Supplementary materials

Supplementary figures and figure legends

Figures S1–S7

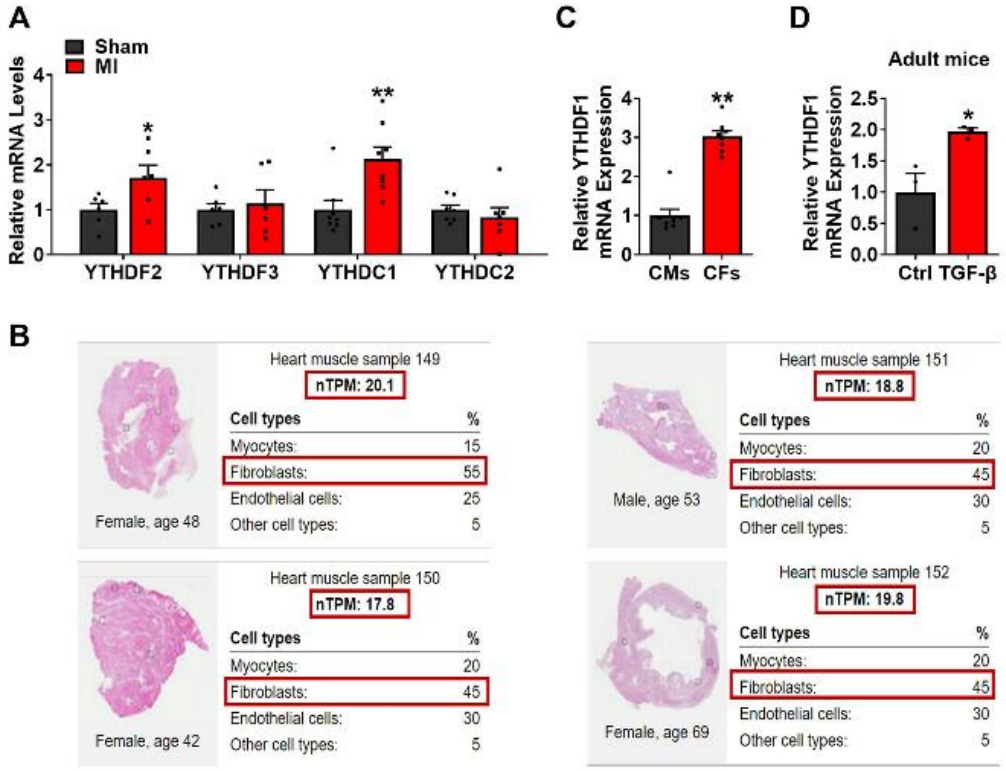

Fig. S1. Expression of m6A readers and YTHDF1 location in human heart cells. (A) The mRNA expression levels of m6A readers in MI mice. n = 6-8. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* Sham. (B) The human protein ATLAS database analyzes the expression of YTHDF1 in various cardiac cells. (C) YTHDF1 mRNA expression level in cardiomyocytes (CMs) and CFs obtained from neonatal mice. n = 8. $^{**}P <$ 0.01 *vs.* CMs. (D) The expression level of YTHDF1 in CFs obtained from male adult C57BL/6 mice. n = 3. $^{*}P < 0.05$ *vs.* Ctrl.

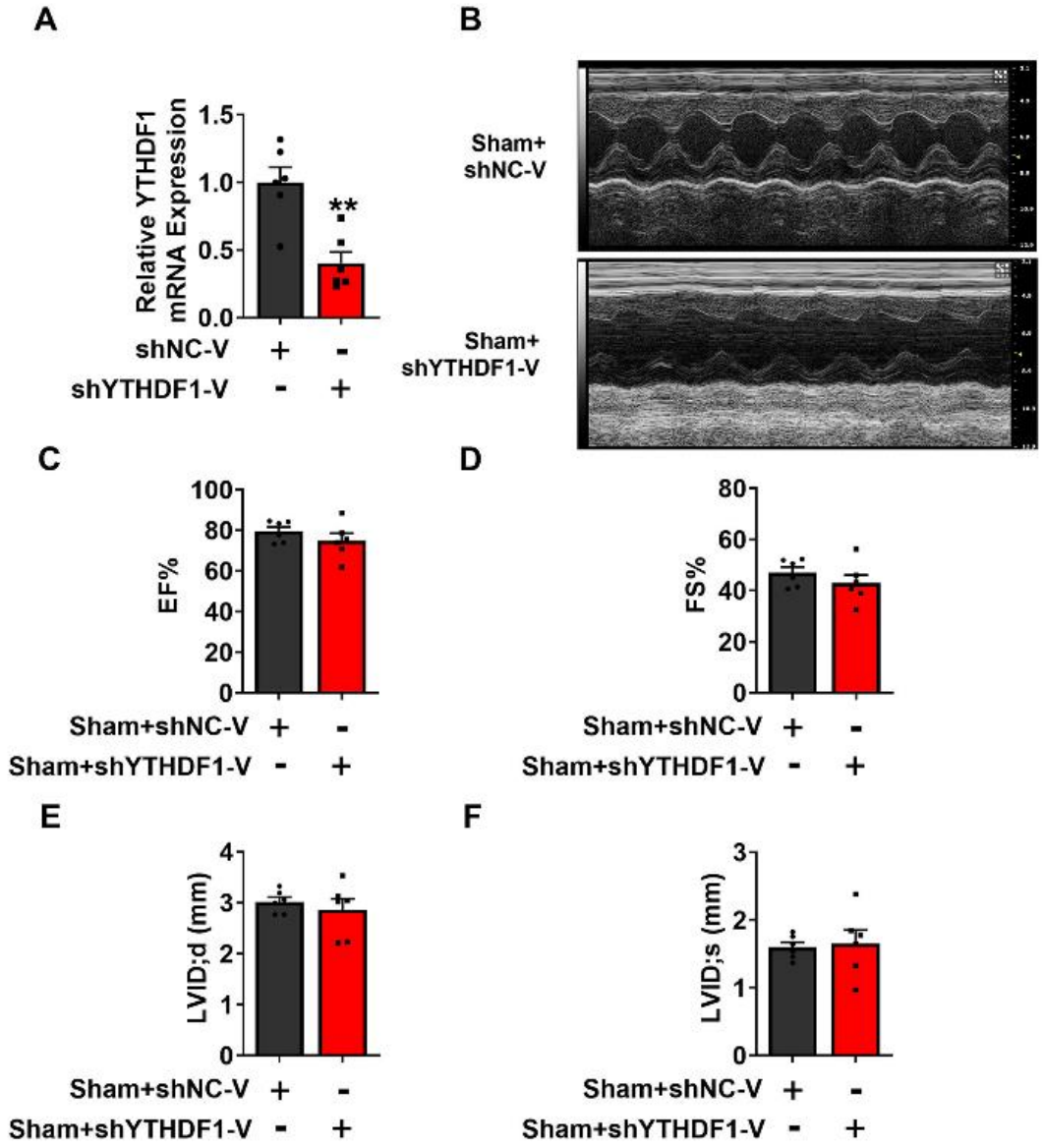

Fig. S2. The silencing efficiency of YTHDF1 and the cardiac function in sham mice. (A) YTHDF1 mRNA expression level in mice was analyzed by qRT-PCR. $n = 6$. $^{**}P < 0.01$ *vs*. shNC-V. (B) Representative echocardiographs and statistics. (C) EF (%), (D) FS (%), (E) LVID;d (mm) and (F) LVID;s (mm). $n = 6$.

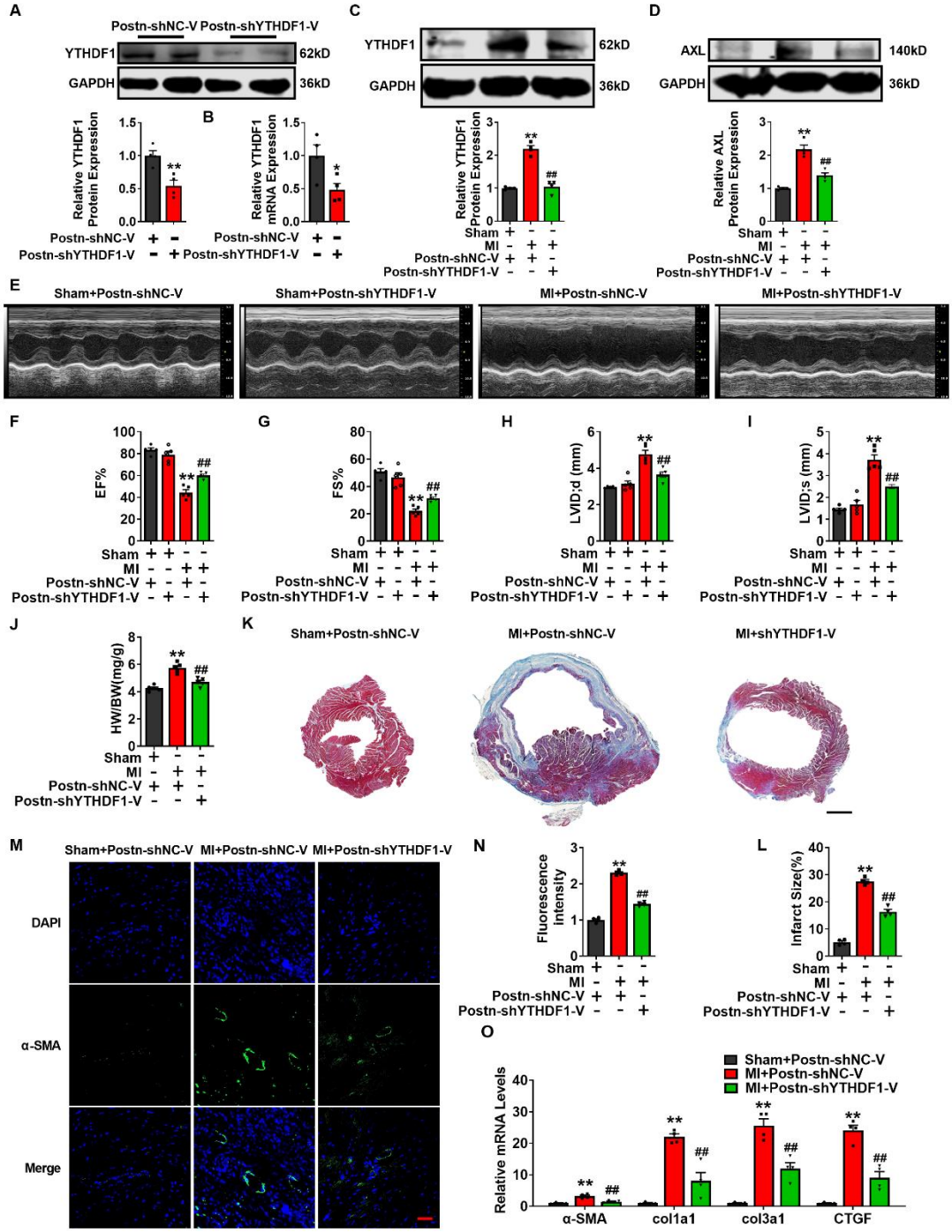

Fig. S3. Inhibiting of YTHDF1 targeting fibroblasts reversed MI induced cardiac fibrosis in mice. (A, B) YTHDF1 knockdown efficiency determination in mouse hearts tissue by delivering Postn-shYTHDF1-V via tail vein. $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. Postn-shNC-V. (C) The protein expression level of YTHDF1 was measured in each group. $n = 4$. $^{**}P < 0.01$ *vs*. Sham+Postn-shNC-V; $^{\#\#}P < 0.01$ *vs*. MI+Postn-shNC-V. (D) AXL protein expression level in each group. $n = 4$. $^{**}P < 0.01$ *vs*. Sham+Postn-shNC-V; $^{\#\#}P < 0.01$ *vs*. MI+Postn-shNC-V. (E) Echocardiography images and

quantitative statistics. (F) EF (%), (G) FS (%), (H) LVID; d (mm) and (I) LVID; s (mm). $n = 5$. $^{**}P < 0.01$ *vs.* Sham+Postn-shNC-V; $^{\#\#}P < 0.01$ *vs*. MI+Postn-shNC-V. (J) Heart weight to body weight ratio. $n = 5$. $^{**}P < 0.01$ *vs.* Sham+Postn-shNC-V; $^{\#\#}P < 0.01$ *vs.* MI+Postn-shNC-V. (K, L) The areas of fibrosis in the infarcted hearts were assessed following Masson staining. Scale bar = 1 mm. $n = 4$. $^{**}P < 0.01$ *vs.* Sham+Postn-shNC-V; $^{\#\#}P < 0.01$ *vs.* MI+Postn-shNC-V. (M, N) Representative images of immunofluorescence assay of α-SMA in mouse hearts. Scale bar = 20 μm. $n = 4$. $^{**}P < 0.01$ *vs.* Sham+Postn-shNC-V; $^{\#\#}P < 0.01$ *vs.* MI+Postn-shNC-V. (O) QRT-PCR analysis showing the mRNA levels of fibrotic markers in mouse hearts. $n = 4$. $^{**}P < 0.01$ *vs.* Sham+Postn-shNC-V; $^{\#\#}P < 0.01$ *vs.* MI+Postn-shNC-V.

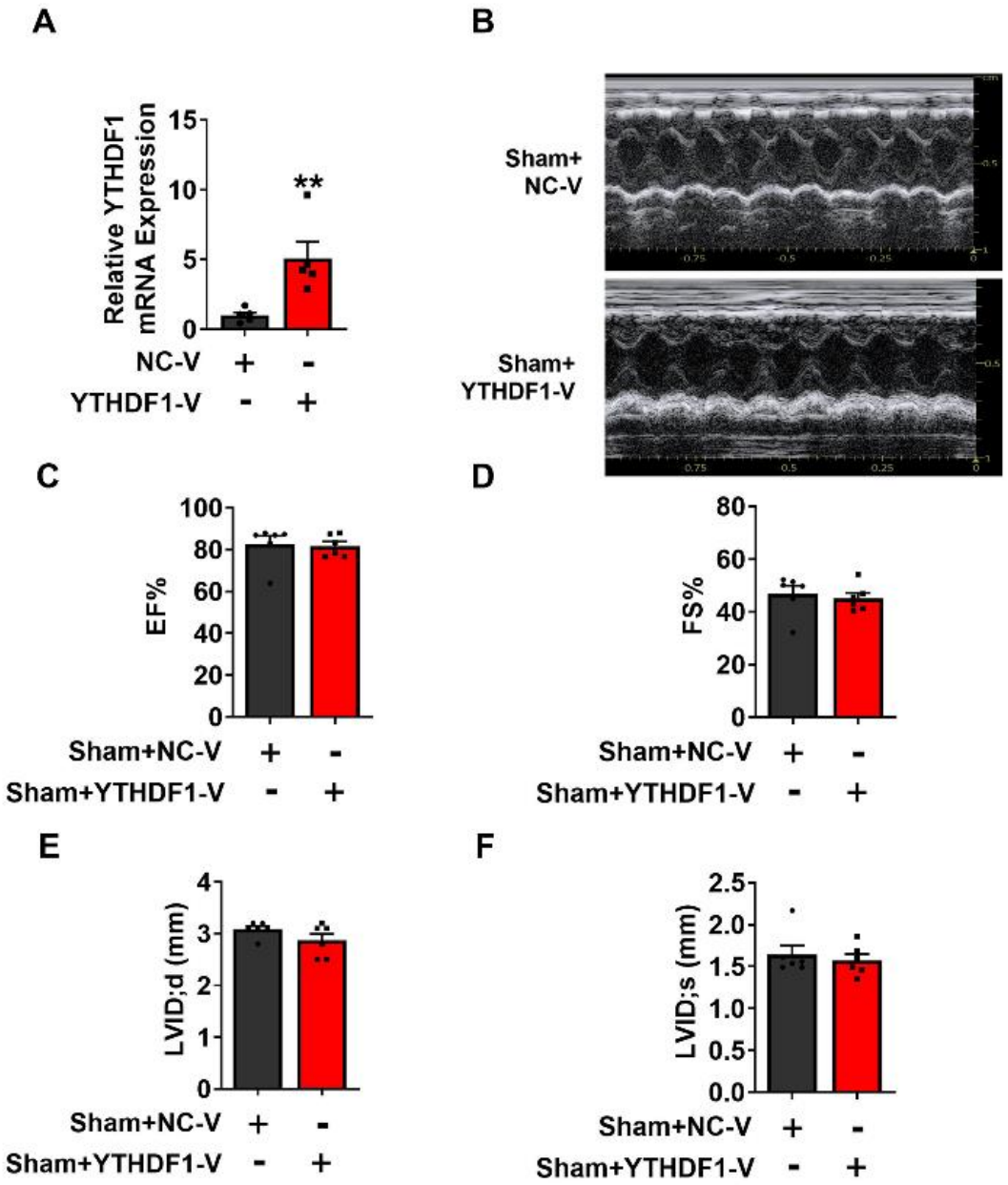

Fig. S4. YTHDF1 overexpression efficiency and the cardiac function in sham mice. (A) The mRNA expression level of YTHDF1 in mice. $n = 5$. $^{**}P < 0.01$ *vs*. NC-V. (B) Representative images of echocardiographs and statistics. (C) EF (%), (D) FS (%), (E) LVID; d (mm) and (F) LVID; s (mm). $n = 6$.

A

TF binding site Prediction Result

CBFB	hTFtarget	YTHDF1	721	745	-	9.19737	1.78e-05	0.0227	AGGACACCAGAGCGAGGCACATGCA
E2F4	hTFtarget	YTHDF1	1863	1874	-	13.0789	1.78e-05	0.039	ATGGGGCGGGGC
EHF	hTFtarget	YTHDF1	2383	2404	+	12.8767	1.78e-05	0.0424	GGACTGGGAACAGGGAGGCCGG
EP300	hTFtarget	YTHDF1	2074	2098	+	11.1818	1.78e-05	0.00145	GCTGCTTCCCGCTGTTCCGGCCCGC
ZBTB7B	database	YTHDF1	2113	2134	-	9.68367	1.78e-05	0.0115	GCGGGTGCGGCCGGGCGCGGCG
BRCA1:USF2	database	YTHDF1	633	640	-	10.2935	1.79e-05	0.0885	TTCTGTTG
ZBED6	database	YTHDF1	1754	1765	-	13.1364	1.79e-05	0.0816	TGGGCTGGCCTC
FOXO3	hTFtarget	YTHDF1	1283	1307	-	-1.40789	1.79e-05	0.00798	ATATATAGACAAGCAGGCACACACA
HDAC2	hTFtarget	YTHDF1	1979	2003	-	10.8657	1.79e-05	0.00204	GACGTCAGCGCGCGGGGCAGGCCGG
MYC	hTFtarget	YTHDF1	2092	2101	+	13.3714	1.79e-05	0.0536	GGCCCGCGTG
POU5F1	hTFtarget	YTHDF1	2205	2226	-	12.2368	1.79e-05	0.00156	CGCGGCGCGGGACCCGGGCGCC

Fig. S5. Transcription factor prediction. (A) The potential binding sequence site for the ZBED6 and YTHDF1 promoter regions were predicted by AnimalTFDB3.0.

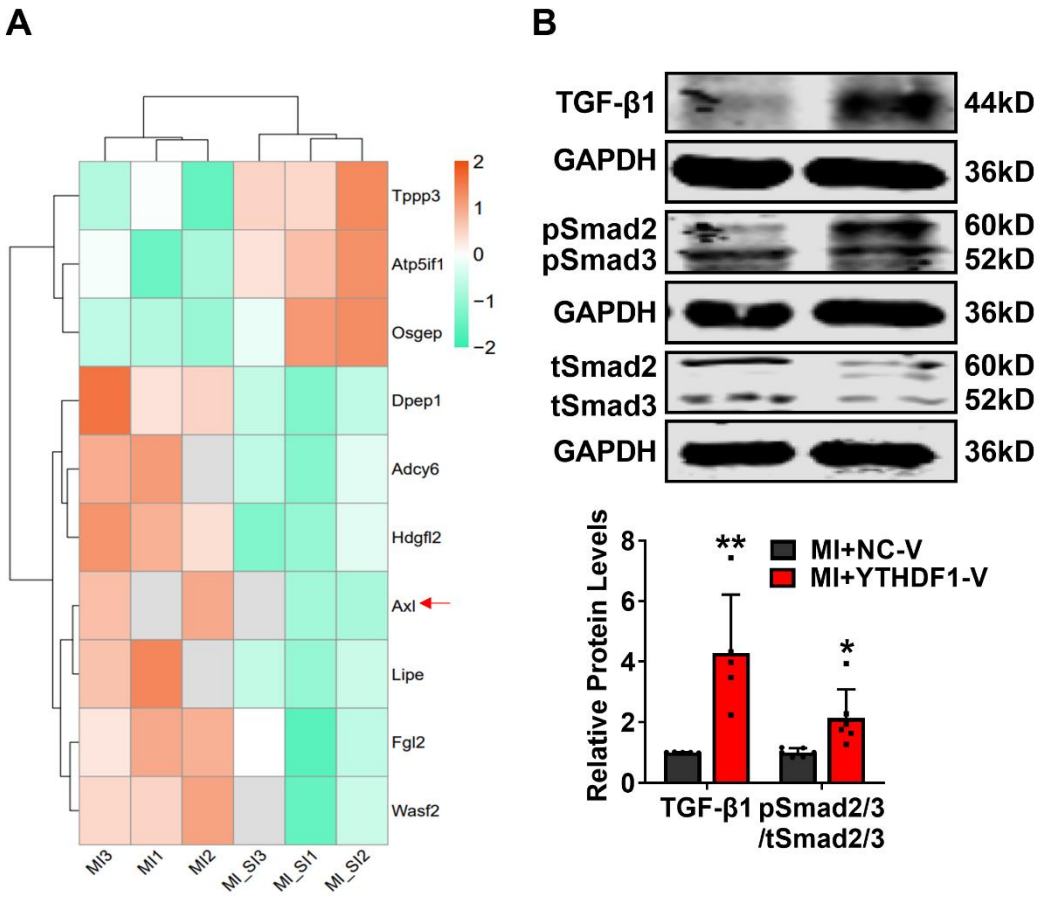

Fig. S6. Proteomics and downstream protein expression level. (A) The heatmap exhibited differentially expressive genes in MI+shNC-V and MI+shYTHDF1-V in mice heart. $n = 3$. (B) The protein expression level of TGF-β and phosphorylated Smad2/3 to total Smad2/3 ratio in YTHDF1-overexpression-MI-mice. $n = 5$-6. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. MI+NC-V.

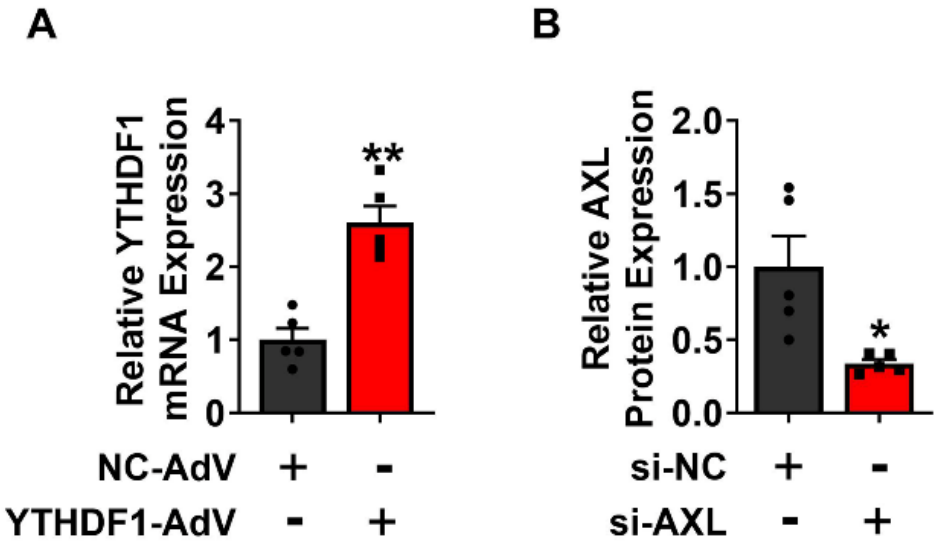

Fig. S7. Transfection efficiency verification. (A) The efficiency of YTHDF1-AdV was detected by qRT-PCR. $n = 5$. $^{**}P < 0.01$ *vs*. NC-AdV. (B) The efficiency of AXL knockdown was detected by qRT-PCR. $n = 5$. $^{*}P < 0.05$ *vs*. si-NC.

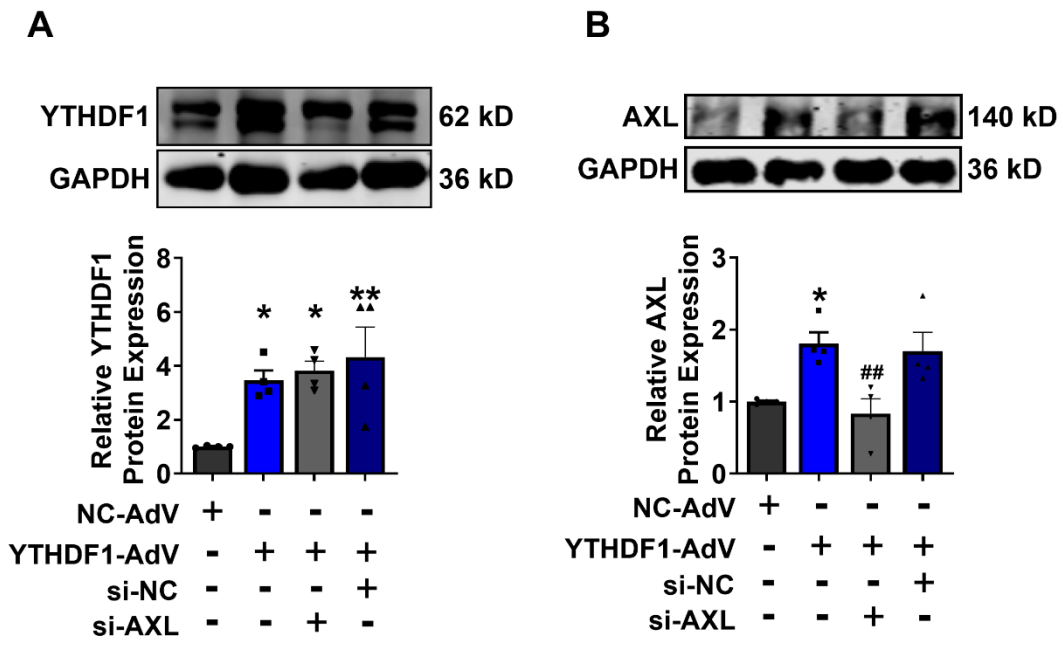

Fig. S8. Determination of protein expression. (A) The protein expression level of YTHDF1 was detected. $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. NC-AdV. (B) AXL protein expression level was measured in each group. $n = 4$. $^{*}P < 0.05$ *vs*. NC-AdV, $^{\#\#}P < 0.01$ *vs*. YTHDF1-AdV.

Table S1: The primer sequences used for qPCR

Gene		Sequence (5'to 3')
GAPDH	F	AAGAAGGTGGTGAAGCAGGC
	R	TCCACCACCCTGTTGCTGTA
18s	F	CCTGGATACCGCAGCTAGGA
	R	GCGGCGCAATACGAATGCCCC
YTHDF1	F	TGGGAGTGGACATTTCTGTG
	R	TTCTAAGGGCACCTCCTGTG
α-SMA	F	GACGCTGAAGTATCCGATAG
	R	CCACACGAAGCTCGTTATAG
Col1a1	F	GCTCCTCTTAGGGGCCACT
	R	CCACGTCTCACCATTGGGG
Col3a1	F	ACGTAGATGAATTGGGATGCAG
	R	GGGTTGGGGCAGTCTAGTG
AXL	F	CAACTGTGCTACGTCCCCTC
	R	TTTCCATCCTCTTGCCGCTC
ZBED6	F	CTATCTGCCTAGTACCAGAGCC
	R	CCTCCTCCCCATTAGTGACTC

Supplementary file 1 All genes detected in the RNA-sequence.