOXL2*-MUT: plasmid with mutated *LOXL2* possessing miR-4465-binding sites.

*COL1A1*, and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) were also downregulated (Fig. 2c). Immunofluorescence staining yielded similar results (Fig. 2d). We further investigated the signaling pathways involved in *LOXL2* downregulation by detecting the activation of transforming growth factor  $\beta$  receptor 2 (TGF $\beta$ R2)/Smad pathway in LX-2. Western blot analysis revealed a decrease in the protein levels of TGF $\beta$ R2 and phosphorylated Smad2/3 (p-Smad2/3) (Fig. 2e), suggesting that miR-4465 can inhibit collagen expression by downregulating *LOXL2* and the TGF $\beta$ R2/Smad pathway.

### 2.3 Location of transplanted MSC-sEV in the liver of fibrotic mice

In this study, MSC-sEV was successfully isolated from the human umbilical cord and characterized using

nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and western blot techniques (Figs. 3a–3c). The MSC-sEV exhibited a round spherical shape with a diameter of approximately 100 nm. The average concentration of MSC-sEV was found to be approximately  $1 \times 10^{11}$  particles/mL (Fig. 3a). The modification of miR-4465 did not alter the shape of MSC-sEV (Fig. 3b). We conducted western blot analysis on MSC-sEV to detect the presence of markers such as cluster of differentiation 9 (CD9), CD63, and tumor susceptibility gene 101 (TSG101). However, the endoplasmic reticulum membrane protein calnexin was not observed in the analysis (Fig. 3c). In addition, we incubated CM-Dil-labeled MSC-sEV with the HSC cell line LX-2 in vitro and found that after 24 h of incubation, the labeled MSC-sEVs were mainly localized in



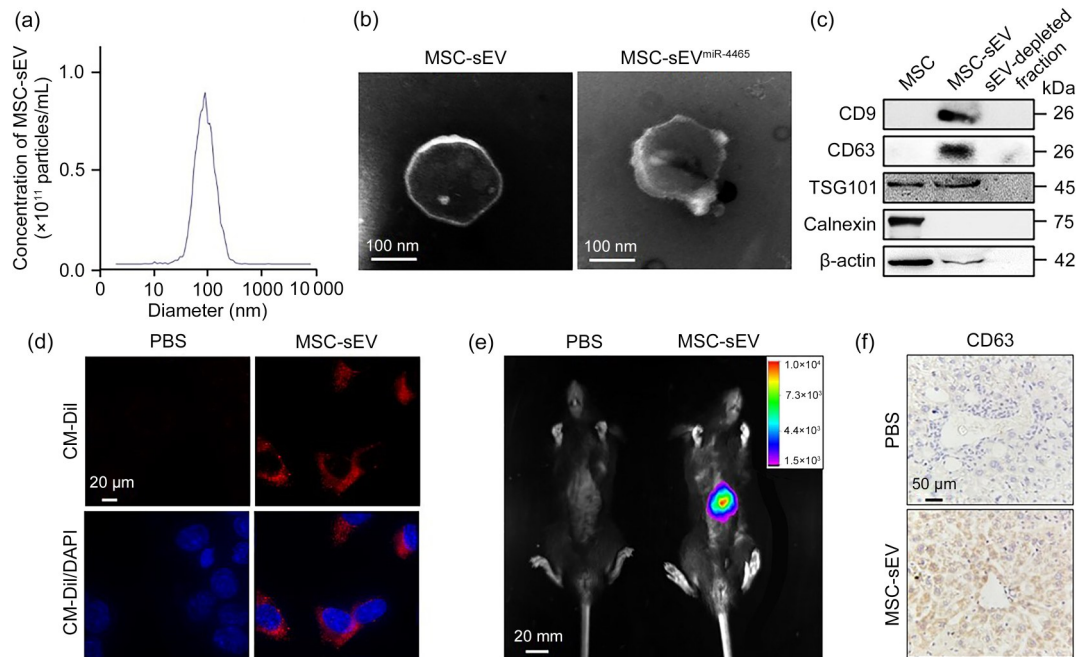
**Fig. 2** Inhibition of collagen deposition by miR-4465 in LX-2 via downregulating LOXL2 expression. (a) qRT-PCR analysis of the transfection efficiency of miR-4465, with U6 as an internal reference; (b) Western blot analysis of LOXL2, COL3A1 and COL4A2, and corresponding quantitative analysis; (c) qRT-PCR analysis of *LOXL2*, *COL1A1*, *COL3A1*, *COL4A2*, and *α-SMA* in LX-2; (d) Representative immunofluorescence images of LOXL2, *α-SMA*, and COL3A1 expression and relative fluorescence intensity in LX-2; (e) Western blot analysis of TGFβ2 and phosphorylated Smad2/3 (p-Smad2/3), and corresponding quantitative analysis. Data are expressed as mean±standard deviation (SD) ( $n=3$ ). \*  $P<0.05$ ; \*\*  $P<0.01$ ; \*\*\*  $P<0.001$ ; ns, no significant difference. MiR: microRNA; LOXL2: lysyl oxidase-like protein 2; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; COL3A1: collagen type III  $\alpha$ 1;  $\alpha$ -SMA:  $\alpha$ -smooth muscle actin; TGFβ2: transforming growth factor  $\beta$  receptor 2; NC: negative control; mRNA: messenger RNA.

the cytoplasm of LX-2, as shown in the confocal results (Fig. 3d). We injected CM-Dil-labeled MSC-sEV into fibrotic mice through the tail vein. In vivo imaging showed that CM-Dil-labeled MSC-sEV could locate in fibrotic livers (Fig. 3e). Additionally, we observed the expression of CD63 in the liver of the mice using immunohistochemistry (Fig. 3f), and the results suggested

that MSC-sEV could be located in LX-2 and the fibrotic liver of mice.

#### 2.4 Inhibition of LOXL2 expression and collagen synthesis in LX-2 by MSC-sEV<sup>miR-4465</sup>

Previous studies have shown that MSC-sEV can transport active molecules, such as miRNA, to specific



**Fig. 3** Location of MSC-sEV in LX-2 and the fibrotic liver of mice. (a) Nanoparticle tracking analysis of the MSC-sEV in PBS. (b) TEM analysis of MSC-sEV and MSC-sEV<sup>miR-4465</sup>. (c) Detection of exosomal marker expression in MSC-sEV by western blot. (d) Confocal microscopic images of CM-Dil-labeled MSC-sEV (red) in LX-2 after 24 h of incubation. (e) Distribution of CM-Dil-labeled MSC-sEV (red) in CCl<sub>4</sub>-injured mice after tail vein administration according to videography. (f) Detection of exosomal marker CD63 in mouse liver by immunohistochemistry. MSC-sEV: mesenchymal stem cell-derived small extracellular vesicle; MSC-sEV<sup>miR-4465</sup>: miR-4465-modified MSC-sEV; PBS: phosphate-buffered saline; TEM: transmission electron microscopy; CD63: cluster of differentiation 63; TSG101: tumor susceptibility gene 101; CM-Dil: 1,1'-dihydro-3,3',3',3'-tetramethylindocarbocyanine perchlorate; DAPI: 4',6-diamidino-2-phenylindole.

target cells or organs (Askenase, 2021). In this paper, the sonication method was utilized to load MSC-sEV (50 µg/mL) with miR-4465 at a concentration of 10 nmol/L. The level of miR-4465 in miR-4465 mimic-loaded MSC-sEV was increased by more than 4636 times compared with control MSC-sEV, as determined by qRT-PCR (Fig. 4a). The data showed that MSC-sEV<sup>miR-4465</sup> consistently induced the downregulation of mRNA and protein levels of LOXL2, COL3A1, and COL4A2, when compared to MSC-sEV (Figs. 4b and 4c). Moreover, the immunofluorescence staining results indicated that MSC-sEV<sup>miR-4465</sup> had a greater tendency to downregulate LOXL2, COL3A1, and COL4A2 than MSC-sEV (Fig. 4d). The above findings suggested that miR-4465 modification can enhance the potential of MSC-sEV in inhibiting LOXL2 and collagen synthesis.

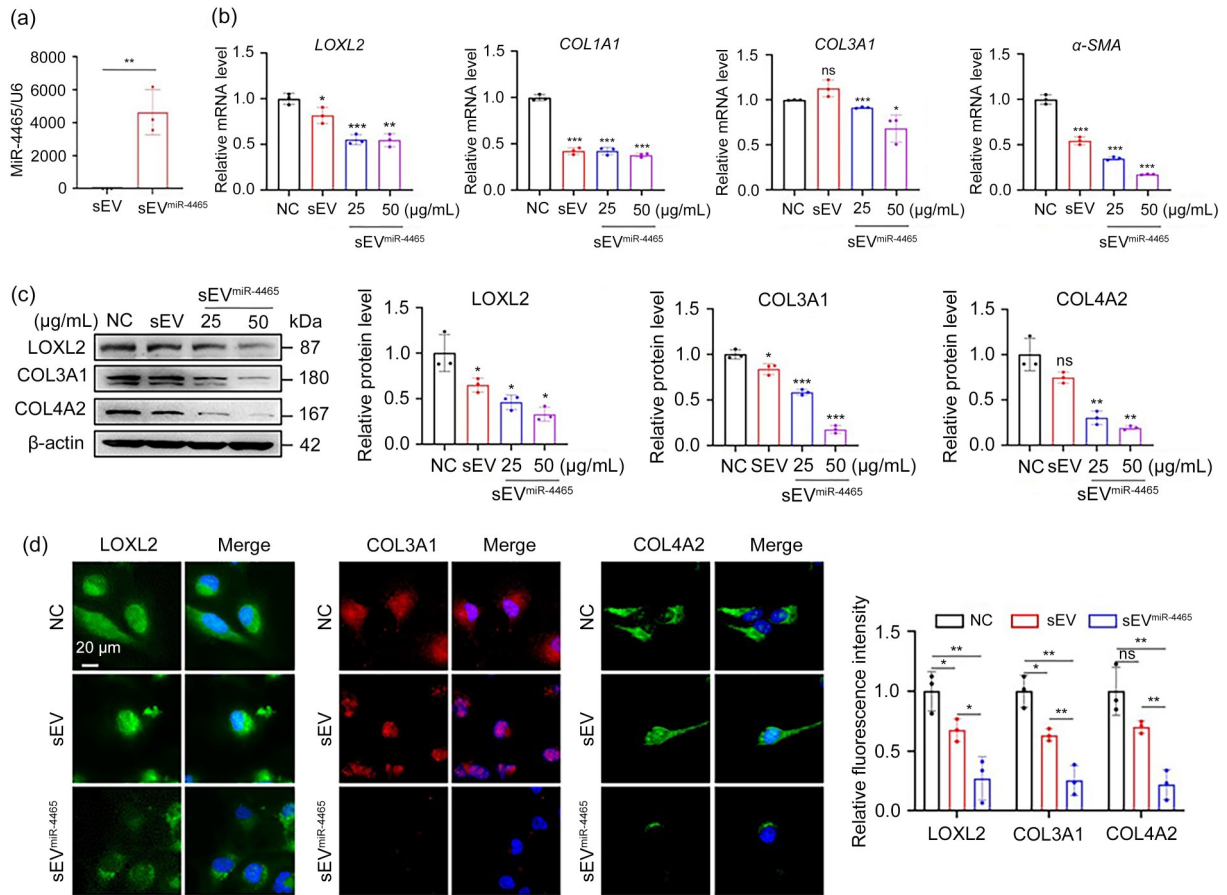
### 2.5 Effect of MSC-sEV<sup>miR-4465</sup> on collagen deposition in CCl<sub>4</sub>-induced fibrotic livers

In order to investigate the effects of MSC-sEV<sup>miR-4465</sup> on liver fibrosis, we established a CCl<sub>4</sub>-induced mouse

liver fibrosis model and injected mice with MSC-sEV and MSC-sEV<sup>miR-4465</sup>. Compared to the group treated with phosphate-buffered saline (PBS), the positive areas of LOXL2 and α-SMA in the livers were significantly decreased in the MSC-sEV and MSC-sEV<sup>miR-4465</sup> groups, with a more significant effect observed in the MSC-sEV<sup>miR-4465</sup> group (Figs. 5a and 5b). The group treated with MSC-sEV<sup>miR-4465</sup> showed a reduction in collagen area in the fibrotic liver compared to the group treated with MSC-sEV alone (Figs. 5c and 5d). This was further confirmed by western blot, which indicated similar changes (Fig. 5e). These findings suggested that MSC-sEV<sup>miR-4465</sup> has the potential to reduce collagen deposition in the CCl<sub>4</sub>-induced mouse model.

### 2.6 Inhibition of the migration and invasion of HepG2 in vitro by MSC-sEV<sup>miR-4465</sup>

Given that liver fibrosis is the most critical risk factor in liver cancer (Jepsen and Younossi, 2021) and LOXL2 also plays a crucial role in its growth and metastasis (Wu et al., 2021), we further investigated the inhibitory effect of MSC-sEV<sup>miR-4465</sup> on the development and



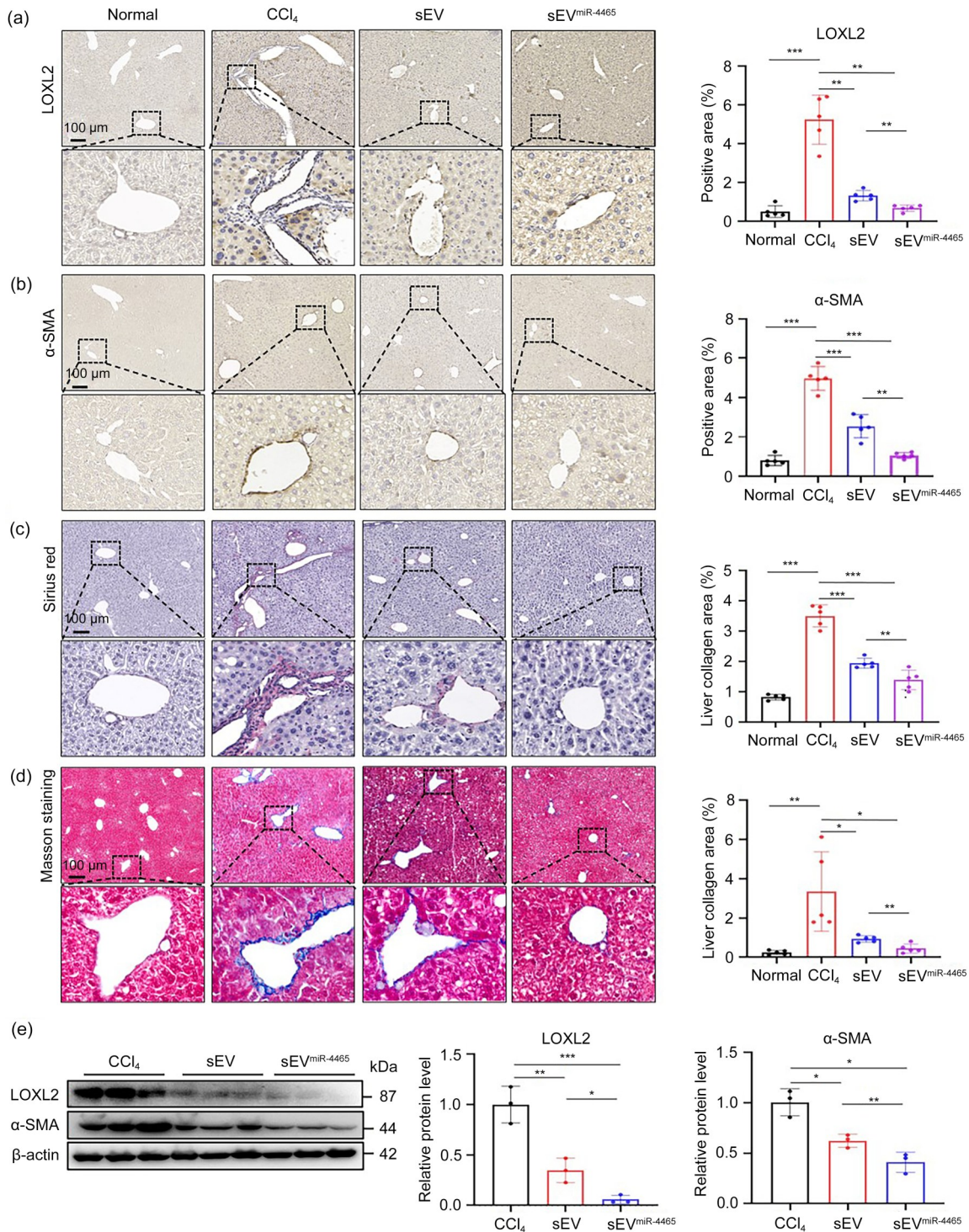
**Fig. 4** Inhibition of LOXL2 expression and collagen synthesis in LX-2 by MSC-sEV<sup>miR-4465</sup>. (a) qRT-PCR analysis of miR-4465 in MSC-sEV after ultrasonic incubation; (b) qRT-PCR analyses of *LOXL2*, *COL1A1*, *COL3A1*, and *α-SMA* in LX-2; (c) Western blot analyses of LOXL2, COL3A1, and COL4A2 in LX-2 and corresponding quantitative analysis; (d) Representative immunofluorescent images of LOXL2, COL3A1, and COL4A2 expression and relative fluorescence intensity in LX-2. Data are expressed as mean±standard deviation (SD) ( $n=3$ ). \*  $P<0.05$ ; \*\*  $P<0.01$ ; \*\*\*  $P<0.001$ ; ns, no significant difference. LOXL2: lysyl oxidase-like protein 2; MSC-sEV: mesenchymal stem cell-derived small extracellular vesicle; MSC-sEV<sup>miR-4465</sup>: miR-4465-modified MSC-sEV; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; COL3A1: collagen type III  $\alpha 1$ ;  $\alpha$ -SMA:  $\alpha$ -smooth muscle actin; NC: negative control.

metastasis of HepG2 liver cancer cells. In vitro experiments were conducted by incubating CM-Dil-labeled MSC-sEV and MSC-sEV<sup>miR-4465</sup> with HepG2. The confocal results showed that the labeled MSC-sEV mainly localized in the cytoplasm of HepG2 after 24 h of incubation (Fig. 6a). After the modification of MSC-sEV with miR-4465, its effects on the migration and invasion of HepG2 were assessed. The transwell assay and wound healing assay results demonstrated that MSC-sEV<sup>miR-4465</sup> significantly inhibited the migration and invasion of HepG2. In contrast, unmodified MSC-sEV had a negligible impact (Figs. 6b–6d). However, the proliferation of HepG2 was not affected by MSC-sEV<sup>miR-4465</sup>, as revealed by the cell counting kit-8 (CCK8) assay (Fig. 6e). The western blot results showed that MSC-sEV<sup>miR-4465</sup>

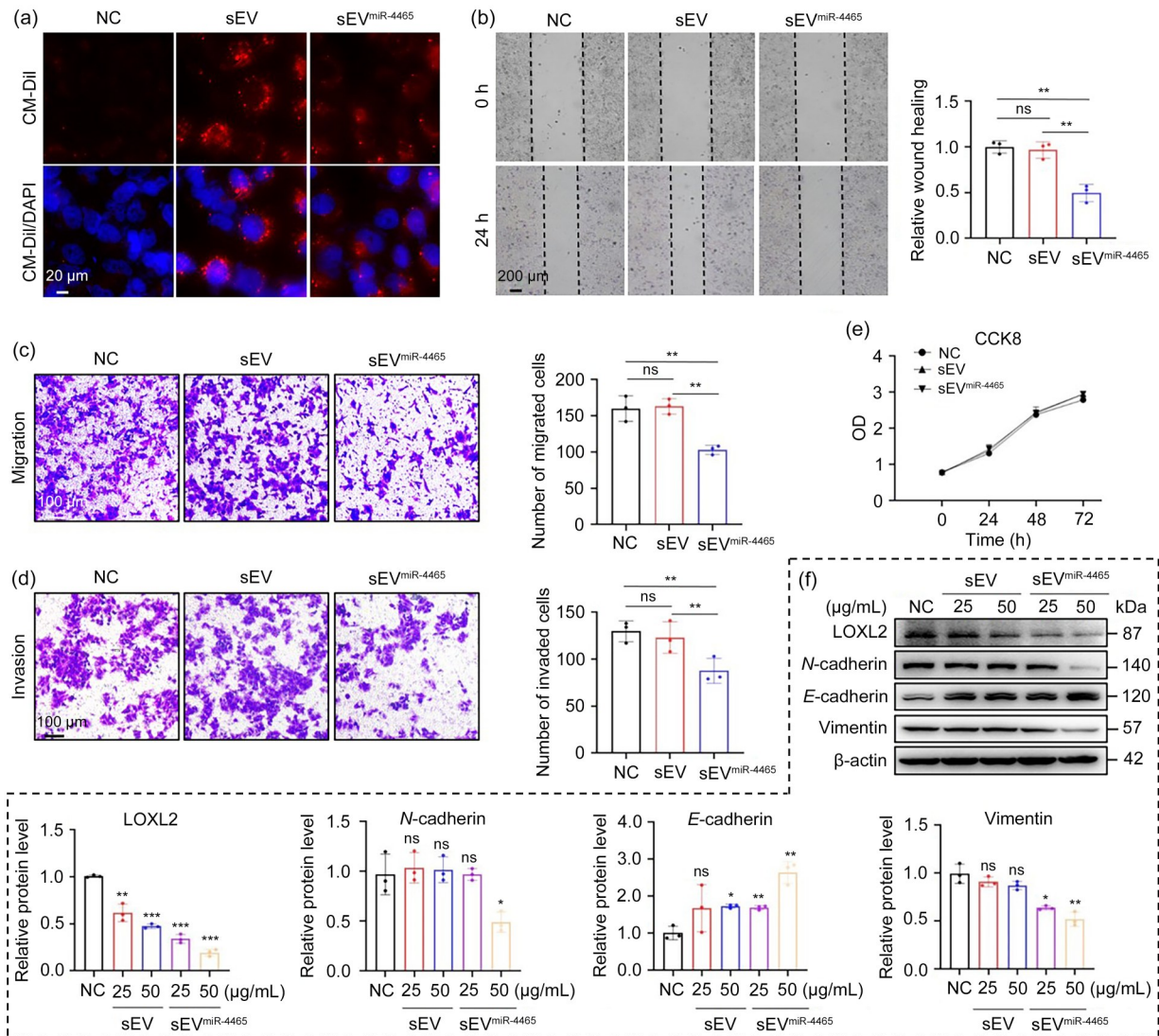
treatment resulted in the downregulation of mesenchymal regulators *N*-cadherin and vimentin, as well as the upregulation of epithelial regulator *E*-cadherin in HepG2 cells compared to the negative control (NC) group (Fig. 6f). Consequently, our findings suggested that MSC-sEV<sup>miR-4465</sup> may hinder the migration and invasion of HepG2 cells by inhibiting epithelial–mesenchymal transition (EMT).

### 3 Discussion

Currently, there is no optimal treatment approach for liver fibrosis. However, in this study, we have discovered that MSC-sEV<sup>miR-4465</sup> can restrict collagen



**Fig. 5** Effect of MSC-sEV<sup>miR-4465</sup> on collagen deposition in the CCl<sub>4</sub>-induced mice model. (a) Immunohistochemical analysis of LOXL2 in three groups and corresponding quantitative analysis of positive area percentage; (b) Immunohistochemical analysis of α-SMA in three groups and corresponding quantitative analysis of positive area percentage; (c) Sirius red staining of collagen in three groups and corresponding quantitative analysis of collagen area percentage; (d) Masson staining analysis of collagen in three groups and corresponding quantitative analysis of collagen area percentage; (e) Western blot analysis of LOXL2 and α-SMA and corresponding quantitative analysis. Data are expressed as mean±standard deviation (SD) (n=3 or 5). \* P<0.05; \*\* P<0.01; \*\*\* P<0.001. MSC-sEV: mesenchymal stem cell-derived small extracellular vesicle; MSC-sEV<sup>miR-4465</sup>: miR-4465-modified MSC-sEV; LOXL2: lysyl oxidase-like protein 2; α-SMA: α-smooth muscle actin.



**Fig. 6** Inhibition of the migration and invasion of HepG2 by MSC-sEV<sup>miR-4465</sup>. (a) Confocal microscopic images of CM-Dil-labeled MSC-sEV and MSC-sEV<sup>miR-4465</sup> (red) in HepG2 after 24 h of incubation; (b) Wound healing assays in HepG2 relative to control cells and corresponding quantitative analysis; (c) The cell migration of HepG2 evaluated by transwell assay and corresponding quantitative analysis; (d) The cell invasion of HepG2 evaluated by transwell assay and corresponding quantitative analysis; (e) The cell proliferation of HepG2 detected by CCK8 assay; (f) Western blot analyses of EMT markers *N*-cadherin, *E*-cadherin, and vimentin, and corresponding quantitative analysis. Data are expressed as mean±standard deviation (SD) ( $n=3$  or  $5$ ). \*  $P<0.05$ ; \*\*  $P<0.01$ ; \*\*\*  $P<0.001$ ; ns, no significant difference. MSC-sEV: mesenchymal stem cell-derived small extracellular vesicle; MSC-sEV<sup>miR-4465</sup>: miR-4465-modified MSC-sEV; CCK8: cell counting kit-8; EMT: epithelial–mesenchymal transition; CM-Dil: 1,1'-diltadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate; DAPI: 4',6-diamidino-2-phenylindole; NC: negative control; LOXL2: lysyl oxidase-like protein 2.

deposition and the development of liver fibrosis. In vitro, the overexpression of miR-4465 in HSC led to the downregulation of LOXL2 and collagen expression. Our findings suggested that miR-4465-loaded MSC-sEVs effectively enhance the suppression of collagen synthesis in HSC by targeting LOXL2. In vivo, the ability of MSC-sEV to inhibit collagen deposition and ameliorate liver fibrosis was increased after the loading of

miR-4465. In addition, MSC-sEV<sup>miR-4465</sup> could inhibit the migration and invasion of the hepatocellular carcinoma cell line HepG2 via EMT. In summary, miR-4465-loaded MSC-sEVs could prevent the collagen deposition of HSC and progression of HCC in vitro and alleviate CCl<sub>4</sub>-induced liver fibrosis by downregulating LOXL2.

Recent studies have shown that miRNAs may play crucial roles in developing liver fibrosis. Zhang

et al. (2019) proposed that miR-378 positively regulates the nuclear factor- $\kappa$ B (NF- $\kappa$ B)/tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) axis, leading to hepatic inflammation and fibrosis. Meanwhile, Liu et al. (2021) identified miR-130a-3p as a potential candidate biomarker and a therapeutic target for schistosomiasis-related liver fibrosis. MiR-4465, a member of the miR-26 family, has been reported to suppress tumor proliferation and metastasis in non-small cell lung cancer (Sun et al., 2017). Despite the above evidences, the role of miR-4465 in liver diseases has not been previously investigated. Our study found that miR-4465 targets the 3' end of *LOXL2* mRNA, as predicted by the bioinformatics software TargetScan, which was also confirmed by the dual-luciferase reporter assay. We found that miR-4465 could target *LOXL2* to inhibit collagen expression in HSC. At the same time, delivering miRNA to target cells or tissues remains a challenging task; therefore, exploring a novel miRNA delivery tool is of great significance.

sEV is a natural carrier for active substances among cells in the human body (Askenase et al., 2021). We utilized MSC-sEV as carriers for miR-4465. MSCs are ideal candidates for production of sEVs for miRNA delivery (Sohrabi et al., 2022). Previous studies have shown that MSC-sEV can effectively target hepatocytes and HSCs in vivo, demonstrating their potential in treating acute and chronic liver diseases (Li et al., 2013). By modifying MSC-sEV with miR-4465, we aimed to enhance their anti-fibrosis effect and establish a novel anti-fibrosis strategy. The extraction of MSCs from umbilical cord tissue offers advantages such as abundant resource, high accessibility, low immunogenicity, and no moral or ethical barriers (Ahani-Nahayati et al., 2022). In addition, umbilical cord MSCs are relatively easy to expand in vitro.

Our research has demonstrated the potential of MSC-sEV<sup>miR-4465</sup> as a novel therapeutic strategy, although additional research is necessary to ensure patient safety. Recent studies have raised concerns regarding the delivery efficiency and capacity of miRNA through sEV to target cells, as noted by Askenase et al. (2021) and Albanese et al. (2021). According to Toh et al. (2018), some researchers have suggested that proteins may play a more significant role than RNA in the function of sEV. Despite the above limitations, delivering miRNA via sEV still holds great promise. The targetability of sEV can be improved by modifying their membrane using chemical or biological methods.

Discovering novel sEV derived from cells may enhance delivery efficiency and reduce protein interference, leading to better physical effects.

Studies have shown that *LOXL2* significantly promotes angiogenesis and the invasive ability of liver cancer cells (Wu et al., 2021). The knockdown of *LOXL2* has been reported to inhibit the function of liver cancer stem cells, making it a potential target for blocking the function of liver cancer cells (Li et al., 2022). Besides, to prevent the metastasis of hepatocellular carcinoma, long noncoding RNA (lncRNA) CARMN (cardiac mesoderm enhancer-associated non-coding RNA) can regulate the miR192-5p/*LOXL2* axis, thereby affecting the prognosis of hepatocellular carcinoma (Wang et al., 2022). Herein, we investigated the effectiveness of using MSC-sEV to transport exogenous miR-4465, which targets *LOXL2*, in order to inhibit the epithelial function of hepatocellular carcinoma cells. The results demonstrated that MSC-sEV<sup>miR-4465</sup> can inhibit HSC activation and suppress liver cancer cell metastasis in vitro. MSC-sEV<sup>miR-4465</sup> also shows a potential as an effective therapy for liver cancer. However, further research is needed to explore its anticancer effect in vivo.

## 4 Conclusions

Our findings suggest that MSC-sEV<sup>miR-4465</sup> can deliver miR-4465 to reduce HSC activation and alleviate liver fibrosis development via downregulating *LOXL2*. Therefore, the modified MSC-sEV may play a significant role in liver diseases and thus hold great clinical value.

## Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

## Data availability statement

Data are available on request from the authors.

## Acknowledgments

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### Author contributions

Yanjin WANG, Yifei CHEN, and Fuji YANG contributed to investigation, validation, formal analysis, and writing – original draft. Xiaolong YU, Ying CHU, and Jing ZHOU performed methodology and software. Jianbo XI and Yongmin YAN contributed to conceptualization, funding acquisition, and writing – review & editing. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

### Compliance with ethics guidelines

Yanjin WANG, Yifei CHEN, Fuji YANG, Xiaolong YU, Ying CHU, Jing ZHOU, Yongmin YAN, and Jianbo XI declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed. The animal study protocol followed the ethical policies and procedures approved by the Jiangsu University Ethics Committee (Approval No. UJS-IACUC-AP-2020033127).

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#### Supplementary information

Materials and methods