Supplementary Figure Legends

Supplementary Figure 1. Schematic of the four steps employed to screen for chondrogenic agents among 2,500 natural and synthetic small compounds. The first and last screenings used the Col2GFP-ATDC5 system. The second and third screenings used toluidine blue staining and *Col2a1* mRNA level measurements by real-time RT-PCR, respectively.

Supplementary Figure 2. (A) Temporal profile of the *COL2A1* promoter activity in Col2GFP-ATDC5 cells transfected with the luciferase reporter gene under the treatment with insulin (10 μg/ml), TD-198946 (10^{-6} M), or the control vehicle. (B) Temporal profile of cell proliferation determined by the WST-8 assay in C310T1/2 cells treated with TD-198946 (10^{-9} and 10^{-6} M) or the control vehicle. All data are expressed as the mean ± s.d. (error bars) for 4 wells/group.

Supplementary Figure 3. Effect of TD-198946 on the transcriptional activities of BMP, TGF-β, and the canonical Wnt signalling pathway. Luciferase assays were performed using reporter plasmids containing the BMP responsive element (12XGCCG), TGF-β responsive element (9XCAGA), and the canonical Wnt signal responsive element (Top flash) in C3H10T1/2 cells treated with TD-198946 (10^{-7} M), BMP2 (100 ng/ml), TGF-β1 (1 ng/ml), or transfected with the plasmid expressing constitutively active TCF (T-cell factor) (TCF-CA). Data are expressed as the mean (bars) ± s.d. (error bars) for 4 wells/group.

Supplementary Figure 4.

Safranin-O stained sections of mouse medial tibial cartilage obtained from normal mouse knee joint of 8-week-old male mice, which were given a 10 μl intra-articular injection of TD-198946 (100 nM) every 5 days for 8 weeks. Scale bars, 100 μm.

Supplementary Figure 5. Identification of target transcriptional factors of TD-198946 for chondrogenesis. (A) Scheme of the screening steps for target factors by a microarray analysis of C3H10T1/2 cells treated with TD-198946 (10^{-7} M) or the control vehicle for 2 days. Genes that showed more than a 10-fold upregulation by TD-198946 were analysed by Gene Ontology. Among genes associated with the most over-represented term, 'Multicellular organismal development,' transcription-related genes were further selected by the GO term. (B) Gene ontology analysis of genes that showed more than 10-fold upregulation by TD-198946. 196 genes were analysed by the Gene Ontology (GO) biological process. The top 20 over-represented terms are shown. The x axis corresponds to *P*-values (logarithmic scale). (C) Six transcription-related genes associated with the Gene Ontology (GO) term, multicellular organismal development, among 196 genes that showed more than 10-fold upregulation by TD-198946.

Supplementary Figure 6.

Effects of TD-198946 treatment on Sox5, Mmp-13 and Col1a1 expression in OA.

Immunostainings of Sox5, Mmp-13 and Col1a1 from the inset box of figure (Figure 4A). Scale bar, 50 μm.

Supplementary Figure 7.

Safranin-O staining and immunostaining of Sox5 in normal and OA regions of tibial cartilage obtained from a total knee arthroplasty patient. Scale bar, 50 μm.

Supplementary Figure 8. Redifferentiating effects of TD-198946 on human articular chondrocytes that had been dedifferentiated by passaging 3 times (P3). *COL2A1* mRNA levels determined by real-time RT-PCR analysis and toluidine blue staining are shown as chondrogenic markers. dedifferentiated human articular chondrocytes were cultured with TD-198946 (10^{-9} and 10^{-6} M) or the control vehicle in DMEM for 7 days. Data are expressed as the mean (bars) ± s.d. (error bars) for 4 wells/group, and representative figures are shown.

Supplementary Figure 9.

Redifferentiating effects of TD-198946 on human OA articular chondrocytes that had been induced to degenerated chondrocytes by interleukin-1β (IL-1β). *COL2A1* and *COL10A1* mRNA levels determined by real-time RT-PCR analysis. Degenerated human articular chondrocytes were cultured with TD-198946 or the control vehicle under interleukin-1β treatment (10 ng/ml) in serum-free media for 7 days. Data are expressed as the mean (bars) ± s.d. (error bars) for 4 wells/group, and representative figures are shown.

*$P<0.01$ vs. the control vehicle under interleukin-1β treatment group

Supplementary Figure 10.

(A) Runx1 protein level determined by immunoblotting using an antibody to Runx1and actin in lysates of primary mouse chondrocytes from *Runx1*-floxed ($Runx1^{fl/fl}$) cells that were adenovirully infected with GFP or Cre.

(B) *Sox5 and Sox6* mRNA levels in primary mouse chondrocytes from *Runx1*$^{fl/fl}$ mice cultured with TD-198946 (10^{-7} M) or vehicle for 5 days after adenoviral (Ax) infection with GFP or Cre. *$P<0.01$ vs. Ax-GFP and TD treatment group.