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## Supplemental Information

# Intra-articular Delivery of Antago-miR-483-5p Inhibits Osteoarthritis by Modulating Matrilin 3 and Tissue Inhibitor of Metalloproteinase 2

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**SUPPLEMENTARY FIGURE LEGENDS**

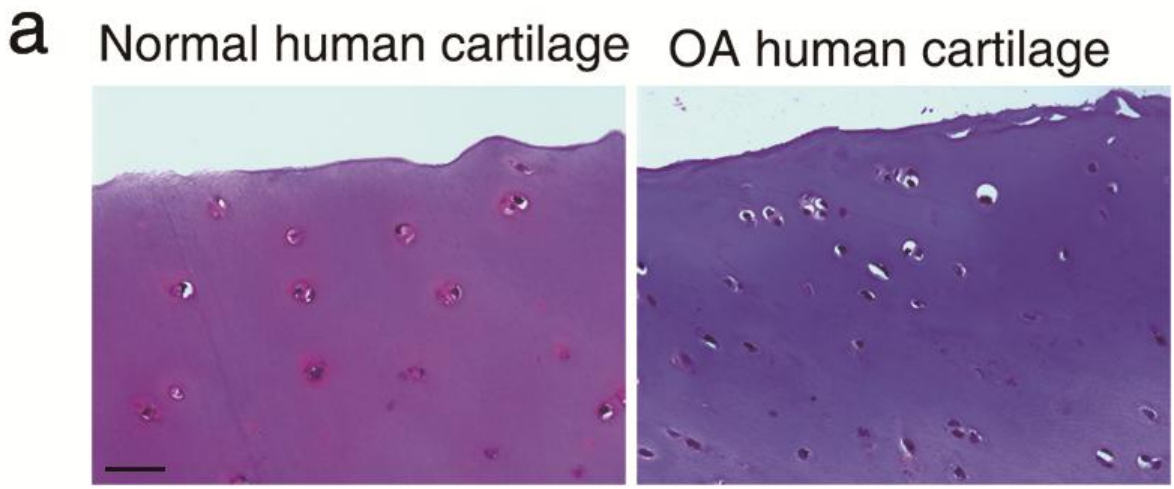
**Supplementary Figure S1. Histologic staining of OA cartilage.** (a) Safranin O-fast green staining of human normal and OA cartilage. Bar = 50 μm.

**Supplementary Figure S2. AntagomiR-483-5p decreased miR-483-5p levels in chondrocytes and synovium.** (a) Immunofluorescence analysis of GFP expression in the tibial plateau of mice intra-articularly injected with LV3-NC or control (n = 10). Bar = 40 μm. (b) Fluorescence *in situ* hybridization analysis of miR-483-5p levels in the articular cartilage (upper, Bar = 40 μm) and synovium (bottom, Bar = 120 μm) of mice injected with antagomiR-483-5p or antagomiR NC after DMM surgery. (c) Quantification of the proportion of positive cells for miR-483-5p in the articular cartilage of mice shown in (b). Error bars represent the mean ±SEM. **P* < 0.05 by independent-sample *t* test for two groups.

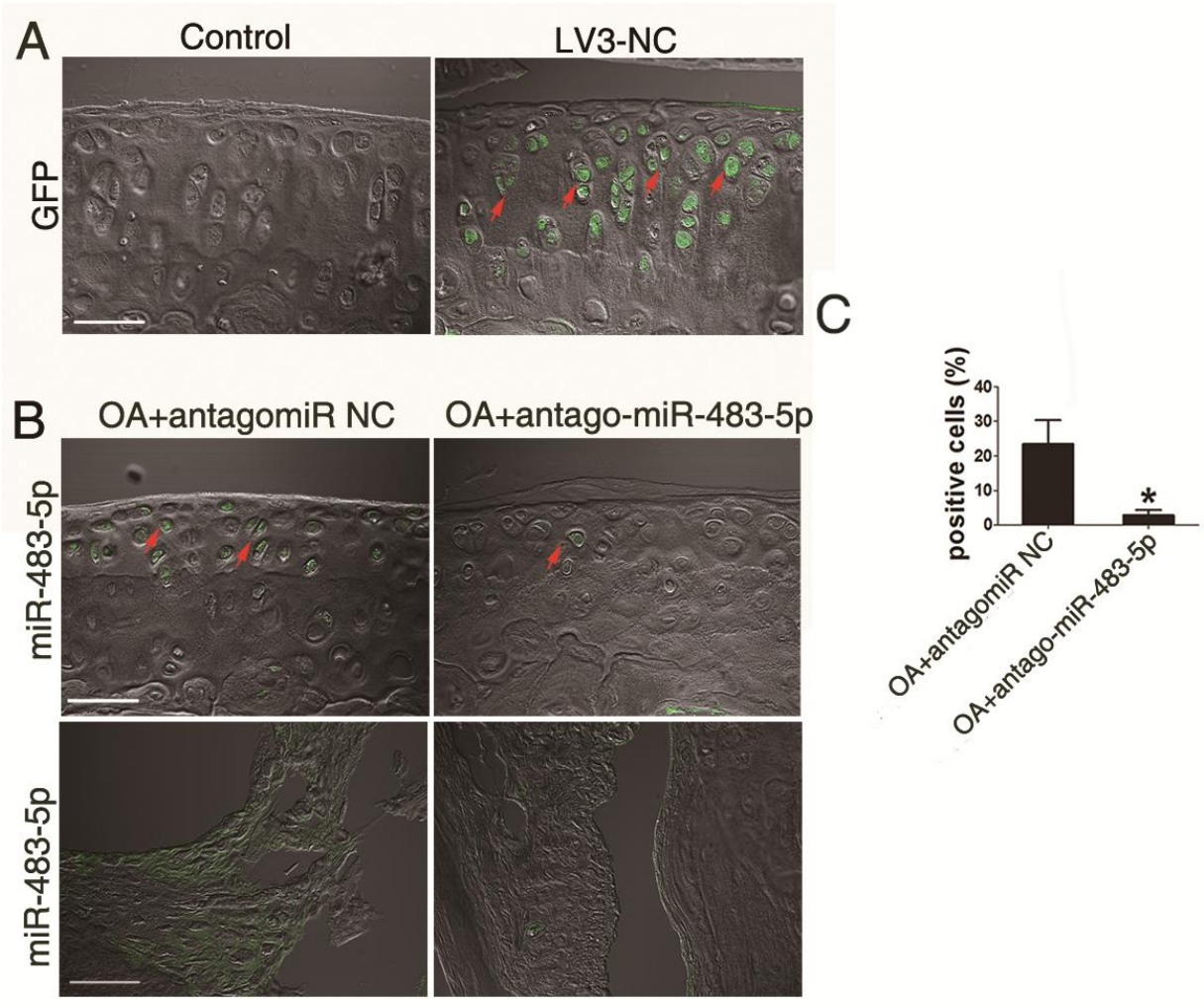
**Supplementary Figure S3. Generation and identification of TG483 mice in genotype.** (a) Schematic representation of the generation of TG483 mice. (b, c) Genomic DNA from tails of mice was subjected to PCR using the indicated primer pairs. Primers miR-483-forward and miR-483-reverse detected a 416 bp product of pri-miR-483 (b). Primers wild type and common detected a 650 bp product from wild-type rtTA DNA, whereas primers mutant and common detected a 340 bp mutant fragment (c). The migration positions of expected DNA products and mass standards in bp are shown to the left and right sides of the gels, respectively. DNA was detected by ethidium bromide staining.

**Supplementary Figure S4. Expression of Timp2 was down-regulated in human OA cartilage.** (a) Immunofluorescence analysis of Timp2 in knee joints from OA and normal human articular cartilage. Red arrows indicate positively-stained cells. Bar = 120 μm.

**Supplementary Figure S5. Downregulation of Timp2 may contribute to OA development through accelerating cartilage angiogenesis.** (a) Western blot analysis of Timp2 in primary chondrocytes infected with *Timp2* expression plasmid and control plasmid for 96 h. (b) HVECs were co-cultured with the supernatant from primary chondrocytes infected with *Timp2* expression plasmid and control plasmid for 12 h and tube formation assay was performed. (c) Western blot analysis of Timp2 in ATDC5 cells infected with LV3-si*Timp2* and control LV3-NC for 96 h. (d) Immunofluorescence analysis of Timp2 (upper, Bar = 40 μm), safranin O-fast green staining (middle, Bar = 40 μm) and immunohistochemical analysis of CD31 (bottom, Bar = 100 μm) in the knee joints from DMM OA mice injected intra-articularly with LV3-si*Timp2* or LV3-NC. (e) Real-time PCR analysis of miR-483-5p level in bone marrow cells (normalized to those in WT mice) from control and TG483 mice exposed to Dox for two weeks. (f) Western blot analysis of Timp2 expression in bone marrow cells from mice shown in (e). Red arrows indicate positively stained cells. Black arrows indicate the tidemark. Error bars represent the mean ±SEM. $^{**}P < 0.01$ by independent-sample t test for two groups.




**Supplementary Figure S1. Histologic staining of OA cartilage.**

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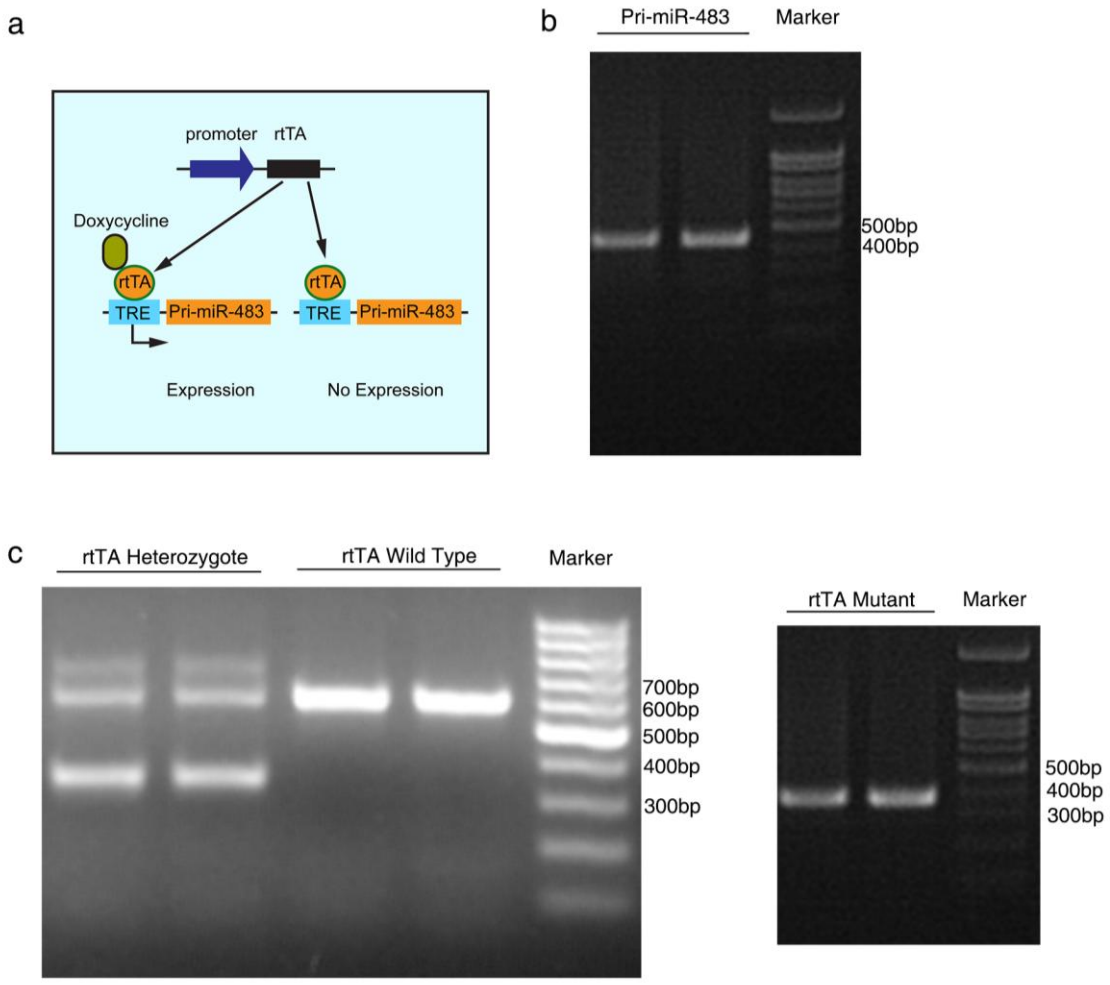


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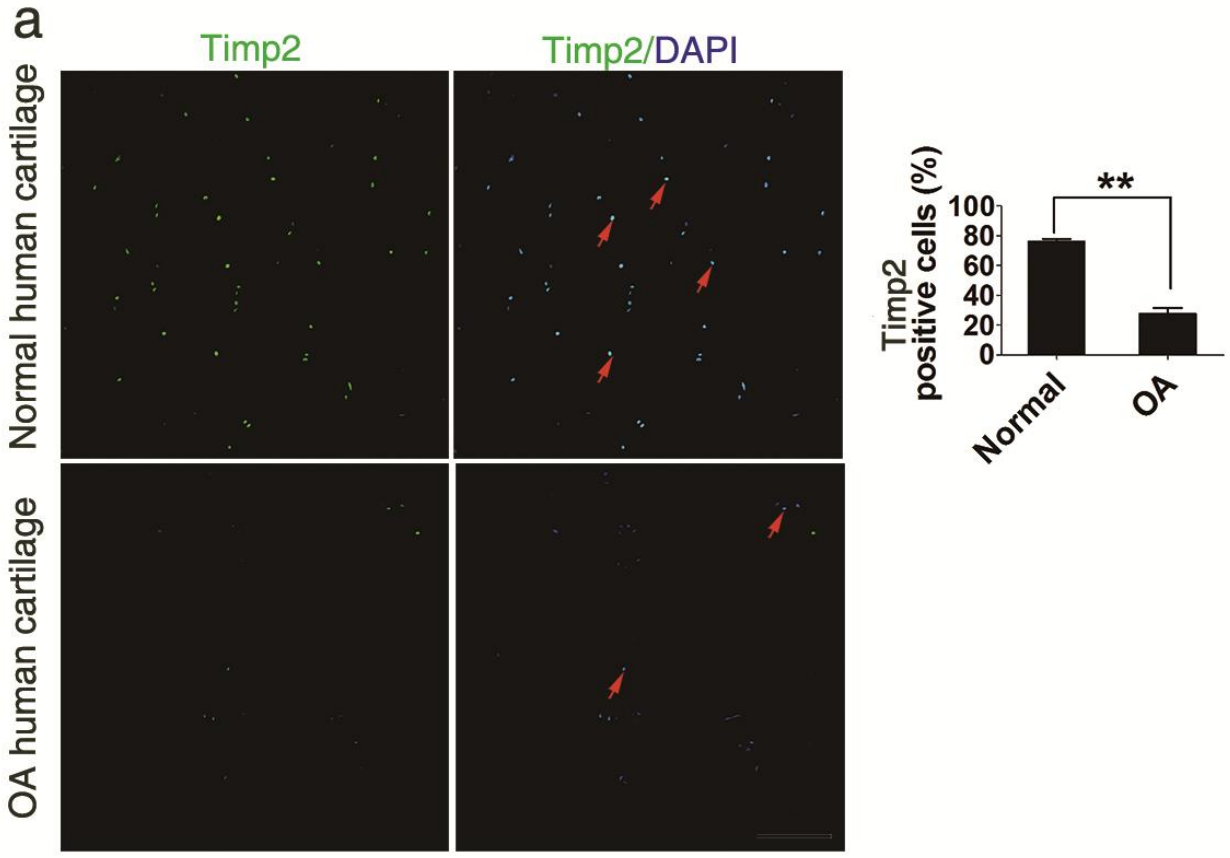


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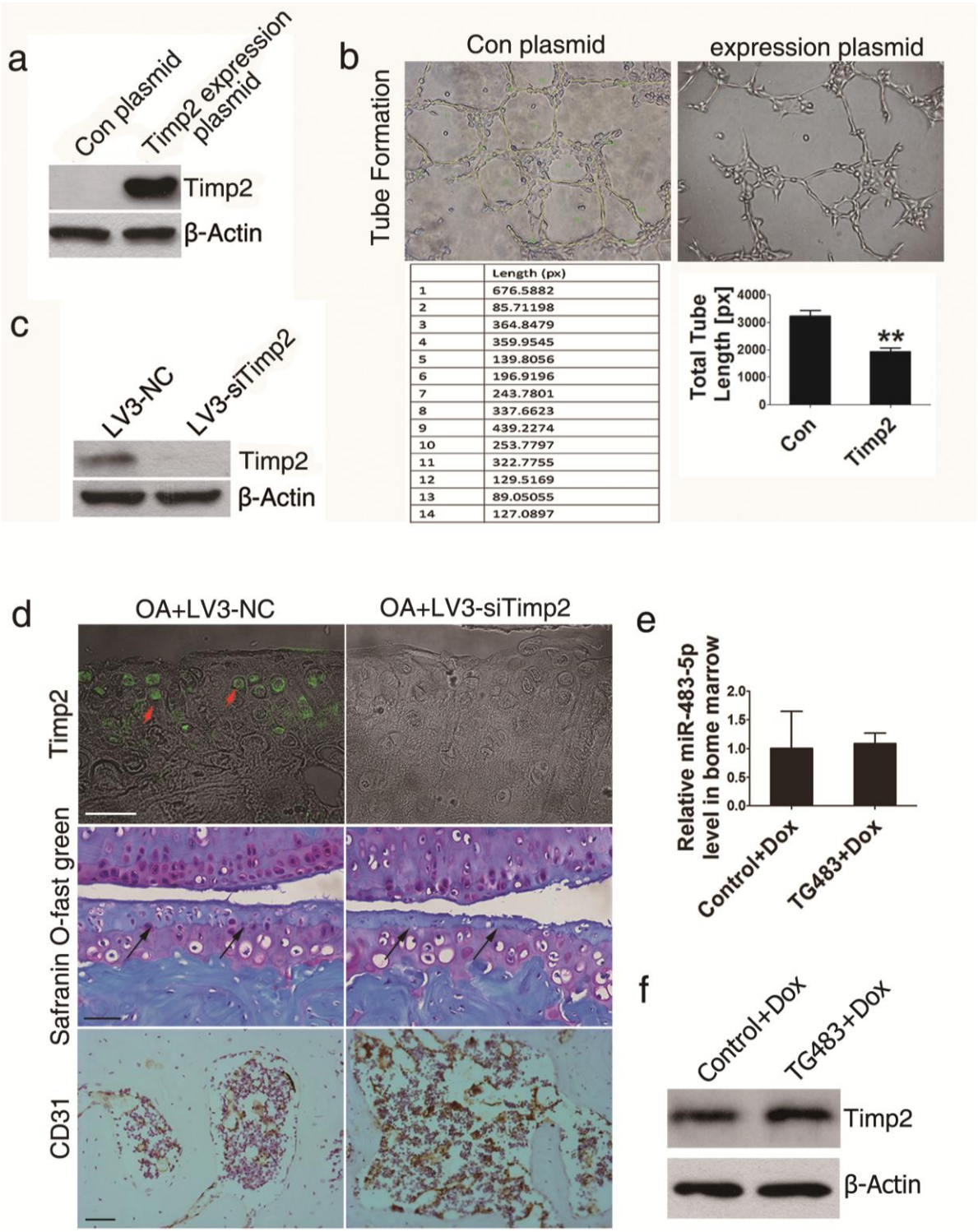


**Supplementary Figure S5. Downregulation of Timp2 may contribute to OA development through accelerating cartilage angiogenesis.**