

Title: Inhibition of transforming growth factor β -activated kinase 1 prevents inflammation-related cartilage degradation in osteoarthritis

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Supplementary Figure S1

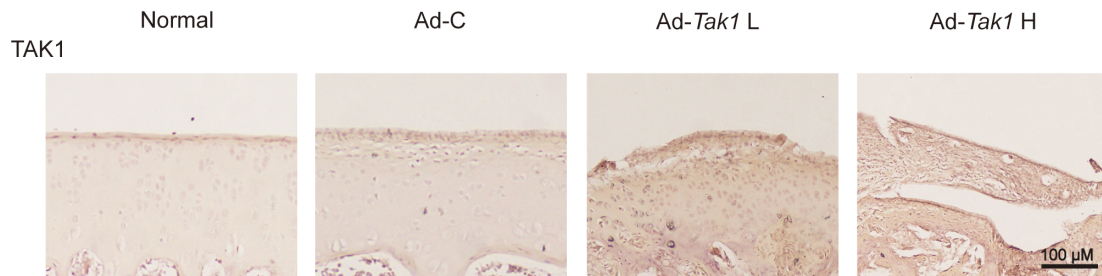


Figure S1 IHC staining of TAK1 for rat cartilage from each group. Scale bar, 100 μ m.

Supplementary Figure S2

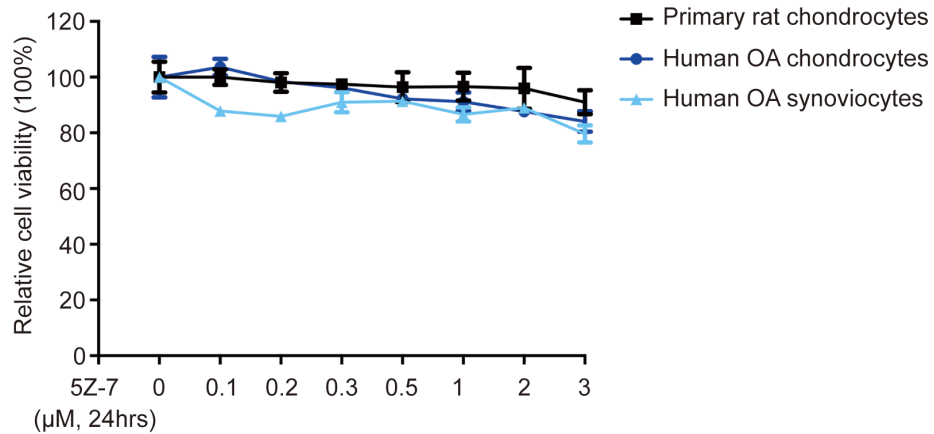


Figure S2 TAK1 inhibitor 5Z-7 exhibited no significant cytotoxicity to chondrocytes and synoviocytes. The relative cell viability of primary rat chondrocytes, human OA chondrocytes and synoviocytes treated with increasing doses of 5Z-7. Data were presented as mean \pm SEM.

Supplementary Figure S3

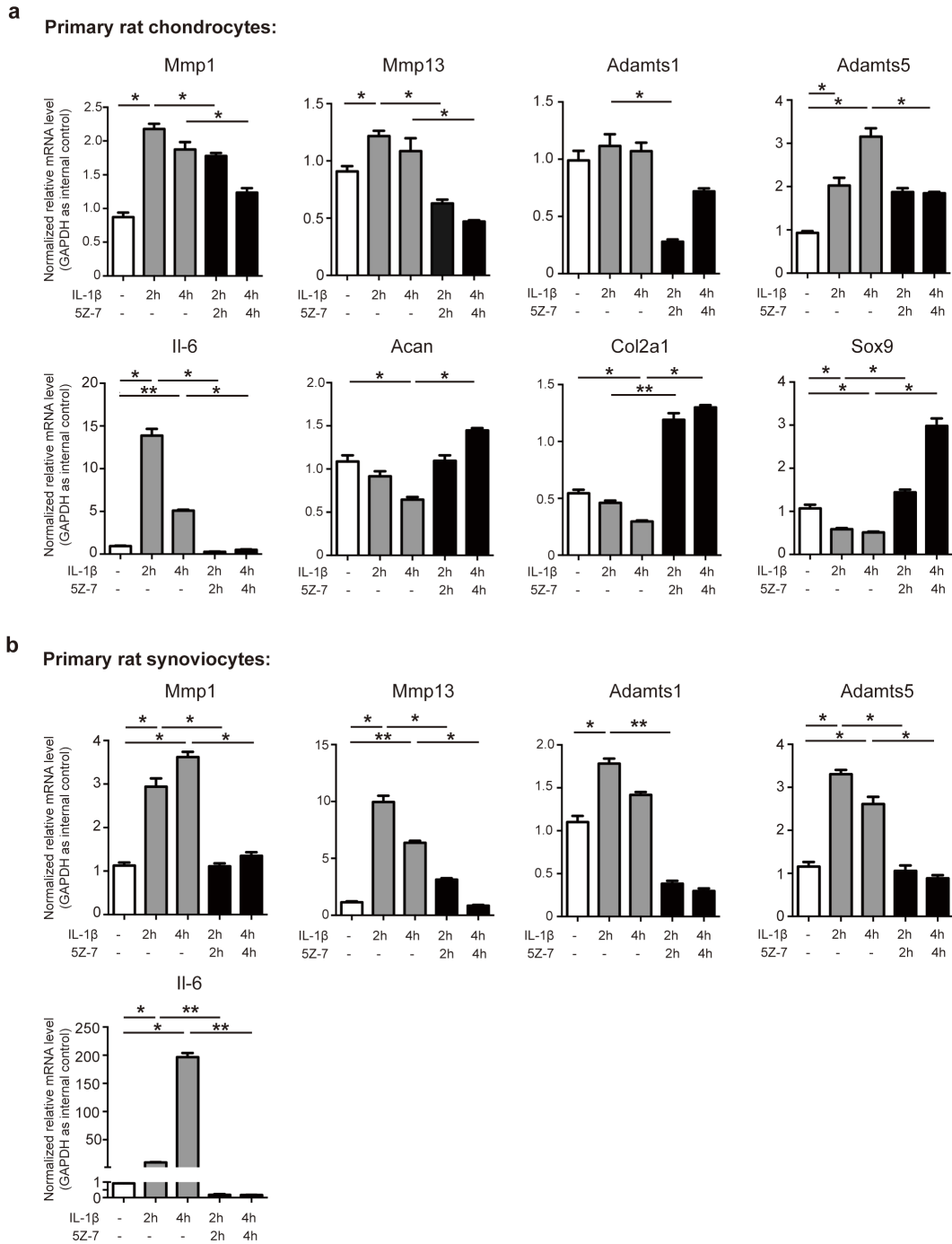


Figure S3 5Z-7 inhibited IL-1 β -induced expression changes of ECM-related genes in chondrocytes and synoviocytes. **(a, b)** The mRNA expression level of classic ECM-related genes in **(a)** primary rat chondrocytes and **(b)** primary rat synoviocytes treated with IL-1 β at indicated time points with or without 5Z-7. * P < 0.05, ** P < 0.01.

Supplementary Figure S4

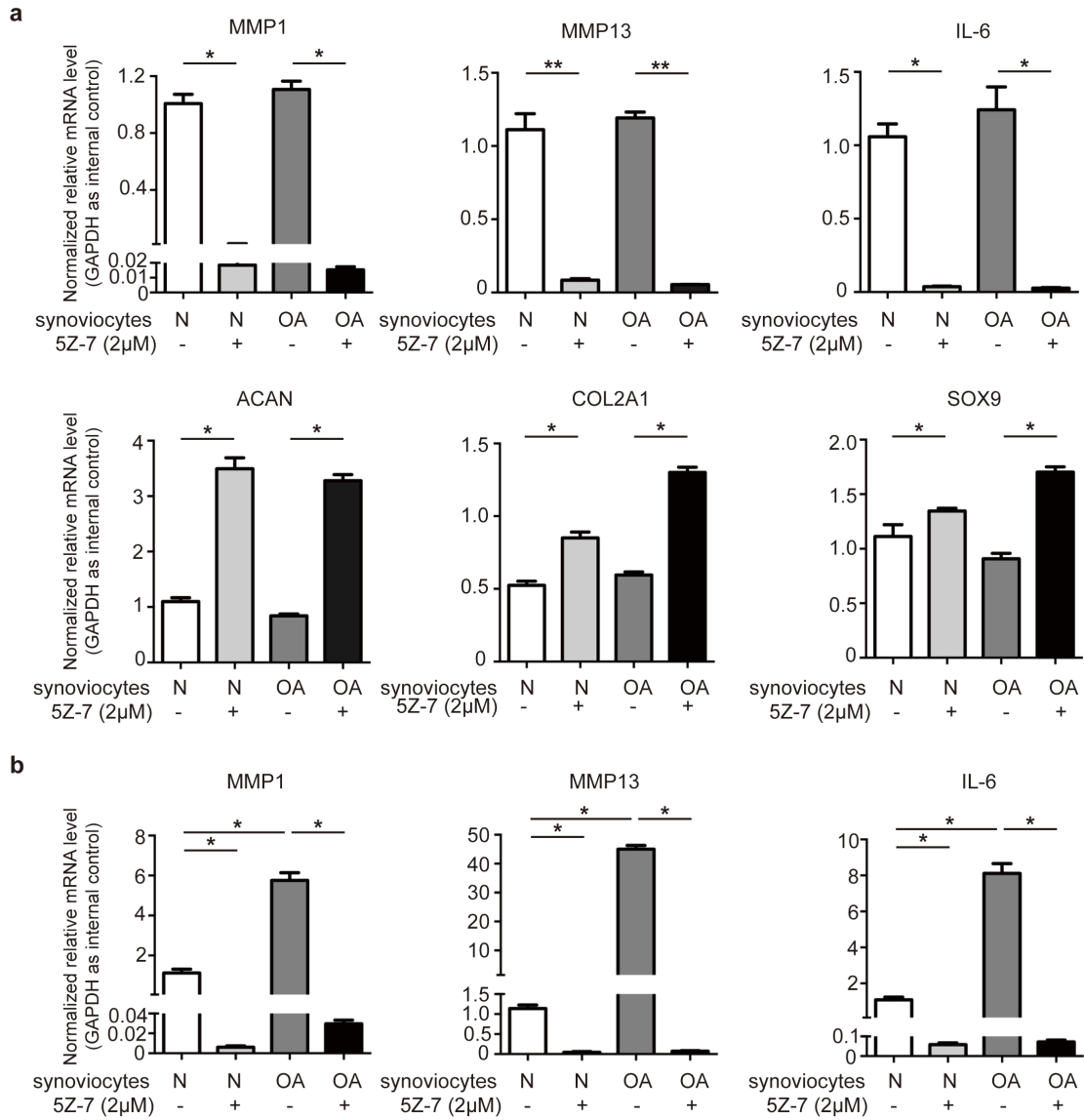


Figure S4 TAK1 inhibition by 5Z-7 modulated microenvironment homeostasis in the co-culture system. **(a, b)** The mRNA expression level of classic ECM-related genes in **(a)** chondrocytes and **(b)** synoviocytes within the co-culture system after 48 hrs. * $P < 0.05$, ** $P < 0.01$.

Supplementary Table S1: The real-time primer sequences for ECM-related genes in rat and human.

Primer sequences for the detection of target genes expression in rats

Gene name	Forward (5'-3')	Reverse (5'-3')
Mmp1	GCAGTGTGACACACATTGAC	GATAACCTGGATCCATGGAC
Mmp13	CTGCGGTTCACTTTGAGGAC	ACAGCATCTACTTTGTCGCC
Adamts1	GCCTACATGGTCACATCATTCT	TGGCGGTTGGCATCGTA
Adamts5	CACGACCCTCAAGAACTTTTGC	TCACATGAATGATGCCACATAA
Il-6	AAGCCAGAGTCATTCAGAGC	TTAGCCACTCCTTCTGTGAC
Acan	CCACTGGAGAGGACTGCGTAG	GGTCTGTGCAAGTGATTGAG
Col2a1	CACCGCTAACGTCCAGATGAC	GGAAGGCGTGAGGTCTTCTGT
Sox9	AGGAAGCTGGCAGACCAGTA	ACGAAGGGTCTCTTCTCGCT
Gapdh	GCAAGTTCAACGGCACAG	GCCAGTAGACTCCACGACA

Note: The annealing temperature of the primers is 60°C.

Primer sequences for the detection of target genes expression in human

Gene name	Forward (5'-3')	Reverse (5'-3')
MMP1	TGAACTCGGCCATTCTCTTG	AACGTCCATATATGGCTTGG
MMP13	ACTGAGAGGCTCCGAGAAATG	GAACCCCGCATCTTGGCTT
ADAMTS1	GTGATCGCATCATAGACTCC	TGGCTCCAGTTGGAATTGTG
ADAMTS5	GAACATCGACCAACTCTACTCCG	CAATGCCACCGAACCATCT
IL-6	TTCGGTACATCCTCGACGGCATC	CAGCTCTGGCTTGTTCCCTCACTAC
ACAN	ACTCTGGGTTTTTCGTGACTCT	ACACTCAGCGAGTTGTCATGG
COL2A1	CCAGATGACCTTCCTACGCC	TTCAGGGCAGTGTACGTGAAC
SOX9	AGCGAACGCACATCAAGAC	CTGTAGGCGATCTGTTGGGG
GAPDH	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA

Note: The annealing temperature of the primers is 60°C.