Supplemental Materials and Methods

Differentiation induction of BMMSCs *in vitro*

For chondrogenesis studies, 1×10^5 cells were placed in a 48-well multiplate (Sumitomo Bakelite Co. Ltd., Tokyo, Japan) and cultured in chondrogenesis medium composed with high-glucose DMEM supplemented with 500 ng/ml bone morphogenetic protein 2 （BMP-2; R&D Systems）, 10 ng/mL transforming growth factor β3 (TGFβ; R&D Systems), 10^{-7}M dexamethasone (Sigma-Aldrich), 50 μg/mL ascorbate-2-phosphate, 100 μg/ml pyruvate, and 50 mg/mL ITS (Thermo Fisher Scientific) [18]. The chondrogenic cultures were fixed with Mildfolm and stained with toluidine blue.

For adipogenesis experiments, 1×10^4 cells were plated in a 48-well multiplate dishes and cultured in adipogenic medium, which consisted of 10%FCS-DMEM with 10^{-7}M dexamethasone, 0.5 mM isobutyl-1-methyl xanthine (Sigma-Aldrich), and 50 μM indomethacin (Wako, Tokyo, Japan) for 14 days. The adipogenic cultures were fixed in 4% paraformaldehyde and stained with fresh oil red O solution.

For osteogenesis studies, 1×10^4 cells were plated in a 48-well multiplate and cultured in calcification medium consisting of complete medium supplemented with 10^{-9}M dexamethasone, 20 mM β-glycerol phosphate (Wako), and 50 μg/mL ascorbate-2-phosphate for 14 days. These dishes were fixed in Mildform and stained with 0.5% alizarin red solution. Differentiation efficiencies were analyzed with ImageJ.

Microarray analysis

The microarray gene expression analysis of DMSO treated BMMSCs and 5zox treated

BMMSCs were performed using the Affymetrix Clariom™ S, mouse (Thermo Fisher Scientific) and the Affymetrix Transcriptome Analysis Console software (Thermo Fisher Scientific).

Quantitative RT-PCR (qRT-PCR)

Total RNA was collected from MSCs using TRI Reagent® (Molecular Research Center Inc., Cincinnati, OH, USA) and reverse-transcribed with the PrimeScript® RT Master Mix Kit (TAKARA Bio Inc., Shiga, Japan). QRT-PCR was performed by using Perfect real-time SYBR green II (TAKARA). To prevent amplification of contaminating genomic DNA, we designed all primers to span at least one intron. Primer sequences are listed in Supporting Information Table S1.

Western blot (WB) analysis

Cells were homogenized in SDS buffer and centrifuged at 9,000 × *g* for 10 min at 4°C to remove debris. Separation of nuclear fraction and cytoplasmic fraction was performed with NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific) following manufacture's instructions. The blotted PVDF membranes were blocked overnight with Block Ace (Dainippon Sumitomo Pharma, Osaka, Japan) and then probed with primary antibodies overnight at 4°C. Detection was performed with horseradish peroxidase (HRP)-conjugated secondary antibodies and Immunostar® LD (Wako) detection reagents. Antibody information is shown in Supporting Information Table S2.

Immunofluorescence

To visualize localization of Tak1 and Yes-associated protein 1 (Yap1)/ Transcriptional coactivator with PDZ domain (Taz), BMMSCs were cultured on coverslips and treated

with 5zox for 48 hours. Cells were then incubated in 4% paraformaldehyde for 15 min, and permeabilized in 0.5% Triton solution for 5 min at room temperature. Samples were blocked with 5% BSA and then incubated with primary antibody for 30 min, which included anti-Tak1 (ADI-KAS-ST009-E, 1:100 dilution, Enzo Life Sciences, Farmingdale, NY, USA) and anti-Yap1 (sc-376830, 1:100 dilution, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) or anti-Taz (560235, 1:100 dilution, BD Biosciences). Samples were washed three times and incubated with secondary antibody for 30 min. Cells were then stained with DAPI and visualized using a Keyence BZX-710 microscope (Keyence, Osaka, Japan).

siRNA treatment *in vivo*

For systemic delivery of the siRNA, the 20 μM siRNA against Tak1 or scrambled RNA were mixed with the AteloGene® (Koken Co., Ltd. Tokyo Japan) and intraperitoneally injected into the juvenile male mice (day 14 after birth) of C57BL/6N strain. The siRNA injection was performed two times every 24 hours. The BMMSC populations were collected as PαS fraction at 24 hours after 2nd injections. Cell cycle status was analyzed with the Vybrant Dye Cycle Violet (Thermo).