Supplemental information

ALKBH5-mediated m^{6}A

demethylation of HS3ST3B1-IT1 prevents

osteoarthritis progression

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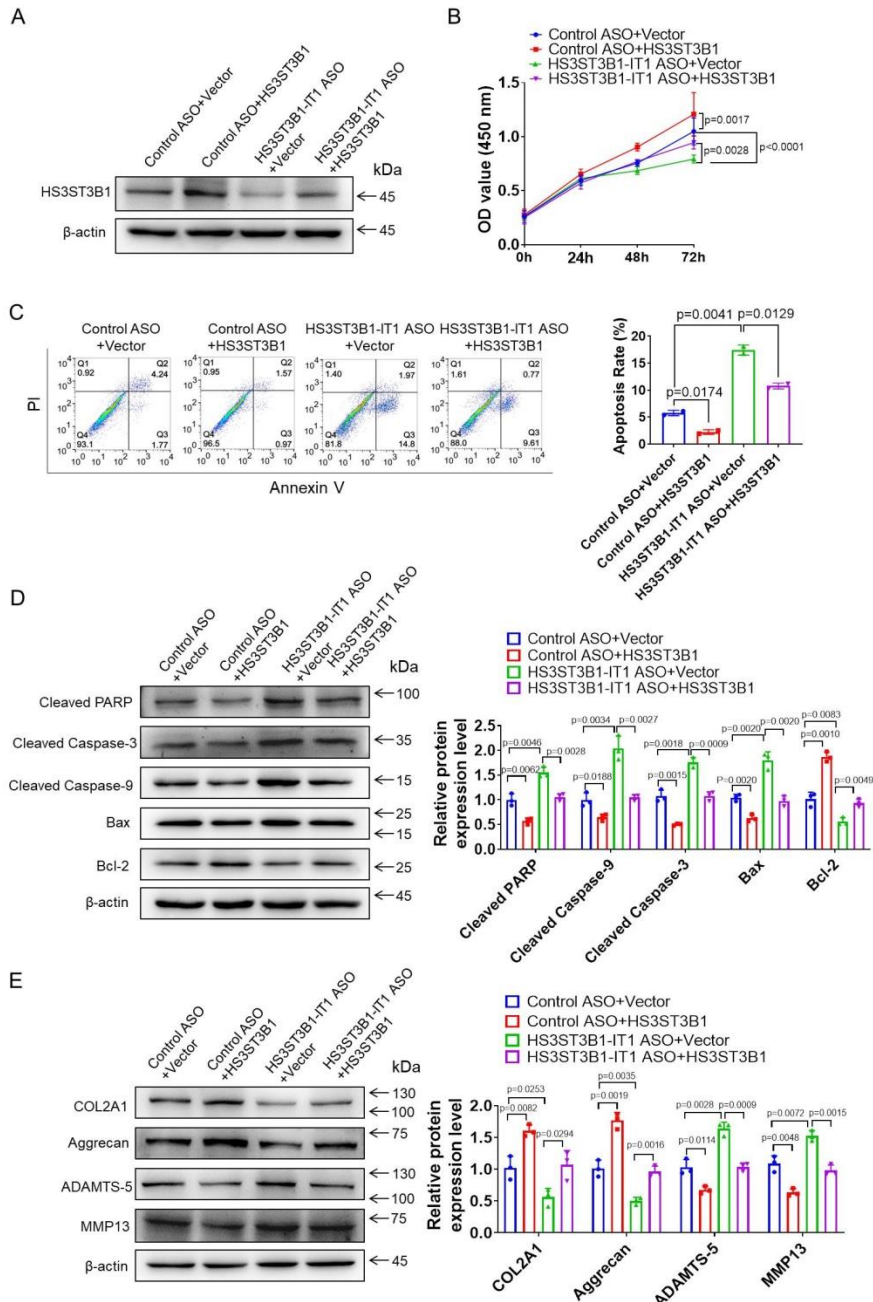## Supplemental materials

Supplemental figures

Fig.S1

Figure S1 Safranin-O staining and IHC staining was performed to assess the histopathological changes in normal and osteoarthritic cartilage samples, related to Figure 1. (A) Safranin-O staining (scale bar=200 μm). (B) IHC staining for COL2A1 and MMP13 (scale bar=200 μm). Representative images from three independent experiments were shown in the left panel. OA, osteoarthritis.

Fig.S2

Figure S2 Characteristics of HS3ST3B1-IT1 predicted by online software, related to Figure 1 and Figure 2. (A) The coding capability of HS3ST3B1-IT1 was determined by CPAT (http://lilab.research.bcm.edu/cpat/). (B) RegRNA 2.0 online software predicted an ORF (315 nt) in the HS3ST3B1-IT1 transcript. (C) The schematic diagram of three GFP fusion constructs (GFPwt, GFPmut, and ORF-GFPmut). (D, E) The expression level of GFP proteins was detected by IF (D) and western blotting assays (E). Scale bar=500 μm.

Fig.S3

Figure S3 HS3ST3B1 has no obvious effects on HS3ST3B1-IT1 expression, related to Figure 5. qRT-PCR was performed to detect the expression levels of HS3ST3B1-IT1 in chondrocytes after transfection with pCMV3-HS3ST3B1, HS3ST3B1 siRNAs and their respective controls. Values were shown as mean $\pm$ SD (n=3). Statistical differences were determined using unpaired Student's two-tailed *t* test.

Fig.S4

Figure S4 Computational analysis for HS3ST3B1-IT: HS3ST3B1 interaction by the RPISeq database (http://pridb.gdcb.iastate.edu/RPISeq/) based on Random Forest (RF) and Support Vector Machine (SVM) classifiers, related to Figure 5.

Fig.S5

A

B

C

D

E

Figure S5 Overexpression of HS3ST3B1 attenuates the effects of HS3ST3B1-IT1 knockdown on chondrocyte viability, apoptosis and ECM metabolism, related to Figure 6. (A) Western blotting was performed to assess the expression levels of HS3ST3B1 in chondrocytes transfected with Control ASO+Vector, Control ASO+pCMV3-HS3ST3B1, HS3ST3B1-IT1 ASO+Vector or HS3ST3B1-IT1 ASO+pCMV3-HS3ST3B1. (B) The viability of chondrocytes was evaluated by CCK-8 assay in each group. Values were shown as mean ± SD (n=3). (C) The apoptosis rates were evaluated by flow cytometry in each group. Values were shown as mean ± SD (n=3). (D) The expression levels of apoptosis-associated proteins were detected by western blotting in each group. Representative blots from three independent experiments were shown (left panel). Densitometric analyses of the blots were presented as mean ± SD (n=3, right panel). (E) The expression levels of COL2A1, Aggrecan, MMP13 and ADAMTS-5 were analyzed by western blotting in each group. Representative blots from three independent experiments were shown (left panel). Densitometric analyses of the blots were presented as mean ± SD (n=3, right panel). Statistical analysis was performed using a two-way ANOVA followed by Tukey's multiple comparison (B) or an unpaired Student's two-tailed *t* test (C-E).

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Fig.S6
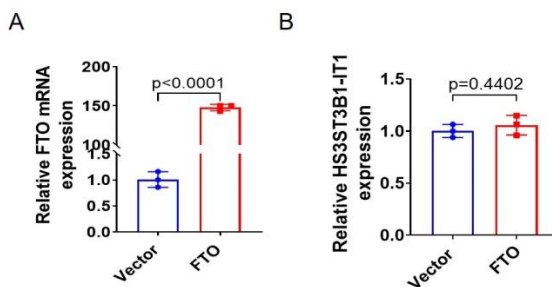
A   B

Figure S6 FTO has no obvious effects on HS3ST3B1-IT1 expression, related to Figure 7.
qRT-PCR was performed to detect the expression levels of FTO (A) and HS3ST3B1-IT1 (B) in
chondrocytes after transfection with FTO expression plasmid. Values were shown as mean
± SD (n=3). Statistical differences were determined using an unpaired Student's two-tailed
t test.

Fig.S7

Figure S7 Computational analysis for HS3ST3B1-IT: YTHDF2 interaction by the RPISeq database (http://pridb.gdcb.iastate.edu/RPISeq/) based on Random Forest (RF) and Support Vector Machine (SVM) classifiers, related to Figure 7.

Fig.S8

A

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                                                          32
MGQRLSGGRSCLDVPGRLLPQPPPPPPVRRKLALLFAMLCVWLY

MFLYSCAGSCAAAPGLLLLGSGSRAAHDPPALATAPDGTPPRLPFR
            102                                          135
APPATPLASGKEMAEGAASPEEQSPEVPDSPSPISSFFSGSGSKQLP
    146 147                                         179
QAIIIGVKKGGTRALLEFLRVHPDVRAVGAEPHFFDRSYDKGLAW
                200                              219   222
YRDLMPRTLDGQITMEKTPSYFVTREAPARISAMSKDTKLIVVVR
                    244                    257
DPVTRAISDYTQTLSKRPDIPTFESLTFKNRTAGLIDTSWSAIQIGIY
278                                                     318
AKHLEHWLRHFPIRQMLFVSGERLISDPAGELGRVQDFLGLKRIIT
324        330 332        338 339              351 353
DKHFYFNKTKGFPCLKKAEGSSRPHCLGKTKGRTHPEIDREVVR
                378
RLREFYRPFNLKFYQMTGHDFGWD
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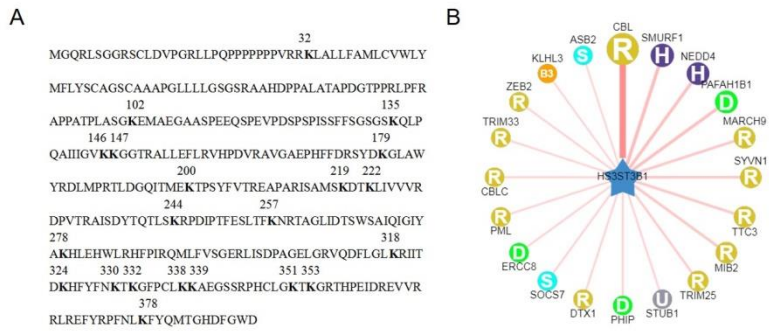
B

Figure S8 Prediction of the Ubiquitination Sites (A) and potential E3 ubiquitin ligases (B) by using UbiBrowser (http://ubibrowser.ncpsb.org/), related to Figure 5.

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Supplemental Tables
Table S3 Logistic regression analysis, related to Figure 1

Variable	B	Std. Error	*p* value	95% CI
Age	-0.058	0.039	0.140	0.874-1.019
BMI	-0.07	0.093	0.448	0.778-1.118
Sex	0.024	0.578	0.967	0.33-3.179

B, coefficient; Std. Error, standard error; CI, confidence interval.

Table S5 Primers used for plasmid construction, related to STAR Methods

Plasmid names	Forward/ Reverse	Sequences 5'-3'
pcDNA-HS3ST3B1-IT1	Forward	CGGGATCCCTGAGATCAGACACACTTGGGCC
	Reverse	CCCTCGAGAAAAGTTACAGATTTAATTTTTGGTC
pEGFP-GFPmut	Forward	GCCACCATTGTGAGCAAGGGCGAGGAGCTGTT
	Reverse	TTGCTCACAATGGTGGCGACCGGCCGGTGGAT
pEGFP-ORF-GFPmut	Forward	CTCAGATCTCGAGATTGCGGCTGGAC
	Reverse	AATCTCGAGATCTGAGTCCGGTAGCG
psi-CHECK2-HS3ST3B1 -IT1 wt	Forward	CCCTCGAGCTGAGATCAGACACACTTGGG
	Reverse	GGGTTTAAACAGCTTGGCAGAGAGGAGAGAAC
psi-CHECK2-HS3ST3B1 -IT1 mut1	Forward	ACACTGCGTTCCTGGCCTGCACCAGC
	Reverse	GCCAGGAACGCAGCGCTTGGGGCCAG
psi-CHECK2-HS3ST3B1 -IT1 mut2	Forward	AACTGACAAGCAGGCCTCCCGGGTCCAGGGGGC
	Reverse	AGGCCTGCTTGTCAGTTCAGGGAGATGGCTCG
psi-CHECK2-HS3ST3B1 -IT1 mut3	Forward	ACTGGCAGGAAAAGGCCTGAACCCTT
	Reverse	GCCTTTTCCTGCCAGTTCCTCCCAAC

Table S6 Sequences of siRNAs and ASOs, related to STAR Methods

Names	Sequences 5'-3'
si-NC target sequence	TTCTCCGAACGTGTCACGT
si-HS3ST3B1 target sequence 1	GCAAATTCTAGCAGTATGT
si-HS3ST3B1 target sequence 2	GCAACAAAGCCATAGGAAA
HS3ST3B1-IT1 ASO sequence	ATTGTACCTCCGCTCTGTCC
LncRNA ASO negative control	lnc6N0000001, RiboBio

Table S7 Primers used for qRT-PCR assay, related to STAR Methods

Gene names	Forward/ Reverse	Sequences 5'-3'
HS3ST3B1-IT1	Forward	CTGAGATCAGACACACTTGGG
	Reverse	CCCAGGTGCTTCAAAACCG
HS3ST3B1	Forward	CCCATCTCCAGCTTTTTCAGTG
	Reverse	CTCCATGGTGATCTGCCCGT
ALKBH5	Forward	AGTTCCAGTTCAAGCCTATTCG
	Reverse	TGAGCACAGTCACGCTTCC
YTHDF2	Forward	CCTTAGGTGGAGCCATGATTG
	Reverse	TCTGTGCTACCCAACTTCAGT
β-actin	Forward	AGATGTGATCAGCAAGCAG
	Reverse	GCGCAAGTTAGGTTTTGTCA