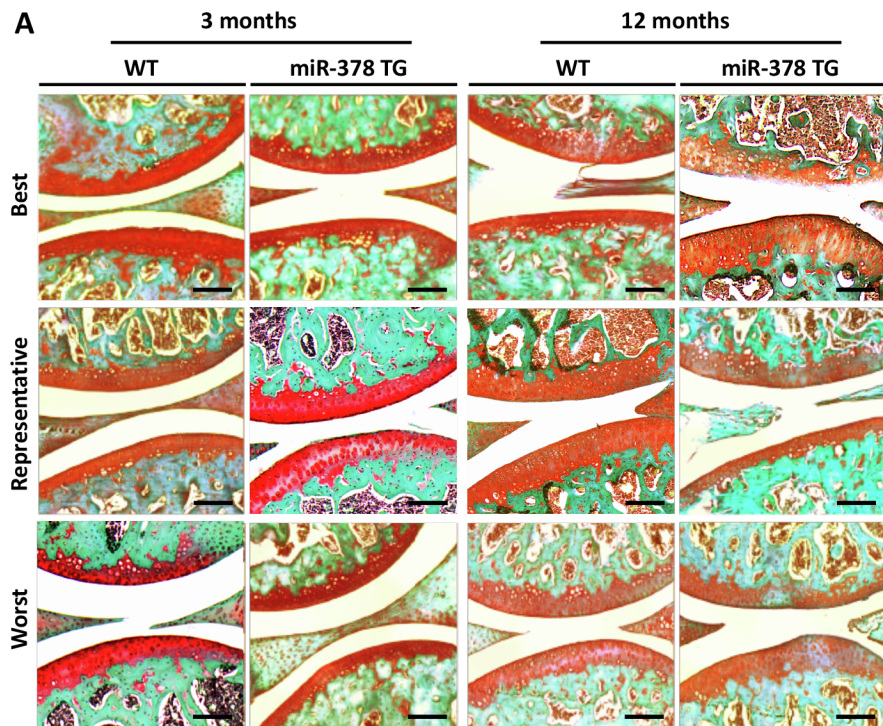# Supplemental information

MicroRNA-378 contributes to osteoarthritis

by regulating chondrocyte autophagy and

bone marrow mesenchymal stem cell chondrogenesis

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A

	3 months		12 months	
	WT	miR-378 TG	WT	miR-378 TG

Rows (left labels): Best, Representative, Worst

B

	WT 3 months	miR-378 TG 3 months	WT 12 months	miR-378 TG 12 months
Best	Stage: 0 Grade: 0 OARSI: 0	Stage: 0 Grade: 0 OARSI: 0	Stage: 0 Grade: 0. OARSI: 0	Stage: 2 Grade: 1 OARSI: 2
Representative	Stage: 1 Grade: 0 OARSI: 0	Stage: 1 Grade: 1 OARSI: 1	Stage: 0 Grade: 0 OARSI: 0	Stage: 3 Grade: 1 OARSI: 3
Worst	Stage: 1 Grade: 1 OARSI: 1	Stage: 1 Grade: 1 OARSI: 1	Stage: 2 Grade: 1 OARSI: 1	Stage: 2 Grade: 2 OARSI: 4

Supplementary Figure 1. A. The best, representative and worst results of Safranin O/fast green staining of OA mice cartilage. **B.** Semi-quantitative analysis of OA score. OA cartilage histopathology grade and stage were evaluated accordingly. OA score=grade X stage.

OA cartilage histopathology grade: Grade 0, cartilage surface intact; Grade 1, surface intact; Grade 2, surface discontinuity; Grade 3, vertical fissures; Grade 4, erosion; Grade 5, denudation; Grade 6, deformation. OA cartilage histopathology stage: Stage 0, No OA activity seen; Stage 1, <10%; Stage 2 10-25%; Stage 3, 25-50%; Stage, 4 >50%.
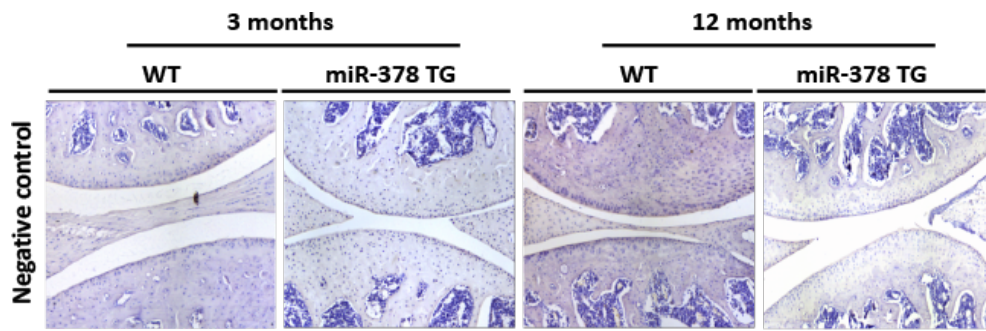
Supplementary Figure 2. Negative control of IHC staining of Col X and Mmp13 positive chondrocytes without primary antibodies application in articular cartilage of WT and miR-378 TG mice at the age of 3-month-old and 12-month-old at low magnitude. Sections were counterstained with hematoxylin (Scale bar=200 μm).
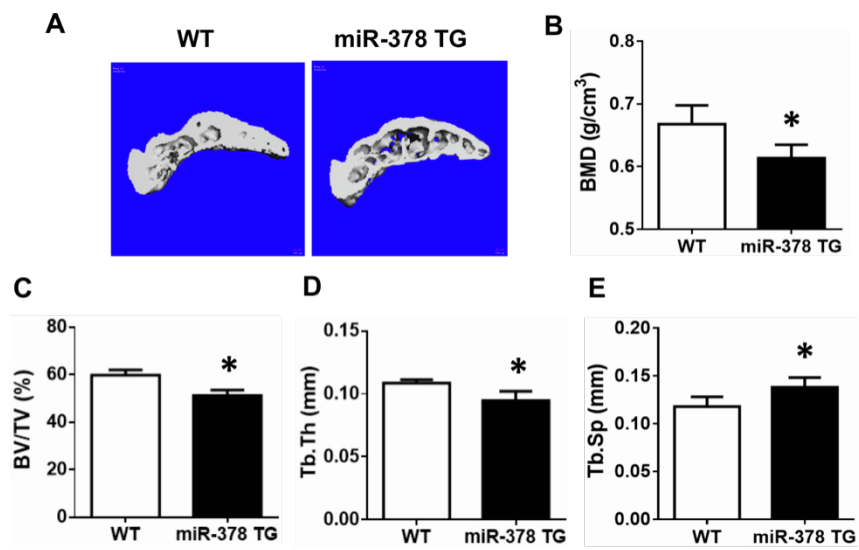
Supplementary 3. A. Micro-CT image reconstruction of subchondral bone in WT and miR-378 TG mice at the age of 12 months. **B-E.** The BMD **(B)**, BV/TV (%) **(C)**, Tb.Th **(D)** and Tb.Sp **(E)** were also evaluated (n=8, *p<0.05).
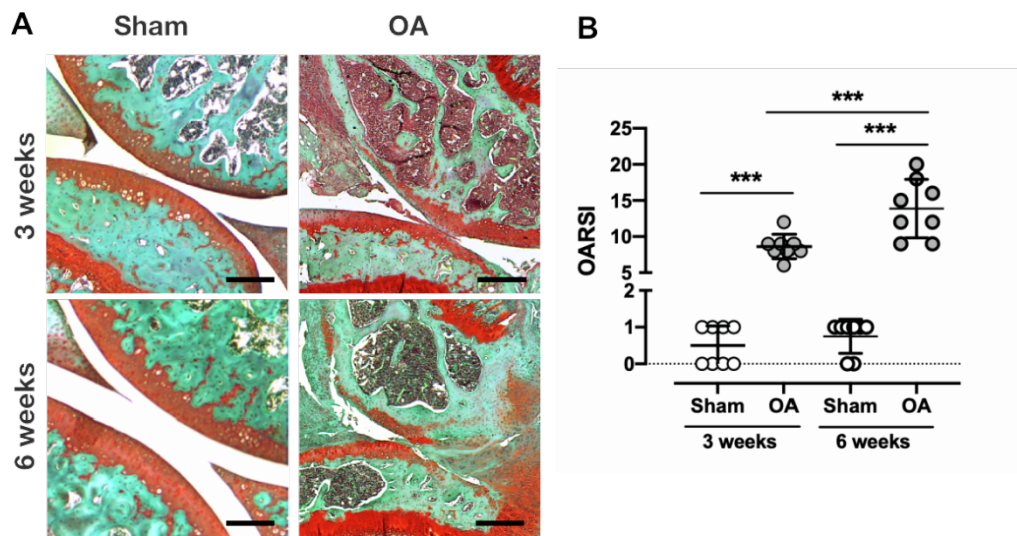
Supplementary Figure 4. A&B. Safranin O&fast green staining **(A)** and OARSI scores **(B)** of knee joint cartilage collected from both Sham and OA groups at week 3 and week 6 post ACLT+DMM surgery (n=8, ***p<0.001).
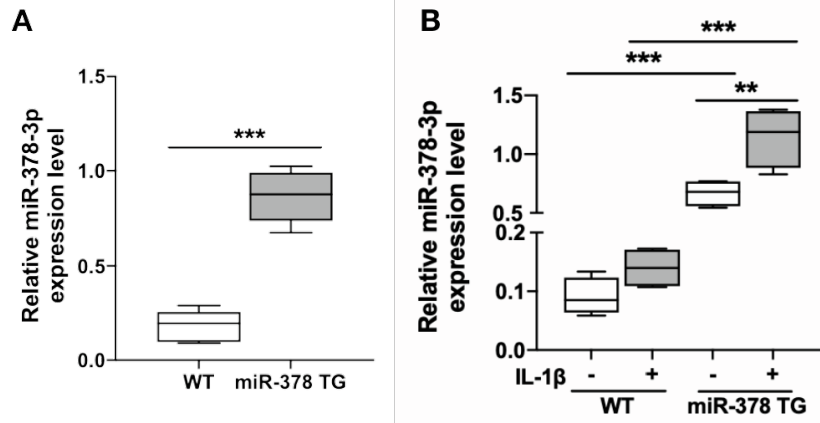
Supplementary Figure 5. A&B. miR-378 expression level in cartilage of WT and miR-378 TG mice **(A)** and in chondrocyte of WT and miR-378 TG mice with/without IL-1β treatment were studied by using Real-time PCR **(B)**. The relative miRNA expression was normalized to mmu-miR-103a-3p (n=4; **p<0.01, ***p<0.001).
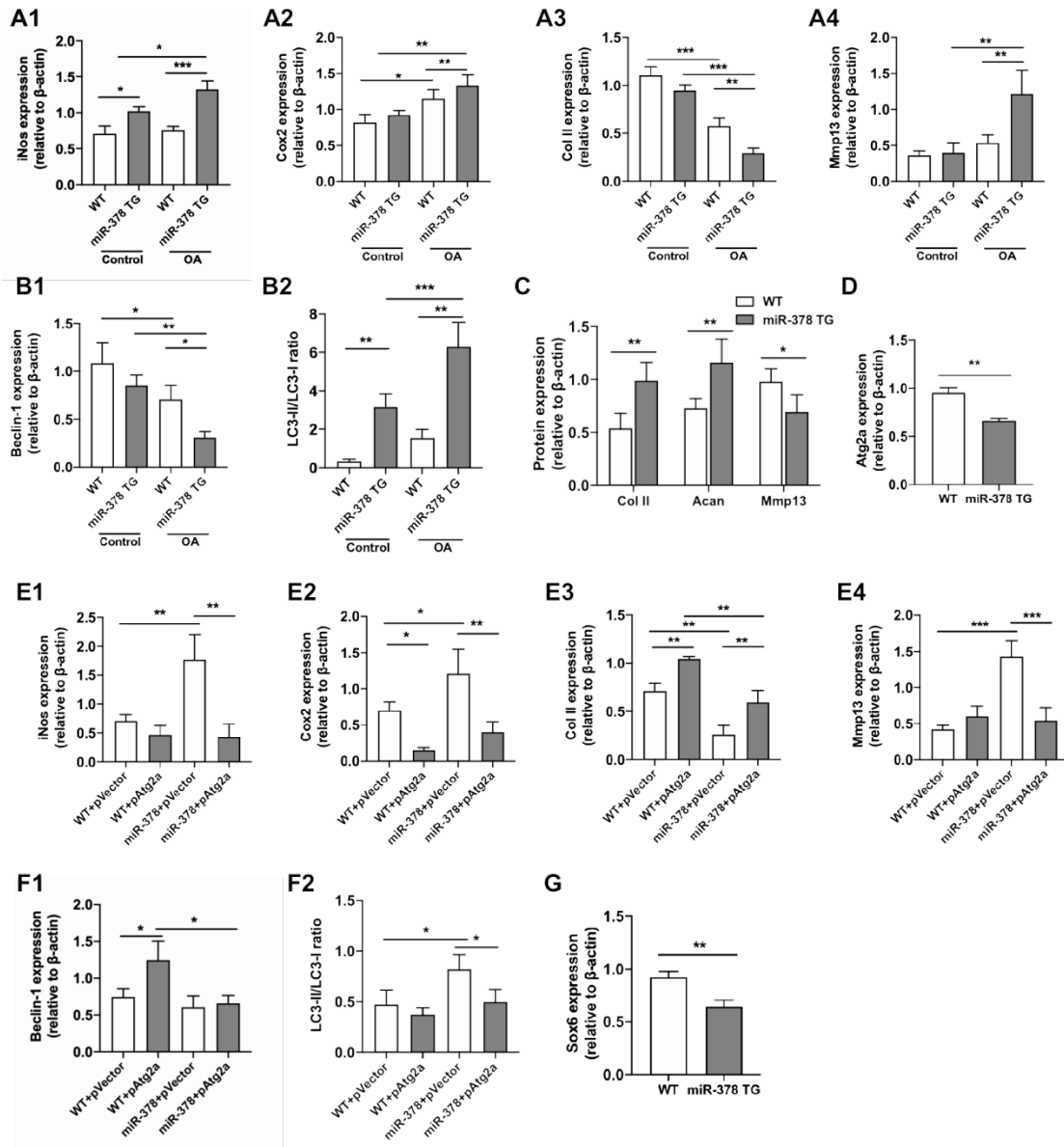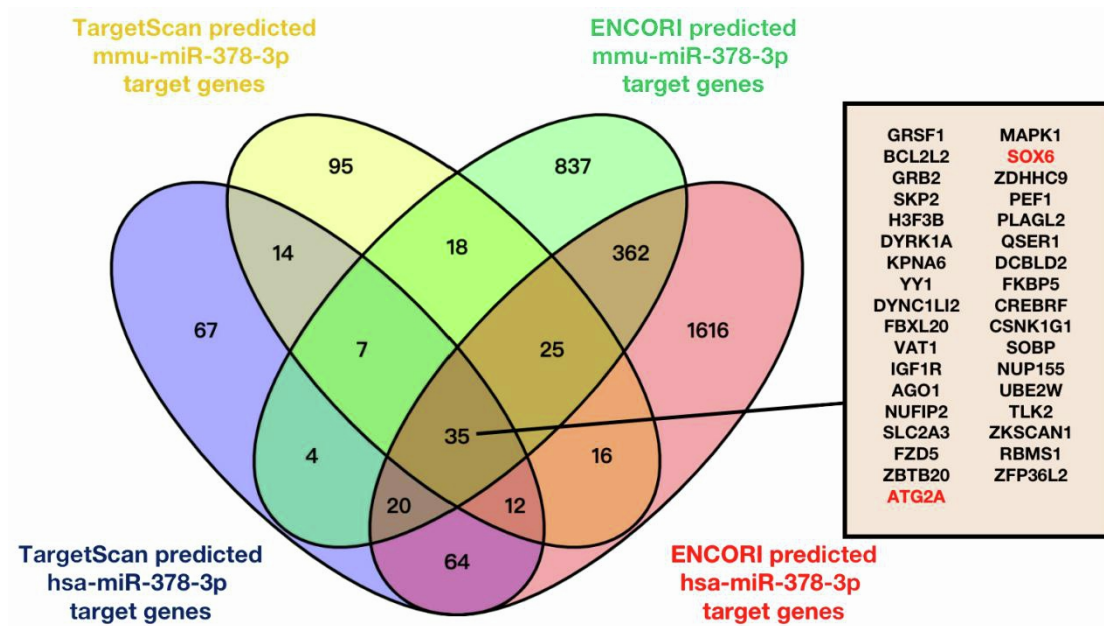
Supplementary Figure 6. A-G. Semi-quantitative analysis of all the Western blot bands in Figure 3F **(A)**, 3G **(B)**, 4G **(C)**, 5C **(D)**, 5I **(E)**, 5J **(F)** and 6C **(G)** (n=3; *p<0.05, **p<0.01, ***p<0.001).
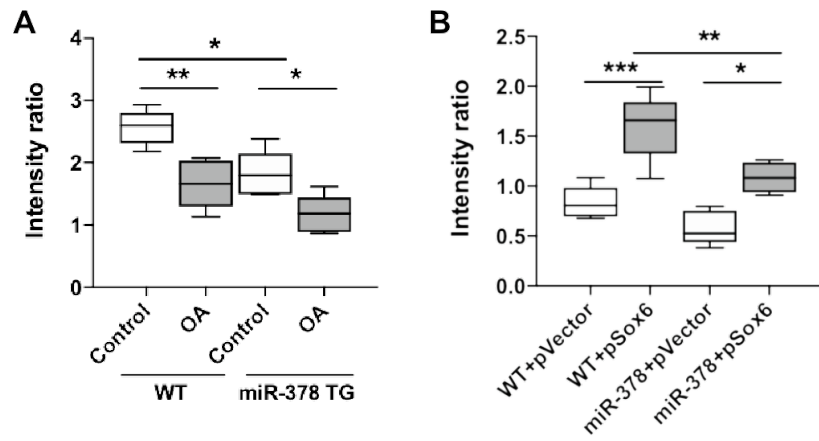
Supplementary Figure 7. A&B. Semi-quantitative analysis of cell fluorescence in Figure 3H **(A)** and 5K **(B)** (n=6; *p<0.05, **p<0.01, ***p<0.001).
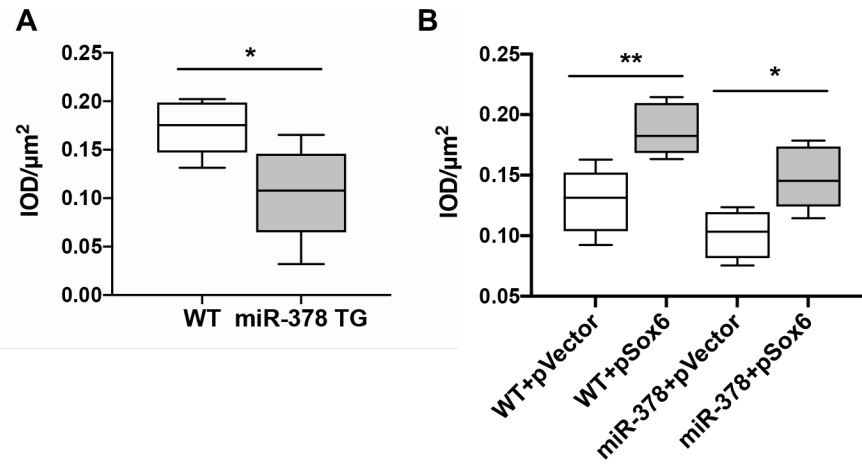
Supplementary Figure 8. A&B. Semi-quantitative analysis of IHC staining in Figure 4A **(A)** and 6I **(B)** (n=6; *p<0.05, **p<0.01).
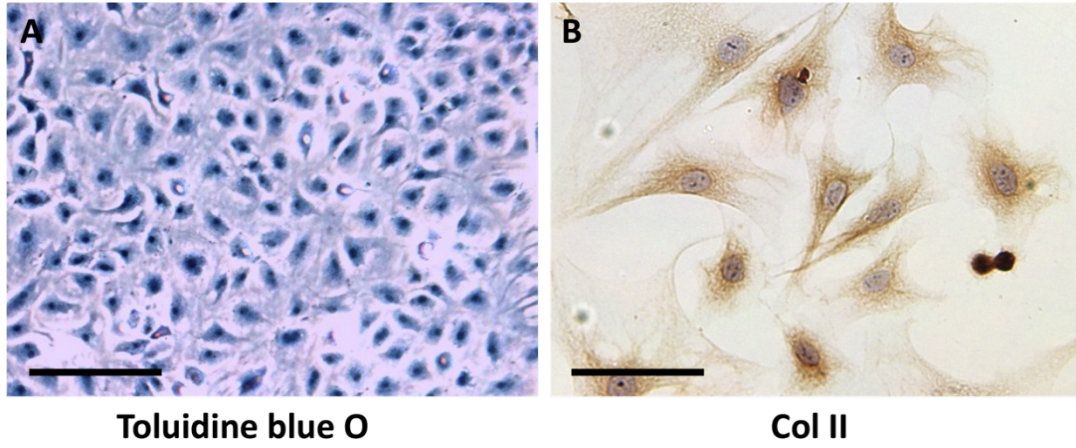
Supplementary Figure 9. Schematic diagram to show the miRNA target gene screening procedure. The TargetScan and ENCORI software were applied for *in silico* analysis of conserved 3'-UTR binding sites of miR-378 target genes respectively. The overlapped genes were listed.
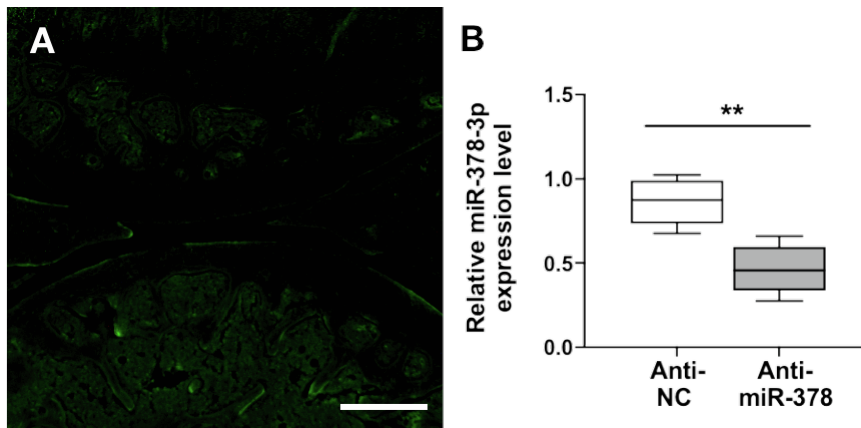
**Supplementary Figure 10. A.** The anti-miR-378-3p mediated lentivirus infected in articular cartilage 4 weeks after OA induction. Scale bar: 100 μm. **B.** The miR-378-3p expression level in cartilage of miR-378 TG mice upon receiving anti-NC and anti-miR-378-3p lentivirus injection was detected by Real-time PCR analysis. The relative miRNA expression was normalized to mmu-miR-103a-3p (n=4; **p<0.01).
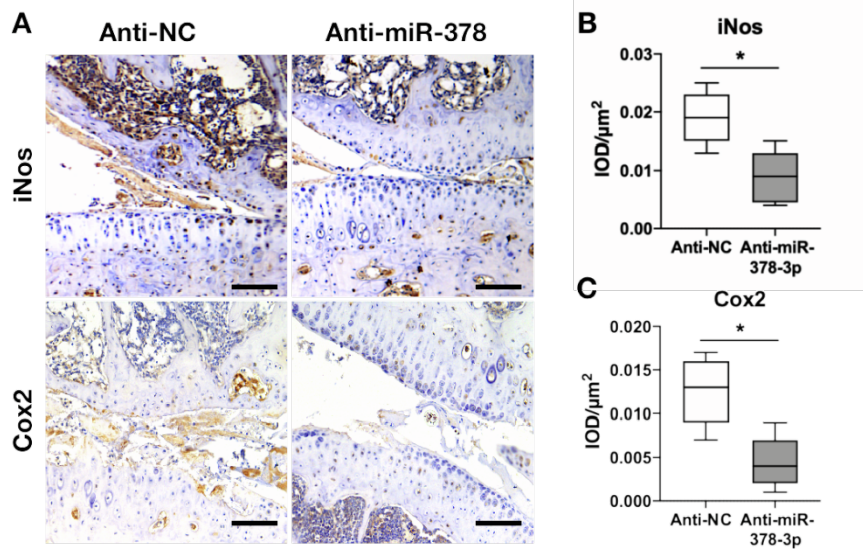
Supplementary Figure 11. IHC staining **(A)** and quantitative analysis of the percentage of iNos **(B)** and Cox2 **(C)** positive areas in articular cartilage from two group of mice at week 4 post ACLT+DMM surgery (n=8; *p<0.05). Sections were counterstained with hematoxylin. Scale bars: 50 μm.

Toluidine blue O   **Col II**

Supplementary Figure 12. A&B. Chondrocytes were characterized by Toluidine blue O staining **(A)** and IHC staining using Col II antibody **(B)**. Scale bar: A, 100 μm; B, 50 μm.
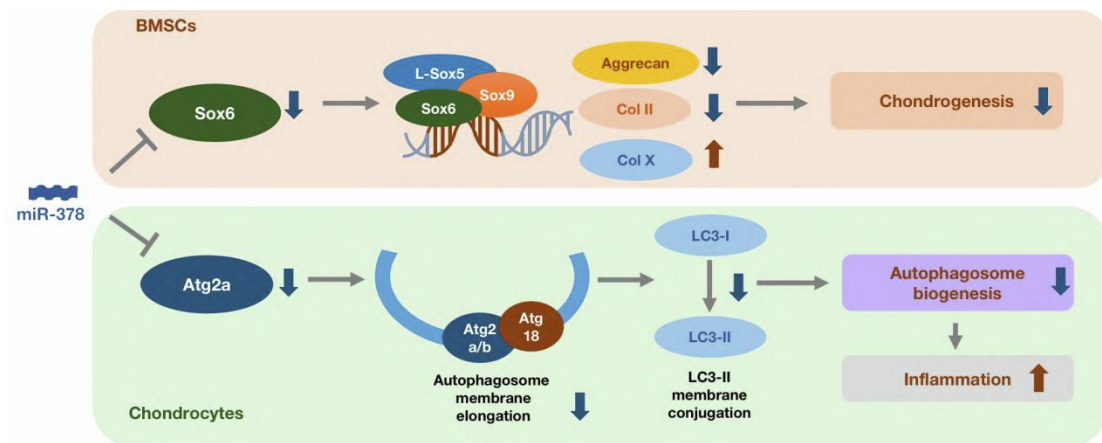
Supplementary Figure 13. Schematic diagram to show the regulation role of miR-378 on autophagy and chondrogenesis during OA progression.

Supplementary Table 1. Primers used for vector construction and Real-time PCR assays

Primers for Real-time PCR				
miNos	NM_010927	F R	CGAAACGCTTCACTTCCAA TGAGCCTATATTGCTGTGGCT	51
mCox2	NM_011198	F R	CAGACAACATAAACTGCGCCTT GATACACCTCTCCACCAATGACC	71
mBeclin-1	NM_019584	F R	TTTTCTGGACTGTGTGCAGC GCTTTTGTCCACTGCTCCTC	171
mAcan	NM_001361500	F R	CAACTATCCAGCCATCC AATAGCTCTGTAGTGGAACA	160
mSox9	NM_011448	F R	AGAAAGACCACCCCGATTACAAGT CGGCGGACCCTGAGATTG	209
mCol2a1	NM_031163	F R	AGAACAGCATCGCCTACCTG CTTGCCCCACTTACCAGTGT	161
mCol10a1	NM_009925	F R	CATCTCCCAGCACCAGAATC GTGTCTTGGGGCTAGCAAGT	152
mMmp13	NM_008607	F R	GCCATTTCATGCTTCCTGAT CTCTGGTGTTTTGGGATGCT	98
mSox6	NM_011445	F R	TGCGACAGTTCTTCACTGTGG CGTCCATCTTCATACCATACG	195
mAtg2a	NM_194348	F R	CCAGCCTAGCAGCCAGTATC CAGCAGAGCCTCTTGAGCTT	331
mYwhaz	NM_0011740	F R	CAGTAGATGGAGAAAGATTTGC GGGACAATTAGGGAAGTAAGT	92
hSOX6	NM_017508	F R	TGAGGAGCTACCAACACTTGTCA TCGGAAGGAATATAGGGAACATAACT	121
hATG2A	NM_015104	F R	TGGCCATGGTCAAACTGTGT TGACCTAAGTAGTGGTGCAGCAA	65
hYWHAZ	NM_003406	F R	ACCGTTACTTGGCTGAGGTTGC CCCAGTCTGATAGGATGTGTTGG	130

"m" stands for "mouse"; "h" stands for "human".