Epigenetic modifications of interleukin-6 in synovial fibroblasts from

osteoarthritis patients

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GAAACCATCCAGCCATCCTCCCCCATTTTCATTTTCACACCAAAGAATCCC AC<mark>CGCG</mark>GCAGAGGACCAC<mark>CG</mark>TCTCTGTTTAGACAAT<mark>CG</mark>GTGAAGAATGGA TGACCTCACTTTCCCCAACAGG<mark>CG</mark>GGTCCTGAAATGTTATGCA<mark>CG</mark>AAACA AAACTTGAGTAAATGCCCAACAGAGGTCACTGTTTTAT<mark>CG</mark>ATCTTGAAGA GATCTCTTCTTAGCAAAGCAAAGAAAC<mark>CG</mark>ATTGTGAAGGTA<mark>ACACCATGT</mark> **TTGGTAAATAAG**TGTTTTGGTGTTGTGCAAGGGTCTGGTTTCAGCCTGAA GCCATCTCAGAGCTGTCTGGGTCTCTGGAGACTGGAGGGACAACCTAGTC TAGAGCCCATTTGCATGAGACCAAGGATCCTCCTGCAAGAGACACCATCC TGAGGGAAGAGGGCTTCTGAACCAGCTTGACCCAATAAGAAATTCTTGGG TGC<mark>CG</mark>A<mark>CGCG</mark>GAAGCAGATTCAGAGCCTAGAGC<mark>CG</mark>TGCCTG<mark>CG</mark>TC<mark>CG</mark>TAG TTTCCTTCTAGCTTCTTTTGATTTCAAATCAAGACTTACAGGGAGAGGGAG CGATAAACACAAACTCTGCAAGATGCCACAAGGTCCTCCTTTGACATCCC CAACAAGAGGTGAGTAGTATTCTCCCCCTTTCTGCCCTGAACCAAGTGG GCTTCAGTAATTTCAGGGCTCCAGGAGACCTGGGGCCCATGCAGGTGCCC CAGTGAAACAGTGGTGAAGAGACTCAGTGGCAATGGGGAGAGCACTGGC AGCACAAGGCAAACCTCTGGCACAGAGAGCAAAGTCCTCACTGGGAGGA $\mathsf{TTCCCAAGGGGTCACTTGGGAGAGGGCAGGCAGCCAACCTCCTCT}$ AAGTGGGCTGAAGCAGGTGAAGAAAGTGGCAGAAGCCA<mark>CGCG</mark>GTGGCA AAAAGGAGTCACACTCCACCTGGAGA<mark>CG</mark>CCTTGAAGTAACTGCA<mark>CG</mark>A AATTTGAGGATGGCCAGGCAGTTCTACAACAGC<mark>CG</mark>CTCACAGGGAGAGC CAGAACACAGAAGAACTCAGATGACTGGTAGTATTACCTTCTTCATAATCC CAGGCTTGGGGGGCTG<mark>CG</mark>ATGGAGTCAGAGGAAACTCAGTTCAGAACAT CTTTGGTTTTTACAAATACAAATTAACTGGAA<mark>CG</mark>CTAAA<mark>TTCTAGCCTGTTA</mark> ATCTGGTCACTGAAAAAAATTTTTTTTTTTTCAAAAAAACATAGCTTTAGC TTATTTTTTTTCTCTTTGTAAAACTT<mark>CG</mark>TGCATGACTTCAGCTTTACTCTTT TAAAGGAAGAGTGGTTCTGCTTCTTAG<mark>CG</mark>CTAGCCTCAATGA<mark>CG</mark>ACCTAA GCTGCACTTTTCCCCCTAGTTGTGTCTTGCCATGCTAAAGGA<mark>CG</mark>TCACATT GCACAATCTTAATAAGGTTTCCAATCAGCCCCACC<mark>CG</mark>CTCTGGCCCCACCC TCACCCTCCAACAAGATTTATCAAATGTGGGATTTTCCCATGAGTCTCAA



Figure S1. Human IL-6 promoter sequence investigated in the current study. The DNA sequence of -1500bp~+500bp around human IL-6 transcription start site was shown. Transcription start site was labeled in red, CpG site was labeled in yellow, forward primer site was labeled in gray and reverse primer site was labeled in green.

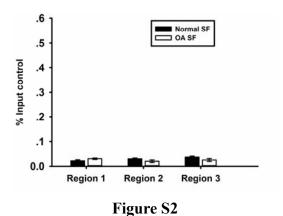


Figure S2. Negative control for ChIP assay. ChIP assays using IgG antibody in the region 1, 2, and 3 of human IL-6 were performed in normal and OA synovial fibroblasts (SF). The results were expressed as the percentage in input control. Data were shown as mean±SD.

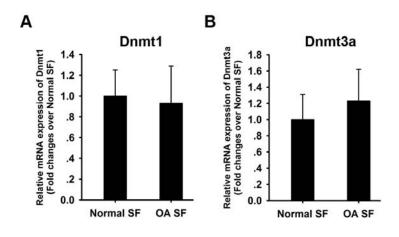


Figure S3

Figure S3. Dnmt1 and Dnmt3a mRNA expression in synovial fibroblasts from OA patients. Synovial fibroblasts (SF) were isolated from non-arthritic donors and OA patients and cultured *in vitro*. Total RNA was isolated for quantitative RT-PCR using Dnmt1 **(A)** and Dnmt3a **(B)** primers. β-actin was used as an internal control. The results are expressed as the fold-change relative to normal SF. Data are shown as the means±SD.

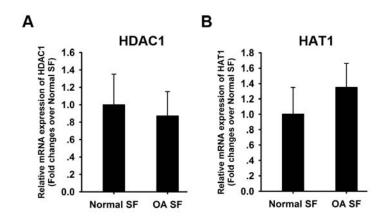


Figure S4

Figure S4. HDAC1 and HAT1 mRNA expression in synovial fibroblasts from OA patients. Synovial fibroblasts (SF) were isolated from non-arthritic donors and OA patients and cultured *in vitro*. Total RNA was isolated for quantitative RT-PCR using HDAC1 (**A**) and HAT1 (**B**) primers. β-actin was used as an internal control. The results are expressed as the fold-change relative to normal SF. Data are shown as the means±SD.

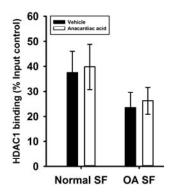


Figure S5

Figure S5. HDAC1 binding in region 2 of the IL-6 promoter in response to anacardiac acid treatment. Normal and OA synovial fibroblasts (SF) were treated with anacardic acid (4 μM). ChIP was performed to measure HDAC1 occupancy in region 2 of the IL-6 promoter. The results were normalized to the percentage of the input control. Data were shown as mean±SD.